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SYSTEMATICS

Ostryopsis intermedia, a new species of Betulaceae from Yunnan, China

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ABSTRACT. *Ostryopsis intermedia* B. Tian & J. Q. Liu, a new species of Betulaceae from northwest Yunnan, China, is described in this study. Morphologically, the new species shows a combination of traits (stable in all examined individuals and populations) of the two recognized species of the genus, which are reproductively isolated from them by differences in flowering phenology and/or geographical distances. In addition, no intraspecific variation was found in the three species' ITS sequences, but there were clear interspecific differences. These results collectively suggest that *O. intermedia* is sufficiently distinct from the other two described species of *Ostryopsis* to be recognized as a new species.

Keywords: *Ostryopsis*; *Ostryopsis intermedia*; New species.

INTRUDUCTION

Ostryopsis Decne. (Betulaceae) is endemic to China and is represented by two species: *O. davidiana* Decne., which is widely distributed in northern China; and *O. nobilis* Balfour & Smith, restricted to Sichuan and northwestern Yunnan (Li and Skvortsov, 1999). This genus has been placed together with *Carpinus* L., *Corylus* L., and *Ostrya* Scopoli in the Coryloideae, largely based on the lack of a perianth in the staminate flowers (Thorne, 1992; Mabberley, 1997). However, relationships among these genera remain unresolved, particularly concerning the phylogenetic position of *Ostryopsis* (Chen et al., 1999; Yoo and Wen, 2007).

In the present study, we report a new species of this endemic genus (Figure 1). During an extensive investigation of most populations of the genus over its entire distribution range, we found a few populations in northwest Yunnan previously ascribed to *O. nobilis* that were morphologically different from the two known species of the genus. In order to evaluate whether these anomalous populations represent a new species, morphological traits, ITS sequences and the flowering phenology of representatives of these populations were analyzed and compared to those of the parapatrically distributed *O. nobilis* and the geographically more isolated species, *O. davidiana*. The results collectively suggest that those morphologically anomalous populations are sufficiently distinct to be recognized as a new species.

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MATERIAL AND METHODS

In the morphological comparisons we examined at least 10 living individuals or herbarium specimens from each of the investigated populations to assess both interspecific morphological differences and the morphological stability of the putative new species and two known species at the population level. The results are shown in Table 1. In addition, we recorded the flowering and fruiting phenology of the three species, by monitoring 10 individuals from each of at least three populations of each species at least once per month from March to August in 2007 and 2008. We also outlined the distributional range of each species according to field investigations and specimen records. Woodworth (1930) reported that chromosome of *O. davidiana* is $2n = 16$. In order to count the chromosome numbers of the three species, seeds were collected from their natural populations and germinated in petri dishes lined with moist gauze. The root tips were incubated in colchicine (0.1% w/v) for 2-3 h when they were 0.5 cm long, fixed in Carnoy's fluid (absolute alcohol:glacial acetic acid; 3:1) at 4°C for at least 30 min, hydrolyzed in 1 mol/L hydrochloric acid at 60°C for 10 min, then washed with water, stained with carbol fuchsin and squashed for observations. The chromosome numbers were determined from observations of 10 somatic cells.

Three representative trees from each of three distantly distributed populations of each species were randomly chosen for molecular (ITS sequence) identification and comparison (Table 2). Leaves of the selected trees were collected and immediately dried in silica gel until total genomic DNA was isolated from them following the CTAB

method of Doyle and Doyle (1987). The ITS regions of nrDNA were amplified with ITS primers 1 and 4 (White et al., 1990), then the PCR products were purified using a TIANquick Midi Purification Kit following the supplier's recommended protocol (TIANGEN). Sequencing reactions were performed with the PCR primers to cover the whole PCR segment using an ABI Prism BigdyeTM Terminator Cycle Sequencing Ready Reaction Kit. The reaction products were analyzed using an Applied Biosystems model 3130xl automated sequencer (Perkin Elmer Applied Biosystems). All sequences were submitted to GenBank (accession numbers: GQ250099 to GQ250101), then aligned using ClustalX version 1.81 (Thompson et al., 1997). *Carpinus turczaninowii* (AF081518) was selected as an outgroup to root all sequenced individuals. The phylogenetic relationships of the three species were assessed by maximum likelihood (ML), Neighbor-Joining (NJ) and maximum parsimony (MP) analyses using PAUP* 4.0b10. All gaps (indels) were coded as binary states (0 or 1). ML exhaustive search parameters were: simple addition of sequences of taxa with TBR branch swapping, MULTREES and COLLAPSE options on. MP analyses (equally weighted characters and nucleotide transformations) involved a heuristic search strategy with 100 replicates of random addition of sequences, in combination with ACCTRAN character optimization, MULPARS+TBR branch swapping and STEEPEST DESCENT options on. In these analyses we used bootstrap values (BS) to assess branch support.

