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Gyrocheilos taishanense: A new species of Gesneriaceae from Guangdong, China

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Abstract

Gyrocheilos taishanense G.T. Wang, Yu Q. Chen & R.J. Wang (Gesneriaceae) from south China is described and illustrated with photographs. It is morphologically similar to *G. microtrichum* W.T.Wang, but it is distinguished by its glabrous or sparsely hairy pedicels, zygomorphic calyx with hairs in unequal length, and slightly bilobed adaxial corolla lips. Molecular phylogenetic analysis based on nuclear ITS and plastid *trn*L-F sequences strongly supported this new species as sister to a clade comprising the previously described *Gyrocheilos* species (100% posterior probability and bootstrap support). The new species is evaluated here as Least Concern (LC) according to the IUCN Red List Categories and Criteria.

Keywords: *Didymocarpus*, Didymocarpoideae, phylogeny, taxonomy

Introduction

The genus *Gyrocheilos* W.T.Wang (1981: 28) (Gesneriaceae) currently includes five species and two varieties, distributed in South China and Vietnam (Middleton 2015, Möller 2019). It is morphologically similar to *Didymocarpus* Wallich (1819: 378), but differs mainly in having an undivided adaxial corolla lip, broader corolla tubes and exserted pistils (Wang 1981, Wang *et al.* 1998). Möller *et al.* (2011) revealed that *Gyrocheilos* formed a monophyletic lineage nested within *Didymocarpus*, but they did not present a revised taxonomic treatment due to inadequate sampling of *Didymocarpus* in their phylogenetic analysis. Based on the analysis of a larger number of molecular sequences and pollen characters, Li *et al.* (2016) confirmed that the delimitation of the two genera was inconsistent and supported the merger of *Gyrocheilos* with *Didymocarpus*, as previously proposed by Weber & Burtt (1998), although they both did not validate any nomenclatural changes. We here followed the taxonomic treatment of Gesneriaceae in China by recognizing both *Gyrocheilos* and *Didymocarpus* (Wen *et al.* 2019).

During our fieldwork in Mt. Nanfengshan, near Taishan, Jiangmen City, Guangdong province, China, we found an unrecognized plant growing on moist mountain slopes. It was characterized by a broad corolla tube and exserted pistils, consistent with *Gyrocheilos*, but its corolla lips were slightly bilobed adaxially, as in *Didymocarpus*. Our subsequent molecular phylogenetic analysis based on nuclear ITS and plastid *trn*L-F revealed that the newly collected species was closed to but did not correspond with any known *Gyrocheilos* species described previously. It is hereafter recognized as a new species, *Gyrocheilos taishanense* G.T. Wang, Yu Q. Chen & R.J. Wang.

TABLE 1. The taxa, GenBank accession numbers of ITS and *trn*L-F sequences and data sources for phylogenetic analysis.

