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Primulina rubella sp. nov. (Gesneriaceae) from a limestone area in Guangdong, China

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A new species of *Primulina* (Gesneriaceae) from a limestone area in southern China, *P. rubella* L.H. Yang & M. Kang, is described and illustrated. The new species has purple-red flowers and is thus similar to *P. lijiangensis*, but differs by its petiole, leaf blade, cyme, filament, stigma and ovary. In addition, a molecular phylogenetic reconstruction indicated that *P. rubella* and *P. lijiangensis* belong to quite different clades. Instead, *P. rubella* appears to be closely related to the sympatric *P. qingyuanensis*, although these two species are obviously differentiated both in terms of flower color and other vegetative and floral characters. The conservation status of *P. rubella* is assessed as ‘Critically Endangered’ (CR) according to the IUCN red list categories and criteria.

The previously monotypic *Primulina* has been expanded both by a redefinition based on molecular phylogenetic studies (Wang et al. 2011, Weber et al. 2011) and by the discovery of numerous new species from southern China in recent years. Hereby, *Primulina* has become one of the largest genera in Didymocarpoideae with more than 150 species distributed in the karst areas of southern China and northern Vietnam (Möller et al. 2016). Flower color in this genus shows a wide range of diversity and has even been used as the main diagnostic characteristics for new species, such as *P. lutea* (Yan Liu & Y.G. Wei) Mich. Möller & A. Weber, *P. purpurea* Fang Wen, Bo Zhao & Y.G. Wei (Wen et al. 2012) and *P. heterochroa* F. Wen & B. D. Lai (Wen et al. 2015). However, the flower color is difficult to accurately describe, and its perception is easily affected by the condition of the material (such as fresh or dried), and can also vary between observers. Therefore, for an accurate description it is recommendable to compare living material with a colorimetric card. At present, most *Primulina* species have been described as having purple-blue corolla, but there are also some species with a yellow corolla (Pan et al. 2016). However, in a few species, such as *P. lijiangensis* (B. Pan & W.B. Xu) W.B. Xu & K.F. Chung (Xu et al. 2012) the corolla color was described as purple in the original protologue (but this is inaccurate) and in *P. heterochroa* the corolla color is essentially purple-red.

During our field work in the karst area of Qingxin County, Guangdong Province, we found an unknown species of *Primulina*. The plants grow on moist rock surfaces, with

no more than 15 individuals found in 2012. We introduced several living individuals from the field into South China Botanical Garden. When flowering, to our surprise, this species displayed a purple-red and slender corolla. On more detailed morphological observation and comparison, we found that the flower of this species is similar to that of *P. lijiangensis*, which also has a purple-red and slender corolla, but the new species differs from the latter by details of its petiole, leaf blade, filament, stigma, ovary, etc. To further elucidate the phylogenetic affinities of the new taxon, we reconstructed a phylogeny of 90 *Primulina* species based on the internal transcribed spacer (ITS) and three plastid markers (*trnL-trnE*, *rpl32-trnL*, *atpB-rbcL*). The sequence data was retrieved from the phylogenetic reconstruction described by Kang et al. (2014). DNA sequences generated in this study were deposited in GenBank (accession no. KX639688–KX639691). The molecular phylogenetic analysis, together with the morphological, biogeographic and literature analyses (Wang et al. 1998, 2011, Li and Wang 2004, Wei et al. 2010, Weber et al. 2011, Xu et al. 2012), convinced us that our plant represents a new species of *Primulina*.

