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A new species of *Claderia* (Orchidaceae)

M.A. Niissalo, P.K.F. Leong, F.E.L. Tay,
L.M. Choo, H. Kurzweil & G.S. Khew

Singapore Botanic Gardens, National Parks Board,
1 Cluny Road, Singapore 259569, Singapore
matti_asser_niissalo@nparks.gov.sg

ABSTRACT. A little-studied orchid genus, *Claderia* Hook.f., has until now contained just one or two species, *Claderia viridiflora* Hook.f. and its possible synonym, *C. papuana* Schltr. We describe a new species from Singapore, which differs from these *Claderia* in having small, cream-yellow flowers that are nodding and presented in a long, unbranched terminal inflorescence, mostly with two flowers open simultaneously. We name the new species *Claderia leontocampus* Niissalo. Plants of the new species have been collected or recorded in Sumatra, Peninsular Malaysia and possibly Peninsular Thailand. We carried out population genetics analyses to show distinction between the new species and *Claderia viridiflora*. The two species occur in the same habitat in Singapore. We used phylogenetic analyses to find out the phylogenetic position of *Claderia* within Orchidaceae; the genus is here considered to belong to subfamily Epidendroideae Kostel., tribe Epidendreae Lindl., subtribe Claderiinae Szlach. We publish the plastome and ITS sequences of *Claderia viridiflora*. Two names are lectotypified.

Keywords. Epidendroideae, ITS, plastome, population genetics, Singapore

Introduction

Claderia Hook.f. has so far consisted of only two published names, *C. viridiflora* Hook.f. and *C. papuana* Schltr., which are sometimes considered synonyms, with *C. viridiflora* having priority. We collected a specimen of *Claderia* from Singapore in a vegetative state in 2020. It later flowered in cultivation and we noted that the plant differed from *Claderia viridiflora* in having smaller, nodding, cream-yellow flowers, and we suspected that it was a new species. There is no name available for this newly discovered *Claderia* and we therefore describe it here as *Claderia leontocampus* Niissalo. Both *Claderia viridiflora* and the new species occur in Singapore. We conducted surveys to collect DNA, vouchers and living material from *Claderia* across Singapore to see if the two species are genetically distinct and to bring all possible clones of the new species into cultivation. We also studied herbarium material to find any further material of the new species.

Claderia is a genus best known for its unusual habit. The plants start their life as terrestrial plants, but their long rhizomes allow them to climb up trees, and they may become epiphytic in later stages. This climbing habit is rare amongst orchids. A partially terrestrial, partially climbing or epiphytic habit is known for example in some

species of the unrelated genera *Dipodium* R.Br. and *Vanilla* Mill., these being stem climbers, whereas at least some species of *Bulbophyllum* Thouars, such as *B. virescens* J.J.Sm., are terrestrial plants with climbing rhizomes.

While the genus *Claderia* has been universally accepted, its evolutionary position in Orchidaceae has not been firmly established. Dressler & Dodson (1960) included it in subtribe Thuniinae Schltr. under tribe Epidendreae Lindl. with no further comment, except that it is 'possible that further study will show [that Thuniinae is] unnatural'. In a later account, Dressler (1983) placed *Claderia* in subtribe Cyrtopodiinae Benth. under tribe Cymbidieae Pfitzer, with a comment that the genus may deserve its own subtribe, or that it 'may belong with the Eulophiinae'. Szlachetko (1995) placed *Claderia* in its own subtribe, Claderiinae Szlach. in tribe Polystachyeae Pfitzer, while stating that 'the flowers of *Claderia* are reminiscent of *Coelogyne* and the genus could be related to Collabiinae and/or Bromheadiinae'. Pridgeon et al. (2009) placed it in subtribe Cymbidiinae Benth. in tribe Cymbidieae. Chase et al. (2015) placed the genus in subtribe Eulophiinae Benth. in tribe Cymbidieae. We know of only one published phylogeny that includes *Claderia*; the position of *Claderia* within Cymbidieae had 64% bootstrap support based on barcoding regions (Pridgeon et al., 2014). As the position of *Claderia* needs to be clarified, we conducted phylogenetic analyses using the entire plastome and ITS sequences from *C. viridiflora*, which we have analysed together with a comprehensive selection of species across Orchidaceae.

Methods

Type collection

The new species was first detected during routine DNA collection surveys. Vegetatively it appeared identical to *Claderia viridiflora* and the differences were only seen when it flowered in cultivation in late 2021 or early 2022 (date not recorded). The plant remained in flower until November 2022, when it was preserved as a herbarium specimen.

Population genomics

We have been recording the populations of *Claderia* in Singapore since 2014. When the new species was identified, we surveyed further suitable habitats and visited all recorded localities and collected DNA, vouchers and living material from them. We found a total of nine clumps. Due to the fast vegetative growth of the species, we assumed that each clump consisted of one genetically distinct individual. We sampled more stems from two clumps, and our analyses confirm that the stems from each clump belonged to the same clone, which supports our assumption. It is likely that we did not detect all *Claderia* clumps in Singapore. The plants are found in all forest types in Singapore, from freshwater swamp forest and primary lowland dipterocarp forest to degraded secondary forest. As the area of suitable habitat is quite large, it makes comprehensive surveys hard to conduct, even in the fragmented landscape of small forest areas in Singapore. Fortunately, the plants are large and relatively easy

to detect. All collections were from the Central Catchment Nature Reserve and Bukit Timah Nature Reserve; there are no recent collections of *Claderia* from other parts of Singapore.

DNA was extracted from fresh or silica-dried leaves using DNeasy Plant Mini Kit (Qiagen) with two ethanol washes. DNA extraction was easier from dry material. We prepared double-digest restriction enzyme associated DNA (ddRADseq) libraries with the enzymes *ApeK*I and *Pst*I, with c. 1 GB data per library and two libraries for each sample; we followed the methods by Peterson et al. (2012), modified as specified in Niissalo et al. (2018), except for using 600 ng starting DNA and a size range of 350–1000 bp (inclusive of adapters). A duplicate library was made for each sample. The samples were sequenced paired-end with 150 bp read length using Illumina sequencing (Novogene-AIT, Singapore).

We sorted the sequences using Stacks v.1.37 (Catchen et al., 2013). We did a clonality check and data quality check as outlined in Niissalo et al. (2020). We ensured that duplicate libraries had more than 90% similarity in heterozygotic SNPs, and then concatenated the reads for final analysis. We outputted alignments for RAxML v.8.2.11 (no missing data allowed in Stacks, options in Geneious: nucleotide model GTR GAMMA, algorithm: rapid bootstrapping and search for best-scoring ML tree, 100 bootstrap replicates and random seed of 2451; Stamatakis, 2014) and for Structure v.2.3.4 (30% of samples were allowed to miss data in Stacks; Pritchard et al., 2000). We used 20 runs for six values of K in Structure (random starting numbers 12345–12364 for each value of K = 1–6) and calculated the Fst statistics between the populations suggested by Structure using Stacks. For the phylogenetic analysis, we added an outgroup to the sequence alignment by extracting matching sequences from a chromosome-level assembly of *Dendrobium huoshanense* Z.Z.Tang & S.J.Cheng (Han et al., 2020, NCBI bioproject: PRJNA597621).

We used Smudgeplot v.0.2.3dev (Ranallo-Benavidez et al., 2020), modified for use with ddRAD data as described in Niissalo et al. (2020) to estimate the ploidy levels of the samples based on kmer-pair frequencies.

Phylogenetic analyses

We assembled the complete plastome and ITS (plus partial ETS) sequences of the type specimen of *Claderia leontocampus* and a specimen of *Claderia viridiflora* from Bukit Timah (*Niissalo et al. SING2022-801*) using Illumina sequencing. The assembly was done using initial mapping to sequences of *Eulophia* and iterative rounds to fill gaps. A final assessment was done to check the plastome and ITS sequences for completeness and even coverage. No rearrangements were necessary.

We collated whole plastome sequences from GenBank, by searching for every subtribe within Orchidaceae and selecting one representative for every genus. We used Hypoxidaceae as the outgroup. We also searched Internal Transcribed Spacer 1–2 and associated ribosomal DNA sequences (ITS) and sequences of *maturase K–transfer RNA K* (*matK–trnK*) as barcode sequences for a more comprehensive taxonomic coverage.

