

The Exploration of Gingers in SE Asia – Some Milestones and Perspectives

Symposium keynote lecture

K. LARSEN

Department of Systematic Botany, University of Aarhus, Building 1540 DK-8000
Aarhus C, Denmark

The international symposia on Zingiberaceae are fora for the communication of new research and meeting places for all who are interested in Zingiberaceae and related families to exchange ideas and establish new contacts. Sometimes, it is also worthwhile to look back to former times and re-evaluate earlier researchers' works and to consider what we have achieved and what should be done. How can we, in the light of what has been done, do better in the future? How can we make the most of what are the available resources - human and economic. That is what I will try to do here today, hoping that it may be useful in our exchange of ideas during this meeting here in Singapore, which is and has been, for more than a century, one of the centres of research in taxonomy of Zingiberaceae in SE Asia, the region I know best and on which I will concentrate my speech.

One of the first scientific collections of gingers was made by the German medical doctor Engelbert Kaempfer who visited Thailand in 1690 on his way to Japan. We do not know much about his collections but Linnaeus described two small plants after him, *Kaempferia galanga* and *Kaempferia rotunda*. Linnaeus enumerated rather few species of Zingiberaceae in his *Species Plantarum* (1753), the starting point for botanical nomenclature. Besides these two species of *Kaempferia* we find *Zingiber zerumbet* which he referred to the genus *Amomum*, *Elettaria cardamomum* also mentioned as an *Amomum* species, and two *Curcuma* species, *Curcuma longa* and *Curcuma rotunda*, the latter now referred to *Boesenbergia*.

Johan Gerhard Koenig was another early explorer in SE Asia. He was German-Baltic by birth, studied for a few years under Linnaeus and became a Danish citizen before being sent as a medical doctor to the Danish colony Trankebar in India in 1767 where he undertook large collections. In 1779 he went to Siam and in 1781 to Ceylon. He became a good friend of Roxburgh who also looked after him during his last illness. He was one of the most important collectors in the 18 century even though he published little himself. He described 21 Zingiberaceae based on living material. The descriptions were sent to his friend Retzius for publishing in 1783. K. Schumann

characterized Koenig's descriptions as excellent. Koenig established 4 new genera, *Hura* (*Globba*), *Languas* (*Alpinia*), *Hedychium* (*H. coronarium*) and *Banksea*, later changed to *Costus* and *Costus malaccensis* Koenig. His Siamese collections were mainly from the island Yunk Ceylon, not to be mistaken for Sri Lanka, it is what today is known as Phuket in Thailand. His large collection of manuscripts was, thanks to Roxborough, given to Sir Joseph Banks, and is now bound in 21 large volumes in the British Museum; herbarium specimens are found there and in Copenhagen. In literature at least up to 1980, and maybe later, we read that Koenig's collection from Siam was lost. They were, however, rediscovered in Copenhagen Botanical Museum some 20 years ago.

Real exploration of the family Zingiberaceae began in the 19th century with important works, such as Roscoe's *Monandrian plants* from 1828. Roscoe was an English historian, and a botanist in Liverpool. He was born in 1753 and became partner in a bank where he lost his money and became bankrupt in 1820. He was also the founder of the botanic garden in Liverpool. In 1824-28 he published his magnificent work: *Monandrian plants of the order Scitamineae*. It was issued in 150 copies, including 112 hand-coloured lithographs. He treated 66 species of Scitamineae most of which were cultivated in the Botanic Garden.

Important descriptions were undertaken also by Roxburgh at the Calcutta Botanic Garden and by his successor, the Danish born Nathaniel Wallich (born Nathan Wulff in Copenhagen) who in his magnificent work, *Plantae Asiaticae Rariores*, published excellent illustrations of plants from British India including British Burma, among these several Zingiberaceae, e.g., *Kaempferia candida* and *Kaempferia elegans*, both described from Burma.

In the Dutch colonies, today's Indonesia, several botanists were active, among them C. L. Blume, a German born botanist, who worked for many years and collected several thousand numbers in Java at the beginning of the 19th century and later became director of the Rijksherbarium in Leiden. The Dutch botanist, Friederich Miquel, also made important contributions and described numerous species from SE Asia. He was a medical doctor from Groningen, and later director of the botanical garden in Rotterdam and professor in Amsterdam, finally succeeding Blume as director of Leiden Rijksherbarium from 1862. From the beginning of the 20th century, Valetton, another Dutch botanist educated at the university of Groningen, became one of the most important explorers of the Zingiberaceae mainly from Java. He first worked in Java at the Dutch sugar cane experimental station but finally became director of the herbarium in Buitenzorg, now Bogor. He took a great interest in Zingiberaceae and described numerous species with very careful and detailed drawings and descriptions from all over the region

as far eastward as New Guinea. His drawings are among the finest and most detailed in the family at that time and also his descriptions are extremely careful and precise. In his later years he worked at the Rijksherbarium in Leiden.

Another fine work from the middle of the 19th century is that of the Russian botanist, Paul F. Horaninov: *Prodromus monographiae Scitaminearum* from 1862. This work, although mainly a compilation of known literature, is still important for its time. He described species not only from Java, but also from New Guinea and other parts of the Dutch colonies in SE Asia.

The Italian botanist, Odoardo Beccari, is indeed admirable. He was born in Firenze in 1843 and died there in 1920. For more than 10 years he collected in Borneo, the Moluccas, Sumatra and Java, amassing several thousands of collections. He is perhaps best known for his extensive palm collections, though his zingiberaceous collections are as important. They are among the finest and most carefully prepared specimens found in herbaria. When one takes into consideration the extremely difficult conditions under which he worked in the field for months they are indeed excellent. They are today found as a special collection in the herbarium in Firenze where he became Director in 1876.

If we now try to summarize our knowledge towards the end of the 19th century and look at the comprehensive work of the De Candolles' *Prodromus systematis naturalis regni vegetabilis* we find the treatment of the Zingiberaceae from 1883. Here are enumerated 21 genera with in all ca 250 species. The largest genera are *Amomum* with 50, and *Alpinia* with 40 species, though the circumscription of *Amomum* does not correspond to ours.

Six years later, in 1889, we get an overview of the Zingiberaceae in a form that we can compare with today's systematics. That was done by a Danish systematist O. G. Petersen, a professor in Botany at the Royal Danish School of Forestry and Agriculture in Copenhagen, in the first edition of the "Die natürliche Pflanzenfamilien" edited by A. Engler and K. Prantl. Here, Petersen, who mainly worked with Neotropic Zingiberaceae, treated 24 genera with a total of ca 240 species including what we today recognize as Costaceae. Just to give an idea of how poorly the SE Asian tropics were known, the genus *Alpinia* was estimated to contain about 40 species, while we today recognize ca 250 species, whilst the other similarly large genus *Amomum* had 50 species including what we today treat as *Etlingera*. Finally the genus *Zingiber* was estimated to comprise ca 20 species, about one third of what we find today in Thailand alone.

The great breakthrough came with three botanists working about the turn of the century or around 1900, namely Schumann, Ridley, and Gagnepain.

I shall begin with Karl Moritz Schumann. The standard work on the family for about a century has been the large monograph on the Zingiberaceae from 1904 by Schumann in A. Engler's monumental world flora: "Pflanzenreich", in which he recognizes over 800 species. About half of these were described by Schumann himself from the German colonies in the eastern part of the area, New Guinea, and the Bismark Archipelago, but also from Borneo, Sulawesi and other areas. It is remarkable that in the 15 years from 1889 to 1904, the number of species almost tripled. In the subsequent 100 years we have been able to describe almost twice as many species. Holttum, in his treatment of the Zingiberaceae of the Malay Peninsula or rather Peninsular Malaysia, expressed a very critical attitude towards Schumann's work. This is impossible for me to understand and can only be caused by his anti-German feeling. Schumann's work is admirable even if we find today that many of the genera he treated have another circumscription than the one we now recognize. Let me add that in many works also in taxonomic literature we can read that Schumann's types were lost during the destruction of the Berlin Herbarium in World War II. That is true only for the herbarium material. The large collections of spirit material were miraculously saved and many types of Zingiberaceae are still preserved in the Berlin Herbarium in perfect condition.

In the French colonies several collectors worked all over Indochina in today's Cambodia, Laos and Vietnam. The same year as Schumann published his work, François Gagnepain wrote his account for "Flore Générale de l'Indo-Chine". He based his descriptions on very careful dissections and fine line drawings. In the herbarium in Paris we still find small envelopes with the material, the remains of his dissections, very often nicely arranged and glued to a piece of paper with fine pencil sketches. Gagnepain described Zingiberaceae not only from Indochina, but from all over tropical Asia.

In 1899, Henry Nicolas Ridley, Director of the Botanic Garden Singapore, published his account of the Scitamineae of the Malay Peninsula based on his own and several others' collections. He described more than 100 species, several of them from the botanical gardens in Singapore and Penang. But his contribution to the Scitamineae did not end with his work on the Ginger flora of the Malay Peninsula. He also contributed to our knowledge of the Zingiberaceae of Borneo in 1906 and the Philippines in 1909. He also described species from Indochina and Africa. All in all he described over 300 species. His work was critically discussed in the excellent paper by I. M. Turner, published in the year 2000, who writes, "His hurry to describe the myriad of undescribed taxa he encountered frequently led to scrappy, inaccurate, or even erroneous descriptions and nomenclatural and other taxonomic muddles. The mistakes Ridley made during his publishing career could probably provide all the examples needed for the International

Code. However, his achievements far outweigh his misdemeanours”, a sentence that might also be applied to the two other great names, Schumann and Gagnepain. In this connection we should also remember the words of Airy Shaw, who in an article unveiled a mistake made by the founder of Flora Malesiana, the late Prof. Van Steenis, who described a new genus that turned out to be some monocot leaves mounted with a legume flower. Airy Shaw’s conclusion was, “He who publishes nothing makes no mistakes”.

The next great synthesis came with Loesener’s contribution in 1930 to the second edition of “Natürliche Pflanzenfamilien”. It is, naturally, based on Schumann’s monograph with the same illustrations, but with a more “modern” approach to the generic concept.

Around the middle of the 20th century little was published except for Holttum’s revision of the Zingiberaceae of the Malay Peninsula published in Gardens Bulletin Singapore in 1950. It was based on material available there and mainly collected by Corner in 1930-40. Holttum based his work on careful dissections of living material as well as on Ridley’s collections. He paid much attention to inflorescence structures. His descriptions vary much in length and the drawings are often of little use as they are of a rather poor quality. His work is, however, still very useful when it comes to determination of species from the Malay Peninsula even if it also reflects the fact that Holttum, during the war, had very little access to literature that was not available in Singapore, something he was himself very well aware of and that he also told me during many fruitful discussions in the sixties when I worked in Kew and profited much from his knowledge. He expressed it once in the middle of the sixties in words along the lines of: “I wrote down what I knew about the Zingiberaceae of Malaya at that time”.

Between 1970-90, B.L. Burtt and Rosemary Smith from Edinburgh did a remarkable amount of work towards a better understanding of the Zingiberaceae, not least of Borneo. In an early paper they dealt with the taxonomic history of the classification of the Zingiberaceae, in which they pointed out the many nomenclatural problems that exist and suggested solutions. Rosemary Smith contributed several revisions and a fine overview and a new classification of the largest genus, *Alpinia*, a work that is still respected in spite of later molecular work.

The latest overview of the family was published in 1998 in the “Families and Genera of Vascular Plants” edited by Kubitzki, following the tradition of Engler in the new century. Here I treated the Zingiberaceae in collaboration with J.M. Lock, M. and P. Maas, on the basis of what we knew at that time. It was just a couple of years before the turn of the century and the molecular age was just beginning. Two years later Kress and his collaborators published their new approach to the system of the Zingiberales and our knowledge took a turn towards a true phylogenetic

system. That is where we are today and what we shall hear much more about at this symposium.

Now we can ask the questions, what have we achieved? How deep or complete is our knowledge of the Zingiberaceous flora of SE Asia? Let us take a quick look at the regions.

I have concentrated in this lecture on SE Asia, but I shall just mention with a few words the situation on the Indian subcontinent where Indian botanists are now contributing greatly. I am also sure that even if India belongs to a part of tropical Asia which has been rather well studied botanically for three centuries, there are still many areas in which undescribed taxa will be found as we have recently seen, e.g., from the Nicobar Islands, and the vast mountainous regions in the extreme north and northeast.

If we move from India towards the East the next country we meet is Myanmar. This was included in Hooker's "Flora of British India" but was far less collected than India. Today it is hardly possible to do serious collecting work in Myanmar due to the political situation, as the most interesting areas cannot be reached. I am, however, not in doubt that when the time comes that we can freely move around in Myanmar, many new taxa will be found. During my work with the genus *Caulokaempferia* I have seen material collected by George Forrest from the frontier between Myanmar and China representing more than one undescribed species, but the collections are too poor to be described properly. Undoubtedly, some of the old taxonomists would have described these as new species. Still new species have been described as, e.g., *Smithatris myanmarensis* W.J. Kress and *Mantisia wardii* Burtt & Smith. All in all the number of species documented from Myanmar is ca 150 and that is according to my experience very low. There is, in my opinion, no doubt that twice as many species occur in that country.

Let us turn to China where we have a new revision. China is a country where taxonomy has a high priority as a basic science strongly supported by the Academy of Science and the government. Chinese botanists are in these years doing an enormous amount of work collecting, describing and publishing. The great partnership between the Missouri Botanical Garden and the Chinese Academy of Science, which is producing the second edition of the Chinese Flora, now in English, is admirable. I co-authored the Zingiberaceae myself with 216 species in 20 genera. 141 species or over the half are endemic. This contribution was published in year 2000. In the same year my co-author, Dr. Wu Te-lin published one new species of *Alpinia* from the Guangdong province and more have been found since. We have also found recently that some of the supposedly endemic species also occur in Thailand and others will probably be found in northern Vietnam. So collecting gingers in the hilly southern tropical provinces of China is far from complete even if we may regard China as, probably the best studied country

from a Ginger specialist point of view. Also in year 2000 a new species of the hitherto monotypic genus *Vanoverbergia* was found and described by Funakoshi and Ohashi from Taiwan.

The Indochinese countries, Cambodia, Laos and Vietnam, were treated together by Gagnepain in the "Flore générale de l'Indochine" in 1908. Comprehensive collecting of Zingiberaceae has not been undertaken since the publication of that Flora in which 12 genera with, in all, 102 species were recognized; 62 of which were described by Gagnepain. From the numerous unnamed collections in Paris on which I worked with in the 60s and 70s, I described several new species but it was also clear that much old material was inadequate for describing. For several decades it has not been possible to travel to and in these countries. Fortunately the situation has completely changed and a new generation of botanists is now working seriously in exploring these countries with international cooperation, e.g., between Russian and American taxonomists and the National Museum in Hanoi.

From this fruitful partnership between the herbarium in St. Petersburg and Hanoi, the Orchids have been treated. I have seen photographs of numerous unknown Zingiberaceae collected in the north of Vietnam where the limestone region seems to be particularly rich in species. From the central limestone region of the country, Mark Newman several years ago described the new genus *Distichochlamys*. A few years ago a Russian zoologist found a second species, and immediately after, a third species was found. From Laos a beautiful little *Curcuma*-related plant was found by Dr. Jenjittikul at the Chatuchak flower market in Bangkok where loads of plants are brought from across the border at the Mekong River. We described this as *Laosanthus graminifolius*. The plant is now found in nurseries in the USA. Today we have documented over 200 species from these three countries, the question is then, "is the flora of these three Indochinese countries less rich than the Thai flora with 300 species?" I do not think that, and I believe that another 100 species will be added when the rich plant communities, particularly of Vietnam and Laos, are properly explored.

Peninsular Malaysia and Singapore are, as China, well studied botanically, but still new species are found. A beautiful species of *Haniffia* was recently refound and it was suddenly possible to solve a question about the occurrence of the genus in Thailand, first posed by Holttum. The plant mentioned by Holttum is not a *Haniffia*, but at the same time I collected a second species from Thailand. A few years ago we published a popular booklet, which gives an overview of the ginger flora. The time has come, however, where a full treatment of the Zingiberaceous flora of Peninsular Malaysia should be published. I am here thinking of a book similar to that of Gunnar Seidenfaden & Jeffrey Wood: The Orchids of Peninsular Malaysia

and Singapore. With the tradition of ginger research in the Singapore Botanic Gardens going back to Ridley and Holttum, it would be a fine way to commemorate these two pioneers.

From these more or less well documented countries we shall move to a region which is far more difficult to overlook. Coming from Peninsular Malaysia it might be natural to continue to Eastern Malaysia: Sarawak and Sabah. Here the situation is different. There are still vast areas to explore as seen from the many new species described of, e.g., *Boesenbergia*, *Zingiber*, *Etlingera*, *Alpinia*, and the new genus, *Tamijia*. This last genus was found by molecular studies to constitute its own subfamily Tamijioideae. There is still a vast field for exploration in this part of Malaysia and I have no doubt that many new taxa will be found on each new collection expedition there.

Indonesia is even more difficult to deal with, even though we now have an excellent overview in the checklist published by Newman, Lhuillier and Poulsen from 2004 covering the whole Malesian area. This vast country is centred around the island of Java with one of the best documented floras of the region, not much new could be expected from the national parks here. Quite different is the situation in Sumatra, which is one of the islands that should attract more attention. Several new *Globbas* have been described and I am aware of new *Boesenbergia* species which cannot yet be described due to the poor state of the existing material. This is indeed a problem with ginger collections made by general collectors who do not know how to preserve or describe the delicate structures of these plants. East of Java the challenge becomes even bigger. Few genera have been revised as, e.g., *Burbridgea*, endemic to Borneo. When I say Borneo it is strange that it seems that there are far more gingers in the northern and eastern Sarawak and Sabah than in Kalimantan. I am not in doubt that this is more due to lack of collecting than to phytogeographic peculiarities. The more we go East in the Malesian region the poorer is our knowledge. New Guinea and the Bismark Archipelago have not been visited for decades by systematic collecting expeditions concentrating on Zingiberaceae. We are here practically at the same level of information as 100 years ago when Schumann published his work. His short descriptions are often difficult to evaluate. Just to give an idea of the situation let me mention the genus *Riedelia* with about 100 species, which has never been revised and with numerous species only known from the type locality. In the genus *Plagiostachys*, much of the old materials are useless as the flower structures in many species cannot be seen due to the deterioration of the inflorescence in a slimy substance after flowering. Even though a survey of the Bornean species was published by Rosemary Smith in 1985, we know that it will be a long time before a revision can be written. What I have said about these two genera could be repeated in the case of *Pleuranthodium*.

The Zingiberaceae of the Philippines were treated by Ridley in 1909 based on specimens in the herbarium in Manila and the numerous collections made by Elmer and Merrill and also the classic collections by Haenke, Cuming and Blanco. Again here many species are only known from the type locality or very few collections.

Let me end this very short presentation of the SE Asian regions with a more comprehensive review of the status of Thai Zingiberaceae, the area I know best, and a flora with which I have worked for half a century. I still remember my first collection of the family from SE Thailand in January 1958. My field notes read, "small, terrestrial orchid with green flowers". It was a *Gagnepainia*.

At about 1960 our knowledge of the Zingiberaceae was mainly based on the old collections of Koenig from Phuket, Johannes Schmidt from Koh Chang, and the Kerr collections. All in all about 70 species were documented.

In 1980 I published the first annotated key to the genera of Zingiberaceae in Thailand. Here the number of species is estimated to ca 150. In 1996 I then published a preliminary list of species and the number had reached 200. In a newly published book, K. & S.S. Larsen 2005, "Gingers of Thailand", we have documented over 300 species, among which are also new endemic genera. To some of you the history of these is well known, but I still find it very important to mention it as it shows how much there is still to do all over SE Asia.

But, first a word about *Caulokaempferia*. In 1964 I described the genus *Caulokaempferia* based on a group of species formerly treated as *Kaempferia*'s. In the following years more species were described and 10 years ago five species from Thailand were known. Today the number has reached 18. We shall hear even more exiting news about this genus later today.

Few years ago my friend and collaborator, John D. Mood, came to me with two specimens that did not match any known taxon. One was collected in Southern Thailand on the mountains bordering Malaysia. We described that as *Siamanthis siliquosus*. Molecular studies have shown that it may be related to the Bornean genus *Burbidgea*. Its long silique-like fruits are, however, similar also to those of *Silquamomum tonkinense* from Vietnam. The other plant Mood brought to me looked like a *Kaempferia* but with a yellow *Zingiber*-like flower. I had indeed myself a colour slide of this plant given to me years previously, but I had never been able to identify it. It was a plant that was in the trade in the USA under the name *Boesenbergia aurea*, an illegitimate name as it is a later homonym. That, we also described as a new genus, *Cornukaempferia aurantiflora*. Shortly afterwards a second species turned up and now we have a third species which is about to be

published. Molecular studies have shown that it is related to *Zingiber*. The third new genus described in the last 5 years is *Smithatris*. It has an even stranger history. During an earlier *Heliconia* meeting in Singapore a plant was shown that had an inflorescence showing resemblance to a *Curcuma* and leaves with the look of a Marantaceae. John Kress and I described it as *Smithatris supraneeana* commemorating the late Miss Rosemary Smith, and at the same time Mrs. Supranee Kongpitchayanond from Thailand, who first presented it from her nursery, and thus brought it to scientific recognition. No botanist had ever collected this spectacular plant even though it grows commonly in a small limestone area just north of Bangkok, an area exploited by a cement factory. Furthermore it has been used since time immemorial by the local people to bring to the temples during the celebration of the Buddhist Lent, often together with *Globba*'s. Strangely enough the year after the Director of the Queen Sirikit Botanical Garden in Chiang Mai, Dr. Weerachai Nanakorn, had bought some rhizomes at a market in Myanmar where he was attending a conference, one of these collections turned out to be a second species, which had almost simultaneously been collected in the wild by John Kress also in Myanmar. This species was described as *S. myanmarensis*. So, three new spectacular new genera described over the last five years.

But also the number of species in the larger genera found in Thailand has astonished us. Two genera illustrate this: in my checklist from 10 years ago I estimated the number of *Zingiber* species to be ca 25. At that time I had a Danish Ph D student who undertook a revision of the genus; she came to the result that the number was 26, but that there were probably two or three undescribed species. Dr. Ida Theilade, however, became engaged in a different line of research and handed over her material to Dr. Pramote Triboun from the Bangkok Herbarium who then started a thorough collecting programme all over the country for three years. We now know, as we shall also hear later, that there are over 50 species of *Zingiber* in Thailand.

A similar result was reached by Dr. Charun Maknoi who has worked for years on the genus *Curcuma* where over 10 new species have been discovered during the last few years, some by John Mood and myself, some by Professor Puangpen Sirirugsa, others by Dr. Maknoi. Besides all the new species, many of the species treated as endemic in the Flora of China in the year 2000 have now been found also to occur in northern Thailand.

So much for the Flora of Thailand, which we regard as one of the better known regions in tropical Asia. Let me finally add that the genus *Amomum* in our checklist in "Gingers of Thailand" comes up to 16 species but according to a team of Thai botanists working on a revision of this genus the number may be twice as high.

I have tried here in this short overview of the history and the progress

of exploration of the zingiberaceous flora of SE Asia to give you an idea of how far we have reached and how much we still do not know. And then the questions come, "What are the priorities of research in the future? Should it be molecular studies or should we go out and collect more, or are there other fields that need to be explored?"

I find that there are three equally important fields:

1. The molecular approach has been shown to be important for a better understanding of the generic boundaries and relationships between species and the evolution of the Zingiberaceae as a whole. It has brought us a big step forward. This is laboratory work that must be based on a sound knowledge of the identity of the taxa. And even if there is still much to do it seems to me that the cream has already been skimmed off the milk.
2. Alpha taxonomy is important and it seems that we are, at the present time, exploring the last unknown frontiers of SE Asian biodiversity. New collectors to the region should be aware that expeditions visiting a tropical country are often taken to the so-called interesting localities by local botanists. This is where collectors have grazed repeatedly - and we know from Thailand that it is outside these areas that all the new discoveries are made.
3. Finally, there is a field that is much neglected: ecological and biological studies. Pollination and dispersal biology are unknown in the majority of species, even the fruits and seeds of many species are unknown. Here local botanists have a vast research field. But it seems that there is more prestige in being in the laboratory in a white coat and working with a big computer than getting out in the jungle.

Is there anything we can do here at this meeting to speed up the exploration of gingers? As funds are limited it might be a good idea to establish a kind of advisory board for Zingiberaceae research, a group of experienced local people who could point out areas to which collecting activities should be directed, taking also regional political aspects into consideration. Furthermore organizing research groups of young botanists with a strong scientific leadership that could attract funds. These activities might be facilitated through a newsletter, probably electronically distributed. These could be the ways to bring us forward by working together and avoiding duplication of research. If we can agree here on broad collaboration, to be open in our research and respect each others work, then, this meeting will be a big step forward and not only a statement of our knowledge today.

Selected Zingiberaceae Species Exhibiting Inhibitory Activity Against *Mycobacterium tuberculosis* H₃₇Rv: A Phytochemical Profile

A.M. AGUINALDO

Research Center for the Natural Sciences, College of Science, Graduate School
University of Santo Tomas, España, Manila 1023, The Philippines

Abstract

As part of the research efforts to identify plant species which may have potential against tuberculosis, a study was earlier conducted in collaboration with the Institute for TB Research, University of Illinois, Chicago, to randomly screen the crude alcoholic extracts of different plant species using the MABA assay, to determine any inhibitory activity against the causative agent, *Mycobacterium tuberculosis* H₃₇Rv. Of the five species belonging to the family Zingiberaceae, four were found to inhibit the growth of *M. tuberculosis* H₃₇Rv. These species included *Alpinia purpurata* (Vieill.) K. Schum., *Alpinia zerumbet* (Pers.) B.L.Burtt. & R.M. Sm., *Etilingera elatior* (Jack) R.M. Sm. and *Zingiber officinale* Roscoe. Each species was collected in bulk and subjected to extraction and several bioassay-directed chromatographic fractionations. The pure constituents obtained were analyzed for their structure using spectroscopic techniques. The bioactivity of the pure isolates, as minimum inhibitory concentration values, was likewise determined. The results showed the antitubercular activity to be present in the nonpolar extracts. Structure elucidation of the pure isolates revealed the presence of sterols (β -sitosterol, stigmasterol), sterol derivatives (β -sitosteryl- β -D-galactoside, β -sitosteryl-3-O-6'-palmityl- β -D-glucoside), phenyldecanoids (6-shogaol and 6-gingerol) and a flavonoid (kumatakenin). Determination of the MIC showed higher activity of the phenyldecanoids than the steroids, the steroidal derivatives and the flavonoid.

Introduction

Tuberculosis is a pandemic that has been afflicting the Philippines and other developing countries. The Philippines is one of six countries with half of all new cases. The number of reported new TB cases keeps rising. Because of

the rapid increase in TB incidence in Africa, there is a yearly 1% growing incidence worldwide. Data show that one third of the world's population is infected with *M. tuberculosis* and every year, nearly 2 million deaths are caused by TB (WHO, 2006a, b).

A number of risk factors affecting TB are HIV/AIDS and multiple drug resistance. For people living with HIV/AIDS, TB is the single biggest killer. Latent TB infection is reactivated to an active disease through HIV. The other risk factor, multiple drug resistance, leads to TB that does not respond to the standard drug treatment. A WHO survey indicated the presence of MDR-TB in 109 countries, with the highest rates in China and former Soviet Union. In 2006, WHO launched a six component "Stop TB Strategy." (WHO, 2006b).

Tuberculosis is caused by *Mycobacterium tuberculosis* (M tb), an intracellular pathogen affecting higher vertebrates. The search for drugs against tuberculosis uses the slow growing M tb in its bioassays. In the late 1980's, the UST Research Center for the Natural Sciences (RCNS) embarked on a natural products program to screen plants using *Mycobacterium* 607 or *M. smegmatis*, a surrogate fast growing and non-pathogenic organism. After a number of comparative tests using both organisms failed to show 100% agreement, it was deemed necessary to screen against the actual etiologic agent, *M. tuberculosis*, a unique organism with unique susceptibilities to drugs.

The Philippines is one of those countries in Southeast Asia with a rich diversity of flora. Though only 858 species were reported to be medicinal (Quisumbing, 1978), there are many more plants whose medicinal properties have not been tested. The general objective of the RCNS TB Group is to provide a scientific rationalization for the use of plants as sources of medicine against TB, either for phytopharmaceutical application or for new drug development. Its specific objectives include the random screening of plants for inhibitory activity against *M. tuberculosis* (done in collaboration with the Institute for TB Research of the University of Illinois in Chicago), identifying plant families / genera exhibiting inhibitory activities, isolating through a bioassay-guided procedure the constituents in the active fractions, identifying the structure of the constituents through a combination of spectroscopic methods, comparing structural characteristics and determining the minimum inhibitory concentration of the pure isolate. This paper collates the results of the different studies done on five species of Zingiberaceae, namely, *Alpinia purpurata* (Vieill.) K. Schum., *Alpinia zerumbet* (Pers.) B.L.Burtt. & R.M. Sm., *Etilingera elatior* (Jack) R.M. Sm., *Hedychium coronarium* J. König and *Zingiber officinale* Roscoe (Aguinaldo *et al.*, 1997; Agbayani *et al.*, 2002; Budoy *et al.*, 2004; Villaflores *et al.*, 2004; Villaflores, 2005).

Materials and Methods

Plant material

The voucher specimens were identified and kept at the UST Herbarium or the National Museum. Table 1 lists the herbarium vouchers, including locality, collection number, and collector.

Table 1. Herbarium voucher information (location, collection date & number, collector, herbarium identity).

	Location	Date of Collection	Collection number	Collector	Where deposited
<i>Alpinia purpurata</i>	Los Banos, Laguna	Feb 2004	USTH 4717	O. Villaflores	UST Herbarium
<i>Etlingera elatior</i>	Los Banos, Laguna	Apr 2002	Ref. No. 2002 - 120	C. Budoy	National Museum (PNH)
<i>Alpinia zerumbet</i>	Los Banos, Laguna	Apr 2002	Ref. No. 2002 - 113	M. Agbayani V. Arbolante	National Museum (PNH)
<i>Zingiber officinale</i>	Tuguegarao, Cagayan	Dec 2001	Ref. No. 2000 - 0481	M. Agbayani	National Museum (PNH)
<i>Hedychium coronarium</i>	Tuguegarao, Cagayan	Dec 2001	Ref. No. 2000 - 0481	M. Agbayani	National Museum (PNH)

Screening for bioactivity

Approximately 100g plant material (air-dried leaves, fresh rhizomes, fresh flowers) was ground and extracted with methanol (or ethanol). The filtrate was concentrated in vacuo at 40°C to give a crude extract. A portion (2 mg) of the crude extract was tested for % inhibitory activity against *M. tuberculosis* H₃₇Rv (Collins and Franzblau, 1997; Fischer *et al.*, 1998). If the crude extract showed activity, it was partitioned between water and hexane, dichloromethane and 1-butanol. Each organic layer was concentrated and a portion similarly tested for bioactivity.

Isolation (bioassay-guided) and structure elucidation

The plant materials (air-dried leaves of *A. purpurata*, fresh rhizomes of *Z. officinale* and *E. elatior*) were collected in bulk and extracted exhaustively with alcohol. The crude extract obtained from each was partitioned as above to obtain the active semicrude extract. The latter was fractionated repeatedly using silica gel column chromatography with gradient elution (hexane-DCM, DCM-MeOH) till pure isolates were obtained. Fractions

from each chromatographic step were assayed for bioactivity. Isolates were analyzed using a combination of spectroscopic techniques such as UV, IR, MS, ^1H -NMR and ^{13}C -NMR, including DEPT, COSY, HMQC and HMBC. These were again assayed for minimum inhibitory concentration (MIC).

Results and Discussion

Table 2 shows the percent inhibitory activity of the crude extracts against *M. tuberculosis* H₃₇Rv at 100 ug/mL. *A. purpurata* leaves exhibited the highest activity (94%, 100 ug/mL), followed by *E. elatior* rhizomes (86%, 100 ug/mL), and *A. zerumbet* (80%, 100 ug/mL). *Z. officinale* showed activity in the rhizomes but absence of activity in the leaves. Among the five species of Zingiberaceae tested, only *H. coronarium* did not exhibit any activity against *M. tuberculosis* H₃₇Rv. Since only the leaves of *H. coronarium* were tested and not the rhizomes, it is worth considering the rhizomes for future tests.

Table 2. Activity of the crude extracts vs. *M. tuberculosis* H₃₇Rv at 100 ug/mL.

	Inhibition (%) - rhizomes	Inhibition (%) - leaves
<i>Alpinia purpurata</i>	Not tested	94
<i>Etlingera elatior</i>	86	38
<i>Alpinia zerumbet</i>	80	Not tested
<i>Zingiber officinale</i>	41	-35
<i>Hedychium coronarium</i>	-59	Not tested

When the crude extracts of *A. purpurata* rhizomes, leaves and flowers were tested, the leaves showed the highest activity at two concentrations (62%, 64 ug/mL; 41%, 32 ug/mL) (Table 3). The percent inhibition values of the rhizomes and flowers were close (34% and 30%, 64 ug/mL; 21% and 17%, 32 ug/mL) and indicated less activity than the leaves.

Table 3. Activity of the crude extracts from the different plant parts of *A. purpurata* vs. *M. tuberculosis* H₃₇Rv.

Plant part	Inhibition (%) at 64 ug/mL	Inhibition (%) at 32 ug/mL	Inhibition (%) at 16 ug/mL
Rhizomes	34	21	12
Leaves	62	41	4
Flowers	30	17	7

Upon separation of the nonpolar, semipolar and polar constituents in the crude leaf extract of *A. purpurata*, the higher activity is in both hexane and DCM semicrude extracts (64-70%, 64 ug/mL; 58-61%, 32 ug/mL; 34-42%, 16 ug/mL) (Table 4). This indicates that the bioactive constituents have a nonpolar character.

Table 4. Activity of the semi-crude extracts from *A. purpurata* leaves vs. *M. tuberculosis* H₃₇Rv.

	Inhibition (%) at 64 ug/mL	Inhibition (%) at 32 ug/mL	Inhibition (%) at 16 ug/mL
Hexane	64	61	34
DCM	70	58	42
n-BuOH	35	10	6

For *Z. officinale* (Table 5), the bioactive constituents are largely nonpolar, being present in the hexane semicrude extract (61%, 100 ug/mL; 19%, 25 ug/mL), with an activity much higher than that in the DCM (9%, 100 ug/mL) or n-BuOH (-10%, 100ug/mL). Table 5 shows that for the rhizomes of *E. elatior*, the active constituents are in the nonpolar (hexane) and semipolar (DCM) extracts (34% and 35%, respectively, 100 ug/mL). The data for *A. zerumbet* show an activity for the DCM extract (76%, 50 ug/mL) which is more than triple that of the hexane extract (23%, 50 ug/mL). Tables 4 and 5 compare activities of the semicrude extracts per plant in order to determine the presence of the active constituents. The different studies used varied concentrations of the semicrude extracts.

Table 5. Activity of the semi-crude extracts from *Z. officinale* rhizomes, *E. elatior* rhizomes and *A. zerumbet* rhizomes vs. *M. tuberculosis* H₃₇Rv.

	Z. officinale rhizomes		E. elatior rhizomes	A. zerumbet rhizomes
	Inhibition (%) at 100 ug/mL	Inhibition (%) at 25 ug/mL	Inhibition (%) at 100 ug/mL	Inhibition (%) at 50 ug/mL
Hexane	61	19	34	23
DCM	9	-4	35	76
n-BuOH	-10	-8	0	Not tested

Extracts from the *Z. officinale*, *A. purpurata* and *E. elatior* were subjected to bioassay-guided isolation till pure isolates were obtained (Fig. 1). The details of the isolation, purification and structure elucidation are written elsewhere. From *Z. officinale* rhizomes, the phenyldecanoids 6-

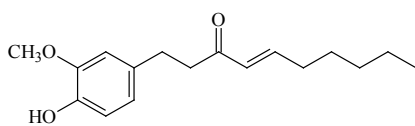
shogaol and 6-gingerol were isolated from the hexane extract. The sterols β -sitosterol and stigmasterol were obtained from the hexane extract of *E. elatior* rhizomes. And from the leaves of *A. purpurata* were obtained β -sitosteryl- β -D-galactoside, β -sitosteryl-3-O-6'-palmityl- β -D-glucoside, and kumatakenin, a flavonoid. The structures were identified upon spectral analysis and comparison of spectral data with the literature (Zaeoung *et al.*, 2005; Wright *et al.*, 1978; Gomes and Alegrio, 1998; Shaiq *et al.*, 2002; Urbatsch *et al.*, 1976; Wang *et al.*, 1989).

Upon bioassay of these isolates, the following MICs were obtained: *Z. officinale*: 6-shogaol (MIC 64 μ g/mL), 6-gingerol (MIC 33 μ g/mL); *A. purpurata*: β -sitosteryl- β -D-galactoside (MIC >128 μ g/mL), β -sitosteryl-3-O-6'-palmityl- β -D-glucoside (MIC >128 μ g/mL), kumatakenin (MIC >128 μ g/mL); *E. elatior*: stigmasterol (MIC >128 μ g/mL), β -sitosterol (MIC >128 μ g/mL). These results show the higher activity of the phenyldecanoid isolates from *Z. officinale* than the sterol glycosides, flavonoid from *A. purpurata* and sterols from *E. elatior*. Furthermore, the high percent inhibitory activity values of the crude extracts do not necessarily correlate with those of the pure isolates. With *Z. officinale*, there was an observed increase in activity as purification progressed. This was not observed with *A. purpurata* or *E. elatior* where constituents seem to exhibit synergism and are therefore more active as a mixture.

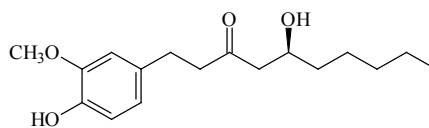
Having observed a common antitubercular property of extracts from Zingiberaceae species randomly selected, it is now worth investigating the other species of Zingiberaceae for reasons of bioactivity targeted search, and probable taxonomic utilization. With the furtherance of investigations on equally or more active species, there is sufficient justification for a comprehensive phytochemical reexamination of natural products elaborated by this family.

Acknowledgements

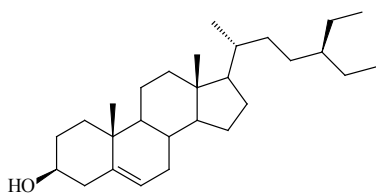
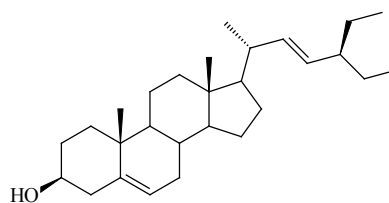
The author thanks Tan Chin Kee Foundation, Dr. Fortunato Sevilla III (UST College of Science), Dr. Corazon Menguito (Chemistry Department, UST CS) for the travel support; Prof. Scott Franzblau for the assays; Prof. Karsten Krohn (Univ. of Paderborn), Dr. Lindsay Byrne (Univ. of W. Australia), Prof. Tatsuo Yamauchi (Fukuoka Univ.), Dr. Fumiko Abe (Fukuoka Univ.), for the spectral analysis; Oliver Villaflores, Christopher Ryan Budoy, Katheryn Mandap, Roland Marcelo, Metchie Gay Agbayani, Vincent Arbolante, for the technical data; Asst. Prof. Rosie Madulid (UST Herbarium) and the National Museum for the botanical identification.

Figure 1. Compounds isolated from Zingiberaceae species.

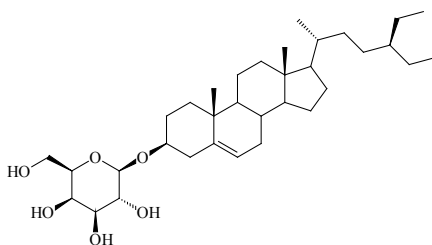
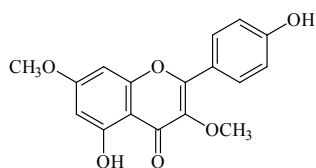
6-shogaol



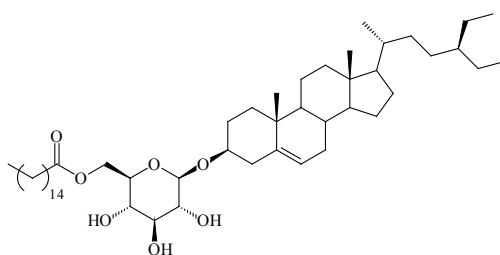
6-gingerol

 β -sitosterol

stigmasterol

 β -sitosteryl- β -D-galactoside

kumatakenin

 β -sitosteryl-3-O-6'-palmityl- β -D-glucoside

References

- Agbayani, M., V. Arbolante, S. Franzblau and A. Aguinaldo. 2002. Phytochemical screening of selected *Zingiberaceae* species inhibitory to *Mycobacterium tuberculosis* H37Rv, p. 28. *Abstracts of Papers*. 7th Annual Convention of the Natural Products Society of the Philippines, United Laboratories, MetroManila, Natural Products Society of the Philippines.
- Aguinaldo, A., M. Bueno, M. Cabanilla, K. Mandap and I. Lapuz. 1997. The chemistry of antitubercular constituents from selected Philippine plants, pp. 74-76. In: Proceedings of the 13th Annual Philippine Chemistry Congress, Asiaworld Hotel, Puerto Princesa, Palawan, Philippine Federation of Chemistry Societies.
- Budoy, C., S. Franzblau and A. Aguinaldo. 2004. Steroids from the antitubercular fraction of the hexane extract of *Etlingera elatior* rhizomes, p. 156. *Abstracts of Papers*, 19th Philippine Chemistry Congress, Sarabia Manor Convention Center, Iloilo City, Philippine Federation of Chemistry Societies.
- Collins, L. and S. Franzblau. 1997. Microplate alamar blue assay vs BACTEC 460 system for high throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrobial Agents and Chemotherapy* **41**: 1004-1009.
- Fischer, N. H., T. Lu, C. L. Cantrell, J. Castaneda-Acosta, L. Quijano and S. G. Franzblau. 1998. Antimycobacterial evaluation of germacranolides in honor of Prof. G. H. Neil Towers 75th birthday. *Phytochemistry* **49**: 559-564.
- Gomes, D., L. Alegrio. 1998. Acyl steryl glycosides from *Pithecellobium cauliflorum*. *Phytochemistry* **49**: 1365-1367.
- Quisumbing, E. 1978. *Medicinal Plants of the Philippines*. Katha Publishing, JMC Press, Quezon City, Philippines.
- Shaiq, Ali M., M. Saleem, W. Ahmad, M. Parvez and Y. Raghav. 2002. A chlorinated monoterpene ketone, acylated β -sitosterol glycosides and flavanone glycoside from *Mentha longifolia* (Lamiaceae). *Phytochemistry* **59**: 889-895.

- Urbatsch, L., T. Mabry, M. Miyakado, N. Ohno and H. Yoshioka. 1976. Flavonol methyl ethers from *Ericameria diffusa*. *Phytochemistry* **15**: 440-441.
- Villaflores, O. 2005. A Flavone and an Acylglucosyl Sterol from the Dichloromethane Leaf Extract of *Alpinia purpurata* (Vieill.) K. Schum. With Inhibitory Activity against *Mycobacterium tuberculosis* H37Rv. M. Sc. Chem. Thesis, University of Santo Tomas, Manila.
- Villaflores, O., A. Macabeo, D. Gehle, K. Krohn, S. Franzblau and A. Aguinaldo. 2004. A flavonoid from *Alpinia purpurata* (Vieill.) K. Schum., pp. 34-35. *Abstracts of Papers*. 9th Annual Convention of the Natural Products Society of the Philippines, U.P. Diliman, Quezon City, Natural Products Society of the Philippines.
- Wang, Y., M. Hamburger, J. Gueho and K. Hostettman. 1989. Antimicrobial flavonoids from *Psiadia trinervia* and their methylated and acylated derivatives. *Phytochemistry* **28**: 2323-2327.
- World Health Organization. 2006a. Tuberculosis Facts. Retrieved June 26, 2006 from the World Wide web: <http://www.who.int/tb/publications/en/>
- World Health Organization. 2006b. Tuberculosis, pp 1-4. Retrieved June 26, 2006 from the World Wide Web: <http://www.who.int/mediacentre/factsheets/fs104/en/index.html>
- Wright, J. L. C., A. G. McInnes, S. Shimizu, D. G. Smith, J. A. Walter, D. Idler and W. Khalil. 1978. Identification of C-24 alkyl epimers of marine sterols by ¹³C nuclear magnetic resonance spectroscopy. *Canadian Journal of Chemistry* **56**: 1898-1903.
- Zaeoung, S., A. Plubrukarn and N. Kewapradub. 2005. Cytotoxic and free radical scavenging activities of Zingiberaceous rhizomes. *Songklanakarin Journal of Science and Technology* **27**: 799.

Ethnobotanical Notes on Gingers of the Huon Peninsula in Papua New Guinea

B.B. BAU¹ AND A.D. POULSEN²

¹Papua New Guinea Forest Research Institute, P.O. Box 314, Lae 411 Morobe
Province, Papua New Guinea

²Royal Botanic Garden, 20A Inverleith Row, Edinburgh EH3 5LR, Scotland

Abstract

Only few studies on useful gingers in Papua New Guinea have been published and we were only able to find information on two commonly used species. We conducted a 2-weeks preliminary study in the Huon Peninsula to document the species of gingers and their local names in the Kote language and their uses by the indigenous people. All species encountered were useful: four species of *Etlingera*, three species of *Amomum* and one species of *Zingiber*. It is recommended that further surveys should be conducted on gingers of Papua New Guinea to understand their taxonomy and ethnobotany in order to devise an appropriate ginger conservation program for the local communities.

Introduction

Papuasiasia, including Papua New Guinea (PNG), harbours approximately 8 native genera and 207 species of gingers (Zingiberaceae) as proposed by Hoft (1992). The family still needs to be thoroughly surveyed before the total species pool can be determined. With more than 800 language groups, PNG is at the same time culturally very diverse. Most of these groups have developed intricate people-to-plant associations including local names and uses for many plants. The uses of plants are still very important for the subsistence strategies employed by the people in many remote villages (Damas, 1998). Detailed ethnobotanical studies in Borneo have found that the ginger family (Zingiberaceae) includes numerous species useful to the local people (Christensen, 2002) and many of these may also be horticulturally important. It is likely that the people of PNG still maintain comprehensive ethnobotanical knowledge but it needs proper documentation as soon as possible before the information disappears

due to the increasing globalisation. The information on gingers and their traditional uses will be important in order to facilitate proper management and conservation strategies either *in situ* or *ex situ*.

Several papers and books have already been published on traditionally important plants of Papua New Guinea as medicines but had made less emphasis on other uses (Paijmans, 1976; Woodley, 1991). Only two gingers have so far been reported as being commonly used: *Amomum aculeatum* Roxb. and *Zingiber officinale* Roscoe (Holdsworth and Mahana, 1983), both of which are used against fever or influenza. The latter is also used throughout Papua New Guinea by the indigenous people to relieve cough (Holdsworth, 1977; Holdsworth and Damas, 1986).

Study Area

The study was conducted in November 2001 inland from Finschhafen on the Huon Peninsula at Jivevaneng Village (6°30'S 147°47'E) at 300–500 m above sea level and at Nanduo Village (6°26'S 147°40'E) at 600–700 m above sea level (Fig. 1.). Both of these villages are inhabited by people speaking the Kote language and have a patrilineal society (R. Banka, 2001, pers. comm.). The Finschhafen area has many coastal and inland villages that are separated by the rugged terrains comprising the Cromwell and the Saruwaged Ranges. The geological aspects of the topography are a major factor that makes the villages inaccessible to basic governmental services. Because of inaccessibility the people still rely largely on forest resources for their survival. The habitat surrounding the village includes old garden sites, and the degraded roadside areas are composed of common weeds species. More pristine forest is found a few kilometres away from the villages.

Materials and Method

The following standard methods were used for plant collecting. The vegetation was explored to locate the conspicuous leafy shoots of ginger. The search was subsequently intensified to find flowers and fruits. Photographs were taken of these before the rhizomes were dug up. This was done carefully to avoid breaking any attached inflorescences or infructescences. The specimens were collected in three sections: 1) the base with the rhizomes and flowers or fruits attached (if any), 2) the mid-section with 2–3 leaves, and 3) the top of the shoot with 4–5 leaves depending on size of leaf.

Interviews with guides from the nearby villages were conducted during the collecting and notes were taken on local names in the Kote language and uses. The collections were deposited at the Papua New Guinea



Figure 1. The study sites are situated approximately 100 km E of Lae in Morobe Province, Papua New Guinea. (Map courtesy Encarta Encyclopaedia of 1988–1997).

National Herbarium (LAE) with duplicates sent to the Royal Botanic Gardens, Kew (K) and University of Aarhus (AAU), Denmark.

Results

All collected species (eight) of gingers were found to have known uses by the Kote speaking villagers at the Huon Peninsula: four species from the genus *Etlingera*; three species from the genus *Amomum*, and one species from the genus *Zingiber*. Each species has one to four uses. The information from seven of these species is documented for the first time in the present paper, whereas the information on one species has already been published.

Description of species

***Amomum aculeatum* Roxb.**

Material collected: *Bau et al.* LAE 86303, Jivevaneng, 9 Nov 2001 (LAE).

Vernacular name: *Asabareng*

Uses: 1) The pseudostem of young leafy shoot is beaten and the extracted juice is rubbed externally on the body and head and has a cooling effect in easing pain. 2) The fruits have a sweet taste and are eaten.

Description: Terrestrial herb to 2.5 m; rhizome thick; leafy shoots to 20 cm apart; base to 5 cm diam.; sheath brown at base - green at top, glabrous and shiny; ligule slightly bilobed; petiole 1.5 cm, base slightly purple; lamina obovate, plain green, pale green beneath, glabrous, midrib pale or white, apex acuminate, base \pm oblique. Inflorescence radical, peduncle to 10 cm long; corolla lobes pale pink; labellum white with red lines in centre basally and yellow above; stamen white, anther crest with two extended wings. Infructescence globular; fruits dark purple, indehiscent; lobes extended above, with yellow lines to base. *Fig. 2.*

Habitat: ridge top near an old garden near village.



Figure 2. Inflorescence of *Amomum aculeatum* Roxb. This species has very juicy fruits when ripe and they are edible. Photo: A.D. Poulsen

***Amomum maximum* Roxb.**

Material collected: *Bau et al. LAE 86308*, Jivevaneng. 9 Nov 2001 (LAE).

Vernacular name: *Sâsiric*

Uses: 1) The inner part of the young leafy shoot is scraped and applied externally on the knee against aches. 2) The fruits are eaten by bandicoots and therefore the plant is grown as an attractant to facilitate hunting near the village.

Description: Terrestrial herb to 2 m; leafy shoots clumped; leafless to 1.3 m, leaves clustered at top, 8–10 leaves per shoot; base of leafy shoot 5 cm diam. gradually decreasing to 2 cm; sheath yellowish at base, pale green to the top; ligule membranous, bilobed, caducous; petiole 5 cm; lamina plicate,

plain green, dull pale beneath, pubescent, almost corrugated, base oblique. Inflorescence radical from rhizome; peduncle 6–11 cm long, bractless, pale green, bracts in spike not persistent. Flowers not seen. Infructescence, globular; fruits irregularly winged, immature, yellow-green, remnants of persistent calyx. *Fig. 3.*

Habitat: Common in old gardens near village at about 300 m elevation.



Figure 3. *Amomum maximum* Roxb. The fruits attract the bandicoots which is a desired game species. Thus the *A. maximum* is planted to make hunting easier. Photo: A.D. Poulsen

Amomum sp. 1

Material collected: *Bau et al. LAE 86358*, Nanduo, 14 Nov 2001 (LAE).

Vernacular name: *Bareng-bafu*

Uses: The flesh inside the leafy shoot is scraped off, squeezed, strained and drunk to treat colds, flu, stomachache and headache.

Description: Terrestrial herb, 2.6–3.8 m tall, leafy shoot with 10–28 leaves, clumped; base of leafy shoot white; sheath green, glabrous, ligule bilobed pale green; petiole short; lamina to 45 x 8.5 cm, green, glabrous above, beneath pale green. Inflorescence radical, peduncle 10–12 cm long; calyx brown, persistent; corolla lobes pale yellow; labellum tunnel-shaped, yellow with red stripes forming into the corolla, apical lobe shorter than style; anther crest white. Infructescence green, immature seeds white. *Fig. 4.*

Habitat: Disturbed forest area near village.



Figure 4. *Amomum* sp. 1, rhizomes, fruits, flowers and leaves. The flesh of the leafy shoots are used to treat colds and flu, stomach ache and body ache. Photo: A.D. Poulsen

***Etlingera labellosa* (K. Schum.) R.M. Sm.**

Material collected: *Bau et al.* LAE 86301, Jivevaneng, 9 Nov 2001 (LAE).

Vernacular name: *Barengopo*

Uses: 1) Leaves are used to cover tubers of *Colocasia antiquorum* Schott when these are boiled in water with added oil to prepare a meal. 2) The beaten pseudostem (using hard sticks) is squeezed to extract juice, which is applied externally against body ache. 3) The twisted and beaten pseudostem is used for making climbing ropes. 4) Fruits are sweet and eaten.

Description: Terrestrial herb to 4 m; rhizome long-creeping; unpleasant smell of cabbage and soap when cut; scales on rhizome white or greenish brown when exposed; base of leafy shoot to 8 cm diam.; sheath green and glabrous; petiole 1.5–2.5 cm, green; lamina to 78 x 15 cm, plain green beneath; base unequal. Inflorescence radical from rhizome; peduncle 3–7 cm long, pale brown to white; calyx white to pale pink at apex; central lobe of labellum emarginate, lateral lobes pale red to white; filament white, anther white with central pink and pink at crest; stigma dark pink. Infructescence subterranean, with 1–2 fruits; fruit ca 2.5–3 cm diameter; densely covered by pale brown hairs. *Fig. 5.*

Habitat: Near forest trail in an old garden area near village.



Figure 5. The leafy shoots of *Etlingera labellosa* (K. Schum.) R.M. Sm. are used as climbing ropes for coconut trees. The flesh is also used to treat all kinds of body aches. Photo: A.D. Poulsen

***Etlingera* sp. 1**

Material collected: *Bau et al. LAE 86300*, Jivevaneng, 9 Nov 2001 (LAE).

Vernacular name: *Safanang*

Uses: The leaves are covered with the bark of *Paraserianthes falcataria* (L.) Nielsen and cooked over the fire; the cooked leaves produce a very strong aromatic smell and are worn as traditional decorations during *sing sings* (traditional dances) and folk celebrations.

Description: Terrestrial herb to 1.5 m; rhizome long-creeping, when cut with strong taste and smell of anis seed (*Pimpinella anisum* L.); base of leafy shoot to 2 cm diameter; sheath green, reticulate, not pubescent; ligule at least 5 mm, green; lamina plain green, young leaves reddish brown. Inflorescence from rhizome some distance from base; peduncle 2–8 cm long; bracts pale pink, calyx pale red, petals dark pinkish red; labellum pink; stamen pale pink; stigma white.

Habitat: The population was found near a bush track in an old garden area. It was growing amongst *Bambusa* sp. below several cultivated *Cocos nucifera* L.

***Etlingera* sp. 2**

Material collected: *Bau et al. LAE 86302*, Jivevaneng, 9 Nov 2001 (LAE).

Vernacular name: *Gamiong*

Uses: The fruits are chewed as a substitute for betel nut (*Areca catechu* L.).

Description: Terrestrial herb to 2.5 m; rhizome ± long-creeping, scales brown;

leafy shoot with c. 20 leaves; base of leafy shoot to 3 cm diam., brownish green; sheath reticulate, greenish brown, pubescent on cross-ribs; petiole 0.5 cm; lamina plain green. Young inflorescence pink, flower plain red, anther brown, stigma white. Smell of cut rhizome and crushed leaves faintly like *Etlingera elatior* (Jack) R.M. Sm.

Habitat: Old garden site near village.

***Etlingera* sp. 3**

Material collected: *Bau et al.* LAE 86337, Bembavaneng Hill, Nanduo, 14 Nov 2001 (LAE).

Vernacular name: *Zunzun*

Uses: 1) The fruit is chewed as betel nut (*Areca catechu* L.). 2) The stems and leaves are used to make small shelters to hide in when hunting bush fowls.

Description: Terrestrial herb 1–2 m; diameter 3 cm, rhizome 1 cm diameter, long creeping; scales reddish, sheath olive-green, reticulate; sheath purple at top; leaves plain green, glabrous above, pubescent below, young purplish brown below, inflorescence less than 5 cm long; flowers plain red; anther pale pink; stigma white.

Habitat: Forest near village.

***Zingiber zerumbet* (L.) Sm.**

Material collected: *Bau et al.* LAE 86304, Jivevaneng, 9 Nov 2001 (LAE).

Vernacular name: *Zazamang*

Uses: Ornamental plant in village and garden areas.

Description: Terrestrial herb to 1 m; rhizome thick and short, yellow in centre when cut; base of leafy shoot fleshy, reddish, to 2 cm; sheath purplish; ligule to 2 cm, membranous; petiole swollen; lamina plain green, soft, beneath pale green and puberulent. Inflorescence radical, to 20 cm long; bracts reddish. Flowers not seen.

Habitat: In a cluster below several planted *Cocos nucifera* L. trees along trail in village garden.

Discussion

The total species pool of the study area is no doubt larger than the eight species collected in the present study, but a more intensive survey would need to be conducted to document more species. This should include more focus on the dominant species in the old garden sites, which were poorly covered in our preliminary survey. Likewise, some common cultivated species like *Zingiber officinale* Roscoe were not sighted as the survey took place away from the most likely areas of its cultivation.

The uses of gingers in Papua New Guinea have, to date, been poorly documented, and the results of this preliminary survey indicates that the species have a wide range of uses from ethnic dressings in traditional dances (*sing sings*) to an alternative for betel nut chewing, and thus have a large potential.

Previous publications only highlighted the genera *Amomum* and *Zingiber* as useful to the villagers in the Huon Peninsula (Holdsworth, 1977; Holdsworth and Damas, 1986; Holdsworth and Mahana, 1983; Paijmans, 1975; Peekel, 1984; Woodley, 1991). But this study also documents uses for the genus *Etilingera* which was found to have several uses. In the present paper, we are only able to give specific epithets for one species, because *Etilingera* is still poorly known in PNG and is still being revised for the Flora Malesiana by the second author.

In the present study, *Zingiber zerumbet* (L.) Sm. is only used as an ornamental plant but in Malaysia it is also used as medicine (Holtum, 1950; Burkill, 1966). In the Bismarck Archipelago, however, Peekel (1984) noted it to be 'less spicy' than *Z. caninum* Peekel and *Z. foliatum* Peekel but no specific notes of its edibility was presented.

Most of the useful species are common around the home gardens, which means that they are either cultivated to some degree or associated with the secondary forest vegetation. Also, in Borneo many gingers thrive in disturbed or human influenced vegetation (Christensen, 2002; Poulsen, 2006).

A more extensive survey of the gingers of Papua New Guinea commenced in Dec 2006 during which additional areas were visited to obtain more collections and ethnobotanical information. This will provide an essential basis for a comprehensive systematic treatment. Combined with ethnobotanical information this may be used to identify endangered and/or endemic species where particular conservation efforts have to be conducted. This will eventually contribute towards a more meaningful and constructive conservation avenue whether it is *in-situ* or *ex-situ*. Such a recommended conservation program should be community-based and economically attractive for the local villagers. The conservation program should be a source of income for the local people who will then be encouraged to propagate the wild seedlings in smaller nurseries for potential horticultural uses nationally and internationally.

Acknowledgements

The authors thank the Organizing Committee of the 4th International Symposium on the Family Zingiberaceae and the Papua New Guinea Forest

Research Institute for providing funding for their participation, and for the first author to present this paper, at the ginger symposium in Singapore in 2006. We are also grateful to Mr. Roy Banka for the logistics and technical advice provided while conducting the survey, the Papua New Guinea Forest Research Institute for the use of the facilities, the councillors and the people of Jivevaneng and Nanduo villages for their support and sharing of their knowledge.

References

- Burkill, I.H. 1966. *A Dictionary of the Economic Products of the Malay Peninsula*. Ministry of Agriculture, Kuala Lumpur, Malaysia.
- Christensen, H. 2002. *The Ethnobotany of the Iban & the Kelabit*. - A joint publication by Forest Department of Sarawak, Malaysia, Nepcon, Denmark & The University of Aarhus, Denmark.
- Damas, K. 1998. The present status of plant conservation in Papua New Guinea. Rare, threatened and endangered Floras of Asia and the Pacific Rim, *Academia Sinica Monograph Series* **16**: 171–179.
- Holdsworth, D. 1977. *Medicinal plants of Papua New Guinea*. Noumea, South Pacific Commission.
- Holdsworth, D. and K. Damas. 1986. Medicinal plants of Morobe Province, Papua New Guinea. Part III. The Finschhafen Coast. *International Journal of Crude Drug Research* **24**: 217–225.
- Holdsworth, D. and P. Mahana. 1983. Traditional medicinal plants of the Huon Peninsula, Morobe Province, Papua New Guinea. *International Journal of Crude Drug Research* **21**: 121–133.
- Holttum, R.E. 1950. The Zingiberaceae of the Malay Peninsula. *Gardens' Bulletin Singapore* **13**: 1–249.
- Hoft, R. 1992. *Plants of Papua New Guinea and the Solomon Islands - Dictionary of the Genera and Families of Flowering Plants and Ferns*. Wau Ecology Institute Handbook No. 13. Wau, Papua New Guinea.
- Paijmans, K. (ed.) 1995. *New Guinea Vegetation*. Australian National University Press, Canberra, Australia.

- Peckel, P.G. 1984. *Flora of the Bismarck Archipelago for Naturalists*. Division of Botany, Lae, Papua New Guinea.
- Poulsen, A.D. 2006. *Etlingera of Borneo*. Natural History Publications (Borneo). 263 pp.
- Woodley, E. (ed.) 1991. *Medicinal Plants of Papua New Guinea; Part 1: Morobe Province*. Wau Ecology Handbook No. 11, Verl. Margraf, Weikersheim.

Rapid *In Vitro* Propagation of *Hornstedtia reticulata* (K. Schum.) K. Schum.