Primulina rubella L.H. Yang & M. Kang sp. nov. (Fig. 1–2)

A species differing from *P. lijiangensis* by its flattened and 11–17 mm wide petiole (vs semi-cylindrical, 5–9 mm wide), broadly ovate to broadly elliptic leaf blade (vs rhomboid-ovate to

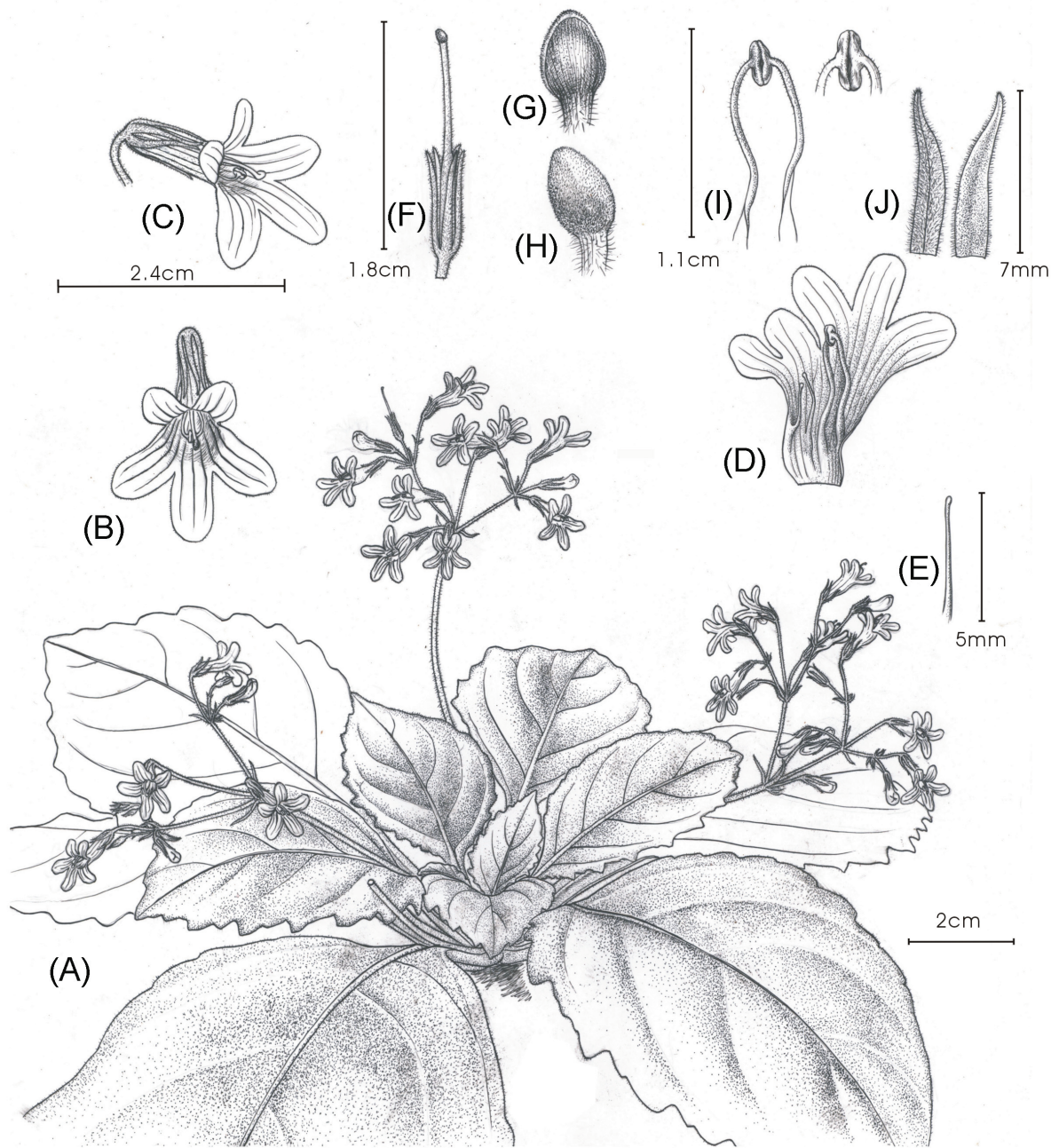


Figure 1. *Primulina rubella* sp. nov. (A) habit, (B) flower in front view, (C) flower in side view, (D) opened corolla, showing stamens and staminodes, (E) staminode, (F) pistil, (G), (H) stigma, (I) fertile stamens and anther, (J) bract. Drawn by Yun-Xiao Liu based on cultivated individual collected from type locality.

