



Morphological and molecular evidence for a new species from China: *Piper semi-transparentes* (Piperaceae)

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Abstract

We conducted morphological and molecular analyses to describe a new species of Piperaceae, *Piper semi-transparentes*, which was observed in Hainan, Guangdong and Guangxi Provinces, China. Detailed morphological comparisons between the newly discovered species and other members of *Piper* indicate that *Piper semi-transparentes* is similar to *P. bonii* and *P. bonii* var. *macrophyllum*, from which it differs in having glabrous leaf blades; stem densely short and unbranched pubescent when young, glabrous when mature; veins 5(–7); stamens 2; stigmas 3, rarely 4; spikes color white when mature; floral bracts glabrous; pericarp white and semi-transparent. Molecular analyses based on the ITS marker of nuclear ribosomal DNA strongly support that *P. semi-transparentes* is a new species in the genus *Piper*, most closely related to *P. bonii* and *P. bonii* var. *macrophyllum*.

Keywords: diversity, ITS, taxonomy, tropical flora

Introduction

Piper Linnaeus (1753: 28) is the nominate genus of the family Piperaceae, comprising approximately 2,000 species, mainly distributed in the tropics (Gentry 1982, Kubitzki *et al.* 1993, Marquis 2004, Quijano-Abril *et al.* 2014), and it is also one of the most diverse lineages among basal angiosperms (Tebbs 1993, Soltis *et al.* 1999). *Piper* species are rather uniform morphologically, with simple, alternate leaves, jointed stems with enlarged nodes, and perianthless flowers arranged in condensed terminal spikes (Tebbs, 1993).

Asian taxa of *Piper* have been studied in numerous publications and current estimates include more than 600 species (Wallich 1824–1849, Blume 1826, Hooker 1886, De Candolle 1910, 1912, 1923, Ridley 1924, Backer & Bakhuizen van den Brink 1963, Long 1984, Huber 1987, Gardner 2006, Suwanphakdee *et al.* 2006, 2008, 2011, 2012, 2014). Specifically in China, more than 60 *Piper* species are represented, half of which are endemic (Gilbert & Xia 1999, Cheng *et al.* 1999, Gajurel *et al.* 2001, Hao *et al.* 2012, Hao *et al.* 2015, Hao *et al.* 2017, Yang *et al.* 2017).

During floristic investigations of *Piper* in South China between 2014 and 2018, an interesting new species was found in the Hainan, Guangdong and Guangxi Provinces, which is described and illustrated herein. A molecular analysis of the ITS region was also performed to determine the proper placement of this species in a genetic context. Here we describe this new species based on morphological and molecular studies.

Materials and methods

Morphological studies

Living material of fifteen individuals from different populations were morphologically investigated for this study. Photographs of the plants in the wild were taken with a digital camera with 55–210 mm macro-lens. All morphological characters were studied with a Leica M50 stereomicroscope and Dino-Lite digital microscope. A detailed examination of the morphological characters of this plant and its possible relatives was conducted, including specimens of several herbaria (A, E, HITBC, IBK, IBSC, K, KUN, P, PE, and VNM).

Molecular methods and genetic relationship analyses

Individuals of the sampled *Piper* specimens and 20 other *Piper* taxa were included in the genetic relationship analysis. Potential outgroup taxa for this analysis included three species of the sister genus *Peperomia* Ruiz & Pavón, namely *Peperomia dindygulensis* Miq. (1843: 122), *Peperomia heyneana* Miq. (1843: 123), and *Peperomia pellucida* (L.) Kunth (1815: 64) (Table 1). Genomic DNA of the accessions was extracted from young leaves using the D2485-01 E.Z.N.A. HP Plant DNA Kit (OMEGA, Stamford, CT, USA), according to the manufacturer instructions (Wu *et al.* 2016). Amplification of the nuclear *r*DNA ITS region was conducted according to Jaramillo *et al.* (2001). DNA sequencing was performed at Sangon Biotech (Shanghai) Co., Ltd.