H.H. BAY ^{1*}, H.B. SANI ¹, P.C. BOYCE ² AND S.L. SIM ¹

¹ Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak 94300 Kota Samarahan, Sarawak, Malaysia

² Malesiana Tropicals, suite 9-04, Tun Jugah Tower, No.18 Jln Tunku Abd Rahman, 90100, Kuching, Sarawak, Malaysia

* Email of corresponding author: bay_ivy@yahoo.com

Abstract

Seeds of *Hornstedtia reticulata* (K. Schum.) K. Schum. collected from the wild were double surface sterilised with 30% Clorox, followed by 15% Clorox, each for 20 minutes. The sterilised seeds were sown on Gamborg B5 medium. The meristems of 12 weeks old seedlings, including the basal parts of leaf sheath, were used to induce multiple shoots in Gamborg B5 media incorporated with 6-benzylaminopurine (BAP) alone (2mg/L and 3mg/L) and in combination with α -naphthalena acetic acid (NAA) at different concentrations (0.5mg/L and 0.1mg/L). Observation showed that all the treatments were able to produce multiple shoots while the highest number of shoots was obtained from explants that were treated with 3mg/L BAP after three subcultures.

Introduction

Hornstedtia Retz. is a well-defined genus characterized by the rigid involucre of sterile bracts, which encloses the entire inflorescence from the uppermost part of the open flowers. Valetton (Bull. Jard. Bot Buitenz. Ser. 3, 3: 150-179, 1921) cited by Smith (1985) had subdivided *Hornstedtia* into three subgenera, *Hornstedtia*, *Elettariostemon* and *Rosianthus*. The Bornean plants all fall within the first two groups.

Hornstedtia reticulata (K. Schum.) K. Schum. is very distinctive. The cyathiform inflorescence is borne on stilt roots, and the sterile bracts, which are the most strongly reticulated of all Bornean *Hornstedtia*, are scabrid to the touch. Study on species of *Hornstedtia* is scarce; hence, information beyond taxonomy study is still unavailable.

Up to this moment, several species of Zingiberaceae with established

uses as condiments, spices and as ornamental plants have been investigated for *in vitro* multiplication (Borthakur *et al.*, 1999; Jasrai *et al.*, 2000; Salvi *et al.*, 2000; Rout *et al.*, 2001; Khatun *et al.*, 2003; Prakash *et al.*, 2004; Wondyifraw *et al.*, 2004). So far, no successful micropropagation protocol for *Hornstedtia* spp. has been reported. For this reason, this study served as a preliminary research on *in vitro* propagation of *Hornstedtia reticulata* to mass produce genetically uniform plantlets for future conservation plan and ornamental purpose.

Materials and Methods

Materials

Seeds of *Hornstedtia reticulata* were collected at Kampung Segong, Bau, Kuching, Sarawak, Malaysia.

Methods

(a) Media preparation

The culture media Gamborg B5 was used in the study (Gamborg *et al.*, 1968). The medium contains 30g/L sucrose and vitamins. After adjusting to pH 5.8±1, medium was solidified using 3g/L gelrite. Culture media were sterilised by autoclaving at 104kPa at 121°C for 20 minutes.

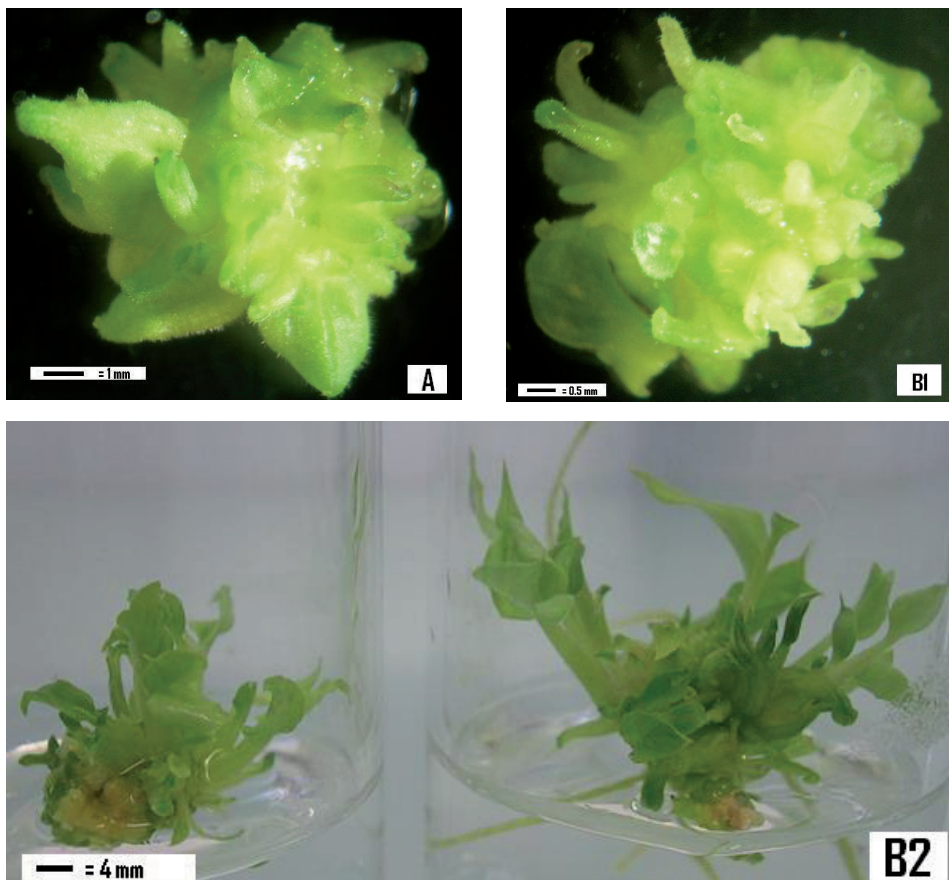
(b) Surface sterilisation and sowing of seed

The seeds were soaked in distilled water overnight to remove the mucilage layers. After that, the seeds were soaked in 75% ethanol for one minute before double surface sterilised with 30% Clorox, followed by 15% Clorox, each for 20 minutes. They were then rinsed three times in sterilised distilled water before cultured in B5 media incorporated with 5ml/L PPM and 5mg/L Tetracyclin for seven days. After seven days, the seeds were sown on Gamborg B5 medium

(c) Induction of multiple shoot formation

After 12 weeks of culture, 30 seedlings, each 4-5 cm in height, were randomly selected for study on the effects of 6-benzylaminopurine (BAP) alone or with the combination of α -naphthalene acetic acid (NAA) in different concentration. Three replicates were used for each treatment. The roots, the leaves, and the leaf sheaths of seedlings were removed. Then, the meristems of 12 weeks old seedlings, which were cut into approximately 1 cm in length, were used to induce multiple shoots. The explants were monthly subcultured in fresh media with growth regulators incorporated for the first two months and thereafter in the plant growth regulator-free medium. Observation on

the number of explants forming shoots was recorded. Data were subjected to factorial analysis using General Linear Model.



Figures A – B2. Shoot multiplication of *Hornstedtia reticulata*. A. A clump of adventitious shoot buds with primordia excised from meristem explants after 6 weeks of subculturing in media with 3mg/l BAP; B1. A clump of adventitious shoot buds from the basal portion of meristem region in media supplemented with 3mg/l BAP + 0.1mg/l NAA after 8 weeks of subculturing; B2. The clump was cut into two halves and both continued to form cluster of shoots after subcultured onto basal medium.

Results and Discussion

All the treatments having BAP alone, or with the combination of NAA in different concentrations, were able to generate multiple shoots. However, the rate of bud multiplication was significantly different according to the BAP + NAA formulations. Based on Fig. 1, number of shoots produced from explants in 3mg/l BAP was significantly different from the explants

cultured in 2mg/l BAP. Frequency of shoot proliferation was highest at 3mg/L BAP alone. The average number of shoots was 9.67 ± 2.31 . Numerous adventitious shoots were observed near the basal portion of the shoot cluster after 12 weeks of subculturing. Multiplication rate in media incorporated with 2mg/L BAP was lowest among the treatments with the mean reading of 4.33 ± 2.31 .

In this study, addition of NAA into medium with BAP was proven not efficient in increasing number of multiple shoots. Similar effect was showed in *Zingiber petiolatum*, where maximum shoots regeneration from terminal buds explants was obtained on medium with 4.4 μ M BAP alone, while lesser shoots were obtained from the explants that cultured in the media with addition of 0.5 μ M NAA (Prathantharug *et al.*, 2004). Furthermore, similar result was also encountered when the apical meristems of *Curcuma amada* Roxb. and *Zingiber officinale* Rosc. were explanted on media supplemented with BAP alone and BAP incorporated with NAA where the percentage of bud growth were decreased 50% and 67% respectively (Jasrai *et al.*, 2000). Hence, addition of NAA is not recommended for most of the Zingiberaceae plant species.

Observation also illustrated that the explants were started to show significantly increase in number of shoots produced after they were subcultured onto basal medium without PGR. The same finding was found in other Zingiberaceae plant species, such as *Curcuma longa* L. and *Zingiber petiolatum* (Prathantharug *et al.*, 2003, 2004). Medium for induction of rooting of shoots was not required as the regenerated plantlets produced plentiful roots in the same growth regulators media. Similar observation was seen in plantlets produced from shoot tip explants of *Zingiber officinale* Rosc. in MS media supplemented with BAP + Kinetin treatments (Khatun *et al.*, 2003).

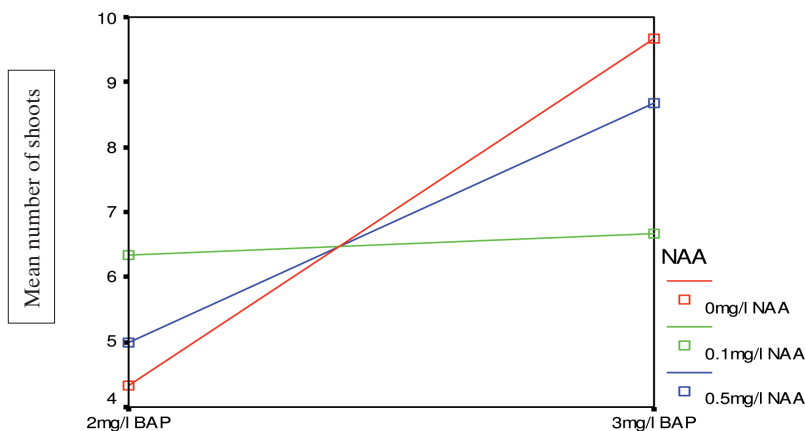


Figure 1. Marginal Means for number of shoots produced.

Conclusion

This report had demonstrated the preliminary result of using tissue culture method as a possible mean for producing large number of true-to-type plantlets of *Hornstedtia reticulata*. BAP alone in concentration of 3mg/l is sufficient enough to micropropagate the plantlets which can be exploited for further research of this species. Currently, the acclimatization has not yet been performed because the regenerated plantlets are kept for further molecular study. Hence, acclimatization will be performed later.

Acknowledgements

The authors would like to acknowledge the IGS fund (Grant no. IGS (R&D) 16/03) granted by The Ministry of Science, Technology and Innovation (MOSTI), Malaysia, for financial support of the research and the first author (H.H. Bay) would like to thank MOSTI for awarding her the MOSTI scholarship.

References

- Borthakur, M., J. Hazarika and R.S. Singh. 1999. A protocol for micropropagation of *Alpinia galanga*. *Plant Cell, Tissue and Organ Culture* **55**: 231-233.
- Gamborg, O.L., R.A. Miller and L. Ojima. 1968. Nutrient requirements of suspension culture of soybean cells. *Experimental Cell Research* **50**: 150-158.
- Jasrai, Y.T., K.G. Patel and M.M. George. 2000. Micropropagation of *Zingiber officinale* Rosc. and *Curcuma amada* Roxb. *Centennial Conference on spices and Aromatic Plants* **1**: 52-54.
- Khatun, A., S. Nasrin and T.M. Hossain. 2003. Large scale multiplication of ginger (*Zingiber officinale* Rosc.) from shoot-tip culture. *Online Journal of Biological Sciences* **3**: 59-64.
- Salvi D., L. George and S. Eapen. 2000. Direct regeneration of shoots from immature inflorescence cultures of turmeric. *Plant Cell, Tissue and Organ Culture* **62**: 235-238.

- Prakash, S., R. Elangomathavan, S. Seshadri, K. Kathiravan and S. Ignacimuthu. 2004. Efficient regeneration of *Curcuma amada* Roxb. plantlets from rhizome and leaf sheath explants. *Plant Cell, Tissue and Organ Culture* **78**: 159-165.
- Prathanturarug S., N. Soonthornchareonnon, W. Chuakul, Y. Phaidee and P. Saralamp. 2003. High-frequency shoot multiplication in *Curcuma longa* L. using thidiazuron. *Plant Cell Reports* **21**: 1054-1059.
- Prathanturarug S., D. Angsumalee, N. Pongsiri, S. Suwacharangoon and T. Jenjittikul. 2004. *In vitro* propagation of *Zingiber petiolatum* (Holtum) I. Theilade., a rare Zingiberaceous plant from Thailand. *In Vitro Cell Development Biology-Plant* **10**: 317-320.
- Rout, G.R., S.K. Palai, S. Samantaray and P. Das. 2001. Effect of growth regulator and culture conditions on shoot multiplication and rhizome formation in ginger (*Zingiber officinale* Rosc.) *in vitro*. *In Vitro Cell Development Biology - Plant* **37**: 814-819.
- Smith, R.M, 1985. A review of Bornean Zingiberaceae: I (Alpineae p.p.). *Notes from the Royal Botanic Gardens Edinburgh* **42**: 261-314.
- Wondyifraw, T. and S. Wannakrairoj. 2004. Micropropagation of Krawan (*Amomum krervanh* Pierre ex Gagnep). *Science Asia* **30**: 9-15.

Variations in Tissue Development and Secondary Product Elaboration of *Hedychium coronarium* J. König Floral Cultures Grown on Different Media

L.B. CARDENAS

Institute of Biological Sciences, University of the Philippines Los Baños,
College, Laguna, Philippines 4031

Abstract

The studies on the variations in tissue development and secondary productelaboration of *Hedychium coronarium* J. König, locally known as camia, in culture on different growth media, using the floral tube part, are reported.

Introduction

The use of biotechnology in the study and harvest of important natural products is a valued approach especially now that the natural resources are fast dwindling. Fascination for plant essential oils, for instance, dates back to antiquity and their production in plant tissue cultures has long been sought for. However, experience shows that not all natural products produced by the plant could be synthesized in its unorganized tissue culture or callus. Most times, tissue differentiation is a requirement. Previous work on essential oils obtained from Apiaceae tissue culture indicated that some components of the essential oil like the phenylpropanoids can be synthesized in the callus but not the mono- nor the sesquiterpenes (Cardenas, 1993). Thus, the essence produced in tissue culture can only approximate the full scent of the plant depending partly on the degree of tissue differentiation attained.

“Camia”, *Hedychium coronarium* J. König of the Zingiberaceae, carries short-lived, white, sweet scented flowers. A study on the tissue culture of camia flowers was attempted to check on any natural products it can synthesize and store. A growth medium previously proven to sustain alkaloid production in *Catharanthus roseus* (L.) G. Don floral tissue culture was used (Cardenas, 1983).

Materials and Methods

Floral tube of yet unopened camia flowers was used as explant for the experiment. Whole unopened flower buds were surface sterilized in 3% CaOCl aqueous solution for 5 mins. The floral tube was excised after three consecutive rinsing in sterile distilled water and cut into 1-mm sections. These were inoculated into two different growth media (Table 1). The first was “MS” Murashige and Skoog medium (Murashige and Skoog, 1962) and the second “mWB” was a modification of the Wood and Braun medium (Braun and Wood, 1962). The two media differed only in their macroelements. Their microelement and vitamin composition are similar following those recommended for MS medium. Both media were supplemented with 3 ppm NAA (naphthalene acetic acid) and 0.5 ppm Ki (kinetin), 3% sucrose and 0.2% Gelrite. The pH was adjusted to 5.8 prior to autoclaving.

The cultures were maintained at ambient room temperature of 28°C with 8-hr daylight provided by a west-facing window. Low diffused light, with the highest value at 5.20 μmolS⁻¹ m⁻² recorded in the early afternoon, was observed. Observation on the basic anatomy of the growing callus was made after 6 weeks of culture under the light microscope. The orange pigment produced by the callus was extracted with absolute methanol in the dark and the absorption spectra in visible light (380-780 nm) determined using Labomed® spectro dual split beam UV-VIS spectrophotometer, USA.

Table 1. Comparison of the MS and mWB growth media used in the experiment.

MS (<i>Physiol. Plant.</i> 18: 100) in mg/l:		mWB (<i>PNAS</i> 48: 1776) in mg/l:	
370	MgSO ₄ -7H ₂ O	MgSO ₄ -7H ₂ O	1,368
		Na ₂ SO ₄	200
440	CaCl ₂ -2H ₂ O	Ca(NO ₃) ₂	288
170	KH ₂ PO ₄	KCl	925
		NaH ₂ PO ₄	316.5
1900	KNO ₃	KNO ₃	80
		NaNO ₃	1,800
1650	NH ₄ NO ₃	(NH ₄) ₂ SO ₄	790

Results and Discussion

The floral tube segments produced callus slowly in two months from inoculation with the 3ppm NAA and 0.5 ppm Ki supplements. Unexpectedly, though, the white explant on mWB changed to orange in color and this color was maintained in the callus that developed. Succeeding subcultures of the callus to media of the same composition proved that the pigment was, indeed, synthesized and sequestered in all cells of the tissue culture. Seldom is chlorophyll produced (Fig. 1). In contrast, MS-grown callus remained mostly unpigmented, typical of cultures from unpigmented explants. As the latter matured, roots were initiated (Fig. 2).

For the Zingiberaceae, the most studied pigments are the curcuminoids: curcumin, monodemethoxycurcumin and bisdemethoxycurcumin of the *Curcuma* species. These are cinnamoyl pigments produced and stored in the rhizome with recorded biological activities, particularly for curcumin (Wagner and Bladt, 1996). On the other hand, no pigment analysis for *H. coronarium* was encountered. Fruit set among the local populations elsewhere is apparently low, unlike the plants of *H. coronarium* at the Singapore Botanic Gardens that produced big orange fruits during the time of the 4th International Symposium on the Zingiberaceae in July 2006. The fruit of the local species populations in the Philippines, encountered only once by this researcher in October 2006, is small and hidden inside the floral bract.

The orange color of fruits is mostly attributed to carotenoids and there are over 600 natural carotenoids known, including those in algae, fungi and bacteria (Rodriguez-Amaya, 1999). It can not be discounted that the orange pigment(s) synthesized in *camia* callus is of the carotenoids.

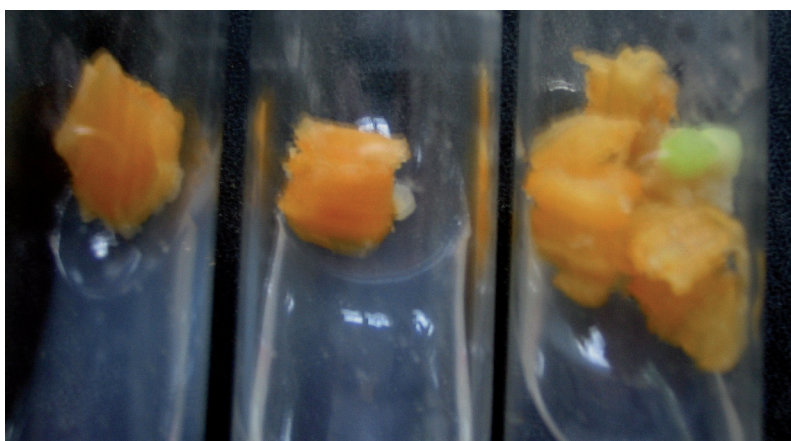


Figure 1. *H. coronarium* callus from floral tube explant two months after inoculation on mWB medium supplemented with 3ppm NAA and 0.5 ppm Ki.

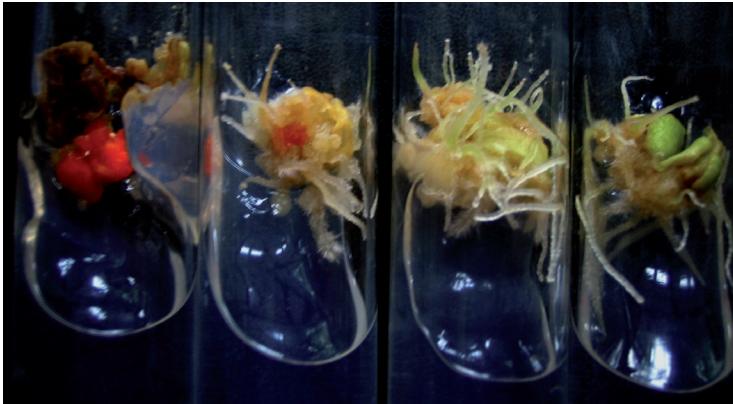


Figure 2. *H. coronarium* callus from floral tube explant two months after inoculation on MS medium supplemented with 3ppm NAA and 0.5 ppm Ki.

Anatomical observations of the callus showed some short trichomes that might be a carry over from the explant. Unorganized callus growth was evident. Compared with carrot and squash that are well studied for their carotenoids, the camia callus methanol extract exhibited a different TLC (thin layer chromatography) profile. Likewise, there are differences in the visible absorption spectra of the methanol extracts of the three plant species (Fig. 3).

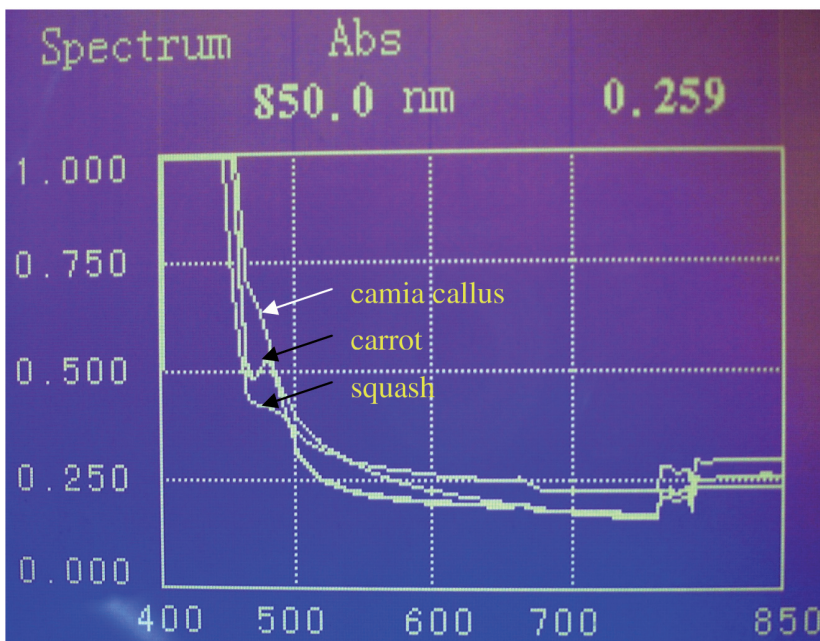


Figure 3. Absorption spectra of the methanol extracts of camia callus, squash and carrot.

The production of the pigment in camia callus might be due to the composition of the macroelements in the medium as this was the only difference between the media. [Succeeding camia floral tube cultures on mWB medium initiated and maintained in growth room of 16-hr light at $16.0 \mu\text{molS}^{-1}\text{m}^{-2}$ provided by fluorescent lamps and in controlled temperature range of 17-24 °C also produced orange pigmented callus.] The possibility of osmotic value difference, however, was not discounted. An experiment using mannitol as osmolyticum will be pursued to check this factor.

The pigment in camia callus grown on mWB medium is likely mainly carotenoids, but this is yet to be ascertained. Lately, the researcher was able to secure a flowering and fruiting specimen of *H. philippinense*. The flowers of this epiphyte are yellow and the fruits are orange reaching 6 cm at maturity. In the absence of *H. coronarium* fruit, pigment of this species' fruit will be used as reference in the further analysis of the camia callus pigment. Modifications in the TLC procedure and in the protocol to obtain absorption spectra of the pigment will be pursued.

Acknowledgements

The author would like to thank the organizers, Singapore Botanic Gardens, of the 4th International Symposium on the Family Zingiberaceae for enabling her to participate in the symposium and PCARRD-DOST (Philippine Council for Agricultural Research and Development – Department of Science and Technology) for partial funding support of this work.

References

- Braun, A.C. and H.N. Wood. 1962. On the activation of certain essential biosynthetic systems in cells of *Vinca rosea* L. *Proceedings of the National Academy of Sciences (Washington)* **48**:1776.
- Cardenas, L.B. 1983. Alkaloid production in intact parts and floral tissue culture of *Catharanthus roseus* (L.) Don. Kalikasan, *Philippine Journal of Biology* **12**: 375-384.
- Cardenas, L.B. 1993. Somatic Embryogenesis and the Production of Essential Oil Components in some *Apiaceae* species. Dr. rer. nat. Thesis, Germany (unpublished).

- Murashige, T. and F. Skoog. 1962. A revised medium for the rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**: 473-497.
- Rodriguez-Amaya, D.B. 1999. *A Guide to Carotenoid Analysis in Foods*. ILSI Press, USA. 64 pp.
- Wagner, H. and S. Bladt. 1996. *Plant Drug Analysis. A Thin Layer Chromatography Atlas*. Springer Verlag, Germany. 384 pp.

Total Phenolic Content and Antioxidant Activity of Leaves and Rhizomes of Some Ginger Species in Peninsular Malaysia

E.W.C. CHAN, Y.Y. LIM AND T.Y. LIM

School of Arts and Sciences, Monash University Sunway Campus,
Bandar Sunway, 46150 Petaling Jaya, Selangor, Malaysia

Abstract

The total phenolic content (TPC) and antioxidant activity (AOA) of leaves and rhizomes of five wild and six cultivated ginger species belonging to seven genera were compared. Altitudinal variation in leaf TPC and AOA of four species of *Etlingera* Giseke was also studied. TPC was measured using the Folin-Ciocalteu method. AOA was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and expressed as ascorbic acid equivalent antioxidant capacity (AEAC). Of the 11 wild and cultivated species screened, leaves of *Etlingera* had the highest TPC and AEAC, which were seven to eight times higher than those of rhizomes. Eight species had significantly higher leaf TPC and/or AEAC than rhizomes. Leaves of highland populations of *Etlingera* species had higher values than those of lowland counterparts.

Introduction

Rhizomes of gingers (Zingiberaceae) are widely consumed as spice or condiments (Larsen *et al.*, 1999; Sirirugsa, 1999). Major commercially cultivated species in Peninsular Malaysia are *Zingiber officinale* Roscoe, *Curcuma longa* L. and *Alpinia galanga* (L.) Willd. As traditional medicine, rhizomes are consumed by women during ailment, illness and confinement (Larsen *et al.*, 1999; Ibrahim *et al.*, 2006). They are also taken as carminative for relieving flatulence.

Leaves of gingers have also been used for food flavouring. In Peninsular Malaysia, leaves of *Curcuma longa* are used to wrap fish before steaming or baking (Larsen *et al.*, 1999). The leaves of *Kaempferia galanga* L. and *Curcuma longa* are ingredients of spicy fish and meat dishes. Some tribal natives use leaves of *Elettariopsis slahmong* C.K. Lim to flavour cuisine of wild meat and fish (Lim, 2003). In Okinawa, Japan, leaves of *Alpinia*

zerumbet (Pers.) B.L. Burtt & R.M. Sm. are traditionally used to wrap rice cakes and are commercially sold as herbal tea.

Past studies on the antioxidant activity of wild and cultivated ginger species were confined to rhizomes (Jitoe *et al.*, 1992; Habsah *et al.*, 2000; Zaeoung *et al.*, 2005). Although their leaves have been used for food flavouring, hardly any research has been done on their antioxidant activity.

Antioxidants are molecules that are able to scavenge free radicals or prevent their generation. Phenolic compounds, in general, are able to scavenge free radicals or chelate metal ions to prevent generation of free radicals. Free radicals have been implicated in the pathogenesis of more than 50 diseases (Percival, 1996). Currently, there is much interest in herbs and spices as sources of antioxidants.

In our present study, the total phenolic content (TPC) and antioxidant activity (AOA) of leaves and rhizomes of five wild and six cultivated ginger species were compared. Altitudinal variation in leaf TPC and AOA of species of *Etlingera* was also studied.

Materials and Methods

Species studied

Five wild and six cultivated ginger species were screened for TPC and AOA. Wild species studied were *Etlingera maingayi* (Baker) R.M. Smith, *Alpinia malaccensis* var. *nobilis* (Ridl.) I.M. Turner, *Elettariopsis slahmong* C.K. Lim, *Zingiber spectabile* Griff. and *Scaphochlamys kunstleri* (Baker) Holttum. Cultivated species studied were *Etlingera elatior* (Jack) R.M. Smith, *Alpinia galanga* (L.) Willd., *Zingiber officinale* Roscoe, *Curcuma longa* L., *Curcuma zanthorrhiza* Roxb. and *Boesenbergia rotunda* (L.) Mansf. For each species, leaves and rhizomes of three plants were sampled.

For wild species, leaves and rhizomes of *Alpinia malaccensis* var. *nobilis*, *Zingiber spectabile* and *Scaphochlamys kunstleri* were sampled from plants growing at Forest Research Institute Malaysia (FRIM) in Selangor, those of *Elettariopsis slahmong* from Bukit Lagong in Selangor, and those of *Etlingera maingayi* from Janda Baik in Pahang. Voucher specimens of wild species studied were deposited at the FRIM herbarium (KEP).

For cultivated species, leaves and rhizomes of *Etlingera elatior* and *Curcuma longa* were sampled from plants found at FRIM, those of *Alpinia galanga* and *Zingiber officinale* from Bukit Maluri in Kepong, and those of *Curcuma zanthorrhiza* from Damansara Utama in Petaling Jaya. Plants of *Boesenbergia rotunda* were purchased from a nursery in Sungai Buluh in Selangor. Rhizomes of *Alpinia galanga*, *Zingiber officinale* and *Curcuma longa* purchased from the supermarket were also screened. Voucher

specimens of cultivated species studied were deposited at KEP.

TPC and AOA of leaves of lowland and highland populations of four *Etlingera* species were compared. The species studied were *Etlingera elatior* (Jack) R.M. Sm., *Etlingera fulgens* (Ridl.) C.K. Lim, *Etlingera littoralis* (J. König) Giseke and *Etlingera rubrostriata* (Holtum) C.K. Lim. Their identification was based on taxonomic descriptions and photographic illustrations of Lim (2000 & 2001) and Khaw (2001). Leaves of highland populations were sampled from Janda Baik and Genting Highlands in Pahang and from Ulu Gombak in Selangor, while those of lowland populations were sampled from FRIM. For each location, mature leaves were sampled from three different plants per species. Voucher specimens of *Etlingera* species studied were deposited at KEP. Altitude of locations, where the populations were sampled, was measured using a Casio altimeter (Model PRG-70-1VDR).

Extraction of samples

Fresh leaves and rhizomes (1 g) were powdered with liquid nitrogen in a mortar and extracted by methanol (50 ml), with continuous swirling for one hour at room temperature. Extracts were filtered and stored at -20°C for further use. Analysis of methanol extracts was done in triplicate for each species.

Total phenolic content

Total phenolic content (TPC) was measured using the Folin-Ciocalteu method (Kahkonen *et al.*, 1999). Samples (300 µl in triplicate) were introduced into test tubes followed by 1.5 ml of Folin-Ciocalteu's reagent (10 times dilution) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were allowed to stand for 30 min before absorption at 765 nm was measured. Total phenolic content was expressed as gallic acid equivalent (GAE) in mg/100 g material. The calibration equation for gallic acid was $y = 0.0111x - 0.0148$ ($R^2 = 0.9998$).

Antioxidant activity

Antioxidant activity (AOA) was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay used by Leong and Shui (2002) and Miliauskas *et al.* (2004) with slight modification. Defined amounts of the extract were added to 3 ml of DPPH (3.9 mg/100 ml methanol). The DPPH solution was then allowed to stand for 30 min before absorbance was measured at 517 nm. All spectrophotometric measurements were made with methanol as blank. An appropriate dilution of the DPPH solution was used as negative control, i.e., methanol in place of the sample. Results

were expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg/100 g calculated from the IC₅₀ (inhibitory concentration in mg/ml of plant material necessary to reduce the absorbance of DPPH by 50%) using the following formula:

$$\text{AEAC (mg AA/100 g)} = \text{IC}_{50(\text{ascorbate})} / \text{IC}_{50(\text{sample})} \times 100,000$$

The IC₅₀ of ascorbate used for calculation of AEAC was 0.00387 mg/ml.

Results and Discussion

Leaves and rhizomes of wild and cultivated species

Results from screening of five wild species showed that leaves of *Etlingera maingayi* had significantly higher TPC and AEAC than those of *Alpinia malaccensis* var. *nobilis*, *Elettariopsis slahmong*, *Zingiber spectabile* and *Scaphochlamys kunstleri* (Table 1). Rhizomes of *Alpinia malaccensis* var. *nobilis* had the highest values. Leaves of *Elettariopsis slahmong*, *Etlingera maingayi* and *Scaphochlamys kunstleri* showed significantly higher values at $P < 0.05$ than rhizomes. Leaves of other wild species were only marginally higher than rhizomes.

For six cultivated species screened, leaf and rhizome TPC and AEAC were highest in *Etlingera elatior* and *Curcuma longa*, respectively (Table 2). In five species, leaves had significantly higher TPC and/or AEAC at $P < 0.05$ than those of rhizomes. Exceptions were AEAC of *Alpinia galanga*, and TPC and AEAC of *Curcuma longa* where rhizomes showed higher values than leaves. The values of *Curcuma longa* were highly variable between rhizomes. For *Alpinia galanga*, *Curcuma longa* and *Zingiber officinale*, differences existed between collected rhizomes and those purchased from the supermarket. This implies that there is variability in TPC and AEAC between different cultivars.

In general, leaves of wild and cultivated *Etlingera* species contain the most antioxidants by having the highest TPC and AEAC. Values were 1110 mg GAE/100 g and 963 mg AA/100 g for *Etlingera maingayi* (Table 1), and 2390 mg GAE/100 g and 2280 mg AA/100 g for *Etlingera elatior* (Table 2) respectively. The outstanding leaf TPC and AEAC of both *Etlingera maingayi* and *Etlingera elatior* were seven to eight times higher than those of rhizomes.

There are very few studies comparing between the AOA of leaves and rhizomes of ginger species. Agnani *et al.* (2004) reported that essential oils extracted from leaves of *Aframomum giganteum* K. Schum. had higher AOA than rhizomes. Contrary to our results, Katsube *et al.* (2004) reported higher TPC and AOA in rhizomes of *Zingiber officinale* than leaves. It is

Table 1. Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of leaves and rhizomes of five wild ginger species.

Species and location	Voucher number	Plant part	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
<i>Alpinia malaccensis</i> var. <i>nobilis</i> - FRIM	EC01	Leaves	744 ± 61 ^a	800 ± 62 ^a
		Rhizomes	564 ± 209 ^a	745 ± 342 ^a
<i>Elettariopsis slahmong</i> - Bukit Lagong	EC02	Leaves	346 ± 45 ^a	269 ± 67 ^a
		Rhizomes	219 ± 57 ^b	197 ± 76 ^a
<i>Etlingera maingayi</i> - Janda Baik	EC06	Leaves	1110 ± 93 ^a	963 ± 169 ^a
		Rhizomes	160 ± 52 ^b	122 ± 53 ^b
<i>Scaphochlamys kunstleri</i> - FRIM	EC08	Leaves	203 ± 21 ^a	171 ± 33 ^a
		Rhizomes	73 ± 3 ^b	14 ± 2 ^b
<i>Zingiber spectabile</i> - FRIM	EC09	Leaves	242 ± 7 ^a	121 ± 24 ^a
		Rhizomes	157 ± 100 ^a	124 ± 109 ^a

Values of TPC and AEAC are means ± SD ($n = 3$). For column of each species, values followed by the same letter (a-b) are not significantly different at $P < 0.05$ measured by the Tukey HSD test. ANOVA does not apply between species.

not known whether their comparisons were based on samples from same or different plants.

This is probably the first study where TPC and AOA of leaves and rhizomes of gingers have been systematically compared. For most of the species screened, TPC and/or AEAC of leaves were significantly higher than rhizomes.

Antioxidants are secondary metabolites, which form part of the plant's protective mechanism against free radicals. In Zingiberaceae, it is generally believed that antioxidants and other secondary metabolites are transported to the rhizomes where they are accumulated. This implies that rhizomes would have higher AOA than other plant parts. However, results of this study showed that this might not be the case.

Photosynthesis and respiration are physiological processes comprising several free radical intermediates. Exposure to sunlight can also increase the amount of free radicals. Leaves therefore require much more free radical scavengers than other plant parts. Similarly, Frankel and Berenbaum (1999) found that foliage of tropical forest plants produced more antioxidants when exposed to elevated light conditions. This observation may also apply to species of *Etlingera*, which have the highest leaf TPC and AEAC. *Etlingera* plants grow in gaps of disturbed forest and are continually

Table 2. Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of leaves and rhizomes of six cultivated ginger species.

Species and location	Voucher number	Plant part	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
<i>Alpinia galanga</i> - Bukit Maluri	EC10	Leaves	366 ± 15 ^a	72 ± 4 ^a
		Rhizomes	150 ± 22 ^b	96 ± 6 ^b
Supermarket		Rhizomes	214 ± 20	168 ± 13
<i>Boesenbergia rotunda</i> - Sungai Buluh	EC11	Leaves	260 ± 8 ^a	157 ± 2 ^a
		Rhizomes	197 ± 50 ^a	89 ± 7 ^b
<i>Curcuma longa</i> - FRIM	EC12	Leaves	230 ± 19 ^a	113 ± 18 ^a
		Rhizomes	534 ± 205 ^b	390 ± 127 ^b
Supermarket		Rhizomes	386 ± 219	275 ± 183
<i>Curcuma zanthorrhiza</i> - Damansara Utama	EC13	Leaves	503 ± 57 ^a	287 ± 39 ^a
		Rhizomes	250 ± 52 ^b	134 ± 21 ^b
<i>Etlingera elatior</i> - FRIM	EC14	Leaves	2390 ± 329 ^a	2280 ± 778 ^a
		Rhizomes	326 ± 76 ^b	295 ± 96 ^b
<i>Zingiber officinale</i> - Bukit Maluri	EC15	Leaves	291 ± 18 ^a	96 ± 7 ^a
		Rhizomes	157 ± 18 ^b	84 ± 3 ^a
Supermarket		Rhizomes	184 ± 11	107 ± 9

Values of TPC and AEAC are means ± SD ($n = 3$). For column of each species, values followed by the same letter (a-b) are not significantly different at $P < 0.05$ measured by the Tukey HSD test. ANOVA does not apply between species.

exposed to direct sunlight (Poulsen, 2006). Furthermore, leaves of *Etlingera* are long lasting and do not abort. This may be due to an efficient protective mechanism delaying senescence in leaves, which is partly attributed to oxidative stress.

Altitudinal variation in leaves of Etlingera species

Leaves of all four species of *Etlingera* sampled from highland populations were found to have higher TPC and AEAC than lowland counterparts (Table 3). Leaves of *Etlingera rubrostriata*, *Etlingera elatior* and *Etlingera fulgens* showed significantly higher values at $P < 0.05$, while *Etlingera littoralis* was marginally higher. Highest TPC and AEAC were found in the leaves of highland populations of *Etlingera elatior* with values of 3550 mg GAE/100 g and 3750 mg AA/100 g, and of *Etlingera rubrostriata* with values of 3480 mg GAE/100 g and 3540 mg AA/100 g, respectively.

Table 3. Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of leaves of four *Etlingera* species sampled from highland and lowland locations.

Species and location	Voucher number	Altitude (m asl)	Moisture content (%)	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
<i>Etlingera elatior</i> - Janda Baik FRIM	EC03	400 100	66.1 ± 2.0	3550 ± 304 ^a 2390 ± 329 ^b	3750 ± 555 ^a 2280 ± 778 ^b
<i>Etlingera fulgens</i> - Janda Baik FRIM	EC04	400 100	74.3 ± 0.1	2270 ± 31 ^a 1280 ± 143 ^b	2030 ± 126 ^a 845 ± 158 ^b
<i>Etlingera littoralis</i> - Genting Highlands FRIM	EC05	800 100	71.2 ± 0.8	2810 ± 243 ^a 2340 ± 386 ^a	2930 ± 220 ^a 2220 ± 913 ^a
<i>Etlingera rubrostriata</i> - Ulu Gombak FRIM	EC07	300 100	71.6 ± 2.8	3480 ± 390 ^a 2430 ± 316 ^b	3540 ± 401 ^a 2640 ± 508 ^a

Values of TPC and AEAC are means ± SD ($n = 3$). For columns of each species, values followed by the same letter (a-b) are not significantly different at $P < 0.05$ measured by the Tukey HSD test. ANOVA does not apply between species.

Higher altitudes seem to trigger an adaptive response in the species of *Etlingera*. The higher leaf TPC and AEAC of highland populations over lowland counterparts might be due to environmental factors such as higher UV-B radiation and lower air temperature. Plants protect themselves from oxidative damage due to UV exposure by producing antioxidative compounds (Larson, 1988). Enhanced UV-B radiation induces greater production of phenolic compounds (Bassman, 2004). Enzymes associated with the synthesis of phenolics are produced in greater quantities or show increased activity (Chalker-Scott & Scott, 2004). Phenylalanine ammonia lyase (PAL) of the phenylpropanoid pathway is up-regulated resulting in the accumulation of flavonoids and anthocyanins, which have free radical scavenging ability (Jansen *et al.*, 1998). Low temperatures have also been shown to enhance PAL synthesis and activity in a variety of plants, leading to an increase in flavonoids and other phenolics (Chalker-Scott & Scott, 2004).

Conclusion

Based on five wild and six cultivated ginger species belonging to seven genera, highest TPC and AOA were found in the leaves of *Etlingera*. For

most species screened, leaf TPC and/or AEAC were significantly higher than those of rhizomes. Rhizomes from different cultivars showed variability in TPC and AEAC. Leaves of highland populations of *Etlingera* had higher values than lowland counterparts. Of the four species studied, highest TPC and AEAC were found in the leaves of highland populations of *Etlingera elatior* and *Etlingera rubrostriata*.

Acknowledgements

The authors are thankful to botanists in FRIM, Saw, L.G. and Lau, K.H., who assisted in the identification of wild ginger species. Chung, R.C.K. and Sam, Y.Y. facilitated the deposition of voucher specimens in the FRIM herbarium. The financial support by Monash University Sunway Campus is gratefully acknowledged.

References

- Agnaniet, H., C. Menut and J.M. Bessiere. 2004. Aromatic plants of tropical central Africa. Part XLIX: chemical composition of essential oils of the leaf and rhizome of *Aframomum giganteum* K. Schum from Gabon. *Flavour and Fragrance Journal* **19**: 205–209.
- Bassman, J.H. 2004. Ecosystem consequences of enhanced solar ultraviolet radiation: secondary plant metabolites as mediators of multiple trophic interactions in terrestrial plant communities. *Photochemistry and Photobiology* **79**: 382–398.
- Chalker-Scott, L. and J.D. Scott. 2004. Elevated ultraviolet-B radiation induces cross-protection to cold in leaves of *Rhododendron* under field conditions. *Photochemistry and Photobiology* **79**: 199–204.
- Frankel, S. and M. Berenbaum. 1999. Effects of light regime on antioxidant content of foliage in a tropical forest community. *Biotropica* **31**: 422–429.
- Habsah, M., M. Amran, M.M. Mackeen, N.H. Lajis, H. Kikuzaki, N. Nakatani, A.A. Rahman, Ghafar and A.M. Ali. 2000. Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. *Journal of Ethnopharmacology* **72**: 403–410.

- Ibrahim, I., N. Khalid and K. Hussin. 2006. *Cultivated gingers of Peninsular Malaysia: Utilization, profiles and micropropagation*. Special lecture presented at the Fourth International Symposium of the Family Zingiberaceae, 3–6 July 2006, Singapore Botanic Gardens, Singapore (this volume).
- Jansen, M.A.K., V. Gaba and B.M. Greenberg. 1998. Higher plants and UV-B radiation: balancing damage, repair and acclimation. *Trends in Plant Science* **3**: 131–135.
- Jitoe, A., T. Masuda, I.G.P. Tengah, D.N. Suprpta, I.W. Gara, and N. Nakatani. 1992. Antioxidant activity of tropical ginger extracts and analysis of the contained curcuminoids. *Journal of Agricultural and Food Chemistry* **40**: 1337–1340.
- Kahkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala and M. Heinonen. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry* **47**: 3954–3962.
- Katsube, T., H. Tabata, Y. Ohta, Y. Yamasaki, E. Anuurad, K. Shiwaku and Y. Yamane. 2004. Screening for antioxidant activity in edible plant products: Comparison of low-density lipoprotein oxidation assay, DPPH radical scavenging assay and Folin-Ciocalteu assay. *Journal of Agricultural and Food Chemistry* **52**: 2391–2396.
- Khaw, S.H. 2001. The genus *Etlingera* (Zingiberaceae) in Peninsular Malaysia including a new species. *Gardens' Bulletin Singapore* **53**: 191–239.
- Larsen, K., H. Ibrahim, S.H. Khaw and L.G. Saw. 1999. *Gingers of Peninsular Malaysia and Singapore*. Natural History Publications (Borneo), Kota Kinabalu. 135 pp.
- Larson, R.A. 1988. The antioxidants of higher plants. *Phytochemistry* **27**: 969–978.
- Leong, L.P. and G. Shui. 2002. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry* **76**: 69–75.
- Lim, C.K. 2000. Taxonomic notes on *Etlingera* Giseke (Zingiberaceae) in Peninsular Malaysia: the “*Nicolaia*” taxa. *Folia Malaysiana* **1**: 1–12.

- Lim, C.K. 2001. Taxonomic notes on *Etlingera* Giseke (Zingiberaceae) in Peninsular Malaysia: the “*Achasma*” taxa and supplementary notes on the “*Nicolaia*” taxa. *Folia Malaysiana* **2**: 141–178.
- Lim, C.K. 2003. Taxonomic notes on *Elettariopsis* Baker, and new taxa from Peninsular Malaysia and Thailand. *Folia Malaysiana* **4**: 205–226.
- Miliauskas, G., P.R. Venskutonis and T.A. van Beek. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry* **85**: 231–237.
- Percival, M. 1996. Antioxidants. *Clinical Nutrition Insights* **1/96**: 1–4.
- Poulsen, A.D. 2006. *Etlingera Giseke* of Java. Special lecture presented at the Fourth International Symposium of the Family Zingiberaceae, 3–6 July 2006, Singapore Botanic Gardens, Singapore (this volume).
- Sirirugsa, P. 1999. Thai Zingiberaceae: species diversity and their uses. <http://www.iupac.org/symposia/proceedings/phuket97/sirirugsa.html>.
- Zaeoung, S., A. Plubrukarn and N. Keawpradub. 2005. Cytotoxic and free radical scavenging activities of Zingiberaceous rhizomes. *Songklanakarin Journal of Science and Technology* **27**: 799–812.

Studies on the Rhizome Oils from Four *Hedychium* Species of South India: A Chemotaxonomic Approach

M. DAN ¹, B. SABULAL ¹, V. GEORGE ¹ AND P. PUSHPANGADAN ²

¹Tropical Botanic Garden and Research Institute,

Pacha-Palode, Thiruvananthapuram - 695 562, Kerala, India

²Amity Institute for Herbal and Biotech Product Development,

Noida, New Delhi, India

Abstract

The genus *Hedychium* J. König (Zingiberaceae) with about 80 species has only four members in south India, viz., *H. coronarium* J. König, *H. flavescens* Carey ex Roscoe, *H. spicatum* var. *acuminatum* (Roscoe) Wall., and the endemic, *H. venustum* Wight. The chemical compositions of essential oils from the rhizomes of these four *Hedychium* species and their morphologies have been compared. The rhizome oils were characterized by analytical gas chromatography and gas chromatography-mass spectroscopy. The number of identified constituents in the rhizome oils of *H. coronarium*, *H. flavescens*, *H. spicatum* var. *acuminatum* and *H. venustum* were 24 (99.7%), 27 (98.8%), 41 (98.9%) and 57 (99.1%) respectively. 1,8-cineole, β -pinene and linalool constituted 70-75% of these rhizome oils. 1,8-cineole was the single major constituent in the rhizome oils of *H. coronarium* (48.7%), *H. venustum* (45.4%), and *H. spicatum* var. *acuminatum* (44.3%). β -pinene (43.6%) was the major component in the rhizome oil of *H. flavescens*. The percentages of sesquiterpenes in these oils were *H. venustum* (24.0%), *H. spicatum* var. *acuminatum* (22.1%), *H. coronarium* (3.1%) and *H. flavescens* (1.3%). Oil yields from the rhizomes of *H. venustum*, *H. spicatum* var. *acuminatum* and *H. coronarium* were comparable (0.13-0.16%), but that from the rhizomes of *H. flavescens* was substantially low (0.05%). *H. venustum* and *H. spicatum* var. *acuminatum* are morphologically similar and significantly different from *H. flavescens*. The chemical data on essential oils are in good agreement with the relative morphological features of these four *Hedychium* species and thus chemotaxonomically significant.

Introduction

The genus *Hedychium* J. König (Zingiberaceae) represents the spectacular

‘Ginger lilies’ comprising over 80 species distributed in India, China and South East Asia. In India it has about 50 species with about 17 endemics (Sabu, 2000). The genus is represented in south India by only four species, viz., *H. coronarium* J. König, *H. flavescens* Carey ex Roscoe, *H. spicatum* var. *acuminatum* (Roscoe) Wall. and *H. venustum* Wight, of which *H. venustum* is endemic to this region. *H. coronarium* is widely cultivated as a garden plant and the rest are restricted to hilly slopes at altitudes ranging from 1000 to 1800 m. All the species have fleshy, branched rhizomes characteristically aromatic due to the presence of essential oils. Essential oils are complex, volatile, aromatic, heterogeneous mixtures containing mostly mono- and sesquiterpenes and their derivatives. Inter-relationships of these four South Indian taxa of *Hedychium* were established by correlating their morphological characters and the percentages and distribution of mono- and sesquiterpenes in their rhizome oils.

Materials and Methods

Flowering specimens and rhizomes of *Hedychium flavescens*, *H. spicatum* var. *acuminatum* and *H. venustum* were collected from established populations in natural habitats at Devikolam, Munnar (Idukki District) and Ponmudi (Thiruvananthapuram District), Kerala State in South India. *H. coronarium* was collected from an established population in Tropical Botanic Garden and Research Institute (TBGRI), Thiruvananthapuram. All specimens were collected in September of 2004, and their vouchers (Mathew 54620, 54609, 54622 and 54624, respectively) were deposited in the Herbarium of TBGRI (TBGT). Seventeen morphological characters from each species were compared (Table 1), of which quantitative characters are the mean values of six measurements taken from different shoots within a clump. The measurements of leaves (length, breadth and area) were determined from middle leaves of different shoots.

Essential oils of these four taxa were isolated from fresh rhizomes by hydrodistillation in a Clevenger-type apparatus for 3 hrs. Chemical constituents of these oils were analysed by analytical gas chromatography (GC-FID) and gas chromatography-mass spectroscopy (GC-MS) as described in Sabulal *et al* (2007). GC-FID of rhizome oils were carried out on a Nucon 5765 gas chromatograph with a SE-30 10% Chromosorb-W packed stainless steel column (2 m x 2 mm). Oven programme consisted of 60°C (5 min), 60°-260°C (5°C/min), 260°C (10 min); carrier gas – nitrogen, flow rate 40 ml/min; injector temperature 240°C; detector temperature 240°C. GC-MS analysis of these oils were performed by splitless injection of 1.0 µl of each oil on a Hewlett Packard 6890 gas chromatograph fitted with a

Table 1. Comparison of morphological characters of four species of *Hedychium*. See text for the species names.

Characters	<i>H. cor.</i>	<i>H. fla.</i>	<i>H. spi. ac.</i>	<i>H. ven.</i>
Shoot length (cm)	120 - 180	200- 260	100-140	100- 180
Leaf length (cm)	40-50	35-45	24-30	35-40
Leaf breadth (cm)	8-12	10-14	6-8	10-12
Leaf area (cm ²)*	206	258	187	210
Ligule length (cm)*	3.5	3.0	0.94	1.6
Petiole length (cm)*	1.0	1.5	1.0	3.5
Spike length (cm)*	10.3	15.6	19	23
Spike nature	Dense	Dense	Lax	Lax
Peduncle length (cm)*	1.5	1.5	2.0	2.0
Bract length (cm)*	6.0	5.0	2.3	2.5
Bract breadth (cm)*	3.1	2.5	1.0	1.0
Corolla lobe colour	White	Yellow	Yellow	Yellow
Staminode colour	White	Yellow	White	White
Lip length (cm)*	6.0	5.0	2.5	2.5
Lip shape	Obovate	Obovate	Ob lanceolate	Ob lanceolate
Lip colour	White	Yellow	White	White
Stamen colour	White	Yellow	Red	Red

*Values are mean of six measurements

cross-linked 5% PH ME siloxane HP-5 MS capillary column, 30 m x 0.32 mm, 0.25 μ coating thickness, coupled with a model 5973 mass detector. GC-MS operation conditions: injector temperature - 220°C; transfer line - 240°C; oven temperature programme - 60° to 243°C (3°C/min); carrier gas -He at 1.4 ml/min. Mass spectra: Electron Impact (EI⁺) mode 70 eV, ion source temperature 240°C. Individual components were identified by Wiley 275.L database matching, comparison of mass spectra with published data and by comparison of their relative retention times. Morphological inter-relationship of the four species was established by calculating the Simple Similarity Coefficient (Sr.) (Table 2) using the following formula proposed by Sokal & Sneath (1963). The Sr. of chemical interrelationship was also calculated using the same formula (Table 3).

$$\text{Sr} = \frac{\text{common characters of two species}}{\text{common characters of two species} + \text{characters specific to one species} + \text{characters specific to other species}} \times 100$$

Results and Discussion

Morphological interrelationship based on Simple Similarity Coefficient of these four *Hedychium* species showed a maximum relationship between *H. spicatum* var. *acuminatum* and *H. venustum* with Sr. 36% (Table 2). The chemical inter-relationships between these four taxa were also determined based on the constituents identified from their rhizome oils by GC-MS analysis (Sabulal *et al*, 2007) (Table 3). *H. flavescens* is morphologically and chemically distinct from all others and showed maximum relationship to *H. coronarium* with Sr. 21% and 50% (Tables 2 & 3). The percentage of oil yields was calculated based on fresh weight of rhizomes. The oil yield was highest in *H. venustum* (0.16%) and lowest in *H. flavescens* (0.05%). The oil yields in *H. spicatum* var. *acuminatum* and *H. coronarium* were 0.13% and 0.13%, respectively.

Table 2. Simple Similarity Coefficient on morphological data between four species of *Hedychium* in South India. See text for the species names.

	<i>H. cor.</i>	<i>H. fla.</i>	<i>H. spi. ac.</i>	<i>H. ven.</i>
<i>H. cor.</i>	100	21	10	10
<i>H. fla.</i>		100	10	3
<i>H. spi. ac.</i>			100	36
<i>H. ven.</i>				100

Table 3. Simple Similarity Coefficient on chemical data between four species of *Hedychium* in South India. See text for the species names.

	<i>H. cor.</i>	<i>H. fla.</i>	<i>H. spi. ac.</i>	<i>H. ven.</i>
<i>H. cor.</i>	100	50	33	29
<i>H. fla.</i>		100	28	24
<i>H. spi. ac.</i>			100	66
<i>H. ven.</i>				100

Fifty-seven constituents (99.1%) were identified in the rhizome oil of *H. venustum*, forty-one constituents (99.2%) from *H. spicatum* var. *acuminatum*, twenty-five constituents (98.2%) from *H. flavescens*, and twenty-three constituents (97.5%) from *H. coronarium*. Thirteen constituents were found common in all the four species. The major constituents in these species were the monoterpenes 1,8-cineole, β -pinene and linalool, constituting 70-75% of these oils (Sabulal *et al*, 2007). 1,8-Cineole is the major constituent in three species, while β -pinene is the major one in *H. flavescens* (Fig. 1) showing the divergence of monoterpene metabolic pathways in *H. flavescens* in agreement with its morphological and chemical dissimilarity with others. Out of the twenty-five constituents identified from *H. flavescens*, sixteen are common to *H. coronarium* and thus, the Sr. on chemical data between the two is 50% (Table 3). Chemical relation of *H. flavescens* with *H. spicatum* var. *acuminatum* is 28% because, constituents found common to them are fifteen out of the total forty-one constituents identified from the latter and with *H. venustum*, it is only 24% (Table 3) as sixteen constituents are common out of the total fifty-seven. Lower percentage of β -pinene and higher percentage of 1,8-cineole in *H. spicatum* var. *acuminatum* and *H. venustum* (Fig. 1) support their close similarity in morphological and chemical inter-relationships. The monoterpenes, viz., myrcene, limonene, p-cymene, camphene and γ -terpinene, were identified from all the four taxa studied here (Fig. 2). These monoterpenes were reported as common constituents in all *Hedychium* species previously studied (Medeiros *et al.*, 2003). The relative percentages of these monoterpenes are highest in *H. flavescens* (Fig. 2).

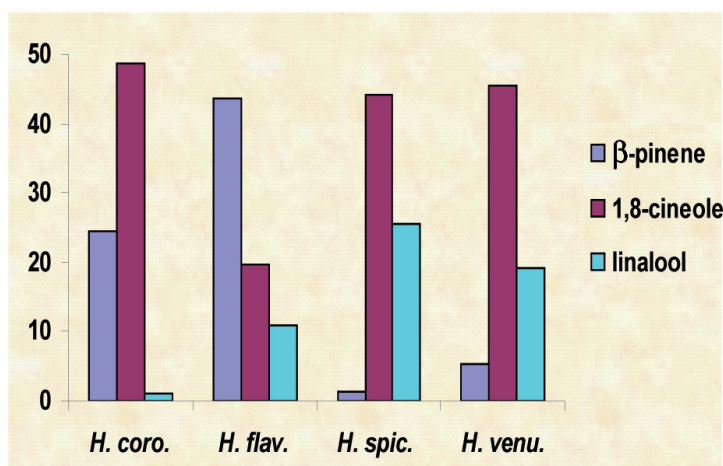


Figure 1. Percentage of major monoterpenes found in four species of *Hedychium* in South India.

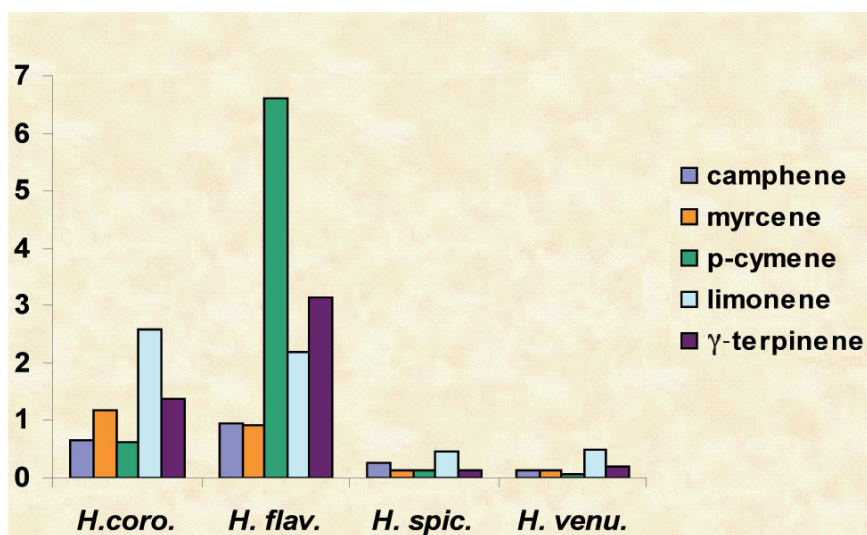


Figure 2. Percentage of minor monoterpenes common to four *Hedychium* species in South India.

Sesquiterpenes, with farnesyl pyrophosphate (FPP) as its precursor, are more evolved in the biosynthetic pathway than monoterpenes, with geranyl pyrophosphate (GPP), as its precursor. The sesquiterpene distribution is more dominant in *H. venustum* compared to others. The number and percentage of sesquiterpenes and their derivatives in *H. venustum* were 38 and 24.0%, respectively. The number and percentages of sesquiterpenes in *H. spicatum* var. *acuminatum*, *H. coronarium* and *H. flavescens* were 23 (22.1%), 4 (3.1%) and 6 (1.3%) respectively (Fig. 3). The role of essential oil constituents as markers in chemotaxonomic studies is described by Hegnauer (1982) and very well proved by many botanists as well as chemists. Among these four south Indian taxa of *Hedychium*, *H. venustum* closely resembles *H. spicatum* var. *acuminatum* morphologically as well as in mono- and sesquiterpene distribution indicating their phylogenetic affinity. Thirty-nine constituents are common in the essential oils of these two species and resulted in 66% chemical similarity between them (Table 3). The current data also suggest that transformation of the farnesyl pyrophosphate to various sesquiterpenes by terpenoid synthases is most advanced in *H. venustum*, followed by *H. spicatum* var. *acuminatum*. The major monoterpene component, β -pinene, and relatively very low numbers of sesquiterpenes in the oil suggest the sharp difference in mono- and sesquiterpene pathways in *H. flavescens* and also indicate its primitiveness in phylogeny.

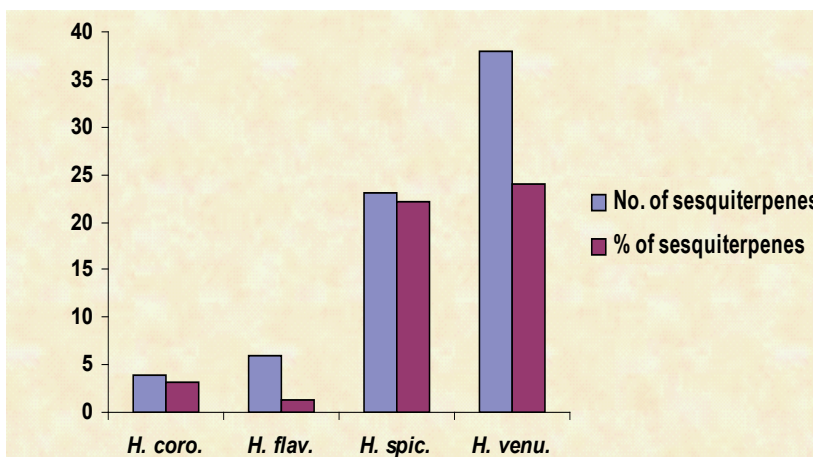


Figure 3. Number and percentage of sesquiterpenes found in four species of *Hedychium* in South India.

Conclusion

The major constituents of the rhizome oil of all the four south Indian *Hedychium* species are monoterpenes, viz., 1,8- cineole, β -pinene and linalool (70-75%). The inter-relationship of the four taxa based on chemical data is well in tune with that of morphological data. *H. venustum* is morphologically closer to *H. spicatum* var. *acuminatum* and they also show 66% chemical similarity to each other. *H. flavescens* is distinct from others morphologically as well as chemically. The species-specific chemical profiles of essential oils from these four *Hedychium* taxa are chemotaxonomically significant.