elliptic), with acute to subacute apex (vs obtuse to round) and dentate margin (vs repand to crenate), (12–)18–32(–64)-flowered cyme (vs 10–20-flowered), white filament (vs purple-red), 5.8–7.6 mm long ovary (vs ca 4 mm long), ovoid to narrowly ovoid stigma (vs obtrapeziform), with undivided apex (vs emarginate). From *P. qingyuanensis* it differs by its densely appressed puberulent leaf blade (vs densely glandular manicate), purple-red corolla (vs purple-blue), slender tube (vs narrowly funnel-formed), elliptic anther (vs flabellate),

ovoid to narrowly ovoid stigma (vs cuneal), with undivided apex (vs 2-lobed).

Type: China. Guangdong Province, Guangzhou City, cultivated in South China Botanical Garden, introduced from Guangdong Province, Qingxin County, Shitan Town, elevation ca 100 m a.s.l., 24.15°N, 112.73°E, growing on the moist rock surfaces of limestone hills. 19 May 2016 (flowering), Li-Hua Yang, YLH297 (holotype: IBSC!).



Figure 2. *Primulina rubella* sp. nov. (A) habit, (B) cyme, (C) flower in side view, (D) flower in front view, (E) opened corolla, showing stamens and staminodes, (F) leaf, (G) pistil, (H) calyx lobes. Photo by Li-Hua Yang.

Etymology

The specific epithet refer to the purple-red corolla of the species.

Description

Perennial herb. Rhizome subterete, 2.0–4.0 cm long, ca 2 cm in diameter. Leaves 12–16, all basal; petiole flattened, 6.1–8.1 × 1.1–1.7 cm, appressed puberulent, eglandular; leaf blade slightly fleshy when fresh, chartaceous as dried, broad-ovate to broad-elliptic, 11.2–16.4 × 8.7–11.8 cm, with both surfaces densely appressed puberulent, acute to subacute at apex, cuneate to broadly cuneate at base, with dentate margin; lateral veins 4 on each side, abaxially conspicuous. Cymes 6–11, axillary, with 2–3 branches and

(12–)18–32(–64) flowers. Peduncles 7.8–17.5 cm long, 1.1–2.4 mm in diameter, densely puberulent. Bracts 2, opposite, lanceolate-linear, 7.9–14.1 × 1.1–2.2 mm, with entire margin, acute at apex, densely pubescent on outer surface, glabrescent inside. Pedicel 12.3–20.3 mm long, 0.6–0.8 mm in diameter, densely pubescent. Calyx 5-parted to near base; lobes narrowly lanceolate, 7.8–10.9 × 1.0–1.6 mm, densely villous outside, glabrescent inside, with entire margin. Corolla purple-red with ca 15 dark longitudinal striae inside, 2.3–2.5 cm long, pubescent on outer surface outside, glabrous inside; corolla tube slender, 1.2–1.4 cm long, 4.1–5.0 mm in diameter at the mouth, 3.8–4.9 mm in diameter at the base; limb distinctly 2-lipped; adaxial lip 2-parted, its lobes oblong, 6.3–7.2 × 4.0–4.6 mm, rounded

