Hyoscyamus labiatus sp. nov. (Solanaceae: Hyoscyameae) from Henan, China: implications for the delimitation of *Hyoscyamus*

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Abstract

Background

Hyoscyamus, the largest genus in the tribe Hyoscyameae, harbors more than 20 species. Although the monophyly of *Hyoscyamus* is supported by both morphological and molecular phylogenetic studies, the delimitation of *Hyoscyamus* has been hotly debated in different classifications. Here, we report a new species of *Hyoscyamus* (Solanaceae) from Henan Province, China, and discuss the delimitation of *Hyoscyamus*.

Results

This species is morphologically similar to the known species of *Hyoscyamus* and its close related genus *Archihyoscyamus*, but can be distinguished by corolla 2-lipped, adaxial lip 3-lobed and much longer than abaxial lips, tube slender at base, and stamens 5, inserted on inner side of disk, free, obviously unequal, and exceeding corolla. Phylogenetic analysis based on four chloroplast markers including *rbcL*, *ndhF*, *trnC-psbM* and *trnL-trnF*, strongly suggested that the new species was sister to a monophyletic group containing all species of *Hyoscyamus* and *Archihyoscyamus* previously described.

Conclusions

Both the morphological observations and molecular phylogenetic analyses support the recognition of *Hyoscyamus labiatus* as a new species. Our study also showed that *Archihyoscyamus* should be a synonym of *Hyoscyamus*. The delimitation of *Hyoscyamus* is thus revised in our study.

Background

Solanaceae has approximately 21 tribes, 100 genera and 2,500 species (Olmstead et al. 1999; Hunziker 2001; Olmstead et al. 2008), including several economically important crops and ornamentals. Within this family, the tribe Hyoscyameae, containing more than 40 species, was strongly supported to be monophyletic (Olmstead et al. 2008; Tu et al. 2010; Sanchez-Puerta and Abbona 2014). It was also the only group mainly distributed in Eurasia and centered on both the Hengduan Mountains and Mediterranean-Turanian region (Tu et al. 2010). According to previous studies, seven genera, *c. Anisodus* Link et Otto, *Atropa* L., *Atropanthe* Pascher, *Hyoscyamus* L., *Physochlaina* G. Don, *Przewalskia* Maxim., and *Scopolia* Jacq. were widely accepted in this group (Lu and Zhang 1986; D'Arcy and Zhang 1992; Olmstead et al. 2008). All the Hyoscyameae taxa are characterized by circumscissile capsular fruits except *Atropa*, which possess a berry fruit (Knapp 2002) and with a basal-most phylogenetic position in the molecular phylogenetic analyses (Olmstead et al. 1999; Yuan et al. 2006; Tu et al. 2010; Sanchez-Puerta and Abbona 2014; Ghahremaninejad and Riahi 2021; Lei et al. 2021).

Hyoscyamus, the largest genus in the tribe Hyoscyameae, harbors more than 20 species (Yousaf et al. 2008; Satil et al. 2015). Although the monophyly of *Hyoscyamus* is supported by both morphological (usually sessile solitary flowers and spiny tips of fruit calyces) (Tu et al. 2010) and molecular phylogenetic studies (Hajrasouliha et al. 2014; Ghahremaninejad and Riahi 2021). However, the delimitation and infrageneric classification of *Hyoscyamus* has been hotly debated in previous studies. In *Flora Iranica*, 23 species have been reported in *Hyoscyamus*, which divided into two subgenera (Subg. *Dendrotericon* and Subg. *Hyoscyamus*) and four sections (Sect. *Hyoscyamus*, Sect. *Pumilio*, Sect. *Euhyoscyamus*, and Sect. *Chamaehyoscyamus*) (Schönbeck–Temesy 1972). However, the recognized subgenera and sections did not form monophyletic groups in the phylogenetic analysis (Hajrasouliha et al. 2014; Ghahremaninejad and Riahi 2021). Khatamsaz (1998) treated some species of *Hyoscyamus* in Iran as synoyms, and introduced a new species, leaving a total of 13 species in *Hyoscyamus* only. Otherwise, a monotypic genus *Archihyoscyamus* A.M. Lu was described based on one species transferred from *Hyoscyamus* (*H. leptocalyx* Stapf) (Lu 1997).