Taxa	ITS	*trn*L-F	Source
Allocheilos guangxiensis H.Q.Wen, Y.G.Wei & S.H.Zhong	HQ632994	HQ632897	Möller *et al*. (2011)
Didymocarpus cortusifolius (Hance) H. Lév.	KR336990	KR476537	Li *et al*. (2016)
Didymocarpus dissectus F.Wen, Y.L.Qiu, Jie Huang & Y.G.Wei	KR336991	KR476538	Li *et al*. (2016)
Didymocarpus glandulosus (W.W.Sm.) W.T.Wang	KR336992	KR476539	Li *et al*. (2016)
Didymocarpus glandulosus var. *minor* (W.T.Wang) W.T.Wang	KR336993	KR476540	Li *et al*. (2016)
Didymocarpus grandidentatus (W.T.Wang) W.T.Wang	KR336994	KR476541	Li *et al*. (2016)
Didymocarpus heucherifolius Hand.-Mazz.	KR336996	KR476543#	Li *et al*. (2016)
Didymocarpus heucherifolius var. *yinzhengii* J.M.Li & Shi J.Li	KR336997	KR476544#	Li *et al*. (2016)
Didymocarpus leiboensis Z.P.Soong &W.T.Wang	KR336998	KR476545#	Li *et al*. (2016)
Didymocarpus mengtze W.W.Sm.	KR336999	KR476546#	Li *et al*. (2016)
Didymocarpus praeteritus B.L.Burtt & R.Davidson	KR337002	—	Li *et al*. (2016)
Didymocarpus pseudomengtze W.T.Wang	GU444003	GU444002	Wang *et al*. (2010)
Didymocarpus salviiflorus Chun	KR337004	KR476549	Li *et al*. (2016)
Didymocarpus silvarum W.W.Sm.	KR337005	KR476550	Li *et al*. (2016)
Didymocarpus sinoprimulinus W.T.Wang	KR337006	—	Li *et al*. (2016)
Didymocarpus stenanthos C.B.Clarke	KR337008	KR476552	Li *et al*. (2016)
Didymocarpus stenanthos var. *pilosellus* W.T.Wang	KR337009*	KR476553	Li *et al*. (2016)
Didymocarpus stenocarpus W.T.Wang	KR337007*	KR476551	Li *et al*. (2016)
Didymocarpus tonghaiensis J.M.Li & F.S.Wang	KR337010	KR476554	Li *et al*. (2016)
Didymocarpus yuenlingensis W.T.Wang	KR337011	KR476555	Li *et al*. (2016)
Didymocarpus yunnanensis (Franch.) W.W.Sm.	KR337012	KR476556	Li *et al*. (2016)
Gyrocheilos chorisepalus W.T.Wang	KR337014	KR476558	Li *et al*. (2016)
Gyrocheilos chorisepalus var. *synsepalum* W.T.Wang	HQ632997	HQ632900	Möller *et al*. (2011)
Gyrocheilos lasiocalyx W.T.Wang	HQ632998	HQ632901	Möller *et al*. (2011)
Gyrocheilos microtrichum W.T.Wang	KR337015*	KR476560	Li *et al*. (2016)
Gyrocheilos retrotrichum W.T.Wang	HQ632999	HQ632902	Möller *et al*. (2011)
Gyrocheilos retrotrichum var. *oligolobus* W.T.Wang	KR337016	KR476561	Li *et al*. (2016)
Gyrocheilos retrotrichum var. *oligolobus* W.T.Wang	HQ633000	HQ632903	Möller *et al*. (2011)
Gyrocheilos taishanense G.T. Wang, Yu Q. Chen & R.J. Wang	MK335699	MK335699	This paper
Gyrocheilos taishanense G.T. Wang, Yu Q. Chen & R.J. Wang	MK335700	MK335700	This paper
Gyrocheilos taishanense G.T. Wang, Yu Q. Chen & R.J. Wang	MK335701	MK335701	This paper
Petrocodon dealbatus Hance	KR337020	KR476565	Li *et al*. (2016)
Petrocodon hancei (Hemsl.) Mich.Möller & A.Weber (syn.: *Didymocarpus hancei* Hemsl.)	KR336995	KR476542#	Li *et al*. (2016)
Petrocodon hechiensis (Y.G.Wei, Yan Liu & F.Wen) Y.G.Wei & Mich.Möller (syn.: *Lagarosolen hechiensis* Y.G.Wei, Yan Liu & F.Wen)	KR337018	KR476563	Li *et al*. (2016)
Petrocodon mollifolius (W.T.Wang) A.Weber & Mich.Möller (syn.: *Didymocarpus mollifolius* W.T.Wang)	KR337000	KR476547#	Li *et al*. (2016)

.....continued on the next page

TABLE 1. (Continued)

Taxa	ITS	*trn*L-F	Source
Petrocodon niveolanosus (D.Fang & W.T.Wang) A.Weber & Mich.Möller (syn.: *Didymocarpus niveolanosus* D.Fang & W.T.Wang)	KR337001	KR476548#	Li *et al*. (2016)
Petrocodon scopulorus (Chun) Yin Z. Wang (syn.: *Tengia scopulorum* Chun)	KR337023	KR476567	Li *et al*. (2016)
Primulina hedyotidea (Chun) Yin Z. Wang (syn.: *Chirita hedyotidea* Chun)	KR336988	KR476535	Li *et al*. (2016)
Primulina polycephala (Chun) Mich.Möller & A.Weber (syn.: *Chirita polycephala* Chun)	KR336989	KR476536	Li *et al*. (2016)
Ornithoboea wildeana Craib	FJ501313	FJ501462	Möller *et al*. (2009)
Paraboea rufescens (Franch.) B.L.Burtt	DQ865196	DQ872825	Li *et al*. (2007)

Notes: "—" indicates that this sequence is absent; "*" indicates spurious sequences that were excluded from our analyses; "#" indicates that this accession number was copied from the GenBank rather than Li *et al*. (2016) because of conflict between the two.

