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## Nuclear ITS Sequences Help Disentangle *Phyllanthus reticulatus* (Phyllanthaceae), an Asian Species not Occurring in Africa, but Introduced to Jamaica

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**Abstract**—*Phyllanthus* comprises approximately 1,270 species, making it one of the world's largest plant genera. Molecular data so far are of limited value for understanding *Phyllanthus* because of the sheer size of the genus. They can, however, help sort-out cryptic species and provide information on the origin of suspected introduced species. One of the seemingly most widespread species is *P. reticulatus*, which has been recorded from Asia, Australia, Africa, and Jamaica. The name is based on a mixed collection from tropical Asia now in the Lamarck herbarium, and we lectotypify it here. We use nuclear ITS sequences to test the broad treatment of *P. reticulatus* in recent floras, identify records of "*P. reticulatus*" from Africa, and investigate the origin of *P. reticulatus* on Jamaica. A maximum likelihood tree for accessions of *P. reticulatus* from throughout its supposed range (plus relevant outgroups) shows that the Jamaican plants represent the Asian species, that the African plants called *P. reticulatus* belong to a separate clade for which *P. polyspermus* is the oldest available name, and that the sensu lato treatment of *P. reticulatus* in recent floras is unjustified. Treating the Asian entities *P. reticulatus* and *P. microcarpus* as separate species appears justified, and identical ITS sequences in Asian and Jamaican *P. reticulatus* indicate a recent introduction. The first "island botanists," J. Macfadyen and N. Wilson, introduced many plants from India in the 1800s, and theirs are the oldest Jamaican collections of *P. reticulatus*. Since the species was introduced to Jamaica without its obligate *Epicephala* moth pollinators, it does not set fruit, persisting instead by vegetative growth.

**Keywords**—Anthropogenic introduction, ITS, Jamaica, lectotypification, *Phyllanthus* phylogenetics, Phyllanthaceae.

*Phyllanthus* L. (Phyllanthaceae) is a cosmopolitan genus of trees, shrubs and herbs, comprising at least 1,270 species (Kathriarachchi et al. 2006). The genus is most diverse in the tropics and subtropics, and relatively poorly represented in temperate regions. The high species number, paired with minute unisexual flowers and confusingly similar habits in unrelated species, make *Phyllanthus* a taxonomically challenging group. Molecular studies have shown that several long-established genera are nested inside *Phyllanthus*, including *Breynia* J. R. Forster & G. Forster, *Glochidion* J. R. Forster & G. Forster, *Reverchonnia* A. Gray, and *Sauropus* Blume (Kathriarachchi et al. 2005, 2006). GenBank currently contains molecular data for about 110 species, all with herbarium vouchers (Samuel et al. 2005; Kathriarachchi et al. 2005, 2006; Kawakita and Kato 2009). This < 10% sampling is too limited to confidently infer species relationships, or to use a bar-coding approach for species identification. China alone harbors 32 species of *Phyllanthus* (Li and Gilbert 2009), of which only a few have been sequenced (including those sampled for this study). Nevertheless, molecular sequences can help differentiate morphologically similar species, even without dense global sampling.

While carrying out fieldwork on the pollination biology of *Phyllanthus* in tropical China, the identity and geographical distribution of *P. reticulatus* Poir. caught our attention. Poirét (1804) described this species based on a collection made by an unknown collector in tropical Asia and deposited in the Lamarck herbarium in Paris (P-LA [P00381823]; Fig. 1A). Although Poirét's protologue mentions that the species grew "dans les Indes," the sheet has no locality information, and it is therefore unclear from where and from whom Lamarck received this plant. Given that the collection must have been made before 1804, one can nevertheless exclude Jamaica, in the West Indies, as the place of origin because the first botanical collections from Jamaica date to 1825 (Fawcett 1897; Morris 1906). *Phyllanthus reticulatus* ranges throughout India, Sri Lanka, the Himalayas, China, Indochina, Malesia, into

tropical Australia and is supposed to also occur in tropical Africa (Müller 1866; Radcliffe-Smith 1987; van Welzen 1997; Govaerts et al. 2000; Balakrishnan and Chakrabarty 2007; Li and Gilbert 2009). It has long been recorded as introduced in Jamaica (Webster 1957). Govaerts et al. (2000) list 22 synonyms of *P. reticulatus* at the species level (in addition to several invalid names). They also accept three partly sympatric varieties of *P. reticulatus*. Other authors (e.g. Chantharanonthai 2005, 2007) have a narrower concept of *P. reticulatus*.