References

- Hegnauer, R. 1982. Distribution and chemotaxonomy of essential oils, pp. 1-23. In: Atal, C. K. and Kapur, B. M. (eds.) *Cultivation and Utilization of Aromatic Plants*. RRL, CSIR, Jammu-Tawi.
- Medeiros, J. R., L.B. Campos, S.C. Mendonca, L.B. Davin and N.G. Lewis. 2003. Composition and antimicrobial activity of the essential oils from invasive species of the Azores, *Hedychium gardnerianum* and *Pittosporum undulatum*. *Phytochemistry* **64**: 561-565.
- Sabu, M. 2000. *Hedychium spicatum* Ham. ex Smith var. *acuminatum* (Roscoe) Wall. – a new record for peninsular India. *Rheedea* **10**: 73-76.

Sabulal, B., V. George, M. Dan and N.S. Pradeep. 2007. Chemical composition and antimicrobial activities of the essential oils from the rhizomes of four *Hedychium* species from south India. *Journal of Essential Oil Research* **19**: 93-97.

Sokal, R. R. and P.H.A. Sneath. 1963. *Principles of Numerical Taxonomy*. Freeman, San Fransisco & London.

Micropropagation of *Boesenbergia pulchella* (Ridl.) Merr., a Potential Ornamental Plant

M.N. HAMIRAH¹, H.B. SANI¹, P.C. BOYCE² AND S.L. SIM¹

¹ Institute of Biodiversity and Environmental Conservation, University of Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

² Malesiana Tropicals, Suite, 9-04, Tun Jugah Tower, No 18 Jln Tunku Abd Rahman, 90100 Kuching, Sarawak, Malaysia

Abstract

Shoot tips of *Boesenbergia pulchella* (Ridl.) Merr. were cultured on Gamborg B5 medium containing 30% (w/v) sucrose and 2.8% (w/v) Gelrite. Various concentration of plant growth regulators (PGR) were supplemented to B5 media, i.e., 6-benzylaminopurine (BAP) at 1-4 mg/l alone or in combination with α -naphthalene acetic acid (NAA) at 0.1 mg/l or thidiazuron (TDZ) at 0.1-0.7 mg/l. Multiple shoot formation were found on both media supplemented with TDZ and BAP. A maximum of 11 shoots were produced after treatment with TDZ at 0.3 mg/l, which were the highest among other treatments. Acclimatization were conducted on (1:1 v:v) soil and vermiculite.

Introduction

Boesenbergia pulchella (Ridl.) Merr. is a small herb known as 'jerangau' locally. This plant is a member of one of the most advanced monocotyledonous plant family, Zingiberaceae. Commonly, this attractive plant can be found on the forest floor. They have beautiful glossy green leaves and the inflorescences are lanceolate on separate leafless shoot. The flower is small, open from apex to bottom with white and red labellum. Since they possess beautiful flower and foliage, they can be introduced as ornamental plants and have the potential to be commercialized, either as indoor potted plant or garden plant, even for landscaping purposes.

To achieve the goal of commercializing this plant, a large number of planting materials are needed. Propagation through conventional technique via rhizome cutting is very slow. This plant will produce shoot once they flower, which takes about three to four months after the shoot emerges. The highest number of shoot produced is two per mother plant, usually it is just one, and this restricts the use of conventional means. Micropropagation

enables mass production of clone and thus could satisfy the demand for planting material. The other benefit of using this technique is that the plantlet is disease-free and true-to-type.

Successful micropropagation of a number of species in Zingiberaceae such as *Zingiber officinale* Roscoe, *Curcuma longa* L., *Kaempferia galangal* L. and *Alpinia galanga* (L.) Willd. were reported (Bhagyalakshmi and Singh, 1988; Shirkumar *et al.*, 2001; Shirin *et al.*, 2000; Borthakur *et al.*, 1999). However, to date, no successful micropropagation protocol has been reported for this species.

The present investigation is an attempt towards establishing a reliable *in vitro* regeneration protocol for *Boesenbergia pulchella* Ridley for use in large-scale propagation. Different concentrations of BAP with or without NAA and TDZ were tested to find the best PGR that can induce the optimum multiplication rate for this species.

Materials and Methods

Explants sources and sterilization

The stock plants for this study were collected from Gunung Ampungan in Kota Samarahan district. Rhizome buds between 1-1.5 cm were selected as initial explants. The fresh buds collected were cleaned of soil dirt and left under running tap water for one to one and a half hour. Then the buds were immersed in 75% (w/v) ethanol for one minute. Without rinsing, they were agitated in 30% (w/v) Clorox (5.25% w/v sodium hypochlorite) added with 0.1ml/l Tween 20 for 20 minutes with constant agitation. After that they were rinsed with sterile-distilled water four times. Under aseptic condition the buds scale were peeled-off and they were trimmed to about 0.5 cm long.

Establishment of axenic culture

The trimmed buds were cultured on Gamborg B5 medium, gelled with 2.8g/l Gelrite, 30% sucrose. The pH was adjusted to 5.7-5.8 with 1N KOH or 0.1 N HCL prior to autoclaving. Tetracycline at 10 mg/l and 1 ml/l Plant Preservative Mixture (PPM) were added to the medium to reduce contamination. After 15 days, the axenic culture were cut into half and subcultured onto B5 medium supplemented with BAP at 1 mg/l for 4 weeks to induce more shoots. The shoots were subcultured on B5 media for 2 weeks before they were used in subsequent experiment.

To study the effects of different types and concentrations of PGR on shoot multiplication, different treatments were used, i.e., BAP at 1, 2, 3 and 4 mg/l alone or each added with 0.1 mg/l NAA and TDZ at 0.1, 0.3, 0.5 and 0.7 mg/l.

Rooting and acclimatization

Rooting was induced on B5 medium without plant growth regulator. Plantlet at about 6-8 cm height were taken out from the vessel and washed thoroughly with tap water before they were transferred to plastic pot containing 1:1 soil and vermiculite. The plants were covered with plastic to retain moisture.

Results and Discussion

After one week, the explants turned greenish from white. For establishment of axenic culture, B5 medium with and without tetracycline and PPM were used. However, addition of tetracycline and PPM did not reduce contamination satisfactorily if compared with explants cultured on B5 medium only. About 50% of the explants were discarded for bacterial contamination. The result showed that tetracycline was not beneficial to control bacterial contamination for this species.

Induction of multiple shoots

In this study, excised shoot obtained from *in vitro* raised plants were used as explants. Shoots were divided into half prior to culture on different treatment of PGR. Treatment with BAP alone or in combination with NAA showed variability in terms of number of shoots produced per explant (Table 1). Optimum concentration for shoot multiplication was found on medium supplemented with BAP at 3 mg/l, which produced 6.6 shoots per explants. However, in terms of number of days the first bud sprouted, the duration was not really different between one treatment to another. After two weeks, at least one bud sprouted for each treatment. The basal part of the explant was enlarging before the new buds sprouted from the lateral side.

For treatment with BAP added with NAA 0.1 mg/l, the number of shoots produced was not much different with the treatment using BAP alone. The highest number of shoots was obtained on B5 medium supplemented with BAP at 3 mg/l added with NAA at 0.1 mg/l with a mean of 6.8 shoots per explant. Hence, this proved that addition of NAA was not beneficial in increasing the number of shoots. Miachir *et al.* (2004) also reported a similar finding on *Curcuma zedoaria* (Christm.) Roscoe where addition of NAA with BAP was not effective for shoot multiplication. However, results from this study showed that explants that were subjected to this treatment produced roots faster than treatment with BAP alone or TDZ. This was probably due to the fact that NAA is an auxin that helps in promoting root development.

In TDZ supplemented media, highest number of shoots obtained was on medium incorporated with TDZ at 0.3 mg/l. Eleven shoots per

explants were developed in the above medium and this was the highest among other treatments. However, more shoot clusters were formed on media supplemented with TDZ. These clusters were later subcultured on fresh medium and were able to regenerate into multiple shoots. The clusters were first separated into smaller clump, since the regeneration was faster if compared to larger clump based on the observation.

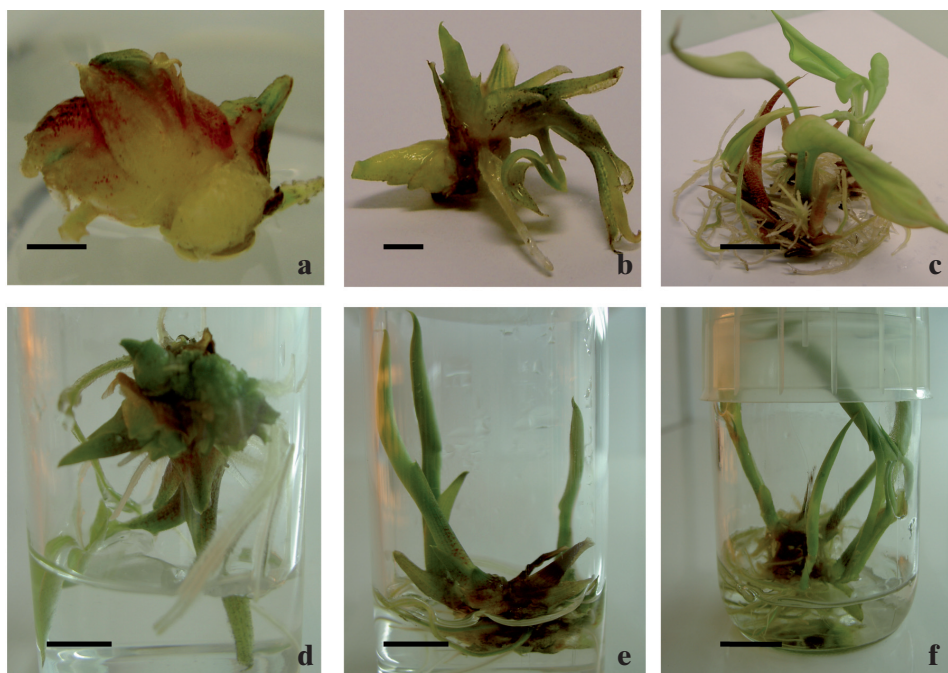
Table 1. Shoots multiplication under different growth regulators after 12 weeks of culture.

Growth regulator (mg/l)	*No of shoots/explant	Days to induction of new shoots
BAP (1)	3.60 ± 0.68	13
BAP (2)	4.40 ± 0.86	15
BAP (3)	6.60 ± 1.03	13
BAP (4)	4.20 ± 1.20	15
BAP (1) + NAA (0.1)	4.60 ± 0.75	13
BAP (2) + NAA (0.1)	4.00 ± 0.84	15
BAP (3) + NAA (0.1)	6.80 ± 1.24	15
BAP (4) + NAA (0.1)	3.96 ± 1.77	12
TDZ (0.1)	9.20 ± 3.44	12
TDZ (0.3)	11.00 ± 1.52	15
TDZ (0.5)	9.80 ± 1.74	11
TDZ (0.7)	6.00 ± 1.14	20

* Data expressed as mean ± SE from 5 replicates

Among the shoot multiplication studies conducted, it is shown that TDZ was able to regenerate a high number of shoots even at lower concentration. This accords with Tefera and Wannakrairoj (2000) where they managed to obtain 15.52 shoots on treatment with TDZ at 0.5 mg/l for multiplication of *Curcuma longa*. However for BAP, a higher concentration is needed to obtain more shoots. In fact, previous studies on other Zingiberaceae species did use a high BAP concentration. Nayak (2000) obtained the highest shoot multiplication of *Curcuma aromatica* Salisb. on medium supplemented with BAP at 5 mg/l and Samsudeen *et al.* (2000) used 10 mg/l BAP to induce organogenesis in *Zingiber officinale*.

Rooting was relatively easy for this species. Vigorous roots were formed on B5 medium without growth regulator. Plants were successfully acclimatized with survival rate of 80% after two months.



Figures a-c. Shoots multiplication on B5 medium supplemented with BAP at 3 mg/l after 4, 8 and 12 weeks of culture (bar = 0.5, 1 and 2 cm); **Figures d-f.** Shoots multiplication on B5 medium supplemented with TDZ at 3 mg/l after 4, 8 and 10 weeks of culture (bar = 1, 1.5 and 2.5 cm).

Conclusions

In vitro technique is a useful approach for propagating plants on large scale. For ginger species, propagation through conventional technique is time consuming and prone to spread disease by rhizome cuttings.

Shoots multiplication of *Boesenbergia pulchella* can be obtained using TDZ and BAP. While TDZ can produce shoots at a lower concentration, BAP is needed at higher concentration to produce similar results. Addition of NAA is not beneficial for shoot multiplication of this species.

Acknowledgement

The authors would like to acknowledge the IGS fund (Grant no. IGS (R&D) 16/03) granted by The Ministry of Science, Technology and Innovation (MOSTI), Malaysia for financial support of the research. We wish to express our thanks for the collaboration and support of the Sarawak Forestry Department, notably Datu Cheong Ek Choon, the Sarawak Biodiversity

Centre, in particular Datin Eileen Yen Ee Lee and the Forest Research Centre (Kuching), notably L.C.J. Julaihi Abdullah. The tertiary author wishes to thank Datuk Amar (Dr) Leonard Linggi Tun Jugah, Graeme Brown and Dr Timothy Hatch of Malesiana Tropicals Sdn Bhd for their considerable support and funding of fieldwork in Sarawak

References

- Bhagyalakshmi and N.S. Singh. 1988. Meristem culture and micropropagation of a variety of ginger (*Zingiber officinale* Rosc.) with a high yield of oleoresin. *Journal of Horticultural Science* **63**: 321-327.
- Borthakur, M., J. Hazarika and R.S. Singh. 1999. A protocol for micropropagation of *Alpinia galanga*. *Plant Cell, Tissue and Organ Culture* **55**: 231-233.
- Miachir, J.I., V.L.M. Romani, A.F. de Campos Amaral, M.O. Mello, O.J. Crocomo and M. Melo. 2004. Micropropagation and callogenesis of *Curcuma zedoaria* Roscoe. *Scientific Agriculture* **61**: 427-432.
- Nayak, S. 2000. In vitro multiplication and microrhizome induction in *Curcuma aromatica* Salisb. *Plant Growth Regulation* **32**: 41-47.
- Samsudeen, K., K. Nirmal Babu, M. Divakaran and P.N. Ravindran. 2000. Plant regeneration from anther derived callus cultures of gingers (*Zingiber officinale* Rosc.) *Journal of Horticultural Science and Biotechnology* **75**: 447-450.
- Shirgukur, M.V., C.K. John and R.S. Nagdauda. 2001. Factors affecting in vitro microrhizome production in turmeric. *Plant Cell, Tissue and Organ Culture* **64**: 5-11.
- Shirin, F., S. Kumar and Y. Mishra. 2000. In vitro plantlet production system for *Kaempferia galanga*, a rare Indian medicinal herb. *Plant Cell, Tissue and Organ Culture* **63**: 193-197.
- Tefera, W. and S. Wannakrairoj. 2004. A micropropagation method for Korarima (*Aframomum corrorima* (Braun) Jansen). *Science Asia* **30**: 1-7.

Cultivated Gingers of Peninsular Malaysia: Utilization, Profiles and Micropropagation

H. IBRAHIM ¹, N. KHALID ¹ AND K. HUSSIN ²

¹ Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

² School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, National University of Malaysia, 43600 Bangi, Malaysia

Abstract

There are approximately 160 species of Zingiberaceae belonging to 18 genera in Peninsular Malaysia. Roughly 16-20% are traditionally utilized by the indigenous folks as spices, condiments, vegetables, food flavours and medicines. The resurgence of interest in herbs and the potential lucrative anticipated revenues from the herbal industry have spurred renewed interest in exploiting traditional knowledge and practices into scientific realities. Current research priorities offer promising development of natural resources into nutraceuticals, cosmeceuticals and biopharmaceuticals. Hence the need to profile or fingerprint species for quality control and consistency of the species utilized. It is also important to establish protocols for micropropagation as a means of providing consistent supply of stable and elite materials for mass propagation and commercialization. Selected examples of indigenous uses, species profiles and successful micropropagation of cultivated gingers are discussed.

Introduction

Besides Thailand, Malaysia represents one of the richest region in terms of Zingiberaceous species in South East Asia. Approximately 60 % of the total land area of Malaysia is reportedly covered by tropical rainforests and an estimate of 15,000 species of flowering plants has been recorded. Of these, there are more than 320 species of Zingiberaceae (excluding many undescribed taxa) representing 21 genera. In Peninsular Malaysia, there are roughly 160 species of Zingiberaceae belonging to 18 genera (Larsen *et al.*, 1999). Worldwide there are more than 1,200 species of Zingiberaceae belonging to more than 50 genera. Hence the total count for Malaysia

account for at least 20 % of the world taxa and 40 % of the world genera. The Zingiberaceae species have been utilized for various purposes worldwide and have been a part of the Asian culture since time immemorial. In Malaysia Zingiberaceae species are used as spice, condiment, food flavour, vegetable, beverage, medicine, ornamental as well as in rituals associated with beliefs, customs and traditions. Of late some cultivated gingers are exploited for the cosmeceutical, neutraceutical and pharmaceutical industry. Between 16-20 % of the Peninsular Malaysian gingers are edible and these are consumed fresh, cooked, pickled or boiled. The plant parts consumed are mainly rhizomes but the inflorescences, fruits, seeds, young shoots and rarely leaves are also utilized.

Utilization

The growth rate in global herbal industry is estimated at 7 % annually with an estimated value of USD \$183 billion in 2005. Blessed with a rich biodiversity, Malaysia has identified the herbal industry as another source of economic engine of growth and as such has provided the relevant national policies for the development of this important industry. In the Malaysian scenario, ginger has been identified as one of the 10 most popular local herbs that have great commercial potential (data from Malaysian Herbal Corporation). In maximising the economic potential of our rich bio-resources, ethnic knowledge and practices need to be exploited and developed into scientific realities. One of the significant contribution of such knowledge is the practices of traditional complementary medicine (TCM). Global market surveys have revealed that TCM plays a major role in the healthcare market both in developing and industrialized nations, most prominent being China (100%), followed by Africa (70-80%) and India (70%) (data from Malaysian Herbal Corporation). Our ethnobotanical surveys of selected states in Peninsular Malaysia representing East, West and South West region of Peninsular Malaysia revealed some interesting findings with regards to TCM.

In general, the practices of TCM in the villages surveyed are influenced by the following factors:

- socio – economic status;
- availability of modern medicine;
- remoteness of the village;
- availability of the herbs used in TCM;
- the age of the population;
- the traditional knowledge of the population.

The results of our ethnobotanical surveys also indicated that Zingiberaceae species are among the most frequently used herbs in folk-medicine. For instance, many medicinal gingers are utilized for women-related ailments or healthcare, such as post- partum medicine, post-natal care treatment in the form of tonic, herbal extracts, decoctions, ointment, aromatic herbal bath, etc. These gingers are also reported to be carminative. Various species are used either as single plant or in herbal mixtures with several Zingiberaceae species or other herbs for treatment of arthritis, skin infections, inflammation, stomach-ache, muscle pains and strains etc. Selected examples of indigenous uses are presented as follows:

1. Post partum medicine and Post-natal health care

- rhizomes of *Boesenbergia rotunda* (L.) Mansf. eaten raw or pickled.
- rhizome juice of *Curcuma longa* L. and *Zingiber ottensii* Valeton drunk.
- leaves of various combinations of Zingiberaceae species, such as *Alpinia galanga* (L.) Willd., *Curcuma longa*, *Amomum compactum* Sol. ex Maton, *Etlingera elatior* (Jack) R.M. Sm., *Zingiber montanum* (J. König) A. Dietr., *Curcuma mangga* Valeton & Zijp in combination with other aromatic herbs such as *Pandanus amaryllifolius* Roxb., *Cymbopogon nardus* (L.) Rendle etc. are boiled and used as an aromatic herbal bath for ladies in confinement. This is practised for 2 weeks or throughout the confinement period (42 days).

2. Dysmenorrhea

- rhizome juice of *Alpinia conchigera* Griff. mixed with water and drunk.

3. Treatment for skin fungal infection (*panau*)

- ground rhizomes of *Alpinia conchigera* mixed with vinegar or kerosene and rubbed on infected parts.

4. Treatment for jaundice

- *Zingiber officinale* Roscoe boiled with *Alpinia galanga*, *Vigna radiata* (L.) R. Wilczek (mung bean), garlic and vinegar and the decoction drunk.

5. To relief flatulence/stomachache/colic

- rhizome juice of *Zingiber officinale* drunk.
- rhizome juice of *Curcuma zedoaria* (Christm.) Roscoe drunk.
- rhizome of *Curcuma mangga* eaten raw with rice.
- rhizome juice of *Alpinia galanga* mixed with other herbs and drunk.

6. Treatment for muscle pains & strains

- decoction of whole plant of *Curcuma aeruginosa* Roxb. drunk.
- oil of *Alpinia conchigera* applied topically.

7. Treatment for sprain

- poultice rhizome of *Kaempferia galanga* L. with rice, applied topically.
- poultice leaves of *Zingiber zerumbet* (L.) Sm., applied topically.

8. Health drink/treatment for lethargy

- rhizome juice of *Alpinia conchigera* and fresh milk drunk in the morning.

9. Treatment for aching joints (e.g., knee joints)

- rhizome juice of *Zingiber officinale* Roscoe var. *rubrum* Theilade and vinegar, applied topically.
- rhizome juice of *Zingiber officinale* drunk.

10. Treatment for hypertension

- rhizome of *Kaempferia galanga* eaten raw.
- rhizome of *Zingiber zerumbet* eaten raw.

11. Flavour

- leaves of *Elettariopsis curtisii* Baker for flavouring fish dish.
- leaves of *Curcuma longa* for flavouring vegetable, fish and meat dishes.
- rhizomes of *Alpinia galanga*, *Curcuma longa*, *Zingiber officinale* for flavouring various dishes.

12. Cosmetic powder

- rhizome of *Curcuma zedoaria* ground finely with glutinous rice and 100 types of flowers and soaked in water.
- leaves of *Kaempferia galanga* mixed with rice and several aromatic plant parts, ground and soaked in water; residue used as cosmetic powder.

Utilization: nutritional value of edible gingers

Realizing the diverse utilization of the cultivated gingers, studies have been carried out to investigate the nutritional composition of these species. Generally our result showed that the moisture content of the rhizomes is high exceeding 70% and low in crude fibre content. The low crude fibre content renders these species suitable as spices. The fat and carbohydrate content are relatively low in the species studied (Table 1). Table 2 shows the

data on thiamine, riboflavin and vitamin C of 5 cultivated gingers. Vitamin C content is generally low except for peeled rhizome of *Zingiber officinale* (11mg/100g) and young rhizome of *Curcuma mangga* (15.46 mg / 100g). This may justify the consumption of the young rhizome *Curcuma mangga* as a fresh vegetable in Peninsular Malaysia. Our studies on some mineral content of *Alpinia galanga*, *Curcuma longa*, *Kaempferia galanga* and *Etlingera elatior* (Table 3), indicated that ferum content is quite high in the roots of *K. galanga* (78.30mg /100g) and in rhizomes of *E. elatior* (67.10mg/100g). This result supports the development of *K. galanga* as a health drink as the rhizomes and the roots are usually taken as a whole when consumed. The screening on the anti-nutritional content showed that no cynogenic glycosides were detected in the twelve cultivated gingers studied (Rahim *et al.*, 1991; Ibrahim *et al.*, 1994).

Table 1. Proximate composition of some common Zingiberaceae species (per 100 g) [Hashim *et al.*, 1988; *Tee *et al.*, 1988; **English and Lewis, 1991; ***Zanariah *et al.*, 1997]

Species	Part	Energy kcal	Moisture (g)	Protien (g)	Fat (g)	Carbohydrate (g)	Fibre (g)	Ash (g)
<i>Z. officinale</i>	Young rhizome	-	88.5	-	-	2.1	-	-
<i>Z. officinale</i> *	Young rhizome	52	86.1	2.1	1.0	8.6	1.7	0.5
<i>Z. officinale</i> **	Peeled rhizome	26	90.4	0.8	0.4	4.8	-	-
<i>Z. officinale</i> ***	Mature rhizome	43	87.9	0.7	0.9	8.1	1.7	0.7
<i>C. domestica</i>	Fresh rhizome	-	83.9	-	-	6.0	-	-
<i>C. domestica</i> *	Dried rhizome	335	14.2	2.3	5.0	70.1	3.2	5.2
<i>C. domestica</i> ***	Rhizome	35	89.3	0.9	0.5	6.9	1.7	0.8
<i>Z. zerumbet</i>	Young rhizome	-	89.1	-	-	2.8	-	-
<i>Z. zerumbet</i> ***	Rhizome	45	88.5	0.4	0.7	8.9	1.3	0.3
<i>A. galanga</i> *	Rhizome	72	89.9	0.9	0.7	6.5	1.6	0.4
<i>A. galangal</i> ***	Rhizome	71	81.5	0.8	0.7	13.0	1.6	1.4
<i>C. mangga</i>	Young rhizome	-	81.1	-	-	3.8	-	-
<i>C. mangga</i> ***	Rhizome	47	88.1	0.4	1.2	8.6	1.1	0.5

Table 2. Vitamin composition of some Zingiberaceae species (mg/100g weight) [Hashim *et al.*, 1988; *Tee *et al.*, 1988; **English and Lewis, 1991; ***Zanariah *et al.*, 1997]

Species	Part	Thiamine	Ribo flavin	Ascorbic acid
<i>Z. officinale</i>	Young rhizome	-	-	2.83
<i>Z. officinale</i> *	Young rhizome	0.04	0.04	5.30
<i>Z. officinale</i> **	Peeled rhizome	0.09	0.06	11.00
<i>Z. officinale</i> ***	Mature rhizome	0.04	0.06	5.78
<i>C. domestica</i>	Fresh rhizome	-	-	1.83
<i>C. domestica</i> *	Dried rhizome	0.03	0.12	0.00
<i>Z. zerumbet</i>	Young rhizome	-	-	6.38
<i>Z. zerumbet</i> ***	Rhizome	0.02	0.02	1.65
<i>A. galanga</i> *	Fresh rhizome	0.02	0.40	0.00
<i>A. galanga</i> ***	Rhizome	0.03	0.01	0.93
<i>C. mangga</i>	Young rhizome	-	-	15.46
<i>C. mangga</i> ***	Rhizome	0.03	0.04	1.95

Ginger products

The popularity of herbal products has increased greatly in recent years due to the consumer's preference for natural ingredients in their medicine, food and personal care products. Several cultivated gingers in particular, *Zingiber officinale*, have been developed commercially (globally and locally) into various herbal products. Selected examples are given below:

1. Medicine

- Arthritis (e.g., Zinaxin).
- Nausea –tablets.
- Anti-inflammatory products.
- Flatulence and indigestion (e.g., Eno).
- Post partum medicine including the Indonesian *jamu* (capsules, tablets, tonic, powder, poultice).
- Medicinal oils / ointment.
- Balm.

2. Health products

- Ginger and ginseng capsules.
- Ginger and garlic capsules.
- Dietary supplement (vitamin C supplement).

Table 3. The levels^a of K, Na, Ca, Mg, Fe and Zn in the rhizomes, roots, leaves and inflorescence of *A. galanga*, *C. domestica*, *K. galanga* and *E. elatior* (Ibrahim and Rahim, 1988)

Part of the Plant	Zingiberaceae species	(g/100g) ^b				(mg/100g) ^b	
		K	Na	Ca	Mg	Fe	Zn
Rhizomes	<i>A. galanga</i>	1.53 ± 0.15	0.013 ± 0.001	0.09 ± 0.004	0.25 ± 0.02	0.28 ± 0.03	0.06 ± 0.06
	<i>C. domestica</i>	0.94 ± 0.08	0.023 ± 0.002	0.13 ± 0.003	0.16 ± 0.01	4.11 ± 0.30	13.90 ± 1.80
	<i>K. galanga</i>	0.50 ± 0.02	0.697 ± 0.010	0.26 ± 0.01	0.37 ± 0.02	4.20 ± 1.20	8.50 ± 0.76
	<i>E. elatior</i>	1.59 ± 0.17	0.135 ± 0.006	0.35 ± 0.003	0.15 ± 0.001	67.10 ± 15.30	0.09 ± 0.01
Roots	<i>A. galanga</i>	1.95 ± 0.74	0.06 ± 0.004	0.47 ± 0.01	0.30 ± 0.004	0.38 ± 0.09	0.10 ± 0.03
	<i>C. domestica</i>	4.89 ± 0.90	0.032 ± 0.008	0.15 ± 0.03	0.05 ± 0.01	N.D.	N.D.
	<i>K. galanga</i>	1.88 ± 0.43	1.130 ± 1.010	0.41 ± 0.01	0.62 ± 0.02	78.30 ± 9.80	5.90 ± 0.75
	<i>E. elatior</i>	2.58 ± 0.19	0.046 ± 0.002	0.26 ± 0.01	0.16 ± 0.007	0.12 ± 0.01	0.05 ± 0.01
Leaves	<i>A. galanga</i>	1.80 ± 0.20	0.015 ± 0.001	1.14 ± 0.07	0.43 ± 0.03	0.28 ± 0.02	0.19 ± 0.01
	<i>C. domestica</i>	2.88 ± 0.18	0.064 ± 0.004	0.84 ± 0.02	0.22 ± 0.02	10.70 ± 1.40	3.90 ± 0.21
	<i>K. galanga</i>	1.53 ± 0.10	0.067 ± 0.004	0.96 ± 0.02	1.20 ± 0.06	1.87 ± 1.00	14.30 ± 2.50
	<i>E. elatior</i>	3.20 ± 0.20	0.014 ± 0.001	0.16 ± 0.02	0.15 ± 0.01	0.19 ± 0.01	0.07 ± 0.001
Inflorescence	<i>A. galanga</i>	3.11 ± 0.32	0.005 ± 0.003	0.42 ± 0.01	0.25 ± 0.01	0.15 ± 0.001	0.09 ± 0.001
	<i>E. elatior</i>	1.12 ± 0.01	0.020 ± 0.001	0.17 ± 0.01	0.16 ± 0.01	N.D.	2.62 ± 0.37

a = as is dry weight basis, b = mean ± standard deviation,
n = 3, N.D. = non-detectable

- Herbal teas.
 - Functional beverages.
3. Personal care
- Aromatic soaps.
 - Aromatherapy products (e.g. massage oils, scrubs, bath oils, etc.).
 - Perfumes.
 - Shampoo and conditioner.
 - Various cosmetics.
4. Skin care
- Moisturizers and toner.
 - Skin lightening/whitening cream.
 - Anti-aging/anti-wrinkle cream.
 - Anti-acne cream.
 - Anti-eczema cream (e.g., *Psoriasis*).
5. Food, confectionery and sweets
- Biscuits / cookies/cakes.
 - Ice cream.
 - Chocolates and sweet preserves.
 - Jam, chutney.

Profiles

In developing plant based products, research and quality control measures on the potential natural resources need to be intensified and established. In this respect, species profiling is considered as a useful tool in maintaining quality, especially when there are problems of presence of adulterants. Several techniques of species profiling are usually used such as chemical profiling, DNA fingerprinting and other botanical techniques. Our studies have revealed that DNA fingerprinting technology is useful in authentication of not only species but also varieties and variants (Plate 1 and Plate 2). For instance, based on RAPD primer OPA 4 as shown in Plate 1, *Zingiber zerumbet* and its three variants could be differentiated quite easily. In another study (Plate 2), local varieties of *Zingiber officinale*, namely *Zingiber officinale* var. *rubrum* (*halia padi*) and *Zingiber officinale* var. *rubrum* (*halia bara*), were shown to differ very slightly in their DNA profiles based on RAPD primers OPA 1, OPA 8 and OPA 20.

Some botanical methods are also useful in identifying species, such as anatomy of leaves and petioles and SEM studies on reproductive structures of plants. These botanical techniques are useful as ancillary tools

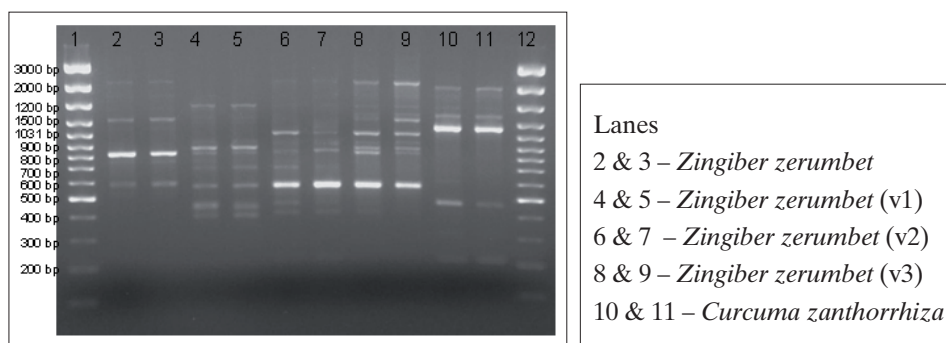


Plate 1. DNA Profile of variants of *Zingiber zerumbet* and *Curcuma zanthorrhiza* (RAPD primer OPA4).

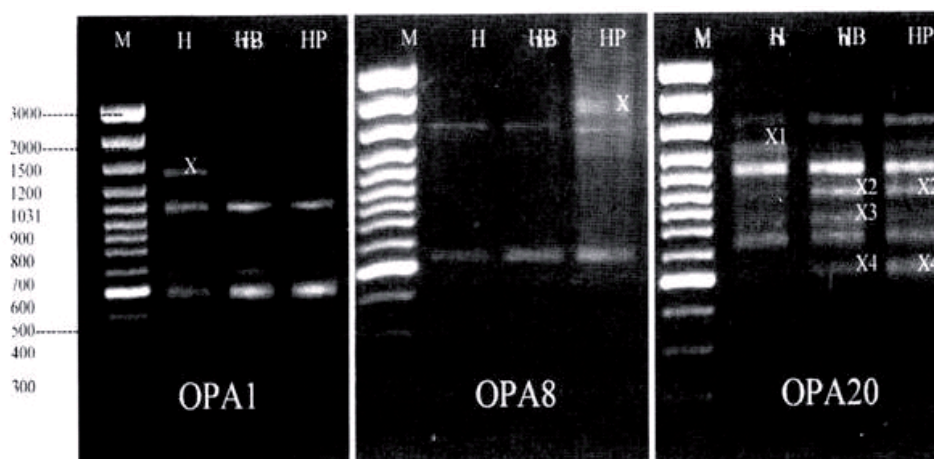
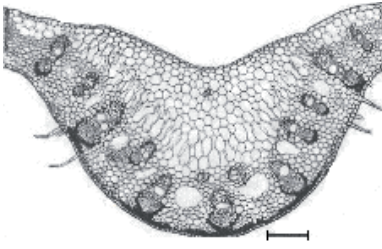
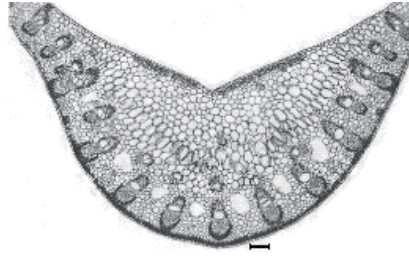
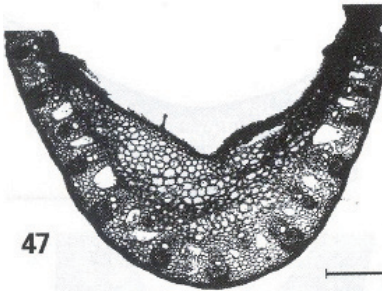
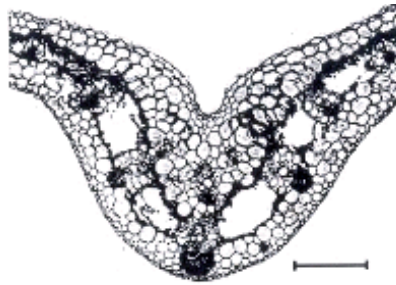
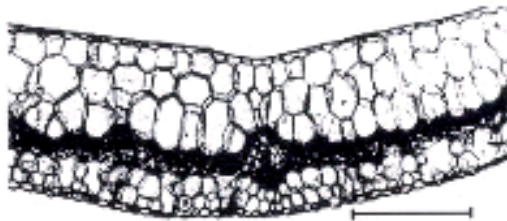
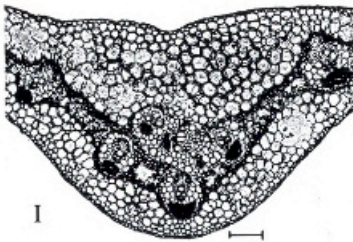
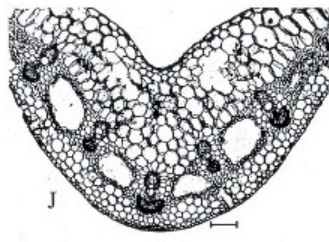


Plate 2. DNA profiles of *Zingiber officinale* and its varieties (RAPD primers OPA1, OPA8 & OPA20). 'X' shows the polymorphic banding patterns distinguishing all varieties studied; (OPA1: X=1500bp, OPA8: X=2000bp, OPA20: X1=1350bp, X2=900bp, X3=800bp, X4=550bp) H = *Z. officinale* var. *officinale* (halia), HB = *Z. officinale* var. *rubrum* (halia bara), HP = *Z. officinale* var. *rubrum* (halia padi), M= Marker 100bp Ladder Plus.

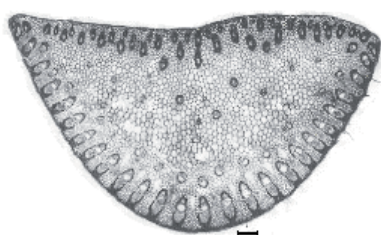
in authentication of species used in product development. Plates 3, 4 and 5 exhibit clearly the differences between selected gingers in their transverse sections of leaf midribs, petioles and margins respectively. Although SEM features of pollen, stigma and labellum of some gingers may not be as distinct, in most cases these data are also useful in species identification as shown in Plate 6.

A) *Alpinia conchigera*B) *A. galanga*C) *A. galanga* - ChinaD) *Boesenbergia rotunda*E) *Kaempferia galanga*F) *K. parviflora*G) *K. pulchra*H) *K. rotunda*

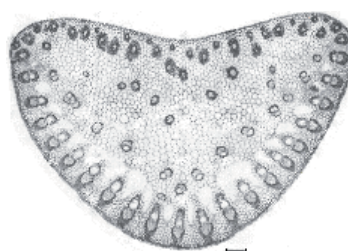
Scale bars = 500 μ m (A,B,C)

Scale bars = 200 μ m (D,E,F,G,H)

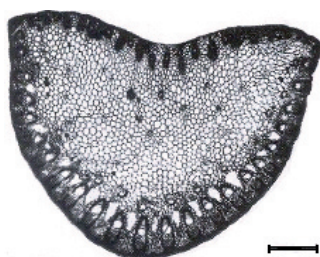
Plate 3. Transverse sections of leaf midribs.



A. galanga



A. conchigera



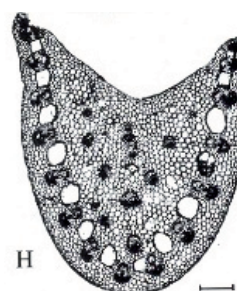
A. galanga – China



Boesenbergia rotunda



Kaempferia galanga



K. parviflora



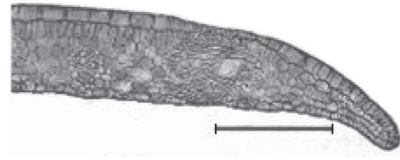
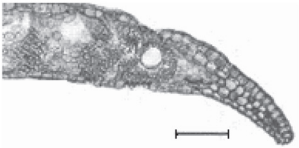
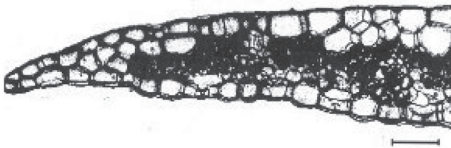
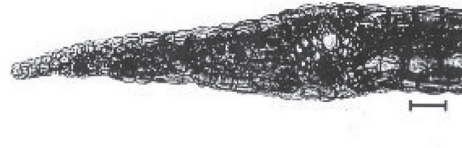
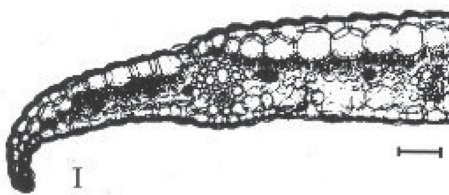
K. pulchra



K. rotunda

Scale bars = 500 μ m

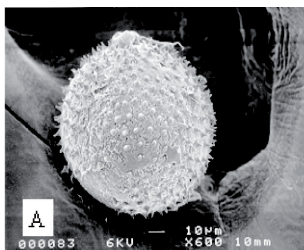
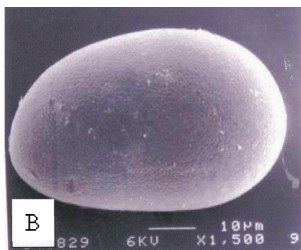
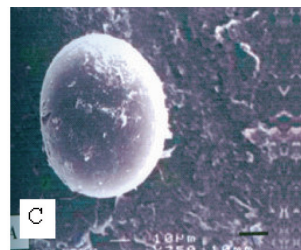
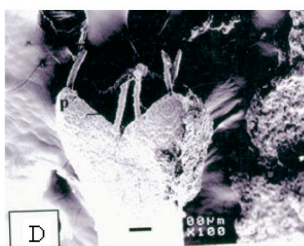
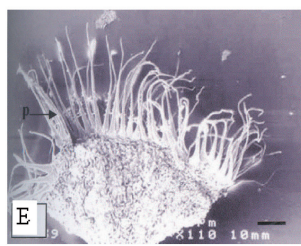
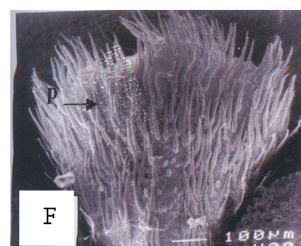
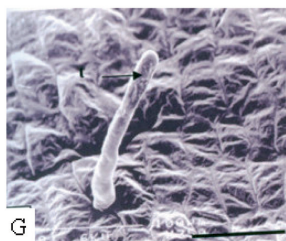
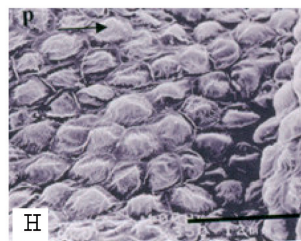
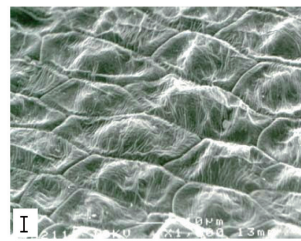
Plate 4. Transverse sections of petioles.

A) *Alpinia conchigera* – leftB) *A. conchigera* – rightC) *A. galanga* – leftD) *A. galanga*- rightE) *A. galanga* – ChinaF) *Boesenbergia rotunda*G) *Kaempferia galanga*H) *K. parviflora*I) *K. pulchra*J) *K. rotunda*

Scale bars = 200 μm (A,B,C,D,E)

Scale bars = 50 μm (F,G,H,I,J)

Plate 5. Transverse sections of leaf margins.

Pollen*Boesenbergia plicata**Scaphochlamys kunstleri**Hedychium coronarium***Stigma***Boesenbergia rotunda**Kaempferia galanga**Scaphochlamys klosii***Labellum***Boesenbergia plicata*
(Middle labellum)*Boesenbergia rotunda*
(Middle labellum)*Hedychium coronarium*
(Lower labellum)

A, B, C (x 10μm)

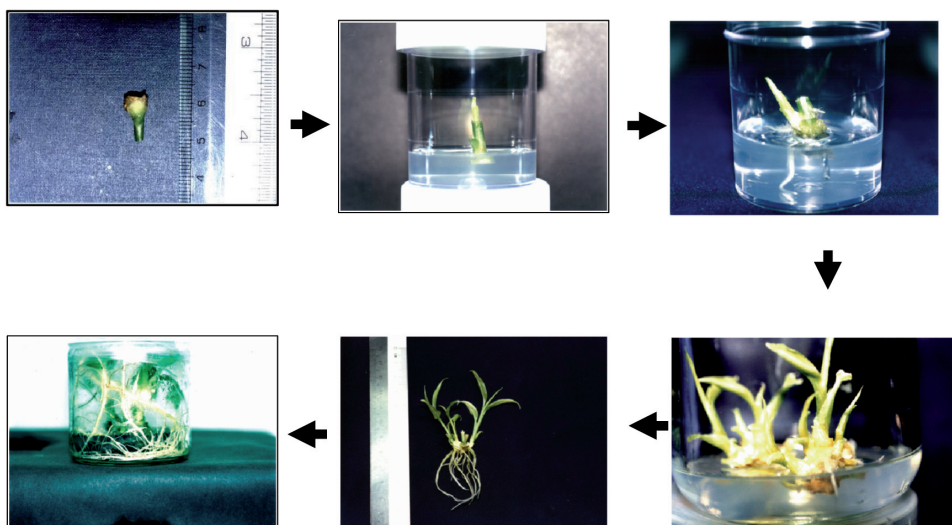
D, E, F (x 100μm)

G, H, I (x 100μm)

Plate 6. SEM studies of flower parts.

Micropropagation

One of the common problems in the development of herbal products is the sufficient supply and consistency of the source materials. Micropropagation by tissue culture technique is a means of providing consistent supply of stable and elite materials for mass propagation. For the last ten years, our team has managed to establish protocols for micropropagation of at least fifteen cultivated gingers most of which are of medicinal importance. In general, an average of 3-5 shoots per explant were successfully regenerated on MS medium supplemented with 3.0 % (w/v) sucrose, 0.2 % (w/v) phytigel and 1.0 – 3.0 mg/L BAP (Plate 7).



Production of multiple shoots

Plate 7. Micropropagation of *Zingiber officinale* and its varieties

Our field studies on the *in vitro* and normal plants of *Zingiber officinale* and its varieties showed that after transplanting, the field performance of *in vitro* plants were found to be superior compared to the control plants based on five quantitative traits as stated in Table 4. This result implicates the significance of establishing micropropagation protocols for the sustainable utilization of commercially important herbs such as the cultivated gingers.

Table 4. Field performance of micropropagated and control plants for five quantitative traits (Muda *et al.* 2004)

Parameter Study	<i>Z. officinale</i> (Halia)		<i>Z. officinale</i> var. <i>rubrum</i> (Halia bara)		<i>Z. officinale</i> var. <i>rubrum</i> (Halia padi)	
	<i>in vitro</i>	control	<i>in vitro</i>	control	<i>in vitro</i>	control
No. of tillers	6.0 ± 1.0	4.6 ± 0.9	4.8 ± 0.8	3.4 ± 0.6	6.6 ± 0.6	4.4 ± 1.1
Plant height (cm)	56.3 ± 5.2	57.2 ± 9.6	26.3 ± 2.3	24.5 ± 3.9	24.3 ± 6.1	24.8 ± 4.7
No. of leaves	64.8 ± 16.3	54.8 ± 7.4	35.0 ± 6.4	31.4 ± 3.1	52.0 ± 15.8	33.0 ± 4.7
Leaf area (cm ²)	27.6 ± 1.2	25.9 ± 1.4	20.5 ± 0.4	18.2 ± 0.6	17.4 ± 0.7	17.2 ± 0.3
Fresh weight of rhizome (g)	50.7 ± 23.3	38.4 ± 20.0	19.3 ± 3.2	12.0 ± 5.0	21.8 ± 4.5	11.3 ± 4.9

Acknowledgements

The authors thank the Ministry of Science, Technology and Environment of Malaysia for the major research grants and University of Malaya for the short termed research grants. The authors wish to thank all collaborators, students and technicians involved in the various projects.

References

- English, R. & J. Lewis, J. 1991. Nutritional values of Australian foods. Cited in Zanariah *et al.* (1977). *Journal of Tropical Agriculture and Food Science* **25**(2): 225-229.
- Hashim, H.M., H. Ibrahim and Z.H.A. Rahim. 1988. Preliminary studies on some nutritional composition of the edible gingers, pp. E1-2. In: A. Sipat *et al.* (eds.). *Advances In Biochemistry And Biotechnology In Asia And Oceania*. Kuala Lumpur, Malaysia.
- Hussin, K.H. and H. Ibrahim. 1989. Taxonomic implications of several *Zingiber* species (family Zingiberaceae) based on morphological and anatomical characteristics. *Malaysian Applied Biology* **18**: 155-161.
- Hussin, K.H., T.S. Chuah, H. Ibrahim, Q.G. Wu, J.P. Liao and N. Liu. 2000. Comparative leaf anatomy of *Alpinia* Roxb. (Zingiberaceae) from China. *Botanical Journal of Linnean Society* **133**: 161-180.
- Hussin, K.H., H. Ibrahim, H.A. Ali D. Aminah, J.P. Liao and N. Liu. 2001. Anatomical variations in leaves of *Boesenbergia* O. Kuntze and *Kaempferia* L. (Zingiberaceae). *Journal of Tropical and Sub-tropical Botany* **9**: 49-54.
- Ibrahim, H. & Z.H.A. Rahim. 1988. Distribution of K, Na, Ca, Mg, Fe, and Zn in four species of the Zingiberaceae. *Malaysian Journal of Science* **10**: 25-29.
- Ibrahim, H., S. Mohd. Nor and Z.H.A. Rahim. 1994. Medicinal gingers (Zingiberaceae) - analysis of oxalate, tannin and cyanogenic glycosides, pp. 125-127. In: S.H. Ton *et al.* (eds). *Proceedings of the 19th Malaysian Biochemical and Molecular Biology Society Conference*. Bangi, Malaysia.

- Ibrahim, H., H.C. Ong and R. Hassan. 2000. Ethnobotanical survey of the ginger family in selected Malay villages in Peninsular Malaysia. *Malaysian Journal of Science* **19**: 93-99.
- Larsen, K., H. Ibrahim, S.H. Khaw and L.G. Saw. 1999. *Gingers of Peninsular Malaysia and Singapore*. Natural History Publications (Borneo), Kota Kinabalu. 135 pp.
- Mohd. Azmi, M., N. Khalid and H. Ibrahim. 1999. Pendekatan baru dalam propagasi spesies-spesies halia ubatan terpilih. *Transaction of Malaysian Society of Plant Physiology* **8**: 55-58.
- Muda, M.A., N. Khalid and H. Ibrahim. 2004. Micropropagation study on three varieties of *Zingiber officinale* Rosc. *Malaysian Journal of Science* **23**(2): 7-10.
- Muda, M.A., H. Ibrahim and N. Khalid. 2004. Differentiation of three varieties of *Zingiber officinale* Rosc. by RAPD fingerprinting. *Malaysian Journal of Science*. **23**(2): 135-139.
- Noraini, T., H.H. Khatijah and H. Ibrahim. 2005. Comparative Leaf Anatomy of *Alpinia* Roxb. species (Zingiberaceae) in Peninsular Malaysia. *Nordic Journal of Botany* **23**: 463-483.
- Nordiana, M., M. Yusoff and H. Ibrahim. 1997. Scanning electron microscope study on selected species of the tribe Hedycheae (Zingiberaceae), pp. 251-253. In: A.R. Razak *et al.* (eds.). *Proceedings of the First ASEAN Microscopy Conference*. Johor, Malaysia.
- Nordiana, M., M. Yusoff and H. Ibrahim. 1998. Ultrastructure of leaf surfaces of *Scaphochlamys* spp. (Zingiberaceae), pp. 118-121. In: *Proceedings of the 7th Scientific Conference of the Electron Microscopy Society Malaysia*. Ipoh, Malaysia.
- Nordiana, M., H. Ibrahim and M. Yusoff. 2000. Floral characteristics of *Boesenbergia* O. Kuntze., *Kaempferia* Linn. and *Scaphochlamys* Bak., pp. 215-217. In: *Proceedings of the 9th Scientific Conference of EMSM 2000*. Malaysia.
- Rahim, Z.H.A., H. Ibrahim and H.M. Hashim. 1991. Edible ginger (Zingiberaceae family) – determination of tannin, oxalate and cyanogenic glycoside content, pp. 183-187. In: A. Wan Azlina (ed.). *Proceedings of*

the 16th annual conference of the Malaysian Biochemical Society. Johor, Malaysia.

Tee, E.S., N. Mohd. Ismail, A. Mohd. Nasir and I. Khatijah (compilers). 1988. Cited in Zanariah *et al.* (1977). *Journal of Tropical Agriculture and Food Science* **25**: 225-229.

Zanariah, J., A. Noor Rehan and O. Rosnah. 1997. Nutritional composition of common Zingiberaceae species used in traditional medicines and cooking. *Journal of Tropical Agriculture And Food Science* **25**: 225-229.

An Introduction to the New Guinea Database, with Notes on the Zingiberaceae, Specifically *Riedelia* Oliv.

R. JOHNS

Botanical Research Institute of Texas [BRIT], 509 Pecan St., Fort Worth, Texas,
46102 – 4060, U.S.A.

Email: mountjaya1@yahoo.com

Abstract

The entries for the family Zingiberaceae in the New Guinea database include records of over 1700 collections. Based on this information an overview of the family is presented. The extensive records in the database highlight several problems. A considerable number of collections in the family have not been identified at generic level (190 collections) and many collections in each genus are not identified at the species level. This particularly applies to the larger New Guinea genera: *Alpinia* Roxb. and *Riedelia* Oliv., respectively with 256 and 236 collections. In this paper particular attention is paid to the neo-endemic genus *Riedelia*, which is represented by 85 described taxa in New Guinea. The genus has not been revised since 1916.

Introduction

The objective of the New Guinea program at BRIT is to instigate a period of intensive botanical collecting of the flora through expeditions to both Papua and Papua New Guinea. These expeditions will be carried out in association with staff from National and Regional Herbaria. It is essential to collect 'high quality' herbarium materials, photographs, and living collections for the scientific study of the flora. The top set of collections from New Guinea will be deposited locally in the major National herbaria with duplicates sent to important international herbaria. Only this will provide an adequate basis for future studies of the flora of New Guinea.

A database is being developed to facilitate the study of the flora of New Guinea. It will not only enable the mapping of plant distributions but will also highlight areas where the flora is unknown or poorly known and will thus act as a guide for future expeditions. The database will also enable the production of species lists for given regions. Another benefit will be to

provide information for younger botanists and ecologists, for the study of plant diversity in New Guinea and also to access the major literature on the New Guinea flora. The database, which so far includes ca 300,000 herbarium collections, has considerable potential for use in herbarium management. It will also be an essential tool to identify conservation areas and to highlight biodiversity priorities for all levels of government in New Guinea.

1. The New Guinea database

The New Guinea database includes collections from the Indonesian Province of Papua, previously Irian Jaya (now divided into two Provinces) and Papua New Guinea. Because of its floristic associations with New Guinea, collections from the Aru (Aroe) Islands (Maluku Province) are included. Records are also included from the North Solomons Province (Buka and Bougainville), politically, part of Papua New Guinea. Extension of the database to the Solomon Islands would include all records for the biogeographical region known as Papuasias.

The New Guinea database is arranged to include:

A species database. The type collection of each species is recorded. The herbarium in which the type occurs is also recorded (holotype and isotypes where known), its place of publication, important literature, and general notes on each species and their ecology. The distribution is recorded and, where the species is endemic to New Guinea, this is recorded as a regional or a local endemic. A note is included if there are obvious nomenclatural problems. Synonyms are also recorded.

A specimen database. It is proposed to list all vascular plant collections from New Guinea in the database. So far nearly 300,000 records of vascular plants have been entered into the database(s). Each collection will be geo-referenced so that its location can be mapped.

Using the entries in the database it will be possible to produce distribution maps of expeditions and collections at the family, genus and species levels. This will be a valuable guide so that areas, which have been poorly collected, can be targeted for expeditions. Locality data will also enable the generation of species lists at provincial and sub-provincial levels as a basis for regional planning and conservation. From the database it will be possible to produce:

- a) Lists of all plant species known from New Guinea, the Provinces and smaller regions within New Guinea;
- b) Lists of species from selected plant families, and genera. It will

- also be possible to map these.
- c) Lists of plants endemic to the New Guinea region, and to map their patterns of distribution.

2. The family Zingiberaceae in New Guinea

The New Guinea region is one of the major centres of diversity for the family Zingiberaceae. Larsen *et al.* (1998) give the diversity of the family, worldwide, as ca 52 genera and 1,300 species. Based on the estimates of Newman (2007), New Guinea probably has over 300 species. In total New Guinea includes over 20 percent of the species worldwide. Over 1700 collections of Zingiberaceae have been databased from New Guinea; the database includes records of many species new to science. The database includes 240 species in 18 genera from New Guinea (Table 1). Newman (2007) lists six major genera from New Guinea: *Alpinia* Roxb., *Amomum* Roxb., *Etlingera* Giseke, *Hornstedtia* Retz., *Pleuranthodium* (K. Schum.) R.M.Sm., and *Riedelia* Oliv. He also lists several genera for New Guinea, which he thinks are cultivated or possibly naturalised, these include *Curcuma* L., *Globba* L., and *Zingiber* Mill. Many of these genera have 'endemic' species listed from New Guinea (Newman *et al.*, 2004). Their status will require detailed taxonomic studies as all genera and species are poorly studied. Potentially many new species remain to be collected and described from the large areas that are still un- or under-collected. In Table 1 the number of collections in a genus, percentage of endemism, and the number of species recorded from Papua, Papua New Guinea and the whole island is enumerated for selected genera. These figures will change as our knowledge of the family in New Guinea increases.

3. History of research on Zingiberaceae in New Guinea

The Zingiberaceae in New Guinea were revised in several important papers by Schumann (1899, 1904), Valeton (1913a, b, 1914, 1917), and Ridley (1886, 1916, 1923). Few general papers have been published since the work of Valeton and Ridley. The recent works of B.L. Burt and R.M. Smith are the first modern attempt to examine the family in Malesia (Burt & Smith, 1972a, b; Smith, 1975, 1990a, b, c).

To date, the family in New Guinea has received little attention. With the exception of *Alpinia*, *Pleuranthodium*, and *Riedelia*, the family is poorly represented in New Guinea. Of the 223 species recorded from New Guinea by Newman *et al.* (2004), 90 taxa are referred to *Riedelia* and 46 to *Alpinia*.

4. The collections of Zingiberaceae in New Guinea

In Papua the majority of species are described from several key areas (Fig. 1). The Kepala Burung (= Vogelkop or Bird's Head, see Fig 1A). L.S. Gibbs

Table 1. Collection data on selected genera of Zingiberaceae from New Guinea.

	No of taxa	No of collect. ¹	Endemism %	Species Recorded		
				Pap.	PNG	Both
<i>Alpinia</i>	63	473 [256] ³	96	31	23	8
<i>Amomum</i>	14	91 [37]	92	6	7	1
<i>Curcuma</i>	6	73 [20]	57	3	1	3
<i>Etlingera</i>	16	69 [23]	100	8	6	2
<i>Globba</i>	1	9 [4]	0	-	1	-
<i>Guillainia</i>	4	18 [-]	100	1	-	-
<i>Hornstedtia</i>	3	108 [24]	66	2	-	1
<i>Pleuranthodium</i>	22	63 [10]	100	5	14	3
<i>Riedelia</i>	85	686 [251]	100	42	32	11
<i>Zingiber</i>	6	19 [-]	50	1	2	3
<i>Zingiberaceae indet.</i>	191 ²					
Totals	231*			99	85	33

¹ Total number of collections in the category.

² Number of collections not identified to genus.

³ [] Number of collections not identified to species level.

Nine species have been added from database records for 'doubtful' genera in Papua New Guinea:

Hedychium [1 species - *H. coronarium*]

Phaeomaria [2 species - *P. anthokaphina*, *P. novoguineensis*]

Thylacophora [1 species - *T. pognocheila*]

Naumannia [1 species - *N. insignis* (*Riedelia insignis*)]

In addition Riswan and Setyowati (1996) listed *Kaempferia* (3), and *Nanochilus* (1) from Papua. *Pleuranthodium* (*Psychanthus*) is also not listed by Riswan and Setyowati (1996) from New Guinea.

organised an expedition to the Arfak Mountains in 1913-1914. Gjellerup made additional collections of gingers from the area in 1912. These ginger collections were described by Valeton in Gibbs (1917). More recent expeditions to this area contain many collections that have not been critically studied. These include collections by staff of the Forestry Department (BW series), collections by P. van Royen and H. Sleumer from the Kebar Valley,

and the expeditions to the N.E. Kepala Burung, funded by the MacArthur Foundation, which were made in the 1990's. Staff from Herbarium Bogoriense and the Manokwari Herbarium have also collected in the area.

The other key areas in Papua are Mt Jaya (Carstensz) (Fig. 1B) and the Lorentz River Basin (Fig. 1C), both to the S of the central Ranges. C.B. Kloss was the botanist/collector for both of the Wollaston Expeditions to Mt Carstensz. The second expedition resulted in the collection of many 'new' species of gingers (Ridley 1916). Some of these species have been recently recollected by the staff of Freeport (Environmental Department) and also during the recent expeditions to Mt Jaya by expeditions organised from the Royal Botanic Gardens, Kew (Johns *et al.* 2006). Collections have also been made in the same area by staff from Herbarium Bogoriense and the Manokwari Herbarium. The Lorentz basin was collected by several expeditions and many species were described by Valetton (1913b). The main collectors were Lorentz, von Roemer, and Versteeg. Some species have also been described from the early collections in the vicinity of Jayapura (= Hollandia, see Fig. 1D). The extensive collections from Papua by H.J. Lam in 1920-1921, and L.J. Brass in 1938-1939, and collectors in the BW series all postdated the most recent revisions of the gingers from Papua.

The history of Zingiberaceae research in Papua New Guinea parallels that of Papua. In 1875 Nauman collected in the Bismark Archipelago (Fig. 1L) as did several latter collectors, including Nymann in 1899 and Peekel from 1908 to 1938. In 1885 Forbes made extensive collections (Ridley 1886) from the Sogeri Division (Fig. 1I), which included many Zingiberaceae. The last collections from Papua New Guinea studied by specialists in the family (Valetton 1914) were those of Schlechter in 1909 from the Toricelli Mts. (Fig. 1E) and various collectors in the Madang and Morobe Provinces (Fig. 1F, G, H). There have been extensive collections from Papua New Guinea since 1916, especially in the Central Highlands (Fig. 1J) and the upper reaches of the Fly River (Fig. 1K) but all postdated any specialist studies of the Zingiberaceae.