Six genera of Hyoscyameae with approximately 16 species and three varieties were found in China, including *Anisodus* Link et Otto (four species, three varieties), *Atropanthe* Pascher (one species), *Hyoscyamus* L. (three species), *Physochlaina* G. Don (seven species) and *Przewalskia* Maxim (one species) (Zhang et al. 1994; Tu et al. 2010). Only two species of the tribe, *Physochlaina infundibuluris* Kuang and *Hyoscyamus niger* L., occur in Henan Province, China. In 2017, during a survey of forest germplasm resources in Henan Province, China, we encountered an unfamiliar taxon, which shares similar vegetative attributes and occurs in similar habitats with *Triaenophora rupestris* (Hemsl.) Soler in Scrophulariaceae Juss. In 2020 and 2021, we collected its flowers and found that this taxon was morphologically similar to the species of *Hyoscyamus*.

To clarify the systematic position of the new taxon, four widely used plastid markers are sequenced and the phylogeny of Hyoscyameae is reconstructed. Based on all these evidence, a new species, *Hyoscyamus labiatus*, is described here, and also the delimitation of *Hyoscyamus* is discussed.

Methods

Morphological analyses

Morphological observations and measurements of the new species were carried out, based on the study of living plants and herbarium specimens. Measurements of flowers and rhizomes were taken from living plants. Photographs of *Hyoscyamus labiatus* were taken in the field and are showed in Fig. 1. The voucher specimens of the new species were deposited in Henan Agricultural University Herbarium (HEAC).

Dna Extraction, Amplication And Sequencing Of Four Plastid Loci

Specimens and silica-dried leaf material of *Hyoscyamus labiatus* were collected from the only known population. Total genomic DNA was extracted from silica gel-dried leaves with CTAB method. To infer the systematic position of the new species in the tribe Hyoscyameae, four plastid markers, including *ndhF*, *rbcL*, *trnL-F* and *trnC-psbM*, were amplified and sequenced. Primers for these four genes followed Olmstead and Sweere (1994), Dong et al. (2014), Taberlet et al. (1991), and Tu et al. (2008), respectively (Table S1). PCR amplification was conducted with a reaction volume of 25 uL containing 10-50 ng of DNA template, 1×Ex Buffer (Mg2+ plus), 200 umol/L of each dNTP, 6.25 pmol of each primer pair, and 0.75 U of Taq DNA polymerase (TakaRa Biotech Co., Dalian, China). PCR cycles were as follows: 3 min at 94 ℃, 36 cycles of 30 s at 94 ℃, 30 s at 50-58 ℃, and 1-5 min at 72℃, with a final elongation for 10 min at 72 ℃. The PCR products were directly sequenced with an ABI Prism 3730xl sequencer (PE Applied Biosystems). The sequences reported in this study were deposited in GenBank (https://www.ncbi.nlm.nih.gov/) under accession numbers MT997072- MT997075. The same four plastid gene sequences of other taxa in the tribe Hyoscyameae were retrieved from GenBank and the accession number are given in Table S2.

Phylogenetic Analysis (Dup: Abstract ?)

The sequences of single plastid loci were firstly aligned using Clustal X (Thompson et al. 1997) and further manually adjusted with BioEdit v7.2.5 (Hall 1999). Phylogenetic trees were reconstructed individually for each plastid loci and the combined gene dataset. Maximum likelihood (ML) analyses were performed under the GTRGAMMA model by RaxML v8.1.17 (Stamatakis 2014) with 100 bootstrap replicates. *Lycium chinense* and *Solanum lycopersicum* were chosen as outgroups.

Results

Taxonomy

Hyoscyamus L.Sp. Pl. 1: 179. 1753. – Type: *Hyoscyamus niger* L.

Archihyoscyamus A.M. Lu, Adansonia 19(1): 135–138. 1997. –Type: *Hyoscyamus leptocalyx* Stapf.