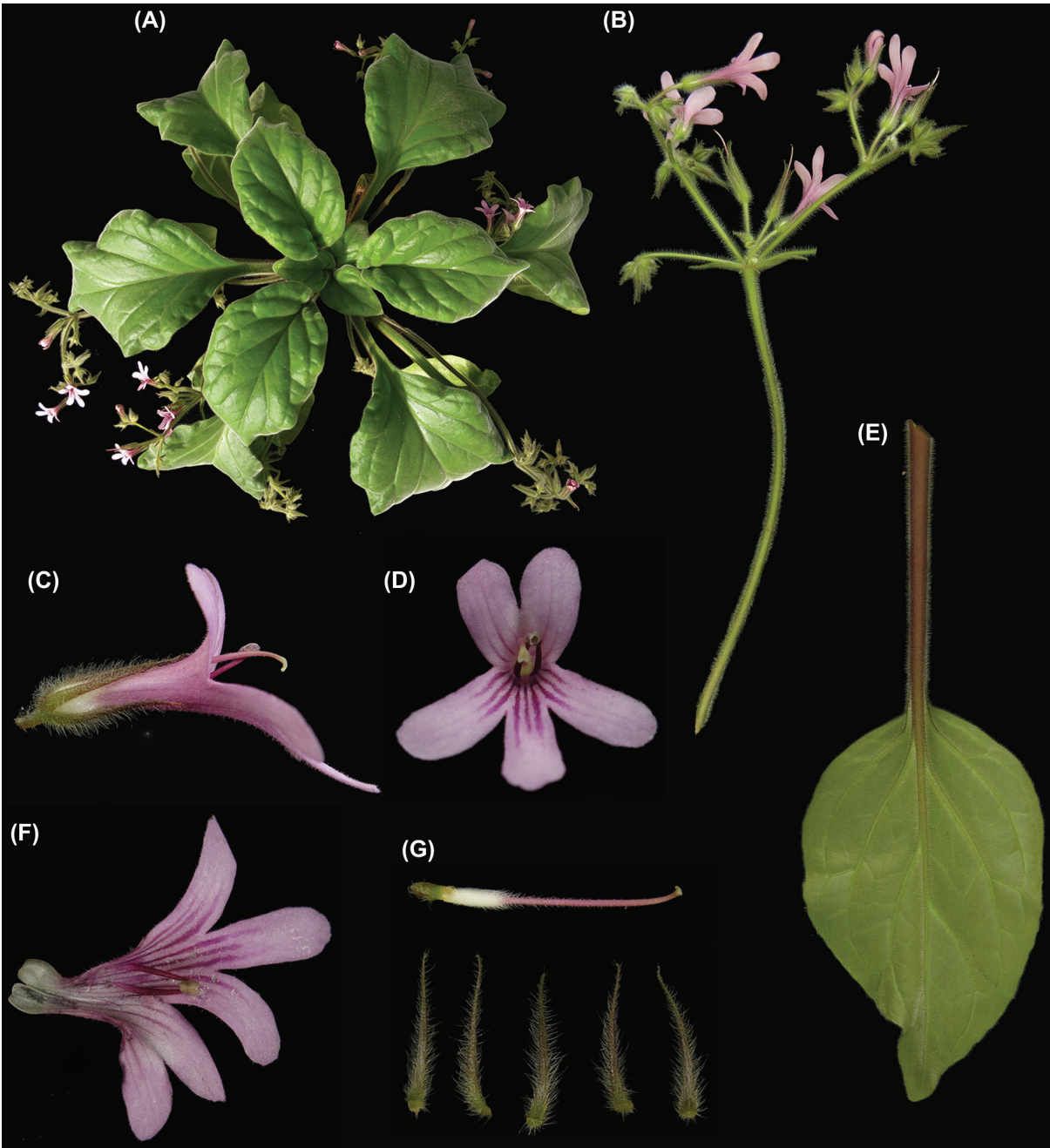


Figure 3. *Primulina lijiangensis*. (A) habit, (B) cyme, (C) flower in side view, (D) flower in front view, (E) leaf, (F) opened corolla, showing stamens and staminodes, (G) pistil and calyx lobes. Photo by Li-Hua Yang.