The wide geographic range of *P. reticulatus* is surprising because the species is neither weedy nor commercially important (but see Discussion for its potential horticultural interest). In the field and when checking herbarium specimens we found small but consistent morphological differences between populations in southern China, Hainan, Taiwan, and Vietnam, and observations over several years (2002–2009) convinced the first author that Indochinese and Chinese material identified as *P. reticulatus* represents two species. To test which of these species might represent the true *P. reticulatus* we matched plants to the type material of *P. reticulatus* at P, which revealed the need for lectotypification given the mixed type collection. We also generated nuclear ribosomal ITS DNA sequences for specimens from Asia, Africa, and Jamaica. Sequences were added to the vouchered *Phyllanthus* ITS sequences from GenBank plus relevant outgroups from the study of Kathriarachchi et al. (2006). The results reveal that the supposedly Asian/Africa species *P. reticulatus* is a polyphyletic complex of several clades, while at the same time showing that Asian *P. reticulatus* is indeed widely distributed, not in Africa, but instead in the New World, namely in Jamaica.

### MATERIALS AND METHODS

**Herbarium Work, Field Observations, Morphological Data**—Relevant specimens were studied at HAST, IBSC, M, and MO, and digital images

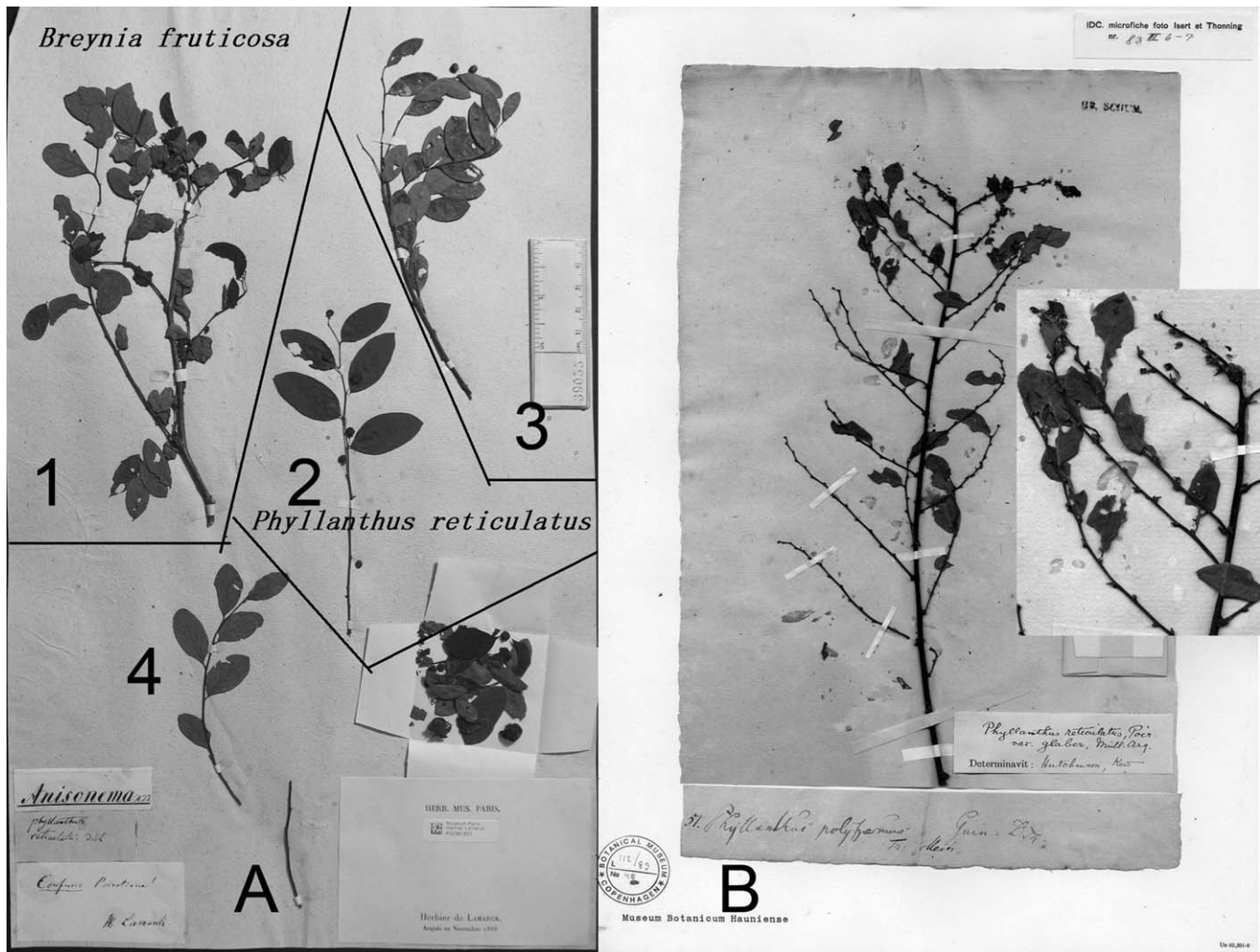


FIG. 1. Holotypes of *Phyllanthus reticulatus* Poir. and *Phyllanthus polyspermus* (Benth.) Müll. Arg. A. The type of *P. reticulatus* (P-LA P00381823) is a mixed gathering collected by an unknown collector at an unknown site in Asia, comprising a branchlet of *Breynia fruticosa* (L.) Hook. f. (1), a branchlet of *P. reticulatus* (2), here selected as the lectotype of the name *P. reticulatus*, and branchlets 3 and 4, which are difficult to assign to either species. B. Thonning 51 (C) from Ghana, one of two syntypes of *Phyllanthus polyspermus*, with fertile branchlets shown enlarged in the inset.

in online databases or provided by the curators of relevant herbaria (cited below). Measurements were taken from living plants in the field and from dried herbarium specimens, and pollen was studied under a scanning electron microscope (SEM, JSM-6360LV, JEOL, Japan). The first author observed flowering and fruiting *P. reticulatus* and *P. microcarpus* (Benth.) Müll. Arg. in Guangdong, Fujian, Hainan, Hong Kong, Taiwan, and Vietnam, monitoring several populations closely between 2002 and 2009.