Herbs annual, biennial, or perennial, erect or sprawling; pubescence of simple glandular hairs. Leaves solitary, paired but not opposite or in whorls, sometimes forming a rosette; leaf blade sinuate, coarsely dentate or pinnately lobed or parted, rarely entire, apex acute. Inflorescences of solitary axillary flowers, sometimes condensed into scorpioid racemes or spikes. Calyx tubular-campanulate, urceolate, or obconical, becoming enlarged, lobes erect or spreading, needlelike or obtuse-rounded. Corolla actinomorphic or zygomorphic, campanulate or funnelform. Stamens inserted in corolla tube, usually slightly exserted; anthers dehiscing longitudinally. Disc sometimes indistinct. Ovary 2-locular; ovules numerous. Fruiting calyx unequal or equal, completely or incompletely enveloping and longer than capsule, lobes with strong or unobvious marginal veins produced into mucros. Capsules dehiscent by an operculum slightly distal to middle. Seeds reniform or discoid, minutely pitted; embryo ringlike or coiled.

Hyoscyamus labiatus Y.Y. Liu, Q.R. Wang & J.M. Li, sp.nov. (□□□□□) (Figs.1 and 2)

Type: China, Henan Province: Yellow River Grand Canyon, Mianchi County, Sanmenxia City, alt. 258.4 m,111°40.5' E, 34°56.64' N, 22 May, 2020, *Li Jiamei* et *Wang Qirui 20205221* (holotype, HEAC!). ibid. 14 July, 2017, *Li Jiamei* et *Liu Yao 20177141*; 14 April, 2021, *Li Jiamei, Wang Qirui* et *Wang Fuqian 20214141*, (Paratype: HEAC!).

Diagnosis

Hyoscyamus labiatus is similar to *H. leptocalyx* by the capsules dehiscent around slightly distal, with apical "lid" deciduous and basal portion persistent, pedicels much longer than leaves, but differs in having corolla 2-lipped, adaxial lips 3-lobed and much longer than abaxial lips, tube slender at base, and stamens 5, inserted on inner side of disk, free, obviously unequal, and exceeding petals (Table 1).

Table 1
The morphological comparison between *Hyoscyamus labiatus* and other similar species in *Hyoscyamus*

Characters	*H. labiatus*	*H. leptocalyx*	*H. senecionis*	*H. aureus*
Stem	30-50cm, dense non-glandular and glandular hairs.	10-15cm, pubescent.	30-70cm, pubescent.	30-70cm, non-glandular and glandular hairs.
Leave	alternate, often paired but not opposite or in whorls; leaf blade 3-5×3.5-5.5cm	alternate; leaf blade 1-2×1.5-2.7cm	alternate, often paired but not opposite; leaf blade 2-4×3-5cm	alternate, leaf blade 3-5×3-5cm
Pedicel	5- 6.5cm long	to 2.8 cm long	to 3 cm long	to 1.5 cm long
Calyx	lobes unequal, 5mm long, apex obtuse-rounded	lobes equal, 2mm long, apex obtuse-rounded,	lobes unequal, 5mm long, apex acute	lobes equal, 5mm long, apex acute
Corolla	Zygomorphic	Actinomorphic	Zygomorphic	Zygomorphic
Tube	ca. 1.2 cm long, ca.1.2 cm wide	ca. 0.2 cm long, ca.0.6cm wide	ca. 1 cm long, ca.0.8 cm wide	ca. 1.3 cm long, ca.0.8 cm wide
Stamens	unequal, filament 1.5-2.5cm long	equal, filament 1cm long	equal, filament 1.5cm long	equal, filament 1.1cm long

Description: Perennial herbs, 30–50 cm tall; Stems 30–45 cm long, apparently from a deep and stout rhizome which is not collected in the stone seam, at first spreading but later erect, bases somewhat woody with age, well-branched below. Stems, leaves, petiole, pedicel and calyx dense non-glandular and glandular hairs, long and short hairs intermixed in varying proportions; Leaves alternate, sometimes paired but not opposite or in whorls; petiole 1.5–4 cm long, the longer petioles below and the shorter ones