at apex; abaxial lip 3-lobed, its lobes oblong, $8.6\text{--}9.7 \times 3.8\text{--}4.9$ mm, rounded at apex. Stamens 2, adnate to $6.0\text{--}6.3$ mm above the corolla tube base; filaments linear, $8.2\text{--}9.3$ mm long, white, conspicuously geniculate below middle, sparsely pubescent; anthers elliptic, fused by their entire adaxial surfaces, ca 2 mm long, glabrous. Staminodes 3; lateral ones $3.9\text{--}4.7$ mm long, adnate to $6.2\text{--}6.5$ mm above the corolla tube base; middle one ca 1 mm long, adnate to $3.7\text{--}4.5$ mm above the corolla tube base. Disc annular, 1.0–1.2 mm high. Pistil $17.2\text{--}18.2$ mm long; ovary cylindrical, $5.8\text{--}7.6$ mm long, ca 1 mm in diameter, densely pubescent; style $10.2\text{--}12.3$ mm long, densely pubescent; stigma ovoid to narrowly

ovoid, undivided, ca 1.1 mm long, ca 0.8 mm wide. Capsule linear, 1.0–1.5 cm long, densely pubescent.

Phenology

Primulina rubella is flowering from May to June.

Distribution, ecology and conservation status

Primulina rubella L.H. Yang & M. Kang is only found at the type locality, growing on moist and shady limestone rock surfaces. About 15 mature individuals were found in 2012, but only 1 mature individual could be found in 2015. Such a severe reduction in population size was perhaps due to

Table 1. Morphological comparison of *Primulina rubella* sp. nov., *P. lijiangensis* and *P. qingyuanensis*.

Characters	<i>P. rubella</i>	<i>P. lijiangensis</i>	<i>P. qingyuanensis</i>
Petiole	flattened, 11–17 mm wide	semi-cylindrical, 5–9 mm wide	flattened, ca 5 mm wide
Leaf blade shape	broadly ovate to broadly elliptic	rhomboid-ovate to elliptic	ovate or broadly ovate
Leaf blade size (cm)	11.2–16.4 × 8.7–11.8	6–20 (30) × 3–10 (15)	5–10 × 4–7
Leaf blade apex	acute to subacute	obtuse to rounded	acute to obtuse
Leaf blade margin	dentate	repand to crenate	irregularly repand-crenate to serrate and revolute
Indumentum on leaf blade	densely appressed puberulent	densely pilose	densely glandular manicate
Cymes	(12–)18–32(–64)-flowered	10–20-flowered	3–9-flowered
Corolla	tube slender, purple-red	tube slender, purple-red	tube narrowly funnel-shaped, purple-blue
Filament	white, glabrous	purple-red, glabrous	white, densely purple piloglandulose
Anther	elliptic	elliptic or reniform	flabellate
Style (mm)	white, 10.2–12.3	purple-red, 12–14	green, 8–10
Ovary (mm)	5.8–7.6	ca 4	4–5
Stigma	ovoid to narrowly ovoid, apex undivided	obtrapeziform, apex emarginate	cuneal, apex 2-lobed
Anthesis	May to June	July to August	May to June

recent human activities. Based on the current information, *P. rubella* is considered as ‘Critically Endangered’ (CR): B2a, B2b(v), C2a(ii), D, E, following the IUCN categories and criteria (IUCN 2016).

Similar species and relationships

The new species is mostly similar to *P. lijiangensis* (Fig. 3), but differs by its petiole, leaf blade, cyme, filament, stigma and ovary (Table 1). According to our study of living individuals, the petiole of *P. lijiangensis* should be described as semi-cylindrical (Fig. 3E), but it was inaccurately described as applanate in the original protologue (Xu et al. 2011). Both these species are distributed in limestone areas, but with non-overlapping distributions (Fig. 4). Furthermore, the analysis of DNA sequences revealed that *P. rubella* and *P. lijiangensis* belong to different clades of the molecular phylogeny of the genus (Fig. 5), indicating

