A new species of *Nervilia* (Orchidaceae) from Singapore

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ABSTRACT. The only species of *Nervilia* Comm. ex Gaudich. included in national checklists and redlists of the Singapore flora is *Nervilia punctata* (Blume) Makino. This species is treated as presumed nationally extinct. There are three historic collections from Singapore, all collected by H.N. Ridley during the late 19th and early 20th centuries. However, the identity of these specimens has recently been cast into doubt as the *Nervilia adolphi/punctata* species alliance has become the subject of taxonomic scrutiny. The lack of visible characters on the existing specimens has so far made it impossible to pinpoint the correct identity of the Singapore specimens. We recently discovered a small population of *Nervilia* in Bukit Timah Nature Reserve in Singapore, which in our opinion is the same taxon that was collected by Ridley. The plants do not agree with other species in the *Nervilia adolphi/punctata* species alliance and the taxon is here described as a new species based on the clinandrial tissue surrounding the anther cap as well as the narrow oblong and truncate labellum with curled sides. We currently consider the species to be endemic to Singapore.

**Keywords.** Bukit Timah, conservation assessments, *Nervilia punctata*

**Introduction**

*Nervilia* Comm. ex Gaudich. (Orchidaceae) is a widespread genus with c. 70 species, found in Africa, Asia and Australasia (Pridgeon et al., 2005). The genus is likely closely related to a few mycoheterotrophic orchids (e.g., *Epipogium* J.G.Gmel. ex Borkh.) and in flower *Nervilia* may appear to be fully mycoheterotrophic as they are often leafless at the time of flowering. However, in *Nervilia* the flowers are followed by a vegetative stem bearing a single photosynthetic leaf. The genus is unusual among orchids in having palmately-veined, often polygonal or lobed leaves.

Within the genus, the *Nervilia adolphi/punctata* species alliance in *Nervilia* sect. *Linervia* Schltr. has been the subject of recent taxonomic attention. This complex is characterised by glabrous polygonal or cordate leaves and inflorescences bearing single flowers with a narrow, white labellum with purple spots. The number of species in the complex is c. 30 (Gale et al., 2018). In Asia, the alliance is found across the distribution of the genus. It has been closely studied and taxonomically treated in Thailand (Gale & Watthana, 2014; Gale et al., 2018), and a few species have also been described in Japan, Taiwan, Laos and India (references in Gale et al., 2018). In Malesia, however, there have been no recent taxonomic revisions on the alliance, though several species have been described. In Peninsular Malaysia, the genus was
most recently treated by Seidenfaden & Wood (1992), who include most material in *Nervilia punctata* (Blume) Makino except for specimens from Langkawi, which they refer to *N. calcicola* Kerr (stating that there is confusion between that species and *N. infundibulifolia* Blatt. & McCann). National redlists and checklists in Singapore only recognise *Nervilia punctata* (e.g. Davison et al., 2008; Chong et al., 2009). *Nervilia punctata* was described from Java and has been traditionally considered widespread, extending north via Sumatra and Borneo to Singapore, Peninsular Malaysia, Thailand and India. However, the actual distribution of *Nervilia punctata* s.s., including its presence in Singapore and Peninsular Malaysia, remains questionable and the name has likely been misapplied in much of the above distribution. In order to help clarify the identity of *Nervilia punctata*, we lectotypify the name below.

We located three old collections of *Nervilia* from Singapore, all at SING, all collected by H.N. Ridley and all identified by him as *Nervilia punctata* (at the time known as *Pogonia punctata* Blume). The only fertile collection, over two sheets, is said to be from Bukit Timah, collected in 1891. However, published (citations below) and unpublished notes by Ridley make it clear that the plant was collected from Bukit Timah in 1889 and vouched only after it flowered in cultivation. Its discovery was described by Ridley in detail (Ridley, 1891), and further field notes exist alongside an illustration he made during the initial collection (in the archives in Kew). There are two sheets of this collection in SING, clearly parts of the same plant, one of which explicitly states that it was cultivated in Singapore Botanic Gardens (“HBS”, Hortus Botanicorum Singaporensis). Ridley (1891) wrote that, “The flowers have a faint smell of cucumbers. It never seems fully to open. Probably it is not so rare as it would appear, for, without doubt, it is an exceedingly difficult plant to find, as it is very inconspicuous and occurs apparently quite sporadically. I have several times since hunted in the spot where I found this little plant, and never seen any more”. According to the notes in Kew, the original collection was from a “stream on Bukit Timah on Tiger’s nest”. Ridley’s description of the flower shape and colour is generic, and he doesn’t provide details of labellum structure. The species was included in further treatments by Ridley (1896, 1900, 1924; in the 1924 work he provided a rarely used combination, *Aplostellis punctata* (Blume) Ridl. – *Nervilia* is now conserved against the earlier *Aplostellis* Thouars), but with little additional detail.