above; leaf blade broadly ovate, deltoid subreniform, 3–5 cm long, 3.5–5.5 cm wide, with teeth generally 3–4 deltoid per side; leaf bases unequal, cordate, rounded, or truncate. Flowers solitary, axillary; pedicel 5–6.5cm long, pubescent. Calyx 1cm long, 5-lobed to halfway, ovate, apex obtuse-rounded. Corolla double longer than the calyx, up to 2.5 cm long, greenish yellow, with dark green reticulations. Corolla 1.5–1.8 cm in diam; tube ca. 1.2 cm long, ca.1.2 cm wide in throat. Stamens 5, inserted at the corolla base, exserted, 1.5–2.5 cm long, arranged in three levels: one long, two middle, and two shorter; tube slender at base, exserted, dilated to throat; upper lip 3-lobed, lower lip entire. Filament glabrescent, inserted at the corolla base. Anther 2–6 mm long. Ovary ovoid, 2-locular with many ovules, style glabrescent, 2 cm long; stigma capitate. Fruiting calyx subrotate, 8–10 mm in diam., the lobes reflexed, 5 mm long, 3 mm wide. Fruiting pedicels elongating, to 15–18 cm long, densely pubescent. Capsule oval elliptic, 6 mm in diam in widest part. Seeds subovoid-oblong to irregularly rectangular, yellow red, 1.2 mm long, 1mm wide, densely papillate.

Etymology

The specific epithet indicates the corolla 2-lipped, which is the most obvious unique character of the new species in the tribe.

Distribution, habitat and conservation status: Currently, *Hyoscyamus labiatus* is known only from Yellow River Grand Canyon in Mianchi County, Henan, China. The type population consisted of only about 1000 individuals in nearly inaccessible sites on the high limestone cliffs. A preliminary assessment of the risk of extinction was conducted according to the International Union for Conservation of Nature (IUCN) Red List Categories and Criteria (https://www.iucn.org/), we thereby consider that *H. labiatus* should be classified as near threatened (NT).

Phylogenetic Analysis

The four plastid loci (*ndhF*, *rbcL*, *trnL-F* and *trnC-psbM*) were successfully amplified and directly sequenced for two individuals of *Hyoscyamus labiatus*. Seventy-five sequences from twenty-three species of seven genera of Hyoscyameae were obtained from the GenBank (Table S2). Sequence characteristics of these DNA fragments are presented in Table 2. Of the four cpDNA markers, *ndh*F and *trn*C-*psb*M were characterized by high levels of length variation within the tribe Hyoscyameae (Table 2). The combined matrix of the four plastid markers from 25 samples comprises 5907 positions, of which 307 are variable (5.2%) and 171 are parsimony-informative (2.9%).

Table 2
Statistics for the alignments of *ndhF*, *rbcL*, *trnC-psbM*, *trnL-F* and combined dataset

	ndhF	*rbcL*	*trnC-psbM*	*trnL-F*	Combined
No.	25	23	22	21	25
Aligned length (bp)	1990	1100	1920	896	5907
V	112	20	99	69	307
PI	60	12	70	29	171

No., number of sequences (outgroups were not included). V, number of variable sites. PI, number of parismony-informative sites.

Phylogenetic trees were firstly reconstructed based on four plastid markers with extensive taxon sampling. The phylogeny based on the combined four plastid loci supported the tribe Hyoscyameae as a monophyletic group and the monophyly of each genus was highly supported (Fig. 3). The new species was sister to all other *Hyoscyamus*, including *Hyoscyamus leptocalyx* (*=Archihyoscyamus leptocalyx*), with high support values (Fig. 3). For other genera within the tribe, *Atropa* was sister to the rest of the genera of the tribe, *Przewalskia* showed a close relationship with *Anisodus* (Fig. 3). But the relationships among the seven genera of the tribe Hyoscyameae were essentially unresolved due to low statistical support (Fig. 3). Phylogenetic trees based on individual plastid locus were not fully resolved due to the low phylogenetic signal of each molecular marker, especially for *rbcL* (Fig. S1).

Discussion

Within the Hyoscyameae, the delimitation of *Hyoscyamus* has been hotly debated, mainly due to the controversial position of *H. leptocalyx* in different classifications. In 1972, Schöenbeck-Temesy transferred *H. leptocalyx* from sect. *Chamaehyoscymus* to sect. *Pumilio* within *Hyoscyamus*, due to its possession of flowers solitary, pedicel longer and calyx apex obtuse-rounded. In 1998, Khatamsaz described a new subgenus *Parahyoscyamus*, including *H. leptocalyx* and *H. malekianus*. In 1997, a monotypic genus *Archihyoscyamus* was described by Lu (1997) based on one species transferred from *Hyoscyamus* (*H. leptocalyx*). *Archihyoscyamus* was accepted by some later studies (Hunziker 2001; Zhang et al. 2005; Tu et al. 2010; Sanchez-Puerta and Abbona 2014; Ghahremaninejad and Riahi 2021). However, *Archihyoscyamus leptocalyx* (Stapf) A.M. Lu, is consistent to the other members in *Hyoscyamus* with the opercular dehiscence of the capsule due to a glandular enlargement on the summit of the ovarium (Miers 1850). Therefore, many taxonomists still considered *A. leptocalyx* as a member of *Hyoscyamus* (Sheidai et al. 2000; Hajrasouliha et al. 2014; Mabberley 2018).

