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Stillingia: A newly recorded genus of Euphorbiaceae from China

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Abstract

Stillingia (Euphorbiaceae) contains ca. 30 species from Latin America, the southern United States, and various islands in the tropical Pacific and in the Indian Ocean. We report here for the first time the occurrence of a member of the genus in China, *Stillingia lineata* subsp. *pacifica*. The distribution of the genus in China is apparently narrow, known only from Pingzhou and Wanzhou Islands of the Wanshan Archipelago in the South China Sea, which is close to the Pearl River estuary. This study updates our knowledge on the geographic distribution of the genus, and provides new palynological data as well.

Key words: Island, Hippomaneae, South China Sea, *Stillingia lineata*

Introduction

During the last decade, hundreds of new plant species or new species records have been added to the flora of China. Nevertheless, newly described or newly recorded plant genera are not discovered and reported very often, suggesting that botanical expedition and plant survey at the generic level may be advanced in China. As far as we know, only six and eight angiosperm genera respectively have been newly described or newly recorded from China within the last ten years (Qiang *et al.* 2007, Li *et al.* 2008, 2011, 2015, Liu *et al.* 2008, 2012, Liu & Yang 2011, German *et al.* 2012, Wang *et al.* 2012, Xie *et al.* 2012, Deng *et al.* 2013, Zhai *et al.* 2013), adding up to the number of angiosperm genera from China of 3,120.

Since the middle of the last century, great progress has been made in exploring plants in China and in compiling a Flora for China. In 1998 and in 2013 respectively, the enormous Chinese masterpiece *Flora Reipublicae Popularis Sinicae* (FRPS) (Editorial Committee of FRPS 1959–2004) and the English-language revision *Flora of China* (Wu *et al*. 1994–2013) were fully compiled. However, detailed surveys of plant biodiversity on the oceanic islands are still rare. Of the more than one million plant specimens from the herbarium of South China Botanical Garden (IBSC), only 2,643 collections are from the ca. 6,500 oceanic islands of China (excluding Hainan and Taiwan) from 1927 to 2013. Most Chinese oceanic islands have never been explored for plant biodiversity. Floristic investigations on the oceanic islands are badly needed because islands may be biological refugia and may represent unique fragile ecosystems on the global scale (Pablo 2007).

Since 2013, we have explored the flora of 18 islands of the Wanshan Archipelago in the South China Sea, and have found an unusual plant on two islands, which is clearly different from any known plant species from China. Detailed morphological, palynological and phylogenetic studies of this plant suggested that it should be a member of *Stillingia* Garden ex Linnaeus (1767: 19, 126), namely *S. lineata* subsp. *pacifica* (Müller Argoviensis 1866: 1156) Steenis (*in* Steenis & Balgooy 1966: 302).

Consisting of ca. 30 species, *Stillingia* occurs mostly in the New World's tropical and subtropical regions, ranging from southern North America to South America (Argentina) and Pacific Islands. There are only one species and one subspecies occurring in Madagascar, Mauritius, Réunion, Fiji, and several parts of Malesia (Esser 1999). Woody basal part of fruits, remaining on the plant as a carpidiophore, fused sepals and 2 stamens are diagnostically important for *Stillingia*. The discovery and study of *Stillingia* in South China Sea not only adds a new generic record to China, but also upgrades our knowledge on the distribution of the genus and the palynology of the species.

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Material and methods

The morphological studies were carried out using herbarium specimens (from IBSC) as well as living individuals in the field. The field studies were conducted at May 2015 in the Wanshan Archipelago, Zhuhai, Guangdong Province. The pollen grains for palynological observation were collected from living plants. More than 30 pollen grains were selected to calculate their size. Protocols for scanning electron microscopic (SEM) study followed Xie *et al.* (2014).