One of the two sheets from Bukit Timah includes the note “fig. by Alwis”, indicating that the plant was illustrated. We were able to trace a painting by James de Alwis from the archives of Singapore Botanic Gardens (Fig. 1). This painting is undoubtedly based on Ridley’s collection as it matches the herbarium material in shape and structure of the leaves and the inflorescence. The flowers are not fully open, leaving some diagnostic characters hidden. The labellum and columns were painted in additional detail. Unfortunately, Ridley’s herbarium specimens are in poor condition and the flower morphology could not be examined, which meant that the species could not be reliably identified to species.

We recently had an opportunity to further investigate the identity of this plant. We discovered a population of *Nervilia* in Bukit Timah Nature Reserve on 11 July 2019. A drought in July to September 2019 caused most of the plants to become
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dormant, after which the population was monitored for flowers. In early November 2019, several developing fruits and flower buds were found. Subsequently a small amount of material was collected over the duration of the flowering period.

The flower morphology of the newly discovered plants was practically identical to the plant painted by de Alwis. The poorly opening flower, flower colour (light brown-green spotted with purple, with lighter spotted petals) and shape (narrow flower, with narrow perianth parts becoming slightly ajar on aging flowers), relative labellum length (shorter than sepals) and labellum shape and colour (oblong labellum with a truncate tip, white, spotted colour with a central green stripe), the cleft tip of the anther cap and the colouration of the column (white with large purple spots) and the presence of subterranean lateral inflorescences match the painting. We therefore have no doubt that the newly discovered population is of the same species that was collected from Bukit Timah by Ridley. The plants are, however, clearly distinct from Nervilia punctata s.s. which has a labellum mid-lobe that is broadest in the middle and acute,

Fig. 1. Nervilia singaporensis Niissalo by James de Alwis, as Pogonia punctata (1891), digitally reconstructed. A. Flowering plant and vegetative stems. B. Labellum detail. C. Column detail, dorsal view. D. Inflorescence base with lateral inflorescences. Reproduced with permission of the Singapore Botanic Gardens.
as clearly seen from illustrations of the lectotype. It is also likely a montane species with flowers that open widely, though re-collection from the type locality would help a better circumscription of that species. *Nervilia punctata* also differs from our material in the column details seen in illustrations of the type material.

We compared the morphology of the Bukit Timah plants to other species in the alliance using protologues, specimens and illustrations available online and in the literature. We also compared two genetic regions to sequences available online. We sequenced ribosomal DNA from the 18S to 26S (partial sequences), including the full length of 5.8S, ITS1 and ITS2 with the primers AB101 and AB102 (Sun et al., 1994; Fazekas et al., 2012; Fig. 2; GenBank ID MT152902), and chloroplast DNA from *trnL*-CAA to *trnF* (partial sequences), including their intergenic region, with primers *trnFf* and *trnLc* (Taberlet et al., 1991; Fig. 3; GenBank ID MT152903). Phylogenetic analyses using these two regions support our view that, within the alliance, our species is not close to *Nervilia punctata* from Java (voucher *Comber 1114*, K). Phylogenetic reconstructions suggest that the species is closely related to *Nervilia trangensis* S.W.Gale, Suddee & Duangjai, a species described from Peninsular Thailand (Gale et al., 2018). The Bukit Timah plants, however, differ from that species substantially in flower details and in having apparently cleistogamous flowers. Other genetically similar species include *Nervilia lanyuensis* S.S.Ying, *N. marmorata* S.W.Gale, Suddee & Duangjai and *N. infundibulifolia*, but they are also morphologically distinct from our collection. The samples included in Gale et al. (2018) as *Nervilia cf. punctata* need closer taxonomic scrutiny. Our samples fit within the morphological variation they report in the appendices (and in Gale & Watthana, 2014), but the specimen illustrated in Gale & Watthana (2014) differs from our plant in having open flowers and a differently coloured perianth, whereas the sample included in their phylogenetic reconstruction (*Chanhormhual 05*, BKF) is genetically distinct from our plants and from *Nervilia punctata* in Java. There are several other species with flowers that do not fully open (Carr, 1933; Hsieh et al., 2013, 2018; Lin, 2014; Gale et al., 2015; Gale & Phaxaysombath, 2017), but none match the Singapore material in flower details as listed in the notes, and where DNA sequences are available, appear to be phylogenetically distinct (sequences are currently available for *Nervilia brevilobata* C.S.Leou, C.L.Yeh & S.W.Gale, *N. futago* S.W.Gale & T.Yukawa and *N. nipponica* Makino). We, therefore, describe our plants as a new species, *Nervilia singaporensis* Niissalo.