We stripped all the plastomes from annotations, and re-annotated them using Geneious v.2022.2.2 (Biomatters Ltd, New Zealand) annotation tool at 80% similarity threshold; as reference we used the CDS annotations from *Eulophia flava* (Lindl.) Hook.f. (GenBank accession MK855051). The annotations were not manually adjusted, but any annotations with premature stop codons were removed from the analyses. We extracted the CDS sequences for downstream analyses. If multiple copies were present, the longest copy was chosen.

We aligned each gene set with MAFFT v.7.475 (option --auto; Katoh & Standley, 2013), removed poorly aligned sequences with trimAl (option -automated1; Capella-Gutiérrez et al., 2009) and re-imported all alignments to Geneious. No manual adjustments were made—all alignments appeared to be of high quality when visually assessed. We then concatenated all alignments by sequence name, and conducted a phylogenetic analysis with RAxML (same parameters as for the ddRADseq dataset).

The ITS and *matK–trnK* datasets were aligned using MAFFT in Geneious and trimmed manually to remove poorly aligned regions or regions with extensive amounts of missing data. A conservative approach was taken, meaning that only the most complete regions were retained. Only *Claderia viridiflora* was included in the ITS analysis.

Results and discussion

Population genetics in Singapore

We received a minimum of 3.7 gigabases of adapter-free, full-length paired reads for each clone. The quality of all duplicated sequencing libraries was very high, with no more than 10% differences in heterozygous loci between duplicates. Each clump consisted of one clone, where this was tested. At most, two samples were collected c. 15 m apart from the same clump.

When we allowed any locus to miss data in 30% of the samples (with no population assumptions), we obtained 12,358 variable ddRAD loci across the nine samples. A delta K analysis using Structure Harvester (Earl & Von Holdt, 2012) unambiguously suggested that there are three populations (Fig. 1B). All 20 replicate runs with K = 3 had the same population assignment, with each sample assigned to a single population in full (Fig. 1A). The population assignment assigned both plants of *Claderia leontocampus* to one population and *C. viridiflora* to two populations (one with five plants, “population 1”, one with two, “population 2”).

The Fst values between the three populations showed strong fixation between *Claderia leontocampus* and both populations of *C. viridiflora* (Fst 0.442 and 0.395), and also relatively strong difference between the two populations of *C. viridiflora* (Fst 0.196).

When we allowed no missing data, we got 3600 variable ddRAD loci across the nine samples. The phylogenetic analysis of the ddRADseq data suggested that *Claderia leontocampus* is sister to the rest of the *Claderia* in Singapore. The two populations of *Claderia viridiflora*, which form a clade, were also separated by considerable genetic distance (Fig. 1C).

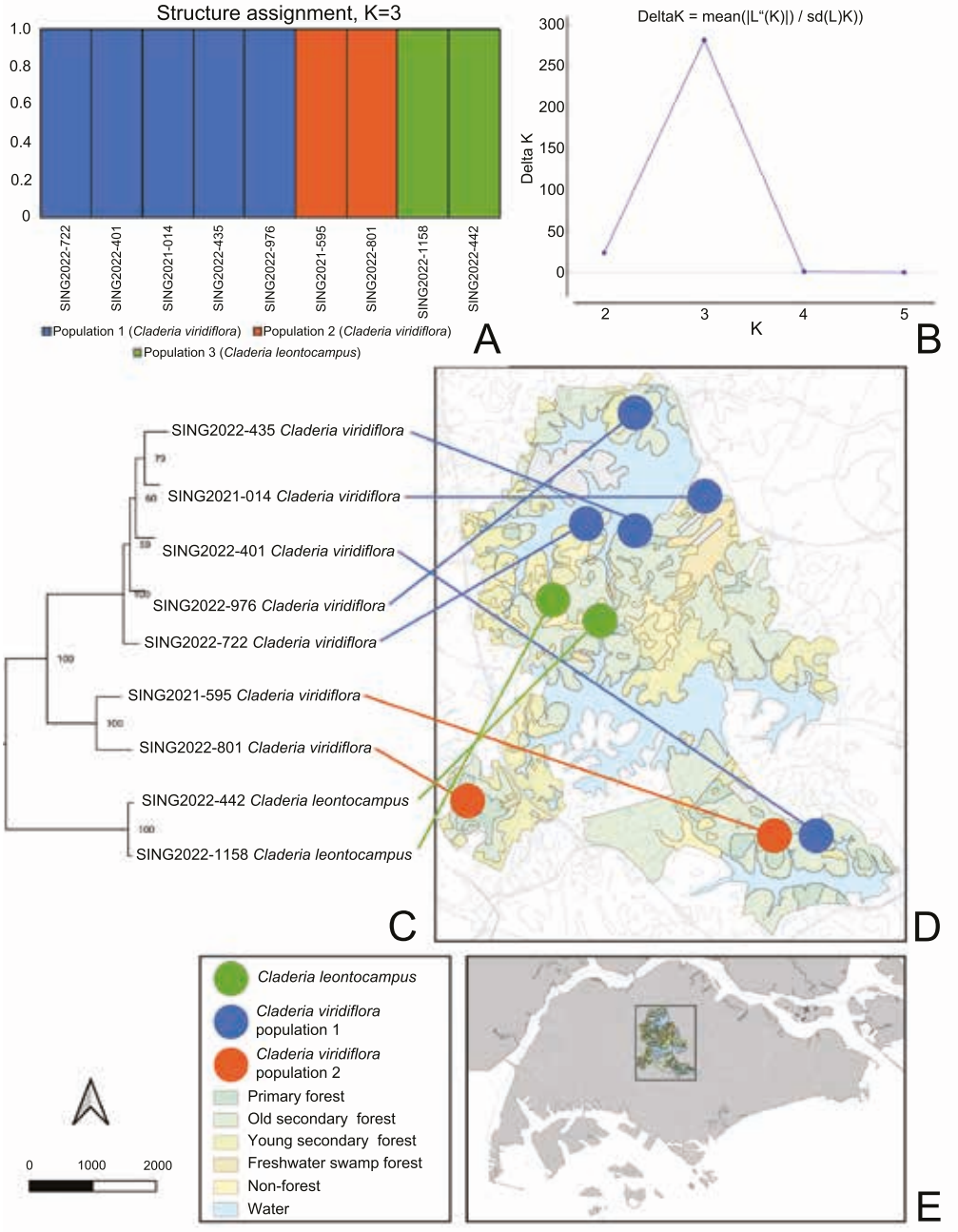

Fig. 1. Population genomics of *Claderia* Hook.f. in Singapore. **A.** Output of Structure analysis. **B.** Delta K for Structure replicates at different values of K. **C.** Phylogeny of *Claderia* in Singapore based on ddRADseq data, outgroup (*Dendrobium*) not shown. **D.** Collection localities of samples, rounded to 0.001 degrees. **E.** Map legends and inset map showing the collection localities in Singapore.

It is clear that *Claderia leontocampus* is isolated from other *Claderia* in Singapore, as expected from the morphological differences. The presence of two populations of *Claderia viridiflora* is not as easily explained and needs further study. The flowers of *Claderia viridiflora* illustrated here (Fig. 2B) are from population 1. These two populations need to be examined in more detail in case there is further undescribed diversity of *Claderia* in Singapore.

All three populations had Smudgeplot-derived k-mer ratios at 50%, suggesting that they are all effectively diploid (plots not shown).

***Phylogenetic position of* Claderia**

The structure of the plastome of *Claderia viridiflora* is shown in Fig. 3. In the whole plastome analysis, *Claderia* is fully supported as sister to *Agrostophyllum* Blume (subtribe Agrostophyllinae in tribe Epidendreae; see Freudenstein & Chase, 2015). Together, *Claderia* and *Agrostophyllum* form a clade that is sister to the rest of tribe Epidendreae (Fig. 4). In previous studies (Freudenstein & Chase, 2015), this clade has only included two genera, both in Agrostophyllinae (*Agrostophyllum* and *Earina* Lindl.).

This position is also supported when only ITS (Fig. 5) and *matK–trnK* (Fig. 6) data are analysed. However, most basal nodes in these analyses remain unresolved. In these analyses, we have been able to include the second genus of Agrostophyllinae (*Earina*, for which there is no published plastome). In both cases, *Claderia* resolves as sister to Agrostophyllinae, albeit with low support (53% in the ITS dataset and 56% in the *matK–trnK* dataset).