that the two species only have a distant relationship. The phylogenetic analysis demonstrated that *P. rubella* is a sister species to *P. qingyuanensis* (Fig. 5), which was recently discovered in the same locality (Ning et al. 2013). These two species usually display similar leaf blade shape, but differs by their indumentum, cyme, corolla, anther and stigma (Table 1). It is probable that the two species might have experienced genetic introgression during their divergence. Introgressive hybridization has frequently been reported in Gesneriaceae (Puglisi et al. 2011). In *Primulina*, Xu et al. (2013) recently described a new species, *P. cardaminifolia*, which could possibly be a homoploid hybrid. Alternatively, the two species may have diverged in sympatry through natural selection enforced by pollinators. However, our current data cannot confirm these hypothesis and further work is needed to determine the evolutionary mechanism of the speciation.

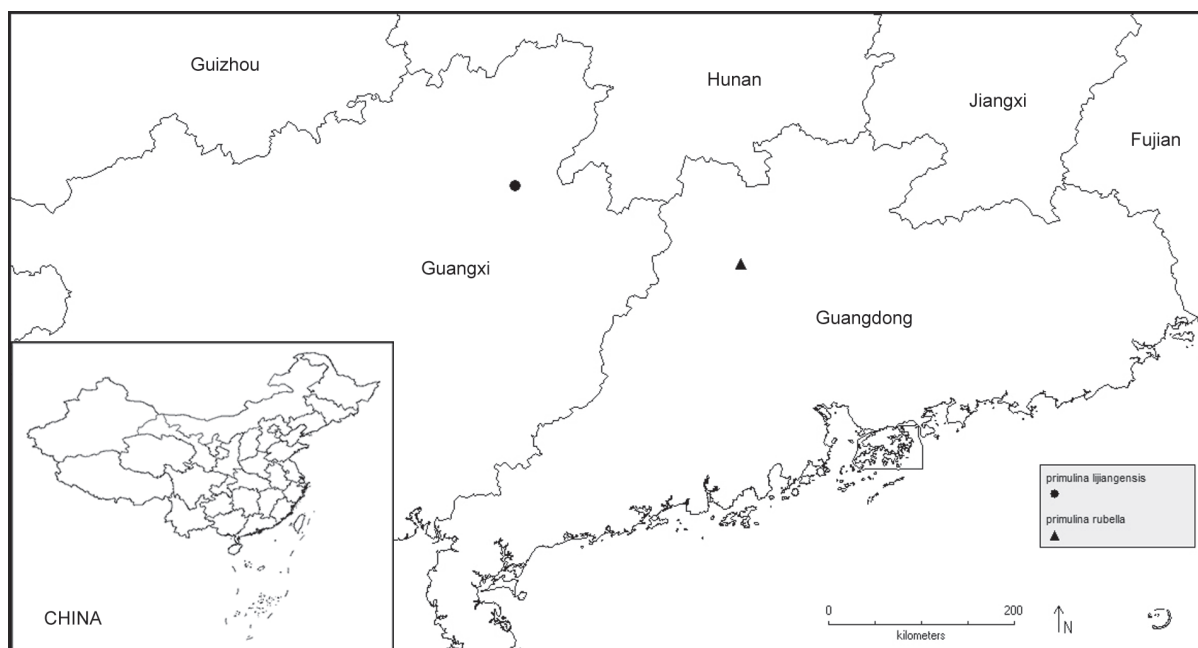


Figure 4. Distribution of *Primulina rubella* sp. nov. (triangle) and *P. lijiangensis* (circle).