With the rapid development of molecular systematics, the sister relationship between *H. leptocalyx* and all other previously described *Hyoscyamus* species was clearly revealed (Hajrasouliha et al. 2014; Ghahremaninejad and Riahi 2021). Our phylogenetic analysis also suggested that *H. leptocalyx* and other *Hyoscyamus* species formed a monophyletic clade with strong support (BS=96%) and *H. labiatus* is

sister to this group with maximal support (BS=100%) (Fig. 3). The treatment of *H. labiatus* in the genus is also supported by morphological characteristics. *H. labiatus* is most morphologically similar to *H. leptocalyx* by numerous, patent stems; solitary flowers on elongate pedicels in the leaf axils; spreading, subrotate fruiting calyx with reflexed lobes and without strong marginal veins, and incompletely enveloping the fruit. *H. labiatus* is also morphologically similar to other species in *Hyoscyamus*, such as *H senecionis* Willd. and *H. aureus* L., by tall perennial herbs, 'labiate' flower with long tube, adaxial lips 3-lobed and much longer than abaxial lips which are obviously different from *H. leptocalyx*, but differs in having lower corolla lip entire and fruit calyx with obtuse-rounded apex (Table 1).

Conclusion

Based on the above evidence, there is no doubt that the new species (described in the below), the controversial species (*H. leptocalyx*) and all other *Hyoscyamus* species have close relationships. But the affiliates among them did not mean *H. labiatus* and *H. leptocalyx* should be treated as separate monotypic genera, which inflates the taxonomy needlessly. Given their similarity in morphological characters (Table 1), we strongly suggested both *H. labiatus* and *H. leptocalyx* should be treated as members of *Hyoscyamus*, rather than separate monotypic genera. The new species described in this study, breaks down the difference between *Archihyoscyamus* and *Hyoscyamus*, so we propose to synonymize *Archihyoscyamus* with *Hyoscyamus*.

Declarations

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Author's contributions

JML designed the research. YYL performed experiments. YYL, JML and ZLM analyzed the data. JML and QRW performed field investigations and collected the specimens. JML and YYL wrote the manuscript.

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Availability of data and materials

All DNA sequences generated in this study have been registered to GenBank.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