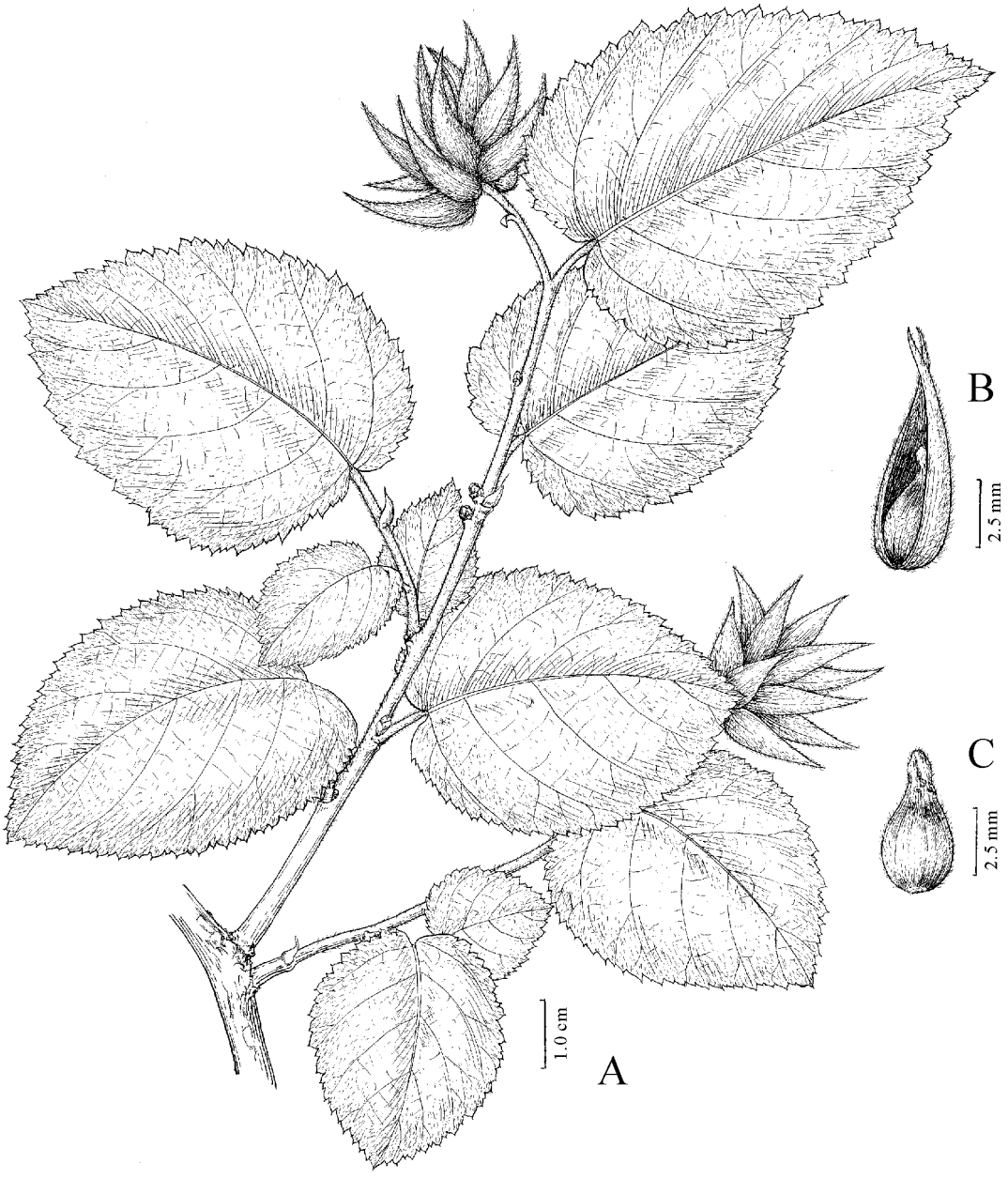

Figure 1. *Ostryopsis intermedia* B. Tian & J. Q. Liu A, fruiting branch; B, fruit; C, seed. Drawn by *A. L. Li from B. Tian & J. Q. Liu 2007003* (LZU).

RESULTS

The new species (*Ostryopsis intermedia*) differs from the two known species of the genus, *O. nobilis* and *O. davidiana*, in a number of morphological characters (listed in Table 1). All these characters are stable within each species. The habit and leaf shape of *O. intermedia* are similar to those of *O. nobilis*, except that the new species has doubly serrate leaves with acuminate or acute apices (Figure 1), while *O. nobilis* has irregularly serrate leaves with obtuse or rounded apices (Table 1). *Ostryopsis intermedia* flowers and sets fruits early, from April to May, while both *O. nobilis* and *O. davidiana* start to flower in June or July (Table 1). Geographically, the new species is found in southwest China and parapatric to *O. nobilis*, but distantly isolated from *O. davidiana* (Figure 3). In the only known sympatric site, the new species and *O. nobilis* showed no overlapping of flowering and fruiting periods (Table 1). More than 1000 mature individuals were found at each of the six recorded sites, and more than half of them can normally flower and set seeds. The total distribution range of the new species is similar to that of *O. nobilis*, and both have limited distributions (Figure 3), although the range of *O. davidiana* (in northern China) is more extensive. All three species were found to have the same chromosomes number of $2n = 16$ (Figure 2).

No intraspecific variation in ITS sequences was found among the nine examined individuals of each species (three from each of three different populations). However, all these sequences are species-specific, with distinct interspecific differences, including a total of eight mutations and three indels (Table 