In common with many plant families in New Guinea the species of Zingiberaceae are very poorly known. While many tree species have been widely collected (both lead herbaria in New Guinea were part of the Forestry Departments; Papua – BW series; PNG – NGF and LAE series), the majority of herbaceous species, including the gingers, are poorly known. As shown in Table 2 most species of gingers are known from only the types, or two to three collections. This is also characteristic of many herbaceous plant families in New Guinea, where over sixty percent of the species are often represented by a single collection. Another fifteen to twenty percent of species are usually represented by only two to three specimens.

Figure 1. Critical sites to be visited for the recollection of the duplicates of types of *Riedelia* in New Guinea. For location of sites see text.

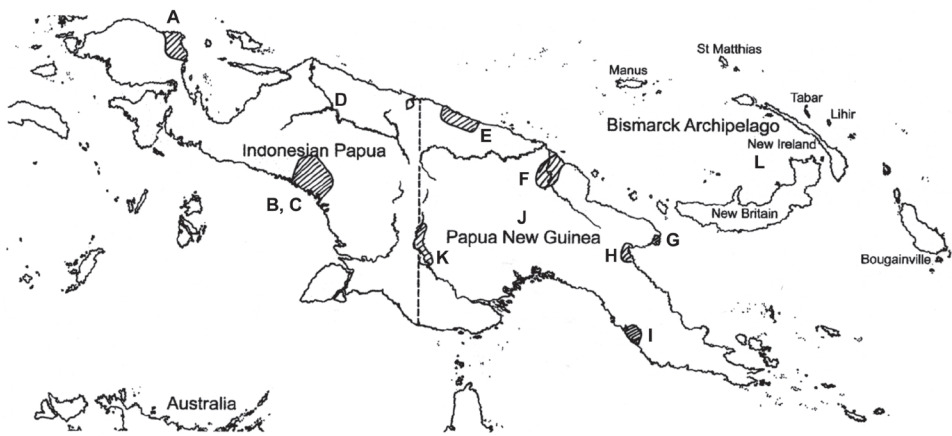


Table 2. Number of specimens collected (1-10+) per species from selected genera of Zingiberaceae from New Guinea.

Collections per species	1	2	3	4	5	6	7	8	9	10+
<i>Alpinia</i>	46	13	11	8	5	1	-	-	1	3
<i>Amomum</i>	15	1	-	-	-	-	-	-	-	-
<i>Curcuma</i>	4	1	-	-	-	-	-	-	-	2
<i>Pleuranthodium</i> (<i>Psychanthus</i>)	13	8	6	8	-	-	-	1	-	-
<i>Riedelia</i>	32	10	5	7	4	2	-	-	2	8
<i>Zingiber</i>	2	-	-	1	-	1	1	-	-	-

5. The genus *Riedelia* nom cons. in New Guinea and Maluku

The genus *Riedelia* was described by Oliver in *Hooker's Icon. Pl.* 15: 15 (1883), based on a collection by J.G.F. Riedel from Pulau Buru in the Maluku. The type species is *Riedelia curviflora* (Plate 1). *Riedelia* was conserved against *Riedelia* Cham. described in the Verbenaceae in 1832, while another

homonym *Riedelia* Meisn., a Brazilian genus described in Ericaceae in 1863 was not validly published (Rickett & Stafleu, 1959). Valeton (1914) incorrectly lists *Riedelia curviflora* Oliv. as a synonym of *Riedelia lanata* (Scheff.) Valeton, a widespread species from both Papua and Papua New Guinea.

Newman *et al.* (2004) list two genera as synonyms of *Riedelia*, *Nyctophylax* Zipp. described by Zippelius in 1829, based on *N. alba* Zipp. a nomenclatural synonym (Greuther *et al.* 2000) and *Thylacophora* Ridl. (1916) based on *T. pogonocheila* Ridl., collected by C.B. Kloss from Mt Carstensz (Mt Jaya) in 1912. *Hedychium lanatum* Scheff. was described in 1876 based on a collection by J.E. Teysmann (*Teysmann 6741*), collected from the MacCleur Gulf in New Guinea in August of 1871.

The monotypic genus *Naumannia* Warb. [*Bot. Jahrb.* 13: 452 (1891)] was based on *N. insignis*. The genus was distinguished by its petaloid lateral staminodes and the flower lacks a labellum. It is possibly related to *R. corallina*. Further collections are required from the type locality, Sattelburg, Morobe Province, to establish its status. Readers should also note that the status of *Nyctophylax alba* Zipp. (1829), the first record listed as a synonym of *Riedelia* by Newman *et al.* (2004), is at present not understood.

The publication of the 90 taxa (species and varieties) of *Riedelia* from New Guinea is summarised in Table 3. The major papers were published by K. Schumann (1904) and a series of papers by Valeton (1907, 1913a, 1913b, 1914, 1917). Ridley (1916) described the collections made by C.B. Kloss from Mt Carstensz (Mt Jaya). There have been no detailed studies of the genus *Riedelia* since the publications of Valeton and Ridley. In 1979 P. van Royen published five species from the subalpine and alpine regions of New Guinea, and A. Gilli (1983) published two new species from the highlands of Papua New Guinea (Table 2).

6. Distribution of *Riedelia*

The genus *Riedelia* is distributed from the Maluku to New Guinea, extending to the Solomon Islands (Bougainville) and is represented by ninety taxa in New Guinea (Fig. 2). Of the 686 collections of *Riedelia*, 251 still remain unidentified. All species are endemic to New Guinea, but *R. curviflora* possibly extends its range to Papua from the Maluku. Forty-two species are known to be restricted to Papua, 32 species to Papua New Guinea, and 11 species occur throughout the island. A more detailed understanding of their distribution patterns must await more detailed study.

Species identified as *Riedelia* are also recorded from Borneo (Sarawak [Ashton 17713; Meijer 21217], and Kalimantan [Kostermans 5122]), Sulawesi [Burley 3511, 3667I], and the Philippines (Newman, *pers. com.*). The taxonomic identity of these collections requires further study.

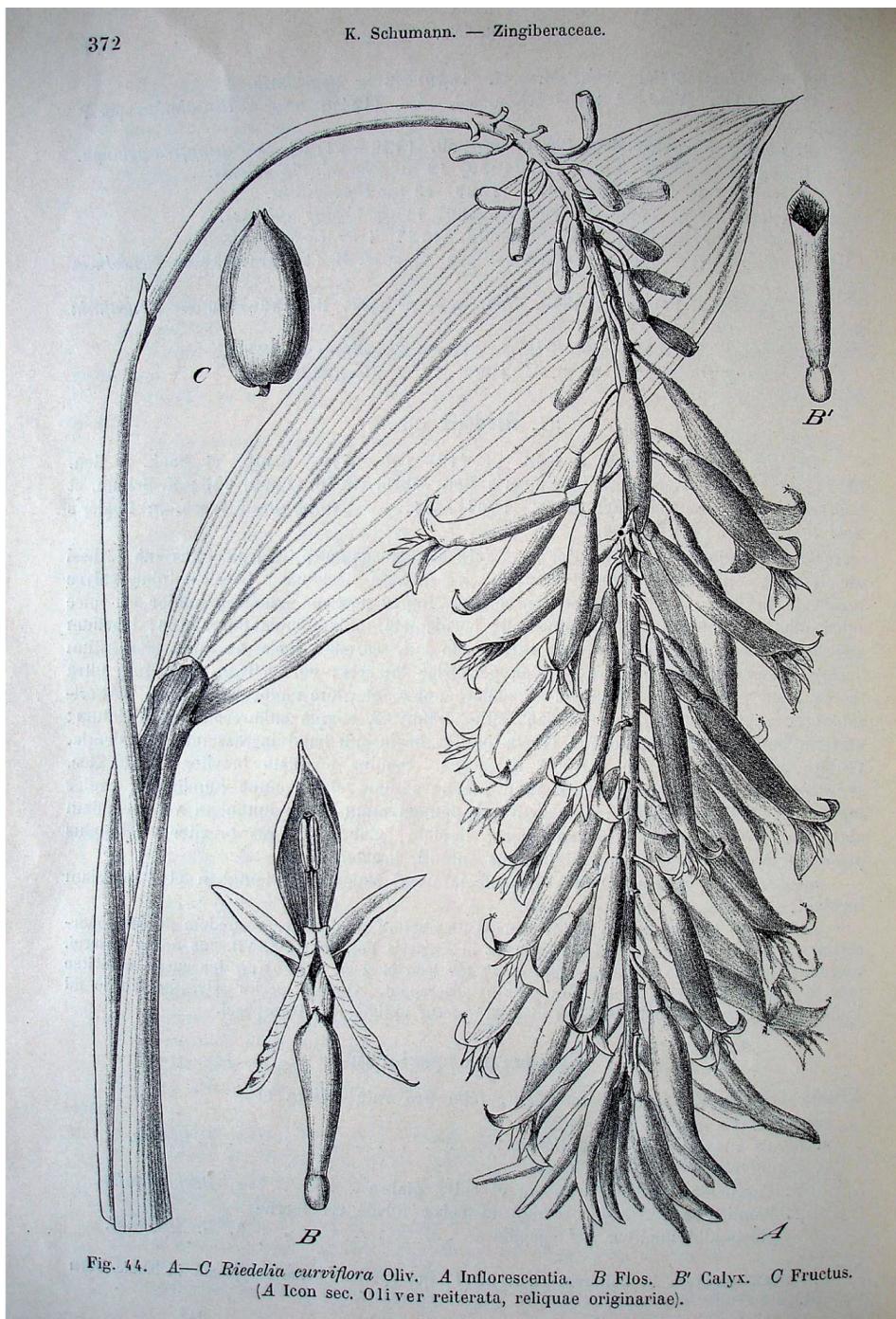


Plate 1. *Riedelia curviflora* Oliver. A. Inflorescence; B. Flower; B'. Calyx; C. Fruit. A-C from Riedel s.n., Pulau Buru, Maluku. Reproduced from: Schuman, 1904.

Table 3. Publication dates for the species of *Riedelia* [86 taxa]. Source of Type collection: ° Moluccas, ¹ Papua, ² Papua New Guinea

Date	Author	Publication
1883	Oliv.	Hooker's Icon. Pl., Pl. 15: 15. [1 taxon] <i>R. curviflora</i> ° [Type species for genus].
1904	K. Schum.	Pflanzenr. IV, 46: 373-440. [7 taxa] <i>R. affinis</i> (Ridl.) K. Schum. ² , <i>R. albertsii</i> (K. Schum.) K. Schum. ² , <i>R. bismarckii-montium</i> ² , <i>R. macrantha</i> (K. Schum.) K. Schum. ¹ , <i>R. monophylla</i> ² , <i>R. nymanii</i> ² , <i>R. stricta</i> ²
1907	Valeton	Bull. Dép. Agric. Indes Néerl. 10: 2. [1 taxon] <i>R. geanthus</i> ¹
1913a	Valeton	Icon. Bogor. 4(3): t. 373-375. [3 taxa] <i>R. corallina</i> (K. Schum.) Valeton ² , <i>R. erecta</i> ¹ , <i>R. lanata</i> (Scheff.) Valeton ¹
1913b	Valeton	Nova Guinea 8: 962-980. [30 taxa] <i>R. alata</i> ¹ , <i>R. angustifolia</i> ¹ , <i>R. areolata</i> ¹ , <i>R. arfakensis</i> ¹ , <i>R. brachybotrys</i> ¹ , <i>R. branderhorstii</i> ¹ , <i>R. brevicornu</i> ¹ , <i>R. epiphytica</i> ¹ , <i>R. eupteron</i> ¹ , <i>R. fulgens</i> ¹ , <i>R. graminea</i> ¹ , <i>R. graminea</i> var. <i>diversifolia</i> ¹ , <i>R. graminea</i> var. <i>elata</i> ¹ , <i>R. graminea</i> var. <i>nana</i> ¹ , <i>R. hollandiae</i> ¹ , <i>R. lanata</i> (Scheff.) Valeton forma <i>ligulata</i> ¹ , <i>R. macranthoides</i> ¹ , <i>R. maculate</i> ¹ , <i>R. maxima</i> ¹ , <i>R. montana</i> ¹ , <i>R. montana</i> var. <i>arfakensis</i> ¹ , <i>R. montana</i> var. <i>golianthensis</i> ¹ , <i>R. orchidoides</i> (K. Schum.) Valeton ¹ , <i>R. paniculata</i> ¹ , <i>R. pterocalyx</i> ¹ , <i>R. robusta</i> ¹ , <i>R. sessilanthera</i> ¹ , <i>R. sessilanthera</i> var. <i>euodon</i> ¹ , <i>R. subulocalyx</i> ¹ , <i>R. tenuifolia</i> ¹
1914	Valeton	Bot. Jahrb. Syst. 52: 70-96. [21 taxa] <i>R. bidentata</i> ² , <i>R. decurva</i> (Ridl.) Valeton ² , <i>R. dolichopteron</i> ² , <i>R. ferruginea</i> ² , <i>R. flava</i> Lauterb. ex Valeton ² , <i>R. geluensis</i> ² , <i>R. geminiflora</i> ² , <i>R. grandiligula</i> ² , <i>R. latiligula</i> ² , <i>R. longifolia</i> ² , <i>R. longirostra</i> ² , <i>R. macrantha</i> var. <i>grandiflora</i> ² , <i>R. macrothyrsa</i> ² , <i>R. microbotrya</i> ² , <i>R. minor</i> ² , <i>R. monticola</i> ² , <i>R. rigidocalyx</i> Lauterb. ex Valeton ² , <i>R. schlechteri</i> ² , <i>R. umbellate</i> ² , <i>R. urceolata</i> ² , <i>R. urceolata</i> var. <i>sessilifolia</i> ²
1916	Ridley	Trans. Linn. Soc. London, Bot. 9: 222-226. [11 taxa] <i>R. aurantiaca</i> ¹ , <i>R. bicuspis</i> ¹ , <i>R. ferruginea</i> ² (nom. illeg.), <i>R. hirtella</i> ¹ , <i>R. klossii</i> ¹ , <i>R. ligulata</i> ¹ , <i>R. longisepala</i> ¹ , <i>R. pulcherima</i> ¹ , <i>R. purpurata</i> ¹ , <i>R. triciliata</i> ¹ , <i>R. wollastonii</i> ¹
1917	Valeton	see Gibbs, Fl. Arfak Mts. 102. [2 taxa] <i>R. exalata</i> ¹ , <i>R. montana</i> var. <i>puberula</i> ¹
1923	Ridley	Proc. Roy. Soc. Queensland 34: 19. [2 taxa] <i>R. lanatiligulata</i> ² , <i>R. whitei</i> ²
1979	P. Royen	The Alpine Flora of New Guinea 2: 860-866. [5 taxa] <i>R. curcumoidea</i> ¹ , <i>R. marafungensis</i> ² , <i>R. rosacea</i> ² , <i>R. subalpina</i> ² , <i>R. suborbicularis</i> ²
1983	Gilli	Ann. Naturhist. Mus. Wien, B 84: 46-47 ("1980"). [2 taxa] <i>R. capillidens</i> ² , <i>R. geluensis</i> Valeton var. <i>microflora</i> Gilli ²
1990	R.M. Smith	Edinburgh J. Bot. 47: 65. [1 taxa] <i>R. cordylinoides</i> (Ridl.) R.M. Sm. ¹

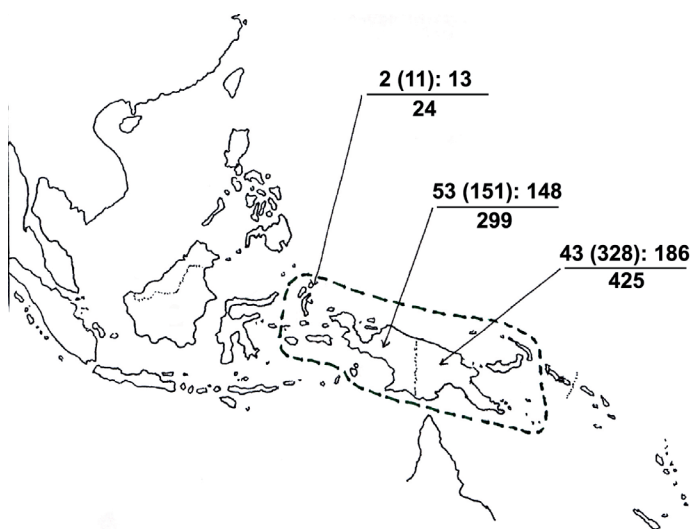


Figure 2. Distribution and diversity of *Riedelia*. Distribution of *Riedelia* (-----). Diversity of *Riedelia* in Maluku, Papua and Papua New Guinea expressed as:

$$\frac{\text{No. of species (no specimens) : No. indet collections}}{\text{Total number collections}}$$

7. Ecology of *Riedelia* in New Guinea

Most species of *Riedelia* grow as terrestrial species in the rainforest from sea level to 3,200 to 3,700 m. Data is presented in Table 4 to show the altitudinal zonation of the common genera of Zingiberaceae from New Guinea. The three most common genera in New Guinea, namely, *Alpinia*, *Pleuranthodium*, and *Riedelia*, occur at most altitudes up to 3,700 m (Table 5). At their upper altitudes species can be locally common in subalpine forest. Most collections however do not have the altitude recorded. Although both *Alpinia*, and *Pleuranthodium* are recorded above 3,500 m altitude, both have fewer collections above 1000 m. *Riedelia* in contrast is more strongly represented by collections from the mid- and upper montane forests, particularly so in PNG. It is difficult to interpret these Tables as they may merely reflect collection patterns rather than actual zonation patterns. Higher altitude forests are more accessible in PNG than in Papua. Mid-montane forests, being wetter than the lower montane forests, are probably more suitable for members of the genus.

The lowland tropical rain forest is diverse with many species of *Riedelia* including *R. albertisii*, *R. aurantiaca*, *R. brevicornu*, *R. erecta*, *R. geanthus*, *R. grandiligula*, *R. longifolia*, *R. macranthoides*, *R. maxima*, *R. nabirensis*, *R. pterocalyx*, *R. stricta*, *R. subulocalyx*, and *R. tenuifolia*.

As stated above, many species of *Riedelia* grow in the montane zone. Species have been subjectively allocated to a vegetation type based on the altitude of the collections. Due to the Massenerhebung effect resulting in vegetation zones being lower on smaller mountain masses, the simple use of altitude as a guide to the vegetation type has probably led to inaccuracies. The vegetation data on labels of collections of *Riedelia* is inadequate. Probably several of the 'lower montane' species will, with more detailed study, be placed as largely mid montane in their distribution ecology.

The following species of *Riedelia* have been recorded from the lower montane zone: *R. affinis*, *R. bicuspis*, *R. dolichopteron*, *R. decurva*, *R. ferruginea*, *R. fulgens*, *R. klossii*, *R. ligulata*, *R. longisepala*, *R. macrantha*, *R. monophylla*, *R. pulcherima*, *R. robusta*, *R. umbellata*, and *R. urceolata*.

The mid-montane forest is probably more diverse than the lower montane forest. Here, species of *Riedelia* include *R. arfakensis*, *R. bidentata*, *R. capillidens*, *R. geluense*, *R. hirtella*, *R. hagenii*, *R. monticola*, *R. orchiodes*, *R. paniculata*, *R. purpurata*, *R. rosacea*, *R. schlechteri*, *R. sessilanthra*, *R. triciliata*.

The upper montane and subalpine forests are also diverse. Species of *Riedelia* known from these forests include *R. bidentata*, *R. curcumoidea*, *R. exalata*, *R. macrantha*, *R. marafungensis*, *R. microbotrya*, *R. montana*, *R. monticola*, *R. rosacea*, *R. suborbicularis*, and *R. subalpina*. The family is represented at its highest altitudes by *Riedelia montana* Valetton var. *montana* (Johns 2006), and by *Riedelia montana* Valetton var. *goliathensis* Valetton (Newman, in press).

The altitudinal database includes several problematic species too, which will require detailed study. Probably the species identifications are incorrect on herbarium sheets. These species include *Riedelia corallianae* (60-500 m and 1800-2250 m), *R. epiphytica* (40-1000 m and 2200-2750 m), *R. graminea* (250-900 m and 2400-3460 m), and *R. lanata* (10-90 m and 1500-1900 m). Another problematic species is *R. macrantha*, which is collected over an altitudinal range from 10 to 3660 m.

8. Brief notes on other genera of Zingiberaceae in New Guinea

To recapitulate, the basic information on species number, number of collections (including collections not identified), percentage of endemism, and the distributions in Papua, Papua New Guinea and number of species recorded from both regions is given in Table 1. Notes are included only on the more poorly known genera. Several genera have many undescribed species in New Guinea.

Boesenbergia Kuntze is known from a single sheet from New Guinea collected in the Central Highlands of Papua New Guinea. *W. Vink 16553* was collected in secondary forest at 1960 m from the Minj-Nona Divide in the

Table 4. Altitudinal zonation of collections from herbarium specimens of selected genera of Zingiberaceae from New Guinea. Altitudinal Zones: 1. 0 – 499 m; 2. 500 – 999 m; 3. 1000 – 1499 m; 4. 1500 – 1999 m; 5. 2000 – 2499 m; 6. 2500 – 2999 m; 7. 3000 – 3499; 8. 3500 m+.

	1	2	3	4	5	6	7	8
<i>Alpinia</i>	+	+	+	+	+	+	+	+
<i>Amomum</i>	+	+	-	-	-	-	-	-
<i>Boesenbergia</i>	-	-	-	-	-	+	-	-
<i>Curcuma</i>	+	+	+	-	-	-	-	-
<i>Etlingera</i>	+	+	+	+	-	-	+	-
<i>Globba</i>	+	-	-	-	-	-	-	-
<i>Guillainia</i>	-	+	-	-	-	-	-	-
<i>Hedychium</i>	+	-	-	-	-	-	-	-
<i>Hornstedtia</i>	+	+	+	+	-	-	-	-
<i>Pleuranthodium</i>	+	+	+	-	+	-	+	+
<i>Riedelia</i>	+	+	+	+	+	+	+	+
<i>Thylacophora</i>	+	-	-	-	-	-	-	-
<i>Zingiber</i>	+	-	-	-	-	-	-	-

Table 5. Altitudinal zonation of *Alpinia*, *Pleuranthodium*, and *Riedelia*. The number of collections is recorded for each altitude. (a) Collections with no recorded altitudes. Altitudinal Zones: 1. 0 – 499 m; 2. 500 – 999 m; 3. 1000 – 1499 m; 4. 1500 – 1999 m; 5. 2000 – 2499 m; 6. 2500 – 2999 m; 7. 3000 – 3499; 8. 3500 m+.

	a	1	2	3	4	5	6	7	8
All Zingiberaceae	599	392	96	125	180	124	93	46	6
<i>Alpinia</i>									
Papua	65	10	11	7	4	6	-	-	-
Papua New Guinea	82	24	12	2	2	4	-	4	1
<i>Pleuranthodium</i>									
Papua	13								
Papua New Guinea	28	5	1	1	-	1	-	3	1
<i>Riedelia</i>									
Maluku	7	14	2	1	-	-	-	-	-

highlands of Papua New Guinea. Vink noted it was planted but according to field notes 'it was not introduced by Europeans'. It was named Singa Manga in the Loowi Language of Papua New Guinea.

As presently known, *Pleuranthodium* is endemic to New Guinea, but collections from C Sulawesi and southern Philippines could extend its range. At present 22 species are known (Smith, 1991). Sixty three collections are entered in the database of which 11 have not been identified. All species are endemic. Only five endemics are known from Papua, fourteen from Papua New Guinea, and three species occur throughout New Guinea. With the exceptions of *Pleuranthodium piundaundensis* (P. Royen) R.M Sm., which has nine collections, all other species are known from only 1-4 collections.

Conclusions

There are many problems in the study of the Zingiberaceae in New Guinea. The family is very poorly collected from most areas in New Guinea; indeed, large areas are uncollected. As botanical explorations proceed we can expect a large increase in the number of species, as many appear quite local in distribution. The existing collections include over 1700 specimens of which 190 are only identified to family level. All the larger genera include significant numbers of specimens not identified at the species level.

Many duplicates of the *Riedelia* collections from the former German New Guinea were sent for study to Valeton in Bogor. Also, Herbarium Bogoriense has many duplicates of the *Riedelia* types based on the collections of Schlechter, which were described by Valeton. The problems encountered by many specialists working on the New Guinean flora, the destruction of critical types collections in Berlin, does not apply to this genus. Recent ginger specimens are often poorly collected, many in fruit but lacking flowers. The quality of the collections reflects the difficulties of collecting in New Guinea.

A detailed taxonomic understanding, particularly of *Riedelia* and *Alpinia*, will probably require the recollection (preferably from their type localities) of most New Guinea species. Many of the collections are poor (often due to insect damage in preservation) and have not been, probably cannot be, identified at the level of genus and species.

The major requirements for collecting specimens of gingers are outlined by Burt and Smith (1976). Particular care should be made when collecting the flowers of gingers. Spirit material and materials for DNA studies could prove critical for an understanding of the larger genera in New Guinea. Photographs of the plants and flower details will be critical. The careful recording of ecological data is important. Future work should

also include living plant collections for growing in the botanic gardens at Lae and Bogor, with duplicate plants sent to Singapore and Edinburgh for additional planting, provided proper export permits are obtained.

No revision of the genera of the Zingiberaceae of New Guinea should be attempted until a sustained effort has been made to recollect adequate materials of the species from the type localities in order that the many names can be properly applied. This applies not only to *Riedelia* but to all the New Guinea genera of Zingiberaceae. Areas where the family is not, or is under collected, should also be targeted for future expeditions. Copies of the New Guinea database of the Zingiberaceae have been given to National Herbarium in Lae, the Singapore Herbarium, and the Herbarium of the Royal Botanic Gardens in Edinburgh.

Acknowledgements

I am very grateful to the Christensen Fund who provided a grant for studies on the flora of New Guinea. Without this support the studies would not be possible. I wish to express my sincere thanks to Dr. Jana Leong-Skornickova (SING) who encouraged my interests in the Zingiberaceae while working on the New Guinea database at the Singapore Botanic Gardens. Thanks are due to the Directors of the following Herbaria for permission to examine their collections: BO, BRI, CANB, LAE, SING. I also wish to thank to Dr. Mark Newman (E) for sending me a copy of his unpublished paper on the Zingiberaceae prepared for the 'Ecology of Papua', and also to thank the two reviewers for their constructive comments.

References

- Burtt, B.L. and R.M. Smith. 1972a. Key species in the taxonomic history of Zingiberaceae. *Notes from the Royal Botanic Garden, Edinburgh* **31**: 177–227.
- Burtt, B.L. and R.M. Smith. 1972b. Notes on Malesian Zingiberaceae. *Notes from the Royal Botanic Garden, Edinburgh* **31**: 307–316.
- Burtt, B.L. and R.M. Smith. 1976. Notes on the collection of Zingiberaceae. *Flora Malesiana Bulletin* **29**: 2599–2601.
- Gilli, A. 1980 [publ.1983]. Beiträge zur Flora von Papue-New Guinea, III Monocotyledones. *Annalen des Naturhistorischen Museums in Wien*, B, **84**: 5–47.

- Johns, R.J., P.J. Edwards, T.M.A. Utteridge and H.C.F. Hopkins. 2006. A guide to the alpine and subalpine flora of Mount Jaya. Kew Publishing, Royal Botanic Gardens, Kew.
- Larsen, K., J.M. Lock, H. Maas and P.M. Maas. 1998. Zingiberaceae, pp. 474-495. In: Kubizki, K. (ed.): *The Families and Genera of Vascular Plants*, Vol. IV. Springer-Verlag, Berlin, Heidelberg.
- Loesener, T. 1930. Zingiberaceae. *Die Natürlichen Pflanzenfamilien* (2nd ed.) **15a**: 541-640, 654-693.
- Newman, M.F. 2007. Zingiberaceae. pp. 473-476 In: A.J. Marshall and B. Beehler (eds.). *The Ecology of Papua*. 5. Periplus (HK) Ltd Editions, Singapore.
- Newman, M., A. Lhuillier and A.D. Poulsen. 2004. Checklist of the Zingiberaceae of Malesia. *Blumea Supplement* **16**: 1-166.
- Rickett, H.W. and F.A. Stafleu. 1959. Nomina generica conservanda et rejicenda spermatophytorum. *Taxon* **8**: 213-243.
- Ridley, H.N. 1886. New species collected by Forbes. *Journal of Botany* **24**: 356-358.
- Ridley, H.N. 1916. Report of the Botany of the Wollaston Expedition to Dutch New Guinea 1912-1913. *Transactions of the Linnean Society, Botany* **9**: 1-269.
- Ridley, H.N. 1923. A contribution to our knowledge of the Flora of Papua (British New Guinea). *Proceedings of the Royal Society, Queensland* **34**: 19-21.
- Riswan, S. and F.M. Setyowati. 1996. Ethnobotanical study of Zingiberaceae in Indonesia, pp. 196-218. In: Wu, T.L., Wu, Q.G. & Chen, Z.Y. (eds.). *Proceedings of the 2nd Symposium on the Family Zingiberaceae*. Zhongshan University Press.
- Royen, P. van 1979. Zingiberaceae. In: *The Alpine Flora of New Guinea* **2**: 860-866.
- Schumann, K. 1899. Monographie der Zingiberaceae von Malaisien und Papuasien. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **27**: 259-350, t. 2-6.

- Schumann, K. 1904. Zingiberaceae. In Engler, A. & Prantl (eds.), *Das Pflanzenreich* IV, **46 (20)**: 1–458, figs. 1–52.
- Smith, R.M. 1975. A preliminary review of the large bracteate species of *Alpinia*. *Notes from the Royal Botanic Garden, Edinburgh* **34**: 149–182.
- Smith, R.M. 1990a. *Alpinia* (Zingiberaceae): a proposed new infrageneric classification. *Edinburgh Journal of Botany* **47**: 1–75.
- Smith, R.M. 1990b. *Psychanthus* (K. Schum.) Ridley (Zingiberaceae): its acceptance at generic level. *Edinburgh Journal of Botany* **47**: 77–82.
- Smith, R.M. 1990c. *Pleuranthodium* replaces the illegitimate name *Psychanthus* (Zingiberaceae). *Edinburgh Journal of Botany* **48**: 63–68.
- Valeton, T. 1913a. Zingiberaceae. *Icones Bogoriensis* **4(3)**: t. 373–375.
- Valeton, T. 1913b. Zingiberaceae. *Nova Guinea* **8**: 923–988, t. 162–179.
- Valeton, T. 1914. Die Zingiberaceen Deutsch-Neu-Guineas. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **52**: 41–100, figs. 1–11.
- Valeton, T. 1917. Zingiberaceae. In: Gibbs, L.S., *A Contribution to the Phytogeography and Flora of the Arfak Mountains*. Taylor & Francis, London.

Morphology and Palynology of *Amomum* Roxb. in Thailand

W. KAEWSRI ¹ AND Y. PAISOOKSANTIVATANA ²

¹Department of Biotechnology, Faculty of Science, Mahidol University,
Kanchanaburi 71150, Thailand

Email: kawks@mahidol.ac.th

²Department of Horticulture, Faculty of Agriculture, Kasetsart University,
Chatuchak, Bangkok 10900, Thailand

Email: agryyp@ku.ac.th

Abstract

Morphological characteristics and pollen morphology of Thai *Amomum* Roxb. were studied in order to aid identification and classification. Thirty-one species collected during our field expedition, only 13 species could be identified to species, and 18 species will be proposed as new to science. Investigation of both vegetative and reproductive organs reveals that leaf, flower and fruit are useful for identification/separating the species of *Amomum*. Pollen grains of 14 representatives were examined using a scanning electron microscope (SEM) in order to reveal their morphology and usefulness for infrageneric classification. Two types of exine sculpture, psilate and echinate, were found. Classification by using pollen morphology does not support grouping by the previous authors that emphasized fruit shapes.

Introduction

Amomum Roxb. is one of the largest genera in the ginger family (Zingiberaceae) with about 150-180 species. They are widely distributed in Southeast Asia from the Himalayas to Northern Australia and extend into the central Pacific (Kiew, 1982; Smith, 1985). Sirirugsa (2001) estimated 15-20 *Amomum* species in Thailand. They are generally evergreen herbs inhabiting wet forests, especially in light gaps and at forest margins (Sakai and Nagamasu, 1998). Many species of *Amomum* are used as medicine, spice, condiment or a vegetable. Even though the plants from this genus have been long utilized, the identification is still confusing because of the absence of good specimens in many herbaria. Moreover, there are often misidentified either infragenerically and intergenerically. These lead to

many changes in taxonomic status. For instance, many species of *Amomum* were transferred to the other genera: *Aframomum* K. Schum., *Elettariopsis* Baker, *Alpinia* Roxb., *Etlingera* Giseke and *Hornstedtia* Retz. Besides, there are frequent changes in species identification. These problems need an intensive study for clarification.

Thailand has a very complex biogeography due to its topography and geographic position. Thus the southern Chinese flora reaches its southern limits in Chiang Mai and Nan provinces, while the Malesian flora covers the southern part of peninsular Thailand, the Burmese flora spills over the western limestone mountains, and the central table mountains harbour a rich endemic flora (Larsen, 2003). The diverse ecological habitats also contribute to a rich diversity of plants including species of *Amomum*.

At present, the country forested area decreases rapidly due to deforestation, urbanization and agricultural land expansion. Many plant species in the forest include *Amomum*, are at risk of extinction. In order to set conservation priorities for these potentially endangered species, the aim of the present study is to examine the vegetative and reproductive parts, as well as pollen morphology, useful for classification.

Materials and Methods

Indigenous species of *Amomum* were collected from all parts of Thailand during April 2003-June 2005 (Table. 1). Inflorescences, infructescences, vegetative parts and habitats, were photographed, and field notes were made. Flowers and fruits were preserved in 70% ethanol. The dried vegetative part and inflorescences were deposited at the herbaria BK (Department of Agriculture, Bangkok) and BKF (Royal Forest Department, Bangkok), Thailand. Living specimens are cultivated at the Department of Horticulture, Kasetsart University. Morphological characters were examined either from dry, spirit collections or living plants. Anthers of 14 species of Thai *Amomum* were taken from live plants at mature stage and stored in 70% ethanol. Pollen grains were collected and kept also in 70% ethanol. The samples were passed through an ethanol dehydration starting with 10-15 min in 90% ethanol with three subsequent changes in absolute alcohol at 10 min each. The Critical Point Dryer (BALZERS LINION CPD-020) was used to dry the samples. The pollen were then mounted on SEM stub using double-sided sticky tape and sputter-coated. Photographs were taken with a JSM Jeol 5410LV scanning electron microscope.

Table 1. List of *Amomum* species collected in Thailand during April 2003-June 2005.

No.	Botanical Name	Collector No.	Locality
1.	<i>Amomum aculeatum</i> Roxb.	Kaewsri 2, 6, 20, 65, 74	Kanchanaburi
2.	<i>Amomum biflorum</i> Jack	Kaewsri 52, 58, 66	Chanthaburi
3.	<i>Amomum dealbatum</i> Roxb.	Kaewsri 110	Chiang Mai
4.	<i>Amomum hastilabium</i> Ridl.	Kaewsri 37	Ranong
5.	<i>Amomum koenigii</i> J.F. Gmel.	Kaewsri 03, 29, 136	Kanchanaburi
6.	<i>Amomum micranthum</i> Ridl.	Kaewsri 63, 84	Chanthaburi
7.	<i>Amomum pierreanum</i> Gagnep.	Kaewsri 122	Nakhon Nayok
8.	<i>Amomum repoeense</i> Pierre ex Gagnep.	Kaewsri 64, 103, 121	Chanthaburi
9.	<i>Amomum rivale</i> Ridl.	Kaewsri 04, 23, 142	Kanchanaburi
10.	<i>Amomum siamense</i> Craib	Kaewsri 14, 116, 123	Tak
11.	<i>Amomum testaceum</i> Ridl.	Kaewsri 15, 16, 96	Tak (Cultivated)
12.	<i>Amomum uliginosum</i> König	Kaewsri 30, 32, 33, 92	Nakhon Nayok
13.	<i>Amomum cf. villosum</i>	Kaewsri 12, 13	Tak
14.	<i>Amomum</i> sp.1	Kaewsri 01, 93	Kanchanaburi
15.	<i>Amomum</i> sp.2	Kaewsri 10, 139	Kanchanaburi
16.	<i>Amomum</i> sp.3	Kaewsri 19, 75	Prachuap Khiri Khan
17.	<i>Amomum</i> sp.4	Kaewsri 22	Kanchanaburi
18.	<i>Amomum</i> sp.5	Kaewsri 24, 147	Kanchanaburi
19.	<i>Amomum</i> sp.6	Kaewsri 25, 88, 89, 113	Kanchanaburi
20.	<i>Amomum</i> sp.7	Kaewsri 27	Uthai Thani
21.	<i>Amomum</i> sp.8	Kaewsri 35	Ranong
22.	<i>Amomum</i> sp.9	Kaewsri 38	Ranong
23.	<i>Amomum</i> sp.10	Kaewsri 50, 54, 104, 105, 107	Sakon Nakhon
24.	<i>Amomum</i> sp.11	Kaewsri 68, 79	Chanthaburi
25.	<i>Amomum</i> sp.12	Kaewsri 70	Chumphon
26.	<i>Amomum</i> sp.13	Kaewsri 81	Ranong
27.	<i>Amomum</i> sp.14	Kaewsri 93, 94	Tak
28.	<i>Amomum</i> sp.15	Kaewsri 97, 108	Chiang Mai
29.	<i>Amomum</i> sp.16	Kaewsri 111, 114, 137	Chiang Mai
30.	<i>Amomum</i> sp.17	Kaewsri 134, 135	Nan
31.	<i>Amomum</i> sp.18	Kaewsri 151	Kanchanaburi

Results and Discussion

1. Morphological characters of vegetative and reproductive parts

Thirty-one species of *Amomum* collected from all over Thailand including 13 identified species and 18 unidentified species (Table 1) were examined. The sheath surface was either reticulate (Fig. 1A) or striate (Fig. 1B), and the ligule, either bilobed (Fig. 1C) or entire (Fig. 1D). These characters have only limited application in species determination whereas reproductive parts are of more useful to aid identification. The latter includes the differences in shape of the stigma (Fig. 1E-G), lateral staminodes (Fig. 1H-J), labellum (Fig. 1K-N), anther crest (Fig. 1O-S), and fruit (Fig. 1T-W).

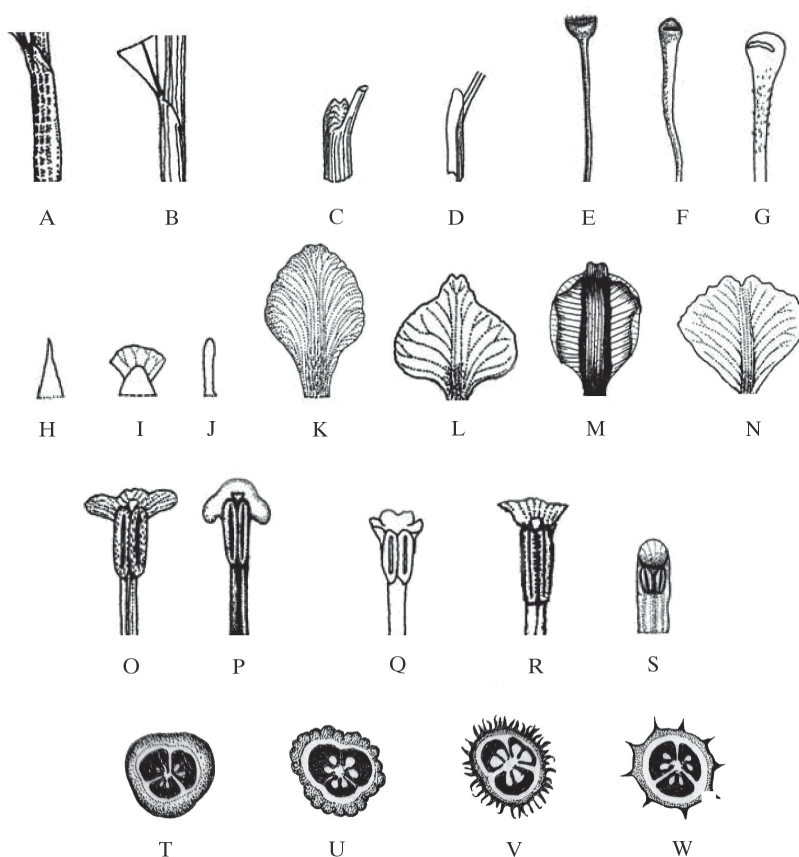


Figure 1. Morphological characteristics of some organs of the genus *Amomum* Roxb.

Leaf sheath: A. reticulate; B. striate. Ligule: C. bilobed; D. rounded. Stigma: E. cup shaped; F. ampullate; G. clavate. Lateral staminode: H. subulate; I. fin-shaped; J. linear. Labellum: K. ovate; L. deltoid; M. orbiculate and hooded; N. flabellate. Anther crest: O. reniform; P. auriculate; Q. trilobed or horn-shaped; R. truncate; S. rounded. X-section of fruit: T. smooth; U. ridged; V. spiny; W. wing.

Due to the closely resemblance in their floral morphology, it is recommended that the fruit types be examined. Three major types of fruits (smooth, hairy and winged) are found among Thai species of *Amomum*. Some variation in the degree of hairiness and smoothness of the fruit are also observed. The fruit shape is highly variable and can be globose, ellipsoid, obovoid, or obconical (Fig. 2A-M).

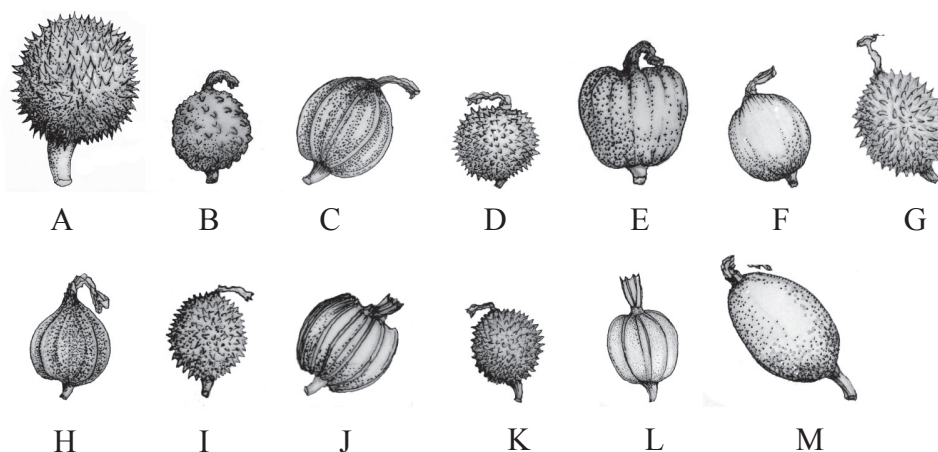


Figure 2. Fruit shapes of *Amomum*: A. *Amomum aculeatum*; B. *A. biflorum*; C. *A. dealbatum*; D. *A. rivale*; E. *A. siamense*; F. *A. testaceum*; G. *A. uliginosum*; H. *Amomum* sp.3; I. *Amomum* sp.4; J. *Amomum* sp.6; K. *Amomum* sp.11; L. *Amomum* sp.12; M. *Amomum* sp.15.

2. Palynological study

The SEM results revealed that the pollen grains of Thai *Amomum* are spherical to sub-spherical, (except for some grains of *A. rivale* Ridl. that are ovoid), 30-70 μm in diameter and the intine layer is 1-7 μm thick. These general results agree with Mangaly and Nayar (1990) which reported that the pollen shape of *Amomum hypoleucum* Thwaites and *A. pterocarpum* Thwaites are sub-spheroidal to ovoid and spheroidal, the diameter being 30-90 and 35-75 μm , respectively and the intine layer 1.25-2.5 μm thick in *Amomum hypoleucum*.

Our results revealed that the pollen can be divided into two groups either by intine thickness (<4 μm ; ≥ 4 μm), and by exine sculpture (echinate or psilate; Table 2, Fig. 3). The results do not coincide with the classification proposed by previous authors, in particular, Schumann (1904) who subdivided the genus (section *Euamomum*) into two series, namely, 1. ser. *Lobulatae* (anther crest with two or three lobes) and 2. ser. *Integrae* (anther crest margin with entire lobe). Our study reveals that most pollen grains are echinate in sculpture, which was consistent in both series and are of less use in classification.

As stated above, the results from palynological study are not useful for classification of Thai species of *Amomum*. Most data from pollen shapes and size characteristics are apparently homoplasious although exine sculpture can divide the species into two groups: echinate and psilate. The echinate group consists of *A. aculeatum*, *A. biflorum*, *A. dealbatum*, *A. siamense*, *A. uliginosum*, *Amomum* sp. 3, *Amomum* sp. 4, *Amomum* sp. 6, *Amomum* sp. 11, *Amomum* sp. 12, *Amomum* sp. 15, *Amomum* sp. 16. The psilate group includes only two species: *A. rivale* and *A. testaceum*.

Table 2. Pollen morphology of Thai *Amomum* species.

Species	Shape and size (dia.)	Intine thickness	Exine sculpture
1. <i>A. aculeatum</i>	Spherical, 46-60 μm .	3 μm	Echinate; spine uniformly distributed, sharp apex, having collared base, 1.5-3 μm tall, 2-3 μm thick at base.
2. <i>A. biflorum</i>	Spherical, 35-46 μm .	1-2 μm	Echinate; spine uniformly distributed, usually sharp apex, having collared base, 2.5-3 μm tall, 3-3.5 μm thick at base.
3. <i>A. dealbatum</i>	Spherical, 52-58 μm .	3 μm	Echinate; spine uniformly distributed, blunt and interrupted by sharp apex, 3-3.5 μm tall, 3 μm thick at base.
4. <i>A. rivale</i>	Subspherical or ovoid, ca. 60 μm .	1 μm	Psilate; exine 0.5 μm thick
5. <i>A. siamense</i>	Spherical, 43-53 μm .	5 μm	Echinate; spine uniformly distributed, sharp apex, 2.5-3 μm tall, 3 μm thick at base, surface reticulate.
6. <i>A. testaceum</i>	Spherical to subspherical, 60 μm .	2 μm	Psilate; exine 1 μm thick.
7. <i>A. uliginosum</i>	Spherical, 30-35 μm .	1-2 μm	Echinate; spine irregularly distributed, blunt interrupted by sharp apex, having collared base, 1 μm tall, 2 μm thick at base.
8. <i>Amomum</i> sp.3	Spherical, 63-70 μm .	4-5 μm	Echinate; spine uniformly distributed, distributed, sharp and interrupted by blunt apex, 4 μm tall, 6 μm thick at base.

9. <i>Amomum</i> sp.4	Spherical or subspherical, 50-58 μm .	1 μm	Echinate; spine uniformly distributed, sharp or blunt apex, having collared base, ca 2 μm tall, 3 μm thick at base.
10. <i>Amomum</i> sp.6	Spherical to subspherical, 45-50 μm .	4-5 μm	Echinate; spine uniformly distributed, blunt and interrupted by sharp apex, 2-2.5 μm tall, 2-3 μm thick at base.
11. <i>Amomum</i> sp.11	Spherical, 56-60 μm .	4-7 μm	Echinate; spine uniformly distributed, sharp apex, having collared base, 3 μm tall, 3 μm thick at base.
12. <i>Amomum</i> sp.12	Spherical, 60-64 μm .	1 μm	Echinate; spine uniformly distributed, sharp apex, 3-4 μm tall, 2-2.3 μm thick at base.
13. <i>Amomum</i> sp.15	Spherical, 50-60 μm .	1 μm	Echinate; spine uniformly distributed, sharp apex, 4 μm tall, 3.5 μm thick at base.
14. <i>Amomum</i> sp.16	Spherical, 60-63 μm .	2-3 μm	Echinate; spine uniformly distributed, sharp apex, 4 μm tall, 4 μm thick at base.

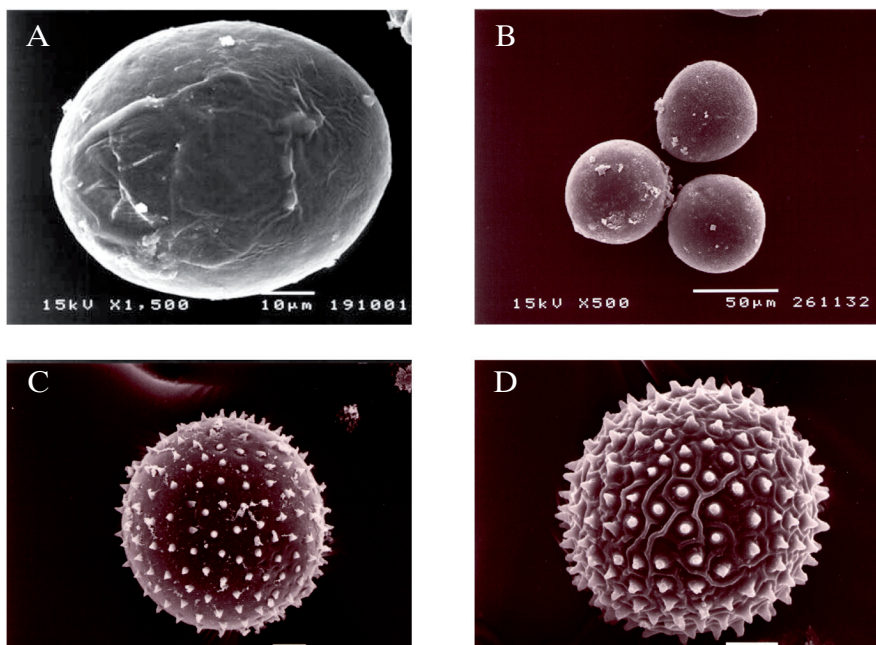


Figure 3. Pollen characteristics of *Amomum*. Psilate type: A. *A. rivale*; B. *A. testaceum*. Echinate type: C. *A. aculeatum*. Echinate-reticulate type: D. *A. siamense*.

Conclusions

1. Morphology of reproductive parts is more useful in aiding precise species identification of *Amomum*, especially the fruit shape.
2. The pollen grains of *Amomum* are spherical to subspherical, inaperturate, and the exine sculpture is either echinate or psilate. Pollen characteristics agree with the previous reports but do not correspond with previous classification based on morphological characteristics. Therefore, the pollen morphology is less useful for subgeneric classification of *Amomum*.

References

- Kiew, K. Y. 1982. The genus *Elettariopsis* (Zingiberaceae) in Malaya. *Notes from the Royal Botanical Garden Edinburgh* **42**: 295-314.
- Larsen, K. 2003. The Zingiberaceae in Flora of Thailand, pp. 1-5. In: P. Chantaranothai, K. Larsen, P. Sirirugsa and D. Simpson (eds.). *Proceedings of the 3rd Symposium on the Family Zingiberaceae*. Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University.
- Mangaly, J. K. and J. Nayar. 1990. Palynology of south Indian Zingiberaceae. *Botanical Journal of Linnaean Society* **103**: 351-366.
- Sakai, S. and H. Nagamasu. 1998. Systematic Studies of Bornean Zingiberaceae I. *Amomum* in Lambir Hills, Sarawak. *Edinburgh Journal of Botany* **55**: 45-64.
- Schumann, K. M. 1904. Zingiberaceae. In: A. Engler (ed.), *Das Pflanzenreich IV*, **46**: 1-458.
- Sirirugsa, P. 2001. Zingiberaceae of Thailand, pp. 63-77. In: V. Baimai and R. Kumhom. *BRT Research Reports 2001*. Biodiversity Research and Training Program. Jirawat Express Co., Ltd., Bangkok. (in Thai)
- Smith, R. M. 1985. A review of Bornean Zingiberaceae: 1 (Alpineae). *Notes from the Royal Botanical Garden Edinburgh* **42**: 295-314.

An analysis of generic circumscriptions in tribe Alpinieae (Alpiniodeae: Zingiberaceae)

W. J. KRESS¹, M. F. NEWMAN², A. D. POULSEN²
AND C. SPECHT³

¹ Department of Botany, MRC-166, United States National Herbarium, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington, D.C. 20013-7012 USA

² Royal Botanic Garden, 20A Inverleith Row, Edinburgh EH3 5LR, Scotland, UK

³ Department of Plant and Microbial Biology, University of California, Berkeley, 431 Koshland Hall, MC 3102, Berkeley, CA 94720 USA

Abstract

Recent investigations based on molecular phylogenies have resulted in new insights into the evolutionary relationships and classification of the Zingiberaceae and various genera within the family, e.g., *Globba*, *Hedychium*, *Roscoea*, *Etilingera*, *Alpinia*, and *Amomum*. At the same time taxonomic boundaries of many traditionally recognized genera have been challenged, e.g., *Curcuma*, *Boesenbergia*, *Caulokaempferia*, *Alpinia*, and *Amomum*. Within the subfamily Alpinoideae the results of our analyses will require the recircumscription of many of the genera included in the tribe Alpinieae. These phylogenetic results are based on a supermatrix analysis of ITS and *matK* sequence data and are discussed in the context of complementary morphological features and geographic distributions. Seventeen clades are recognized at the generic level although some remain tentative and in need of additional analysis before final taxonomic circumscriptions can be made. A revised classification will require that many species be placed in new or different genera, which will greatly facilitate identification and our understanding of morphological evolution in the family as well as species and genera therein.

Introduction

During the last few years molecular data have been routinely used in determining generic boundaries and evolutionary relationships of the genera in the ginger family, the Zingiberaceae (e.g., Harris *et al.*, 2000; Ngamriabsakul *et al.*, 2000; Rangsiruji *et al.*, 2000a, b; Searle and Hedderson,

2000; Wood *et al.*, 2000; Kress *et al.*, 2002; Pedersen, 2004; Williams *et al.*, 2004; Xia *et al.*, 2004). The study by Kress *et al.* (2002) is the most thorough paper to date addressing relationships among genera in the Zingiberaceae. In that study, sequence data from both the Internal Transcribed Spacer (ITS) and *matK* regions were used to establish well-resolved phylogenetic relationships among the genera, and a new classification of the Zingiberaceae was proposed that recognized four subfamilies and four tribes. Kress *et al.* (2002) also demonstrated that a number of the larger genera in the family (*Amomum*, *Alpinia*, *Boesenbergia*, and *Curcuma*) may be para- or polyphyletic and suggested that more extensive sampling was necessary for these taxa. Subsequent studies have been carried out in some of them (*Alpinia*: Kress *et al.*, 2005; *Amomum*: Xia *et al.*, 2004).

With respect to the tribe Alpinieae, the results of investigations by Rangsiruji *et al.*, (2000a, b), Kress *et al.*, (2002, 2005), and Xia *et al.* (2004) are most pertinent. In the first study, in which 47 species of *Alpinia* and a small number of outgroup taxa were sampled, the authors demonstrated significant statistical support for several monophyletic groups of species of *Alpinia*, but suggested that the genus itself may not be monophyletic. In a broader analysis of genera of Alpinioideae, Kress *et al.* (2002) identified four separate groups of alpinias (*Alpinia* I–IV) for the 11 *Alpinia* species sampled (Fig. 1). These four groups did not form a monophyletic assemblage, were scattered throughout the tribe, and corresponded to at least some of the clades recognized in the molecular analyses of Rangsiruji *et al.* (2000b). Further sampling in the tribe was also conducted by Xia *et al.* (2004) in their analysis of the generic boundaries of *Amomum*. They identified at least three major non-monophyletic groups of species within the current circumscription of *Amomum*. Within Alpinieae, the phylogenetic position of the presumed extinct *Leptosolena* and a second species of the formerly monotypic *Vanoverberghia* were determined by Funakoshi *et al.* (2005) based on molecular sequence data, providing more information on generic relationships within the tribe.

Kress *et al.* (2005) conducted the most exhaustive phylogenetic analysis of tribe Alpinieae to date sampling 99 species in the Alpinioideae with an emphasis on the genus *Alpinia*. Their results (Fig. 2) demonstrated six polyphyletic clades of *Alpinia* and at least two clades of *Amomum* while resolving the evolutionary relationships among a number of genera in the Alpinieae with a slightly different topology than earlier analyses had indicated (Kress *et al.*, 2002). Although their analyses confirmed the division of both *Alpinia* and *Amomum* into numerous polyphyletic groups, the authors were reluctant to propose a new classification until a number of issues, especially taxon sampling, were resolved.

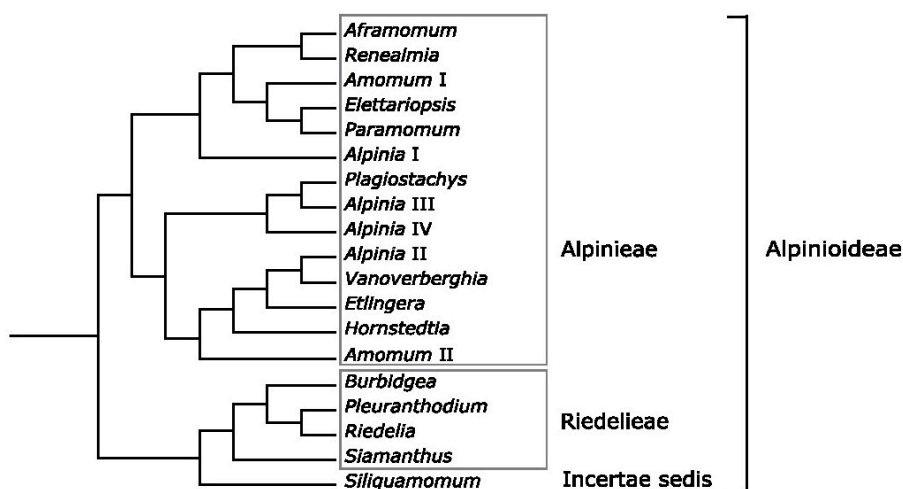


Figure 1. Early results on the phylogenetic relationships among the genera of the subfamily Alpinioideae of the Zingiberaceae based on a parsimony analysis of ITS and matK sequence data for 45 species in the subfamily (from Kress *et al.*, 2002). Note the four polyphyletic clades of *Alpinia* and two clades of *Amomum*.

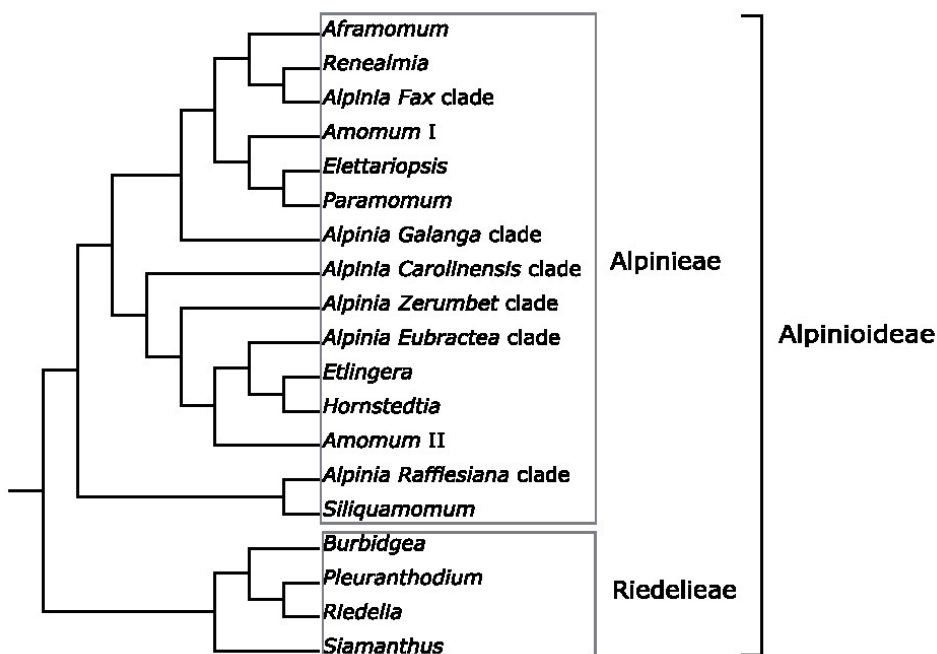


Figure 2. Most recent results on the phylogeny of the Alpinioideae resulting from further analysis of the combined ITS and matK sequence data for 99 species in the subfamily (from Kress *et al.* 2005). Note the six clades of *Alpinia*. The *Alpinia* Zerumbet clade includes the genus *Plagiostachys*; the *Alpinia* Eubractea clade includes the genera *Vanoverberghia* and *Leptosolenia*.

The goals of our current analyses are 1) to combine molecular sequence data from previously published analyses with new original sequences to build a “supermatrix” for Alpinieae; 2) to identify major clades defined by molecular data and assess their correspondence to existing generic boundaries; and 3) to provide new circumscriptions of genera and apply new names if necessary. Goals one and two are addressed in this paper. The results of these analyses have established the major lineages within the tribe, have confirmed the validity of previously recognized genera, and have identified clades which will require new generic circumscriptions. Although our sampling is extensive, we will be adding several key taxa to a final analysis to be published in the near future. At that time we will address goal three and provide a new revised classification of the Alpinieae that will recognize more finely circumscribed genera.

Materials and Methods

Taxon and Character Sampling

Taxon sampling was designed to include the full diversity (taxonomic, morphologic, and biogeographic) of the Alpinioideae with a focus on phyletic and biogeographic diversity in *Alpinia* and *Amomum*. A total of 230 taxa are used in the analysis, including 23 outgroup taxa representing the Zingiberoideae, Tamijiodeae and Siphonochiloideae. Previously published sequence data from our own work as well as additional data downloaded from GenBank were combined with new original sequences in the current analysis. Two independent gene regions were sampled for this analysis: the ITS (internal transcribed spacer) region of nuclear ribosomal DNA (White *et al.*, 1990) and the intron of the chloroplast transfer RNA gene for lysine *trnK*, including the maturase (*matK*) coding region and 5′ and 3′ flanking introns (Johnson and Soltis 1994; Mohr *et al.* 1993). ITS was obtained for all 230 taxa included in this analysis, while the entire *trnK* region is missing from 84 taxa.

DNA Isolation and Manipulation

Whole genomic DNA was extracted from plants with the Plant DNAeasy kit protocol (Qiagen). DNA fragments were amplified and sequenced for each of the gene regions using the primers and protocols previous published for Zingiberales (Kress *et al.*, 2002, 2005; Specht, 2006; Specht *et al.*, 2001). Sequencing was carried out on an ABI 3700 automated sequencer equipped with ABI PRISMTM sequencing analysis software. Sequences were analyzed and edited using Sequence Navigator (Applied Biosystems) and GeneJockey (Taylor, 1994) or Se-Al (Rambaut, 1996). Alignments

across taxa were performed using CLUSTAL X (Thompson *et al.* 1994) as a Multiple Alignment option in GeneJockey with both fixed and floating gap penalties set to 10. All manual alignment adjustments follow the criteria of Zurawski and Clegg (1987) in which gaps are considered as characters and the number of evolutionary events (insertions or deletions) is minimized. All characters are treated as unordered and gapped regions are treated as missing, however insertion-deletion events (indels) are coded as additional individual binary characters using the simple indel coding method (Simmons and Ochoterena 2000). A total of 25 and 17 gaps were coded from within the *trnK* and ITS regions, respectively.