**Taxonomic treatment**

*Nervilia singaporensis* Niissalo, **sp. nov.**

Similar to *Nervilia punctata* (Blume) Makino, but differing in having a truncate lip (not acute) and a cleft anther cap that is surrounded dorsally by clinandrial tissue for over half of its length. – TYPE: Singapore, Bukit Timah Nature Reserve, 3 December 2019, M.A. Niissalo SING2019-1365 (holotype SING [SING0273892], an inflorescence
with a manually spread flower with anther cap removed, an infructescence and a leafy stem). (Fig. 1, 4 & 5).

Glabrous deciduous terrestrial herb, flowering when leafless, up to 6 cm tall in leaf, up to 9 cm tall in flower and up to 26 cm tall in fruit. **Tuber** whitish, approximately spindle-shaped, 10–23 mm long, 7–12 mm in diameter, partially covered in pale brown, soon degrading sheaths and sometimes with a few vestigial roots emerging over the entire length. **Subterranean stem** from the apical node of the tuber, 2–4.7 cm long, rootless or rarely producing a few lateral roots or short stolons (c. 1 mm thick, up to 1.5 cm long) and covered in a fine plume of root-like hairs. Runners and the development of new tubers not seen. **Leaf** pseudopetiolute; pseudopetiole erect, 1.5–4 cm long, 0.7–1.8 mm in diameter, bright green, sulcate, sheathed by 1 or 2 brown-green, membranous, translucent (except near major veins) cataphylls 1.2–1.6 cm long, which are sometimes deciduous; leaf blade plicate, opening almost flat, held a short distance above ground level, approximately parallel to the ground, cordate-polygonal, with 7 main veins diverging palmately, 3.2–5.8 cm long, 2.9–5.3 cm wide, cordate at base, margin entire, uniformly green, glossy on both surfaces, leaf lobes usually rounded, apical lobe sometimes acute. **Inflorescence** emerging from apical node of tuber, whitish-brown, underground part 1.7–2 cm long, 2–2.2 mm in diameter, c. 6-noded, bearing a c. 0.7 mm long, membranous, sheathing bract at each of the upper nodes, sometimes with lateral branches emerging at nodes producing lateral inflorescences (up to 2 lateral inflorescences emerging below soil level), above-ground parts of the inflorescence 6–9 cm tall in flowering specimens, 0.8–1.7 mm in diameter, pale brownish green, spotted with pink, bearing 2 membranous sheathing cataphylls 0.8–2 cm long, 1-flowered; floral bract enclosing the pedicel at base, 3.5–4.5 mm long, 1.5 mm wide, acute. **Flower** resupinate, erect in bud, becoming horizontal over a period of one day, and over two days after that gradually nodding, only slightly opening at the apex and remaining in this stage until slowly withering as the fruit develops, 18–25.5 mm long (flower gradually elongates as pedicel grows, but perianth parts remain approximately the same size), shrinking somewhat on drying, sepals pale green-brown covered in purple spots, lateral petals, petals white to pale yellow-brown with purple spots, labellum pale pink, covered throughout in bright violet spots, with a light green central line on the disk, column white with conspicuous purple spots; scent not detected; pedicel and ovary in a fully developed flower 3.7–11 mm long (elongating over time). **Sepals** narrowly elliptic, acute, 3-veined; dorsal sepal 14–15.5 mm long, c. 1.8 mm wide; lateral sepals slightly oblique, 14–15.5 mm long, 1.8–2.0 mm wide. **Petals** narrowly elliptic, 12–13.8 mm long, 2–2.4 mm wide, acute, 3-veined. **Labellum** trilobed, narrowly oblong, 12–13.8 mm long, not saccate or swollen at the base, basal disk 4.5–5 mm long, 2.2–3 mm wide at its widest, margins rolled upwards at the base and embracing the column (impossible to fully spread flat, causing a secondary constriction in the mid-part of the disk when spreading is attempted), sides less strongly incurved towards the apex; lateral lobes acute, 0.9–1.1 mm long, appressed to the column; mid-lobe narrowly oblong, 7–7.5 mm long, 2–2.5 mm wide,