3). The new species differs from *O. nobilis* at nine ITS sites, and from *O. davidiana* at just three sites (pairwise differences: 1.5% and 0.5%, respectively). All phylogenetic analyses produced a similar topology with high bootstrap supports for all nodes (100% in all analyses), indicating that the new species is sister to the northerly species, *O. davidiana* (Figure 4).

DISCUSSION

The results of our molecular investigation suggest that the new species is a sister species to *O. davidiana*, and *O. nobilis* is sister to the clade composed of *O. davidiana* and the new species. *Ostryopsis davidiana* and *O. intermedia* share several morphological characters, including acuminate or acute leaf apices, doubly serrate leaf margins, and capitate infructescences. However, *O. intermedia* differs from the northern species in having larger, more densely pubescent leaves that lack resinous glands. The new species resembles *O. nobilis* in leaf size, habit and habitat; the main differences between them are in the characters shared

Table 1. Morphological and phenological differences between Ostryopsis intermedia and two known species.

Character	*O. davidiana*	*O. intermedia*	*O. nobilis*
Leaf blade	Ovate or elliptic-ovate, rarely broadly ovate or broadly obovate, margin doubly serrate and usually incised above middle, apex acuminate or acute	Broadly ovate or ovate-orbicular, margin doubly serrate and usually incised above middle, apex acuminate or acute	Broadly ovate or ovate-orbicular, rarely ovate, margin irregularly serrate, apex obtuse or rounded
Adaxial leaves	Sparsely white pubescent	Densely white pubescent	Densely yellow tomentose
Leaf size	1.5-5×1-4 cm	3-9 × 2-6 cm	3-10 × 2-6 cm
Leaf resinous gland	Yes	No	No
Infructescence	Capitate	Capitate	Racemose
Fruiting period	July-August	April-May	June-July

Table 2. Locations of populations used for ITS sequence comparison.