We consider the phylogenetic position we retrieved to be robust, even though it contradicts Pridgeon et al. (2014). Our phylogenetic reconstruction based on plastome data had full bootstrap support for this position, and even the ITS and *matK –trnK* analyses support this position. Based on our results, the genus *Claderia* should no longer be associated with Cymbidiinae, nor any of the other relationships previously suggested. *Claderia* is vegetatively distinct from Agrostophyllinae: *Claderia* are terrestrial climbers with broad plicate leaves and hairy roots whereas Agrostophyllinae are epiphytic or rarely terrestrial herbs with duplicate leaves and glabrous roots. They are similar in (usually) lacking pseudobulbs. In flower morphology, Agrostophyllinae is diverse, and there are no consistent differences from *Claderia*, except for a somewhat more reduced lip and glabrous flowers in Agrostophyllinae. *Claderia* is particularly similar to *Earina* in that they both lack a column foot and stelidia and they both have four pollinia. The flowers differ in the cymbiform, fairly deeply lobed lip in *Claderia* and in the excavate clinandrium in *Earina*, as well as their glabrous flowers. In our view, *Claderia* is best maintained in a separate subtribe due to the lack of synapomorphies with Agrostophyllinae; we consider it a member of the monotypic subtribe Claderiinae within tribe Epidendreae; Claderiinae is sister to Agrostophyllinae but there is no formal name for this clade. Given the isolated position of these two subtribes, it is unlikely that any excluded taxa would change this relationship; that said, the possible relationship of *Claderia* to the poorly known genus *Devogelia* (Schuiteman, 2004) should be investigated when material becomes available.

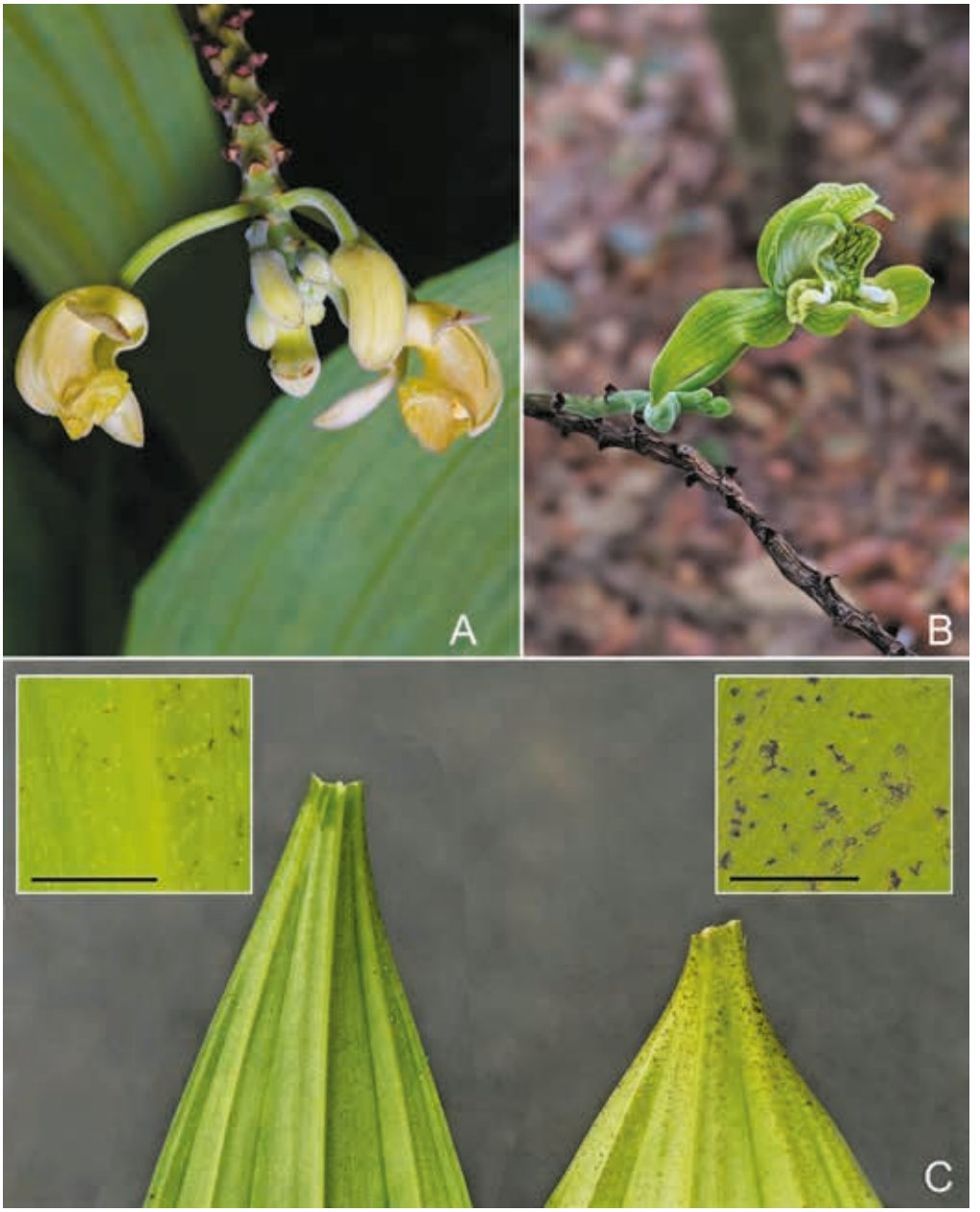

Fig. 2. Differences between *Claderia leontocampus* Niissalo and *C. viridiflora* Hook.f. in Singapore. **A.** *Claderia leontocampus*, with multiple buds developing in quick succession and two small, nodding, cream-yellow flowers open simultaneously. **B.** *Claderia viridiflora*, with flower buds developing individually and only one large, upright green flower with dark green markings open at any one time. **C.** Underside of young leaf of *Claderia leontocampus*, on the left, showing the green colour with no visible brown exudate and *C. viridiflora*, on the right, showing the green base with a brown scale-like exudate. Inset scale bars: 1 mm. A from *Niissalo SING2022-1158*; B from *Leong et al. SING2021-014*; C showing *Niissalo et al. SING2022-442* on the left and *Leong et al. SING2021-014* on the right. (Photos: M. Niissalo)