To reconstruct the phylogenetic relationships, we selected two sequences of the plastid DNA, the *trn*L-F region (Taberlet *et al.* 1991) and the *rbc*L 1F/724R region (Fay *et al.* 1997). The *trn*L-F region was amplified using the primers c (5'-CGAAATCGGTAGACGCTACG-3') and f (5'-ATTTGAACTGGTGACACGAG-3'). The *rbc*L1F/724R region was amplified using the primers 1F (5'-ATGTCACCACAAACAGAAAC-3') and 724R (5'-TCGCATGTACCTGCAGTAGC-3'). The PCR amplification used a 5 min denaturing step at 94°C followed by 30 cycles of denaturing for 1 min at 94°C, primer annealing for 30 sec at 52°C, and elongation step for 1 min at 72°C, with a final 8 min elongation step at 72°C. Sequencing was conducted using the same PCR primers at the Invitrogen Corporation (Shanghai, China). Sequences were assembled using SEQUENCHER 5.3 (Gene Codes Corporation 2015) and aligned initially with ClustalX version 1.83 (Thompson *et al*. 1997), followed by manual adjustments on Se-Al v2.0a11 (Rambaut 2007). Phylogenetic trees based on the maximum parsimony (MP) analysis were reconstructed using PAUP* 4.0b10 (Swofford 2000). The GenBank accessions of sequences published in a previous study (Wurdack *et al.* 2005) and newly produced in this study are provided in Table 1.

TABLE 1. Species, vouchers and GenBank accession numbers analyzed here.

Taxon	Vouchers or references	*trn*L-*trn*F	*rbc*L
Adenopeltis serrata (W.T. Aiton) I.M. Johnst.	(Wurdack *et al.* 2005)	AY794633	AY794844
Bonania cubana A. Rich.	(Wurdack *et al.* 2005)	AY794613	AY794833
Colliguaja integerrima Gillies & Hook.	(Wurdack *et al.* 2005)	AY794617	AY794836
Colliguaja odorifera Molina	(Wurdack *et al.* 2005)	AY794618	AY794837
***Excoecaria agallocha* L.**	***X.X. Huang 15412* (IBSC)**	**KY496709**	**KY501144**
***Excoecaria acerifolia* Didr.**	***X.X. Huang 15760* (IBSC)**	**KY496710**	**KY501145**
***Excoecaria cochinchinensis* Lour.**	***S.C. Li 183* (IBSC)**	**KY496711**	**KY501146**
Excoecaria grahamii Stapf	(Wurdack *et al.* 2005)	AY794623	
***Excoecaria venenata* S.K. Lee & F.N. Wei**	***S.C. Li 182* (IBSC)**	**KY496712**	**KY501147**
Grimmeodendron eglandulosum (A. Rich.) Urb.	(Wurdack *et al.* 2005)	AY794612	AY794832
Gymnanthes lucida Sw.	(Wurdack *et al.* 2005)	AY794653	AY794858
Hippomane mancinella L.	(Wurdack *et al.* 2005)	AY794616	AY794835
Homalanthus nutans (G. Forst.) Guill.	(Horn *et al.* 2012) and (Tokuoka 2007)	JN249590	AB267957
Homalanthus populneus (Geiseler) Pax	(Wurdack *et al.* 2005)	AY794650	AY380350
Hura crepitans L.	(Wurdack *et al.* 2005) and (Tokuoka & Tobe 2006)	AY794636	AB233886
***Microstachys chamaelea* (L.) Müll.Arg.**	***S.C. Li 129* (IBSC)**	**KY496713**	
Ophthalmoblapton pedunculare Müll. Arg.	(Wurdack *et al.* 2005)	AY794638	AY794848
Pachystroma longifolium (Nees) I.M. Johnst.	(Wurdack *et al.* 2005)	AY794637	AY794847
Pleradenophora bilocularis (S. Watson) Esser & A. L. Melo	(Wurdack *et al.* 2005)	AY794614	AY794834
Sapium glandulosum (L.) Morong	(Wurdack *et al.* 2005)	AY794626	AY794841
Sapium laurocerasus Desf.	(Wurdack *et al.* 2005)	AY794628	AY794842
Sebastiania commersoniana (Baill.) L.B. Sm. & Downs	(Wurdack *et al.* 2005)	AY794640	AY794850

...Continued on next page

TABLE 1. (Continued)

Taxon	Vouchers or references	*trn*L-*trn*F	*rbc*L
Sebastiania pavoniana (Müll. Arg.) Müll. Arg.	(Wurdack *et al.* 2005)	AY794625	AY794840
Spegazziniophytum patagonicum (Speg.) Esser	(Wurdack *et al.* 2005)	AY794635	AY794846
Spirostachys africana Sond.	(Wurdack *et al.* 2005)	AY794621	AY794838
Stillingia lineata (Lam.) Müll. Arg.	(Wurdack *et al.* 2005)	AY794629	
***Stillingia lineata* subsp. *pacifica* (Müll. Arg.) Steenis**	***X.X. Huang 18528* (IBSC)**	**KY496714**	**KY501148**
***Stillingia lineata* subsp. *pacifica* (Müll. Arg.) Steenis**	***X.X. Huang 18376* (IBSC)**	**KY496715**	
Stillingia paucidentata S. Watson	(Wurdack *et al.* 2005)	AY794634	AY794845
Stillingia sylvatica L.	(Wurdack *et al.* 2005)	AY794631	AY794843
Stillingia texana I.M. Johnst.	(Wurdack *et al.* 2005)	AY794630	
Tetraplandra sp.	(Wurdack *et al.* 2005)	AY794639	AY794849
***Triadica cochinchinensis* Lour.**	***S.C. Li 181* (IBSC)**	**KY496716**	**KY501149**
***Triadica sebifera* (L.) Small**	***S.C. Li 180* (IBSC)**	**KY496717**	**KY501150**