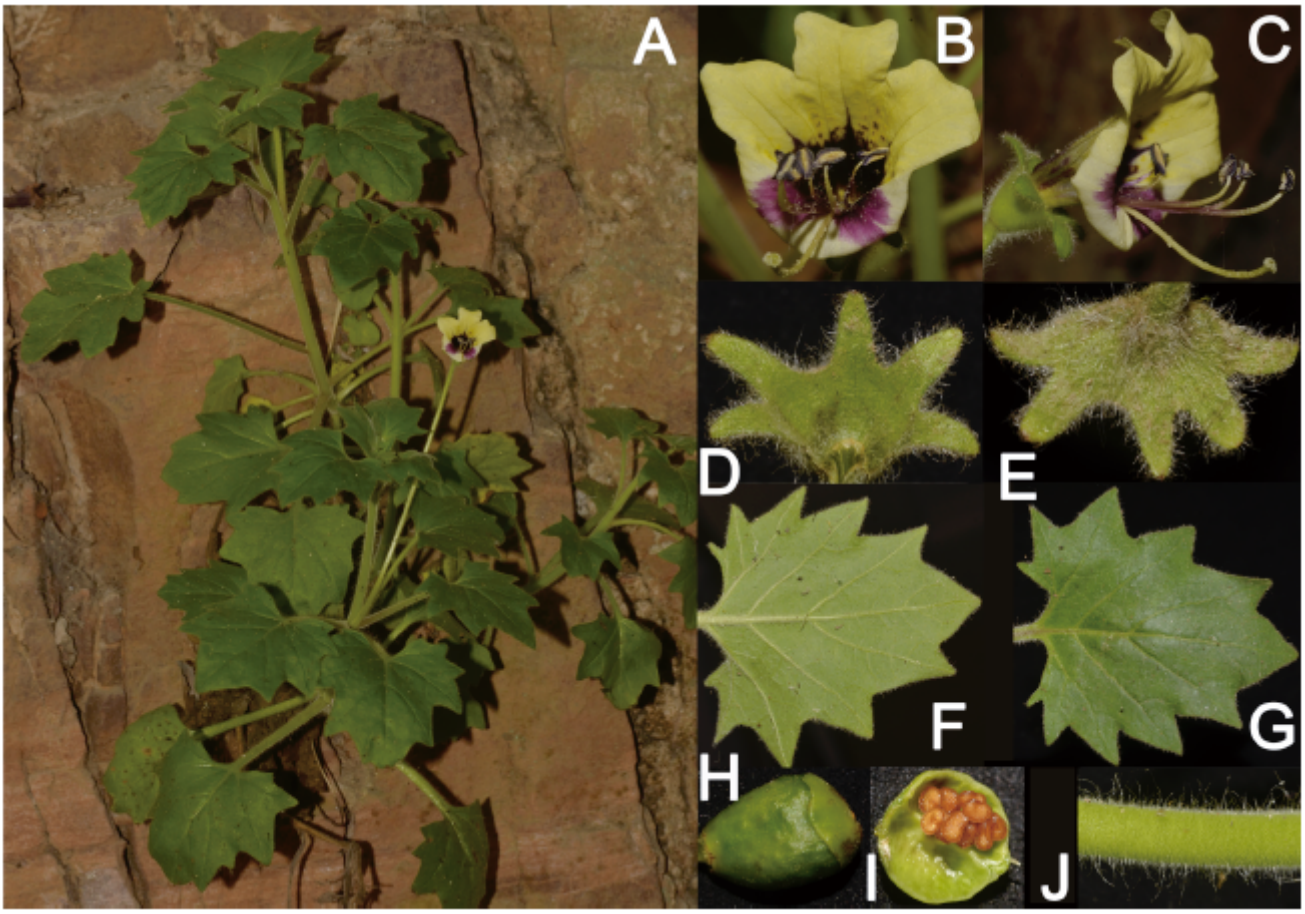

Figure 1

Hyoscyamus labiatus Y.Y. Liu, Q.R. Wang and J.M. Li sp. nov. A habitat B Frontal view of a flower C Lateral view of a flower D Inside of calyx E Outside of calyx F Abaxial leaf surfaces G Adaxial leaf surfaces H Fruit I Open fruit showing seeds J Stem. All photos by J.M. Li. (colored)

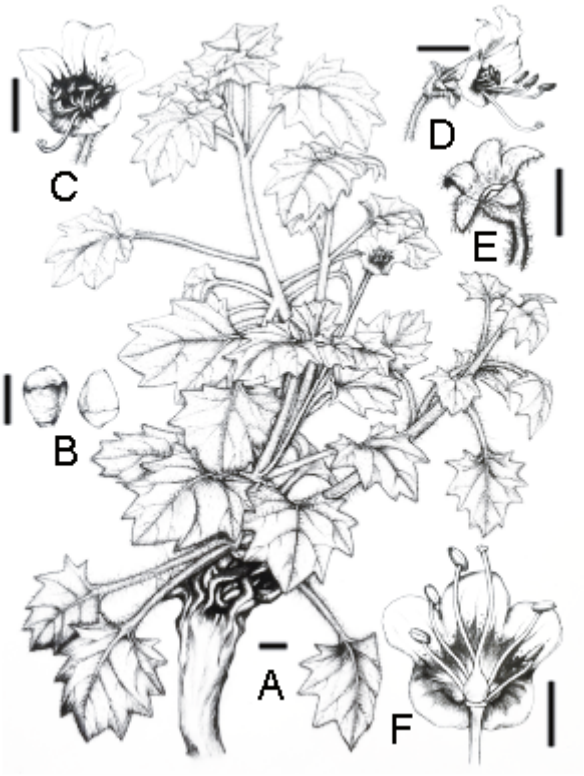

Figure 2

Hyoscyamus labiatus Y.Y. Liu, Q.R. Wang and J.M. Li sp. nov. A Habit B Up and down view of fruits C Frontal view of a flower D Lateral view of a flower E Calyx F Open flower. scale bar=1cm. Drawn by Mi ZengLou from Li Jiamei and Wang Qirui 20205221.