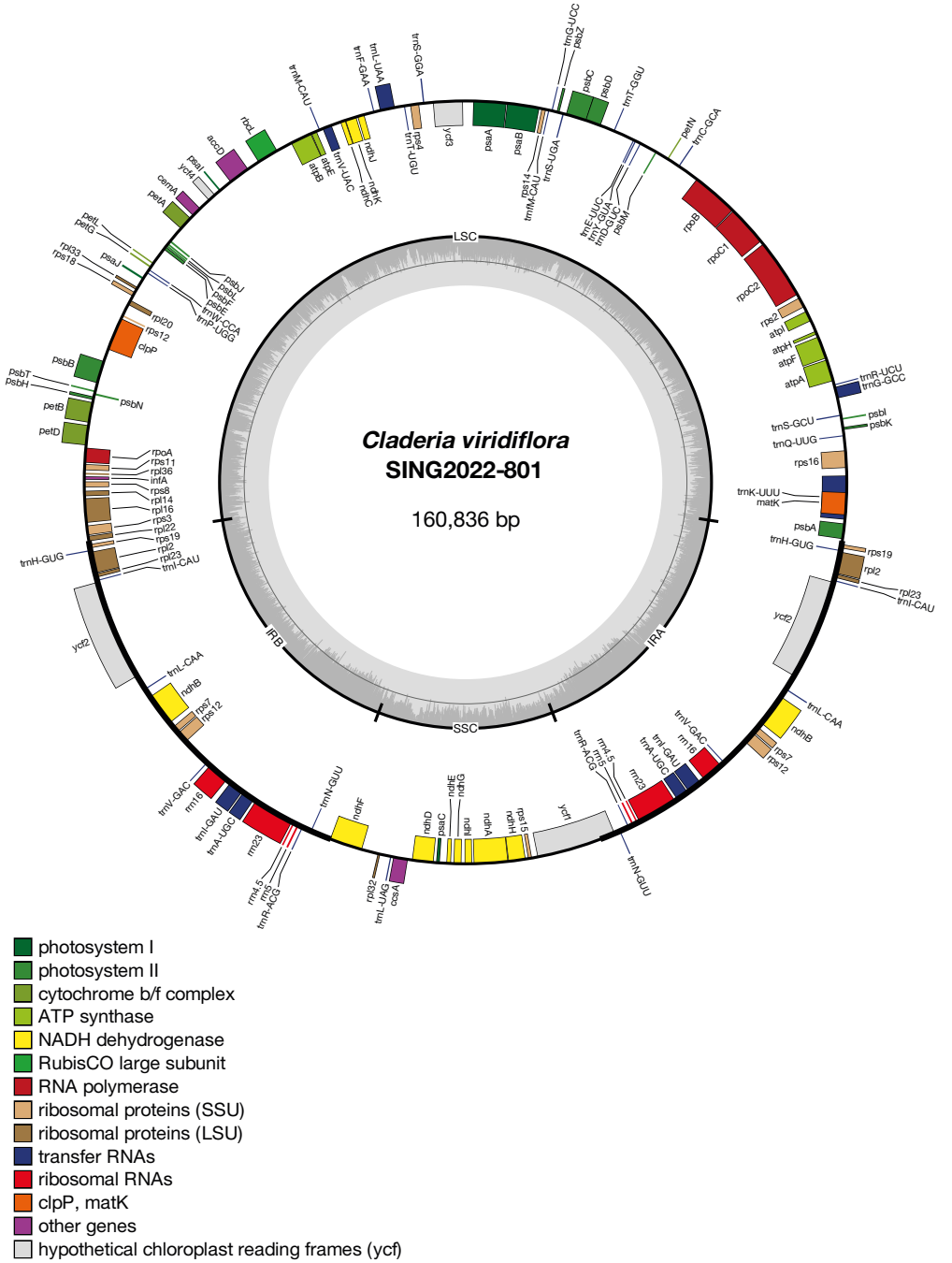

Fig. 3. Plastome structure of *Claderia viridiflora* Hook.f. illustrated using OGDRAW (Greiner et al., 2019).

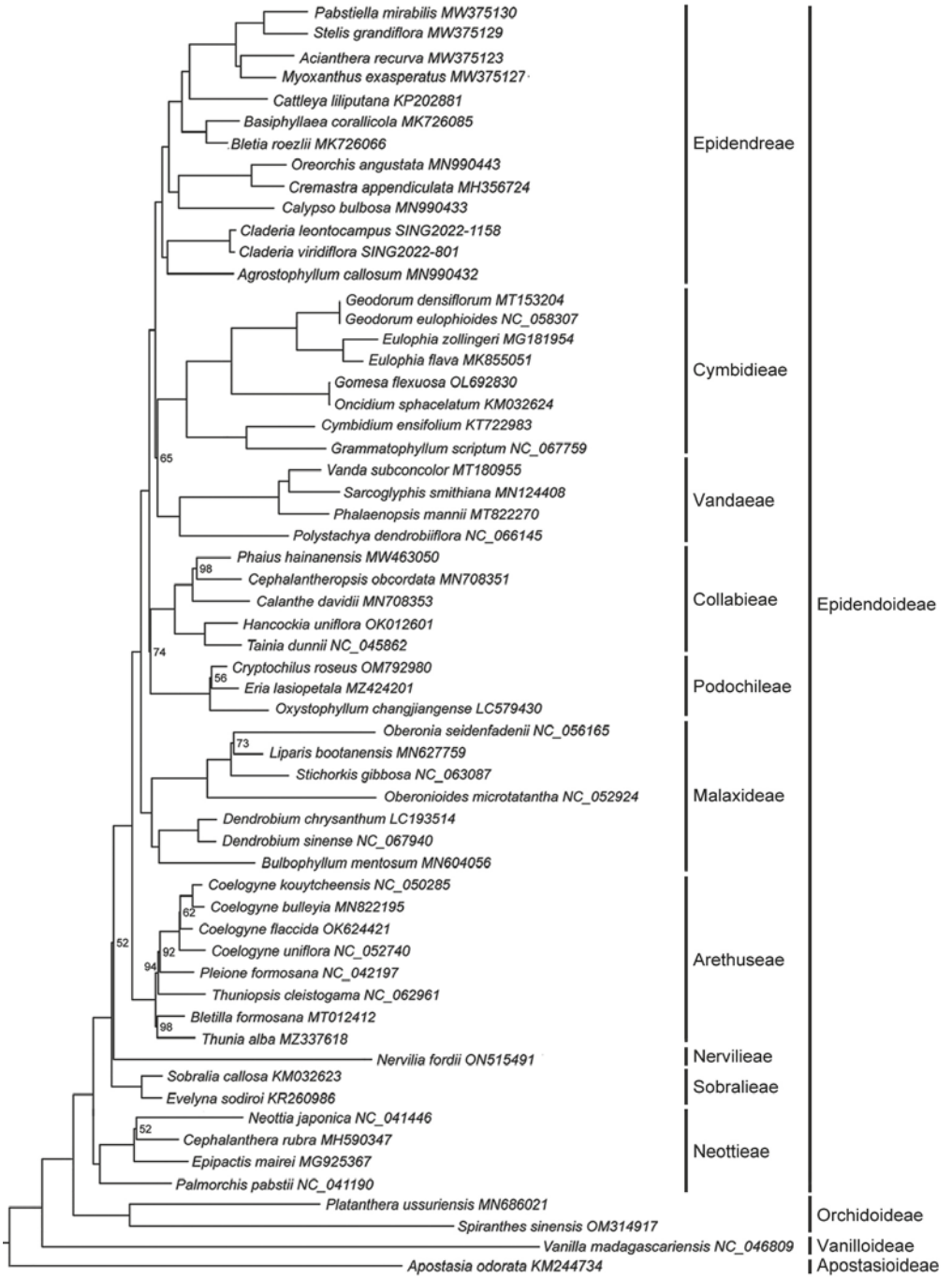

Fig. 4. Phylogenetic position of *Claderia* Hook.f. based on plastome CDS sequences. RAxML bootstrap support below 100% are shown. Tips have associate GenBank accession numbers. The subfamily, tribe and subtribe names follow Freudenstein & Chase (2015).

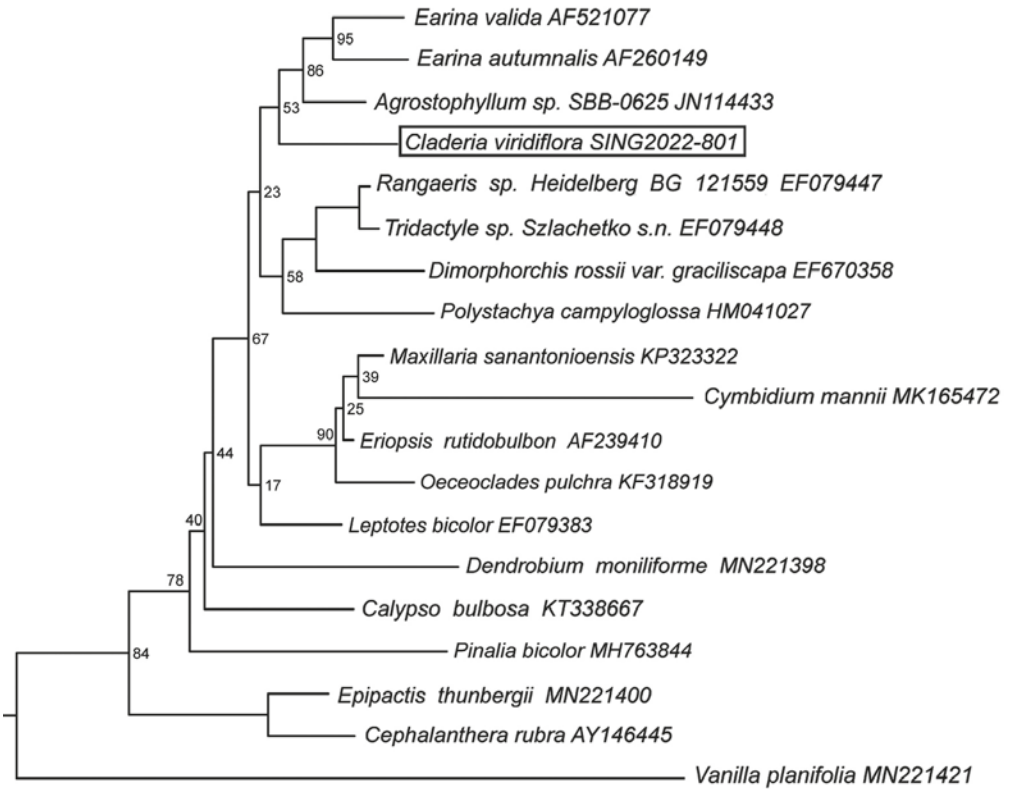

Fig. 5. Phylogenetic position of *Claderia* Hook.f. based on the ITS dataset. RAxML bootstrap support below 100% are shown.