TABLE 1. Specimens and associated GenBank accession numbers for the taxa used in the genetic relationship analysis.

Species	GenBank No.	Specimen / voucher	Locality
<i>Piper austrosinense</i> Y.C. Tseng	MG208076	Hao 2011199 (HITBC)	Guangdong, China
<i>Piper bambusifolium</i> Y.C. Tseng	MG208082	Qin 2012042 (HITBC)	Shanxi, China
<i>Piper betle</i> L.	MG208083	Hao 2011041 (HITBC)	Yunnan, China
<i>Piper boehmeriifolium</i> (Miq.) C. DC.	MG208064	Hao 2015135 (HITBC)	Yunnan, China
<i>Piper bonii</i> C. DC.	MG208080	Hao 2012030 (HITBC)	Yunnan, China
<i>Piper bonii</i> var. <i>macrophyllum</i> Tseng	MN820446	Hao 2012079 (HITBC)	Hainan, China
<i>Piper cathayanum</i> M.G. Gilbert & N.H. Xia	MG208072	Qin 2014305 (HITBC)	Guangxi, China
<i>Piper flaviflorum</i> C. DC.	MG208075	Hao 2015167 (HITBC)	Yunnan, China
<i>Piper hancei</i> Maxim.	MG208079	Hao 2011202 (HITBC)	Hainan, China
<i>Piper infossibaccatum</i> A. Huang	MG208085	Hao 2013258 (HITBC)	Hainan, China
<i>Piper jianfenglingense</i> C. Y. Hao & Y. H. Tan	MG208070	Hao 2015066 (HITBC)	Hainan, China
<i>Piper laetispicum</i> C. DC.	MG208078	Hao 2013147 (HITBC)	Hainan, China
<i>Piper lingshuiense</i> Tseng	MG208069	Hao 2014009 (HITBC)	Hainan, China
<i>Piper macropodium</i> C. DC.	MG208074	Hao 2015112 (HITBC)	Yunnan, China
<i>Piper methysticum</i> Forst.	MG208063	Hao 2015083 (HITBC)	Suva, Fiji
<i>Piper mutabile</i> C. DC.	MG208071	Hao 2012027 (HITBC)	Guangdong, China
<i>Piper nigrum</i> L.	MG208084	Hao 2011014 (HITBC)	Hainan, China
<i>Piper pedicellatum</i> C. DC.	MG208073	Hao 2015121 (HITBC)	Yunnan, China
<i>Piper polysyphonum</i> C. DC.	MG208065	Hao 2015116 (HITBC)	Yunnan, China
<i>Piper semi-transparente</i> C. Y. Hao & Y. H. Tan	MN818871	Hao 2015028 (HITBC)	Hainan, Guangdong, Guangxi, China
<i>Piper thomsonii</i> (C. DC.) Hook. f.	MG208068	Hao 2014013 (HITBC)	Yunnan, China
<i>Piper umbellatum</i> L.	MG208077	Yang 2012024 (HITBC)	Bogor, Indonesia
OUTGROUP			
<i>Peperomia dindygulensis</i> Miq.	MG208067	Tan 01879 (HITBC)	Guangxi, China
<i>Peperomia heyneana</i> Miq.	MG208066	Tan 01658 (HITBC)	Yunnan, China
<i>Peperomia pellucida</i> (L.) Kunth	MG208081	Tan 01845 (HITBC)	Hainan, China

Sequences were aligned using Clustal X version 1.8 (Thompson *et al.* 1997) and manually adjusted to provide maximum alignment and minimize gaps. To ensure the stability and reliability of genetic relationships among strains used in this study, genetic relationship analysis was performed using the maximum parsimony (MP) method with

PAUP*4.0b10 (Swofford, 2002). Bootstrap resampling (1,000 pseudoreplicates) was done for each method and a bootstrap consensus tree produced. All methods generated trees with similar topology and bootstrap values.