Fig. 2. RAxML tree of *Nervilia singaporenensis* in relation to other closely related *Nervilia* species, based on ITS sequences. Bootstrap values of more than 75 are indicated at their respective nodes. Outgroups are not shown.
Fig. 3. RAxML tree of *Nervilia singaporensis* in relation to other closely related *Nervilia* species, based on *trnL-trnF* sequences. Bootstrap values of more than 75 are indicated at their respective nodes. Outgroups are not shown.
Fig. 4. *Nervilia singapurensis* Niissalo at the type locality. 
A. An emerging leaf. 
B. A manually opened flower. 
C. A lateral view of a fully developed flower. 
D. Column, ventral side. 
E. A dissected perianth. (Photos: M.A. Niissalo).
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Fig. 5. *Nervilia singaporensis* Niissalo. **A.** Inflorescence. **B.** A leafy shoot. **C.** Infructescence with a lateral inflorescence. **D.** A manually opened flower, front view. **E.** Dorsal sepal. **F.** Petal. **G.** Lateral sepal. **H.** Flower with sepals and petals removed. **I.** Labellum spread out. **J.** Details of a papillae in the depression in the central ridge of the labellum. **K.** Column, bottom view. **L.** Column, oblique top view. **M.** Column, side view. **N.** Anther cap with incomplete pollinia still attached, side view. **O.** Column with anther cap removed, oblique front view. **P.** Ripe capsule prior to dehiscence. **Q.** Seeds. Scale bars: a–c: 10 mm; d–l & k–o: 1 mm; j: 0.2 mm; p–q: 0.5 mm. Drawn by Evonne Tay Koh from *M.A. Niissalo SING2019-1365.*
flat at the base but with sides rolled or folded up towards the apex, margin entire but slightly irregularly rounded-dentate and with short papillae, apically obtuse, truncate or shallowly emarginate; upper surface shortly papillose throughout, with papillae gradually getting longer and more clustered along the disk; the longer papillae form a more obvious Y-shaped ridge from the end of the disk to the tip of the labellum, the ridge with a narrow central depression extending to about 1/5 the length of the mid-lobe, the depression apparently not papillate; outer surface of the labellum with only a few scattered papillae. **Column** clavate, 4.5–4.7 mm long, c. 1 mm in diameter at the base, dilating to c. 1.4 mm at the apex, ventral side covered in short hairs, with a pronounced, irregularly lobed clinandrium, c. 1.4 mm long at the dorsal side (covering most of the anther, including the anther cap), absent on the ventral side or present as a few short lobes; **stigma** pillow-shaped, c. 1.2 mm wide; **anther cap** oblong, c. 1.4 mm long and 1.1 mm wide, bifid and deeply concave or cleft at the apex; pollinia 1.1–1.2 mm long, reaching the stigma at anthesis, not easily removed from the flower; **rostellum** not present. **Capsule** ovoid to fusiform, dehiscing almost round, drooping, dehiscent, 9–10 mm long, c. 4.2 mm wide, expanding to c. 12 mm wide when fully dehisced and comprising only 6 narrow strips, perianth on capsule persistent even after dehiscence. **Seeds** fusiform, terete, curved (often crescent-shaped), c. 0.9 mm long, c. 0.15 mm wide, transparent, whitish, with a minute orange-yellow embryo in the middle.