Taxonomic treatment

Claderia leontocampus Niissalo, **sp. nov.**

Similar to *Claderia viridiflora* Hook. f. but differs from it in having narrower flowers (2.5 cm vs 5 cm in C. *viridiflora*), which are pendulous and cream-yellow (upright and bright green in C. *viridiflora*) with narrower lip midlobe (7 mm wide vs 12 mm wide or more in C. *viridiflora*). The inflorescences mostly have two flowers open simultaneously, along with many developing buds, instead of one flower developing and open at a time in *Claderia viridiflora*. – TYPE: Originally from Singapore, Upper Seletar Reservoir, grown in cultivation and vouchered on 4 November 2022 as *Niissalo SING2022-1158* (holotype SING [SING0291161]). (Fig. 2A, 2C, 7–9)

Sympodial herbs, erect to suberect, 25–35 cm tall; initially growing terrestrially, becoming scandent, climbing to at least 2 m up small tree trunks. ***Rhizomes*** terete, bright green, 20–30 cm long, terminating in a leafy stem which produces a new rhizome or up to three new rhizomes from the base; nodes more than 10, internodes 2–3 cm long,

with transparent, red-tinged, pale green sheaths, drying papery, maturing light brown, apex obtuse. ***Roots*** adventitious, emerging from most nodes, initially unbranched, branching when reaching a substrate, with short hairs (c. 1 mm long). ***Stems*** 2–6 cm long, covered with 1 or 2 basal sheaths or small leaves and fully overlapping foliage leaf bases; sheaths imbricate, soon drying and degrading, 4–7 cm long. ***Leaves*** 3–5, mostly dichotomous, arching to one side; narrow base sheathing, 2–4 cm long, clasping the stem; blade suberect, arching, plicate with 4 or 5 prominent veins and several smaller, parallel veins, veins sunken adaxially, raised abaxially, blade narrowly elliptic, thinly coriaceous, glossy, bright yellow-green on both surfaces, young leaves slightly glaucous abaxially, 12–32 × 2.5–5 cm, basal-most and terminal leaves smaller (to 5 × 0.5 cm), margin entire, apex acuminate; blade glabrous, leaf base with minute amount of dark brown exudate on the abaxial surface, not visible to the naked eye. ***Inflorescences*** terminal, with c. 160 flowers, at first erect, then arching and pendulous towards the tip, mostly racemose, rarely with lateral inflorescences emerging from the base, sometimes persisting longer than the leaves; peduncle terete, slender, c. 1.5 mm diam., 10–19 cm long, glabrous, with one larger sterile bract, 5–15 × c. 1.5 cm, and 2 or 3 subsequent smaller bracts; rachis gradually elongating to 13–17 cm long, 2–3 mm diam., pubescent, with persistent floral bracts; floral bracts triangular, c. 1.5 mm long, patent, stiff, puberulous, brown, apex obtuse; two flowers open simultaneously, with many developing buds. ***Flowers*** pendulous, c. 1.3 cm high, c. 2.5 cm wide; pedicel and ovary puberulous, c. 1.5 cm long, non-resupinate, but the inflorescence pendulous, and the lip therefore lowermost. Flowers lasting c. 3 days, lacking fragrance. ***Sepals*** and ***petals*** free, pale cream-yellow. Median sepal arcuate over column, obovate, apex subacute, c. 1.7 × 0.5 cm, shortly pubescent on the abaxial side and glabrous on the adaxial side; lateral sepals arcuate to slightly spreading, broadly oblong to obovate, base gibbous, apex subacute, c. 1.3 × 0.6 cm, pubescence as on median sepal, margin entire. Petals falcate, oblong to oblanceolate, apex obtuse, 1.4–1.6 × 0.3–0.4 cm, glabrous. ***Lip*** free, immobile, fleshy, glossy, 3-lobed, cymbiform, c. 1.5 cm long, c. 1 cm at its widest (measurements taken when spread out; in original condition c. 1.5 cm long, c. 0.8 cm at its widest), abruptly narrowed towards base with two central ridges in the lowest c. 0.9 cm of the lip, cream-yellow, with weak pale greenish reticulation, glabrous abaxially and adaxially except for the central ridges, which are densely hairy along their entire length; side lobes erect, incurved, c. 1 × 0.3 cm; midlobe reniform, reflexed, apex recurved and retuse, margin entire, c. 0.2 × 0.7 cm; spur globose, c. 4 × 4 mm, filled with nectar; the hairy ridges at the centre of the lip c. 1 mm broad towards the apex (combined), c. 2.5 mm broad at the base of the flower (combined), continuing all the way through the inside of the spur to the base of the flower. ***Column*** 1.3–1.5 cm long, c. 0.2 cm at its widest, fleshy, stout, inverted boat-shaped, with long hairs at the base (to 1 mm long), otherwise glabrous; rostellum well-developed; stelidia and column-foot absent, clinandrium entirely covered with anther cap. ***Pollinia*** four, in two pairs, one of each pair smaller than the other, the larger c. 0.4 × 0.2 mm, concave and encapsulating the smaller, the smaller c. 0.3 × 0.1 mm, viscidium poorly developed or absent. ***Capsule*** fusiform, c. 5 × 1.5 cm.