Phylogenetic Analysis

The combined ITS and *trnK/matK* phylogenetic analyses were performed using the parsimony optimality criterion, in each case considering all positions of equal weight for evaluating phylogenetic relationships. Analyses were conducted with PAUP*4.0b4a (Swofford, 2001) for a total of 3820 aligned characters with 28% of the matrix scored as missing as the result of either inability to acquire sequence data for certain taxa or the presence of gap characters. For all parsimony analyses, heuristic searches were performed with TBR as the branch-swapping algorithm; starting trees were obtained using stepwise random addition with 100 replicates and one tree held at each step. Jackknife support values were calculated with 37% deletion and the “emulate Jac resampling” option selected (Farris *et al.*, 1996).

Results and Discussion

The first goal of our analysis was addressed by the parsimony consensus tree of the combined ITS and *trnK/matK* sequence data. The consensus tree represents 9,624 equally parsimonious trees with a length of 4,277 steps, including 32 coded gaps (Figs. 3, 4). A total of 920 characters were parsimony informative (2433 were constant, 467 were uninformative). The majority of primary clades defining recognized or tentative genera, including *Etlingeria*, *Renealmia*, *Aframomum*, the *Alpinia rafflesiana* clade, the *Alpinia zerumbet* clade, the *Alpinia carolinensis* clade, the *Alpinia eubractea* clade, the *Alpinia galanga* clade, the *Alpinia fax* clade, and the *Amomum tsao-ko* clade, are strongly supported with jackknife values ranging between 99-100%. Monotypic genera or genera with only a single species sampled in our analysis, including *Siliqueamomum*, *Geostachys*, and *Geocharis*, are clearly differentiated from their sister taxa. The *Amomum villosum* clade, the *Amomum maximum* clade and the *Elettariopsis* clade have slightly lower jackknife values (76-89%) while the seven species of *Hornstedtia* sampled

in our analysis form a grade with *Etlingera*. The relationships of only a few of the major lineages are not fully resolved, such as the placement of the *Silquamomum-Alpinia rafflesiana* clade, and the polytomy formed by the *Geostachys* - *Amomum tsao-ko* clade, the *Alpinia zerumbet* clade, and the *Alpinia carolinensis* clade.

The second goal of our analyses, to assess the correspondence of the major clades defined by the molecular data to existing generic concepts, has also been accomplished. The 17 clades recognized here (Fig. 4) can be classified into three categories: 1) well-supported lineages that correspond to formerly recognized genera whose taxonomic names should be maintained, 2) well-supported lineages in need of new (or previously used) taxonomic names, and 3) problematic lineages in need of additional data prior to making final taxonomic decisions.

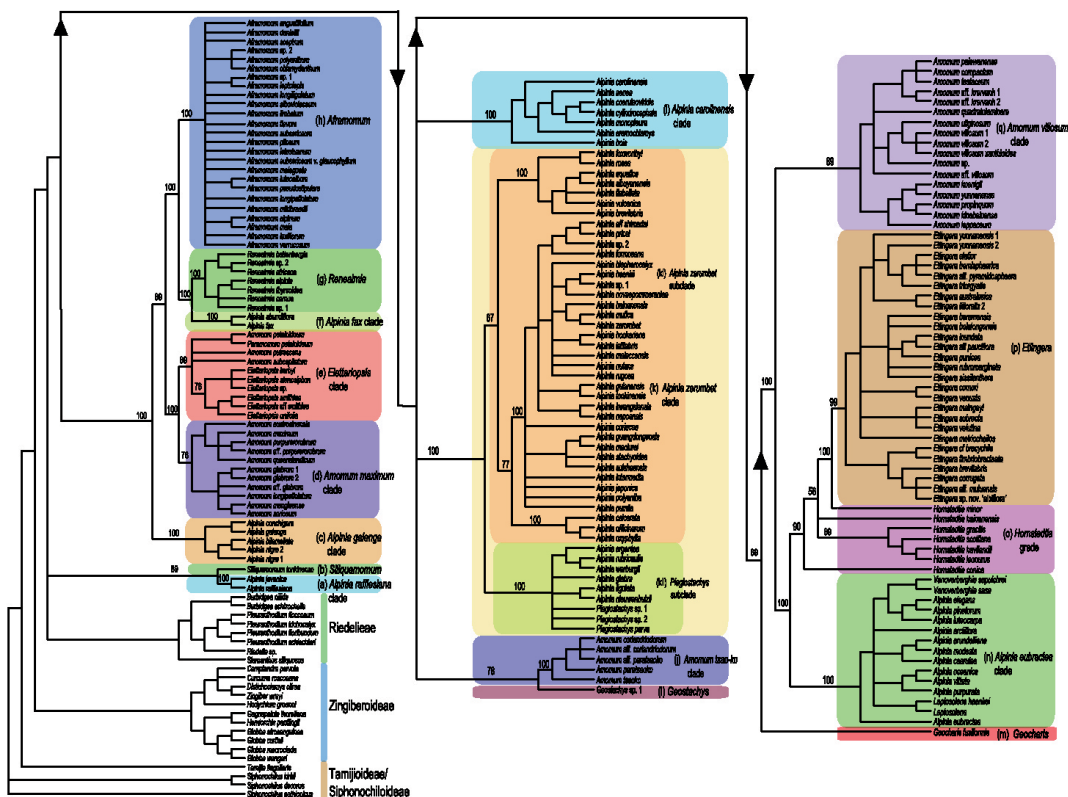


Figure 3. The strict consensus supertree of 9,624 equally parsimonious trees of the Alpinioideae (with an emphasis on tribe Alpinieae) in the analysis of combined ITS and *trnK/matK* sequence data for 207 species in the subfamily (length = 4,277 steps) showing bootstrap values from the parsimony analysis. The seventeen major clades in Alpinieae are indicated with small letters (a through q) and variously color shading. For discussion of each clade see text.

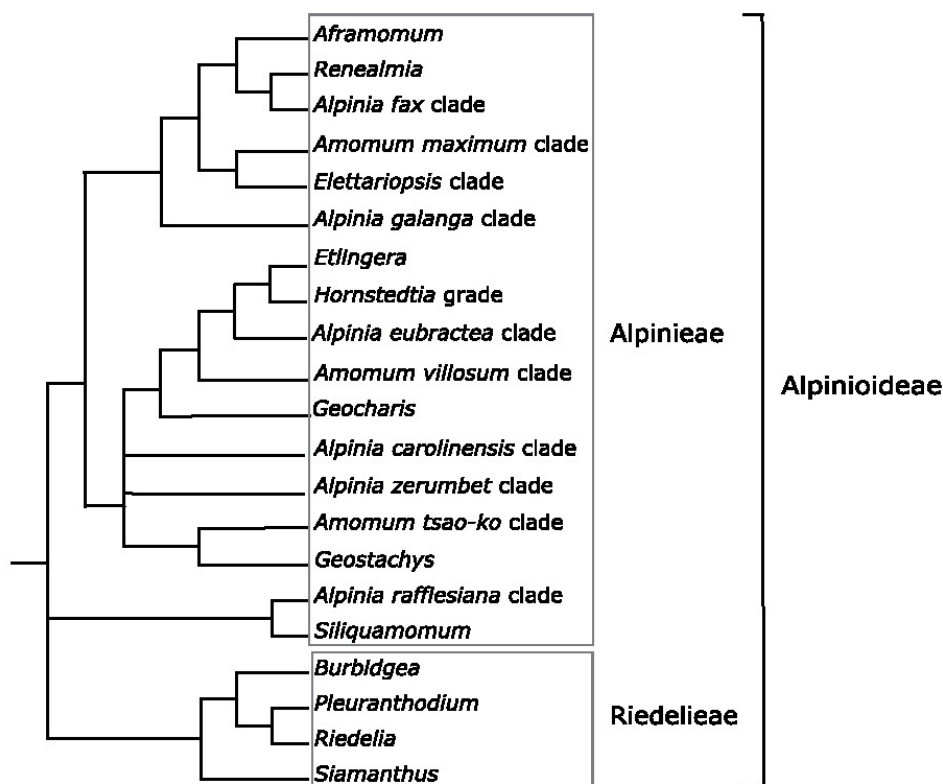


Figure 4. Condensed tree of the Alpinioideae resulting from the analysis of the combined ITS and *trnK/matK* sequence data (see Fig. 3) in which the major clades/grades have been collapsed into single branches for clarity.

(1) Well-supported lineages that correspond to formerly recognized genera whose taxonomic names should be maintained.

Aframomum. As earlier demonstrated by Harris *et al.* (2000), this African genus is strongly supported as monophyletic by molecular data. The flask-shaped fruit is a distinct synapomorphy of the genus. The basal inflorescence radical to the leafy shoot is shared with its sister taxon *Renealmia* from which it is distinguished by the presence of scale-like trichomes on the vegetative structures in *Aframomum*.

Renealmia. This genus is one of the few genera in the order Zingiberales with an amphi-Atlantic distribution with species in the tropical forests of the Americas and Africa. The clade is well-supported as monophyletic, and although it shares the distinctive basal inflorescence with *Aframomum*, it is

distinguished by stellate, rather than scale-like, trichomes. It should be noted that at least a few species on both sides of the Atlantic have inflorescence terminal on the leafy shoots (e.g., the neotropical *R. cernua* (Sw. ex. Roem. & Schult.) J. F. Macbr., *R. helenae* Maas, and *R. pyramidalis* (Lam.) Maas, and the African *R. battenbergiana* Cummins).

Alpinia galanga clade. The type species of the large conglomerate genus *Alpinia* is *A. galangal* (L.) Willd., which is placed in this small clade in the molecular analyses. For this reason, the generic name *Alpinia* is best applied to this group of four species. Both the placement of this clade in relationship to other genera and the monophyly of the four species have strong jackknife support (100%). Branched inflorescence with small flowers, open bracteoles, a clawed labellum, and thin-walled fruits are characteristic of the species in the *A. galanga* clade. Members of this clade are distributed primarily in continental Asia with the wide distribution of *A. galanga* most likely due to its important culinary use by local peoples.

Etlingera. The often large involucre of sterile inflorescence bracts and the fusion of the corolla tube to the labellum and single stamen filament, forming a staminal tube beyond the insertion of the corolla lobes, are characteristic of this monophyletic genus. Species are spread throughout the wet lowland tropics of Southeast Asia. The genus *Etlingera* is closely related and apparently paraphyletic with *Hornstedtia*.

Geocharis. Although only one of the six species of *Geocharis* was sampled in our molecular analysis, this genus appears to be distinctive in the tribe being differentiated by the radical, sometimes lax, inflorescence and the stem venation marked by prominent white hairs between major veins. However, increased sampling of species of this genus and other taxa placed in the genus *Amomum* are needed to determine the monophyly of *Geocharis*. Species are found in the Philippines, Peninsular Malaysia, Sumatra, and Borneo.

Geostachys. As in *Geocharis*, only one of the 20-25 species of this genus was included in our analysis. The stilt roots, lax inflorescence, non-imbricate bracts, and two or more flowers per cincinnus are characteristic of species of *Geostachys* and suggest that the genus is monophyletic. Many endemic species are found in this genus distributed in peninsular Malaysia, Sumatra, northwestern Borneo, and Thailand to Cambodia.

Siliquamomum. With only a single species found in northern Vietnam and bordering regions of tropical China, this genus has unique cylindrical torulose fruits resembling siliques. The phylogenetic placement of *Siliquamomum* has

been problematic and often unresolved within the Alpinioideae (see Kress *et al.*, 2002, 2005). Our current analysis allies it to the *Alpinia rafflesiana* clade although this placement may change in future analysis. It is best maintained as a distinctive monotypic genus in the subfamily.

(2) Well-supported lineages in need of new (or previously used) taxonomic names.

Alpinia fax clade. The three species placed in this clade (only two sampled in the molecular study; the third species, *A. rufescens* (Thw.) K. Schum., is only known from the type specimen) form a well-supported monophyletic group characterized by a radical capitate inflorescence often borne on a long leafless peduncle with conspicuous sterile bracts (Sabu, 2006). This clade is distributed in Sri Lanka and southern India and is sister to the clade containing the genera *Renealmia* and *Aframomum* (see above). Together with these two genera the *A. fax* clade constitutes a strongly supported monophyletic lineage stretching from tropical America through Africa to south Asia, which may represent both vicariant and long-distance dispersal events. All three species of this clade were originally described in the genus *Elettaria*, which has not been included in the present analysis. A new generic name is required for this clade.

Alpinia eubractea clade. This clade is made up of species found primarily in the Pacific Ocean, including the Philippines, Oceania, and Australia, and includes taxa earlier described in three genera (*Alpinia*, *Leptosolena*, and *Vanoverberghia*). The *A. eubractea* clade is strongly supported (bootstrap = 100%) in the molecular analysis, but the morphological apomorphies of this group of species are not immediately obvious. This clade will require a new generic name after additional taxa are added to the phylogenetic analysis, especially species of *Alpinia* from New Guinea.

Amomum villosum clade. Although it may be premature to recognize segregate genera for species that were earlier described in the genus *Amomum*, the results of our analyses as well as earlier investigations by Xia *et al.* (2004) support at least three distinct lineages of taxa formerly placed in *Amomum*. The *A. villosum* clade is characterized by echinate fruits and a trilobed anther appendage. Species in this clade are distributed primarily in Indochina, peninsular Malaysia, and Borneo. Additional species samples throughout this distribution will provide a more complete picture of the taxonomic breadth of this clade.

Alpinia carolinensis clade. Species in this clade tend to be plants large in

stature, with a caducous primary inflorescence bract, and a narrow fleshy labellum adpressed to the stamen. Members of the *A. carolinensis* clade are concentrated in Sulawesi and generally east of Wallace's Line in the Pacific Ocean. Additional species from Sulawesi and New Guinea should be added to the molecular analysis in anticipation of applying a new generic name to this clade.

Alpinia zerumbet clade. The great bulk of species named in the genus *Alpinia* are found in this well-supported clade with a broad geographic distribution in tropical Asia. The absence of a primary inflorescence bract and the presence of short one-three flowered cincinni characterize most of the species included in the *A. zerumbet* clade. At least four subclades with strong (= 100%) jackknife support are apparent within the *A. zerumbet* clade, including the *A. aquatica* subclade, the *A. nutans* subclade, the *A. calcarata* subclade, and the *Plagiostachys* subclade. It may be appropriate to recognize each of these well-supported subclades at the subgeneric level. However, if subgenera are to be established, at this time we advocate for the purpose of simplicity the recognition of only two subgenera corresponding to the *A. zerumbet* subclade and the *Plagiostachys* subclade as circumscribed in Fig. 3.

Amomum tsao-ko clade. The species included in this clade are primarily Chinese in distribution. They are characterized by leaves with pleasant and distinctive aromatic oils, a bi- or tri-lobed anther appendage, and smooth fruits. In the molecular analysis this clade of *Amomum* is only moderately supported as sister to *Geostachys*, which is morphologically distinct. As more species of *Amomum* are added to the overall analyses, additional species may be included in the *A. tsao-ko* clade.

Alpinia rafflesiana clade. Only two of the over 200 species in tribe Alpinieae that we sampled are contained in this well-supported clade found in peninsular Malaysia and southern Thailand. The two species are characterized by a broadly spread labellum and/or drooping inflorescence. It is possible that after additional sampling other species will be included in this clade, such as *A. capitellata* Jack from Borneo, but the molecular distinctiveness of the lineage suggests that a separate generic name is warranted. Our analysis has placed the *A. rafflesiana* clade sister to the unique monotypic *Siliqueamomum*, which should be maintained as a separate genus (see above).

(3) Problematic lineages in need of additional data prior to making final taxonomic decisions.

Amomum maximum clade. Species possessing an entire anther appendage, orange and yellow labellum, and winged fruits are contained within this clade, which was also recognized by Xia *et al.* (2004) in their molecular analysis of *Amomum*. Most of the taxa are distributed in China, India, and Australia. In our current sample of taxa the molecular data only moderately support the monophyly of the *A. maximum* clade. However, jackknife support is strong (100%) for the node joining the *A. maximum* clade with the *Elettariopsis* clade (see below). Although we have not yet obtained sequence data for *A. subulatum* Roxb., the type of the genus *Amomum* with winged fruits, we expect this species to be included in one of these clades and not in the *A. tsao-ko* clade or *A. villosum* clade. More sampling of species of *Amomum* with winged fruits, including the type species, is needed before a final decision can be made on taxonomic alignments.

Elettariopsis clade. This clade contains species of *Elettariopsis*, *Amomum*, and the monotypic *Paramomum* and, similar to the *A. maximum* clade, it is only moderately supported by our molecular data (jk = 89%). *Elettariopsis* has long been recognized as a genus because of the distinctive two-to-three-leaved shoots with characteristic aromatic oils, an elongated underground inflorescence with subterranean fruits, and a trilobed anther crest. Some of these features are also shared with the species of *Amomum* and *Paramomum* contained in the same clade. If sampling additional taxa does not provide better support for this clade, it may be justified to recognize the *Elettariopsis* clade together with the *A. maximum* clade as a single genus.

Hornstedtia grade. The characteristic stilt roots and elongated corolla tubes are found in most species of the widespread tropical Asian genus *Hornstedtia*. Although the close relationship between this genus and *Etlingera* has been recognized for over a half century (Holtum, 1950; Smith, 1985), the monophyly of *Hornstedtia* had not been challenged until recently. Pedersen (2004) in her analysis of *Etlingera* demonstrated that the species of *Hornstedtia* that she sampled formed a paraphyletic group. Our analyses with broader sampling confirm her result. At this point we suggest that *Hornstedtia* and *Etlingera* remain as separate genera until further analyses clarify the issue.

Several interesting genera in the Alpinieae were not sampled in our molecular analyses because of the unavailability of sufficient tissue samples or problems encountered in generating DNA sequences. *Cyphostigma*, a monotypic genus endemic to Sri Lanka and distinctive in its lax inflorescence, resupinate flowers, and large petaloid anther crest, may be related to *Elettariopsis* or the *Amomum maximum* clade because of similarities

in inflorescence and floral features (Holtum, 1950). *Elettaria*, which has a broad distribution in India, peninsular Malaysia, Java, and Borneo and contains the economically important spice cardamon (*E. cardamomum* (L.) Maton), was also not sampled in our analysis. Its position in the Alpinieae is as yet unclear.

Conclusions

As DNA sequence data for additional taxa have been added to the phylogenetic analysis of the Alpinioideae, a more detailed and refined understanding of evolutionary relationships and generic boundaries in the subfamily has been obtained (Figs. 1-4). As outlined above, our investigations are centered on three goals, two of which are addressed in this paper. We will address the third goal (to provide new circumscriptions of genera and apply new names where necessary) in a future publication that will include a more complete sampling of critical taxa necessary to make final decisions on meaningful taxonomic units in the Alpinioideae and especially the tribe Alpinieae.

Acknowledgements

We wish to thank Ray Baker, Mike Bordelon, Mark Collins, Heather Driscoll, David Harris, Qing-Jun Li, and Ida Lopez for assistance in preparing this manuscript. Funding was provided by the Smithsonian Institution.

References

- Farris, J. S., V. A. Albert, M. Kallersjö, D. Lipscomb and A. G. Kluge. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* **12**: 99-124.
- Funakoshi, H., W. J. Kress, J. Skornickova, A.-Z. Liu and K. Inoue. 2005. Return from the lost: rediscovery of the presumed extinct *Leptosolenia* (Zingiberaceae) in the Philippines and its phylogenetic placement in the gingers. *Acta Phytotaxonomica et Geobotanica* **56**: 41-53.
- Harris, D. J., A. D. Poulsen, C. Frimodt-Møller, J. Preston, and Q. C. B. Cronk. 2000. Rapid radiation in *Aframomum* (Zingiberaceae): evidence from nuclear ribosomal DNA internal transcribed spacer (ITS) sequences. *Edinburgh Journal of Botany* **57**: 377-395.

- Holttum, R. E. 1950. The Zingiberaceae of the Malay Peninsula. *Gardens' Bulletin of Singapore* **13**: 1–249.
- Johnson, L. A. and D. E. Soltis. 1994. matK DNA Sequences and Phylogenetic reconstruction in Saxifragaceae s. str. *Systematic Botany* **19**: 143–156.
- Kress, W. J., L. M. Prince and K. J. Williams. 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. *American Journal of Botany* **89**: 1682–1696.
- Kress, W. J., A.-Z. Liu, M. Newman and Q.-J. Li. 2005. The molecular phylogeny of *Alpinia* (Zingiberaceae): a complex and polyphyletic genus of gingers. *American Journal of Botany* **92**: 167–178.
- Mohr, G., P. S. Perlman and A. M. Lambowitz. 1993. Evolutionary relationships among group II intron-encoded proteins and identification of a conserved domain that may be related to maturase function. *Nucleic Acids Research* **21**: 4991–4997.
- Ngamriabsakul, C., M. F. Newman and Q. C. B. Cronk. 2000. Phylogeny and disjunction in *Roscoea* (Zingiberaceae). *Edinburgh Journal of Botany* **57**: 39–61.
- Pedersen, L. B. 2004. Phylogenetic analysis of the subfamily Alpinioideae (Zingiberaceae) with special emphasis on *Etlingera* Giseke, based on nuclear and plastid DNA. *Plant Systematics and Evolution* **245**: 239–258.
- Rambaut, A. 1996. *Sequence Alignment Editor*. Available at <http://evolve.zoo.ox.ac.uk/>.
- Rangsiruji, A., M. F. Newman and Q. C. B. Cronk. 2000a. Origin and relationships of *Alpinia galanga* (Zingiberaceae) based on molecular data. *Edinburgh Journal of Botany* **57**: 9–37.
- Rangsiruji, A., M. F. Newman and Q. C. B. Cronk. 2000b. A study of the infrageneric classification of *Alpinia* (Zingiberaceae) based on the ITS region of nuclear rDNA and the *trnL-F* spacer of chloroplast DNA. In: K. L. Wilson and D. A. Morrison [eds.], *Monocots—Systematics and evolution*, pp. 695–709. CSIRO Publishing, Collingwood, Australia.
- Sabu, M. 2006. *Zingiberaceae and Costaceae of South India*. Indian Association for Angiosperm Taxonomy. Kerala, India.

- Searle, R. J. and T. A. J. Hedderson. 2000. A preliminary phylogeny of the Hedychieae tribe (Zingiberaceae) based on ITS sequences of the nuclear rRNA cistron. In: K. L. Wilson and D. A. Morrison [eds.], *Monocots—Systematics and evolution*, 710–718. CSIRO Publishing, Collingwood, Australia.
- Simmons, M. P. and H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* **49**: 369–381.
- Smith, R. M. 1985. A review of Bornean Zingiberaceae: 1 (Alpinieae p.p.). *Notes from the Royal Botanic Garden Edinburgh* **42**: 261–314.
- Specht, C. D. 2006. Systematics and Evolution of the tropical monocot family Costaceae (Zingiberales): a multiple data set approach. *Systematic Botany* **31**: 88–105.
- Specht, C. D., W. J. Kress, D. W. Stevenson and R. DeSalle. 2001. A molecular phylogeny of Costaceae (Zingiberales). *Molecular Phylogenetics and Evolution* **21**: 333–45.
- Swofford, D. L. 2001. PAUP*. *Phylogenetic Analysis Using Parsimony* (*and other methods). Sinauer Associates, Sunderland, Massachusetts.
- Taylor, P. L. 1994. *Genejockey II Sequence Processor software*. Biosoft, Cambridge, UK.
- Thompson, J. D., D. G. Higgins and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673–4680.
- White, T. J., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: *A Guide to Methods and Applications*, Academic Press.
- Williams, K. J., W. J. Kress and P. S. Manos. 2004. The phylogeny, evolution, and classification of the genus *Globba* and tribe Globbeae (Zingiberaceae): appendages do matter. *American Journal of Botany* **91**: 100–114.
- Wood, T. H., W. M. Whitten and N. H. Williams. 2000. Phylogeny of *Hedychium* and related genera (Zingiberaceae) based on ITS sequence data. *Edinburgh Journal of Botany* **57**: 261–270.

- Xia, Y.-M, W. J. Kress and L. M. Prince. 2004. A phylogenetic analysis of *Amomum* (Alpinioideae: Zingiberaceae) using ITS and *matK* DNA sequence data. *Systematic Botany* **29**: 334–344.
- Zurawski, G. and M. T. Clegg. 1987. Evolution of higher-plant chloroplast DNA-encoded genes: Implications for structure-function and phylogenetic studies. *Annual Review of Plant Physiology* **38**: 391-418.

Materials for a Taxonomic Revision of *Geostachys* (Baker) Ridl. (Zingiberaceae) in Peninsular Malaysia

K.H. LAU ¹, C.K. LIM ² AND K. MAT-SALLEH ³

¹Tropical Forest Biodiversity Centre,
Biodiversity and Environment Division, Forest Research Institute Malaysia,
52109 Kepong, Selangor, Malaysia.

²215 Macalister Road, 10450 Penang, Malaysia.

³School of Environmental and Natural Resource Sciences,
Faculty of Science and Technology, Universiti Kebangsaan Malaysia,
43600 Bangi, Selangor, Malaysia.

Abstract

Materials for a taxonomic revision of the *Geostachys* (Baker) Ridl. in Peninsular Malaysia, resulting from recent fieldwork are presented, with notes on the threat assessment of extant species. Twelve of the 13 previously known species were studied in situ, and two newly described species have also been found (*Geostachys belumensis* C.K. Lim & K.H. Lau and *G. erectifrons* K.H. Lau, C.K. Lim & K. Mat-Salleh), bringing the current total to 15 taxa, all highland species, found in hill, sub-montane and upper montane forests ranging from 600 m to 2000 m a.s.l. Thirteen out of 15 of the known species are believed to be hyper-endemic, found so far only in their respective type localities.

Introduction

Geostachys (Baker) Ridl. is a relatively small genus within the Zingiberaceae family, with only 21 species previously recorded. Its distribution ranges from Vietnam, Thailand, Sumatera, Peninsular Malaysia and Borneo. Peninsular Malaysia is the home for most of the species, with 15 taxa scattered in the rain forest of this country (Holtum, 1950; Stone, 1980; Lau *et al.*, 2005).

The name *Geostachys* was introduced by Baker (1892) as a subgenus of *Alpinia* when he first described two species, *Alpinia decurvata* Baker and *A. secunda* Baker, both from Perak. In his pioneering work, The Scitamineae of The Malay Peninsula, Ridley (1899) elevated *Geostachys* as a genus, with five species, adding three new ones: *G. elegans* Ridl., *G. rupestris* Ridl. and *G. penangensis* Ridl. In 1920, Ridley described two other new species, namely, *G. primulina* Ridl. and *G. densiflora* Ridl., bringing the total to seven. He had

earlier also described two taxa under separate genera: *Carenophila montana* Ridl. in 1909 and *Conanomum sericeum* Ridl. in 1915, both of which were subsequently transferred to *Geostachys* by Holttum (1950) in his important monograph, *The Zingiberaceae of The Malay Peninsula*. Holttum further added three new species: *G. megaphylla* Holttum, *G. taipingensis* Holttum and *G. tahanensis* Holttum, all relatively unknown or not recollected until recently. Stone (1980) discovered another taxon, *G. leucantha* B.C. Stone, making a total of 13, prior to our studies.

As a consequence of fieldwork to study the genus in their type localities, all but *G. montana* (Ridl.) Holttum have been recollected, and two new species found and published by the authors (Lau *et al.*, 2005): *Geostachys belumensis* C.K. Lim & K.H. Lau and *G. erectifrons* K.H. Lau, C.K. Lim & K. Mat-Salleh.

Holttum's account of *Geostachys* within the Zingiberaceae of Peninsular Malaysia was made more than 50 years ago, and he intimated that there were still several taxa based on incomplete data, also mentioning that several species seemed rather closely allied, while other new species may yet be discovered. We were encouraged to work on the revision of this genus, to provide fresh data and updates on conservation status in the wild. Further extension studies on the Bornean records of the genus may follow, currently outside the scope of our study.

Studies on the *Geostachys* in other parts of the region have been carried out by Gagnepain (1906), Valetton (1921) and Larsen (1962), in which they had done various studies on this genus in Indo-China, Sumatera and Thailand respectively.

Materials and Method

Field study and living plant collections

Observations were carried out during the recorded flowering and fruiting season of the *Geostachys* species. Fieldwork was conducted at the type or other known localities (Fig. 1) of each species to study and collect living specimens and herbarium topotypes, and the *in situ* information proved valuable in ascertaining characters and other attributes, such as coloration and morphological variations, with reference to particular populations and/or those in different localities. Field observations were also important to analyze the habitat of each species.

Comparative morphology based on herbarium collections

Herbarium specimens or images of collections from the year 1884 to 2001 (in addition, the authors' recent collections) preserved at five major herbaria: K, KEP, KLU, SING and UKMB, were scrutinized. A total of 98 specimens,

including types were examined. Selected main morphology characters of each species were investigated and compared. The characters include habit of the plant, root and rhizome, leafy shoot, leaves, peduncle, stalk of cincinni, bracts, pedicel, calyx, corolla tube, labellum, staminode, anthers, stamen, fruit and seed where available.

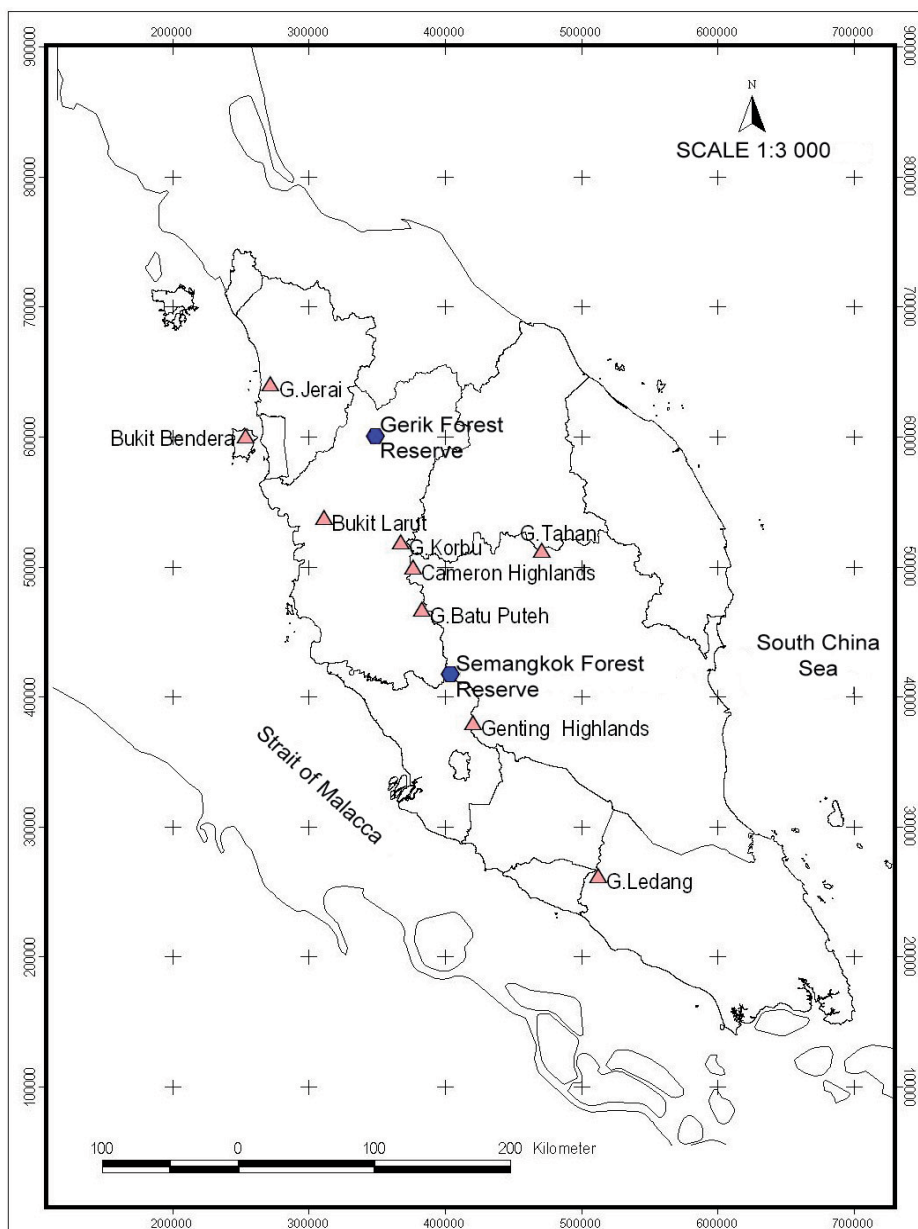


Figure 1. Study areas at type localities of species of *Geostachys*.

Results and Discussion

Typification of the generic name Geostachys

Studies made on literature (Baker, 1892; Ridley 1899, 1920, 1924; Holttum, 1950) and as reported by Turner (2000) and Newman *et al.* (2004), have shown that no type species has been designated for the genus to date. To remedy this, and after due consultations, one of the two early taxa recognised by Baker, *Geostachys decurvata* (Baker) Ridl., is proposed as the type species. It displays all the essential characters of the genus, and can still be conveniently referred to in its original type location at Bukit Larut, Perak.

GEOSTACHYS (Baker) Ridl., J. Straits Branch Roy. Asiat. Soc. 32 (1899) 158; *Alpinia* subg. *Geostachys* Baker, in Hook. f. Fl. Brit. India 6 (1892) 257. TYPE: *Geostachys decurvata* (Baker) Ridl. (selected here).

A checklist and distribution of the Geostachys

Fourteen of the 15 species were found and studied by us in the field. *Geostachys montana* could not be found in the type area, and remains in doubt, as to whether it is truly distinct. A checklist of all the species in Peninsular Malaysia is provided in Appendix 1.

All *Geostachys* species are highland species found in hill, sub-montane and upper montane forests ranging from 600-2000 m a.s.l. in the forest of Gunung Jerai (Kedah), Gunung Korbu (Perak), Gunung Ledang (Johore), Gunung Mering (Malacca), Gunung Tapis (Pahang), Gunung Benom (Pahang), Gunung Tahan (Pahang), Gunung Brinchang (Pahang), Gunung Berembun (Pahang), Bukit Bendera (Penang), Bukit Larut (Perak), Bukit Kedondong (Malacca), Bukit Fraser (Pahang), Genting Highlands (Pahang), Cameron Highlands (Pahang), and on the hills of Semangkok Forest Reserve (Selangor) and Belum Forest Reserve (Perak).

There have been records of *G. penangensis* from Borneo (Sarawak) and some other *Geostachys* spp. in Sabah (C.K. Lim and K. Mat-Salleh, pers. comm.). Initially, it was believed that *G. penangensis* was endemic to Penang (Lau, 2004). However, the current revision only targetted on species from Peninsular Malaysia. Nonetheless, the authors feel that further investigation on the genus should also include other species from Borneo, as well as in Thailand and Sumatera.

Morphological observations

Generally, all the species have stilt roots, or at least stilt roots-like coming out from the rhizomes. Some species have true stilt roots, whereas some are just having long and reticulated roots.

As for the leaves, there are few characters that are quite useful especially for field identification. The colours of the upper and lower surfaces of the leaves are important as there are few species with maroon colour underneath. The lamina comprises of four different types; widely elliptic, narrowly elliptic, lanceolate and oblong. The other less prominent but useful character is the presence of hairs at the ligule.

The structure of the inflorescence consists of two very different types: decurved and erect. As for the decurved inflorescence, the curving of the inflorescence starts at the peduncle and run through the whole rachis. Each of the flowers curved at a very peculiar upwards manner, as if all of the flowers are growing at one side only. However, for the erect type, the flowers grow closely and in whorled-like manner. The flowers can either be single, 1-2 per cincinnus, 1-3 per cincinnus, or 1-5 per cincinnus.

The flowers of *Geostachys* can be either yellow or white. However, majority of the species have yellow labellum with only 3 species having white. Some of the species have flowers with crumpled margin whereby some have smooth margin. Few species have staminodes on their flowers and can be seen with red markings. Another distinct attribute that is quite useful in recognizing between species is the presence of the anther crest. The anther crest can be very prominent and is trilobed-like.

The shape of the fruits of this genus is either ovoid or ellipsoid. The shape can be sometimes not so obvious, but in a particular species, the shape is more or less constant. Table 1 summarizes the characteristic of each species.

Endemism of Geostachys

Among the 15 recognised species, 13 are hyper-endemic to their respective type localities. Some are observed as rare, and several species were found only in few and small clumps at their habitats, e.g., *G. primulina*, *G. tahanensis*, *G. secunda*, *G. taipingensis*, *G. megaphylla*, *G. sericea*, *G. leucantha*, *G. decurvata* and *G. belumensis*. Attempts have been made to search for more populations of these endemic species, but as yet to no avail. Adding to this critical situation is the threat to their habitat. Except for *G. tahanensis*, *G. sericea* and *G. erectifrons* whose habitats are within a National Park, those of other species are rather exposed to hazards that entire populations could be wiped out in a short period of time. As an example, the population of *G. taipingensis* was no longer to be found on the second visit to a known locality near the type area. Conversely, however, other successful rediscoveries of the *Geostachys* have been made, such as that of *G. primulina* after 80 years since it was first collected (Lau, 2006 and C.K. Lim, pers. com.). A particular study of the hyper-endemics, *G. rupestris* and *G. penangensis*, at their type areas have shown that they are well able to survive under relatively protected

Table 1. Summary of important characters of species of *Geostachys*.

Species	Stilt roots	Leaf abaxial	Leaf adaxial	Leaf shape	Ligule	Inflorescence, flower	Labellum	Staminode	Anther crest	Fruit
<i>G. primulina</i>	long & reticulated	maroon	glaucous	broadly elliptic	broadly glabrous	decurved, single flowered	yellow, margin crumpled	absence	none	ovoid & brownish black
<i>G. erectifrons</i>	long & reticulated	maroon	green	lanceolate	glabrous	decurved, single flowered	yellow	not known	not known	ovoid & brownish red
<i>G. belumensis</i>	long & reticulated	maroon	green	lanceolate	glabrous	decurved, single flowered	yellow with red spots, margin crumpled	presence, with red bands	absence	ellipsoid & dark red
<i>G. tahanensis</i>	long & reticulated	green	green	broadly elliptic	glabrous	decurved, single flowered	yellow	not known	not known	ovoid & chilly red
<i>G. penangensis</i>	stilt roots	green	green	narrowly elliptic	glabrous	decurved, single flowered	yellow, margin smooth	absence	not known	ellipsoid & brownish black
<i>G. secunda</i>	long & reticulated	green	green	broadly elliptic	glabrous	decurved, single flowered	yellow, margin smooth	absence	absence	ellipsoid & dark red
<i>G. elegans</i>	stilt roots	green	green	narrowly elliptic	glabrous	erect, 1-2/cincinnus	yellow	not known	not known	ovoid & orange
<i>G. taipingensis</i>	stilt roots	green	green	oblong	glabrous	erect, single flowered	yellow with red spots, margin crumpled	absence	presence	ovoid & brownish black

<i>G. megaphylla</i>	stilt roots	green	green	oblong	hairy	erect, 1-3/cincinnus	white with pink spots & red spots, margin crumpled	absence	presence	ovoid & brownish black
<i>G. sericea</i>	stilt roots	green	green	oblong	hairy	erect, 1-2/cincinnus	yellow with red spots, minutely- hairy silky surface, margin smooth	presence, with red spots	presence	ovoid & light green
<i>G. montana</i>	not known	green	green	elliptic- oblong	hairy	erect- decurved, single flowered	white	presence	not known	ellipsoid & dark red
<i>G. leucantha</i>	stilt roots	green	green	oblong	glabrous	erect- decurved, 1-5/cincinnus	white with pink spots, margin crumpled	absence	presence	ovoid & dark brown
<i>G. rupestris</i>	stilt roots	green	green	broadly elliptic	glabrous	decurved, single flowered	yellow with red marks, margin smooth	absence	absence	ellipsoid & brownish red
<i>G. densiflora</i>	stilt roots	green	green	narrowly elliptic	glabrous	decurved, 1-2/cincinnus	yellow, margin crumpled	absence	absence	ovoid & dark purple
<i>G. decurvata</i>	stilt roots	green	green	narrowly elliptic	glabrous	decurved, single flowered	yellow, margin crumpled	absence	absence	ellipsoid & brownish red

situations. The two widespread species, *G. elegans* and *G. densiflora*, are relatively common and not threatened.

Conservation status of Geostachys

A threat assessment on the genus is being carried out based on the IUCN Red List Categories and Criteria Version 3.1 (IUCN, 2001) and Malaysia Plant Red List (Chua and Saw, 2006). The final result of the assessment is expected to be out together with the full revision of the *Geostachys* which is currently in preparation by Lau *et al.*

List of species of Geostachys in Peninsular Malaysia.

G. belumensis C.K. Lim & K.H. Lau

G. decurvata (Baker) Ridl.

G. densiflora Ridl.

G. elegans Ridl.

G. erectifrons K.H. Lau, C.K. Lim & K. Mat-Salleh

G. leucantha B.C. Stone

G. megaphylla Holttum

G. montana (Ridl.) Holttum

G. penangensis Ridl.

G. primulina Ridl.

G. rupestris Ridl.

G. secunda (Baker) Ridl.

G. sericea (Ridl.) Holttum

G. tahanensis Holttum

G. taipingensis Holttum

Acknowledgements

Thanks are due to Dato' Musa Nordin, the Director-General, Department of Wildlife and National Parks for assistance and encouragement for the trips to Gunung Tahan, and to his field guides, Baharim Selat, Tajuddin Mohd. Amin, and Juhari Basri; also to Imin Kamin of FRIM for field assistance. Part of the research program was conducted at Tropical Forest Biodiversity Centre (TFBC) in FRIM; the first author expresses his thanks to TFBC Director, Dr. Saw Leng Guan, and to his staff for their support. Further acknowledgements go to Dr. Lillian Chua and Hamidah Mamat, also from FRIM for their assistance in the Red List Threat Assessment of the genus. The second author acknowledges the contribution by his field team: Azizan Busu, Alus Sarip and Zaidee Hitam. The first author's research and fieldwork

were funded in part by IRPA Research Grant 09-02-02-EA0035. Further thanks are due to the herbarium curators at K, KEP, KLU, SING and UKMB for access to specimens, and also to the Penang Botanic Gardens.

References

- Baker, J.G. 1892. Scitamineae. In J.D. Hooker, Flora of British India, vol. 6. Reeve, London. 257pp.
- Chua, L.S.L. and L.G. Saw. 2006. Malaysia Plant Red List. Guide for Contributors. Forest Research Institute Malaysia, Malaysia.
- Gagnepain, M.F. 1906. Zingibéracées nouvelles de l'herbier du Muséum. *Bulletin de la Société Botanique de France* **53**: 132-147.
- Holttum, R.E. 1950. The Zingiberaceae of the Malay Peninsula. *Gardens' Bulletin, Singapore* **13** (1): 224-236.
- IUCN. 2001. *Red List Categories and Criteria*, Version 3.1. Gland, Switzerland.
- Larsen, K. 1962. Studies in Zingiberaceae 1. The genus *Geostachys* in Thailand. *Botanisk Tidsskrift* **58**: 43-49.
- Lau, K. H. 2004. Observations on the endemic *Geostachys* (Baker) Ridl. of Penang Hill and its environment. *Folia malaysiana*. **5**: 109-114.
- Lau, K.H., Lim, C.K. and K. Mat-Salleh. 2005. Two new species of *Geostachys* (Zingiberaceae) from Peninsular Malaysia. *Folia malaysiana* **6**: 83-94.
- Lau, K.H. 2006. A ginger lost and found. *Conservation Malaysia*, FRIM **2**: 3.
- Newman, M., Lhuillier, A & Poulsen, A, D, 2004. Checklist of the Zingiberaceae of Malesia. *Blumea Supplement* **16**. Nationaal Herbarium Nederland.
- Ridley, H.N. 1899. The Scitamineae of the Malay Peninsula. *Journal of the Straits Branch Royal Asiatic Society* **32**: 157-160.

- Ridley, H.N. 1909. The Flora of the Telom and Batang Padang Valleys. *Journal of the Federated Malay States Museums* **4(1)**: 1-78.
- Ridley, H.N. 1915. Botany of Gunong Tahan. *Journal of the Federated Malay States Museums* **6**: 1-185.
- Ridley, H.N. 1920. New and Rare Species of the Malayan Plants. *Journal of the Straits Branch Royal Asiatic Society* **82**: 1-201.
- Ridley, H.N. 1924. *The Flora of the Malay Peninsula*. Vol. 4. London: Reeve & Co. Ltd.
- Stone, B.C. 1980. A new *Geostachys* (Zingiberaceae) from Gunung Ulu Kali, Pahang, Malaysia. *Malaysian Journal of Science* **6(A)**: 75-81.
- Turner, I.M. 2000. The Plant Taxa of H.N. Ridley, 3. The Zingiberales. *Asian Journal of Tropical Biology* 4(1), 1-47.
- Valeton, T. 1921. *Geostachys sumatrana*. *Bulletin du Jardin Botanique de Buitenzorg Ser. III*, **3**: 146-147.

Materials Towards a Revision of *Aulotandra* Gagnep. (Zingiberaceae)

M.F. NEWMAN

Royal Botanic Garden, 20A Inverleith Row
Edinburgh EH3 5LR, Scotland, UK

Abstract

Aulotandra Gagnep. has recently been transferred from the subfamily Alpinioideae, tribe Alpinieae, to the subfamily Siphonochiloideae. Materials towards a revision of *Aulotandra* and *Siphonochilus* J.M. Wood & Franks are presented.

Introduction

Harris et al. (2006) have included *Aulotandra* Gagnep. in a phylogenetic analysis in order to determine its correct place in the new classification of Zingiberaceae by Kress *et al.* (2002).

Aulotandra was, until recently, the only African genus of Zingiberaceae which had not been included in a molecular systematic study. Two molecular datasets, chloroplast and nuclear, placed *Aulotandra* closest to *Siphonochilus* J.M. Wood & Franks, showing that genetic divergence levels were smaller between accessions of *Aulotandra* and *Siphonochilus* than between *Aulotandra* and any other taxon included in the analysis.

In addition, phylogenetic analyses of the two data matrices showed that the species of *Aulotandra* and *Siphonochilus* sampled in that study formed a monophyletic group. It was clear from the shared synapomorphies and the high branch support for the clade containing these genera that this relationship was very close. The two data sets showed some discrepancy as to the relationships between these two genera - the ITS analysis indicating that *Aulotandra* was monophyletic, but the *trnL*-F results suggesting that the two genera were paraphyletic.

Accepting that *Aulotandra* and *Siphonochilus* form a monophyletic group led to the transfer of *Aulotandra* from subfamily Alpinioideae, tribe Alpinieae, to subfamily Siphonochiloideae. Taking this study further, it is clear that the species in subfamily Siphonochiloideae must be revised together.

Materials and Methods

A list of the names in *Aulotandra*, *Siphonochilus* and *Kaempferia* L. in Africa was compiled using the International Plant Names Index (www.ipni.org) and the World Checklist of Monocotyledons (<http://www.kew.org/wcsp/home.do>). Protologues were consulted and details of the type of each name were added. Where possible, the collector, collection number and herbarium location are given but, in some cases, it is not clear from the literature where types are to be found.

Results

In total, there are 30 names to be revised in the Siphonochiloideae, eight in *Aulotandra*, 12 in *Siphonochilus*, and 10 in *Kaempferia*. The majority of names are based on type specimens held at the Muséum national d'Histoire naturelle, Paris. A few are yet to be located; those collected by German botanists may have been lost.

Names in *Aulotandra* Gagnep.

1. *Aulotandra angustifolia* H. Perrier, Bull. Soc. Bot. France 86: 178. 1939.
Type: *Perrier de la Bâthie* 7264 (P).
2. *Aulotandra humbertii* H. Perrier, Bull. Soc. Bot. France 86: 180. 1939.
Type: *Humbert s.n.* (P).
3. *Aulotandra kamerunensis* Loes., Bot. Jahrb. Syst. 43: 389. 1909.
Type: *Zenker* 3696 (US, WU, WRS�).
4. *Aulotandra madagascariensis* Gagnep., Bull. Soc. Bot. France 48: LXXIX. 1901.
Type: *Humblot* 448 (P).
5. *Aulotandra trialata* H. Perrier, Bull. Soc. Bot. France 86: 181. 1939.
Type: *Perrier de la Bâthie* 1021 (P).
- 6a. *Aulotandra trigonocarpa* H. Perrier var. *trigonocarpa*. Bull., Soc. Bot. France 86: 179. 1939.
Type: *Perrier de la Bâthie* 19014 (P).
- 6b. *Aulotandra trigonocarpa* H. Perrier var. *calcicola* H. Perrier, Bull. Soc. Bot. France 86: 180. 1939.
Type: *Perrier de la Bâthie* 1672, 1687 (P).
- 6c. *Aulotandra trigonocarpa* H. Perrier var. *gypsicola* H. Perrier, Bull. Soc. Bot. France 86: 180. 1939.
Type: *Perrier de la Bâthie* 15943 (P).

Names in *Siphonochilus* J.M. Wood & Franks

1. *Siphonochilus aethiopicus* (Schweinf.) B.L.Burt, Notes Roy. Bot. Gard. Edinburgh 40(2): 372. 1982.
Type: *Cienkowski s.n.*, *Steudner s.n.*
2. *Siphonochilus bambutiorum* A.D.Poulsen & Lock, Kew Bull. 54: 203. 1999.
Type: *Poulsen & Liengola 1146* (holo, C; iso BR, E, K, MO).
3. *Siphonochilus brachystemon* (K.Schum.) B.L.Burt, Notes Roy. Bot. Gard. Edinburgh 40(2): 372. 1982.
Type: *Volkens 201* (B), *Holst 3100* (B).
4. *Siphonochilus carsonii* (Baker) Lock, Kew Bull. 39: 841. 1984.
Type: *Carson s.n.* (K).
5. *Siphonochilus decorus* (Druten) Lock, Kew Bull. 54: 346. 1999.
Type: *Schweickert s.n.* (PRE).
6. *Siphonochilus evae* (Briq.) B.L.Burt, Notes Roy. Bot. Gard. Edinburgh 40(2): 372. 1982.
Type: *Prosch 12* (G).
7. *Siphonochilus kilimanensis* (Gagnep.) B.L.Burt, Notes Roy. Bot. Gard. Edinburgh 40(2): 372. 1982.
Type: *Le Testu s.n.* (P).
8. *Siphonochilus kirkii* (Hook.f.) B.L.Burt, Notes Roy. Bot. Gard. Edinburgh 40(2): 372. 1982.
Type: *Kirk s.n.* (K).
9. *Siphonochilus natalensis* (K.Schum.) J.M.Wood & Franks, Natal pl. 6(3): plates 560-561. 1911.
Type: *Wood 544* (K).
10. *Siphonochilus nigericus* (Hepper) B.L.Burt, Notes Roy. Bot. Gard. Edinburgh 40(2): 372. 1982.
Type: *Dalziel 276* (K).
11. *Siphonochilus parvus* Lock, Kew Bull. 46: 269. 1991.
Type: *Congdon 46* (K).
12. *Siphonochilus rhodesicus* (T.C.E.Fr.) Lock, Kew Bull. 39: 841. 1984.
Type: *Fries 1146* (UPS).

Names in *Kaempferia* L. in Africa

1. *Kaempferia aethiopica* (Schweinf.) Benth. var. *angustifolia* Ridl., J. Bot. 25: 131. 1887.
Type: *Welwitsch 683* (K).
2. *Kaempferia ceciliae* N.E.Br. Bull. Misc. Inform. 169. 1906.
Type: *Cecil 248* (K).
3. *Kaempferia dewevrei* De Wild. & T. Durand, Bull. Soc. Roy. Bot. Belgique

- 38: 142. 1899.
Type: *Dewèvre 1021*.
4. *Kaempferia ethelae* J.M. Wood, Gard. Chron. 23: 94. 1898.
Type: not known.
5. *Kaempferia homblei* De Wild., Repert. Spec. Nov. Regni Veg. 13: 195. 1914
Type: *Homblé 851, 907*.
6. *Kaempferia montagui* F.M. Leight., S. African Gard. 22: 57, 59. 1932.
Type: *Montagu 888/21 (K)*.
7. *Kaempferia pleiantha* K. Schum., Bot. Jahrb. Syst. 15(4): 425. 1892.
Type: *Buchner 694, Mechow 559b*.
8. *Kaempferia puncticulata* Gagnep., Bull. Soc. Bot. France 53: 353. 1906.
Type: *Kiener s.n. (P)*.
9. *Kaempferia stenopetala* K. Schum., Pflanzenr. IV 46 (Heft 20): 69. 1904.
Type: *Wood 1942*.
10. *Kaempferia zambesiaca* Gagnep., Bull. Soc. Bot. France 53: 355. 1906.
Type: *Le Testu 563 (P)*.

Recommendations

Preliminary morphological observations and the sequence results presented by Harris *et al.* (2006) suggest that there may be only one genus in subfamily Siphonochiloideae. In order to test this hypothesis, a molecular and morphological study with wider sampling should be carried out to determine the relationships between the species and assess the limits of these two genera. All names listed above should be revised so that the number of accepted species, their distributions and their conservation status may be confidently known.

Acknowledgements

I am grateful to my colleagues, David Harris, Michelle Hollingsworth, Michael Möller and Alexandra Clark, for allowing me to present their results and to build upon them.

References

- Harris, D.J., M.F. Newman, M.L. Hollingsworth, M. Möller and A. Clark, 2006. The phylogenetic position of *Aulotandra* Gagnep. (Zingiberaceae). *Nordic Journal of Botany* **23(6)**: 725-734.

Kress, W.J., L.M. Prince and K.J. Williams, 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. *American Journal of Botany* **89**: 1682-1696.

***Etlingera* Giseke of Java**

A.D. POULSEN

Royal Botanic Garden Edinburgh, 20A Inverleith Row
Edinburgh EH35LR, Scotland

Abstract

Nine species of *Etlingera* Giseke are known in Java, though two of them have not been collected recently. An identification key is given, along with descriptions, illustrations, and notes on local names, uses, and ecology. The conservation status of each species is assessed. Two species remain enigmatic and the remaining seven, including *E. parva*, which is synonymized with *E. brachychila*, are all found in Borneo, Sumatra and/or the Malay Peninsula.

Introduction

The genus *Etlingera* Giseke (Zingiberaceae) is an Indo-Pacific genus especially rich in species in the perhumid forests of Thailand, Malaysia, Indonesia and New Guinea. Many species are useful to man as food, condiment, medicine or as ornamentals. They also play an important role in the understorey as a food source for animals. The leafy shoots can be up to 8 m tall and often dominate gaps in disturbed forests.

In Java, *Etlingera* may be confused with other genera that have radical inflorescences: *Amomum* Roxb., *Hornstedtia* Retz. and *Zingiber* Mill. *Etlingera* is, however, distinguished from all these by having a staminal tube.

Blume (1827), described five ginger species from Java which are included in the present paper as *Etlingera coccinea* (Blume) S. Sakai & Nagam., *E. foetens* (Blume) R.M.Sm., *E. hemisphaerica* (Blume) R.M.Sm., *E. solaris* (Blume) R.M.Sm., an *E. walang* (Blume) R.M.Sm. At that time, *E. elatior* (Jack) R.M.Sm. had already been collected in Sumatra and described. Subsequently, *E. megalochelos* Griff. was described from Peninsular Malaysia (Griffith, 1851) and Valeton (1904) documented its presence in Java with useful information also on other species of *Etlingera* there. Valeton published further observations and clear and informative illustrations in 1906. In 1921, he described what is here included as *E. heyneana* (Valeton) R.M.Sm. and *E. parva* (Valeton) R.M.Sm. Since then no further species of

Etlingera has been described from Java and thus the revision by Bakhuizen f. (1958; 1968) includes a total of nine species. In the present treatment, seven of these at least are good species but two remain somewhat "mysterious".

Material and Methods

Measurements of plants follow Poulsen (2006). Only recent collections are cited in the present paper. As new flowering material will be collected allowing detailed measurements, a wider range can be expected for some of the character measurements included below. This is demonstrated by the variation exhibited by the most commonly collected *E. coccinea*, the species with most collections assessed in the present paper.

Assessments of conservation status were carried out following IUCN (2000), based on current knowledge and using their terminology on categories, criteria and subcriteria.

Key to species of *Etlingera* in Java

1. Ligule 4–8 cm long, deeply bilobed; fruit beaked 7. *E. solaris*
1. Ligule <3.5 cm, \pm entire; fruit rounded, flat-topped or with depressed apex 2
2. Inflorescence spike raised more than 10 cm above ground; peduncle extended above ground, \pm erect 3
2. Inflorescence spike at ground level or partly embedded in soil; peduncle subterranean 4
3. Leaves green beneath; inflorescence erect, 60–200 cm; bracts to 13 cm long, outer ones reflexed when flowering; receptacle extended to 10 cm 3. *E. elatior*
3. Leaves reddish beneath (especially when young); inflorescence erect, 15–100 cm; outer bracts forming a cup-shaped spike not recurved when flowering; receptacle <2 cm 5. *E. hemisphaerica*
4. Labellum short (< 15 mm), extended <5 mm beyond stigma; fruit with soft teeth 1. *E. brachychila*
4. Labellum long (> 35 mm), extended >7 mm beyond stigma; fruit smooth, ridged or roughly papillose 5
5. Labellum extended <32 mm beyond anther; filament <1 mm long 4. *E. foetens*
5. Labellum extended >34 mm beyond anther; filament >3 mm long 6

6. Petiole usually absent; dorsal corolla lobe hooded over the anther; labellum yellow with red margin; anther dehiscing from 1.5 mm above base to apex; fruit flat-topped with roughly papillose ridges 2. *E. coccinea*
6. Petiole 1–4 cm; dorsal corolla lobe not covering the anther; labellum red with yellow margin; anther dehiscing in upper half only; fruit top rounded, smooth or with a few warts 6. *E. megalochelos*

1. *Etlingera brachychila* (Ridl.) R.M. Sm.

Notes Roy. Bot. Gard. Edinburgh 43 (1986) 243; Smith, Notes Roy. Bot. Gard. Edinburgh 43 (1986) 452; Poulsen, Etlingera of Borneo (2006) 66. **Basionym:** *Hornstedtia brachychila* Ridl., J. Straits Branch Roy. Asiat. Soc. 46 (1906) 239. **Type:** Malaysia, Sarawak, Bau, July 1903, flowering, *H.N. Ridley s.n.* [lecto, K (designated by Turner (2000): 34)].

- *Etlingera parva* (Valeton) R.M. Sm., Notes Roy. Bot. Gard. Edinburgh 43 (1986) 248, **syn. nov.** **Basionym:** *Geanthus parvus* Valeton, Bull. Jard. Bot. Buitenzorg ser. 3, 3 (1921) 145. - *Amomum parvum* (Valeton) Bakhuizen f., Bekn. Fl. Java 18 (1958) 30; Bakhuizen f. in Backer, Fl. Java 3 (1968) 56. **Type:** Indonesia, Java, Cipatuja, Bivak Denu, 450 m, 20 Aug 1913, *C.A. Backer* 8887 (holo, BO).

Rhizome long-creeping (80 cm between neighbouring leafy shoots); stilt roots absent. **Leafy shoot** to 3 m, with up to c. 30 leaves; base to 3 cm in diameter, bright red. Sheath striate, margin membranous and glabrous. Ligule to 9.5 mm, entire, truncate to slightly emarginate, greenish, glabrous, margin membranous. Petiole 10–20 mm, hirsute especially adaxially and at base. **Lamina** oblong to narrowly obovate, to 55 x 9 cm, smooth, green, pale beneath, with 0.5 mm long white hairs with swollen base along midrib and near base above; glabrous beneath; average length to width ratio (4–)6(–8); base rounded to cuneate, \pm unequal; apex acuminate 1.5 cm. **Inflorescence** (including peduncle) to 17 cm, embedded in the soil, arising from base or along rhizome, with up to 29 flowers, 5 open at a time. Peduncle to 10 cm, subterranean, ascending, peduncular bracts to 2.5 x 1.8 cm, base with pale hairs, upper just overlapping the base of the spike. Spike 7 x 3 cm, cylindrical, flowers extended 5 cm above the bracts, length only including bracts: 2.5 cm. Sterile bracts 2, distichous, 2.5 x 1.2 cm, elliptic, \pm acute, brownish, pubescent at base. Fertile bracts 2.2–2.5 x 0.3–0.7 cm, narrowly spatulate, boat-shaped, apex rounded, pale brown, pubescent at very base only. Bracteole 2.1 cm, pale reddish, with two fissures of 0.5–1.5 cm, pubescent near base, apex bilobed with a few hairs. **Flower:** Calyx 4.5–6.1 cm, reaching base of filament shorter than corolla lobes, red, with one fissure of ca 3 cm, glabrous, apex \pm tridentate with 3 mucro <1 mm. Corolla tube 5.7 cm, red, glabrous outside and inside.

Lobes red, with a few hairs near apex; dorsal lobe 19–20 x 6–7 mm, almost reaching middle or apex of anther, elliptic, cucullate, margin inrolled, apex rounded; lateral lobes 20–21 x 5 mm, narrowly elliptic, cucullate, margin inrolled, apex rounded; insertion slightly oblique, diverging, 3 mm below dorsal lobe. Staminal tube 10 mm. Labellum broadly ovate, 3-lobed, 14 x 16 mm, red to dark orange, lateral lobes rigid, erect on either side of the stamen (pushing the dorsal lobe apart and exposing the stamen), margin recurved, especially in the distal part, yellow, central lobe rigid, entire, rounded, red or orange, extended 2.5 mm beyond anther. Stamen 11 mm; filament 4–4.5 mm, with a few hairs on the margin especially near base; anther 7.5 x 3.5–5.5 mm, erect, rectangular, red, slightly wider at apex, apex hairy, anther crest truncate; thecae dehiscent *ca* 3.5 mm in the middle (from 2.5 mm above base to *ca* 1.5 mm below apex), pubescent, especially below the slits. Style *ca* 6.5 cm, hairy dorsally near apex. Stigma 3.5–4 mm wide, transverse narrow-elliptic with scattered hairs, red; ostiole transverse 2 mm, facing downwards. Ovary 4 x 3.5 mm, densely sericeous; epigynous glands 4 mm, deeply lobed, each half irregularly lobed. **Infructescence:** head 6 cm, subglobular, *ca* 16 fruits per head; **fruit** 2.5 x 2.5 cm, pyriform with soft, spiny teeth up to 7 mm long, especially developed on the top, dark purple-brown, pubescent. **Seeds** rounded-angular up to 4 mm across. *Plate 1A.*

Local names and uses: None documented.

Etymology: The epithet refers to the short labellum.

Ecology and habitat: Primary lowland (250–450 m) forest.

Distribution: W Java and Borneo.