Results

Genetic relationship analysis:—ITS sequences were obtained for all 21 taxa of *Piper* and three species of *Peperomia*, with GenBank numbers provided in Table 1. Alignment of the 24 samples yielded 743 nucleotide sites distributed in the ITS region as follows: ITS1=233 bp, 5.8S=164 bp, and ITS2=346 bp. A total of 178 (23.6%) informative sites were included in the genetic analysis (ITS1=68, 5.8S=13, ITS2=97). The strict consensus tree is shown in Fig. 1. The results showed that the *Piper* species included here were polyphyletic. The 21 *Piper* species were classified into four distinct clades: clade A with *P. methysticum* and *P. umbellatum*; clade B with *P. semi-transparentes*, *P. bonii* C. DC. (1910: 85), *P. bonii* var. *macrophyllum* Tseng (1979: 31), *P. boehmeriifolium* (Miquel) C. DC. (1869: 348), *P. pedicellatum* C. DC. (1866: 164), *P. infossibaccatum* A. Huang (1990: 295) and *P. thomsonii* (C. DC.) J. D. Hooker (1886: 87); clade C with *P. nigrum* Linn. (1753: 28), *P. flaviflorum* C. DC. (1917: 477), *P. laetispicum* C. DC. (1914: 42), *P. cathayanum* M.G. Gilbert & N.H. Xia (1999: 191), *P. austrosinense* Y. C. Tseng (1979: 36) and *P. betle* Linn. (1753: 28); clade D with *P. polysyphonum* C. DC. (1904: 1026), *P. lingshuiense* Y. C. Tseng (1979: 28), *P. mutabile* C. DC. (1910: 92), *P. macropodum* C. DC. (1904: 1026), *P. bambusaefolium* Y. C. Tseng (1979: 28) and *P. hancei* Maxim. (1886: 94). Our results suggested that *P. semi-transparentes* is most closely related to *P. bonii* and *P. bonii* var. *macrophyllum*, but separable with high support.