Distribution and ecology. We currently consider the species to be endemic to Singapore. We have not found likely conspecific material from Peninsular Malaysia. Malaysian specimens at SING filed under *Nervilia punctata* differ from our species most obviously by their longer and wider labellum. However, a thorough revision of the Peninsular Malaysian material is necessary before we can discount the presence of *Nervilia singaporensis* in Malaysia. A search for photographs of *Nervilia* from Peninsular Malaysia, including in the literature, the internet and social media, did not reveal any material that could be referred to this species.

*Nervilia singaporensis* is found among leaf litter in mature secondary forest, surrounded by coastal hill dipterocarp forest. The habitat is dominated by unidentified *Ficus* spp. and introduced herbs (*Dieffenbachia seguine* (Jacq.) Schott, *Epipremnum aureum* (Linden ex André) G.S.Bunting and *Heliconia psittacorum* L.f.). The historic collection locality in Bukit Timah and published notes suggest that the species once occurred in moist dipterocarp forest. There is no longer intact habitat at the other localities where this species is likely to have occurred (Stagmount and Choa Chu Kang; the two areas are near each other and may in fact have been part of the same collection), but the habitat was probably similar to the nearby forest in Bukit Timah.

Phenology. Flowering was observed in November and December 2019, and probably started in October. This followed an unseasonal drought in July to September of the same year, during which most of the population became dormant for c. 3 months before flowers were observed. The only historic mention of a flowering time is in the de Alwis painting, which seems to state “March” (text partially lost).
The flowers of this species never, or only rarely, open fully. Several flowers that appeared to be approaching anthesis were monitored over several days in November and December 2019, with no more than a few hours between observations during daylight hours. The flowers showed no indication of opening more than slightly at the apex. No pollinators were observed; fruit flies landed on the flowers, but likely only due to nearby decaying figs. The pollinia are firmly attached to the stigmatic surface in an undisturbed flower, as previously reported for *Nervilia nipponica* (Gale, 2007), with no obvious rostellum or other barrier preventing self-pollination (fig. 4 D). We therefore believe that the flowers are usually autogamous and cleistogamous. This also matches the observations of Ridley (1891), who stated that the flowers do not seem to open fully. However, unlike Ridley, who described a cucumber scent, we have not been able to detect any floral scent.

**Etymology.** The species is named after Singapore, where it was discovered.

**Provisional IUCN conservation assessment.** The only known population of *Nervilia singaporesis* contains 20–30 plants in a very small area of a few square metres and there has been a notable reduction in the area of suitable habitat (especially the loss of forest near Choa Chu Kang) and habitat quality (now severely fragmented with considerable edge effects). It should therefore be considered Critically Endangered (CR) globally under multiple categories (B1ab(i,ii,iii), B2ab(i,ii,iii), D) in the IUCN Red List (IUCN Standards and Petitions Subcommittee, 2017). The extent of occurrence (EOO) is below 100 km² and the known area of occupancy (AOO) is below 10 km². The species is very difficult to find, especially when in flower (it sheds its leaf before flowering), and it could have been overlooked at other localities. It does not appear to occur in Peninsular Malaysia (see above). If it is present there, the lack of collections would suggest the species is likely to be very rare. The species is able to survive in secondary forest, which may increase its chances of long-term survival. In Singapore, the species should be considered Critically Endangered following the criteria in Davison et al. (2008). We have harvested a few seed pods for germination trials for *ex situ* conservation purposes and have observed a very low seed set (fewer than 50 seeds per capsule), which could be an indication of a highly inbred population. This could be typical for a selfing species, although autogamous *Nervilia nipponica* has a much higher seed set (Gale, 2007). Seeds have been sown on germination media designed specifically for terrestrial orchids, but they showed no sign of germination after 45 days.