Results

Morphology and species identification

In 2008, Binghui Chen collected a specimen of *Stillingia lineata* subsp. *pacifica* (*B. H. Chen 7914*, IBSC) from the Wanshan Archipelago. The identification of the specimen, however, was not able then because the flowers and the fruits were absent. In the summer of 2015, we fortunately found the species again and collected flowers as well as fruits (Fig. 1 A–H). The plants can be easily identified as *S. lineata* subsp. *pacifica* by having small obovate leaves with obvious serration, a flat midvein, distinct secondary veins and disc-shaped glands at the base of bracts, 3–11-flowered staminate cymules, and the remaining carpidiophore of the opened fruit, which are comparable to the typical individuals of the subspecies from Fiji and the Malesia.

Specimens studied

Here is the information on specimens of *Stillingia lineata* subsp. *pacifica* studied: CHINA. Guangdong: Zhuhai, Wanshan Archipelago, Pingzhou, growing on rocks, 21°48′45″N, 113°58′02″E, 13 m, 1 June 2015, *Xiangxu Huang 18528* (IBSC); Wanzhou, growing in the bush, 21°49′36″N, 113°57′54″E, 61 m, 29 May 2015, *Xiangxu Huang 18376* (IBSC); Wanzhou, 21°49′01″N, 113°57′48″E, 12 January 2008, *Binghui Chen 7914* (IBSC).

Palynology

The pollen grains of the subspecies are monads, spheroidal, radiosymmetric, 31.60 ± 2.62 (P: 25.50–37.48) × 31.00 ± 1.87 (E: 25.69–34.23) μm in size. All the pollen grains are tricolporate with smooth margo. The exine ornamentation is perforate, both polar and equatorial view are circular (Fig. 1 I–L).

Molecular phylogeny

The topology of the strict consensus tree is consistent with those of Wurdack *et al.* (2005). Our data strongly supported the cluster of *Stillingia lineata* (Lamarck 1788: 734) Müller Argoviensis (1866: 1157) as previously reported from Mauritius, and the three newly sampled individuals of *S. lineata* subsp. *pacifica* from China. Thus, our molecular data support the identification of the subspecies. The phylogenetic relationships between *S. lineata* and other species of the genus need further studies using more molecular data and more thorough sampling due to a polytomy among the three clades of *Stillingia* (Fig. 2).