Material and methods

Taxon sampling

Materials for plant morphological study and molecular analysis have been deposited at IBSC herbarium. Additional *Gyrocheilos* specimens were examined mainly at IBSC, as well as E, GXMI, IBK, P and PE herbaria (acronyms following Thiers, 2017).

All *Gyrocheilos* and most *Didymocarpus* species, as well as some *Allocheilos* W.T.Wang (1983: 321), *Petrocodon* Hance (1883: 167) and *Primulina* Hance (1883: 169), were selected as ingroup, with available sequence data downloaded from GenBank (Table 1). Data for *Ornithoboea* Parish ex C.B.Clarke and *Paraboea* (C.B.Clarke 1883: 105) Ridley (1905: 63) were similarly obtained and included as the outgroup for the phylogenetic analysis. New sequences were generated for three accessions of the new species. Some species names in this study followed the new taxonomic treatments (Liu 2017, Xu *et al*. 2017).

DNA extraction and PCR

Total DNA was extracted from silica-gel dried leaves using the Tian quick DNA secure Plant kit (Tiangen Biotech, Beijing) following the manufacturer's protocol. The markers ITS and *trn*L-F were amplified using ITS primers ITS1 and ITS4 (White *et al*. 1990) and *trn*L-F primers *c* and *f* (Taberlet *et al*. 1991) respectively and PCR was following standard protocol (Li *et al*. 2016).

Phylogenetic analysis

Sequences were aligned and adjusted manually using the software Geneious version 11.0.3. ITS and *trn*L-F alignments were analyzed separately in PAUP*4.0b10 (Swofford 2002). The incongruence length difference test (Farris *et al*. 1994) was used in PAUP to assess potential incongruence between the two phylogenies, and an insignificant value ($p<0.01$) was detected between the two datasets. The combined dataset was analyzed using Maximum Parsimony (MP) as implemented in PAUP and Bayesian inference (BI) in MrBayes version 3.2.2 (Ronquist & Huelsenbeck 2003). For BI, the model of sequence evolution detected by IQ-Tree version 1.6.6 was K3Pu+F+G4 (Nylander 2004). 1,000,000 generations were run in two independent analyses, each with four Markov chain Monte Carlo (MCMC) chains. One tree was sampled every 500 generations (=2000 trees). The first 400 trees were discarded as burn-in. Posterior probabilities (PP) obtained from the analysis were used to indicate the support for each branch.

Results

Molecular analysis

The combined matrix had a total of 1596 characters, of which 222 (13.91%) were variable and 341 (21.37%) parsimony informative. The parsimony analysis resulted in 153 trees of equal length (1292 steps; consistency index = 0.627; retention index = 0.780). A strict consensus of 153 most parsimonious trees was generated from the combined ITS and *trn*L-F data. The topology derived from the BI analysis was congruent with the MP trees.

The analyses showed that all *Gyrocheilos* and *Didymocarpus* species sampled, as well as *Allocheilos guangxiensis* H.Q.Wen, Y.G.Wei & S.H.Zhong in Wei *et al*. (2000: 297), form a well-supported clade (PP=100, BS=100). Within this clade, all *Gyrocheilos* species, including the new species, formed a strongly supported monophyletic group (PP=100, BS=100) (Fig. 1).

Discussion

Gyrocheilos taishanense has a slightly bilobed adaxial corolla lip, very similar to that of *G. orbiculatum* D.J.Middleton (2015: 237) and *Didymocarpus* species, indicating that this character may not be effective for generic classification as defined by Wang (1981). In addition, both *Gyrocheilos chorisepalum* var. *synsepalum* and *G. retrotrichum* var. *oligolobum* W.T.Wang (1981: 35) have a zygomorphic calyx, but the leaf shape of the former and the depth of the calyx lobes of the latter enable the new species to be distinguished easily.

The topology of phylogenetic tree presented here, based on 41 selected taxa, is almost same as that of Li *et al*. (2016), that is, *Gyrocheilos taishanense* and congeners clustered into a lineage and was nested within *Didymocarpus*. Although molecular sequences were not available for the Vietnamese species *Gyrocheilos orbiculatum* D.J.Middleton and hence its phylogenetic relationship remains unknown, its actinomorphic calyx with equal and deeply divided lobes can distinguish it from the new species, even if both sharing an almost undivided adaxial corolla lip.

