

Chirita auriculata (Gesneriaceae), a new species from China, placed in sect. *Chirita* based on molecular data

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Chirita auriculata J.M. Li & S.X. Zhu *sp. nova* (Gesneriaceae) is described from Yunnan, China, and illustrated. We investigated its phylogenetic relationships within the *Chirita-Chiritopsis* group using separate and combined data from the nuclear ribosomal Internal Transcribed Spacer (ITS) and the chloroplast *trnL-F* sequence for twenty-three species in different sections of *Chirita* and *Chiritopsis*. *Chirita auriculata* belongs to section *Chirita*. This is an unexpected phylogenetic position because the new species has a calyx divided almost to the base, ovoid ovaries shorter than the styles, small plant bodies, and small flowers, characters that typically define *Chiritopsis*. Its position close to *C. pumila* is supported by its annual stems and membranous leaves.

Key words: *Chirita*, ITS, morphology, new species, phylogeny, taxonomy, *trnL-F*

Introduction

Chirita is one of the most diverse genera of Gesneriaceae, occurring primarily in South China and Vietnam. In the first revision of the genus (including 34 species), it was divided into five sections (Clarke 1883): *Gibbosaccus*, *Microchirita*, *Bilabium*, *Euchirita* and *Liebigia*. Ninety years later it was re-examined by Wood (1974), who retained the former two and combined the latter three of Clarke's sections into sect. *Chirita*. Wood predicted that a large number of new species would be discovered in southern China. *Chirita* now has swelled to contain at least 140 species and new species, especially in sect. *Gibbosaccus* in southern China, are still being

discovered. The section *Gibbosaccus*, the largest section of *Chirita*, contains about 110 species with a distribution range extending from southern China to northern Vietnam.

Wood's (1974) comprehensive classification has later been followed. However, Wang's (1985) distinction between *Chirita* sect. *Gibbosaccus* and *Chirita* sect. *Chirita* differs from Wood's (1974) in that all species of sect. *Gibbosaccus* are annual or perennial herbs with calyx lobes divided to the base and with small stigmas. The section *Liebigia* was recently re-established by Hilliard (2003) to accommodate *C. asperifolia*.

Chiritopsis, a close relative of *Chirita* and endemic to China, was described by Wang (1981) who put the greatest weight on ovary and capsule features in delimiting this genus from

other Old World Gesneriaceae. All members of *Chiritopsis* are perennial herbs, uniform in gross morphology, with small plant bodies, small flowers, ovoid ovaries shorter than the styles, and capsules straight ovoid to ellipsoid. Recent molecular phylogenetic analyses have indicated that *Chiritopsis* is not monophyletic and that its species are distributed within *Chirita* sect. *Gibbosaccus* (Li & Wang 2007).

However, during field work, a puzzling specimen was discovered in Yunnan that was difficult to ascribe to any extant species or even to the section of *Chirita* or *Chiritopsis* according to the key to the infrageneric classifications provided by Wood (1974) and Wang (1981, 1985).

DNA sequencing has become the dominant technique in recent years, as DNA sequences can provide abundant information for phylogenetic analysis and resolve problems where taxonomists have not been able to agree on relationships using traditional characters (Zhang & Ge 2007). We used ITS and *trnL*-F data to determine the phylogenetic placement of the species here described.

Material and methods

Taxon sampling

Sequences for ITS and *trnL*-F for 22 species of *Chirita* sections *Gibbosaccus*, *Chirita* and *Microchirita*, and *Chiritopsis* were used from the previous study (Li & Wang 2007). *Chirita auriculata* was sampled from field-collected material and the voucher specimen is deposited in the Herbarium of Henan Agricultural University (HEAC). *Ornithoboea wildeana* and *Paraboea rufescens* were chosen as the outgroups (Li & Wang 2007). Genbank accession numbers for ITS and *trnL*-F for *C. auriculata* are FJ796197 and FJ796196, respectively.

Molecular analysis

DNA isolation, amplification and sequencing followed Li and Wang (2007). The sequences were aligned using CLUSTAL W ver. 1.83 (Thompson *et al.* 1997) and adjusted manually in BioEdit

5.0.9.1 (Hall 1999). Identification of the start of ITS1 and the end of ITS2 were determined by comparison with various published sequences available in GenBank (AF316900). The resulting ITS and *trnL*-F data sets were subsequently analyzed separately with parsimony methods using PAUP*4.0 ver. b10 (Swofford 2002). Gaps were treated as missing data. Heuristic searches were performed with 1000 replicates of random addition, one tree held at each step during stepwise addition, tree-bisection-reconnection (TBR) branch swapping, MulTrees in effect, and steepest descent off. Characters and character-state changes were weighted equally. To examine the robustness of various clades, bootstrap analysis was performed with 1000 replicates using a heuristic search with 1000 replicates of random sequence addition and TBR branch swapping.