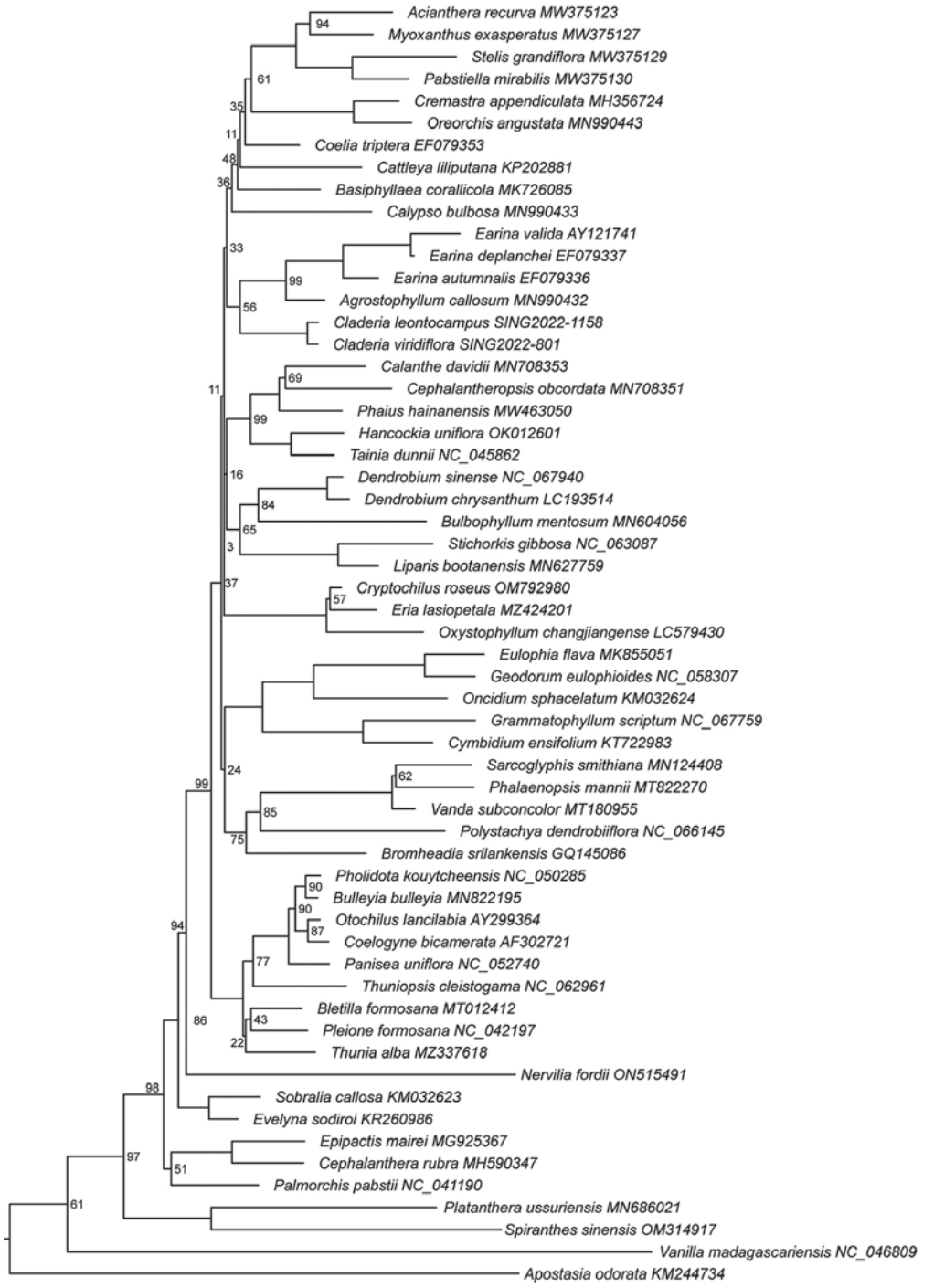

Fig. 6. Phylogenetic position of *Claderia* Hook.f. based on the *matK–trnK* dataset. RAxML bootstrap support below 100% are shown.

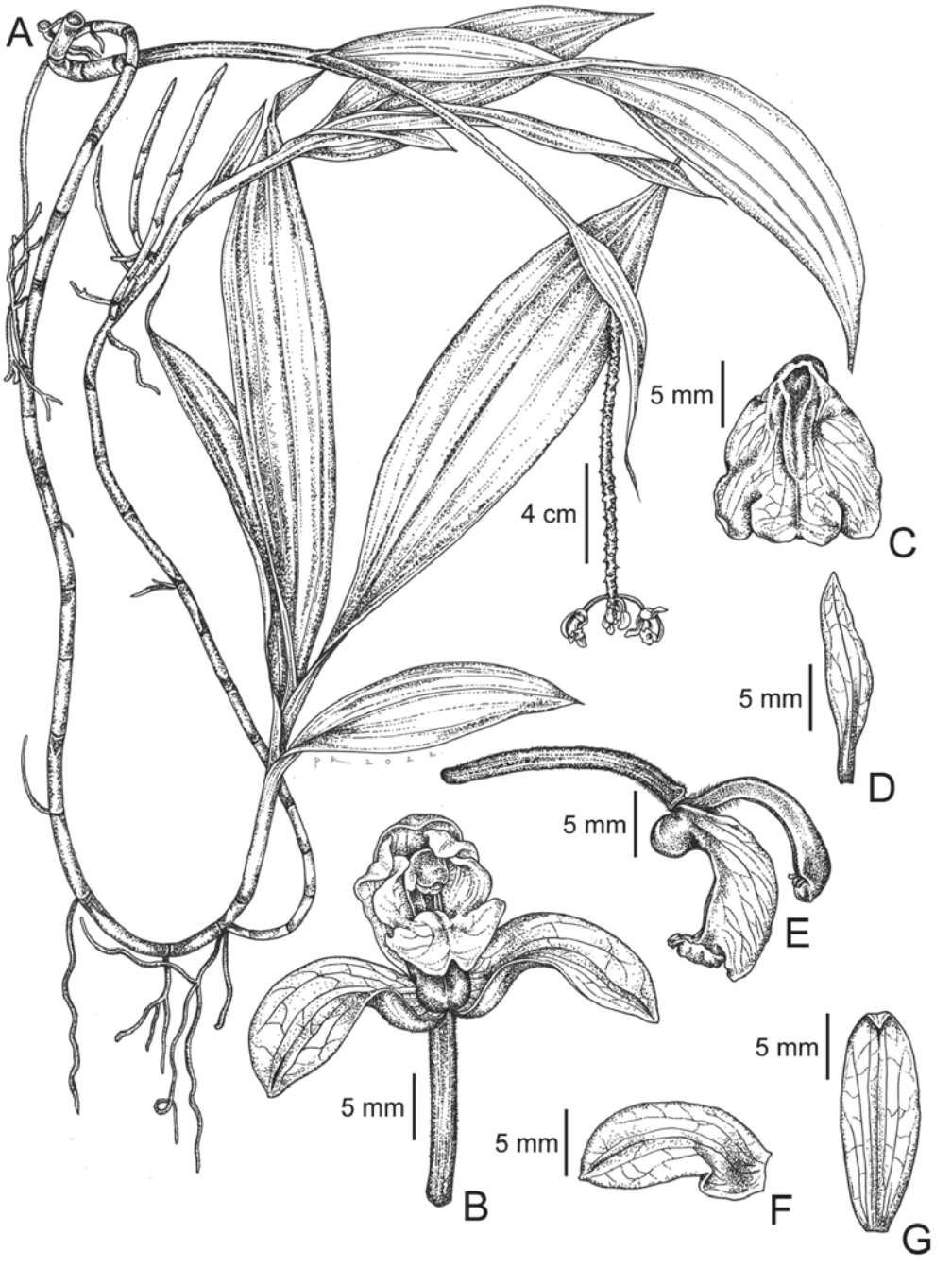

Fig. 7. Line drawing of *Claderia leontocampus* Niissalo. **A.** Habit. **B.** Flower, front view. **C.** Lip, flattened. **D.** Petal. **E.** Flower with sepals and petals removed. **F.** Lateral sepal. **G.** Median sepal. From *Niissalo SING2022-1158*. Drawn by Cheng Puay Koon.