Taxonomy

Gyrocheilos taishanense G.T. Wang, Yu Q. Chen & R.J. Wang, *sp. nov*. (Fig. 2).

Type:—CHINA. Guangdong: Jiangmen City, Taishan, 21°55’26”N, 112°56’22”E, elev. ca. 560 m, 1 May 2018, flowering, *Gang-Tao Wang et al. 669* (holotype IBSC 0855721; isotypes IBSC0855722, KUN1347954, PE02331416).

Gyrocheilos taishanense is morphologically similar to *G. microtrichum* W.T.Wang (1981: 32), but is distinguished by its glabrous or sparsely hairy pedicels (vs. glabrous in *G. microtrichum*), zygomorphic (vs. actinomorphic) calyx with hairs in unequal (vs. equal) length, and slightly bilobed (vs. entire) adaxial corolla lips (Table 2).

Rosulate herb, rosulate, rhizomatous. Petioles 4.5–13.7 cm long, with 0.2–0.5 mm long hispid hairs. Leaf blade 7.5–13.1 × 8.5–12.4 cm, orbicular, rounded at apex, cordate at base, nearly glabrous and green adaxially, densely pubescent along the midribs abaxially and ferruginous when dry; margin irregularly serrate to crenate; secondary veins 7–9 at each side. Inflorescence a lax cyme, axillary, 7.5–13.5 cm long, 4–36 flowers; peduncle 7.2–19.1 cm long, with uneven and 0.2–0.5 mm long hairs; bracts 2, opposite, 6–7 × 3–4 mm, lanceolate, acute at apex, slightly cordate at base, midrib and margins with short and dense hairs; bracteoles narrowly lanceolate, with short hairs at apex. Flowering pedicels 7–28 mm long, glabrous or with sparsely hairs unequally in length; calyx campanulate, zygomorphic; lobes 5, divided to half or deeply to base, unequal, two abaxial lobes 1.8–2.2 × ca. 1 mm, oblong, three adaxial lobes ca. 1 mm × ca. 1 mm, broadly triangular, acute to acuminate at apex, sparsely hispid abaxially. Corolla rose-pink, zygomorphic, ca. 1.2 cm long, tube ca. 8 mm long, broadly tubular; adaxial lip 3–5 × 6–7 mm, slightly bilobed, lobes broadly triangular, recurved; abaxial lip 3-lobed, 4–6 × 2–3 mm, lobes oblong to elliptic. Stamens 2, included; filaments adnate to the corolla base and free at upper part, ca. 3 mm long, slightly curved, glabrous; anthers ca. 0.8 × 1.9 mm, spheroid, glabrous, cohering by adaxial surfaces; staminodes 2, adnate to adaxial side at ca. 3 mm from base of corolla tube. Ovary ca. 7 mm long, linear, glabrous, 1-loculed, placentas 2, parietal, intrusive, bifid, recurved. Styles ca. 4 mm long, glabrous, strongly deflected off center to the left or right; stigma capitate; ovules many. Capsule 15–32 × ca. 3 mm,

straight, linear, loculicidal dehiscing to base, valves 2, not twisted. Seeds many, ca. 0.3 × 0.1 mm, ovoid or ellipsoid, without appendage, brown.