Conservation status: EN B1ab (iii). Deforestation seriously threatens the forest in the southern part of Java, including its type locality at Cipatuja. With only one recent collection from one location, despite intensive searching, it is potentially endangered in Java. Valetton noted as early as 1921(b), that this species is 'apparently very rare as are the primaeval forests, its habitat.'

Additional material examined: **Banten Province**, buffer zone of Ujung Kulon NP, Gunung Honje, 250 m, 17 Nov 1996, flowering and fruiting, *Funakoshi IU 19* (E, BO n.v.).

Notes: The collection by Funakoshi is to my knowledge the only one from Java since the type of *E. parva* was collected in 1913, and the first including the infructescence. The material matches the type of *E. brachychila* from

Sarawak very well, except the calyx and corolla tube being slightly longer and the lateral lobe of the labellum being yellow but these characters are still within the range of *E. brachychila* var. *vinosa* A.D. Poulsen — also from Borneo.

Etlingera brachychila is easily distinguished from other Javanese species with their inflorescence at ground level by its poorly developed involucre bracts, short labellum and its conspicuous soft-toothed fruit somewhat resembling an *Amomum*. It resembles two species so far only known from Borneo: *E. aurantia* A.D. Poulsen and *E. kenyalang* A.D. Poulsen & H. Chr. *Etlingera brachychila* is distinguished from the other two by the smaller and fewer sterile bracts which give less support to the spike. The apex of the calyx is less mucronate. Finally, *E. brachychila* differs in its flower colour and the orientation of the lateral lobes of the labellum (reflexed vs. erect or involute).

In Borneo a variety *E. brachychila* var. *vinosa* has a white-waxy sheath and often more or less bullate leaves which are burgundy beneath. In Java, this variation in leaf colour has not been documented and the leaves are only sparsely hairy. Future collections may demonstrate more pubescent leaves like those *E. brachychila* var. *brachychila* in Borneo.

2. *Etlingera coccinea* (Blume) S. Sakai & Nagam.

Edinburgh J. Bot. 60 (2003) 190; Poulsen, *Etlingera* of Borneo (2006) 88.

Basionym: *Elettaria coccinea* Blume, Enum. Pl. Javae (1827) 53; Hasskarl, Pl. Jav. Rar. (1848) 134; Burt & Smith, Notes Roy. Bot. Gard. Edinburgh 31 (1972) 212. - *Geanthus coccineus* Reinw., *nom. nud.*, Catalogus (1823) 29.

- *Alpinia coccinea* (Blume) D. Dietr., Syn. Pl. 1 (1839) 12. - *Cardamomum coccineum* (Blume) Kuntze, Revis. Gen. Pl. 2 (1891) 686. - *Amomum coccineum* Benth. [Gen. pl. 3 (1883) 644, *nom. inval.*] ex Schumann, Bot. Jahrb. Syst. 27 (1899) 305; Valeton, Bull. Inst. Bot. Buitenzorg 20 (1904) 44; Valeton, Icon. Bogor. 2 (1906) t. 156, t. 157 figs. 21–22; Bakhuizen f., Bekn. Fl. Java 18 (1958) 34; Bakhuizen f. in Backer, Fl. Java 3 (1968) 57; van Steenis, Mt. Flora of Java, (1972) pl. 57: 2. - *Achasma coccineum* (Blume) Valeton, Bull. Inst. Bot. Buitenzorg 20 (1904) 93; Heyne, Nuttige pl. Nederl. Indië 1 (1927) 489. **Type:** Probably Java, C.G.C. Reinwardt s.n., bar code L0193260, accession number 905339126, including only fertile material to the right of the leaf (lecto, L, designated by Sakai & Nagamasu, 2003).

- *Achasma macrocheilos* Griff., Not. Pl. Asiat. 3 (1851) 429, Ic. Pl. Asiat. 3 (1851) t. 357. - *Amomum macrocheilos* (Griff.) Baker, Fl. Brit. India 6 (1892) 235. - *Hornstedtia macrocheilos* (Griff.) Ridl., J. Straits Branch Roy. Asiat. Soc. 32 (1899) 147; Schumann, Pflanzenr. IV, 46 (1904) 198; Ridley, Matr. Fl.

Mal. Penin. 2 (1907) 39; Ridley, Fl. Mal. Penin. 4 (1924) 271. **Type:** Malaysia, Malacca, Ayer Punnus, *W. Griffith s.n.* (not seen, specimen probably lost).

- *Amomum gomphocheilos* Baker, Baker, Fl. Brit. India 6 (1892) 236. **Type:** Malaysia, Perak, *H. H. Kunstler* 1897 (holo, K).

- *Hornstedtia punicea* (Roxb.) K. Schum., Pflanzenr. IV, 46 (1904) 197, pro parte, not including type; *Etlingera punicea* auct. non (Roxb.) R.M. Sm.: Smith, Notes Roy. Bot. Gard Edinburgh 43 (1986) 443; Cowley in Coode et al., Checklist Pl. Brunei (1996) 419; Keng et al., Concise Fl. Singapore 2 (1998) 57; Khaw, Gard. Bull. Singapore 53 (2001) 229.

- *Hornstedtia winkleri* Ridl., Bot. Jahrb. Syst. 44 (1910) 530. **Type:** Indonesian Borneo, SE of Sungai Sibak in Gunung Benanpin, 12 Aug 1908, flowering, *Hubert J.P. Winkler* 3175 (holo, WRS�).

Rhizome long-creeping (70–100 cm between neighbouring leafy shoots), 2.5–4 cm diam., scales to 6 cm long, ensheathing. **Leafy shoot** 5–8 m, with up to to 32 leaves; base to 8–12 cm diam., green or reddish brown. Sheath green, yellowish green or brownish, striate, ± reticulate (especially when young), glabrous or slightly pubescent (especially on cross ribs), margin ciliate. Ligule 10–15 mm, entire, green to purplish brown, ± pubescent. **Lamina** sessile (rarely with a 1–2 cm, petiole-like, winged-attenuate base), oblong to narrowly obovate, to 130 x 23 cm, mid- or dark green, young leaf reddish brown beneath, glabrous (rarely pubescent beneath); average length to width ratio 4–6; base cuneate, sometimes irregularly winged; apex acuminate 1.5 cm. **Inflorescence** (including peduncle) to 47 cm, arising from the rhizome near base of leafy shoot, with 15–27 flowers, 4–14 open at a time. Peduncle 2–33 cm, subterranean, peduncular bracts cream to pale brown. Spike 8–10 x 3–5 cm, ovoid to cylindrical, flowers extending 2–4 cm above the bracts, length only including bracts: 6–8 cm. Sterile bracts: 4–5 forming a dense support, distichous (uppermost one sometimes in the middle), 4–6 x 1.5–3 cm, ± pale reddish brown or cream with ± reddish apex and pale brown margin, pubescent near base. Fertile bracts 4–7 x 0.7–1.5 cm, spatulate, membranous, cream, pale red or brown especially at apex, pubescent especially near base and apex. Bracteole 3.8–5 cm, pale red at least at apex, with two fissures of 6–15 mm, ± pubescent, apex 2-toothed, ciliate. **Flower:** calyx 7–8.3 cm, reaching base of anther and shorter than corolla lobes, pale pink with darker apex, with one fissure of 2.5–4 cm, glabrous, apex 3-toothed. Corolla tube 4.9–6.8 cm, cream, glabrous outside, tube inside with an irregular band c. 1 cm 2 cm below labellum; lobes reddish pink, glabrous, reaching beyond anther; dorsal lobe 21–31 x 9–11 mm, elliptic,

apex rounded, hooded over the anther; lateral lobes 21–25 x 4–7 mm, elliptic, apex rounded, insertion oblique and converging; staminal tube 4–12 mm. Labellum 3-lobed, 50–65 x 21–25 mm, red with yellow in centre, glabrous, lateral lobes folded over stamen, pale to dark red at margin, margin finely plicate, central lobe c. 30 x 16 mm, spatulate, emarginate to 15 mm, rarely entire, dark red extended 40–50 mm beyond anther. Stamen 12 mm long: filament 3–7 x 3.5–5 mm (widest at base), white to pale red; anther 9–10 x 2.5–5.5 mm (widest at apex), emarginate to 2.5 mm, angled 120°, pink; thecae dehiscing from 1.5 mm above base to apex, glabrous. Style 8–8.5 cm, sparsely pubescent, flexistylous. Stigma 2.5 mm wide, white or pale pink, triangular to heart-shaped, ± hairy, ostiole apical, transverse. Ovary 5 mm long, densely pubescent; epigynous glands 5–7 mm long, bipartite, linear. **Infructescence:** head to 12 cm, globose, bracts persistent, 5–15 fruits per head, **fruit** 4.5 x 3.5 cm, pyriform, flat-topped with irregular radiating roughly papillose ridges up to 6 mm high, brownish (red when young), pubescent. **Seeds** 2–3 mm across. *Plate 1B.*

Local names and uses: Blume (1827) listed *tepus*, *tepus gede*, and *mancirian* (Sundanese). Valeton (1904) mentioned that *tepus bener* (genuine) and *tepus leuweung* (forest or wood) as names for subspecies with an entire or emarginate apex to the labellum, respectively. Heyne (1927) also mentioned *tepus bener* but specified that *mancirian* is the name of the flower, and *rongod* refers to the fruit.

The young leafy shoot and the fruits are edible (*Poulsen et al.* 2282). Shoot tastes cabbage-like when young; bitter when old. Even though it is often considered useful I never came across it being planted and cultivated in Java.

Etymology: *Coccinea* means scarlet.

Ecology and habitat: Old field edges, in traditional home gardens (but not planted), secondary forests, or in gaps or near streams of primary forests at 40–1650 m. Valeton (1904) noted that the seeds are frequently dispersed by animals that leave behind a big hole in the top of the empty fruit.

In Sumatra and Borneo, *E. coccinea* is pollinated by bees or spiderhunters (Kato et al., 1993; Sakai et al., 1999). Fruit eaten by rodents.

Distribution: Thailand, Peninsular Malaysia, Singapore, Sumatra, Java and Borneo.

Conservation status: LC (least concern), because of its wide distribution and persistence in very disturbed habitats.

Additional material examined: **Banten Province:** bufferzone of Ujung Kulon NP, Gunung Cibiug, 3 km E of Tamanraya village (6°47'S 105°32'E), 130 m, 27 Apr 2005, flowering and fruiting, *Poulsen et al.* 2343 (AAU, BO, E). **West Java Province:** Gunung Salak (6°40'S 106°44'E), 950 m, 28 Sep 2003, flowering, *Poulsen et al.* 2233 (BO); Halimun NP, Citalahab (6°44'S 106°32'E), 1100 m, 20 Mar 2004, flowering and fruiting, *Poulsen et al.* 2282 (AAU, BO, E); Gunung Kancana, 2 km WSW of Parabuan village (6°55'S 107°03'E), 950 m, 27 Mar 2004, flowering, *Poulsen et al.* 2294 (AAU, BO); Cisarakan (7°05'S 106°35'E), 500 m, 10 Aug 2006, flowering, *Poulsen et al.* 2457 (BO); Cikaso (7°22'S 106°37'E), 40 m, 11 Aug 2006, flowering, *Poulsen et al.* 2458 (BO, E); Gunung Cikuray (7°19'S 107°54'E), 1250 m, 13 Aug 2006, flowering, *Poulsen et al.* 2463 (BO, E).

Notes: In Java, *E. coccinea* can most easily be confused with *E. megalochilos*. *Etlingera coccinea* differs in its (most often) sessile leaves, absence of tufts on the apex of the calyx, the broad, pink dorsal corolla lobe hooded over the stamen, the long-elongate labellum with a broad yellow centre and red margin and thecae dehiscing almost to base. The margins are inrolled and like a tube so that the red at first glance appears to be in the centre of the labellum (like in *E. megalochilos*).

Most often the leaf of *E. coccinea* is glabrous but not always. During my recent survey, one plant was collected in which the lamina was pubescent beneath (*Poulsen et al.* 2457) and *E. megalochilos* also varies regarding the indumentum beneath the lamina. Thus as demonstrated in the Bornean revision of *Etlingera* (Poulsen, 2006) indumentum is not a completely reliable character.

Schumann (1904) regarded *E. coccinea* as a synonym of *E. punicea*, and therefore the latter name was used by Smith (1986) and others. This misconception was finally sorted out by Sakai & Nagamasu (2003).

Valeton (1904) described beautifully how the flowers in one inflorescence opened, in up to three circles of up to 15 flowers, each lasting only one day; he obviously took great care in making precise observations of live plants of *Etlingera* in Java. Valeton (1904) further mentioned that the 'subspecies' (cf. etymology above) with an entire apex to the labellum is much more common than the emarginate one, whereas I found the opposite to be the case. In any case, I do not see any reason to formally recognize this variation in two subspecies.

The local name in Java, *tepus* (Sundanese), is also used in Borneo by many tribes (Poulsen, 2006). Often peoples in Borneo use a generic name for all gingers that seems to be derived from that. The giant form that I collected in several places in Borneo is most similar to wild plants in Java,

such as those on Gunung Salak. All these lack a strong smell. Many local names in Borneo include an epithet that refers to the distinct smell. Some forms may have been selected over centuries and taken into cultivation. These plants are often smaller, but I did not find any floral measurements justifying the recognition of the smaller and smellier plants even at a lower taxonomic rank.

In this context it is interesting that the enigmatic *Etlingera walang* (Blume) R.M. Sm. is considered a very smelly plant. New collections in Java of a smelly *Etlingera coccinea* may provide the necessary evidence for establishing this synonymy.

3. *Etlingera elatior* (Jack) R.M. Sm.

Notes Roy. Bot. Gard. Edinburgh 43 (1986) 244; Smith, Notes Roy. Bot. Gard. Edinburgh 43 (1986) 442; Lim, Folia Malaysiana 1 (2000) 4; Lim, Folia Malaysiana 2 (2001) 168; Khaw, Gard. Bull. Singapore 53 (2001) 206; Sakai & Nagamasu, Edinburgh J. Bot. 60 (2003) 192452; Poulsen, Etlingera of Borneo (2006) 111. **Basionym:** *Alpinia elatior* Jack, Descriptions of Malayan plants 2 (7) (1822) 2; Miquel, Fl. Ned. Ind., 1. Suppl. (1861) 273. - *Nicolaia elatior* (Jack) Horan., Prodr. Monogr. Scitam. (1862) 32; Burtt & Smith, Notes Roy. Bot. Gard. Edinburgh 31 (1972) 210, fig. 17B. **Type:** Nias Island or Ayer Bangy, W coast of Sumatra, W. Jack s.n. 1818 (specimen lost). *Bunga kenchong* (Malay).

- *Geanthus speciosus* Reinw., Catalogus (1823) 29, *nom. nud.*

- *Diracodes javanica* Blume, Enum. Pl. Javae (1827) 55. - *Alpinia javanica* (Blume) D. Dietr., Syn. Pl. 1 (1839) 13. - *Alpinia acrostachya* Steud., Nomencl. Bot. 2 (1840) 62. - *Alpinia diracodes* Loes., Nat. Pflanzenfam. ed. 2, 15, A (1930) 614. **Type:** Java, Hariang, C.G.C. Reinwardt s.n., bar code L0041105 (holo, L).

- *Elettaria speciosa* Blume, Enum. Pl. Javae (1827) 51; Hasskarl, Pl. Jav. Rar. (1848) 133; Valeton, Bull. Inst. Bot. Buitenzorg 20 (1904) 29.

- *Alpinia speciosa* (Blume) D. Dietr., Syn. Pl. 1 (1839) 13. - *Nicolaia speciosa* (Blume) Horan., Prodr. Monogr. Scitam. (1862) 32; Valeton, Bull. Jard. Bot. Buitenzorg ser. 3, 3 (1921) 138; Heyne, Nuttige pl. Nederl. Indië 1 (1927) 488; Bakhuizen f., Bekn. Fl. Java 18 (1958) 47; Bakhuizen f. in Backer, Fl. Java 3 (1968) 64. - *Phaeomeria speciosa* (Blume) Koord., Exkurs.-Fl. Java 1 (1911) 332; Merrill, Enum. Phil. Pl. 1 (1925) 241; Holttum, Gard. Bull. Singapore 13 (1950) 181. **Type:** Java, in forest near Kapang Dungan, H. Kuhl & J.C. van Hasselt s.n., bar code L0193778 (lecto, L, designated by Sakai & Nagamasu, 2003).

- *Alpinia magnifica* Roscoe, Monandr. Pl. Scitam. (1828) t. 75; Hooker, Bot. Mag. 59 (1832) t. 3192. - *Bojeria magnifica* Raf., Fl. Tellur. (1836) 50, *nom. inval.* - *Phaeomeria magnifica* Lindl., Intr. Nat. Syst. Bot., ed. 2 (1836) 446, *nom. inval.* - *Nicolaia imperialis* Horan., Prodr. Monogr. Scitam. (1862) 32, t. 1, *nom. inval.* - *Amomum magnificum* (Roscoe) Benth. [Gen. Pl. 3 (1883) 644, *nom. inval.*] ex Schumann, Bot. Jahrb. Syst. 27(1899) 307; Valetton in Merrill, Interp. Herb. Amboin. (1917) 159. - *Cardamomum magnificum* (Roscoe) Kuntze, Revis. Gen. Pl. 2 (1891) 687. - *Hornstedtia imperialis* (Horan.) Ridl., J. Straits Branch Roy. Asiat. Soc. 32 (1899) 148. - *Nicolaia magnifica* (Roscoe) K. Schum. ex Valetton, Bull. Inst. Bot. Buitenzorg 20 (1904) 35. - *Phaeomeria magnifica* (Roscoe) K. Schum., Pflanzenr. IV, 46 (1904) 262; Loesener, Nat. Pflanzenfam. ed. 2, 15A (1930) 594. - *Phaeomeria imperialis* Lindl. ex Ridl., Fl. Malay Penins. 4 (1924) 272; Elmer, Leaf. Phil. Bot. 8 (1915) 2907. **Type:** Originally from Mauritius, 1825, *C. Telfair s.n.*, cultivated (holo, K).

- *Nicolaia intermedia* Valetton, Bull. Jard. Bot. Buitenzorg ser. 3, 3 (1921) 133. **Type:** Possibly from Java, *Anon. s.n. (HB XI B IV 101)*, cultivated in Bogor (holo, BO).

Rhizome short-creeping. **Leafy shoot** to 6 m, several together in a loose clump; base to 6 cm in diameter, sheath pubescent at the very base, ± reddish. Sheath green, striate when dry, glabrous, pruinose when young. Ligule to 15 mm, entire, obtuse, green, glabrous, margin ciliate. Petiole to 25 mm. Lamina oblong, to 80 x 20 cm, yellowish or mid-green, pale green beneath; average length to width ratio 3.5–6.5; base truncate, ± unequal; apex acuminate to *c.* 1 cm. **Inflorescence** (including peduncle) to 2 m, erect from base of leafy shoot, receptacle elongate to 10 cm in old inflorescences, with up to at least 320 flowers, 10–20 open at a time. Peduncle 0.6–2 m, peduncular bracts to 22 x 2.5 cm (upper, which play the same role as the lower sterile bracts), distichous, green to yellowish green, pubescent near base. Spike to 15 x 15 cm, ovoid, flowers not extended above bracts. Sterile bracts numerous, spirally arranged, lower to 13 x 4.5 cm, lowest one biggest, distinctly reflexed, dark or pale pink with pale (sometimes almost white) margin; turning brown with age, pubescent near base. Fertile bracts 2.5–7 x 0.7–2.5 cm, oblong, concave, apex obtuse, sometimes inrolled, red to pale pink with a white margin, glabrous, apex with ciliate margin. Bracteole 2.2–3.1 cm, membranous, transparent, pale pink to red at apex, with two fissures of 0.3–1.5 cm, glabrous, apex 2-toothed, tufted. **Flower:** Calyx 3–3.5 cm, reaching at least base of anther, pink, fissured 2 cm, glabrous; apex irregularly 3-toothed, tufted. Corolla tube 2.7–2.8 cm, dark red at apex, sericeous ventrally below lobes, tube inside with two elongate, densely

V-shaped hairy cushions 5 mm wide, c. 1 cm below labellum and coinciding \pm with attachment of the lobes on the outside, pubescent dorsally towards filament. Lobes dark pink, glabrous with ciliate apex; dorsal lobe 21–23 x 5.5–6 mm, reaching slightly beyond stigma, narrowly elliptic to spatulate, cucullate, apex obtuse, tufted; lateral lobes 22–24 x 3.5–4 mm, narrowly elliptic to spatulate, cucullate, apex obtuse, slightly emarginate, with ciliate tuft; attached straight, 2 mm below dorsal lobe. Staminal tube 10–12 mm; labellum \pm entire, 20 x 18 mm, red with yellow margin, roughly papillose in centre extending into the corolla tube, lateral lobes \pm erect, meeting above stamen, margin slightly recurved, central lobe slightly emarginate, extended c. 10 mm beyond anther, margins slightly recurved. Stamen 10–11 mm long; filament 2.5–3 x 2.5 mm, white, papillose inside; anther 8–9 x 3.5 mm, \pm oblong, slightly wider at apex, erect, white to pale red, anther crest short; thecae dehiscent in upper 1/2–2/3 almost to apex, pubescent, base with long tuft. Style 3.7–3.8 cm, with scattered long hairs. Stigma 3 mm wide, dark red, club-shaped; ostiole round, facing downwards. Ovary 4–5.5 x 5 mm, sericeous; epigynous gland 4 mm, slightly bilobed, roughly papillose. **Infructescence** remaining erect, head 10–12 (or longer) x 5–9 cm, the lower bracts rot and are not conspicuous, 67–100 fruits per head; **fruit** 2.5–3.5 x 1.5–2.5 cm, pyriform, angled, top rounded, not beak-like, calyx \pm persistent, to 3 mm, reddish, pink, pale orange-red or maroon, pubescent. **Seeds** to 4 mm across, rounded-angular. *Plate 1C*.

Local names and uses: Blume (1827) listed the following names *konje*, *hunje*, *hunje-reuma* (Sundanese). Heyne (1927) mentioned that *rombe* refers to the young inflorescence and *combrang* (*tjombrang* orth. var.) to the flowers (also Sundanese) and in Javanese or *kecombrang*, *cumbrang*, *combrang*.

Hondje hedjo (Sundanese; *hedjo* means green referring to the colour of the leaves as opposed to *E. hemisphaerica* that has reddish leaves beneath; Poulsen *et al.* 2293).

Edible leafy shoots, flowers, and fruits. Often sold at the market.

Etymology: The epithet refers to the raised inflorescence.

Ecology and habitat: It is difficult to say with certainty if *Etlingera elatior* is native to Java as it has been cultivated there for a very long time. Bakhuizen f. (1968) listed its occurrence in primary and secondary forests to 1200 m, though I am yet to encounter it in natural vegetation in Java.

Distribution: Widespread in the tropics (Poulsen, 2006).

Conservation status: LC (Least Concern). Not threatened.

Additional material examined: West Java Province, Gunung Kancana, 800 m, W of Parabuan village (6°54'S 107°03'E), 800 m, 26 Mar 2004, flowering, Poulsen *et al.* 2293 (AAU, BO).

Notes: *Etlingera elatior* is most similar to *E. hemisphaerica* but the most striking differences between the two is the the longer and reflexed lower bracts in the involucre of *E. elatior*. Furthermore, the inflorescence is usually more than 1 m (vs. <1 m), the receptacle extends to 10 cm (vs. <2 cm), and the fruit is reddish (not green).

The description by Bakhuizen f. (1968) mentions that the leaves can be purple beneath which could resemble those of *E. hemisphaerica*. This I have only observed in Java in cultivated plants. The colour of the bracts of *E. elatior* can vary from deep blood-red to white (Poulsen, 2006). In Java, some of the inflorescences sold in the market are deep red whereas the more typical ones are pink.

In rare cases, such as the type of *Diracodes javanica* Blume, the inflorescence in *E. elatior* appears terminally on a leafy shoot.

4. *Etlingera foetens* (Blume) R.M. Sm.

Etlingera foetens (Blume) R.M. Sm., Notes Roy. Bot. Gard. Edinburgh 43 (1986) 245; Poulsen, *Etlingera* of Borneo (2006) 132. **Basionym:** *Elettaria foetens* Blume, Enum. Pl. Javae (1827) 52. *Alpinia foetens* (Blume) D. Dietr., Syn. Pl. 1 (1839) 12. *Cardamomum foetens* (Blume) Kuntze, Revis. Gen. Pl. 2 (1891) 686. *Amomum foetens* (Blume) Benth. [Gen. Pl. 3 (1883) 644, *nom. inval.*] ex Schumann, Bot. Jahrb. Syst. 27 (1899) 322; Valeton, Bull. Inst. Bot. Buitenzorg 20 (1904) 44; Valeton, Icon. Bogor. 2 (1906) t. 157 figs. 16–17, 23, t. 154 fig. 18, t. 162 figs. 10–15; Bakhuizen f., Bekn. Fl. Java 18 (1958) 34; Bakhuizen f. in Backer, Fl. Java 3 (1968) 58. *Achasma foetens* (Blume) Valeton, Bull. Inst. Bot. Buitenzorg 20 (1904) 93; Heyne, Nuttige pl. Nederl. Indië 1 (1927) 489. *Hornstedtia foetens* (Blume) K. Schum., Pflanzenr. IV, 46 (1904) 200. *Geanthus foetens* (Blume) Valeton ex A.W.K. de Jong, Jaarb. Dep. Landb. Nijverh. Handel Ned.-Indië 1917 (1919) 78. **Type:** *C.L. von Blume s.n.*, bar code L0193175 (holo L).

- *Etlingera triorgyalis* auct. non (Baker) R.M. Sm.; *Etlingera triorgyalis* vel sp. aff.: Smith, Notes Roy. Bot. Gard. Edinburgh 43 (1986) 443; Sakai & Nagamasu, Edb. J. Bot 60 (2003) 212.

Rhizome long-creeping (up to 80 cm between shoots), stout, c. 2 cm in diameter, scales large (to 9 cm), cream, pale brown to reddish. **Leafy shoot** 2–5 m, leafless c. 1 m, with up to 22 leaves; base to 7.5 cm in diameter,

reticulate with distinct and tufted cross bars, green or greenish brown. Sheath mid- to yellow-green, roughly reticulate, \pm pubescent, margin ciliate. Ligule 15–21 mm, entire, green, pubescent, margin densely tufted ciliate at apex. Petiole to 25 mm, pubescent. Lamina narrowly obovate, to 83 x 14 cm, slightly plicate, green, glabrous above (rarely scabrid in centre near base), pubescent beneath; average length to width ratio 4.5–6; base cuneate, \pm unequal; apex acuminate to 2 cm. **Inflorescence** (including peduncle) (10–)18–25 cm, with 18–24 flowers, 2–7 open at a time. Peduncle (3–)9–13 cm, subterranean, peduncular bracts to 5 x 5 cm. Spike 6–9 x 3–5 cm, ovate-cylindrical, flowers extended 0–1 cm above the bracts, length only including bracts: 8–9 cm. Sterile bracts 5–7, distichous, lower 4.5–5.5 x 4.5–5.5 cm, upper to 8.5 x 2.3–3.5 cm, ovate to broadly spatulate (upper), rigid, mucronate, red, pubescent in lower third. Fertile bracts 6.5–8 x 0.7–1.5 cm, spatulate, membranous, translucent white with pinkish apex, densely pubescent. Bracteole 5.4–6.5 cm, whitish with pink apex, with two fissures of c. 1 and c. 1.5 cm, densely pubescent; apex bifid both lobes emarginate, aristate 3 mm, densely hairy. **Flower**. Calyx 7–7.5 cm, reaching anther, \pm as long as corolla lobes or slightly shorter, membranous, fissured 3–3.5 cm, densely pubescent; apex irregularly 3-fid, aristate. Corolla tube 5.8–6.7 cm, whitish with pink apex, glabrous or with scattered hairs, inside with 5 mm band of scattered hairs c. 10 mm below labellum. Lobes pale red with bright red apices, with a few hairs; dorsal lobe 20–22 x 6–7 mm, reaching middle or apex of anther, spatulate, cucullate, margin constricted; lateral lobes 18–22 x 5–6 mm, spatulate, \pm cucullate; attached obliquely, 0–2 mm higher or at same level as dorsal lobe. Staminal tube 11–12 mm; labellum 3-lobed, 38–43 x 17 mm, deep red with darker red and roughly papillose centre, glabrous, lateral lobes erect, margins thin, involute over stamen, central lobe broadly spatulate, 14–18 mm wide, \pm emarginate, apex extended 27–31 mm beyond anther; anther subsessile, 10–11.5 x 2.5–5 mm, widest at apex, \pm erect, pale pink or red, darker at crest; thecae dehiscing in upper 60%, few hairs at the base, especially dorsally. Style 6.8–7.3 cm, hairy dorsally in upper part. Stigma 4 mm, rounded-triangularly to pentangular with a rounded back, pale red or red; ostiole transverse, 2 mm, facing downwards. Ovary 5–6 mm, densely pubescent; epigynous glands 6.5 mm, bipartite, apices tooth-shaped. **Infructescence** half embedded in the soil, head 4.5 x 3–8 cm, subglobose, bracts shredding, to ca 22 fruits per head; **fruit** 2.2–3 x 2.5 cm, subglobular, angled, with fine, papillose ridges, pink, densely pubescent. **Seeds** to 3 mm across, rounded. *Plate 1D*.

Local names and uses: *Tepus sigung* (Sundanese). This name was recorded by Blume (1827) and confirmed when *Poulsen et al.* 2296 was collected in 2004. According to Valetton (1904), *sigung* (or *sigeung*) is the local name for the

Javan Skunk Marten (possibly *Mydaeus javanensis*) which is infamous for its revolting smell, resembling asafoetida and fennel. The fruits are eaten.

Etymology: The epithet means smelly.

Ecology and habitat: Lowland primary or disturbed forests at banks of rivers or streams to 950 m.

Distribution: Borneo, Java, Sumatra and possibly Peninsular Malaysia and Thailand.

Conservation status: EN B1ab(iii). Deforestation seriously threatens the forest in Java – especially in the lowlands. Already Bakhuizen f. (1968) noted that *E. foetens* was rare and, during my surveys from 2003–2006, I only found one sterile plant.

Additional material examined: **West Java Province**, Gunung Kancana, 2 km WSW of Parabuan village (6°55'S 107°03'E), 950 m, 27 Mar 2004, sterile, Poulsen *et al.* 2296 (AAU, BO).

Notes: *Etlingera foetens* is easily recognized by its deeply reticulate and broad leaf bases, plain red flowers where the dorsal lobe of the corolla does not cover the anther, the elongate and broad labellum and the strong smell when crushed.

I have not seen flowering plants of *E. foetens* in Java and thus the description of floral characters are based on Bornean measurements, which, however, match nicely those in the Flora of Java (Bakhuizen f., 1968). In Borneo, all flowers observed so far are uniformly red whereas in Sumatra the lateral margins of the labellum are occasionally slightly yellow. Thus it would not be surprising if flowers in Java showed similar variation.

As discussed in detail by Poulsen (2006), *E. triorgyalis* (Baker) R.M. Sm. is similar to *E. foetens*. It is sometimes a taller plant (8 m vs. 5 m) and has larger sterile bracts (with recurved apices making the inflorescence cyathiform), and a greater number of flowers per inflorescence. In addition, several floral measurements (calyx, corolla, and width of apical lobe of the labellum) are larger (Khaw, 2001). These characters seem to separate the material from Peninsular Malaysia (including the type of *E. triorgyalis* from Perak) from that of Java, Borneo and Sumatra.

5. *Etlingera hemisphaerica* (Blume) R.M. Sm.

Etlingera hemisphaerica (Blume) R.M. Sm., Notes Roy. Bot. Gard. Edinburgh 43 (1986) 245. **Basionym**: *Elettaria hemisphaerica* Blume, Enum. pl. Javae (1827) 51. *Alpinia hemisphaerica* (Blume) D. Dietr., Syn. pl. 1 (1839) 13.

Nicolaia hemisphaerica (Blume) Horan., Prodr. Monogr. Scitam. (1862) 32; Bakhuizen f., Bekn. Fl. Java 18 (1958) 46; Bakhuizen f. in Backer, Fl. Java 3 (1968) 63. *Cardamomum hemisphaericum* (Blume) Kuntze, Revis. gen. pl. 2 (1891) 686. *Amomum hemisphaericum* (Blume) K. Schum., Bot. Jahrb. Syst. 27 (1899) 307. *Phaeomeria hemisphaerica* (Blume) K. Schum., Pflanzenr. IV, 46 (1904) 263. **Type:** C.L. von Blume s.n. (holo, L), Java.

- *Elettaria anthodioides* Teijsm. & Binn., Ned. Kruidk. Arch. 3 (1855) 392. *Amomum anthodioides* (Teijsm. & Binn.) Koord., Meded. Lands Plantentuin 19 (1898) 318. *Phaeomeria anthodioides* (Teijsm. & Binn.) Koord., Exkurs.-Fl. Java 1 (1911) 332. *Nicolaia anthodioides* (Teijsm. & Binn.) Valeton, Bull. Jard. Bot. Buitenzorg ser. 3, 3 (1921) 128. **Type:** J.E. Teijsmann s.n. (iso, P).

- *Elettaria atropurpurea* Teijsm. & Binn., Natuurk. Tijdschr. Ned.-Indië 24 (1862) 327. *Phaeomeria atropurpurea* (Teijsm. & Binn.) K. Schum., Pflanzenr. IV, 46 (1904) 266. *Nicolaia atropurpurea* (Teijsm. & Binn.) Valeton, Bull. Jard. Bot. Buitenzorg ser. 3, 3 (1921) 128; Heyne, Nuttige pl. Nederl. Indië 1 (1927) 487. **Type:** J.E. Teijsmann s.n.

- *Nicolaia sanguinea* Valeton, Bull. Inst. Bot. Buitenzorg 20 (1904) 36. *Phaeomeria sanguinea* (Valeton) Koord., Exkurs.-Fl. Java 1 (1911) 332. **Type:** Anon. s.n. (BO).

- *Nicolaia rostrata* Valeton, Bull. Jard. Bot. Buitenzorg ser. 3, 3 (1921) 134. *Phaeomeria rostrata* (Valeton) Loes., Nat. Pflanzenfam. ed. 2, 15,A (1930) 594. **Type:** H.A.B. Bünnemeyer 809 (holo BO), Gunung Talamau ("Ophir")

- *Nicolaia rostrata* var. *talangensis* Valeton, Bull. Jard. Bot. Buitenzorg ser. 3, 3 (1921) 135. **Type:** H.A.B. Bünnemeyer 5298, 5299, 5431 (syn, BO), West Sumatra, Gunung Talang.

Rhizome in clump. Leafy shoot 3–6 m; base to 6–8 cm diam., bright red. Sheath green or yellow-green with reddish blotches, red when young, glabrous. Ligule 12–13 mm, slightly emarginate, green. Petiole to 25 mm. Lamina narrowly elliptic, to 80 x 15 cm, dark green, with pale green midrib above; reddish or brownish beneath, margin undulating; average length to width ratio 4.8–5.4; base cuneate to ± auriculate. **Inflorescence** (including peduncle) 18–81(–120) cm, erect, receptacle 12–15 mm, with 38–49 flowers, 1–4 open at a time. Peduncle to c. 1 m, peduncular bracts not completely covering the green axis, uppermost enclosing spike, pale yellow-green. Spike 6–7 x 3–6 cm, cup-shaped, flowers only extending 5 mm above the bracts in very mature inflorescences. Sterile bracts: 5, to 6 x 3.5 cm, ovate-elliptic, pale

pink tinged green at base or darker red especially towards apex and with a pale margin, glabrous. Fertile bracts 3–6 x 0.9–3.5 cm, cucullate, tinged red, short-lived, glabrous (pubescent at the very base only). Bracteole to 2.5 cm, cream tinged red, with 2 fissures of 0.5–1.5 cm, apex bifid. **Flower:** calyx c. 4 cm, reaching beyond apex of anther and shorter than corolla lobes, red with yellow-green apex, fissured 2 cm, pubescent near base, apex 3-toothed, teeth close together. Corolla tube 2.5 cm, white, glabrous, tube inside densely hairy from point of attachment of dorsal lobe on the outside to base of anther and with distinct hairy cushions at point corresponding to lateral lobe attachment. Lobes reaching beyond stigma, dark burgundy red with white margin and apex; dorsal lobe 25 x 7 mm, cucullate, distinctly rigid mucronate; lateral lobes 22 x 6 mm, attached oblique, converging, 0–3 mm above dorsal lobe. Staminal tube 12 mm. Labellum narrowly ovoid, 17 x 15 mm, dark red, yellowish white in centre, margin yellowish white, central lobe extended 7.5 mm beyond anther. Stamen 11 mm long; filament 1.5 x 2 mm, cream; anther 9.5 x 3 mm, red; thecae dehiscent in upper half, margin hairy. Style 3.5 cm, with scattered hairs. Stigma 2.5 mm wide, purple, ostiole transverse elliptic, facing downwards. Ovary 3 x 3 mm; epigynous glands 3.5 mm, deeply bilobed, papillose. **Infructescence** to 12 x 10 cm, with 2–20 fruits per head, bracts not persistent; **fruit** yellowish green, pubescent, apex truncate to slightly depressed. Mature infructescence with **seeds** not seen in Java. *Plate 1E.*

Local names and uses: *Hondje burem* (Sundanese; referring to the leaves being red beneath; Poulsen *et al.* 2295). *Hunje leuweung* (Sundanese; Blume, 1827 — according to Valetton (1904) *leuweung* means wood; he considered *E. hemisphaerica* the wild origin of *E. elatior*). Heyne (1927) added the names *hondje hedjo* and *hondje laka* (based on what he called *Nicolaia atropurpurea*); these local names are also given for *E. elatior* and *E. solaris*, respectively.

Leafy shoot and fruit edible. According to Bakhuizen f. (1968), cultivated locally.

Etymology: The epithet means hemispherical, probably referring to the cup-shaped inflorescence.

Ecology and habitat: Primary and secondary lowland forests to 950 m. Fruits emptied by rodents.

Distribution: Sumatra, Java, and probably Peninsular Malaysia and Thailand. There are no definite records of this species from the wild in Borneo but it is cultivated at Tenom Agricultural Park in Sabah. A. Lamb (pers. comm.)

found it near Tenon and believes it was introduced by Javanese workers who came to work in the tobacco estates at about 1850 and took useful plants with them from Java.

Conservation status: VU Blab(iii). Vulnerable by extent of occurrence estimated $<20,000 \text{ km}^2$, known from <10 locations, and decline in the extent and quality of lowland forest habitats in Java.

Additional materials examined: **Banten Province:** buffer zone of Ujong Kulon NP, Cikacang ($6^{\circ}48'S$ $105^{\circ}32'E$), 130 m, 28 Apr 2005, fruiting, Poulsen *et al.* 2347 (BO, E). **West Java Province:** Gunung Kancana, 2 km WSW of Parabuan village ($6^{\circ}55'S$ $107^{\circ}03'E$), 950 m, 27 Mar 2004, sterile, Poulsen *et al.* 2295 (AAU, BO); Gunung Tutupan, ($7^{\circ}22'S$ $106^{\circ}42'E$), 150 m, 11 Aug 2006, flowering and fruiting, Poulsen *et al.* 2460 (AAU, BO, E, L).

Notes: Amongst the *Etlingera* presently known in Java, *E. hemisphaerica* is most similar to *E. elatior* from which it differs in its erect bracts (not reflexed) and in having a lamina that is reddish beneath. Floral differences between the two species seem to be minor except for the anther possibly being longer in *E. hemisphaerica*, but more material is needed to test this.

With its leaves being wine-red beneath, *Etlingera pyramidosphaera* in Borneo appears very similar to *E. hemisphaerica* but the former differs in having a narrower inflorescence with fewer flowers, the anther thecae dehiscing for their entire length, and in its beaked fruits.

Bakhuizen f. (1968) described the fruits as globular or spindle-shaped, beaked. In the revision of *Etlingera* of Borneo, Poulsen (2006) emphasized the fruit shape as a reliable character. Thus, in the present account above, the fruit is described as globular — not beaked! More material will be necessary to establish if beaked fruits of *hemisphaerica*-like plants actually occur in Java, and if these plants deserve taxonomic recognition.

6. *Etlingera megalocheilos* (Griff.) A.D. Poulsen

Etlingera megalocheilos (Griff.) A.D. Poulsen, *Etlingera of Borneo* (2006) 167. **Basionym:** *Achasma megalocheilos* Griff., Not. Pl. Asiat. 3 (1851) 426, Ic. Pl. Asiat. 3 (1851) t. 355; Valetton, Icon. Bogor. 2 (1906) t. 188, t. 199 figs. 7–9; Heyne, Nuttige pl. Nederl. Indië 1 (1927) 489; Holttum, Gard. Bull. Singapore 13 (1950) 191, fig. 23; Burt & Smith, Notes Roy. Bot. Gard. Edinburgh 31 (1972) 221, fig. 16. *Amomum megalocheilos* (Griff.) Baker, Fl. Brit. India 6 (1892) 236; Valetton, Bull. Inst. Bot. Buitenzorg 20 (1904) 44; Valetton, Icon. Bogor. 2 (1906) t. 188 figs. 1–8, t. 199 figs. 7–9; Bakhuizen f., Bekn. Fl. Java 18 (1958) 33; Bakhuizen f. in Backer, Fl. Java 3 (1968) 57. *Hornstedtia megalocheilos* (Griff.) Ridl., J. Straits Branch Roy. Asiat. Soc.

32 (1899) 146; Schumann, Pflanzenr. IV, 46 (1904) 199; Ridley, Matr. Fl. Mal. Penin. 2 (1907) 38; Ridley, Fl. Mal. Penin. 4 (1924) 270. **Type:** *W. Griffith s.n.* (specimen not found). Malaysia, Johore, Mt. Ophir, flowering in February.

- *Amomum rubroluteum* Baker, Fl. Brit. India 6 (1892) 236. *Etlingera rubrolutea* (Baker) C.K. Lim, Folia Malaysiana 2(3) (2001) 157. **Type:** *A.C. Maingay 1588* (holo, K), Malaysia, Malacca.

- *Etlingera littoralis* auct. non (J. König) Giseke: Smith, Notes Roy. Bot. Gard Edinburgh 43 (1986) 445; Lim, Folia Malaysiana 2 (2001) 149; Keng et al., Concise Fl. Singapore 2 (1998) 57; Khaw, Gard. Bull. Singapore 53 (2001) 235.

Rhizome long-creeping, subterranean (1.5–25 cm), stout, >2 cm in diameter, cream to pale brown, scales to 6 cm, brown, pubescent at base. **Leafy shoot** to 5 m, with up to 28 leaves; base to 8 cm in diameter, dark green. Sheath striate with some cross bars, especially in upper part of the shoot, glabrous, green when fresh. Ligule to 35 mm, entire, green or tinged reddish brown, glabrous or with a few scattered hairs, margin ciliate. Petiole 25–55 mm, glabrous. **Lamina** to 101 x 16 cm, oblong, broadest above the middle, mid- to dark green, pale beneath, young leaf tinged reddish, glabrous (rarely pubescent); average length to width ratio 3.5–7; base \pm unequal; apex acute. **Inflorescence** (including peduncle) 9–18 cm, embedded in the soil, often some distance from base of leafy shoot, with 10–12 flowers, 2–5 open at a time. Peduncle 2–10 cm, subterranean, peduncular bracts cream, acute, shiny, glabrous. Spike to 10–12 x 2–3 cm, cylindrical, flowers extended 3–4 cm above the bracts, length only including bracts: 5–8 cm. Sterile bracts *c.* 5, loosely and spirally arranged, to 4–7 x 1.5–3.5 cm (upper longest and narrowest), ovate to broadly spatulate (widest above the middle), rigid, mucronate, cream tinged pink or bright red, densely pubescent at least in lower half. Fertile bracts 5–8.5 x 0.6–1.9 cm, linear to spatulate, semitransparent, white, pubescent in lower half; apex cucullate, ciliate. Bracteole 4.5–7 cm, pale pink, membranous, with two fissures of 1.5–2.5 cm, pubescent in lower half, apex 2-toothed, ciliate. **Flower:** Calyx 6.1–9 cm, almost reaching filament, \pm as long as corolla lobes, white to pale red with pinkish apices, fissured 2.5–3.5 cm, pubescent in lower 1/4; apex irregularly 3-toothed, tufted. Corolla tube 5.8–8 cm, pale red, darker at apex, glabrous, tube hairy inside especially in a 10 mm band ending 10 mm from labellum. Lobes pale red or pink, glabrous, delicately membranous; dorsal lobe 21–30 x 7–9 mm, reaching near middle of anther (but pushed to the side by the lateral lobes of labellum leaving the anther \pm exposed), elliptic, broadest below middle, apex slightly ciliate; lateral lobes 21–25 x 4.5–5 mm, linear-elliptic, broadest below middle, apex

slightly ciliate; insertion oblique, converging, 0–3 mm above dorsal lobe. Staminal tube 12–22 mm; labellum hourglass-shaped, 52–70 x 20–22 mm, plain red or red to orange–red with yellow margin, with a longitudinal central ridge, glabrous, lateral lobes erect, adhering to sides of anther, base slightly auriculate, central lobe 40–48 x 17 (measured from apex of anther and when flattened), spatulate, entire or emarginate (to 1.5 mm), margin recurved, apex extended 35 mm beyond anther. Stamen 17 mm; filament 4–7 x 4–5 mm, slightly hairy on outside, pale red; anther 10–11.5 x 5–5.5 mm, broadest at apex, emarginate 1.5–2.5 mm, slightly angled 135–160°, red, darker at crest; thecae dehiscing in upper 1/2–2/3, glabrous with a few hairs at the base. Style 8.5–9.5 cm, glabrous to very sparsely hairy adaxially near apex. Stigma 3–4 mm wide, rounded-triangular with a rounded back, pale or dark red; ostiole transverse, 2.5–3 mm, facing downwards or forwards, perhaps flexistylous. Ovary 3–6 x 3–4 mm, densely hairy; epigynous gland 5–9 mm, deeply bilobed or bipartite, apex sometimes hairy. **Infructescence** embedded in the soil, head *ca* 5 x 7–8 cm, bracts not persistent; **fruit** 2.5–3.5 cm across, rounded, not ridged, sometimes slightly warty at apex, pale brown or pink, densely pubescent. **Seeds** up to 4 mm across, angular. *Plate 1F*.

Local names and uses: *Tepus* (Sundanese; Heyne, 1927). The smell is variable; at least in Borneo (Poulsen, 2006) and in Sumatra the smell is strong and somewhat unpleasant, similar to *E. foetens*.

According to Heyne (1927), *E. megalocheilos* was not cultivated but the fruits searched for in the wild and eaten.

Etymology: The epithet refers to the large labellum.

Ecology and habitat: Often dominant in forest gaps or completely open areas to 1300 m.

Distribution: Malay Peninsula, Singapore, Sumatra, Java, and Borneo.

Conservation status: Least concern (LC). Bakhuizen f. (1968) thought it scarce everywhere, but I have observed it in several very open habitats and consider it rather resilient to disturbance. It may actually have expanded in recent years.

Additional materials examined: **Banten Province**, Ujung Kulon NP, Cibayoni (6°41'S 105°35'E), 100 m, 26 Apr 2005, flowering, *Poulsen et al.* 2341 (AAU, BO, E). **West Java Province**, Cibabi (7°18'S 106°24'E), 50 m, 12 Aug 2006, flowering, *Poulsen et al.* 2461 (BO, E).

Notes: Etlingera megalocheilos is most easily confused with *E. coccinea* that also has the inflorescence embedded in the soil and an elongate, red and yellow labellum. But in *E. megalocheilos* the anther is not covered by the corolla lobe, the margins of the labellum are not inrolled, and the labellum is red with more or less pale red or yellowish lateral lobe margins (not yellow with red margins).

Griffith (1851) described *Achasma megalocheilos* from Peninsular Malaysia – a taxon also mentioned by Ridley (1899), Holttum (1950), and which Khaw (2001) called *E. littoralis* following Burt and Smith (1986).

I have not encountered fruits of *E. megalocheilos* in Java but those I have seen from Borneo (Poulsen, 2006) and Sumatra and also described by Holttum (1950) match Valeton's (1906) description and illustrations of the fruits of *A. megalocheilos* which are rounded and smooth but with a few warty protuberances near the top, based on material from Malabar Mts., Java.

7. *Etlingera solaris* (Blume) R.M. Sm.

Etlingera solaris (Blume) R.M. Sm., Notes Roy. Bot. Gard. Edinburgh 43 (1986) 249; PROSEA 13 (1999) 254. **Basionym:** *Elettaria solaris* Blume, Enum. pl. Javae (1827) 52. *Alpinia solaris* (Blume) D. Dietr., Syn. pl. 1 (1839) 12. *Nicolaia solaris* (Blume) Horan., Prodr. Monogr. Scitam. (1862) 32; Heyne, Nuttige pl. Nederl. Indië 1 (1927) 488; Bakhuizen f., Bekn. Fl. Java 18 (1958) 45; Bakhuizen f. in Backer, Fl. Java 3 (1968) 63. *Cardamomum solare* (Blume) Kuntze, Revis. gen. pl. 2 (1891) 687. *Amomum solare* (Blume) K. Schum., Bot. Jahrb. Syst. 27 (1899) 308; Valeton, Icon. Bogor. 2 (1906) t. 160 figs. 15, 13–15, t. 165 figs. 6–12, 16; *Phaeomeria solaris* (Blume) K. Schum., Pflanzenr. IV, 46 (1904) 264. **Type:** *C.L. von Blume s.n.* (L).

- *Elettaria pallida* Blume, Enum. pl. Javae (1827) 52. *Alpinia pallida* (Blume) D. Dietr., Syn. pl. 1 (1839) 12. *Nicolaia pallida* (Blume) Horan., Prodr. Monogr. Scitam. (1862) 32. *Cardamomum pallidum* (Blume) Kuntze, Revis. gen. pl. 2 (1891) 687. *Phaeomeria pallida* (Blume) K. Schum., Pflanzenr. IV, 46 (1904) 264. **Type:** *C.L. von Blume s.n.* (holo L), Java.

Rhizome short-creeping (10–20 cm between closest pairs of leafy shoots), 4 cm diameter, scales dehiscent, brownish, papery. **Leafy shoot** 5 m; base to 5–7 cm diameter, brownish. Sheath: lower caducous, brownish; upper yellowish green with pubescent reticulation. Ligule 40–80 mm, membranous, caducous, deeply bilobed. Petiole 15–20 mm. **Lamina** to 83 x 19 cm, narrowly elliptic or obovate, slightly plicate, dull mid-green, midrib yellow-green, pale green with yellow-green midrib beneath, glabrous; average length to width ratio 3.25–5.25; base ± unequal, cuneate. **Inflorescence** (including peduncle)

to 50 cm, prostrate or ascending to erect, receptacle 3–8 cm (longest in infructescence), with numerous flowers, *ca* 10 open at a time. Peduncle to 40 cm, peduncular bracts, upper as long as lowest sterile bracts, to 9 x 3–4 cm. Spike 11 x 12 cm, globose, robust, flowers not extending above the bracts. Sterile bracts: lower 7–8 x 2–4 cm, with membranous margin and conspicuous apex (to 25 mm with inrolled margin, horn-like twisted towards centre of inflorescence), red soon turning brown, densely pubescent especially at base and margin. Fertile bracts to 5–7 x 1–1.2 cm, similar in shape to sterile bracts, orange-red. Bracteole 4–6 cm, with red apex, one long fissure to 5–15 mm above base; sometimes a second fissure for 5 mm only, densely pubescent throughout, apex bifid with 2 mucro (thus sometimes appearing 3-toothed). **Flower:** calyx 4.5–6 cm, reaching to apex of anther and beyond corolla lobes, red, fissured 3 cm, pubescent, apex 3-toothed 5–9 mm. Corolla tube 3–4 cm, \pm pubescent in lower half, tube inside with an opposite V-shaped hairy cushion coinciding with dorsal corolla lobe attachment and a V-shaped one coinciding with the lateral lobes on the outside, *ca* 22 mm below labellum. Lobes orange-red, with scattered hairs; dorsal lobe 25–26 x 3–3.5 mm, linear, apex acute, reaching to base of anther; lateral lobes 23 x 2–3 mm, linear, insertion oblique, diverging, 3 mm below dorsal lobe. Staminal tube 17–22 mm. Labellum rounded triangular to ovate, 20–23 x 20 mm, orange-red; lateral lobes, margin yellow, central lobe extended 4–8 mm beyond anther, margin curved outwards. Stamen 16 mm: filament 2–3 mm x 3–3.5 mm, white to pale red; anther 13–14 x 3.5 mm, linear, \pm erect, red, anther crest bilobed; thecae dehiscent in upper half 4–5 mm to 2 mm below apex, pubescent. Style 5–5.5 cm, with scattered hairs. Stigma 3–3.5 mm wide, dark purple, heart-shaped with scattered hairs; ostiole transverse, facing down- or forwards (possibly flexistylous); ovary 5–8 x 5 mm, pubescent. Epigynous glands 4–4.5 mm, with one incision, apex irregular, bilobed, margin curved inwards. **Infructescence** lying on ground (because of the heavy fruits), head 20 x 20–25 cm, globose, bracts persistent (at least the bases); **fruit** 10 x 4 cm, angularly obovoid, beaked, (broadest *ca* 4 cm from base), 3- to 6-sided, with persistent calyx, red and juicy when ripe, pubescent. **Seeds** 4 mm diameter, rounded. *Plate 1G.*

Local names and uses: *Hondje warak* (Sundanese: Blume, 1827; Poulsen *et al.* 2297), *honje laka*, *honje ngoser* (Heyne, 1927). Fruit edible.

Etymology: The epithet means sun-like probably referring to the inflorescence at anthesis. In the Mountain Flora of Java, van Steenis (1972) mentions *E. solaris* as "earth sun".

Ecology and habitat: Montane forests near streams at 800–1750 m.

Distribution: Sumatra, W Java as far east as Gunung Merapi in Central Java.

Conservation status: VU B1ab(iii). Vulnerable by extent of occurrence estimated <20,000 km² of montane forest, known from <10 locations, and decline in extent and quality of habitat.

Additional materials examined: **West Java Province:** Halimun NP, Citalahab (6°44'S 106°31'E), 1100 m, 21 Mar 2004, flowering and fruiting, *Poulsen et al.* 2285 (AAU, BO, E, L); Gede-Pangrango NP, Cibodas (6°45'S 107°59'E), 1750 m, 28 Mar 2004, flowering and fruiting, *Poulsen et al.* 2297 (AAU, BO).

Notes: The peduncle of *Etlingera solaris* is variable in position and direction but the species is easily recognized by the long and deeply bilobed ligule and the horn-shaped, twisted, pubescent bracts. Valeton (1921a, p. 137, plate 6) described *E. solaris* var. *aurantiaca* from Gunung Salak, Java. The variety is supposed to have an erect inflorescence to 20 cm (not procumbent), shorter teeth to the calyx and the lip orange rather than dark red, and possibly the same as *Elettaria pallida* Blume. I have seen this at Halimun and agree with Bakhuizen f. (1968) that it is hardly different. A collection (*Poulsen 2418*) from Gunung Kerinci, Sumatra, had its inflorescence embedded in the ground as the peduncle was subterranean, the stamen was shorter (13–14 mm) but the anther dehiscence matched that in the Javanese material.

Amomum chrysocalyx K. Schum. was listed as a synonym by Bakhuizen f. (1968) but, after inspecting its type, I am convinced that it has no relevance to *E. solaris*.

Incompletely Known Species

8. *Etlingera heyniana* (Valeton) R.M. Sm.

Etlingera heyniana (Valeton) R.M. Sm., Notes Roy. Bot. Gard. Edinburgh 43 (1986) 246. **Basionym:** *Nicolaia heyniana* Valeton, Bull. Jard. Bot. Buitenzorg ser. 3, 3 (1921) 132; Heyne, Nuttige pl. Nederl. Indië 1 (1927) 488. *Phaeomeria heyniana* (Valeton) Burkill, Bull. Misc. Inform. (1935) 318. *Amomum heynianum* (Valeton) Bakhuizen f., Bekn. Fl. Java 18 (1958) 31; Bakhuizen f. in Backer, Fl. Java 3 (1968) 56. - **Type:** Sentiong, Jakarta, cemetery, possibly cultivated, Nov 1918, *K. Heyne s.n.* (holo, BO).

No recent material has been seen and the modified description below is only a summary of what is presented by Valeton (1921a) and Bakhuizen f. (1968).

Leafy shoot to 4 m. Sheath glabrous. Ligule *ca* 8 mm, elliptic, obtuse, glabrous, stout. Petiole to 5 mm. **Lamina** 19–28(–65) x 4.5–5.5(–17) cm, narrowly

obovate, glabrous throughout; length to width ratio 4–6; base acute; apex shortly caudate-acuminate; margin glabrous. **Inflorescence** fusiform, red; receptacle discoid, with <20 flowers (estimated from Valeton, 1921a, Plate 3), *ca* 4 open at a time. Peduncle short, curved, scales obovate, glabrous, red, apex rounded, mucronate. Spike \pm ovoid, only including bracts: to 5.5 x 0.5–1 cm. Sterile bracts 5.5–6.5 x 3 cm, oblong. Fertile bracts to 2–5.5 x 0.5–1 cm. Bracteole *c.* 4 cm, glabrous. **Flower:** calyx 5 cm, glabrous, 3-toothed. Corolla lobes 25 x 5 mm, linear. Labellum 3 x 1.5 cm long, with about equal elliptic upper and lower halves separated by a distinct constriction, red with yellow margin; central lobe *ca* 10 mm wide, margin slightly curled. Filament 7 mm; anther *ca* 10 mm, crest bilobed, divergent. Style glabrous. Stigma discoid, ostiole transverse; ovary pubescent. Epigynous glands 4 mm. **Infructescence** unknown.

Local names and uses: *Hondje* (Heyne, 1927). Valeton (1921a) noted that the entire plant is strongly aromatic like *Nicolaia speciosa* (*E. elatior*).

Etymology: The epithet is in honour of the Dutch botanist Karel Heyne (1877–1947) who collected in Java and Sumatra.

Ecology and habitat: Unknown. Seems to tolerate growing in a rather open habitat.

Distribution: Java.

Conservation status: Unknown.

Notes: The illustration of *Etlingera heyniana* in Valeton (1921a, Plate 3) looks to me more like an inflorescence of *E. megaloscheilos* where the flowers have not fully opened yet, similar to what may be observed in *E. nasuta* (K. Schum.) R.M. Sm. in Borneo. Apart for the labellum being erect and significantly shorter in *E. heyniana*, there are not strong evidence to separate them. At least the colours of the labellum (red with yellow margin) are the same. The type at BO mentions Gunung Honje as the locality— not Sentiong. I thus went to one G. Honje, of which locality there might actually be several in Java. At the locality I visited near Ujung Kulon, *E. megaloscheilos* was very common. A closer study of the floral development of this species may be fruitful.

Even though Valeton (1921a) mention the distinct smell of *E. heyniana*, this is not strong evidence against the possibility of synonymy with *E. megaloscheilos*, as in Sumatra I have experienced that it may sometimes

have a strong smell.

9. *Etlingera walang* (Blume) R.M. Sm.

Etlingera walang (Blume) R.M. Sm., Notes Roy. Bot. Gard. Edinburgh 43 (1986) 251; PROSEA 13 (1999) 254. **Basionym:** *Donacodes walang* Blume, Enum. pl. Javae (1827) 55. *Alpinia walang* (Blume) D. Dietr., Syn. pl. 1 (1839) 13. *Elettaria walang* (Blume) Miq., Fl. Ned. Ind. 3 (1859) 603. *Cardamomum walang* (Blume) Kuntze, Revis. gen. pl. 2 (1891) 687. *Achasma walang* (Blume) Valetton, Bull. Inst. Bot. Buitenzorg 20 (1904) 93. *Amomum walang* (Blume) Valetton, Bull. Inst. Bot. Buitenzorg 20 (1904) 44; Valetton, Icon. Bogor. 2 (1906) t. 162 figs. 1–9; Bakhuizen f., Bekn. Fl. Java 18 (1958) 32; Bakhuizen f. in Backer, Fl. Java 3 (1968) 56. *Hornstedtia walang* (Blume) Koord.-Schum., Syst. Verz. 1 (1911) 21. **Type:** Java, *C.L. von Blume s.n.* (holo, L).

- *Elettaria glaberrima* Zoll. & Moritzi, Syst. Verz. (1845–1846) 84. *Cardamomum glaberrimum* (Zoll. & Moritzi) Kuntze, Revis. gen. pl. 2 (1891) 686. **Type:** *H. Zollinger 670* (isotypes, BM, K, L, P).

No recent material has been seen and the description below is only a summary of what is presented by Valetton (1904; 1906) and Bakhuizen f. (1968).

Rhizome slender. **Leafy shoot** 1.5–2 m. Sheath glabrous. Ligule *c.* 10 mm, finely ciliate, otherwise glabrous, stout. Petiole 5–10 mm. **Lamina** 29–49 x 5–6.5 cm, narrowly obovate, glabrous throughout; average length to width ratio 6–7.5; base \pm unequal, narrowly cuneate; apex shortly acuminate, finely hairy; margin glabrous. **Inflorescence:** receptacle almost flat, with <20 flowers, *c.* 3 open at a time. Peduncle to 8 cm, subterranean, scales to 5 x 1.3 cm, mucronate, finely longitudinally veined. Spike ovoid-cylindrical, only including bracts: to 7 x 2.5–3.5 cm; about 5 sterile bracts, narrowly obovate, acuminate, mucronate: to 6–8 x 1.3 cm. Fertile bracts to 6 cm, narrower than sterile bracts. Bracteole *c.* 5 cm, bifid, densely pubescent at least in lower half. **Flower:** calyx as long as corolla, irregularly 3-toothed. Corolla red; lobes erect, oblong, dorsal lobe longest. Labellum yellow with red margin; lateral lobes, margin curved upwards and conspicuously crenate, central lobe 5 cm long, narrow, ligulate-spathulate, deeply bilobed. Filament 7 mm; anther *ca* 10 mm, crest bilobed, divergent. Style 5.5 cm, hairy below apex. Stigma triangular, hairy; ovary pubescent. Epigynous glands 4 mm. **Infructescence** unknown.

Local names and uses: *Walang* (Sundanese). Leaves are served as a side dish with rice (Valetton, 1904), as a condiment, or the leaves are burnt on rice fields as an insect repellent (Heyne, 1927).

Etymology: The epithet refers to the bad-smelling rice bug, *walang sangit* (*Leptocorisa acuta* Thunb. or *L. varicornis* Fabr.). Bakhuizen f. (1968) noted that all parts – especially the leaves — are ill-smelling.

Ecology and habitat: forests to 1200 m, and cultivated.

Distribution: Java.

Conservation status: Impossible to assess.