To assess character congruence between ITS and *trnL*-F, the incongruence length difference (ILD) test (Farris *et al.* 1994) as implemented in PAUP*4.0b10 (Swofford 2002) was performed with 100 replicates, each with ten random additions with TBR branching swapping. The resulting *p* value was used to determine whether the two data sets had significant ($p < 0.05$) incongruence. Parsimony analysis for the combined data set was conducted using the same methods as those used for ITS and *trnL*-F.

Results

Chirita auriculata J.M. Li & S.X. Zhu *sp. nova* (Fig. 1)

Species nova C. pumilae affinis, a qua differt calycibus 5-partitis, ovariis ovoideis; et C. forestii similis, sed corollis 1.1 cm longis, filamentis 4 mm longis e basi tubi corollae differens.

TYPE: China. Yunnan, Hekou, Nanxi, the shelter of cave-mouths, 16.VI.2008 Li Jia-Mei 08816 (holotype HEAC).

Annual herb; stem up to 5–7 mm long, curving, sparsely hairy, often red, internodes 1–2 mm, numerous roots radiating out from base; leaves, opposite, thin, membranous, viscid, usually a pair of large leaves at base of stem, subsequent leaves clustered apically, rotund to ellipti-

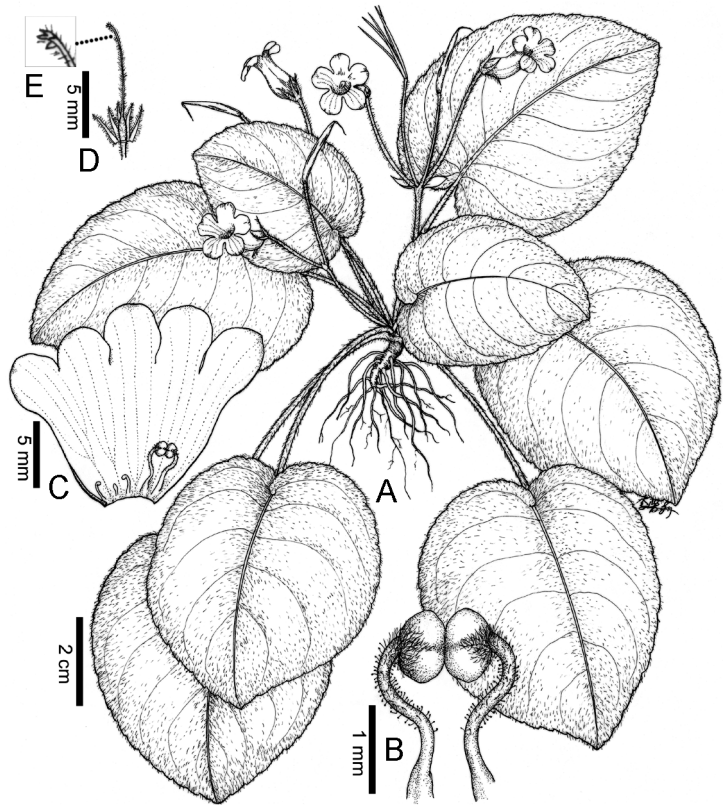


Fig. 1. *Chirita auriculata* (from the holotype, drawn by A. L. Li). — **A:** Flowering plant. — **B:** Stamens. — **C:** Tube of corolla (opened). — **D:** Calyx (opened) and pistil. — **E:** Detail of stigma

cal, 3.5–7 × 2.5–5 cm, base auriculate, shallowly serrate to coarsely dentate, densely hairy on both surfaces, lateral veins 5–7 on each side, grooved upper, prominent below; petiole 1–4 cm, usually pairs of petioles adnate at base, densely hairy on both surfaces; pair-flowered cymes in leaf axils, 2–3 on a plant, each 3–5-flowered, peduncles 2–4 cm long, densely hairy; bracts paired, free, elliptical, 6 mm long, widest up to 2 mm, with eglandular hairs; pedicels 6–11 mm, with eglandular hairs; calyx ca. 0.4 cm long, divided to base, elliptical to long triangular, longicaudate; corolla 1.1 cm long, white or light purple outside, with purple stripes inside, glabrous or sparsely hairy on both sides, tube curved, slightly pouched, 3–4 mm at base, mouth naked, 5 mm wide, adaxial lobes 2, rectangular, 4 mm, abaxial lobes 3, oblong, 5–7 mm; filaments 4 mm, inserted from base of corolla, S-shaped, glabrous below, sparsely upper with glandular hairs (Fig. 1B); anthers coherent face to face, with dense glandular hairs on connective base; staminodes 3, inserted from base of

corolla, central ca. 0.6 mm long, laterals ca. 1.2 mm, apex curved, with glabrous or sparse glandular hairs (Fig. 1C); disc a shallowly lobed ring 0.3 mm deep. Gynoecium 7 mm long, with dense eglandular hairs, ovary long ovate, 2 mm long, style 5 mm long, stigma lamellate, bilobed; fruit 1–1.2 cm long, with sparse hairs; style persistent and curved, calyx not. Flowering April–June.