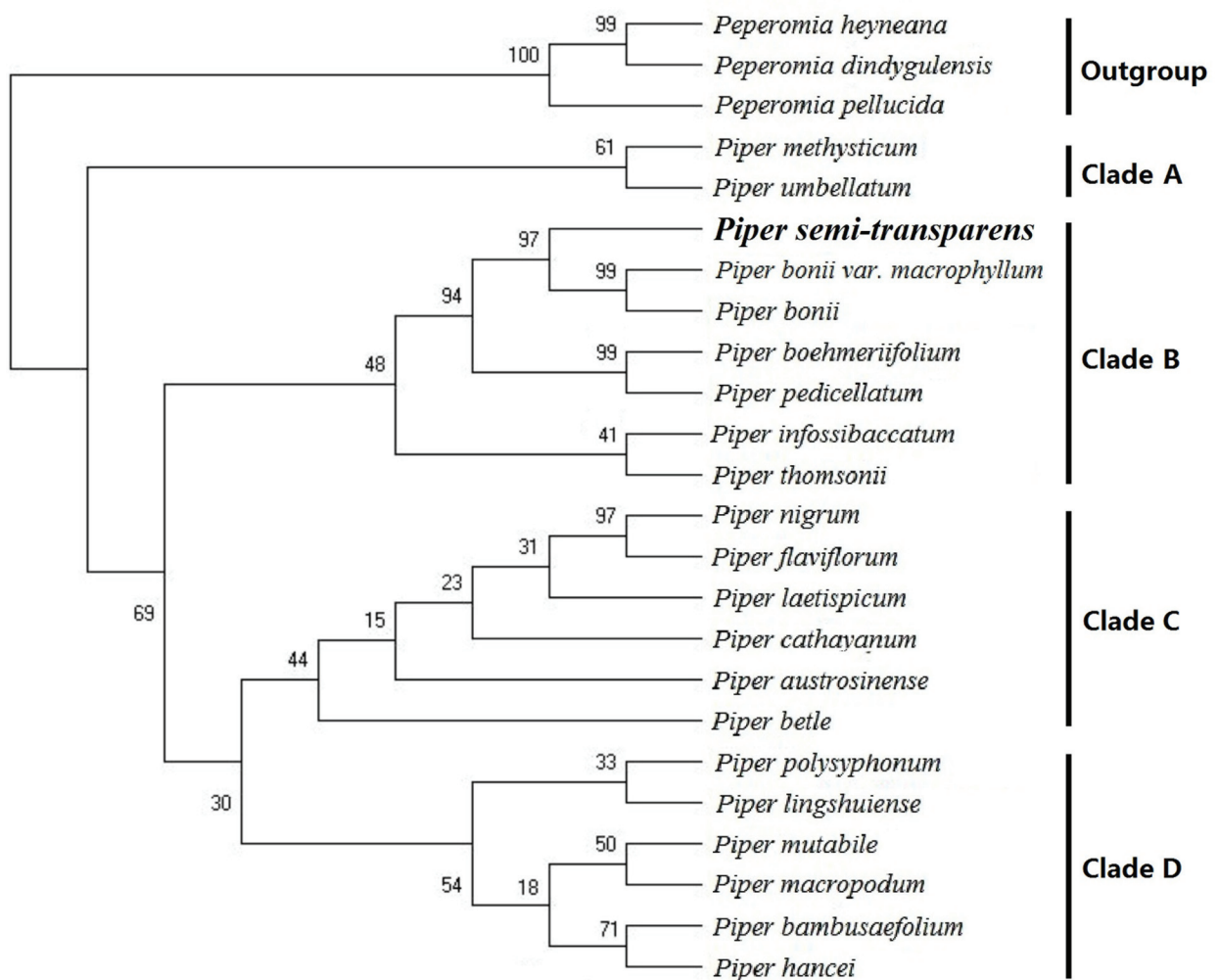


FIGURE 1. Genetic relationships among 21 *Piper* taxa based on ITS gene sequences. Three species of *Peperomia* were used as outgroup. The tree was constructed using the MP method. Numbers at nodes represent levels (%) of bootstrap support from 1000 resampled datasets.

Taxonomy

Piper semi-transparentes C.Y. Hao & Y.H. Tan, *sp. nov.* (Figs. 2–3)

Type:—CHINA. Hainan: Lingshui County, Diaoluoshan National Natural Reserve, tropical low montane moist forests, ca. 1084m, 18°43'46.31"N, 109°51'43.38"E, 9 April 2015, *Chao-Yun Hao 2015028* (holotype HITBC!; isotype HITBC!).

Piper semi-transparentes is morphologically similar to *Piper bonii* and *P. bonii* var. *macrophyllum*, but it is distinguished by glabrous leaf blades, stems that are densely short and unbranched pubescent when young and glabrous when mature, leaves with 5(–7) veins, stamens 2, stigmas 3 or rarely 4, and spikes white when mature; floral bracts glabrous; pericarp white and semi-transparent.

Woody climbers, more than 5 m high, dioecious. Stems pale green, brown when dry, 0.4–1.0 cm in diameter, finely ridged when dry, densely short and unbranched pubescent when young, glabrous when mature, nodes swollen, with climbing adventitious roots. Leaves chartaceous, glandular; adaxial surface green, glabrous; abaxial surface pale green, glabrous; petioles 0.5–1.0 cm long, unbranched pubescent, vaginate at base. Trophophyll leaf blade cordate or ovate, 1.5–3.0 cm long, 1.0–2.5 cm wide, base cordate, symmetric, apex acute; veins 5, apical pair arising 0.3–0.5 cm above base. Gonophyll leaf blade elliptic, ovate or ovate-lanceolate, 5.5–11.0 cm long, 2.5–5.0 cm wide, base rounded, oblique, apex acuminate; veins 5(–7), apical pair arising 1–2 cm above base, adaxial surface dark green, glabrous; abaxial surface pale green or yellow, glabrous. Inflorescence a pedunculate spike, leaf-opposed, solitary, pendulous, cylindrical, green when young, white when mature; the fertile rachis glabrous, with densely compacted flowers; floral bracts orbicular, ca. 1–2 mm diameter, peltate, glabrous, stalk 0.2–0.5 mm long. Male inflorescences 8–15 cm long, 0.2–0.4 cm wide; peduncles 1.0–2.0 cm long, glabrous. Male flowers with 2 stamens; filaments short; anthers subglobose, 2-locular with lateral dehiscence. Female inflorescences 3.0–5.0 cm long, 0.3–0.5 cm wide; peduncles 1.0–2.0 cm long, glabrous. Female flowers with ovary ellipsoid to ovoid; style short, persistent and stiff-pointed; stigma ovoid, apex acute, 3-lobed, rarely 4-lobed, hairy. Infructescences 3.5–5.5 cm long, 0.5–0.7 cm wide, glabrous, pendulous, cylindrical, with an echinate appearance from the persistent styles. Fruit a single-seeded berry, connate with rachis, glabrous, ellipsoid, 2 mm diameter, pericarp white and semi-transparent. Seed dusty brown, ellipsoid, 1.0–1.2 mm long × 0.7–1.0 mm wide, smooth.