Notes: In Blume's protologue (1827) of *Etlingera walang* he placed a question mark after the genus (*Donacodes*). All that is reported of the new species is that the leaves are elongate-linear-lanceolate, acuminate, and glabrous, and it appears he had not seen the flowers. I think the question mark refers to the uncertainty of which genus to place it in, but, without the flowers, one has to wonder why Blume placed it in *Donacodes* (the remaining species of which are presently placed in *Hornstedtia*).

Valeton (1904) did detailed studies around Bogor where he found that *E. walang* was 'one of the most economically important Sundanese plants' and often cultivated, of unknown origin and very easily recognized by its characteristic smell that stays for months with the specimen after drying. He studied the inflorescence in detail (see plate in Valeton, 1906) and thought it was much more narrow than that of *E. coccinea* and *E. foetens* but that these three species formed a natural group and apart from the crenate margin to the labellum, *E. walang* was very similar to *E. coccinea*.

In my opinion what Valeton illustrated is just an *E. coccinea* with a deeply bifid apex to the labellum, similar to *Poulsen et al.* 2343 from Ujung Kulon. It is important to remember, however, that the basis for Valeton's descriptions (1904; 1906) and illustrations (1906; Plate 162, figs. 1–9) are not based on the type.

The issue of smell is, however, very interesting. As mentioned in the notes above on *E. coccinea*, in Borneo a very smelly form of this species – also of unknown origin – is often cultivated and commonly sold in the markets.

More detailed surveys around Bogor may result in the discovery of *walang* and it would then be possible to establish if this just a form of *E. coccinea*.

Conservation of *Etlingera* in Java

None of the seven well-known species of *Etlingera* is endemic to Java. They all display geographical affinity with nearby Borneo or Sumatra to the north and west, but no overlap with species in Wallacea to the east.

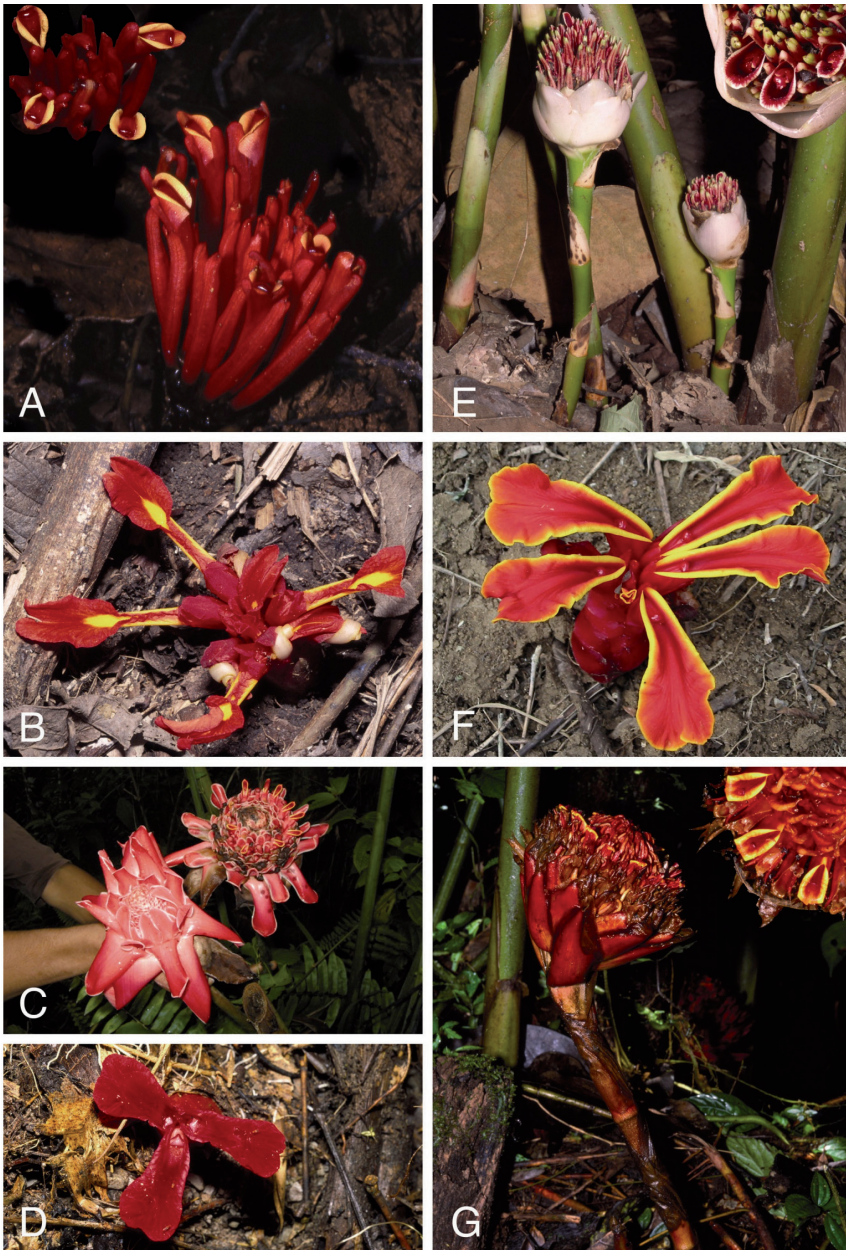


Plate 1. Photographs of Javanese *Etlingera* species. A. *E. brachychila*. Ujung Kulon, Banten Province (Funakoshi IU 19) by H. Funakoshi; B. *E. coccinea*. Cikaso, W Java Province (Poulsen et al. 2458) by A.D. Poulsen; C. *E. elatior*. Batang Gadis NP, North Sumatra Province (not collected) by Lis Maidi Darjo; D. *E. foetens*. Bau, Sarawak, Borneo (Poulsen et al. 1894) by A.D. Poulsen; E. *E. hemisphaerica*. W Java Province (Poulsen et al. 2460) by A.D. Poulsen; F. *E. megaloscheilos*. Baduy Territory, Banten Province (not collected) by M. van Heist; G. *E. solaris*. Halimun NP, W Java Province (Poulsen et al. 2285) by A.D. Poulsen.

Two of the species (*E. coccinea* and *E. megalocheilos*) seem to be common in very disturbed habitats and there is no great concern for their conservation. However, there is reason to fear the future survival in Java of *E. brachychila*, *E. foetens*, *E. hemisphaerica*, and to a lesser extent, of *E. solaris*.

Acknowledgements

I thank the Indonesian Institute of Sciences (LIPI) for permission to conduct research in Java; the Herbarium Bogoriense and its Directors and staff for logistic and other support, especially Marlina Ardiyani, Deden Girmansyah, Ismail Rachman, Agus Sayadi, Aden Muhidin, and Alex Sumadijaya; The National Parks Service of Halimun, Gede-Panggrango, and Ujong Kulon; the local people in W Java for assistance in the field; H. Funakoshi for sharing his important material of *Etlingera brachychila* with me; The National Parks of Singapore for funding my journey to Asia in 2006; The Royal Botanic Garden Edinburgh for providing facilities and contributing to the fieldwork in Java in 2006; Mark F. Newman for giving constructive comments on an earlier stage of this paper; Hidetoshi Funakoshi, Lis Maidi and Miriam van Heist for letting me use their images; and the Carlsberg Foundation, Denmark, for a senior research stipend.

References

- Bakhuizen van den Brink, R.C. 1958. *Beknopte Flora van Java* **18**.
- Bakhuizen van den Brink, R.C. 1968. Zingiberaceae, pp. 41–76. In: C.A. Backer & R.C. Bakhuizen van den Brink, *Flora of Java*, vol. **3**. N.V.P. Noordhoff, Groningen, The Netherlands, 761 pp.
- Blume, C.L. 1827. *Enumeratio Plantarum Javae et insularum adjacentium*. J.W. van Leenwen.
- Burtt, B.L. and Smith, R.M. 1986. *Etlingera*: the inclusive name for *Achasma*, *Geanthus* and *Nicolaia* (Zingiberaceae). *Notes from the Royal Botanic Garden Edinburgh* **43**: 235–241.
- Griffith, W. 1851. *Notulae ad plantas Asiaticas*, vol. **3**. Calcutta.
- Heyne, K. 1927. Zingiberaceae, pp. 473–504. In: De nuttige planten van Nederlandsch Indië **1.2nd** edition. Department van Landbouw, Nijverheid en handel in Nederlandsch Indië.

- Holttum, R.E. 1950. The Zingiberaceae of the Malay Peninsula. *The Gardens' Bulletin Singapore* **13**: 1–249.
- IUCN 2000. *IUCN Red List Categories and Criteria*. Version 3.1.
- Kato, M., Itino, T and Nagamitsu, T. 1993. Melittophily and ornithophily of long-tubed flowers in Zingiberaceae and Gesneriaceae in West Sumatra. *Tropics* **2**: 129-142.
- Khaw, S.H. 2001. The genus *Etlingera* (Zingiberaceae) in Peninsular Malaysia including a new species. *Gardens' Bulletin Singapore* **53**: 191–239.
- Poulsen, A.D. 2006. *Etlingera of Borneo*. Natural History Publications (Borneo), Kota Kinabalu, Malaysia & Royal Botanic Garden Edinburgh, Scotland, 263 pp.
- Ridley, H.N. 1899. The Scitamineae of the Malay Peninsula. *Journal of the Straits Branch of the Royal Asiatic Society* **32**: 85–184.
- Sakai, S., Kato, M. & Inoue, T. 1999. Three pollination guilds and variation in floral characteristics of Bornean gingers (Zingiberaceae and Costaceae). *American Journal of Botany* **86**: 646–658.
- Smith, R.M. 1986. A review of Bornean Zingiberaceae: II (Alpineae, concluded). *Notes from the Royal Botanic Garden Edinburgh* **43**: 439–466.
- Schumann, K.M. 1904. Zingiberaceae. In: A. Engler, *Das Pflanzenreich IV*, **46**: 1–458.
- Valeton, T. 1904. Ueber neue und unvollständig bekannte Zingiberaceae aus West-Java und Buitenzorg. *Bulletin de l'Institut Botanique de Buitenzorg* **20**: 1–99.
- Valeton, T. 1906. *Icones Bogoriensis*, vol.2. Leiden.
- Valeton, T. 1921a. *Nicolaia* Horan. Description of new and interesting species. *Bulletin Jardin Botanique Buitenzorg* **3**: 128–140.
- Valeton, T. 1921b. *Rhynchanthus* Hook. f., *Geanthus* Reinw. and *Geostachys* Ridl. *Bulletin Jardin Botanique Buitenzorg* **3**: 141–147.
- van Steenis, C.G.G.J. 1972. *The mountain flora of Java*. E.J. Brill, Leiden.

Planting Date and Night Break Treatment Affected Off-Season Flowering in *Curcuma alismatifolia* Gagnep.

S. RUAMRUNGSRI^{1,2}, J. UTHAI-BUTRA³, O. WICHAILUX⁴
AND P. APAVATJRUT²

¹ Department of Horticulture, Faculty of Agriculture, Chiang Mai University,
Chiang Mai 50200, Thailand.

² H.M. the King's Initiative Centre for Flower and Fruit Propagation,
Chiang Mai University, Chiang Mai 50200, Thailand

³ Department of Biology, Faculty of Science, Chiang Mai University,
Chiang Mai 50200, Thailand

⁴ Department of Agricultural Extension, Ministry of Agriculture,
Bangkok, Thailand.

Abstract

Off-season flowering of *Curcuma alismatifolia* Gagnep. was studied in Chiang Mai Province of Thailand where the weather in winter is cool with temperatures between 16 to 30 °C, RH from 65 to 70 %, and 10 hrs of daylight. Rhizomes were stored at 15 °C for the 6 months from February to July, 2004. After root emergence, plants were grown under different night break treatments. Night break treatments were conducted by supplying 2 hrs of light daily from 20.00 to 22.00 hrs. The light source was 100 watt incandescent light bulbs. There were three treatments: T1, night breaks supplied from sprouting of the first shoot until the floral spike reached one inch long; T2 as T1, but continued until the first floret opened; T3, was a control treatment with no night break. Each treatment was carried out at different planting dates, i.e., August 9, September 9, October 9 and November 9. Plant height, number of plants per cluster, flowering percentage and flower qualities (number of coma bracts, number of green bracts, spike length and length of flower stalk) were recorded. The results showed that plant growth and flower qualities were similar with and without the night break treatment at the 9 August planting date. However, the September to October planting dates required night break treatments to promote flowering and maintain flower qualities.

Introduction

Curcuma alismatifolia Gagnep. or 'Siam tulip', in family Zingiberaceae is a native plant in Thailand. It is a high potential crop for cut flower and potted plant. Thailand exports about two million rhizomes per year to Japan, EU and USA.

Generally, flower and rhizome production starts from April to May, the plant flowers in July to August during the rainy season in Thailand, when the weather is averaging 27 to 28°C, 12 to 13 hrs of sunshine duration, and 80% RH. Then, it becomes dormant in November to December, the rhizomes are harvested in December to February when the temperature is about 30/16°C (max/min), sunshine is about 10 hrs, and relative humidity (RH) is about 65 to 70%. High demand of flower in the world market is mostly in winter, when the environmental conditions, such as, short day length and low temperature in winter are limiting factors for growth and development of this plant.

All plants need light to use nutrients and manufacturing food. Artificial light is useful when natural light is insufficient. Plant absorbs red and blue lights, both are used in controlling photosynthesis, leaf development and flowering. Incandescent light can supplement natural day light and give a large amount of red light and infrared light (Barkley, 2005).

The responses of plants to day length were classified in three classes i.e. short-day plants (SD), long day plants, and day length neutral plants; however, this original classification has since become considerably more complex with various subclasses. Plants differ in respect of the strictness of dependence on day length were divided into, i.e., (1) qualitative or obligate photoperiodism, where there is an absolute requirement for a particular day length (SD or LD plants), and (2) quantitative or facultative photoperiodism, where a particular day length advances or enhances flowering, but the plants will eventually flower anyway (Hart, 1988). Interruption by light of dark period, called night break, can lead to floral promotion of LD plants (Thomas and Vince-Prue, 1997). Hagiladi *et al.* (1997) reported that *Cucurma alismatifolia* should be classified as quantitative long day plants, since long day condition using supplement light source enhances flowering of this plant. Therefore, the research was aimed to study the effect of planting date and night break treatment on growth and development of *C. alismatifolia* using incandescent light to extend flower production period from the rainy season to winter.

Materials and Methods

Stubbed rhizomes with storage roots of *Curcuma alismatifolia* were stored in cool room at 15 °C, RH from 70 to 80% for 6 months from February to July. The experiment using the storage rhizomes was started from August to November, plants were grown in different conditions at four different planting dates, 9 August; 9 September; 9 October; and 9 November. Before planting, the rhizomes were soaked in water for 3 days to stimulate sprouting, and planted in 6 x 12 inches plastic bags using sand : rice husk : rice husk charcoal (1:1:1) as planting medium. Water was supplied daily and nutrient solution containing 200 mg l⁻¹ of N, 50 mg l⁻¹ of P, 200 mg l⁻¹ of K, 65 mg l⁻¹ of Ca, 20 mg l⁻¹ of Mg, 0.22 mg l⁻¹ of B, 0.54 mg l⁻¹ of Mn, 0.26 mg l⁻¹ of Zn, 0.04 mg l⁻¹ of Mo and 0.45 mg l⁻¹ of Fe was supplied twice a week. For each planting date there were three treatments: T1, night break treatment started from shoot emerged until the flower spike reached one inch long; T2, night break treatment started from shoot emerged until the first floret opened; and T3, control treatment where plants were grown in natural conditions with no night break treatment. The growing plants were exposed to 2 hrs supplement light from 20.00-22.00 hrs. Light source was 100 watt of incandescent light bulbs emitting about 462 μmol s⁻¹ m⁻². Since growth rate of plants were different at different planting dates, therefore T1 and T2 had different light supplement duration depending on the planting dates as shown in Table 1. Plant growth in terms of plant height, number of plants per cluster, and flower quality were collected. The experimental design was a completely randomized design with 10 replications/treatment.

Table 1. Growing time required for starting night break treatments in T1 and T2 from different planting dates.

Planting dates	Time required after planting (wks)	
	T1 (from planting to one inch of flower spike appeared)	T2 (from planting to opening of the first floret)
Aug. 9	9	11
Sep. 9	10	12
Oct. 9	12	14
Nov. 9	14	16

Results and Discussion

Plant growth

The results showed that growing habits of *C. alismatifolia* were not significantly different at planting dates of 9 Aug. and 9 Oct. On the other hand, they were affected by the night break treatments T1 and T2 compared with control (T3) when planted in 9 Sep., and 9 Nov. Heights of plants at late planting dates (9 Sep., 9 Oct. and 9 Nov.) were lower than early planting date in 9 Aug. (Table 2). Assuming that the average temperature in Thailand during that period was 20 to 24°C, which was cooler (26 to 27°C) than the other periods and sunshine duration was from 10 to 11 hrs (Table 3). Lower temperature during later planting dates has a deleterious effect on final plant growth and development. However, night break treatment could stimulate plant height compared with the control (Table 2).

Table 2. Plant height (cm) affected by night break treatments from different planting dates, 12 WAP.

Treatments	Planting dates			
	Aug. 9	Sep. 9 ¹	Oct. 9	Nov. 9 ¹
T1. night break until one inch of spike appeared	47.65	48.12a	42.70	34.40a
T2. night break until the first floret opened	50.08	55.62a	39.68	35.25a
T3. control (no night break)	45.08	32.48b	39.05	24.88b
LSD .05	ns	11.12	ns	6.38

¹ Means followed by different letters within the same column are significantly different among treatments; ns: not significantly different.

Table 3. Meteorological data during August–December in Chiang Mai Province at the Multiple Cropping Research Station, Chiang Mai University.

Month	Air temp. (°C)		Data	Sunshine duration (hrs)
	max	Min		
August	32	23	27	12
September	32	23	27	12
October	32	21	26	11
November	31	19	24	11
December	29	13	20	10

Number of plants per cluster indicated the yield of rhizomes after harvest. The results showed that night break did not affect the number of plants per cluster of plants growing on 9 Aug., 9 Sep., and 9 Oct. (Table

4). However, the number of plants per cluster of the controlled treatment T3 was significantly lower than T1 when planted on 9 Nov. (Table 4). This indicates that the effect of night break was sensitive to low temperature during the growing period.

Table 4. Number of plants per cluster affected by night break treatments from different planting dates, 12 WAP.

Treatments	Planting dates			
	Aug. 9	Sep. 9	Oct. 9	Nov. 9 ¹
T1. night break until one inch of spike appeared	1.25	1.75	1.75	2.75a
T2. night break until the first floret opened	1.50	1.00	1.50	1.50ab
T3 control (no night break)	1.50	1.25	1.50	0.75b
LSD .05	ns	ns	ns	1.98

¹ Means followed by different letters within the same column are significantly different among treatments; ns: not significantly different.

Days to flowering

The number of days from planting to flowering tended to be delayed when plants were grown under night break treatments at the 9 Sep. and 9 Oct. planting dates. However, days to flowering in the 9 Nov. group was less (99.25 days) for T1 compared to the T3 control treatment (103 days) (Table 5). Night break treatments in T1 and T2 also increased flowering percentages, compared to the control (Table 6). The similar results were also found in *Cosmos atrosanguineus* (Hook.) Voss. (Kanellos and Pearson, 2000), *Petunia x hybrida* (Adams *et al.*, 1999) and *Eustoma grandiflorum* (Raf.) Shinn. (Islam *et al.*, 2005), the quantitative (facultative) long-day plants whose flowering was advanced and hastened by long day.

Table 5. Number of days to the first floret opening affected by night break treatments from different planting dates.

Treatments	Planting dates			
	Aug. 9	Sep. 9	Oct. 9	Nov. 9 ¹
T1. night break until one inch of spike appeared	68.75	85.75	98.00ab	99.25b
T2. night break until the first floret opened	79.75	89.50	102.50a	102.00ab
T3 control (no night break)	70.00	75.25	90.00b	103.00a
LSD .05	ns	ns	12.44	2.87

¹ Means followed by different letters within the same column are significantly different among treatments; ns: not significantly different.

Table 6. Flowering percentages affected by night break treatments from different planting dates.

Treatments	Planting dates			
	Aug. 9	Sep. 9	Oct. 9	Nov. 9
T1. night break until one inch of spike appeared	80.00	80.00	80.00	60.00
T2. night break until the first floret opened	86.70	100.00	66.70	86.70
T3. control (no night break)	66.70	53.30	46.70	26.70

Flower qualities

Flower qualities in terms of spike length, number of coma bracts, number of green bracts and length of flower stalk were determined. It showed that both of the night break treatments (T1 and T2) did not affect all flower quality parameters of the plants grown at 9 Aug. (Table 7 to 10). On the other hand, they increased length of spike in general (Table 7), number of coma bract (Table 8, Fig. 1) and length of flower stalk (Table 9) compared to the control treatment at 9 Sep., 9 Oct. and 9 Nov. planting dates. Length of spike in T1 were 14.05, 12.18 and 10.15 cm at 9 Sep., 9 Oct. and 9 Nov., respectively and they were not significantly different from T2, but they were significantly higher than control treatment (T3) (Table 7). Number of coma bracts were significantly higher in T1 (10.75, 12.00 and 10.50 bracts per spike at 9 Sep., 9 Oct. and 9 Nov., respectively) and T2 (13.00 and 10.75 bracts at 9 Sep. and 9 Nov., respectively) than control treatment (T3) (Table 8). The results of flower stalk length were similar to length of spike (Table 9). Chang (2000) also reported that to extend flowering period of *C. alismatifolia* in Taiwan using plastic tunnel and light illumination from 22.00 p.m. to 2.00 a.m. increased quality on length of flower stalk, diameter of stalk and number of coma bract. However, number of green bracts was not significantly different among treatments at each planting date (Table 10). Later planting dates had adverse effect, giving less flower qualities although the plants were supplied with night break and also affected flower morphology as showed in Fig. 1.

For short day plant, such as chrysanthemum, night break is used for floral bud initiation. In case of *C. alismatifolia*, a quantitative long-day plant, night break seems to involve in extending photosynthetic period and stored assimilates required for growth and flowering.

Table 7. Length of spike (cm) affected by night break treatments from different planting dates.

Treatments	Planting dates			
	Aug. 9	Sep. 9 ¹	Oct. 9 ¹	Nov. 9 ¹
T1. night break until one inch of spike appeared	17.13	14.05ab	12.18ab	10.15a
T2. night break until the first floret opened	16.63	16.48a	12.45a	9.32a
T3. control (no night break)	17.38	13.45b	11.10b	7.22b
LSD .05	ns	2.77	1.26	0.97

¹ Means followed by different letters within the same column are significantly different among treatments; ns: not significantly different.

Table 8. Number of coma bracts affected by night break treatments from different planting dates.

Treatments	Planting dates			
	Aug. 9	Sep. 9 ¹	Oct. 9 ¹	Nov. 9 ¹
T1. night break until one inch of spike appeared	12.50	10.75ab	12.00a	10.50a
T2. night break until the first floret opened	12.00	13.00a	9.25b	10.75a
T3. control (no night break)	13.25	8.75c	9.50b	8.25b
LSD .05	ns	1.98	1.93	4.57

¹ Means followed by different letters within the same column are significantly different among treatments; ns: not significantly different.

Table 9. Length of flower stalk (cm) affected by night break treatments from different planting dates.

Treatments	Planting dates			
	Aug. 9	Sep. 9 ¹	Oct. 9 ¹	Nov. 9 ¹
T1. night break until one inch of spike appeared	40.25	54.38ab	31.25a	31.30a
T2. night break until the first floret opened	42.13	62.38a	28.20a	28.38a
T3. control (no night break)	40.50	43.38b	22.75b	16.62b
LSD .05	ns	12.60	5.29	6.13

¹ Means followed by different letters within the same column are significantly different among treatments; ns: not significantly different.

Table 10. Number of green bract affected by night break treatments at different planting dates.

Treatments	Planting dates			
	Aug, 9	Sep. 9 ¹	Oct. 9 ¹	Nov. 9 ¹
T1. night break until one inch of spike appeared	8.75	9.00	8.75	7.00
T2. night break until the first floret opened	8.75	9.75	8.25	9.25
T3. control (not supplied night break)	10.00	9.50	8.50	7.00
LSD .05	ns	ns	ns	ns

¹ Means followed by different letters within the same column are significantly different among treatments; ns: not significantly different.

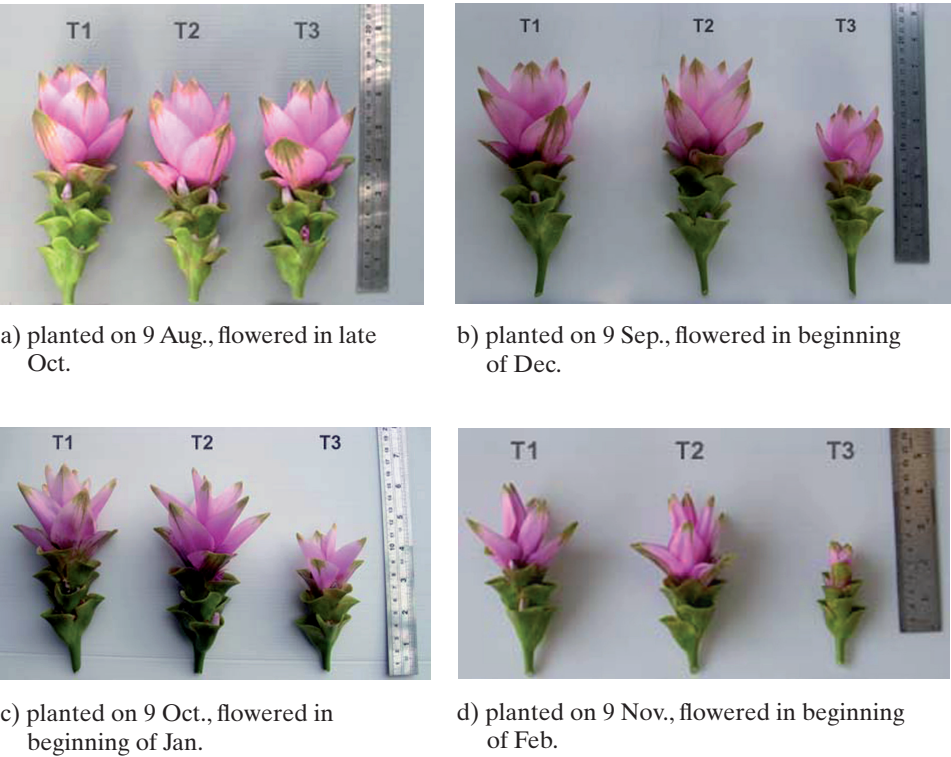


Figure 1. Flower qualities influenced by night break and planting dates.

Conclusion

It was possible to produce off-season flower of *Curcuma alismatifolia* by storing rhizome in a controlled room at 15°C, then stimulate shoot emergence by soaking the rhizome in water for three days. It was not necessary to supply night break when planted on August 9. However, for delayed growing in September to November, night break treatment was necessary to promote flowering, flowering percentage and increased flower qualities in December and January. Duration of night break treatments between T1 and T2 were not significantly different, therefore night break should be supplied from shoot emerged until one inch of tight flower spike appeared (T1) which was sufficient for off-season flowering.

Acknowledgements

The research was supported by the National Research Council of Thailand (2004). We would like to thank H.M. the King's Initiative Centre for Flower and Fruit Propagation for cold room supporting. Thank to the Hitachi Scholarship Foundation for financial support of the oral presentation in this symposium.

References

- Adams, S.R., S. Pearson, P. Hadley and W.M. Patefield. 1999. The effect of Temperature and light integral on the phases of photoperiod sensitivity in *Petunia x hybrida*. *Annals of Botany* **83**: 263-269.
- Barkley, S. 2005. House Plants: Artificial Light at - [http://www.agric.gov.ab.ca/\\$development/septdocs.nsf/all/webdoc1380](http://www.agric.gov.ab.ca/$development/septdocs.nsf/all/webdoc1380).
- Chang, C. S. 2000. Dormancy in *Curcuma* (*C. alismatifolia*) at <http://www.chang.htm>. 17/7/2004.
- Hagiladi, A., N. Umiel and X.-H. Yang. 1997. *Curcuma alismatifolia*. II. Effects of temperature and daylength on the development of flowers and propagules. *Acta Horticulturae* **430**: 755-761.
- Hart, J.W. 1988. *Light and Plant Growth*. Urwin Hyman Ltd. London. 204 pp.

- Islam, N., G.G. Patil and H.R. Gislerod. 2005. Effect of photoperiod and light integral on flowering and growth of *Eustoma grandiflorum* (Raf.) Shinn. *Scientia Horticulturae* **103**: 441-451.
- Kanellos, E.A.G and S. Pearson. 2000. Environmental regulation of flowering and growth of *Cosmos atrosanguineus* (Hook.) Voss. *Scientia Horticulturae* **83**: 265-274.
- Thomas, B. and D. Vince-Prue. 1997. *Photoperiodism in Plants*. Academic Press. London. 428 pp.

Effects of 2,4-D on Callus Induction from Leaf Explants of *Cornukaempferia larsenii* P. Saensouk

P. SAENSOUK, P. THEERAKULPISUT, B. KIJWIJAN
AND S. BUNNAG

Applied Taxonomic Research Center, Department of Biology, Faculty of Science,
Khon Kaen University, Khon Kaen 40002, Thailand.

Email addresses: saensouk@yahoo.com, piyth@kku.ac.th,
bookij@kku.ac.th, sumbun@kku.ac.th

Abstract

Callus was induced from young leaves of *Cornukaempferia larsenii* P. Saensouk on Murashige and Skoog medium supplemented with 3% sucrose and various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) in light and dark conditions. The highest number of callus formation, percentage of callus formation and average weight of callus were obtained from young leaves cultured on the medium supplemented with 0.5 mg/l 2,4-D in the light condition. The callus could not be regenerated to plantlets in media added with various concentrations of NAA and BA.

Introduction

The genus *Cornukaempferia* Mood & K. Larsen is the new genus in Zingiberaceae from Thailand, described by Mood and Larsen (1997, 1999). Two species, *C. aurantiflora* Mood & K. Larsen and *C. longipetiolata* Mood & K. Larsen, have been recognized. This genus is listed as rare and endemic to Thailand and its distribution is restricted to only few provinces in the northeastern and northern part of the country. *C. aurantiflora* has been used by local people in northeastern Thailand to treat infected hemorrhoids and laryngitis common in Thai children. During a floristic survey carried out in May of 2005, a morphologically distinct species of *Cornukaempferia* was discovered and will be named *C. larsenii* in honor of Professor Kai Larsen, University of Aarhus, Denmark (Saensouk *et al.*, 2007).

The new species is propagated vegetatively by pieces of rhizomes. In a vegetatively propagated plant like *Cornukaempferia*, the risk of systemic infections with rootknot nematodes, bacterial wilt and *Fusarium* from the propagules is very high. Thus, the application of tissue culture can be used to produce large amount of disease-free plantlets. The objective of this work

is to establish a system for vegetative propagation of this rare plant species through tissue culture. This is the first report of callus induction from leaf tissue of plants in this genus.

Materials and Methodology

Young leaves of *C. larsenii* (Fig. 1) collected from natural habitats were washed with running tap water, rinsed with 70% (v/v) ethyl alcohol for 30 seconds, sterilized with 0.9 % sodium hypochlorite containing 2 drops of Tween 20 for 15 seconds followed by three washes with sterilized distilled water. The young leaves were cut into 1x1 cm² pieces and cultured on MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose, 0.7% agar, and 0, 0.1, 0.5, 1, 2, 3, 4 and 5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) in both light and dark conditions for 16 weeks. Callus was transferred to regeneration medium, i.e., MS medium, added with 0, 1, 3 and 5 mg/l 1- naphthaleneacetic acid (NAA) and benzyladenine (BA) for 16 weeks. The cultures were incubated at 25 ± 2 °C under white, fluorescent light (2,000 lux) at a 16 h photoperiod or in the dark. All the experiments were conducted using complete randomized design (CRD) with 15 replicates each containing one explant per culture tube. Data were analyzed using ANOVA and the mean separation was achieved by the Duncan's Multiple Range Test (DMRT). The test of statistical significance was performed at the 5% level using the SPSS program (version 11.5).

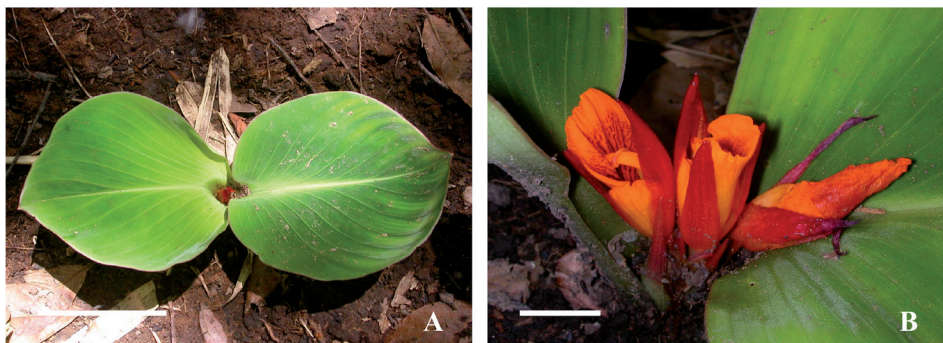


Figure 1. *Cornukaempferia larsenii*. A. Habit; B. Flower. (Scale bars A= 10 cm and B= 1 cm).

Results and Discussion

Preliminary efforts to propagate this plant by culturing shoot tips from underground stems resulted in extremely high level of contamination, therefore, an attempt was made using the leaf blade as explants. Young leaves from natural habitats were cultured on MS medium with various levels of

2,4-D for induction of callus in the light and dark conditions. The callus was soft in texture, friable in structure, and yellowish white (Figs. 2 and 3). Callus did not form on medium lacking 2,4-D. The callus formation occurred on medium added with 0.1, 0.5, 1, 2, 3, 4 and 5 mg/l 2,4-D in the light, and 0.5, 1, 2 and 3 mg/l 2,4-D in the dark, after 8 weeks of culture. The highest percentage of callus formation (99.33%) and the highest average weight of callus (2.61 g) were obtained from young leaves cultured on the medium supplemented with 0.5 mg/l 2,4-D in the light condition for 16 weeks (Table 1). These results differ from that of Babu *et al.* (1992) who reported callus formation on the young leaves of *Zingiber officinale* Rosc. (ginger) cv. Maran cultured on MS medium containing 2-5 mg/l 2,4-D. Kackar *et al.* (1993) induced callus formation from young leaf segments of ginger on MS medium added with dicamba. Samsudeen *et al.* (2000) induced ginger anther to develop callus on MS medium supplemented with 2-3 mg/l 2,4-D. Prakash *et al.* (2004) obtained semi-friable callus from leaf sheath explants of *Curcuma amada* Roxb. on MS medium added with 2 mg/l 2,4-D. Moreover, Salvi *et al.* (2001) induced callus from leaf base of turmeric on MS medium supplemented with 2 mg/l dicamba, 2 mg/l picloram or 5 mg/l NAA in combination with 0.5 mg/l BA. Callus was induced more effectively in the light than in the dark condition. These results differ from Malamug *et al.* (1991) who reported callus induction from shoot tips of ginger on MS medium containing 1 mg/l BA and 0.5 mg/l 2,4-D in the dark condition. High contamination of cultures was reported when rhizomes or vegetative buds are used as explants for initiation of culture. By using the leaf tissue as explants this problem was eliminated almost completely. In *Cornukaempferia*, 2,4-D was used for induction of callus from leaf explants (see Table 1), but when callus was transferred to MS medium added with varying concentrations of NAA and BA and cultured for 16 weeks, plant regeneration failed. Varying types and concentrations of auxin and cytokinin have been successfully used to regenerate plantlets from calli of several other species of Zingiberaceae. In ginger, Malamug *et al.* (1991) reported plant regeneration from shoot tip callus on MS medium added with 1 and 3 mg/l 2,4-D. Callus could also be regenerated from the young leaf explants of ginger on MS medium supplemented with 0.2 mg/l 2,4-D and 5 mg/l kinetin or 5 mg/l BA (Babu *et al.*, 1992). Samsudeen *et al.* (2000) was able to regenerate plantlets from callus of ginger anther on MS medium supplemented with 5-10 mg/l BA and 0.2 mg/l 2,4-D. Prakash *et al.* (2004) cultured semi-friable callus from leaf sheath explants of *Curcuma amada* Roxb. on MS medium containing 2 mg/l BA and 0.5 mg/l NAA and produced optimum shoot initiation and development. In addition, Salvi *et al.* (2001) transferred callus of turmeric (*Curcuma longa* Linn.) to half strength MS medium supplemented with 5 mg/l BA in combination with TIBA or 0.1 mg/l 2,4-D, green shoot primordial were seen to differentiate

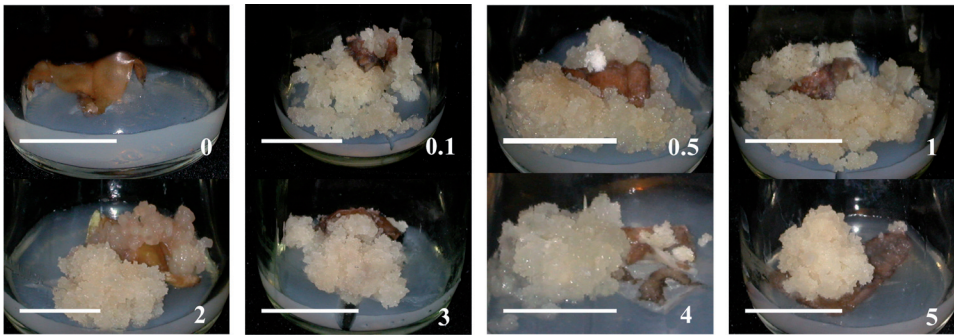


Figure 2. Callus induction from leaf explants of *Cornukaempferia larsenii* on MS medium added with 0, 0.1, 0.5, 1, 2, 3, 4 and 5 mg/l 2,4-D in the light condition (scale bars = 2.5 cm).

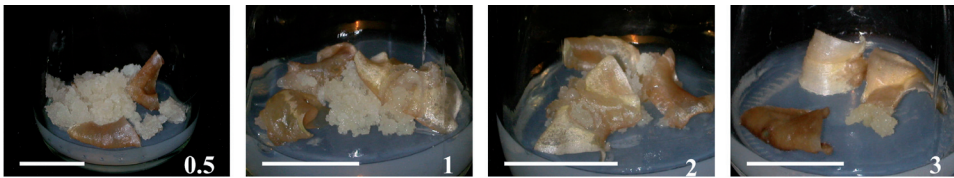


Figure 3. Callus induction from leaf explants of *Cornukaempferia larsenii* on MS medium added with 0.5, 1, 2 and 3 mg/l 2,4-D in the dark condition (scale bars = 2.5 cm).

Table 1. Effect of 2,4-D on callus induction from leaf explants of *Cornukaempferia larsenii* in the light and dark conditions for 16 weeks.

2,4-D (mg/l)	No. of explants	Light			Dark		
		No. of callus formation	% of callus formation	Average weight of callus (g) mean±SE	No. of callus formation	% of callus formation	Average weight of callus (g) mean±SE
0	15	0 a*	0	0 a*	0 a*	0	0 a*
0.1	15	3 ab	20	2.13±0.25 ab	0 a	0	0 a
0.5	15	14 d	93.33	2.61±0.40 d	4 b	26.67	2.20±0.29 b
1	15	11 cd	73.33	2.31±0.48 cd	3 ab	20	1.77±0.25 ab
2	15	8 bc	53.33	2.10±0.36 bc	3 ab	20	1.40±0.40 ab
3	15	7 bc	46.67	2.16±0.35 bc	2 ab	13.33	1.00±0.14 ab
4	15	7 bc	46.67	2.06±0.38 bc	0 a	0	0 a
5	15	6 bc	40	1.95±0.40 b	0 a	0	0 a

*In each column the values with the different letters differ significantly (P = 0.05) as determined by DMRT (see text).

from the surface of the callus. On transfer of regenerating cultures to half MS media supplemented with kinetin, shoot primordial developed into well-differentiated shoots. When shoots were transferred to medium devoid of phyto-hormone, complete rooted plants were obtained. Further experiments are being performed to obtain efficient plant regeneration using different growth regulators and culture conditions.

Conclusion

This is the first report describing tissue culture of *Cornukaempferia larsenii*, a recently discovered and rare species of Thailand. We reported a successful protocol for the efficient and reliable callus induction using cultured leaf explants of this species. Plant regeneration from leaf tissue through an intermediary callus phase may be a possibility of increasing rate of somaclonal variations that can be exploited for crop improvement which are not available by conventional methods. Furthermore, regeneration of plantlets from callus is an important technique, which can be utilized in the application of tissue culture in developing new germplasm.

Acknowledgements

This study was supported by University Staff Development Program, Mahasarakham University and Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Thailand.

References

- Babu, K.N., K. Samsudeenn and M.J. Ratnambal. 1992. *In vitro* plant regeneration from leaf-derived callus in ginger. *Plant Cell, Tissue and Organ Culture* **29**: 71-74.
- Kackar, A., S.R. Bhat, K.P.S. Chandel and S.K. Malik. 1993. Plant regeneration via somatic embryogenesis in ginger. *Plant Cell, Tissue and Organ Culture* **32**: 289-292.
- Malamug, J.J.F., H. Inden and T. Asahira. 1991. Plant regeneration and propagation from ginger callus. *Scientia Horticulturae* **48**: 89-97.
- Mood, J. and K. Larsen. 1997. *Cornukaempferia*, a new genus of Zingiberaceae from Thailand. *Natural History Bulletin of the Siam Society* **45**: 217-221.

- Mood, J. and K. Larsen. 1999. New to cultivation: the genus *Cornukaempferia* in Thailand with description of a second species. *The New Plantsman* **6**: 196-205.
- Prakash, S., R. Elangomathavan, S. Seshadri, K. Kathiravan and S. Ignacimuthu. 2004. Efficient regeneration of *Curcuma amada* Roxb. plantlets from rhizome and leaf sheath explants. *Plant Cell, Tissue and Organ Culture* **78**: 159-165.
- Saensouk, P., P. Theerakulpisut and P. Chantaranothai, 2007. *Cornukaempferia larsenii* sp. nov. (Zingiberaceae): A new species from Thailand. *The Natural History Journal of Chulalongkorn University* **7**: 169-173.
- Salvi, N.D., L. George and S. Eapen. 2001. Plant regeneration from leaf base callus of turmeric and random amplified polymorphic DNA analysis of regenerated plants. *Plant Cell, Tissue and Organ Culture* **66**: 113-119.
- Samsudeen, K., K.N. Babu, M. Divakaran and P.N. Ravindran. 2000. Plant regeneration from anther derived callus cultures of ginger (*Zingiber officinale* Rosc.). *Journal of Horticultural Science & Technology* **75**: 447-450.

Evaluation of anti-oxidant and cytotoxic properties of tropical ginger, *Zingiber montanum* (J. König) A. Dietr.

G.J. SHARMA ¹, P. CHIRANGINI ¹ AND K.P. MISHRA ²

¹ Department of Life Sciences, Manipur University, Imphal-795003, India

² Radiation Biology & Health Sciences Division, Bhabha Atomic Research Centre, Mumbai-400085, India

Abstract

Many members belonging to the family Zingiberaceae are well known for their uses in traditional medicine for curing various ailments since times immemorial. The rhizomes of some medicinal Zingiberaceae are widely used in the dietary intakes. Curcumin present in turmeric and gingerol in ginger have been known to possess anti-oxidant properties. The northeast India, which lies within the Indo-Burmese mega-biodiversity 'hotspot' region, is a genetic treasure house of biological resources with good representation of Zingiberaceous species. The present studies were conducted to assess the free radical scavenging antioxidant properties of rhizome extract of *Zingiber montanum* (J. König) A. Dietr [= *Z. cassumunar* Roxb.] using various chemical assay systems like diphenyl picrylhydrazyl (DPPH), superoxide (O_2^-) and hydroxyl (OH^\cdot) radical scavenging methods. Increased percent of DPPH decoloration from 50-500 $\mu\text{g/ml}$ indicated concentration dependent scavenging activity of DPPH radicals by the crude extract of this species. Even at a low concentration of 1 $\mu\text{g/ml}$, the rhizome extract showed strong (~75%) OH^\cdot scavenging activity. Similarly, the crude extract showed a concentration dependent inhibition of O_2^\cdot radical production where a concentration of 50 $\mu\text{g/ml}$ almost showed 100% inhibition. Cytotoxicity was assessed by MTT assay using NIH 3T3 fibroblast cell line. Only 28% cytotoxicity was observed up to a concentration of 100 $\mu\text{g/ml}$. The results strongly support the therapeutic use of crude rhizome extract of *Z. montanum* for its dietary intake and use as traditional medicine, thereby suggesting its potential as promising radioprotective agent.

Introduction

Manipur, which lies within the Indo-Burmese mega-biodiversity 'hotspot' region in the northeast India, is a genetic treasure house of rich biological resources. This active 'centre of speciation' represents a zone of gene diversity

for a variety of wild as well as domesticated plants, and a secondary centre for several economically important medicinal and aromatic plants. These gene pools are invaluable resources and their sustainable utilization can, through biotechnological interventions, bring about economic growth of the region. Bioprospects of these biological wealth scattered in the potentially useful plants as '*bio-active molecules*' need to be fully explored. These are the molecules, which would help in designing and manufacturing various plant-based drug formulations.

A wide variety of phytochemicals including polyphenolics, carotenoids, terpenoids, coumarins, saponins, phytosterols, curcuminoids, etc., have been identified in several plants. The most publicized phytochemicals with antioxidant profiles have been vitamins C, E and beta-carotene. Flavonoids are widely distributed in plants and other plant products, and are powerful inhibitors of lipid peroxidation, reactive oxygen species (ROS) scavengers, inhibitors of damage by haem protein/ peroxide mixtures, metal ion binding agents and inhibitors of lipoxygenase and cyclooxygenase enzymes *in vitro*. The degree of hydroxylation and relative position of -OH groups are of prime importance in determining antioxidant activity. In whole animals, flavonoids have been reported to exert anti-inflammatory and anti-cancer effects (Read, 1995).

Frankel *et al.* (1995) reported that plant phenols in red wine exerted cardioprotective effect. Keli *et al.* (1996) suggested an inverse relationship in the incidence of coronary heart disease and stroke in elderly men with dietary intakes of flavonoids from tea, fruits and vegetables in human populations. Phenolic substances have been found to possess anti-carcinogenic and anti-mutagenic activities, the majority of these naturally occurring phenolics retain antioxidative and anti-inflammatory properties which appear to contribute to their chemopreventive or chemo-protective activity (Surh, 1999). The human body is constantly under attack from free radicals, which are highly reactive chemical entities and are fundamental to any biochemical processes representing aerobic life. They are continuously produced by the body's normal use of oxygen, such as respiration and cell-mediated immune functions, and are generated through a variety of environmental agents. Free radicals can react readily with various biomolecules, such as DNA, proteins and lipids, to cause cellular lesions, which, in turn, lead to various human diseases (Halliwell and Gutteridge, 1999). *In vitro* generated sulfur free radicals have been suitably used in experiments against a reference molecule such as curcumin, beta-carotene or retinol (Devi *et al.*, 1992; D'Aquino *et al.*, 1994) for rapid evaluation of antioxidant potentials.

The rhizomes of tropical ginger, *Zingiber montanum* (J. König) A. Dietr. (syn. *Z. cassumunar* Roxb.), abundantly found in Manipur are used in diarrhoea, colic, and used as stimulant, carminative, for flavouring food

preparations and substituting for true ginger as antidote for snakebites, and in asthma, ascites, anemia, bruises, bronchitis, dropsy and fever, and for treatment of intestinal disorders, swellings, rheumatism, numb feet and painful parts. Antioxidant molecules already reported from this plant are alflabene, cassumunene, cassumunaquinones I, II, cassumunins A, B, C and cassumunarins A, B, C (Dinter *et al.*, 1980; Masuda and Jitoe, 1994; Jitoe *et al.*, 1992). Our investigations on sulfur free radical reactivity using curcumin as a reference indicator, and its inhibition by various crude extracts of fourteen medicinal Zingiberaceous species *in vitro* showed that *Z. montanum* [as *Z. cassumunar* in the work] exhibited maximum antioxidant property (Chirangini *et al.*, 2004). In this paper, attempts have been made to screen antioxidant potentials using DPPH, hydroxyl and superoxide radical scavenging assays and cytotoxicity using NIH 3T3 mouse fibroblast cell lines.

Materials and Methods

The tropical ginger, *Zingiber montanum*, has rootstocks that are perennial. Rhizomes are bright yellow inside with strong camphoraceous scent. Leaves are oblong-lanceolate and hairy underneath. The spike-like inflorescence is oblong with ovate, reddish bracts. From the bracts, pale yellow colored flowers come out in acropetal succession. The corolla tube is as long as the bract with whitish segments, upper portion being broader and more concave. The most beautiful part of the flower, the lip or the labellum, is yellowish white in coloration with a deeply bifid midlobe. The basal auricles are large, oblong, and obtuse. The male part of the flower, the stamens are yellowish white, but shorter than the lip. The ovary is 3-celled and the ovules are many arranged in the inner angle of the cells. Plants collected from wild natural wetland habitats of Manipur grown in the Experimental Field, Department of Life Sciences, Manipur University, since July 2000, were used in these experiments. Morpho-taxonomic characters were properly recorded. Healthy, uninfected and unbruised fresh rhizomes were used for all the experiments. Herbarium voucher have been collected and deposited at Herbarium of Manipal University, Imphal (MU/LSD/Herb.32): India, Manipur, Imphal, Thoubal & Bishenpur Districts, 22.VI.2000, Chirangini *et al.* 32.

Preparation of the Zingiber montanum extract

Fresh rhizomes were washed and cleaned thoroughly in running tap water. The roots were removed along with the outer scales. After drying in between the folds of the filter paper, rhizomes were weighed and crushed with the

help of mortar and pestle. Then, it was homogenized in absolute methanol (1gm/ml). The crude extracts obtained were centrifuged twice and filtered, using Whatman No. 1 filter paper, till a clear supernatant was obtained. The supernatant was vacuum evaporated till dryness. The residue obtained was kept at 4°C for future use.

I. Antioxidative capacity - The antioxidative capacity of *Z. montanum* extract was examined by comparing it to the activity of known antioxidants, such as ascorbic acid, by the following chemical assays - scavenging of DPPH radical and oxygen radicals such as superoxide, and hydroxyl radicals.

DPPH assay

The DPPH assay was carried out as described by Cuendet et al. (1997) with slight modification. The reaction mixture consisted of 250 µM DPPH in 100% methanol with 50-500 µg/mL of the crude extract of *Z. montanum* or 0.01-0.1 mM of vitamin C. After a 30-min incubation period in the dark at room temperature, the absorbance was read against a blank at 517 nm. Percentage inhibition was determined by comparison with a methanol treated control group. The percentage of DPPH decoloration was calculated as follows:

$$\% \text{ DPPH decoloration} = [1 - \text{O.D. sample} / \text{O.D. control}] \times 100$$

The degree of decoloration indicates the free radical scavenging efficiency of the substances. Values are presented as mean \pm standard deviation of three determinations.

Hydroxyl radical scavenging activity assay

Hydroxyl radical scavenging activity assay was carried out by measuring the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe(II)/ascorbate/EDTA/H₂O₂ system. The attack of the hydroxyl radical to deoxyribose leads to thiobarbituric acid reactive substances (TBARS) formation (Kunchandy and Rao, 1990). Various concentrations of the extracts were added to the reaction mixture containing 2.8 mM deoxyribose, 25 µM FeCl₃, 100 µM EDTA, 100 µM ascorbic acid, 2.8 mM H₂O₂, and 5 mM phosphate buffer (pH 7.4), making up a final volume of 1.0 mL. The reaction mixture was incubated at 37 °C for 1 h. The formed TBARS were measured by the method of Ohkawa et al. (1979). One milliliter of thiobarbituric acid (TBA, 1% w/v in 50 mM NaOH) and 1 mL of trichloroacetic acid (TCA, 2.8% w/v) were added to test tubes and incubated at 100°C for 30 min. After the mixtures cooled, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Reactions were carried out in triplicate.

Inhibition (*I*) of deoxyribose degradation in percent was calculated in the following way:

$$I = (A_0 - A_1)/A_0 \times 100$$

where A_0 is the absorbance of the control reaction (containing all reagents except the test compound) and A_1 is the absorbance of the test compound.

Inhibition of superoxide radical

Superoxide radical generated by the hypoxanthine/xanthine oxidase system was determined spectrophotometrically by monitoring the product of nitroblue tetrazolium (NBT). Various concentrations of the extracts were added to the reaction mixture containing 100ml of 30 mM EDTA (pH 7.4), 10ml of 30 mM hypoxanthine in 50 mM NaOH, 200ml of 1.42 mM NBT and the final volume of 3 ml was made up by 50 mM PO₄ Buffer (pH 7.4). After adding 100ml of 0.5 U/ml xanthine oxidase, the reaction mixture was incubated for 30 min at 25°C. The absorbance was read at 560 nm and compared with control samples in which the enzyme, xanthine oxidase, was not included.

The percent inhibition of superoxide radicals was calculated from the optical density of the treated and control samples.

$$\text{Inhibitory effect (\%)} = [(A_{560 \text{ control}} - A_{560 \text{ sample}})/A_{560 \text{ control}}] \times 100$$

II. Cytotoxicity studies - *In vitro* cytotoxic effect of crude extracts of *Z. montanum* was studied on normal mouse embryo fibroblast cell (NIH/3T3). The methanol extract was dissolved in dimethylsulphoxide (DMSO). The cell line NIH/3T3 was provided by National Centre for Cell Science, Pune, India.

Cell culture conditions

Stock cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal calf serum supplemented with 0.04M NaHCO₃, 0.006% penicillin and 0.025% streptomycin at 37°C in an atmosphere of 5% CO₂ and 95% humidity. The medium was changed every three days. Monolayer cells were plated out at 2x10⁴ cells/well in 96-well microtitre plate. The cell growth was found to be exponential during 2-3 days in the medium.

MTT assay

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] easily

enters cells. The esterases present in viable cells can cleave MTT to form purple-colored formazan crystals which are then solubilized. Color produced is directly proportional to cell viability.

The cytotoxic effect of the crude extracts, expressed as cell viability, was assessed by MTT staining experiment (Mosmann, 1983). Briefly, confluent cultures of NIH/3T3 cells were treated with medium containing the methanol extract of *Z. montanum* at concentrations from 2-400 µg/mL. The extract was first dissolved in absolute DMSO and then in DMEM. The final concentration of DMSO in the test medium and controls was <1%. Cells were exposed for 48 hr to test medium with or without the extracts. The medium was removed and 100 µl of MTT solution (1 mg/ml in PBS) was added to each well of 96 multiwell plates and the plates were incubated for additional 3 hr at 37°C. Finally, 100 µl of 10 % (w/v) sodium dodecyl sulfate in 0.01N HCl was added to each well and the absorbance was measured at 550 nm using the ELISA reader (Biotek System). Each concentration of plant extract was tested in hexaplicate and repeated twice in separate experiments.

Percentage viability was calculated from the following relation:

$$\% \text{ Viability} = [1 - \text{OD}_{\text{sample}}/\text{OD}_{\text{control}}] \times 100$$

Results and Discussion

DPPH Assay: As shown in Fig. 1, Vitamin C and methanol extract of *Z. montanum* were able to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The strongest effect was observed in 0.1mM Vitamin C with 91% DPPH decoloration. Up to 86% DPPH decoloration was observed in case of 500 µg/mL *Z. montanum* extract that is having similar effect with 0.08mM Vitamin C (84%).

Hydroxyl radical (OH[•]) scavenging: When the methanol extract of *Z. montanum* was incubated with the reaction mixture used in the deoxyribose degradation assay, they removed hydroxyl radicals from the sugar and prevented its degradation. As shown in Fig. 2, *Z. montanum* extract, even at a concentration of 1 µg/mL there is 59% scavenging of the OH[•] radical showing very potent activity. The following results show that there is no dose dependent reponse for OH[•] radical scavenging capacity.

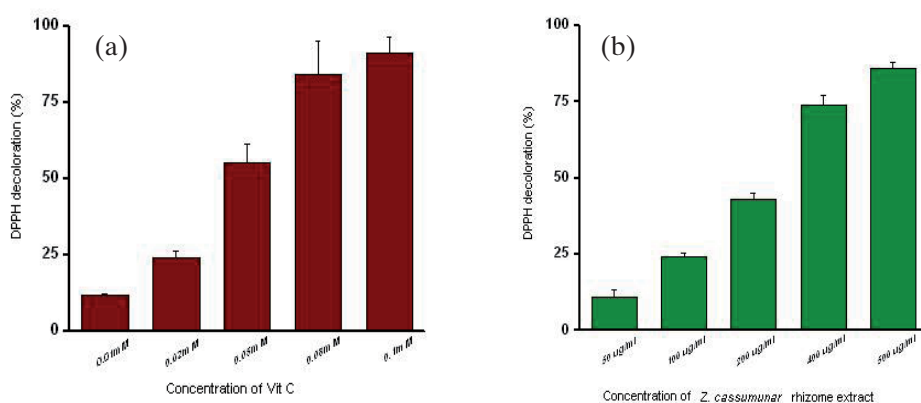


Figure 1. DPPH radical scavenging activity of (a) Vitamin C and (b) *Z. montanum* rhizome.

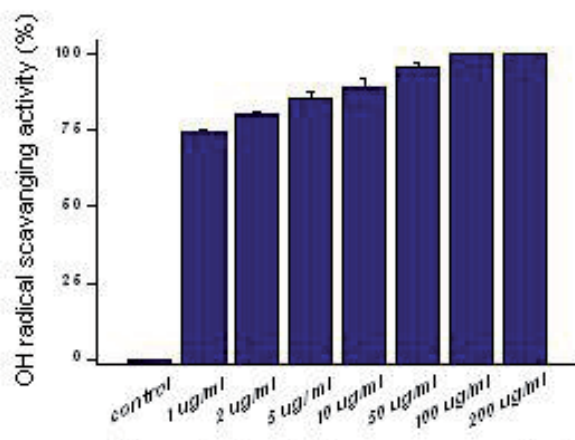


Figure 2. Hydroxyl radical (OH) scavenging activity of *Z. montanum* rhizome extract.

Inhibition of superoxide radical: Methanol extract of *Z. montanum* were found to scavenge the superoxide radicals generated from the hypoxanthine/xanthine oxidase method. There is a dose dependent response of the compound as well as the extract (fig. 3). The results do point towards an increased trend in the response with small increases in the concentration of the extract.

Cytotoxicity testing by MTT assay: Methanol extracts of *Z. montanum* tested for cytotoxicity against normal mouse fibroblast cell line using standard MTT assay showed very low toxicity up to 100µg/ml (fig.4). Only 24% of the cells survive the toxic effect of *Z. montanum* extract at a concentration of 200 µg/mL.

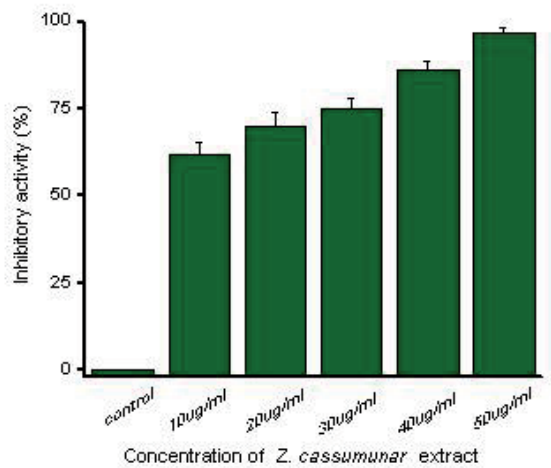


Figure 3. Inhibition of superoxide radical production by *Z. montanum* rhizome.

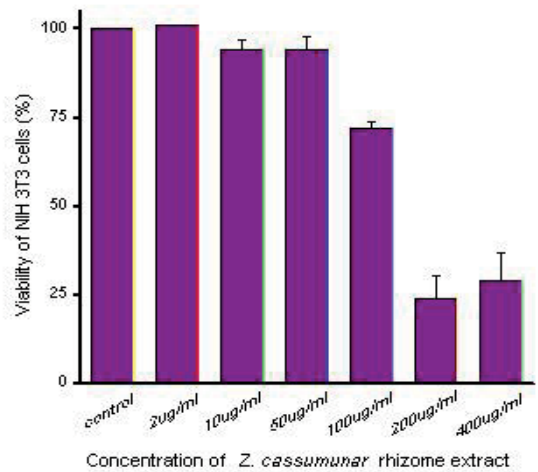


Figure 4. Cytotoxicity of methanolic extracts of *Z. montanum* rhizome.

Sulfur free radicals (GS[•]) formed in gamma irradiated aqueous glutathione (GSH) solution could easily oxidize the chrome orange-yellow compound curcumin - its depletion increasing with increasing dose of radiation. Supplementation of the crude rhizome extract reduced the depletion of curcumin significantly. The inhibition of curcumin depletion in the rhizome extract-added reaction solution varied with the species showing different protective indices (PIs). A relative comparison of PIs at 75 Gy exposure showed *Zingiber cassumunar* [currently accepted name *Z. montanum*] > *Kaempferia galanga* > *Hedychium flavascens* > *Zingiber officinale* >

Hedychium coccineum > *Curcuma caesia* > *Curcuma amada* > *Alpinia allughas* > *Curcuma leucorrhiza* > *Hedychium coronarium* > *Alpinia galanga* (Chirangini *et al.*, 2004).

Free radicals generated either by endogenous metabolism or external environmental agents are harmful to cellular constituents, such as proteins, lipids, DNA and carbohydrates, and result in possible alteration of cell function (Davies *et al.*, 1987; Dezwart *et al.*, 1999; Gebicki & Gebicki, 1999). ROS are known to be carcinogens and act at several stages in malignant transformation (Cerutti, 1994), including permanent DNA sequence changes in the form of point mutations, deletions, gene amplifications, and rearrangements which may result in the activation of proto-oncogenes or the inactivation of tumor-suppressor genes (Hsie *et al.*, 1986; Moraes *et al.*, 1990). The role of these ROS in oxidative damage to the membranes and mechanism of apoptotic death of thymocytes have been well elaborated (Bhosle *et al.*, 2002; Mishra & Hota, 2003; Pandey & Mishra, 2003).