Molecular phylogeny analyses

The topology of the strict consensus trees from ITS and *trnL-F* data set analyses were not in conflict with each other and are basically coincident with Li and Wang (2007). In both trees, representative species of *Chiritopsis* and *Chirita* sect. *Gibbosaccus* cluster in a clade. *Chirita auriculata* is sister to *C. pumila* with medium to high support. The two species of sect. *Microchirita* are included in a separate clade.

The ILD test gave $p = 0.114$, indicating that the data from two distinct marker regions were

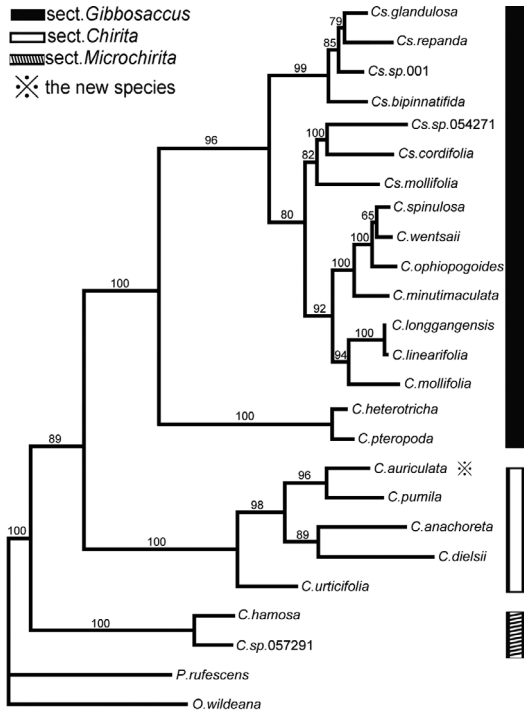


Fig. 2. Single MPT generated from the combined ITS and *trnL-F* data. C = *Chirita*, Cs = *Chiritopsis*, P = *Paraboea*, O = *Ornithoboea*.

congruent, thereby justifying the combined analysis of both loci. The combined matrix included the identical set of taxa as single marker matrices, consisted of 1630 positions, 202 (12.39%) of which were variable but uninformative and 314 (19.26%) parsimony-informative, the rest invariable. The parsimony analysis produced a single tree of 1044 steps with CI = 0.723 and RI = 0.751 (Fig. 2). A single MPT (Fig. 2) provides strong support (BS = 96%) for the placement of *C. auriculata* as sister to *C. pumila*, as well as the monophyly of sect. *Chirita* included here (BS = 100%), albeit only five of 40 currently described species have been sampled.

Discussion

Ongoing studies of *Chirita* have revealed a new species, *C. auriculata*, from Yunnan, South China, which can be distinguished from congeners by the combined characters of caulescent herb, leaves auriculate at the base, calyx divided

almost to the base, anthers fused face to face, ovoid ovaries shorter than the styles, and capsules straight to ellipsoid. However, the combination of characters makes its placement into either sect. *Gibbosaccus* or sect. *Chirita* difficult since it has characteristics of both.

Although *C. auriculata* is clearly assignable to *Chirita* by having lamellate, bilobed-stigmas and elongated capsules, it has a small plant body with small flowers and ovoid ovaries that are shorter than the styles. These characters are traditionally used to circumscribe *Chiritopsis*, which previous phylogenetic analyses have shown to be a part of *Chirita* sect. *Gibbosaccus* (Li & Wang 2007). However, *C. auriculata* has much longer mature capsules, which are not present in species of *Chiritopsis*. Based on personal observations of the author Li from fieldwork, cultivated collections and the literature, some species of *Chirita* have ovoid ovaries shorter than the styles but longer mature capsules, i.e. *C. ronganensis*, *C. secundiflora*, and *C. tenuituba* (Wang 1990, Wei & Fang 2001).

Following the latest circumscription by Wang (1985) who used the presence of a calyx divided to the base as a key character for differentiating sect. *Gibbosaccus* from sect. *Chirita*, *C. auriculata* seems to be ascribed to *Chirita* sect. *Gibbosaccus*. However, following Wood (1974) who circumscribed sect. *Chirita* as annual caulescent herbs, or perennial herbs with the calyx fused, *C. auriculata*, as an annual caulescent herb, should belong to sect. *Chirita*.

Chirita auriculata, an annual herb with a calyx divided to the base, blurs the delimitation between sections *Chirita* and *Gibbosaccus*. However, the molecular phylogenetic results are basically coincident with Wood's (1974) classification. *Chirita auriculata* and *Chirita* sect. *Chirita* comprise a well-supported monophyletic group (BS = 100%). The section *Chirita* consists of caulescent annual herbs and caulescent or acaulescent perennial herbs with anthers fused face to face and with thin membranous leaves, all of which are character states found in *C. auriculata*.

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