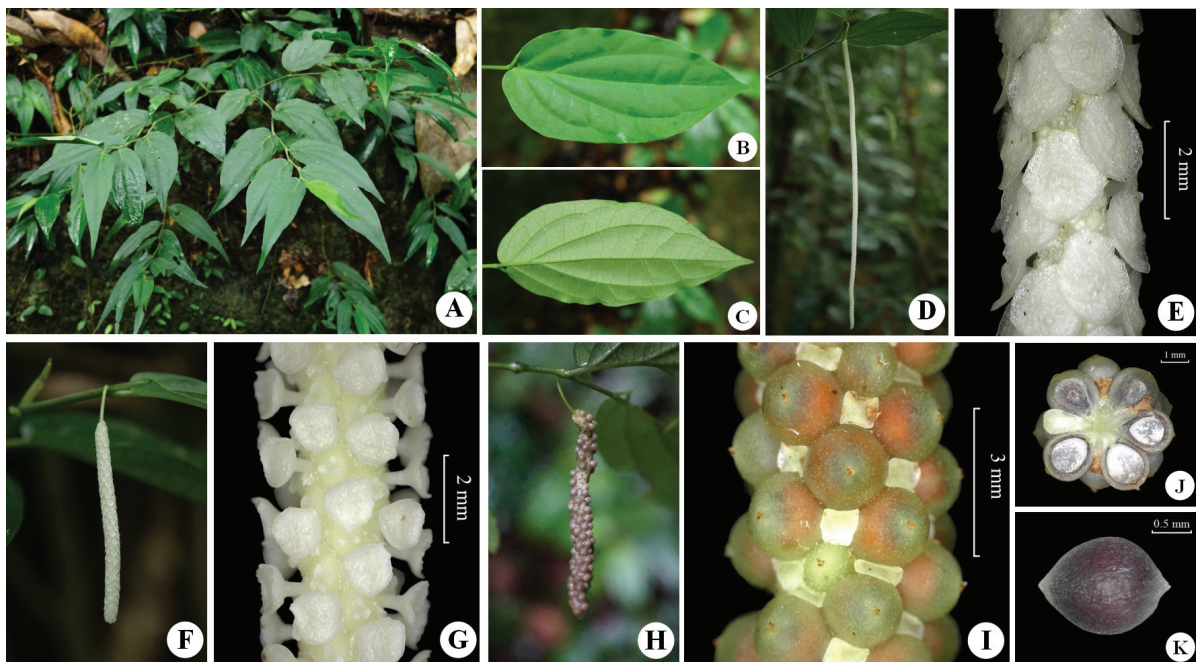


FIGURE 2. *Piper semi-transparentes*. A. Habit; B. Adaxial surface of leaf; C. Abaxial surface of leaf; D. Male spike; E. Detail of male spike; F. Female spike; G. Detail of female spike; H. Infructescence; I. Detail of infructescence; J. Transection of infructescence; K. Seed (side view); Photographed by C.-Y. Hao.