Taxon	Location	Lat. (ºN)	Long. (ºE)	Elevation (m)
Ostryopsis davidiana	Tangchang, GanSu	33º59’	104º28’	1680
	Maoxian, SiChuan	31º39’	103º48’	1570
	Lingyuan, LiaoNing	40º55’	119º16’	720
O. nobilis	Lijiang YunNan	27º16’	100º13’	1910
	Xianggelila, YunNan	27º48’	99º28’	1950
	Muli, SiChuan	27º49’	101º12’	2190
O. intermedia	Weixi, YunNan	27º13’	096º04’	1980
	Lijiang, YunNan	27º16’	100º13’	1910
	Deqin, YunNan	28º22’	98º54’	2870

Table 3. Variable sites of the aligned ITS sequences between the three *Ostropsis* species.

Taxon	Variable positions										
	58	72	82	108	170-172	225	395	484	565	582	586
O. davidiana	G	C	A	C	GT-	C	C	G	C	A	C
O. intermedia	-	T	A	C	GT-	T	C	G	C	A	T
O. nobilis	-	-	G	T	ACA	C	T	C	T	G	T

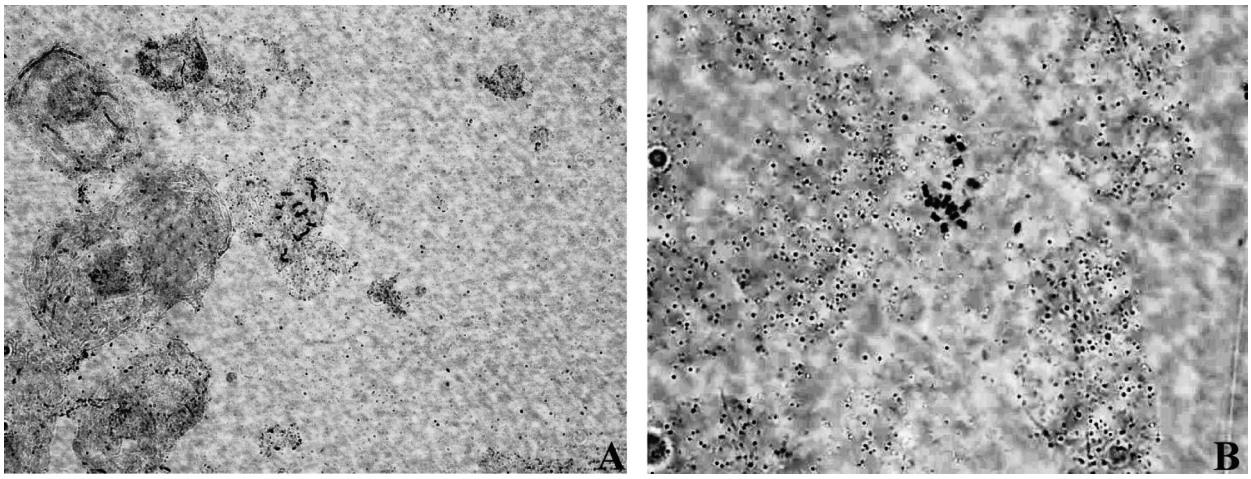

Figure 2. Mitotic chromosomes of *O. intermedia* and *O. nobilis*. A, *O. intermedia* (2*n* = 16); B, *O. nobilis* (2*n* = 16).