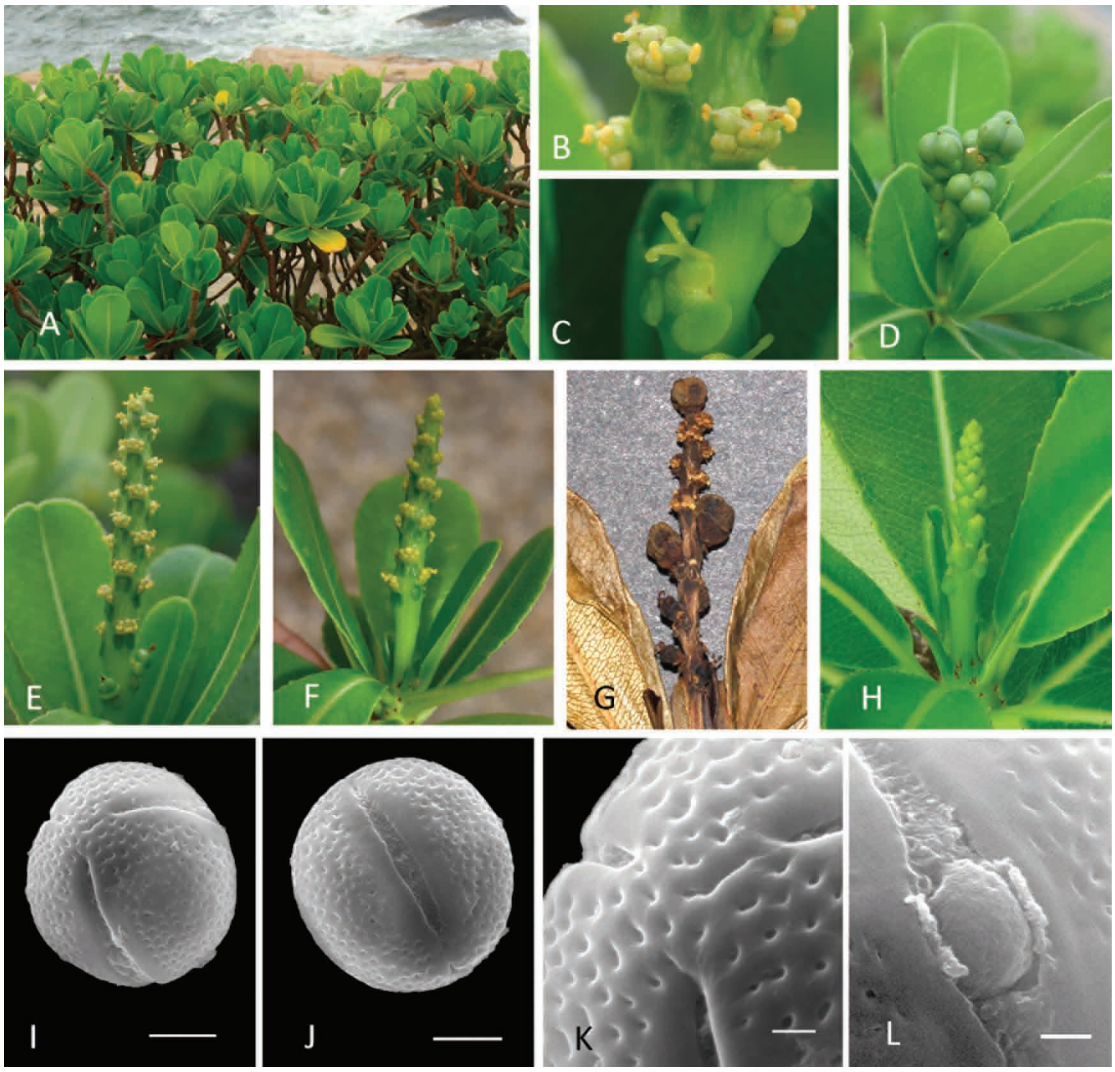

FIGURE 1. *Stillingia lineata* subsp. *pacifica*. A, Habitat; B, Male flowers; C, Female flowers; D, Fruits with carpidiophore; E–H, Four different inflorescences (E, Androgynous inflorescence with female flowers on the lower part; F, Male inflorescence; G, Androgynous inflorescence with female flowers on both lower and upper part; H, Female inflorescence); I, Pollen in polar view; J, Pollen in equatorial view; K, Exine ornamentation at mesocolpium in polar view; L, Pollen in equatorial view, showing aperture. Voucher specimens: *X.X. Huang 18376* (IBSC). Scale bar = 10 μm (I, J, L), 2 μm (K). Photo credits: A–H by X.X. Huang; I–L by S.C. Li.

Key to distinguish *Stillingia* from genera of Hippomaneae in China (in the Flora of China by Li *et al.* 2008, *Stillingia* can be inserted in key step 28 of page 167, corresponding to key step 4 below).

1. Perennial herbs; stems and leaves sparsely puberulent; fruit a capsule, each lobe with 2 lines of conical spines..... *Microstachys*
1. Trees or shrubs; stems and leaves glabrous; fruit a drupe or capsule, not spiny.....2
2. Fruit a many-loculed woody schizocarp, 8–9 cm in diam., eventually breaking into curved segments; female flower with dark purple stigma 1.5–2.5 cm wide; anthers connate; trunk and branches spiny ..... *Hura*
2. Fruit a 2- or 3-loculed capsule or drupe, up to 1.5 cm in diam., female flower with stigma much smaller; anthers free, filaments connate; spines absent 3
3. Male sepals distinct or calyx with 2 largely fused sepals; inflorescences bisexual or unisexual, axillary or terminal; leaves opposite or alternate 4
3. Male calyx shallowly 2–5-lobed; inflorescences bisexual, always terminal or leaf-opposed; leaves always alternate.....5
4. Leaves without glands on petiole apex or lamina base visible from above; staminate flowers 1–3 per bract, with free sepals and 3 stamens, basal part of fruit inconspicuous, not woody ..... *Excoecaria*
4. Leaves with glands on petiole apex or lamina base visible from above; staminate flowers 3–18 per bract, with fused sepals and 2 stamens, basal part of fruits woody, remaining on the plant as a woody carpidiophore ..... *Stillingia*
5. Stipules large, concealing apical bud, deciduous; male calyx laterally compressed, free; leaves with petiolar glands..... ..... *Homalanthus*
5. Stipules small or absent; male calyx not laterally compressed; leaves with or without petiolar glands 6