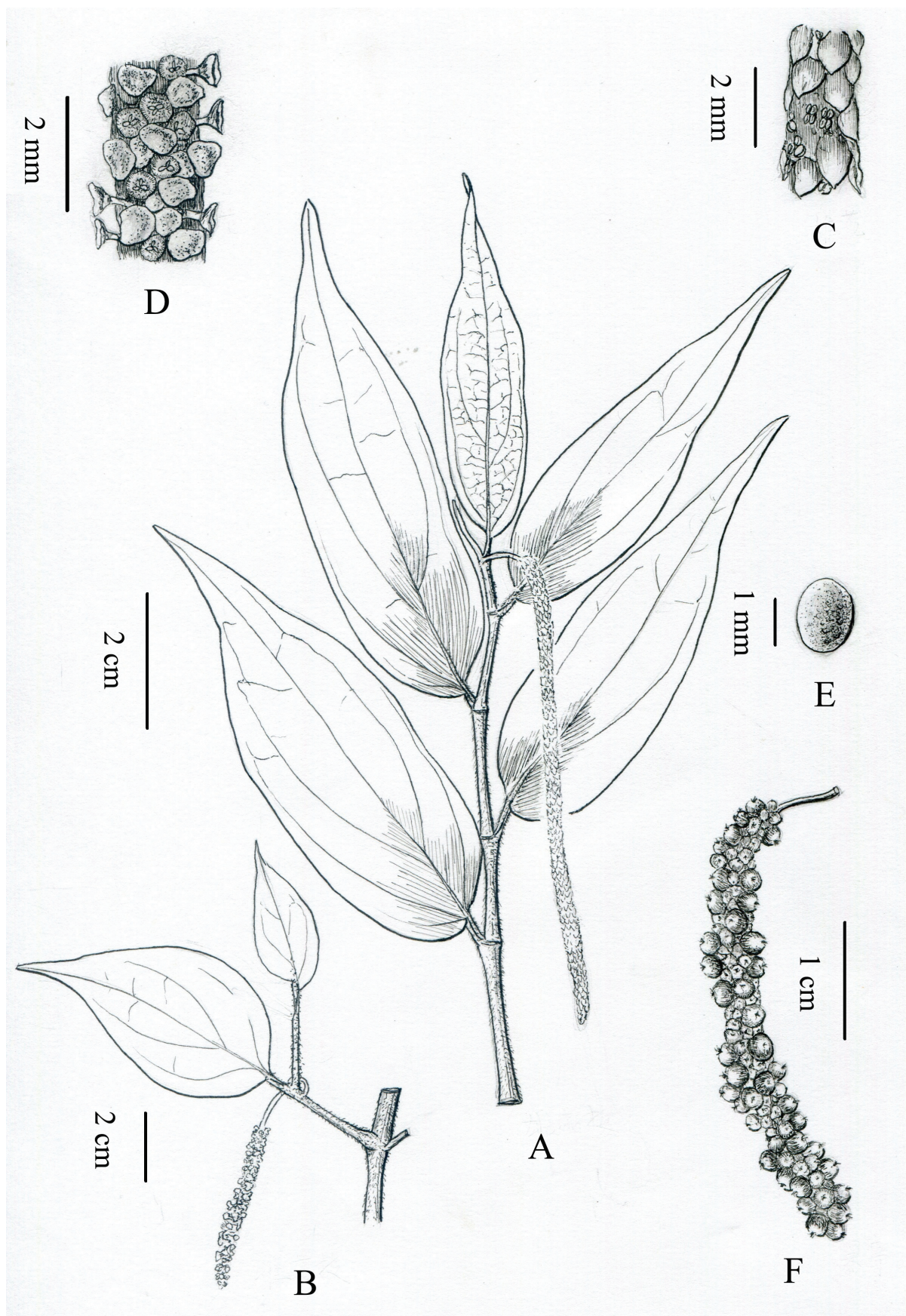


FIGURE 3. *Piper semi-transparente*. A. Branch with male inflorescence; B. Branch with female inflorescence; C. Detail of male inflorescence; D. Detail of female inflorescence; E. Floral bract; F. Infructescence; Illustration based on the holotype *C.-Y. Hao* 2015028 by M.-H. Lin.