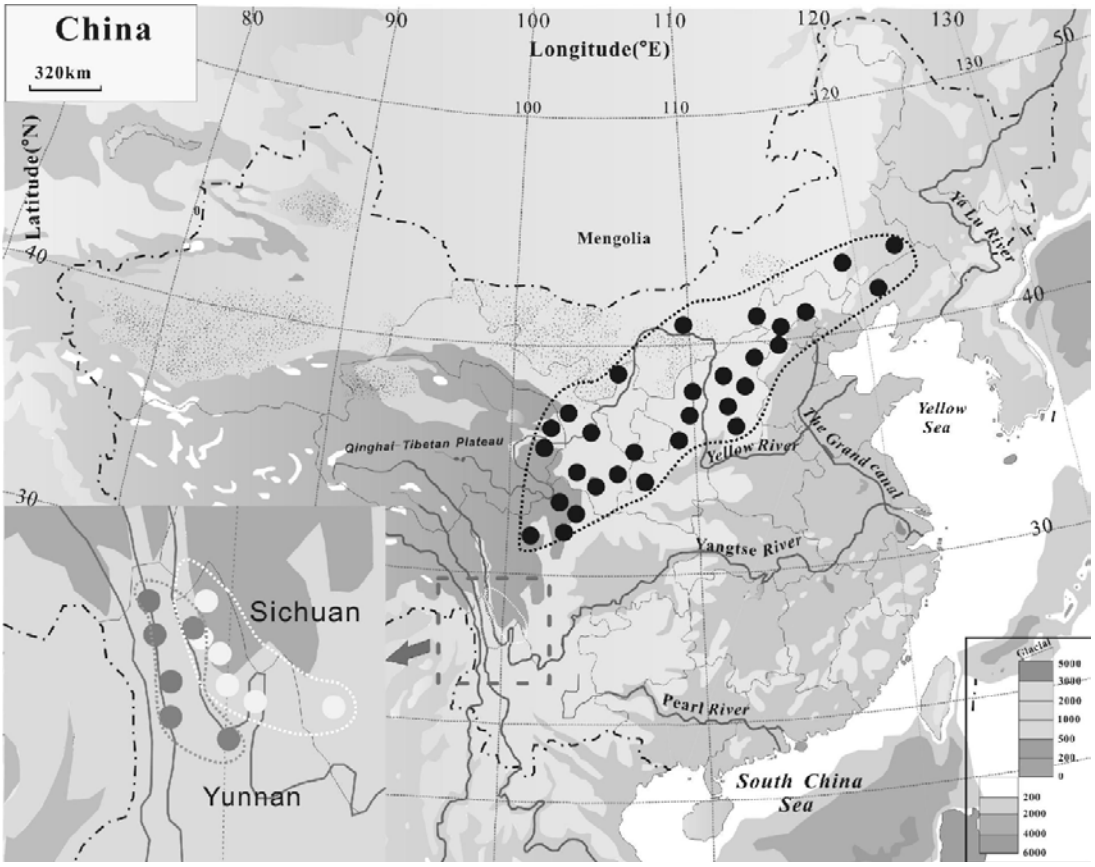

Figure 3. The distributional ranges (lines) and locations of the investigated populations (dots) of the three *Ostyopsis* species: *O. davidiana* (black), *O. nobilis* (white) and *O. intermedia* (gray).

by the new species and *O. davidiana* (Table 1). Overall, the new species shows a combination of morphological traits (stable in all examined individuals and populations) of the two known species, *O. davidiana* and *O. nobilis*, and seems to be an intermediate taxon between them. Hybrids between two differentiated species also generally have intermediate traits between their parental species (Grant, 1981). However, rather than representing a stable and isolated evolutionary lineage (species), hybrid swarms usually show inter-individual morphological variations. In addition, hybrids are usually distributed intermediately between the two assumed parental species and generally have completely or partly overlapping flowering stages with both parents. In contrast, the distributional ranges of the three species studied here suggest that *O. davidiana* is geographically isolated from both the new species and *O. nobilis* (Figure 3); the new species is distributed in more southern regions than *O. nobilis* and although the new species is parapatric to *O. nobilis* (one known population occurs sympatrically with *O. nobilis*) neither their flowering nor fruiting periods overlap (Table 1, Figure 3).

Common mechanisms of reproductive isolation include auto- and allo-polyploidy, which usually lead to immediate isolation from ancestral lineages and strongly promote maintenance of the new evolutionary make-up when the new and ancestral lineages have sympatric or parapatric distributions (Grant, 1981). However, this new species cannot have arisen by polyploidy since all three of the species are diploid (Figure 2). Understanding more about the origins and histories of these species is a target of our future research. In addition, since the new species and *O. nobilis* have limited distributions and wild populations are sparse, both of them warrant high priority for protection for further scientific research.