The body's antioxidant defense system is composed of various antioxidants present in the plasma or biological fluids in a reduced form. While scavenging/neutralizing the free radicals, they are either oxidized or exhausted. An external anti-oxidant can prevent oxidative damage by inhibiting the generation of reactive species, scavenging free radicals, or raising the endogenous level of antioxidant defense. To maintain antioxidant level in the body, external supplementation is necessary for healthy living (Halliwell & Gutteridge, 1989). Supplementation of natural antioxidants through a balanced diet could be more effective, and also more economical than supplementation of an individual antioxidant, such as Vitamin C or E, in protecting the body against oxidative damage under various conditions (Wang *et al.*, 1996). It has been known that several medicinal plants contain 'active principles' possessing antioxidant properties. In Manipur, a number non-conventional and under-used plant-based food, particularly belonging to the family Zingiberaceae, possessing rich antioxidant properties are consumed by the people which perhaps may be the basis for low incidence of cancers (Chirangini *et al.*, 2004).

Although some work has been done on the radioprotective effect of curcumin extracted from *Curcuma longa* (Inano & Onado, 2002) and ginger rhizome (Jagetia *et al.*, 2003), detailed studies have not been carried out as yet on the potential antioxidant properties of *Z. montanum*. The present studies made using the DPPH, hydroxyl and superoxide radical scavenging assays, therefore, have reaffirmed the antioxidant potentials of *Z. montanum* in a much more elaborate manner, and are hence quite relevant. Although the crude extracts of these various plants have numerous medicinal potentials, clinical applications can be made only after extensive research on the bioactivity, mechanism of action, pharmaco-therapeutics

and toxicity studies of the different compounds present in these plants. Recent years have seen an increased enthusiasm in treating various diseases with natural products. Many phytonutrients or phytochemicals having very high antioxidant profile need to be investigated for their application as anti-tumour or radioprotective agents to inhibit acute and chronic effects and even mortality after irradiation. It is expected that some novel compounds may turn out to have very rich radioprotective property which could be comparatively better than that of amifostine, the only agent that reduces radiation induced toxicity during clinical trials.

It can be concluded that *Z. montanum* rhizome extract possesses significant radical scavenging and anti-oxidant properties. Besides being an efficient scavenger, cytotoxicity of *Z. montanum* rhizome extract above 100 µg/ml indicate its significant potential as an anti-tumor agent. The results shown above do strongly support the therapeutic applicability of *Z. montanum* extract for its dietary intake and use in traditional system of medicine. Based upon these significant anti-oxidant properties, there is an urgent need for investigation of the rhizome extract of *Z. montanum* for its radioprotective activity using suitable in vivo mammalian test systems.

Acknowledgement

The authors are thankful to the Board of researches in Nuclear Sciences, Department of Atomic Energy, Government of India, Mumbai for providing financial support [vide Grant No. 2004/37/29/BRNS/2130]. We thank Dr. Jana Leong-Skornickova (SING) for comments regarding the nomenclature of the names *Zingiber montanum* and *Z. cassumunar*.

References

- Bhosle, S.M., B.N. Pandey, N.G. Huilgol and K.P. Mishra. 2002. Membrane oxidative damage and apoptosis in cervical carcinoma cells of patients after radiation therapy. *Methods in Cell Science* **24** (1-4): 65-68.
- Cerutti, P.A. 1994. Oxy-radicals and cancer. *Lancet* **344**: 862-863.
- Chirangini, P., G.J. Sharma, and S.K. Sinha. 2004. Sulfur free radical reactivity with curcumin as reference for evaluating antioxidant properties of medicinal Zingiberales. *Journal of Environmental Pathology, Toxicology and Oncology* **23**: 227-236.
- Cuendet, M., K. Hostettmann and O. Potterat. 1997. Iridoid glucosides

with free radical scavenging properties from *Fragarea blumei*. *Helvetica Chimica Acta* **80**: 1144-1152.

Davies, K.J.A., M.E. Delsingore and S.W. Lin. 1987. Protein damage in degradation by oxygen radicals. *Journal of Biological Chemistry* **262**: 9902-9907.

D'Aquino, M., C. Bullion, M. Chopra, D. Devi, S. Devi, G. James, E. Komuro, S. Kondu, E. Niki, F. Raza, F. Robertson, J. Sharma and R. Willson. 1994. Sulfhydryl free radical formation, enzymatically, by sonolysis, by radiolysis and thermally: vitamin A, curcumin, muconic acid, and related conjugated olefins as references. *Methods in Enzymology* **233**: 34-46.

Devi, M.D., H.S. Devi, G.J. Sharma, G. James, I. Tipping and R.L. Willson. 1992. Curcumin: visible destruction of the conjugated unsaturated compound as a reference indicator or sulphur free radical reactivity. *International Journal of Radiation Biology* **62**: 105.

Dezward, L.L., J.H.N. Meerman, J.N.M. Commandeur and N.P.E. Vermeulen. 1999. Biomarkers of free radical damage: applications in experimental animals and in humans. *Free Radicals in Biology and Medicine* **26**: 202-206.

Dinter, H., R. Haensel and A. Pelter. 1980. Isolation of two phenylbutadiene dimers and one monomeric 4-phenylbut-3-ene from *Zingiber montanum* Roxb. *Zeitschrift für Naturforschung C: Bioscience* **35**: 156-158.

Frankel, E.N., A.L. Waterhouse and P.L. Teissedre. 1995. Principal phenolic phytochemicals in selected California wines and between organisms. *Journal of Agricultural and Food Chemistry* **43**: 890-894.

Gebicki, S. and J.M. Gebicki. 1999. Cross-linking of DNA and protein induced by hydroperoxides. *Biochemistry Journal* **338**: 629-636.

Halliwell, B. and J.M.C. Gutteridge. 1989. *Free Radicals in Biology and Medicine*, Clarendon Press, Oxford.

Hsie, A., I. Recio, D. Katz, C. Lee, M. Wagner and R. Schenley. 1986. Evidence for reactive oxygen species inducing mutations in mammalian cells. *Proceedings of National Academy of Science* **83**: 9610-20.

- Inano, H. and M. Onoda. 2002. Radioprotective action of curcumin extracted from *Curcuma longa* Linn: inhibitory effect on formation of urinary 8-hydroxy-2'-deoxyguanosine, tumorigenesis, but not mortality, induced by gamma-ray irradiation, *International Journal of Radiation Oncology, Biology and Physics* **53**: 735-743.
- Jagetia, G.C., M.S. Baliga, P. Venkatesh and J.N. Ulloor. 2003. Influence of ginger rhizome (*Zingiber officinale* Rosc.) on survival, glutathione and lipid peroxidation in mice after whole-body exposure to gamma radiation. *Radiation Research* **160**: 584-592.
- Jitoe, A., T. Masuda, I.G.P. Tengah, D.N. Suprpta, I.W. Gara and N. Nakatani. 1992. Antioxidant activity of tropical ginger extracts and analysis of the contained curcuminoids. *Journal of Agricultural and Food Chemistry* **40**: 1337-1340.
- Keli, S.O., M.G. Hertog, E.J. Feskens and D. Kromhout. 1996. Dietary flavonoids, anti-oxidant vitamins and incidence of stroke: the Zutphen study. *Archives of Internal Medicine* **156**: 637-642.
- Kunchandy, E. and M.N.A. Rao. 1990. Oxygen radical scavenging activity of curcumin. *International Journal of Pharmacognosy* **58**: 237-240.
- Masuda, T. and A. Jitoe. 1994. Antioxidative and anti-inflammatory compounds from tropical gingers: isolation, structure determination and activities of cassumunins A,B and C, new complex curcuminoids from *Zingiber cassumunar*. *Journal of Agricultural and Food Chemistry* **42**: 1850-1856.
- Mishra, K.P. and H. Ohta. 2003. Radiation membrane damage and apoptotic death of mouse thymocytes *in vitro*: ESR and fluorescence studies. *Memoirs of Graduate School of Science and Technology, Kobe University* **21A**: 43-48.
- Moraes, E.C., S.M. Keyse and R.M. Tyrell. 1990. Mutagenesis by hydrogen peroxide treatment of mammalian cells: a molecular basis. *Carcinogenesis* **11**: 283-293.
- Mossman, T.R. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* **65** (1-2): 55-63.

- Okhawa, H., N. Ohoshi and K. Yagi. 1979. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* **95**: 351-358.
- Pandey, B.N. and K.P. Mishra. 2003. Oxidative membrane damage and its involvement in gamma radiation-induced apoptotic cell death. *Iranian Journal of Radiation Research* **1**: 17-22.
- Read, M.A. 1995. Flavonoids: naturally occurring anti-inflammatory agents. *American Journal of Pathology* **147**: 235.
- Surh, Y. 1999. Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. *Mutation Research* **428**: 305-327.
- Wang, H., D. Cao and P.L. Ronald. 1996. Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry* **44**: 701-705.

The Genus *Curcuma* L. (Zingiberaceae): Distribution and Classification with Reference to Species Diversity in Thailand

P. SIRIRUGSA ¹, K. LARSEN ² AND C. MAKNOI ³

¹ Faculty of Science, Prince of Songkla University, Hat Yai 90112, Thailand.

² Department of Systematic Botany, Aarhus University, Building 540,
Ny Munkegade 116, DK-8000 Aarhus C., Denmark.

³ Queen Sirikit Botanic Garden, The Botanical Garden Organization,
P.O. Box 7, Maerim, Chiang Mai 50180, Thailand

Abstract

The genus *Curcuma* L. is one of the largest genera in the Zingiberaceae, with about 80 species, and distributed throughout tropical Asia from India to South China, Southeast Asia, Papua New Guinea and Northern Australia. In Thailand, thirty-eight species have been found. Taxonomic knowledge of this genus is necessary for citing correctly the species used commercially as spices, ornamentals and medicines. Formerly, *Curcuma* was a member of the tribe Hedychieae. According to the new classification of the Zingiberaceae proposed by Kress *et al.* (2000), this genus belongs to the tribe Zingibereae. This paper presents an overview of the genus *Curcuma* and its species diversity in Thailand. The infrageneric classification of the genus based on morphology and molecular evidences with reference to species diversity in Thailand is discussed. The representative taxa, their distribution and uses are provided.

Introduction

Curcuma is one of the largest genera in the Zingiberaceae which comprises approximately 80 species (Larsen, 2005). It is widely distributed in the tropics of Asia from India to South China, Southeast Asia, Papua New Guinea and Northern Australia (Fig. 1). In Thailand they are normally found in the teak, pine or dipterocarp forests at the altitude 500-900 m. Some species, such as *C. alismatifolia* Gagnep., grows well in the open areas. The most common species, *C. parviflora* Wall., grows in wide range of altitudes from 100 – 1300 m on limestone hills. Generally, most *Curcuma* grows well in loose and sandy soil in shaded areas.



Figure 1. Distribution of the genus *Curcuma*

Morphological Characters of *Curcuma*

The habit of *Curcuma* is a rhizomatous herbaceous plant, comprising of underground parts, leafy shoot and leaf blades (Fig. 2).

Underground parts. At the base of the aerial shoot, the stem consists of erect ovoid or globose structure (primary rhizome), bearing few to many horizontally branches, and roots. However, branched rhizomes are rarely produced in some species. The roots often produce ellipsoid tubers. Inner part of rhizomes varies in various colours, i.e., white, cream, yellow, orange, blue and bluish-green. Some species have a unique colour of rhizomes which are useful for identification, such as the bluish-green rhizome in *C. aeruginosa* Roxb.

Leafy shoots. These are 1-2 m high, forming a pseudostem by the leaf-sheaths and surrounded by the leafless sheaths at the base. Leaf blades are usually large, lanceolate or elliptic, rarely linear with or without the purple stripe along either side of the midrib.

Inflorescence. This occurs either terminally on the leaf-shoot, with the peduncle enclosed by the leaf sheaths, or on the separate shoot with the peduncle enclosed by the bladeless sheaths. The inflorescence can be cylindric, conic or ovoid in shape.

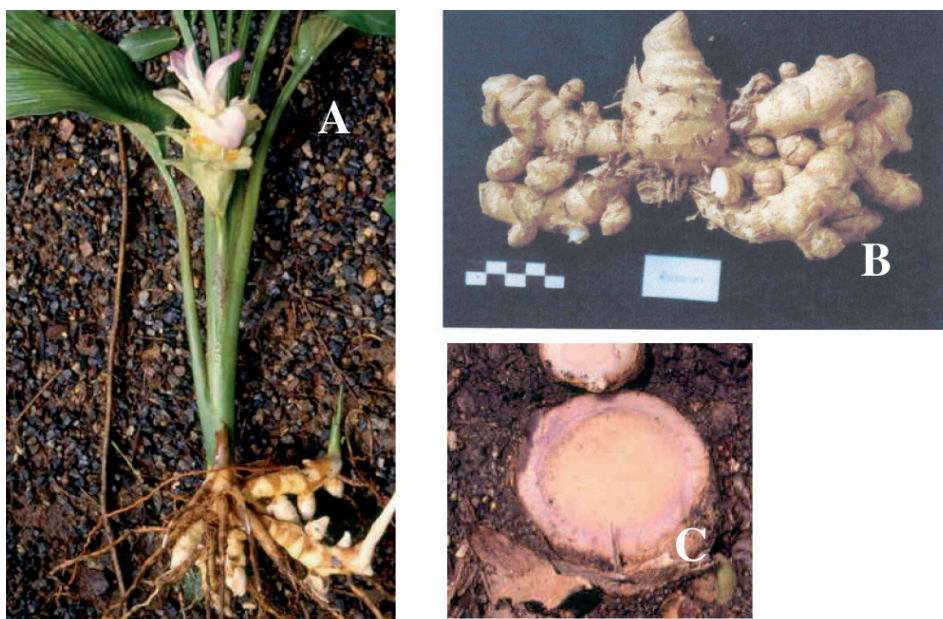


Figure 2. *Curcuma*. A. Habit; B. Primary rhizome with branches; C. Rhizome of *C. caesia*.

Bracts. Bracts are usually large and joined to each other forming pouches at the base, the free ends of the bracts are normally wide spread, each subtending a cincinnus of 2-10 flowers. In many species the uppermost bracts, which are called “coma”, are longer than the rest and differently coloured. They are usually sterile.

Flowers. Flowers are enclosed by bracteoles, comprising of the following floral parts: **Calyx** is tubular, unequally toothed, deeply divided along one side. **Corolla-tube** is more or less funnel shaped; **corolla-lobes** are unequal, the dorsal slightly larger than the lateral ones, and its apex is hooded. **Staminodes** are petaloid, elliptic, oblong or linear. **Labellum** has a thickened middle part and thinner lateral lobes which overlap the staminodes. **Stamen** has a short and broad filament, and a constricted apex. **Anther** is versatile, with or without spurs, and the anther-crest is usually small. **Spurs** vary in several shapes and sizes, and they are important characters for infra-generic classification. **Ovary** is glabrous or pubescent, and 3-lobed. **Stylodes** can be present. **Capsule** is ellipsoid, and **seeds** are arillate.

Flower forms (Fig. 3). The different arrangement of staminodes and corolla-lobes made up the 2-formed flowers, i.e., *closed form*: the staminodes are wrapped by the dorsal corolla-lobe; and the *open form*: the staminodes are free from the dorsal corolla-lobe.

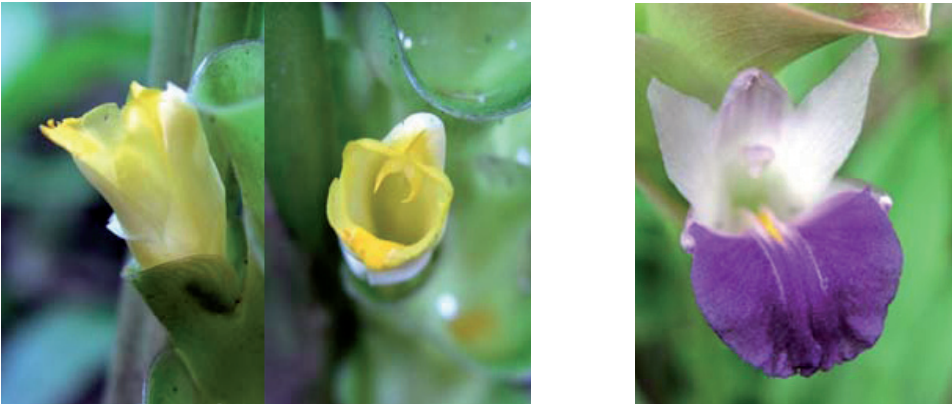


Figure 3. *Curcuma* flowers, showing the closed form (left) and open form (right).

Distinguished Characters

It is easy to distinguish *Curcuma* from other genera of Zingiberaceae by the following characters: the joining bracts to form pouches; flowers borne in cincinni, subtended by bracteoles and bracts; and the sterile and differently coloured coma bracts.

Classification of Zingiberaceae

Zingiberaceae (Burt & Smith, 1972)			Zingiberaceae (Kress <i>et al.</i> , 2002)	
Tribe	Genus	Subfamily	Tribe	Genus
Hedychieae	<i>Curcuma</i>	Siphonochiloideae	Siphonochileae	
Zingibereae		Tamijioideae	Tamijieae	
Alpinieae		Alpinioideae	Alpinieae	
Globbeae			Riedelieae	
		Zingiberoideae	Zingibereae	<i>Curcuma</i>
			Globbeae	

According to the system of Burt & Smith in 1972, which was accepted for many years, the family Zingiberaceae was classified into 4 tribes and *Curcuma* was placed in the tribe **Hedychieae**. In the year 2002 a new system was proposed by Kress *et al.* Zingiberaceae was classified into 4 subfamilies and 6 tribes (as shown above), and *Curcuma* has been placed in the tribe **Zingibereae**.

The genus *Curcuma* shows great morphological variations, the overlapping similarities among them made confusion in the identification of species. Several systems of the infra-generic classification of *Curcuma*

have been developed. Some of them are shown below.

Infra-generic classification of *Curcuma*

Baker (1892)	Schumann (1904)	Valeton (1918)
	Subgenus	Subgenus
	<i>Eucurcuma</i>	<i>Eucurcuma</i>
Section	Section	Section
<i>Exantha</i>	<i>Exantha</i>	<i>Exantha</i>
Section	Section	Section
<i>Mesantha</i>	<i>Mesantha</i>	<i>Mesantha</i>
	Subgenus	Subgenus
Section <i>Hitcheniopsis</i>	<i>Hitcheniopsis</i>	<i>Paracurcuma</i>

Baker (1890) divided *Curcuma* into three sections: Section *Exantha* (the spikes separate from the shoot), Section *Mesantha* (the spikes borne on the shoot either with or without leaves), and Section *Hitcheniopsis* (characterized by autumnal spikes from the centre of the tuft of leaves; bracts are very obtuse, adnate at the sides and spreading at the tip).

Schumann (1904) divided the genus into subgenus *Eucurcuma* and raised the taxonomic rank of *Hitcheniopsis* Baker to subgenus. Subgenus *Eucurcuma* is again divided into section *Exantha* and section *Mesantha*.

Valeton (1918) divided the genus into subgenus *Eucurcuma* and *Paracurcuma* and divided the subgenus *Eucurcuma* into section *Exantha* and *Mesantha*. Subgenus *Paracurcuma* was characterized by bracts connected at least partly beyond the middle and often very numerous. Spike is cylindrical, with comparatively short coma bracts. Anther spurs are very short (not a quarter of the anther) or none.

However, more information for resolving the problems of identification of this genus is still required.

Taxonomic Treatments

The previous taxonomic works on *Curcuma* from whole range of the distribution of the genus are shown below. From these records and also from recent collections from Thailand it can therefore be estimated , that there are over 90 species of *Curcuma* in the world.

Baker (1890) recorded 29 species from India (10 species are not found in Thailand).

Holttum (1950) reported 9 species from Malay Peninsula (1 species is not found in Thailand).

Backer & Bakhuizen Van Den Brink (1963) reported 18 species from Java (11 species are not found in Thailand).

Sabu and Mangaly (1996) presented 18 species from South India (8 species are added to the Baker's list, and these are not found in Thailand).

Wu and Larsen (2000) published 12 species from China (6 species are not found in Thailand).

Newman *et al.* (2004) reported 20 species from Malesia (16 species are not found in Thailand).

***Curcuma* Species in Thailand**

In Thailand, 38 species of *Curcuma* are now recognized. Among them, six species are undescribed (no. 33-38), three species are new records for Thailand (11, 21, 25), three known species are endemic to Thailand (7, 10 and 12) and eight species are cultivated for food and spices (1, 3, 5, 9, 17, 18, 30, and 31).

- | | |
|---------------------------------------|---|
| 1. <i>C. aeruginosa</i> Roxb. | 21. <i>C. pierreana</i> Gagnep. |
| 2. <i>C. alismatifolia</i> Gagnep. | 22. <i>C. rhabdota</i> Siriruga & M.F. Newman |
| 3. <i>C. amada</i> Roxb. | 23. <i>C. roscoeana</i> Wall. |
| 4. <i>C. angustifolia</i> Roxb. | 24. <i>C. rubescens</i> Roxb. |
| 5. <i>C. aromatica</i> Salisb. | 25. <i>C. rubrobracteata</i> Skornick., M.
Sabu & Prasanthk. |
| 6. <i>C. aurantiaca</i> Zijp | 26. <i>C. singularis</i> Gagnep. |
| 7. <i>C. bicolor</i> Mood & K. Larsen | 27. <i>C. sparganiifolia</i> Gagnep. |
| 8. <i>C. cochinchinensis</i> Gagnep. | 28. <i>C. stenochila</i> Gagnep. |
| 9. <i>C. comosa</i> Roxb. | 29. <i>C. viridiflora</i> Roxb. |
| 10. <i>C. ecomata</i> Craib | 30. <i>C. zanthorhiza</i> Roxb. |
| 11. <i>C. flaviflora</i> S.Q. Tong | 31. <i>C. zedoaria</i> (Christm.) Roscoe |
| 12. <i>C. glans</i> K. Larsen & Mood | 32. <i>C. larsenii</i> Maknoi & Jenjitt. |
| 13. <i>C. gracillima</i> Gagnep. | 33. <i>C. sirirugsae</i> (in prep.) |
| 14. <i>C. harmandii</i> Gagnep. | 34. <i>C. sp.</i> |
| 15. <i>C. latifolia</i> Roscoe | 35. <i>C. sp.</i> |
| 16. <i>C. leucorhiza</i> Roxb. | 36. <i>C. sp.</i> |
| 17. <i>C. longa</i> L. | 37. <i>C. sp.</i> |
| 18. <i>C. mangga</i> Valetton & Zijp | 38. <i>C. sp.</i> |
| 19. <i>C. parviflora</i> Wall. | |
| 20. <i>C. petiolata</i> Roxb. | |

Infra-generic Classification of *Curcuma* in Thailand

Based on morphological characters, 38 species of *Curcuma* in Thailand can be divided into 5 groups. Anther types of *Curcuma* are the important distinctive characters for the classification into groups (Fig. 4).

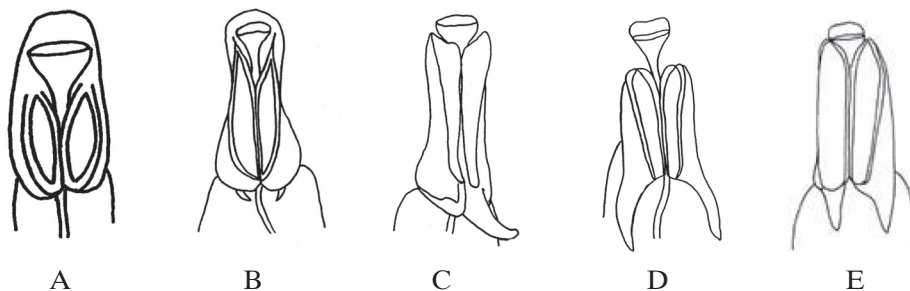


Figure 4. Anther types: A. “*Alismatifolia*” type; B. “*Cochinchinensis*” type; C. “*Ecomata*” type; D. “*Longa*” type; E. “*Petiolata*” type.

Five Groups of *Curcuma* in Thailand

The distinguished characters, representative species, with short information and illustrations of the five groups of *Curcuma* in Thailand are presented below (Fig. 5).

1. “*Alismatifolia*” group

Distinguished characters are:

- Anther spurs absent;
- Stylodes absent.

Eight species are in this group: *C. alismatifolia*, *C. gracillima*, *C. harmandii*, *C. parviflora*, *C. rhabdota*, *C. sparganiifolia* and two new species.

C. alismatifolia Gagnep.

This species is native to Thailand in the northeast and distributed to Laos and Cambodia. It is commonly found in open areas in the pine or deciduous forests at altitude 1300 m above sea level. *C. alismatifolia* has become an important economic plant for the aesthetics of its inflorescences. It is easily identified by the long, slender and stiff peduncle, the large and bright pink coma bracts. The newly improved cultivars of this species, such as the white-bract form, have also appeared in the markets for more than ten years (Wanakrairote in 1996).

C. parviflora Wall.

This species was originally found in Myanmar and distributed throughout tropical Asia. It grows in wide range of altitudes from 300 to over 1000 m above sea level. It is also commercially popular for cut flowers or as potted plants. The plant is small, about 30 cm tall. Its inflorescence is attractive with the white coma bracts.

C. rhabdota Sirirugsa & M. F. Newman

This species was described in 2000 after it has become popular as ornamental plant. It is widely spread both in Thailand and other countries. It was first known from a selling at Chong Mek market at the Laos-Thai border in Ubon Ratchathani province, and was collected from Laos as told by the seller. This plant was brought to grow in the Royal Botanic Garden Edinburgh, from which it was taken as the type specimen. However, it has been found later that this species commonly grows in Ubon Ratchathani Province of Thailand.

C. harmandii Gagnep.

This species is native to Cambodia and distributed to eastern, southeastern and central Thailand. The uniqueness of its bracts with dark green, lanceolate and reflexed apex is attractive and easily recognized for this species.

C. sparganiiifolia Gagnep.

This is a native of Indochina and is distributed to northeastern, eastern and southeastern Thailand. It is a small plant; its leafy shoot is about 15-20(-30) cm tall. This species can be distinguished by its spike with slender peduncle, pinkish-purple and suborbicular bracts.

2. “Cochinchinensis” group

Distinguished characters are:

- Anther spurs filamentous;
- Stylodes shortly cylindrical.

Two species are in this group: *C. cochinchinensis* and *C. pierreana*

C. pierreana Gagnep.

The species is native to Cambodia. In Thailand, it is found only in the northeast. Its sessile inflorescence, white staminodes with large purple blotches apices are distinctive characters for this species.

3. “Ecomata” group

Distinguished characters are:

- Anther spurs broad and blunt;
- Stylodes long and slender.

Seven species are in this group: *C. bicolor*, *C. ecomata*, *C. flaviflora*, *C. glans*, *C. singularis*, *C. stenochila*, and one new species)

C. bicolor Mood & K.Larsen

This species is endemic to Thailand, which was described in 2001. The plant is 40-60 cm tall. It can be distinguished from other species in the “**Ecomata**” group by its yellow staminodes with red blotches at the bases.

C. ecomata Craib

This is also endemic to Thailand. It is easily recognized by its purple labellum with the dark yellow midband.

C. flaviflora S.Q. Tong

This is a Yunnan plant and distributed to northern Thailand. It grows at high altitude from 1200-1400 m above sea level. This species can be distinguished by its bright yellow flowers.

C. glans K.Larsen & Mood

This is another endemic species of Thailand that was recently described in 2001. Its yellow staminodes with red apices covering with densely glandular hairs are the important distinctive characters of this species.

It is noted that the above four species of “**Ecomata**” group are uncommon in Thailand.

4. “**Longa**” group

Distinguished characters are:

- Anther spurs acicular, inwardly curved;
- Stylodes cylindrical;
- Bract apex acute.

Thirteen species are in this group: *C. aeruginosa*, *C. amada*, *C. angustifolia*, *C. aromatica*, *C. comosa*, *C. latifolia*, *C. leucorrhiza*, *C. longa*, *C. mangga*, *C. rubescens*, *C. viridiflora*, *C. zanthorrhiza*, and *C. zedoaria*.

C. aeruginosa Roxb.

This is a native of Myanmar and distributed to India, Indochina, Malaysia, Indonesia and Ceylon. In Thailand, it is commonly found in the dipterocarp forests and is also cultivated. It is distinguished from other species by its bluish-green rhizome and red corolla-lobes. The rhizome is medicinally used throughout its range of distribution.

C. comosa Roxb.

The original country of this species is Myanmar, but is distributed to India, and is cultivated in Malaysia. In Thailand, it is rarely found in the deciduous

and bamboo forests. It is commonly cultivated for medicinal purpose. This species can be identified by its sessile inflorescence, white bracts tinted with pink and white coma bracts with pink apex throughout dorsal midband.

C. longa L.

This species is well known as the “commercial turmeric”. It is widely cultivated in Asia. Turmeric is an important spice, which is used in the preparation of curries in many Asian countries. It is also used in many other ways, such as the source of yellow dye, cosmetic and medicines. Its coma bracts are mostly white but vary to pale yellow or white with pink apices.

C. mangga Valetton & Zijp

This species is cultivated throughout Thailand. It is also commonly cultivated in Malay Peninsula and Java. Coma bracts are pink or white, with a largely pink blotch at the centre. This species is easily recognized by its white rhizome that is pale yellow inside and the smell of mango. It is extensively used as vegetable.

C. rubescens Roxb.

It is native to India, distributed to Myanmar, and uncommonly cultivated in Thailand. Its red petioles and leaf-sheaths are good distinguishing characters for this species.

C. zanthorrhiza Roxb.

This species was described by Roxburgh from a plant said to have been introduced to Calcutta from Amboina, Moluccas (Holttum, 1950). It is widely cultivated throughout SE Asia. Its rhizome is large, dark yellow or yellowish-orange inside. It is used extensively in traditional medicine particularly in Malaysia and Indonesia.

C. zedoaria (Christm.) Roscoe

A native species of India, it is cultivated throughout Southeast Asia. The plant is about a meter tall, the primary rhizome is ovoid, and the inside of the tuber is pale sulphur yellow. It is widely used as medicine in India and Malaysia. The leaves are also used for flavouring fish and other foods in Java.

5. “Petiolata” group

Distinguished characters are:

- Anther spurs shortly acicular, straight;
- Stylodes cylindrical;
- Bract apex truncate or rounded.

Eight species are in this group: *C. aurantiaca*, *C. petiolata*, *C. roscoeana*, *C. rubrobracteata* and four new species.

C. aurantiaca Zipp

This is a native of Ceylon and is also present in Java and Malaysia. In Thailand, it commonly grows in the clearing of evergreen forests and rubber plantations in the south. This species can be recognized by its brownish-green flower bracts, purplish-pink coma bracts, and anther without spurs. It is also a well-known ornamental plant.

C. petiolata Roxb.

This species is distributed in India, Myanmar, Laos, Java and Thailand. It can be identified by its large leaves with cordate base, pink coma bracts, and white flower with yellow midband on the labellum. Its inflorescence may be the largest of the genus. It is highly popular as an ornamental plant. The variegated form of this species is called in Thai “Bua Chun”. Its flower bracts are greenish with pink apices and its inflorescence is narrower. Note: the hybrids between *C. aurantiaca* and *C. petiolata* have produced attractive plants.

C. roscoeana Wall.

It is a native of Myanmar. In Thailand, it commonly grows in deciduous forests and has been well known as an ornamental plant for a long time. This species is easily identified by its orange flower bracts and anthers without spurs.

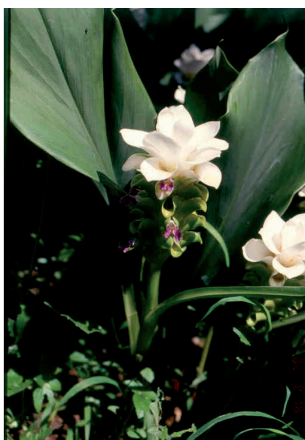
C. rubrobracteata Skornick., M. Sabu & Prasanthk.

This species is distributed in India and Myanmar. In Thailand, it is commonly found in deciduous and dry evergreen forests. The species was described in 2003, and is one of the new records for Thailand in 2005.

Figure 5. A gallery of representative species of *Curcuma* in Thailand.



C. alismatifolia



C. parviflora



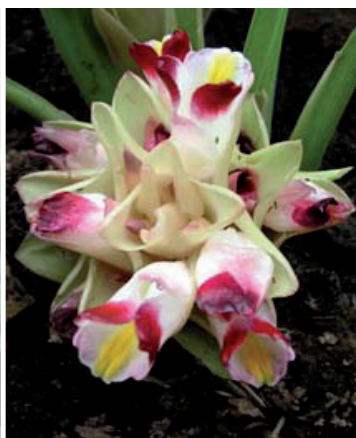
C. harmandii



C. sparganiiifolia



C. rhabdota



C. pierreana



C. ecomata



C. flaviflora



C. glans



C. aeruginosa



C. comosa



C. longa



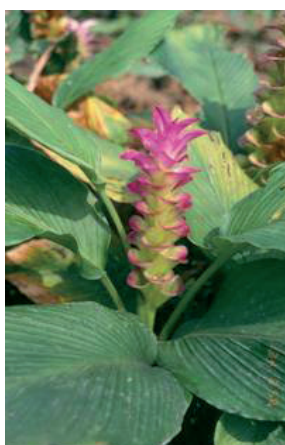
C. rubescens



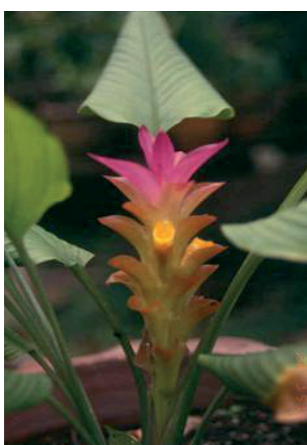
C. zanthorrhiza



C. zedoaria



C. petiolata
(variegated form)



C. aurantiaca



C. petiolata



C. aurantiaca x petiolata



C. roscoeana



C. rubrobracteata

A Summary of Distribution of *Curcuma* in Thailand

“**Alismatifolia**” group has its centre of distribution in the northeastern and eastern regions of the country.

“**Cochinchinensis**” group consists of only 2 species: one (*C. cochinchinensis*) occurs in the north and southwest, another one (*C. pierreana*) has its limited distribution in the eastern region.

“**Ecomata**” group has all its species occurring in the northern region; however, two of them, *C. singularis* and *C. stenochila*, are also distributed in the northeastern, eastern and southeastern parts of the country.

“**Longa**” group has most of its member species cultivated. In Thailand, few of them grow wild in the north.

“**Petiolata**” group has most species distributed from the north to the peninsula along the western ranges (Fig. 6).



Figure 6. Distribution of *Curcuma* in Thailand

Phylogenetic Relationship of *Curcuma* Species in Thailand

The strict consensus of three most parsimonious cladograms, which resulted from ITS sequence analysis (Fig. 7) reveals that three groups (“*Alismatifolia*”, “*Cochinchinensis*” and “*Ecomata*”) are clearly separated from each other and from the “*Longa*” group. The relationship of “*Petiolata*” group is unclear. The study shows that except for “*Petiolata*” group, the remaining four groups proposed by the morphological classification are supported by molecular evidence. It also suggests that the new classification of the genus *Curcuma* should be considered.

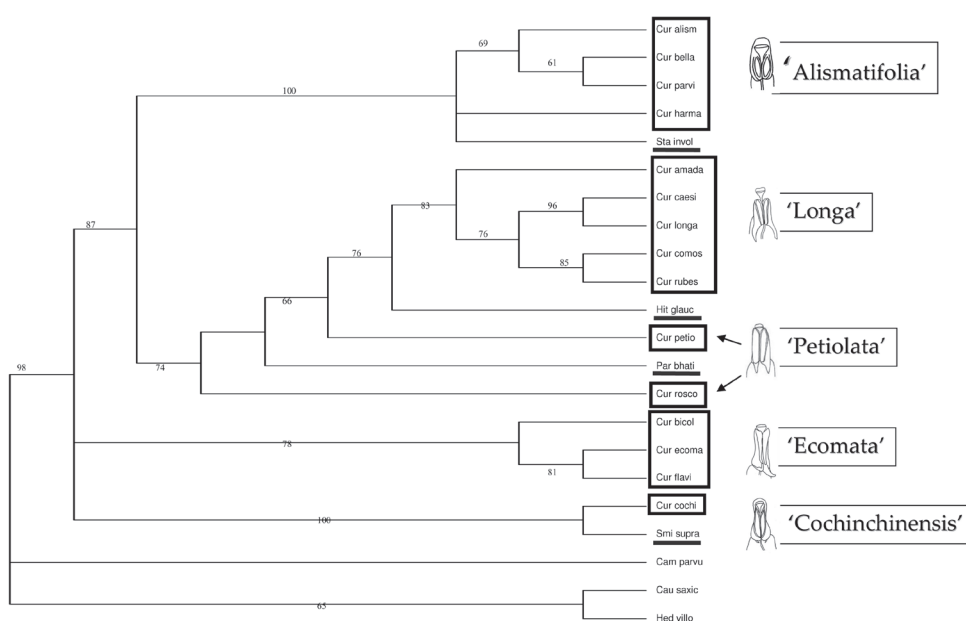


Figure 7. Cladogram showing the relationships of some *Curcuma* species (Maknoi, 2005)

Conclusion

Some suggestions for further studies on the genus *Curcuma* are:

1. Though several classification systems of *Curcuma*, as well as one from this paper, have been proposed, it seems that the taxonomic problems of this genus still exist. Further studies for more information, particularly the phylogenetic relationships of species, are needed and required to support the taxonomy of the grouping of this genus.

2. Regarding the diversity of the genus, it is believed that more undescribed species of *Curcuma* can still be found in natural forests of tropical Asia. Therefore, more explorations for new taxa are suggested.

3. *Curcuma* is a genus very useful to man. Many species are used as medicines, ornamental plants, dyes, cosmetics, foods and spices. It is reported that less than 50 % of the species are used by man. The rest, more than 50 % of *Curcuma* species have not been known of their uses. Therefore, more studies on the biological activities and pharmacological actions of *Curcuma* are needed in order to explore and search for their potential uses. Species with high potential use in decoration are also suggested to be commercially developed.

However, on account of conservation, over exploitation or over collection of plants from the wild may cause the genetic erosion. The awareness and warning of over using some species should be heeded.

References

- Backer, C.A. and Bakhuizen Van Den Brink, R.C. 1963. Monocotyledones, pp. 1-641, In: *Flora of Java*, vol. **3**. N.V.P. Noordhoff-Groningen, The Netherlands.
- Baker, J.G. 1890. Scitamineae, pp. 198-264. In: Hooker, J.D., *Flora of British India*, vol. **6**. London.
- Burt, B.L. and Smith, R.M. 1972. Key Species in the taxonomic history of Zingiberaceae. *Notes from the Royal Botanic Gardens Edinburgh* **31**: 177-227.
- Gagnepain, F. 1908. *Zingiberacées*, pp. 25-121. In: Gagnepain, F.(ed.), *Flore Générale de L' Indo-Chine*. Vol. **6**. Masson et Cie, Paris.
- Holttum, R.E. 1950. The Zingiberaceae of the Malay Peninsula. *Gardens Bulletin Singapore* **13**: 1-249.
- Kress, W.J., L.M. Price and K.J. Williams. 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): Evidence from molecular data. *American Journal of Botany* **89**: 1682-1696.
- Larsen, K. 2005. Distribution patterns and diversity centres of Zingiberaceae in SE Asia. *Biologiske Skrifter* **55**: 219-228.

- Maknoi, C. 2005. *Taxonomy and Phylogeny of the genus Curcuma L. (Zingiberaceae) with particular reference to its occurrence in Thailand*. Ph.D. Thesis. Prince of Songkla University.
- Mood, J.D. and Larsen, K. 2001. New *Curcuma* from Southeast Asia. *The New Plantsman* **8**: 207-217.
- Newman, M., A. Lhuillier and A.D. Poulsen. 2004. Checklist of the Zingiberaceae of Malesia. *Blumea* **16**: 65-69.
- Sabu, M. & Mangaly, J.K. 1996. Taxonomic Revision of South Indian Zingiberaceae, pp. 15-22. In: Wu, D.-L., Wu, Q.-G., & Chen, Z.-Y (eds.). *Proceedings of the 2nd Symposium on the Family Zingiberaceae*. Zhongshan University Press, Guangzhou.
- Schumann, K. 1904. *Zingiberaceae*. In: Engler, A.(ed.), *Das Pflanzenreich*., IV, **46**. Engelmann, Leipzig. 458pp.
- Sirirugsa, P. and Newman, M. 2000. A new species of *Curcuma* L. (Zingiberaceae) from SE. Asia. *The New Plantsman* **4**: 196-198.
- Valeton, T. 1918. New notes on the Zingiberaceae of Java and Malaya. *Bulletin du Jardin Botanique Buitenzorg* ser. 2, **27**: 1-166.
- Wanakrairote, S. 1996. *Curcuma*. Amarin Printing And Publishing Public Co., Ltd. Bangkok.
- Wu, D.-L. and Larsen, K. 2000. *Zingiberaceae*, pp.322-377. In: Wu, Z.-Y. & Raven, P.H.(eds.), *Flora of China*, vol. **24**. Science Press, Beijing & Missouri Bot. Gard. Press, St. Louis.

Floral Ontogeny in *Alpinia oxyphylla* Miq. (Zingiberaceae) and Its Systematic Significance

J.-J. SONG^{1,2}, P. ZOU¹, J.-P. LIAO^{1*}, Y.-J. TANG¹ AND Z.-Y. CHEN¹

¹ South China Botanical Garden, Chinese Academy of Sciences,
Guangzhou 510650, China

² College of life science, Shenzhen University, Shenzhen 518060, China

*Corresponding author: Liaojp@scbg.ac.cn

Abstract

Floral organ development of *Alpinia oxyphylla* Miq. begins with the initiation of sepal primordia, and then the three common primordia comprising petal and inner whorl androecial members. Each common primordium separates into a dorsal petal and a ventral androecial member. The adaxial common primordium begins to separate first to produce the functional stamen and the adaxial petal. Subsequently the two abaxial common primordia separate to form two abaxial inner androecial members and two abaxial petals. After the three common primordia completed their differentiation, three outer androecial members are formed, of which the two adaxial primordia have a slow growth and finally become two lateral staminodes, while the abaxial primordium ceases growth and disappears gradually. The gynoecium is the last floral structure to initiate. Soon after the initiation of gynoecial primordium, the two abaxial inner androecial members form two secondary primordia. Compared to the development of the anther on the fertile stamen, the secondary primordia may be homologous with the primordia of the pollen sacs. This provides new evidence supporting the view that the labellum was derived from the two inner whorled androecial members.

Introduction

The Zingiberales is a natural order of monocotyledons consisting of eight families, namely, Heliconiaceae, Strelitziaceae, Lowiaceae, Musaceae, Zingiberaceae, Costaceae, Cannaceae and Marantaceae (Tomlinson, 1982; Dahlgren and Rasmussen, 1983). Among the eight families, Heliconiaceae, Strelitziaceae, Lowiaceae and Musaceae are often informally referred to as the banana group, and flowers of those four families possess either five or six fertile stamens. The remaining four families form the monophyletic ginger group. Flowers in the ginger families possess either one fertile stamen with

two anther sacs (Zingiberaceae and Costaceae) or one stamen with only one anther sac (Marantaceae and Cannaceae) (Kress *et al.*, 2001; Rudall and Bateman, 2004). A typical flower of angiosperm consists of four whorls of different floral organs, they are from outside to inside: sepals in whorl 1, petals in whorl 2; stamens in whorl 3 and carpels in whorl 4. Nevertheless, there are labellum and lateral staminodes in the whorl 3 besides the stamens in ginger group. Fig. 1 shows a flower diagram of Zingiberaceae and a picture of mature flower of *Alpinia oxyphylla* Miq. to indicate the positions of lateral staminodes and labellum. In spite of several opinions about the nature of the labellum and the lateral staminodes in the ginger family based primarily on morphological and anatomical evidences expressed in the past (e.g. Burrt, 1972; Gregory, 1936; Rao, 1963; Raghavan and Venkatasubban, 1941; Willis, 1948), the recent evidences support labellum being derived by the congenial fusion of two inner staminodes (e.g. Kirchoff, 1997, 1998).

In angiosperms, many structures that are absent in mature flower have vestigial remains that can be observed during the development of floral primordium (Endress, 1994). Accordingly, floral organogenesis and development can reveal not only the early state of the floral structure in the flower, but also the homeosis of floral structure. Kirchoff has described the aspect of floral development of several species in the Zingiberaceae (Kirchoff, 1988a, 1997, 1998) and other Zingiberales (Kirchoff, 1983, 1986, 1988b), and Box and Rudall (2006) have investigated the floral ontogeny in *Globba*.

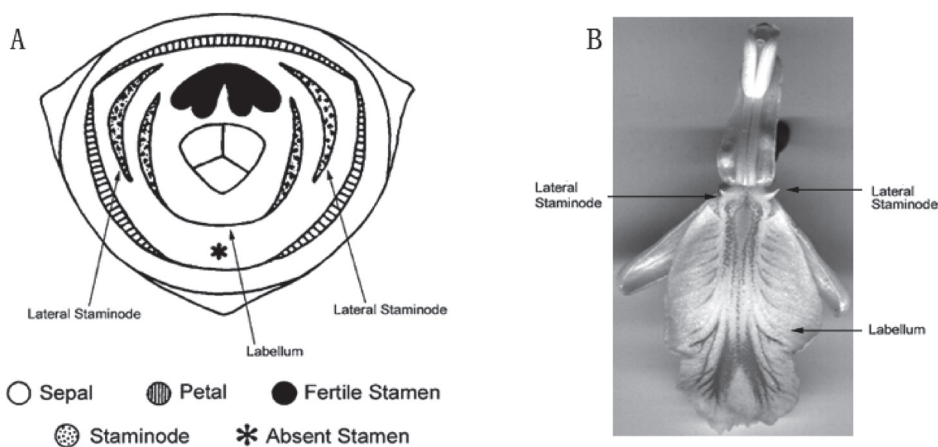


Figure 1. Floral diagram of Zingiberaceae and a mature flower of *Alpinia oxyphylla*. A. Floral diagram of Zingiberaceae (cited from Kress *et al*, 2002); B. mature flower of *Alpinia oxyphylla*.

In the genus *Alpinia* only one species, *Alpinia calcarata* (Haw.) Roscoe, was studied (Kirchoff, 1988a). Here we describe the floral development of *Alpinia oxyphylla* to provide additional information for the species within the genus *Alpinia* and present additional evidence to a better understanding of the origin of the labellum and the lateral staminodes in the family Zingiberaceae.

Materials and Methods

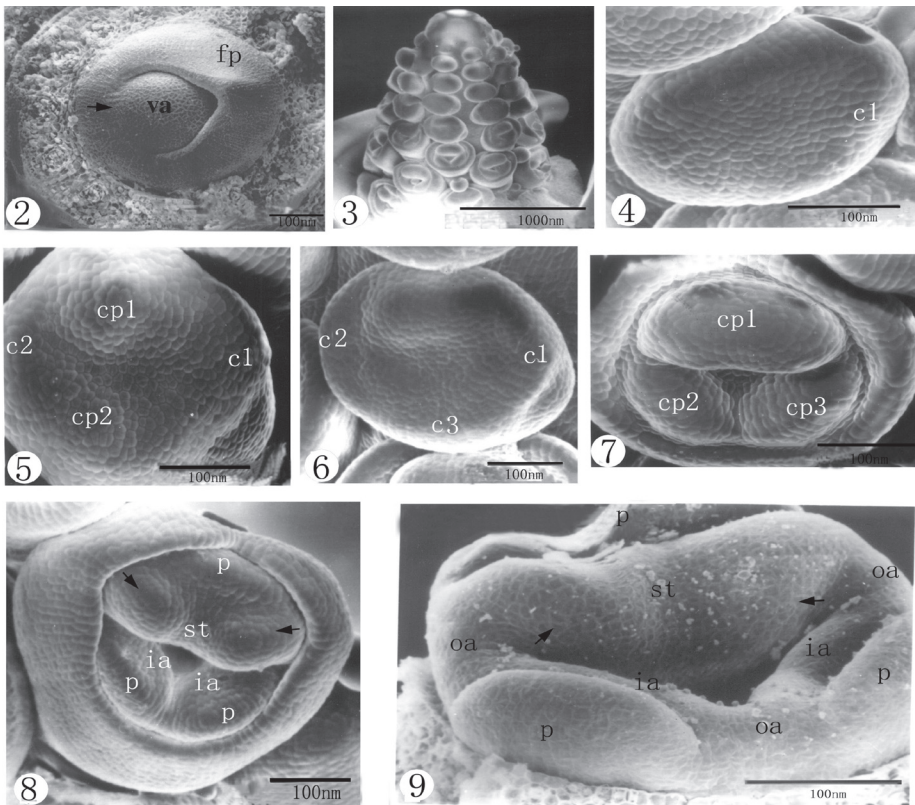
Inflorescences of *Alpinia oxyphylla* were collected from the ginger garden of South China Botanical Garden, Chinese Academy of Sciences. Fresh materials were fixed using formalin-acetic acid-alcohol (FAA) (Berlyn and Miksche, 1976) for two days, and then stored in 70% alcohol. The vouchers (collection number: *J. J. Song 2004-03*) were deposited at SCBG. Specimens used for scanning electron microscope (SEM) were dissected in 70% alcohol under Wild Stereo Microscope, dehydrated in a series of alcohol running up to 100%, treated with isoamyl acetate, and then critical-point dried using CO₂. Gold-sputtered specimens were observed and photographed under the SEM.

Results

The shoot apex of *Alpinia oxyphylla* is a domed shape and more or less symmetrical structure and produces leaves in an alternate arrangement (Fig. 2). A crescent-shaped primordium initiates on one side of the apex, which indicates the initiation of a leaf. This leaf primordium has a rapid growth and forms a cap-like structure; gradually encloses the apex from above. When this cap-like structure encloses half of the shoot apex, another foliar primordium begins to initiate on the other side of the apex and repeats the same growth process (Fig. 2). After about the differentiation of 17-20 leaves, the shoot apical meristem converts itself into an inflorescence meristem.

The inflorescence of *A. oxyphylla* is a raceme (Wu and Larsen, 2000) and there are about 50 to 70 flowers that are initiated in acropetal order in one inflorescence (Fig. 3). Floral organ development begins with the initiation of sepal primordium. The three sepal primordia are initiated sequentially at the three angles of the floral shoot apex (Figs. 4, 5). These three primordia continue to enlarge and gradually separate from the central part of the floral primordia to form a ring of calyx primordium (Figs. 6, 7, 8).

Soon a bulge initiates at the adaxial side of the midsection of the floral primordium after the initiation of the sepal primordium (Fig. 5).



Figures 2-9. Floral organogeny of *Alpinia oxyphylla*: 2. The vegetative apex (va) and the foliar primordium (fp). The black arrow indicates the initiation of a new foliar primordium. 3. The inflorescence shoot. 4. The first sepal (c1) is initiated on a flower bud. 5. Sepals (c1, c2) labeled in order of initiation; cp1, cp2: the two primordia of petal and inner whorl androecium. 6. Three sepals (c1, c2, c3) begin to form a ring calyx primordium. 7. The complete formation of three primordia (cp) labeled in order of initiation. 8. The three primordia separate into petal and inner whorled androecium (ia) [p, petal; st, stamen; ia, abaxial inner whorled androecial member]. Black arrows indicate pollen sac of the anther. 9. Formation of three outer androecial members (oa) [p, petal; st, stamen; ia, abaxial inner whorled androecial member]. Black arrows indicate pollen sacs.

Thereafter, two other bulges form in a counter-clockwise order at the abaxial side of the floral primordium (Figs. 5, 6). These are the common primordia of three petals and the three inner whorl androecial members. The three common primordia arrange triangularly and fuse basally to form the floral cup. However, there is an unequal development of these three primordial: the adaxial one becomes obviously larger than the abaxial two (Fig. 7). The center of the floral cup is depressed, and the depression becomes deeper with the growth of the three common primordia (Figs. 6, 7). The three petals and their associated androecial members are formed from the separation

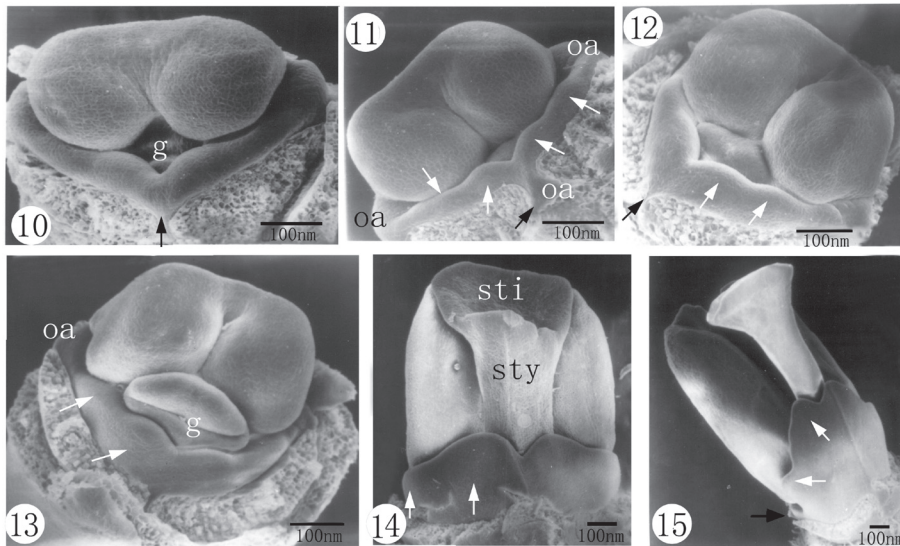
of these three common primordia. Each primordium separates into a dorsal petal and a ventral inner whorled androecial member. The adaxial primordium begins to separate first and produces the functional stamen and the adaxial petal. Subsequently the two abaxial primordia separate more or less simultaneously to form the two abaxial inner androecium and two abaxial petals (Fig. 8).

Additionally, the primordium of the fertile stamen differentiates into two bulges soon after its formation and these two bulges grow rapidly to give rise to the locules (pollen sac) of the anther (Figs. 8, 9, see arrows). The two abaxial inner androecial primordia then fuse with each other, and ultimately form the labellum. After the three common primordia complete their differentiation, three outer androecial members begin to form (Fig. 9): two of them appear beside the stamen primordium, one presents at the position outside the fused part of the two abaxial inner androecial primordia. The two adaxial primordia of the outer androecial members form the lateral staminodes when the flower finishes differentiation (Fig. 15, see black arrow). The abaxial one ceases growth soon after its initiation and disappears gradually (Figs. 10-12, see black arrows).

The three petals undergo rapid growth after their initiation and enclose the inner floral organs gradually. The floral primordium has differentiated calyx, petal, inner and outer whorl androecial members at this stage. The gynoecial primordium is the last one to initiate; it appears in the depression formed by the development of outer whorls (Fig. 10). The gynoecial primordium grows rapidly following initiation and soon forms the stigma and style (Figs. 13-15). Soon after the initiation of gynoecial primordium, the two abaxial inner androecial primordia, which ultimately form the labellum, each begin to produce two secondary primordia respectively (Fig. 11, see white arrows). The size of the four secondary primordia is similar when they are just formed (Fig. 11), but there is an unequal development among them. The abaxial two grow faster than the adaxial two, which results in the difference of their sizes (Fig. 12). Furthermore, the difference between their sizes becomes greater with growth and it is these four secondary primordia eventually form the semi-oval labellum when all floral organs finish the ontogenetic differentiation (Figs. 12-15).

Discussion

Kirchoff (1983, 1986, 1988a, b, 1997, 1998) studied the floral organogenesis in Zingiberales and established the pattern of floral ontogeny in the order, which in general, is highly conserved at the family level. The floral organ development of *A. oxyphylla* fits well into this general pattern.



Figures 10-15. Floral organogeny of *Alpinia oxyphylla*: 10. The initiation of the gynoecium (g). Black Arrow indicates the abaxial outer whorled androecial member. 11. Two abaxial inner androecial members begin to form secondary primordia (white arrows) [oa, outer whorl androecium]. 12-13. Size differentiation (see white arrows) between the secondary primordia of inner androecium. 14. The differentiation of stigma (sti) and style (sty). 15. The adaxial outer whorl androecium with subulate appendage (see arrow).

The initiation sequence of the floral organ is sepal, petal and inner androecium, outer androecium, gynoecial primordium, which resembles the developmental pattern reported for *A. calcarata* (Kirchoff, 1988a). In our study of *A. oxyphylla*, some of the developmental characters observed are like *A. calcarata*: (1) there is a lag between the formation of the inner and outer androecial whorls, that is, the outer androecial members do not begin to initiate until after the petals and the inner androecium are distinctly formed; (2) the three common primordia initially are asymmetric, and originated on the adaxial side of the floral shoot apex; and (3) the shape of the floral cup. However, these characters observed in the floral ontogeny of two species of *Alpinia* are different or partly different from what was described by Kirchoff (1988a) for other members of the ginger group. Kirchoff (1988a) had used the characters of floral ontogeny in his phylogenetic analysis of the family. Our data on the floral ontogeny may add information to the understanding of the relationship between the floral development and evolution of the individual groups in the family.

The formation of the labellum and the lateral staminodes of species in Zingiberaceae has received much attention. Various interpretations have been advanced. Brown (1830) regarded the labellum and the two subulate

appendages as the outer whorled stamens, and the two epigynous glands and the functional stamen as the inner whorled stamens. Raghavan and Venkatasubban (1941) had the same point of view on the basis of work on *A. calcarata*. But Rao (1963) proved that the two epigynous glands of Zingiberaceae are merely an outgrowth from the upper surface of the ovary. Gregory (1936) gave an interpretation that the stamen and the lateral portions of the labellum belong to the inner whorl, while the median part of the labellum and the two subulate appendages belong to the outer whorl on the basis of his work on *Elettaria cardamomum* (L.) Maton. Others, like Willis (1948), believed that the functional stamen and the labellum represented the inner whorled stamens. Liao *et al.* (2006) studied the floral vasculature of *Alpinia hainanensis* and showed that the labellum is supposed to represent five members of the androecium: its two marginal and the median portions are derived from three members of the outer androecial whorl and its two lateral parts represent the two members of the inner whorl. The recent evidences supporting the origin of labellum is derived from the congenial fusion of two staminodes, and the two lateral staminodes represent the outer androecial whorl, the anterior member of this whorl being absent.

The floral development study reported by Kirchoff (1997, 1998) supported the interpretation that the primordia of the two inner staminodes are joined by the intercalary growth to produce the labellum, while the abaxial outer androecial member ceases growth soon after initiation and contributes only initially to the formation of the labellum. The other two outer androecial members form the two lateral staminodes. In our study, the floral development of *A. oxyphylla* also supports this interpretation. Moreover, the two abaxial inner androecial primordial differentiated into two secondary primordia respectively. Compared with the development of the anther on the fertile stamen, the two secondary primordia maybe homologous to the primordia of two locules (pollen sac) of the anther. From this point of view, the four secondary primordia observed by us in *A. oxyphylla* that eventually form the labellum, may represent the fusion of four pollen sacs of two stamens. This brings forth new evidence for the view that the labellum was derived from the two inner whorled androecial members.

Acknowledgements

This research was supported by National Natural Science Foundation of China (39870087, 30370099, 40332021) and National Key Program for Basic Research of China (2001CCA00300).

References

- Berlyn G.P. and J.P. Miksche. 1976. *Botanical Microtechnique and Cytochemistry*. Iowa State University Press, Ames.
- Box M.S. and P.J. Rudall. 2006. Floral structure and ontogeny in *Globba* (Zingiberaceae). *Plant Systematics and Evolution* **258**: 107–122.
- Burrt, B.L. 1972. General introduction to papers on Zingiberaceae. *Notes from the Royal Botanic Garden Edinburgh* **31**:155-165.
- Dahlgren R. and F.N. Rasmussen. 1983. Monocotyledon evolution: characters and phylogenetic estimation. *Evolutionary Biology* **16**: 255-395.
- Endress, P.K. 1994. *Diversity and Evolutionary Biology of Tropical Flowers*. Cambridge University Press, Cambridge.
- Gregory, P.J. 1936. The floral morphology and cytology of *Elettaria cardamomum* Maton. *Botanical Journal of the Linnean Society* **50**: 363-391.
- Kirchoff B.K. 1983. Floral organogenesis in five genera of the Marantaceae and in *Canna* (Cannaceae). *American Journal of Botany* **70**: 508–523.
- Kirchoff B.K. 1986. Inflorescence and development in the Zingiberales: *Thalia geniculata* (Marantaceae). *Canadian Journal of Botany* **64**: 859–864.
- Kirchoff, B.K. 1988a. Floral ontogeny and evolution in the ginger group of the Zingiberales, pp. 45-56. In: P. Liens, S.C. Tucker and P.K. Endress (eds.). *Aspects of Floral Development*. Berlin Stuttgart.
- Kirchoff B.K. 1988b. Inflorescence and flower development in *Costus scaber* (Costaceae). *Canadian Journal of Botany* **66**: 339–345.
- Kirchoff, B.K. 1997. Inflorescence and flower development in the Hedychieae (Zingiberaceae): *Hedychium*. *Canadian Journal of Botany* **75**: 518-594.
- Kirchoff, B.K. 1998. Inflorescence and flower development in the Hedychieae (Zingiberaceae): *Scaphochlamys kunstleri* (Barker) Holttum. *International Journal of Plant Sciences* **159**: 261-273.

- Kress, W.J., L.M. Prince, W.J. Hahn and E.A. Zimmer. 2001. Unraveling the evolutionary radiation of the families of the Zingiberales using morphological and molecular evidence. *Systematic Biology* **50**:926-944.
- Kress, W.J., L.M. Prince and K.J. Williams. 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. *American Journal of Botany* **89**:1682-1696.
- Liao, J.-P., P. Zou, Y.-J. Tang, J.-J. Song, Z.-Y. Xie, Q.-G.. Wu and T.-L. Wu. 2006. Floral vasculature in *Alpinia hainanensis* in relation to the nature of the labellum in gingers. *Nordic Journal of Botany* **23**: 545-553.
- Rao, P.J.M. 1963. The epigynous glands of Zingiberaceae. *New Phytologist* **62**: 342-349.
- Raghavan, T.S. and K.R. Venkatasubban. 1941. A contribution to the morphology and cytology of *Alpinia calcarata* Rosc. with special reference to the theory on Zingiberous flowering. *Proceedings of the Indian Academy of Sciences* **B13**: 300-325.
- Rudall, P.J. and R.M. Bateman. 2004. Evolution of zygomorphy in monocot flowers: iterative patterns and developmental constraints. *New Phytologist* **162**: 25-44.
- Tomlinson, P.B. 1982. Phylogeny of the Scitamineae - morphological and anatomical considerations. *Evolution* **16**: 192- 213.
- Willis, J.C. 1948. *A Dictionary of the Flowering Plants and Ferns*. Cambridge.
- Wu, T.-L. and K. Larsen. 2000. Zingiberaceae, pp. 322-377. In: Wu, Z. and P.H. Raven (ed.). *Flora of China*, vol. **24**. Science Press and Missouri Botanical Garden Press, St. Louis.