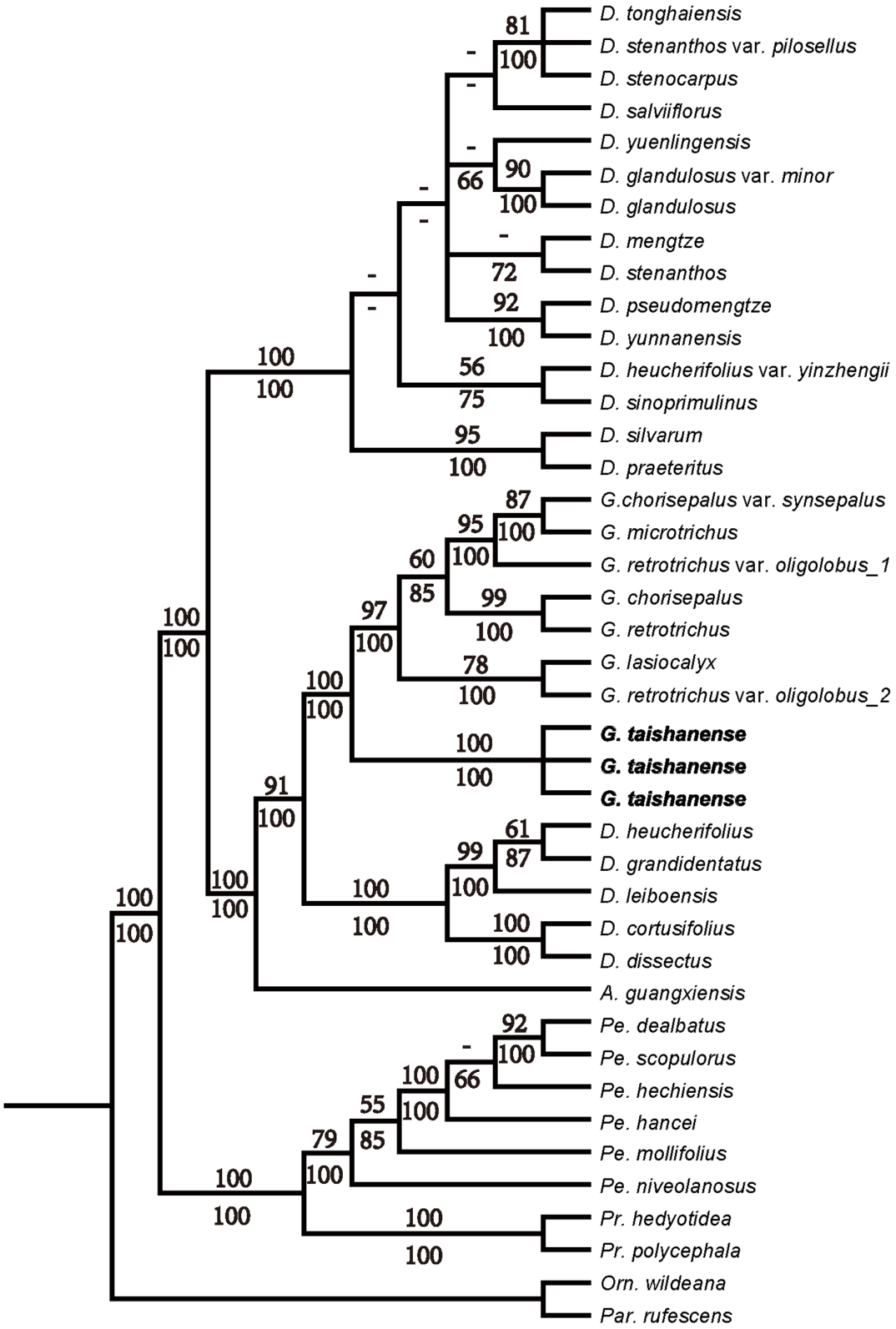

FIGURE 1. Strict consensus of 153 most parsimonious trees generated from the combined ITS and *trn*L-F data. The numbers above and below the branches are bootstrap values and Bayesian posterior probabilities, respectively. "-"means MP-BS support values < 50%. *A*., *Allocheilos*; *D*., *Didymocarpus*; *G*., *Gyrocheilos*; *Orn*., *Ornithoboea*; *Par*., *Paraboea*; *Pe*., *Petrocodon.*