**Molecular Phylogenetics**—The complete internal transcribed spacer of nuclear ribosomal DNA (ITS1–5.8S–ITS2) was sequenced following the methods described in Kathriarachchi et al. (2006). Total genomic DNA was isolated from silica-dried leaves or from herbarium specimens with a commercial plant DNA extraction kit (NucleoSpin, Macherey-Nagel, Düren, Germany), following the manufacturer's manual. Amplification products were purified with a PCR clean-up kit (PCR Wizard, Promega GmbH, Mannheim, Germany), and cycle sequencing was performed with the BigDye Terminator cycle sequencing kit on an ABI automated sequencer (Applied Biosystems, Foster City, California). Chromatograms were edited using Sequencher (4.6; Gene Codes, Ann Arbor, Michigan) and sequences were aligned with the matrix of Kathriarachchi et al. (2006), using MacClade 4.06 (Maddison and Maddison 2003). The final matrix had a length of 821 nucleotides and comprised 43 accessions of which 15 were newly generated for this study. Their GenBank accession numbers and herbarium vouchers are listed in Appendix 1. Voucher information for the remaining sequences is given in Kathriarachchi et al. (2006). Maximum likelihood (ML) analyses under the GTR + G model of substitution were performed using RAxML (Stamatakis et al. 2008), with bootstrap support values estimated from 100 replicates under the same model as used in

the tree searches. Trees have been submitted to TreeBASE (study number S10782).

## RESULTS AND DISCUSSION

Poiret (1804) described *P. reticulatus* based on a mixed collection from tropical Asia, possibly India (Fig. 1A), as already recognized by previous workers (Webster 1957: 59, "The type sheet contains as mixture of *P. reticulatus* and a species of *Breynia*"). On the lower left, the gathering bears a label in the handwriting of Poiret with the name "*Phyllanthus reticulata*, Inde," and a second label in the handwriting of J. A. C. Roeser, saying "Confusio Poiretiana, Hb. Lamarck." The left-hand sprig of the gathering represents *Breynia fruticosa* (L.) Hook. f. (Fig. 1A-1), while the middle sprig represents *Phyllanthus reticulatus* (Fig. 1A-2). The branches labeled 3 and 4 in Fig. 1A cannot securely be assigned from the image alone. Poiret's description could refer to several taxa present on this sheet, but several of the traits mentioned certainly fit *P. reticulatus*. Since a type is defined as a single specimen (ICBN Art. 8.1) representing a single taxon (Art. 8.2), the P-LA sheet represents a mixed collection, and judicious lectotypification