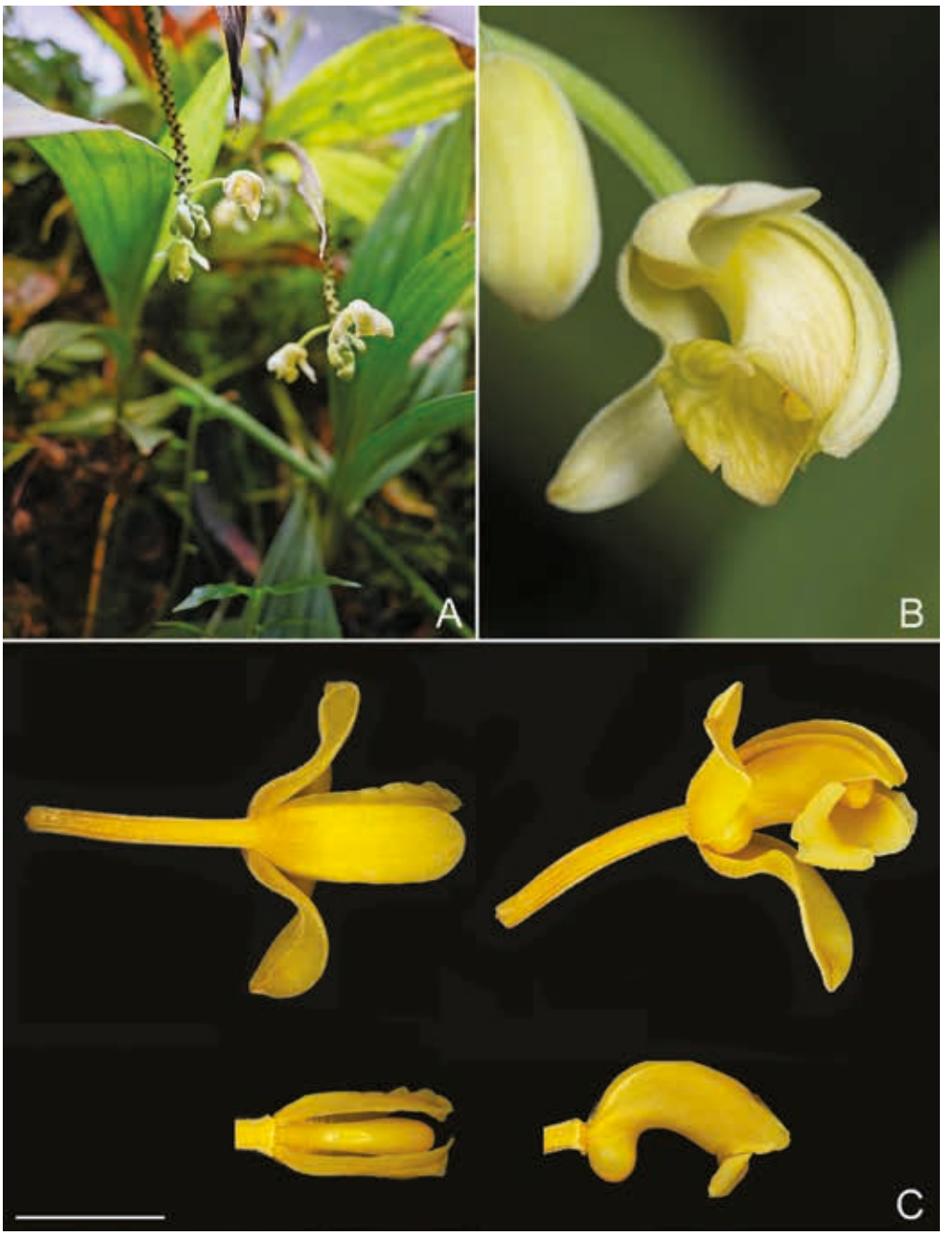

Fig. 8. Photographs of *Claderia leontocampus* Niissalo. **A.** Habit of a plant in cultivation. **B.** Flower. **C.** Details of a flower in spirit. Scale bar: 10 mm. A, B from *Niissalo SING2022-1158*; C from *Niissalo SING2022-1166*. (Photos: M. Niissalo)

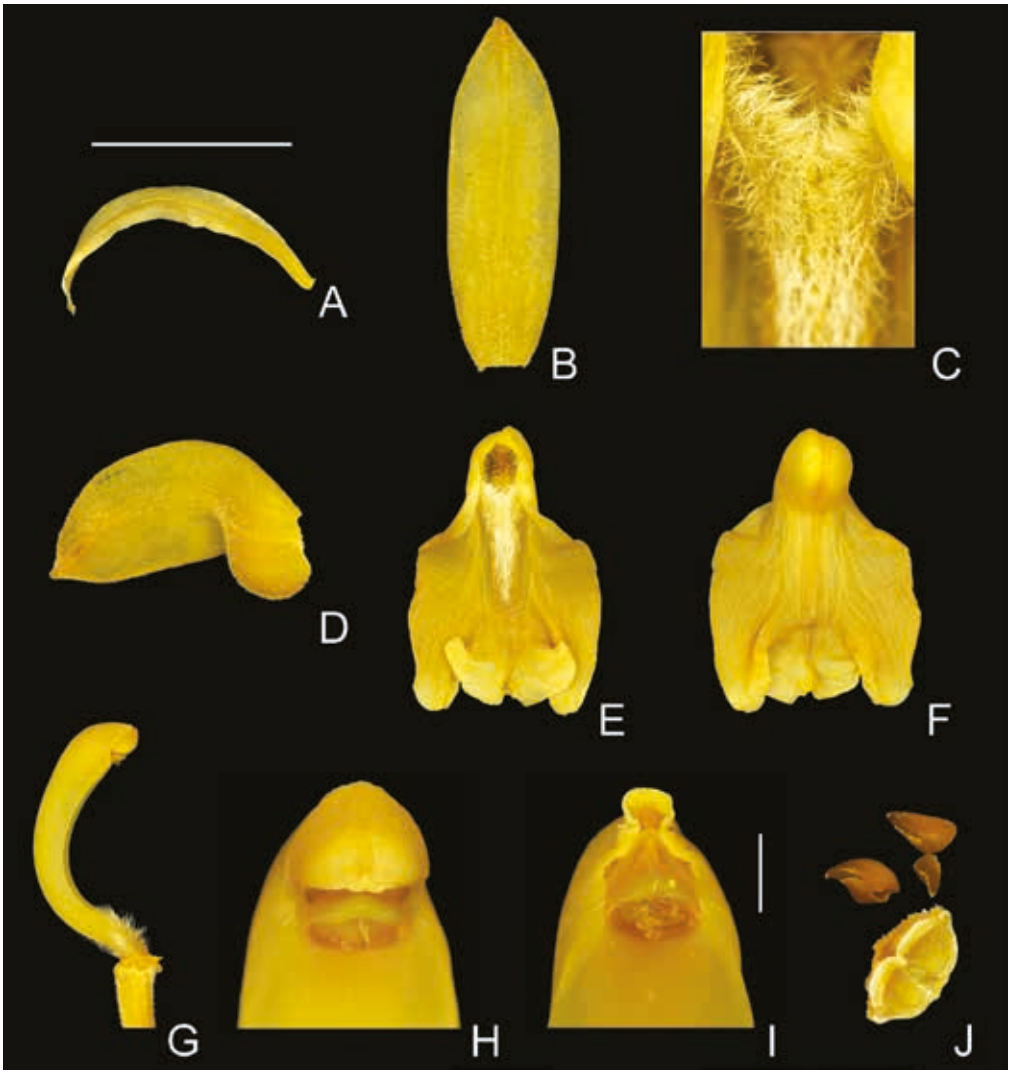

Fig. 9. Photographs of the floral parts of *Claderia leontocampus* Niissalo. **A.** Petal, abaxial surface. **B.** Median sepal, abaxial surface. **C.** Hairs at the base of the lip and the spur mouth. **D.** Lateral sepal, abaxial surface. **E.** Lip, adaxial surface. **F.** Lip, abaxial surface. **G.** Column. **H.** Tip of column, anther cap intact. **I.** Tip of column, anther cap removed. **J.** Anther cap with pollinia. Scale bars: A, B, D–G = 10 mm (upper left corner); C, H–J = 1 mm (lower right corner). All from *Niissalo SING2022-1166*. (Photos: M. Niissalo)

Distribution and ecology. Singapore, Malaysia (Perlis), Indonesia (Sumatra), and possibly Thailand (Nakhon Si Thammarat). In Singapore, it occurs in old secondary lowland forest on well-drained soil, in the area between Upper Seletar Reservoir and Upper Peirce Reservoir.

Etymology. The epithet is our Greek translation of Merlion, a symbol of Singapore, which is the type locality of the species.

Provisional IUCN conservation assessment. Claderia leontocampus appears to be much less common than the frequently collected *C. viridiflora*. The range of *Claderia leontocampus* is completely within the range of the more widespread *C. viridiflora*. The two species appear to occur in similar ecological niches, and they occur sympatrically in Singapore. We know of only five records (of which two are near to each other in Singapore), with an extent of occurrence (EOO) of 220,000 km^2. Of these records, two (*De Wilde & De Wilde-Duyfjes 20520* from Sumatra and *Kerr 0511* from Thailand; the latter a doubtful record) are in areas that no longer have natural forest, suggesting c. 50% population decline in the last three generations (the forest loss has likely been most intense in the last few decades). The species is likely long-lived due to its extensive vegetative spread and generation time is therefore likely to be long. The forest loss still continues in some areas. The area of occupancy is hard to define as we do not know if the species persists in Sumatra and Thailand and how widespread it is in Perlis. Similarly, the population size is unknown. The species is likely under-recorded, as it is almost identical to *Claderia viridiflora* when sterile. We consider it to be Vulnerable under IUCN criterion A2c (IUCN Standards and Petitions Committee, 2022). Fortunately, this species, like *Claderia viridiflora*, is capable of growing in secondary forest, which allows for some hope that it can form sustainable populations in landscapes that increasingly consist of small remnants of degraded forest.

In Singapore, only two individuals are known despite an eight-year period of recording of populations. The species should, therefore, be assessed as Critically Endangered (CR/D) in Singapore. The plant appears to be self-infertile, which means that both individuals need to flower simultaneously in cultivation and be cross pollinated to produce viable seeds for ex-situ conservation. The species is less common in Singapore than *Claderia viridiflora*, which is known from seven individuals.

Additional specimens examined. SINGAPORE: Near Upper Seletar Reservoir, 1 Sep 2020, *Niissalo et al. SING2020-676* (SING [SING0340893, sterile specimen; same individual as the holotype]); ibidem, Apr–Nov 2022, *Niissalo SING2022-1166* (SING [SING0383826, flowers in spirit; same individual as the holotype]); Between Upper Seletar Reservoir and Upper Peirce Reservoir, 26 Apr 2022, *Niissalo et al. SING2022-442* (SING [SING0291160, sterile specimen]).

MALAYSIA: Peninsular Malaysia, Perlis, photograph only (Go et al., 2010).