TAXONOMIC TREATMENT

Ostryopsis intermedia B. Tian & J. Q. Liu, sp. nov.— Type: CHINA. Yunnan: Lijiang, Judian, in thickets on sunny mountain slopes, alt. 1,910 m, 2007-05-09, *B. Tian & J. Q. Liu 2007003* (holotype, LZU; isotype, LZU). Figure 1

Species O. nobilis Balfour & Smith. affinis, sed foliis apice acutis, margine duplicato-serratis, infra dense pubescentibus albus; infructescentiis capitula differtis. Etiam similis O. davidiana Decne, quae foliis infra sparse pubescentibus et glandulosis.

Shrubs to 5 m tall. Branches brown or gray, glabrous; branchlets brown, densely gray pubescent, sometimes with red-brown seta. Petiole 2-5 mm, densely pubescent; leaf blade broadly ovate or ovate-orbicular, 3-9 × 2-6 cm, subleathery, abaxially sparsely white pubescent, adaxially densely white pubescent, base cordate or obliquely cordate, margin doubly serrate and usually incised above middle, apex acuminate or acute; lateral veins 6-9 on each side of midvein, raised abaxially. Male inflorescences 1 or 2; bracts densely white pubescent. Female inflorescence terminal, racemose-capitulate; peduncle 2-3 mm, densely white tomentose; infructescence capitate, bracts forming a tubular sheath, ca. 1-1.5 cm, leathery, densely white pubescent, striate, 3-lobed at apex, dehiscent on 1 side when mature. Nutlet brown, ovoid or subglobose, 4-6 × 4-5 mm, shiny, sparsely pubescent, ribbed; Fl. Mar - Apr, fr. Apr-May; Chromosome number: $2n = 16$.

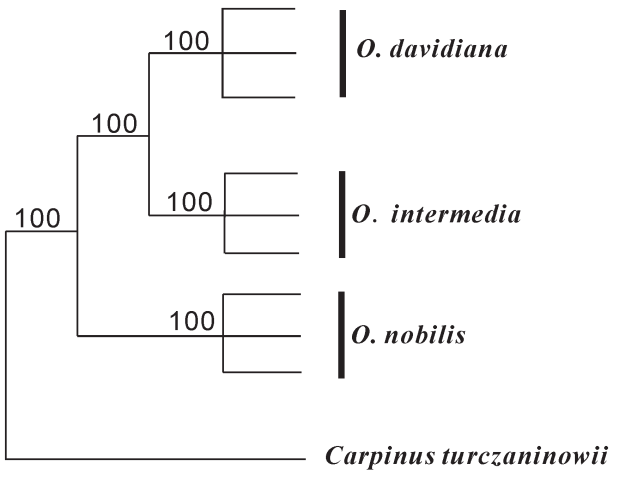

Figure 4. The single most parsimonious phylogenetic tree constructed based on ITS data. Numbers above branches indicate bootstrap supports.

Thickets on sunny mountain slopes; 1,500-2,500 m. NW Yunnan.

Key to three species of *Ostryopsis*

1. Leaf adaxially densely yellow-brown tomentose, margin irregularly serrate, apex obtuse or rounded; infructescence racemose .....*O. nobilis*
1. Leaf adaxially white pubescent, margin doubly serrate and usually incised above middle, apex acuminate or acute, infructescence capitate.
   2. Leaf 1.5-5×1-4 cm, ovate or elliptic-ovate, rarely broadly ovate or broadly obovate, adaxially sparsely white pubescent, with yellow or brown resinous glands.....*O. davidiana*
   2. Leaf 3-9 × 2-6 cm, broadly ovate or ovate-orbicular, rarely ovate, adaxially densely white pubescent, without yellow or brown resinous glands..... .....*O. intermedia*

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中國雲南樺木科一新種：居中虎榛子

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本文報導樺木科虎榛子屬一新種：居中虎榛子。該新種形態上位於虎榛子屬已有兩個物種之間，但這一新的形態特徵組合在所有檢查的個體和居群中都十分穩定。該新種在開花物候以及地理上與已有的兩個物種存在生殖隔離。ITS 序列在種內不存在變異，種間區別明顯並具種特異性；新種與兩個已知種之間都存在顯著的 ITS 序列差異。所有的證據都表明應將居中虎榛子獨立為一個新種。

關鍵詞：虎榛子屬；居中虎榛子；新種。