6. Leaf blade margin conspicuously serrate or undulate 7
6. Leaf blade margin entire or serrulate 8
7. Petiole apex with 2 distinct adaxial glands; axis of inflorescence glabrous; exocarp slightly fleshy ..... *Falconeria*
7. Petiole apex without glands; axis of inflorescence pilose; exocarp thick and hard ..... *Shirakiopsis*
8. Petiole not winged and without glands 9
9. Inflorescence 4–12 cm long; fruit an indehiscent berry ..... *Balakata*
9. Inflorescence ca. 2 cm long; fruit a capsule ..... *Gymnanthes*
8. Petiole winged and/or with apical glands 10
10. Seeds dry; leaf blade with lowermost pair of veins originating distinctly above base, not forming basal margin ..... *Neoshirakia*
10. Seeds enclosed by thin waxy sarcotesta; leaf blade with lowermost pair of veins originating at very base, forming basal margin ..... *Triadica*

Discussion

The taxonomic history of the Asian *Stillingia* is complex considering there is only a single species in this region (Esser 1999). In 1866, Müller Argoviensis described a new and rare species, *S. pacifica* Müller Argoviensis (1866: 1156), from Fiji. In a revision of *Stillingia* from East Malesia, Airy Shaw (1963) provisionally accepted this name and kept it apart from another species in Malesia, *S. lineata*, because of the more markedly crenate leaf margin and the great geographical separation. Steenis (*in* Steenis & Balgooy 1966) studied *Stillingia* from Malesia and found that there is continuous morphological variation among individuals of *S. lineata* and *S. pacifica,* so that he reduced *S. pacifica* as a subspecies under *S. lineata*. The hardened portion at the base of the pericarp has been considered a prominent distinguishing feature of *Stillingia*. According to this feature, we herein adopt Houtuotong (厚托桐) and Haihoutuotong (海厚托桐) as the Chinese name of the genus and of the subspecies respectively.

The palynological characters of *Stillingia lineata* subsp. *pacifica* are studied here for the first time. These characters are comparable to those of *S. treculiana* (Müller Argoviensis 1865: 216) Johnston (1923: 91) and *S. sylvatica* Linnaeus (1767: 126), which have tricolporate pollen and perforate exine (Park & Lee 2013). But the pollen grains of *S. lineata* subsp. *pacifica* (P/E=1.02) are rounder and smaller than those of the other two species (*S. treculiana*: P/E=1.33; *S. sylvatica* P/E=1.20).

The species groups of *Stillingia* from distinct geographical regions are usually morphologically separable and narrow endemics (Esser *et al.* 2012). The taxonomy of the genus is settled now in Asia, but not in the New World (Athie-Souza *et al.* 2016). The New World *Stillingia* can be divided into two groups, the non-succulent shrubs of mostly Andean distribution and the succulent shrubs in the dry regions of northeastern Brazil, both with restricted distributions. The succulent shrub habit of *S. lineata* subsp. *pacifica* with milky juice and robust branches may be correlated with the seasonally dry climate on the islands. On the islands of South China Sea, it often grows on rocks and in the bushes near the coast with other common oceanic island plants.

A number of recent studies have attempted to explain the pantropical disjunctions in plants by long-distance dispersal. Birds capable of long-distance flight and monsoon trade winds coupled with oceanic currents may be important factors related to dispersal (Ali *et al.* 2012). The drivers of the disjunct distribution of *Stillingia lineata* subsp. *pacifica* in the tropical Asian islands may be better explained by oceanic currents. This is because the fruits of the species are capsules and the globose seeds develop a cavity inside when mature, making it floating in the water (own observation). The occurrence of the species only in the islands of Pingzhou and Wanzhou may suggest that it is a new comer to China from southern Asia because the two islands are the outermost ones of the Wanshan Archipelago to the Pearl River estuary.