INDONESIA: Sumatra, P.T. Hargas logging concession, south of the road Sibulussalam–Gelombang, just north of the crossing of the approach road with Lae Batu Batu (a tributary of Alas River), near the abandoned village of Belintang, 2°43′N 97°54′E, c. 16 m, 1 Aug 1985, *De Wilde & De Wilde-Duyfjes 20520* (L [L.1500038, with flowers and fruits; L.1500037, with flowers], P [MNHN-P-P00432969, with flowers], US [US00474468 n.v.]).

Possible additional specimen examined. THAILAND: Nakhon Si Thammarat Province, Ta Samet [possibly Wat Tha Samet, 7°57′19″N 100°00′24″E], 29 Jan 1928, *Kerr 0511* (K [K000482225] – identification uncertain but matches in lip shape and flower size).

Cultivation and horticultural merit. Claderia leontocampus, like *C. viridiflora*, is easy to grow and it grows quickly in suitable conditions. New stems produce further rhizomes before they start to mature, as soon as they sprout leaves, and a plant can produce c. 50 cm to one metre of rhizome per lead per year, each with multiple leafy stems. Each stem often produces multiple new leads. The rhizomes are flexible and in cultivation can be bent back to the pot if a climbing habit is to be discouraged; if allowed to grow uninterrupted, the rhizomes follow any support vertically upwards. Small divisions of both species of *Claderia* are easily rooted in pine bark and sphagnum moss in humid environments. The rhizomes branch when they are cut to take a division, which makes vegetative propagation easy. In *Claderia viridiflora*, we have even observed new rhizomes emerging from cut leafy stems placed in water that have no part of a rhizome attached. The plant seems to be self-infertile, as pollination by hand has been unsuccessful so far. The plant has little horticultural merit. The flowers are small, unscented, and each flower only lasts for c. 3 days, but they are produced continuously for about one year from a single inflorescence. As the genus is distantly related to any other orchids, it likely has little value in hybridisation.

Notes. The fruit description is based on *De Wilde & De Wilde-Duyfjes 20520* from Sumatra. The description is otherwise based on the type specimen.

We have examined herbarium materials from AMES, K (including materials in spirit), L, P and SING. Only one collection, from northern Sumatra, *De Wilde & De Wilde-Duyfjes 20520*, is complete enough to be confidently stated to be conspecific with the new species. The lip shape is not clearly visible in the online image of this collection, but the sheets agree with our species in flower size, in two flowers opening at the same time and multiple buds developing in quick succession, and in colour notes on the sheets. The notes on the sheets state that the plant was found in primary forest at the edge of a peat swamp and marshy forest on flat land with greyish clay-mud, peaty soil, and that it was an 'epiphytic climber against a tree trunk, c. 2 m high. Stems and leaves (dark) green, whole flowers some greenish creamy-white'. Additionally, a *Claderia* photographed from Perlis in northernmost Peninsular Malaysia, not far from the border with Thailand (Go et al., 2010), agrees well with the new species. One collection from Peninsular Thailand, *Kerr 0511*, illustrated in several accounts by Gunnar Seidenfaden (originally in Seidenfaden 1983), also approximately agrees with the new species in lip shape and the size of the flowers. The arrangement of flowers on the inflorescence is not clear on the specimen and we are therefore treating it as a doubtful additional specimen. The notes state: 'flowers greenish yellow, climbing on trees in swamp'.

Vegetatively, *Claderia leontocampus* is very similar to *C. viridiflora*. It has somewhat narrower leaves, but they are within the range of *Claderia viridiflora*. In fresh material, *Claderia viridiflora* has leaves that are somewhat densely covered in a brown, scale-like exudate at the abaxial leaf surface (Fig. 2C). This is visible to the naked eye, especially in young shoots and rhizomes, which appear to be dotted brown. This exudate is much less abundant in *Claderia leontocampus* and is not visible to the naked eye; the young stems and underside of leaves appear bright green. The character

remains constant in common garden conditions. However, this character is usually lost or obscure in dried specimens.

The flowers are presented differently in the two species. In *Claderia viridiflora*, one flower is produced at a time, with only one bud developing at a time and replacing the previous flower as it drops; the other buds remain small as the new flower develops. The inflorescence is often pendulous, but the individual flowers are upright, with the lip often pointing up. The bright green lip midlobe is strongly ornamented in dark green. We do not know how long the individual flowers last in that species. In *Claderia leontocampus*, several flower buds are expanding at any one time. Usually, two flowers are open at all times, and there are multiple developing buds that replace them after they fall. The flowers last c. 3 days each. The inflorescence lasts c. one year, producing c. 160 flowers. The flowers are nodding, with the lip pointing downwards on a pendulous inflorescence; the midlobe is cream-yellow and lacks the dramatically contrasting markings. In the material seen so far, *Claderia leontocampus* has long inflorescences (15–20 cm), whereas in *C. viridiflora*, shorter inflorescences are often produced (< 10 cm). However, the inflorescence length is highly variable in *Claderia viridiflora* (for instance, the stem on the lectotype sheet has a long inflorescence). We have seen both species produce lateral inflorescences from the base of an old spike.

There is considerable variation in the lip shape of *Claderia viridiflora*. This is often poorly preserved in dried specimens and is best seen among the spirit specimens held at K. However, *Claderia leontocampus* is clearly distinct. It has a consistently smaller flower, a unique lip shape, and we have seen no other material of *Claderia* that produces nodding flowers in quick succession as *C. leontocampus* does. We did not attempt to identify sterile material, and many fertile specimens were in too poor condition to assign them to either species. It should be noted that the type material of the poorly understood name *Claderia papuana*, which may be a synonym of *C. viridiflora*, is not similar to *C. leontocampus*, except in having a narrower midlobe than the type of *C. viridiflora*. The central ridges on the lip of *Claderia leontocampus* are densely hairy for their entire length, which makes it appear that there is only one ridge present. In other *Claderia* samples we have seen, the central ridges are glabrous towards the apex of the lip.

The flowers in *Claderia leontocampus* develop approximately symmetrically, and are not distorted between vertical axes, as is often (but not always) the case in *C. viridiflora*. Most authors who have reported pollen numbers from *Claderia* have expressed some uncertainty about the number. For instance, Seidenfaden (1983) stated that there are probably two pollinia (he also cites other references in support of it), but that he wasn't sure, and that there may have been four. Most recently, Pridgeon et al. (2009) states that *Claderia* has two pollinia. This was likely due to the shape of the pollinia. All materials we have seen of both *Claderia viridiflora* and *C. leontocampus* have four pollinia, with the larger pair encapsulating the smaller one.

Nothing is known of pollination in *Claderia*. Given that the new species is sympatric with *Claderia viridiflora*, and that both species are diploid, we suspect that

they might have different pollinators which could act as a barrier for introgression. While *Claderia leontocampus* has no detectable scent, the flowers of *C. viridiflora* have a mild fennel-like scent.

Typification of *Claderia viridiflora* Hook.f. and *Claderia papuana* Schltr.

To typify the pre-existing names in *Claderia*, we selected lectotypes which best show the shape and size of the flowers from the original materials.

Claderia viridiflora Hook.f., Fl. Brit. India 5, fasc. 16: 810 (1890). – Type: [Peninsular Malaysia], Melaka [Malacca], *Maingay 3294* (lectotype K [K000482232, flower only], designated here).

Claderia papuana Schltr., Repert. Spec. Nov. Regni Veg. Beih. 1: 222 (1911). – Type: [Papua New Guinea], Faduna, *Schlechter 19266* (lectotype AMES [AMES00139063], designated here; isolectotypes AMES [AMES00139061, AMES00139064], E [E00394212], GH [GH00139062], K [K000943824], L [L0062383]).

Data availability

The sequences and raw reads generated for this study are available in NCBI (project number PRJNA935056, BioSample accessions SAMN33316546– SAMN33316554). The ITS and plastome sequences are available in GenBank. The raw ddRADseq reads as well as the genome skimming reads are available in SRA. Alignments (ddRADseq sequences, ITS, *matK-trnK* and plastome CDS datasets), ddRAD and plastome phylogenies and structure input file have been submitted to FigShare (10.6084/m9.figshare.22100201). All specimens, including those used in the population genetics study, have been lodged in the Herbarium of the Singapore Botanic Gardens, except *SING2022-435*, for which we currently only have living material.

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