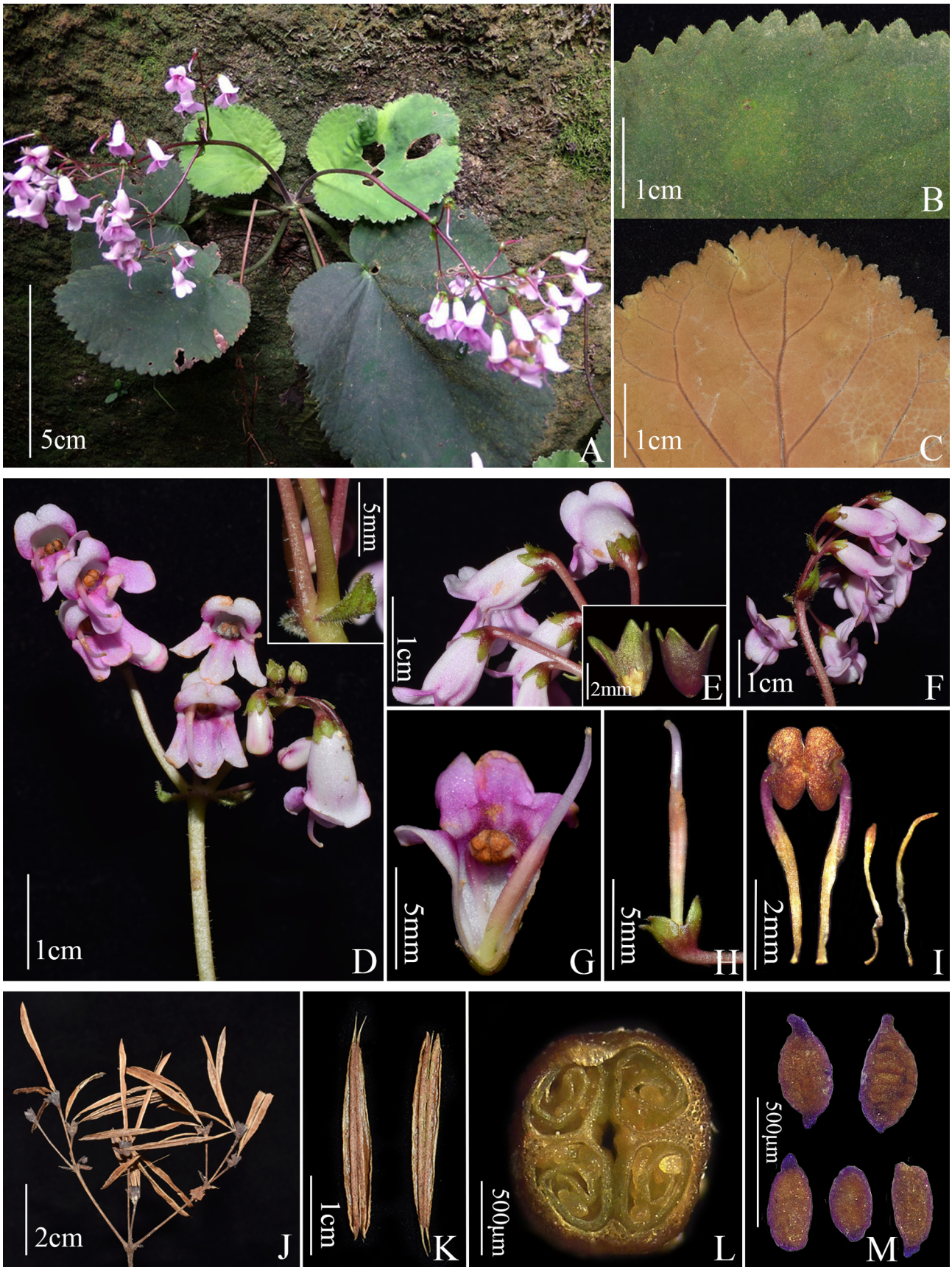

FIGURE 2. *Gyrocheilos taishanense* G.T. Wang, Yu Q. Chen & R.J. Wang, A: Habit, B–C: Adaxial and abaxial side of leaf blade when dry, respectively, D: Inflorescence and peduncle with unequal hairs, E: Zygomorphic calyx, F: Inflorescence, G: Longitudinal section of flower, H: Style and Ovary, I: Stamens (left two) and staminodes (right two), J–K: Capsules, L: Transverse section of capsule, M: Seeds.

TABLE 2. Morphological comparison among *Gyrocheilos taishanense*, *G. microtrichum*, and *G. orbiculatum.*