This study suggests that the tropical oceanic islands may have special floristic elements comparing to the mainland, and are worthy to be explored for plant biodiversity in the future.

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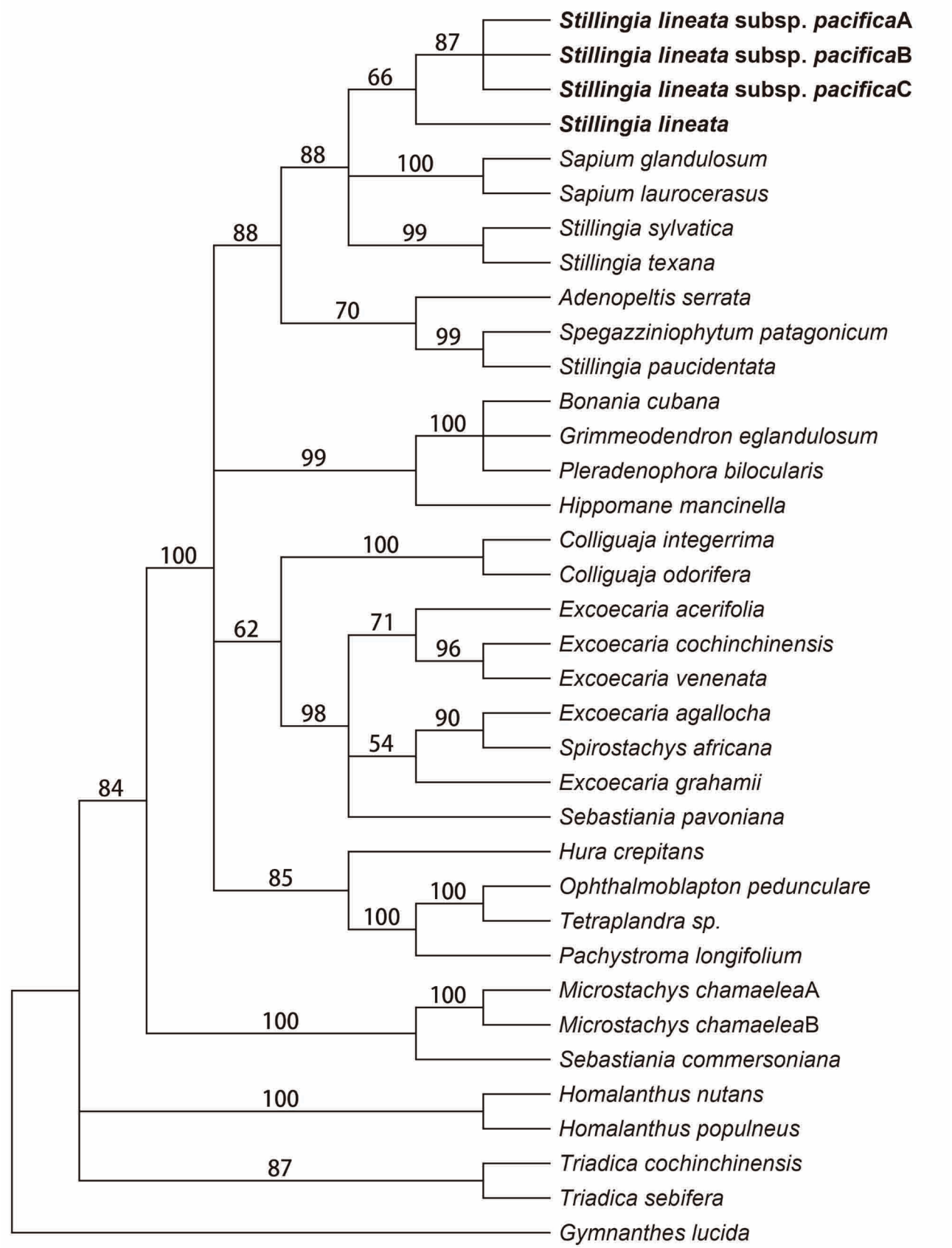

FIGURE 2. Strict consensus of 72 equally most parsimonious trees based on parsimony analysis of the combined matrix of *trn*L-F and *rbc*L sequences. The shortest trees had a length of 441 steps, CI =0.787 and RI =0.665. Numbers above the branches are maximum parsimony bootstrap percentages from a bootstrap test of 1000 runs.

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