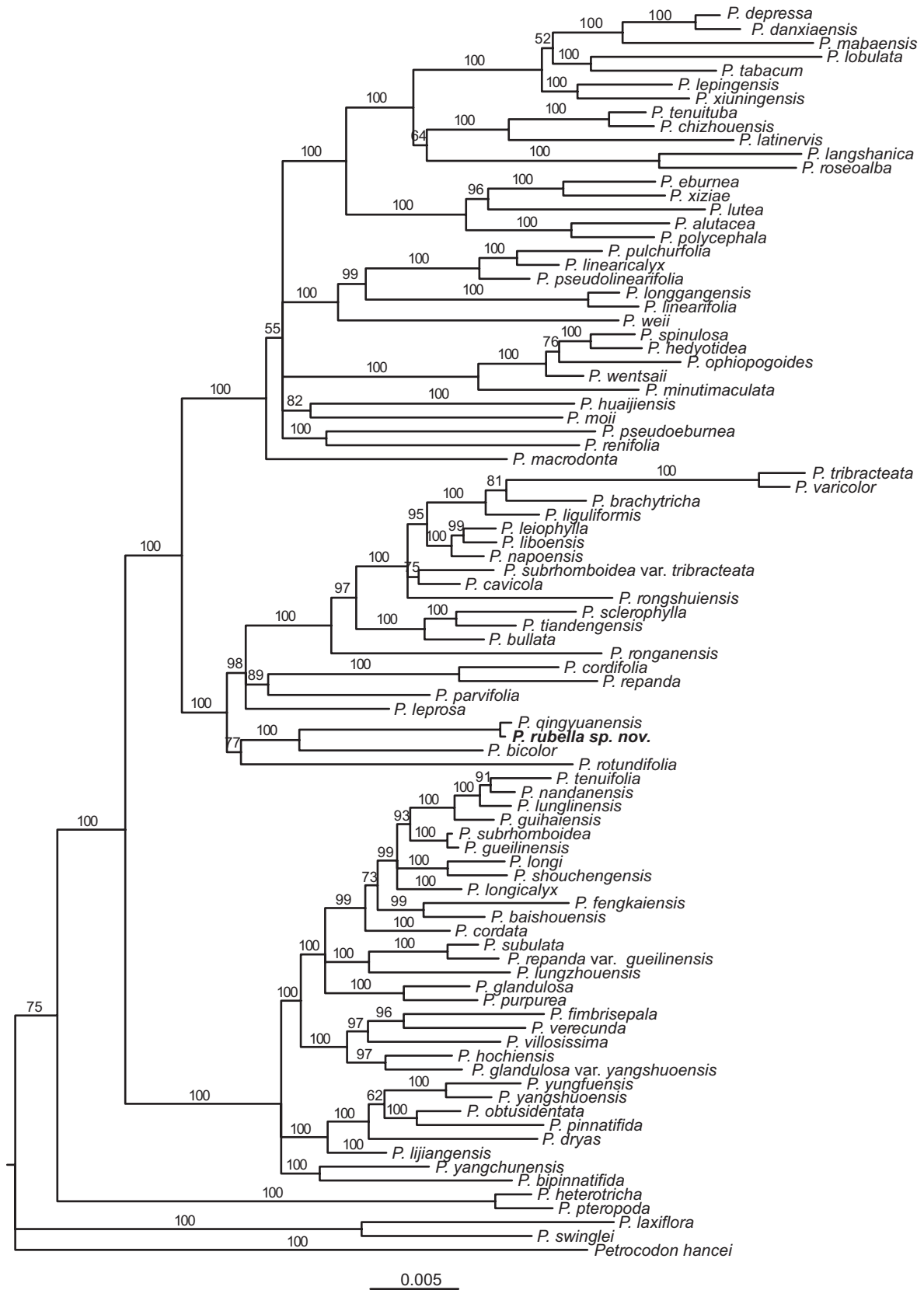


Figure 5. Majority rule consensus Bayesian tree based on combined sequence data from the internal transcribed spacer (ITS) and three plastid markers (*trnL-trnF*, *rpl32-trnL* and *atpB-rbcL*). Numbers above branches are posterior probabilities in percent.

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