**Additional specimens examined.** SINGAPORE: Bukit Timah, 1891 [cultivated material from a specimen originally collected in 1889], Ridley s.n. (SING [SING0010886, one flower only], [SING0010885, vegetative parts and a flowering plant]); ibid., 11 Jul 2019, Niissalo et al., SING2019-653 (SING [SING0286395, leaf]; ibid., 6 Nov 2019, Niissalo SING2019-1362 (SING [SING0273899, flower in spirit, bottle 7852, photograph on herbarium sheet]); ibid., 19 Nov 2019, Niissalo SING2019-1363 (SING [SING0273890, infructescence with an open capsule]); ibid., 25 Nov 2019, Niissalo SING2019-1364 (SING [SING0273891, a single inflorescence with a manually spread flower]).
Possible additional specimens examined. SINGAPORE: Stagmount, 1907, Ridley s.n. (SING [SING0054962]); Choa Chu Kang, undated (sterile), Ridley s.n. (SING [SING0054961]).

Notes. It is not easy to identify species of *Nervilia* with weakly opening flowers. Several floral characters are shared between species with flowers that do not open fully, particularly the loss of a rostellum and a reduction in the size of the labellum. These characters are likely a result of convergent evolution and probably reflect an autogamous mode of pollination. The few *Nervilia* species with weakly opening flowers that have so far been sequenced are not genetically close to one another, and likely represent different evolutionary lineages.

The species with poorly or non-opening flowers are: *Nervilia brevilobata* C.S.Leou, C.L.Yeh & S.W.Gale, *N. futago* S.W.Gale & T.Yukawa, *N. kasiensis* S.W.Gale & Phaxays., *N. linearilabia* T.P.Lin, *N. nipponica* Makino, *N. petaloidea* Carr, *N. septemtrionarius* T.P.Lin and possibly *N. punctata* (Blume) Makino. *Nervilia singaporensis* differs from all of these in leaf and flower details. The clinandrial tissue surrounding the anther cap dorsally is rare and has only been previously illustrated in *Nervilia nipponica*. The short (c. 7–7.5 mm) and narrow (2–2.5 mm) mid-lobe of the labellum that is truncate and slightly emarginate, without an acute tip, is unique to *Nervilia singaporensis*. *Nervilia singaporensis* also differs from *N. futago*, *N. kasiensis*, *N. nipponica*, *N. punctata* and *N. septemtrionarius* in possessing a different leaf morphology and a narrower labellum; from *N. brevilobata* by a longer and narrower labellum mid-lobe; from *N. linearilabia* by having a less acute labellum plus a cleft tip to the anther cap; and from *N. petaloidea* by having a non-petaloid labellum and spotted tepals.

All material of *Nervilia* in Singapore is best treated under *Nervilia singaporensis* (possibly *Nervilia* sp. in the case of the sterile specimens), and *Nervilia punctata* should be considered a misapplied name.

The history of this species demonstrates the value of botanical art to taxonomy. James de Alwis, who painted the species for Ridley, was a resident botanical illustrator at Singapore Botanic Gardens from 1890 to 1908. Together with his brother Charles de Alwis, whose arrival at the Gardens post-dates the painting of *Nervilia singaporensis*, they painted over two hundred works which are an invaluable part of the Singapore Botanic Gardens’ collections. The de Alwis painting enabled us to confirm the identity of Ridley’s collection, which would have otherwise been limited by the lack of details seen in the preserved plant material.

**Lectotypification of Pogonia punctata Blume**

Notes. Blume (1849: 32) described *Pogonia punctata* from the mountains of Western Java. No specimen was cited in the protologue but in a later treatment (Blume, 1858) he provided details that enable the tracing of the original material. He mentions only one fertile specimen from Java. He states that the specimen mentioned was collected by Heinrich Kuhl, which must have happened in 1820 or 1821, before Kuhl died. The species is illustrated in the same publication (t. 54, f. B & t. 49, f. 2A–C). The illustrations, apparently first published in Blume (1858) are from Kuhl’s and Johan Conrad van Hasselt’s material according to Reichenbach (1874). They were associated with notes that match those mentioned by Blume and are almost certainly based on the specimen that we choose as a lectotype. The illustration shows flower characters that are not visible in the dried specimen and it was likely prepared from fresh material; it might be an original illustration by Kuhl or van Hasselt. We have traced an original herbarium sheet of this collection. It is the only sheet of *Nervilia punctata* from Java with Blume’s handwriting at L. Blume has written “K.v.H.” on the sheet, and refers to an illustration (“conf. fig.”). It would have been at Blume’s disposal at the time he wrote the protologue in Leiden. Therefore, the original materials Blume used for the description were very likely this single sheet and the two drawings made of it.

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