Character	*G. taishanense*	*G. microtrichum*	*G. orbiculatum*
Leaf size	7.5–13.1 × 8.5–12.4 mm	7.5–12 × 8–12 mm	8.5–15 × 7.4–12.1 mm
Leaf shape	Leaf blade orbicular	Leaf blade broadly ovate to reniform	Leaf blade orbicular
Leaf indument	Nearly glabrous adaxially	Sparsely pubescent adaxially	Sparsely pubescent adaxially
Petiole length	4.5–13.7 mm	8–23 mm	8–18 mm
Bract shape	Bracts lanceolate, entire	Bracts narrowly ovate or narrowly lanceolate, entire to crenate	Bracts ovate, entire
Bract size	6–7 × 3–4 mm	5–7.5 × 2–5 mm	5.5–7 × 5.5–7 mm
Pedicel indument	Glabrous or sparsely hairy	Glabrous	Glabrous
Calyx symmetry	Zygomorphic	Actinomorphic	Actinomorphic
Calyx lobe length	Unequal	Equal	Equal
Divided degree of calyx lobes	To middle or near base	To base or near base	To base
Shape of calyx lobes	Broadly triangular or oblong	Lanceolate-linear to narrowly ovate or oblong	Elliptic
Corolla length	Ca. 12 mm	Ca. 11 mm	15–15.5 mm
Corolla tube length	Ca. 8 mm	Ca. 6.5 mm	6–7 mm
Shape of adaxial corolla lips	Slightly bilobed	Entire	Almost entire
Stamen position	Adnate to adaxial side at ca. 3.0 mm from base of corolla	Adnate to adaxial side at ca. 2.5 mm from base of corolla	Adnate to adaxial side at ca. 3.2 mm from base of corolla

Phenology:—Flowering from April to May; fruiting from May to July.

Etymology:—The specific epithet refers to the type locality of this species.

Distribution and habitat:—*Gyrocheilos taishanense* is currently only found on Mt. Nanfengshan, Taishan, Jiangmen City, Guangdong Province, China. It grows in secondary broad-leaf forests on mountain slopes with 60–70% canopy density and in mountain valleys. Associated plants mainly include *Castanopsis fissa* (Champ. ex Benth.) Rehder & E.H.Wilson, *Psychotria serpens* L. and *Conchidium pusillum* Griff.

Additional specimens examined for taxa in this study

Gyrocheilos chorisepalum W.T.Wang:—CHINA. Guangxi Zhuang Autonomous Region: Nanning City, Wuming County, *Mus. Guangxi 381* (holotype IBSC0097119), *Xie Guanggan et al. 2-72* (paratype PE00154195); Shanglin County, *Cai Canxing 5149* (paratypes IBK00191640, IBSC0004893).

Gyrocheilos chorisepalum* var. *synsepalum W.T.Wang (1982: 135):—CHINA. Guangxi Zhuang Autonomous Region: Yulin City, Beiliu, *Beiliu Exped. 8-4031* (holotype GXMI050706), *Fabrica Med. Pingzheng 6761* (paratype GXMI050707); Guangdong Province: *Nanzhidi 4099* (paratype IBSC0549587).

Gyrocheilos lasiocalyx W.T.Wang:—CHINA. Guangxi Zhuang Autonomous Region: Guigang City, Guiping, *Guiping Exped. 8-327* (holotype GXMI050708), *Liang Naikuan 10757* (paratype IBSC0549589).

Gyrocheilos microtrichum W.T.Wang:—CHINA. Guangdong Province: Maoming City, Xinyi, *C. Wang 32143* (holotype IBSC0004893; isotypes IBK00054510, IBK00054512), *C.Wang 38118* (paratypes IBSC0549591, IBK00054511).

Gyrocheilos orbiculatum D.J.Middleton:—VIETNAM. *Petelot s.n.* (holotype E00697919; isotypes P04021810, P04021811).

Gyrocheilos retrotrichum W.T.Wang:—CHINA. Guangdong Province: Maoming City, Xinyi, *S. P. Kao 51222* (holotype IBSC0649582; isotypes IBK00191641, IBSC0004871, IBSC0004872, IBSC0004894, PE00030815, PE00154201, PE00030815).

Gyrocheilos retrotrichum* var. *oligolobum W.T.Wang:—CHINA. Guangxi Zhuang Autonomous Region: Liuzhou City, Rongshui, *Chen Shaoqing 8902* (holotype IBSC0004895; isotype IBK00191642); *T.Chen 16* (paratypes IBK00191643, IBSC0549607); *Chen Shaoqing 14122* (paratypes IBK00054514, IBSC0549606).

Conservation status

More than 3000 mature individuals in four subpopulations were found in the mountainous valleys nearby the type locality. The species grows in a well-preserved nature reserve and there are no plausible threats due to its low medicinal and ornamental values. According to the IUCN (2012) and IUCN Standards and Petitions Subcommittee (2019), the Least Concern (LC) category is recommended for *Gyrocheilos taishanense.*

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