TABLE 2. Morphological comparison between *Piper semi-transparentes*, *P. bonii* and *P. bonii* var. *macrophyllum*.

	<i>P. semi-transparentes</i>	<i>P. bonii</i>	<i>Piper bonii</i> var. <i>macrophyllum</i>
Stems	densely short and unbranched pubescent when young, glabrous when mature	tomentose	tomentose
Leaves	Leaf blades glabrous; 5.5–11.0 cm × 2.5–5.0 cm	abaxially tomentose, especially on veins, most hairs forked, adaxially glabrous or sometimes pubescent at base; 4.5–9.0 cm long × 2.2–5.0 cm wide	abaxially tomentose, especially on veins, most hairs forked, adaxially glabrous or sometimes pubescent at base; 12.0–18.0 cm long × 6.0–8.0 cm wide
Male flowers	Veins 5(–7) Stamens 2	7 3	7 3
Female inflorescences	3–5 cm at anthesis, 3.5–5.5 cm in fruit	4–8 cm at anthesis, 5–9 cm in fruit.	4–8 cm at anthesis, 5–9 cm in fruit.
Female flowers	Stigmas 3, rarely 4	4	4
Spikes color	Green when young, white when mature	Green when young, yellow when mature	Green when young, yellow when mature
Floral bracts	Surface glabrous Shape orbicular	glabrous, abaxially with 2–5 long hairs orbicular, base usually serrulate	glabrous, abaxially with 2–5 long hairs orbicular, base usually serrulate
Berries	Pericarp color white and semi-transparent Attachment style connate with rachis	green partly immersed in rachis	green partly immersed in rachis

Phenology:—Flowering from February to June; fruiting from July to November.

Etymology:—The epithet refers to the semi-transparent pericarp, which is unique in Chinese *Piper* species.

Distribution and habitat:—*Piper semi-transparente* is currently known from Hainan, Guangdong and Guangxi Provinces of China. It occurs in wet subtropical and tropical montane forest at elevations of 500–900 m, and often climbs on trees or rocks close to streams. In its distribution area, other *Piper* species such as *P. hancei* and *P. austrosinense* are often associated.

Additional specimens examined:—CHINA. Hainan: Ledong County, Jianfengling National Nature Reserve, ca. 600 m, 16 July 2015, C.-Y. Hao & X.-W. Qin 2015061 (HITBC!). Guangdong: Zhaoqing County, Dinghushan National Nature Reserve, ca. 520 m, 20 August 2016, C.-Y. Hao 2016089 (HITBC!); Huaiji County, Heishuikeng, 25 October 1958, Y.-G. Liu 298514 (IBSC!); Yingde County, Heishuikeng, 7 December 1957, C. Huang 143742 (IBSC!). Guangxi: Rong County, Miunishan, 27 October 1955, D.-M. Lei & Z.-Y. Wei 40438 (IBK!).

Discussion

The molecular analyses suggest that *P. semi-transparente* is genetically close to *P. bonii* and *P. bonii* var. *macrophyllum* based on the studied marker. Morphologically, *P. semi-transparente* and the other taxa differ significantly in their leaf blade indument (glabrous vs. abaxially tomentose, especially on veins, most hairs forked, adaxially glabrous or sometimes pubescent at base), leaf blade vein number [5(–7) vs. 7], stamen number (2 vs. 3), stigma number (3, rarely 4 vs. 4), floral bract surface (glabrous vs. glabrous adaxially, and pubescent with 2–5 long trichomes abaxially), and pericarp color of mature berries (white and semi-transparent vs. green). After comparison with the specimens and literature, we found that the new species could be clearly differentiated from *P. bonii* and *P. bonii* var. *macrophyllum* by many characters, as described in the diagnosis above and summarized in Table 2.

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