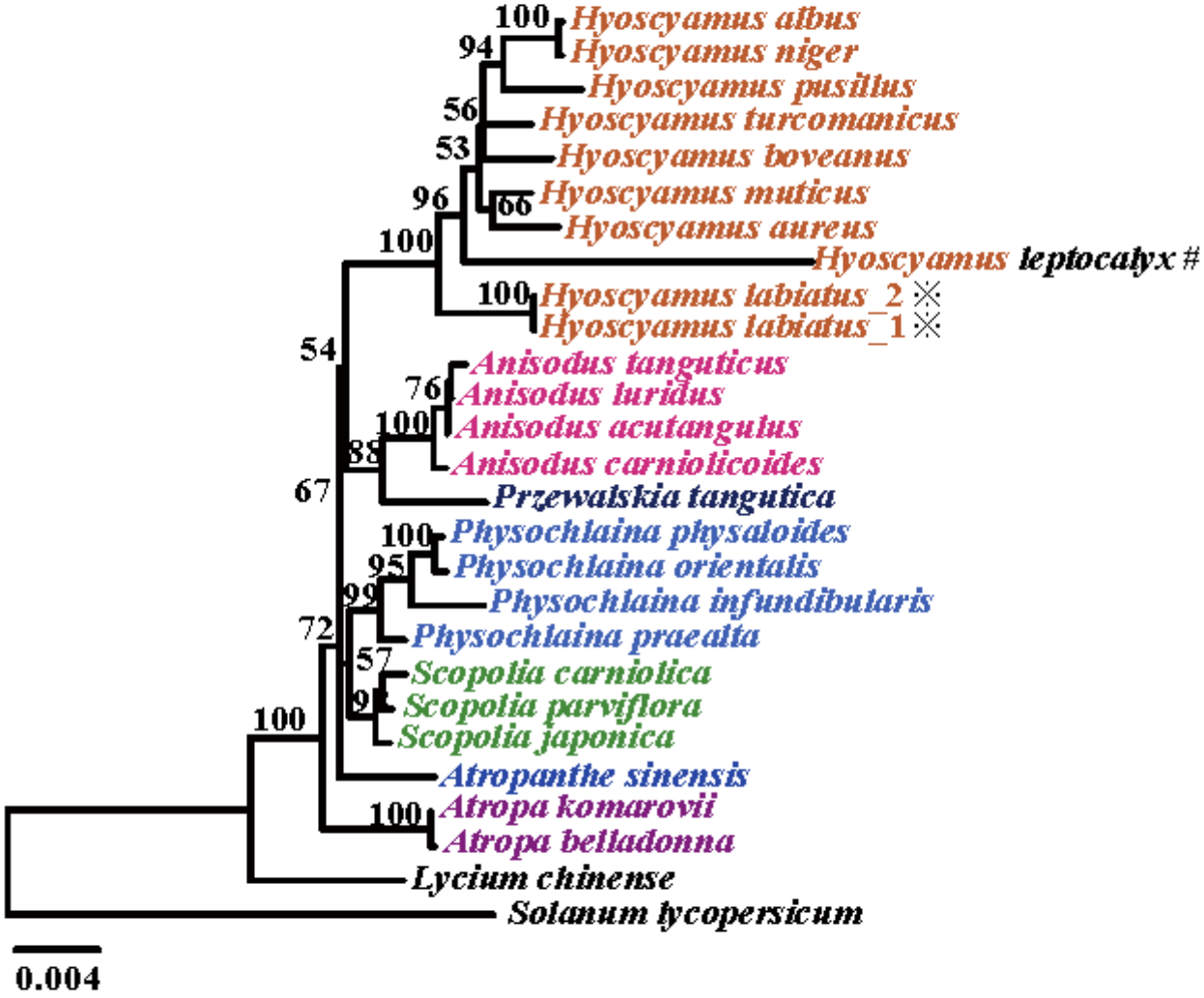

Figure 3

Maximum likelihood tree constructed with the combined four plastid genes. The numbers on the branches indicate bootstrap values > 50%. # Treated as Archihyoscyamus leptocalyx by Lu (1997). ※The new species described in our study. (colored)

Supplementary Files

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- Supplementarydata.docx