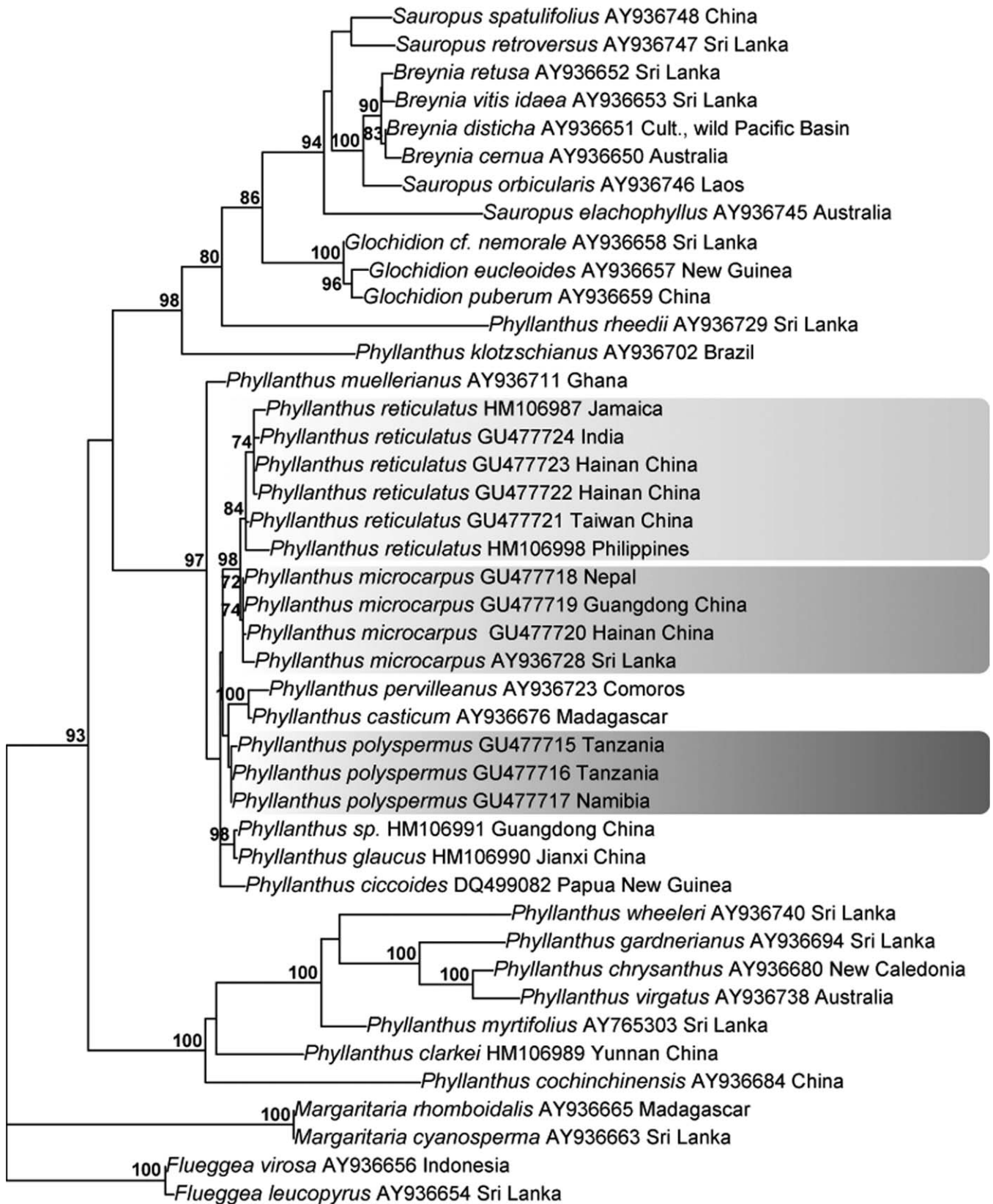


FIG. 2. Maximum likelihood tree based on 821 nucleotides of nuclear ITS sequenced for 43 accessions of Phyllanthaceae and rooted with *Flueggea* and *Margaritaria* following Kathiriarachchi et al. (2006). Bootstrap support values  $\geq 70\%$  are shown at the nodes. The clades marked by grey shading are the *Phyllanthus reticulatus* clade, the *P. microcarpus* clade, and the African *P. polyspermus* clade, consisting of specimens formerly identified as *P. reticulatus*. Herbarium vouchers for newly sequenced material are listed in Appendix 1.

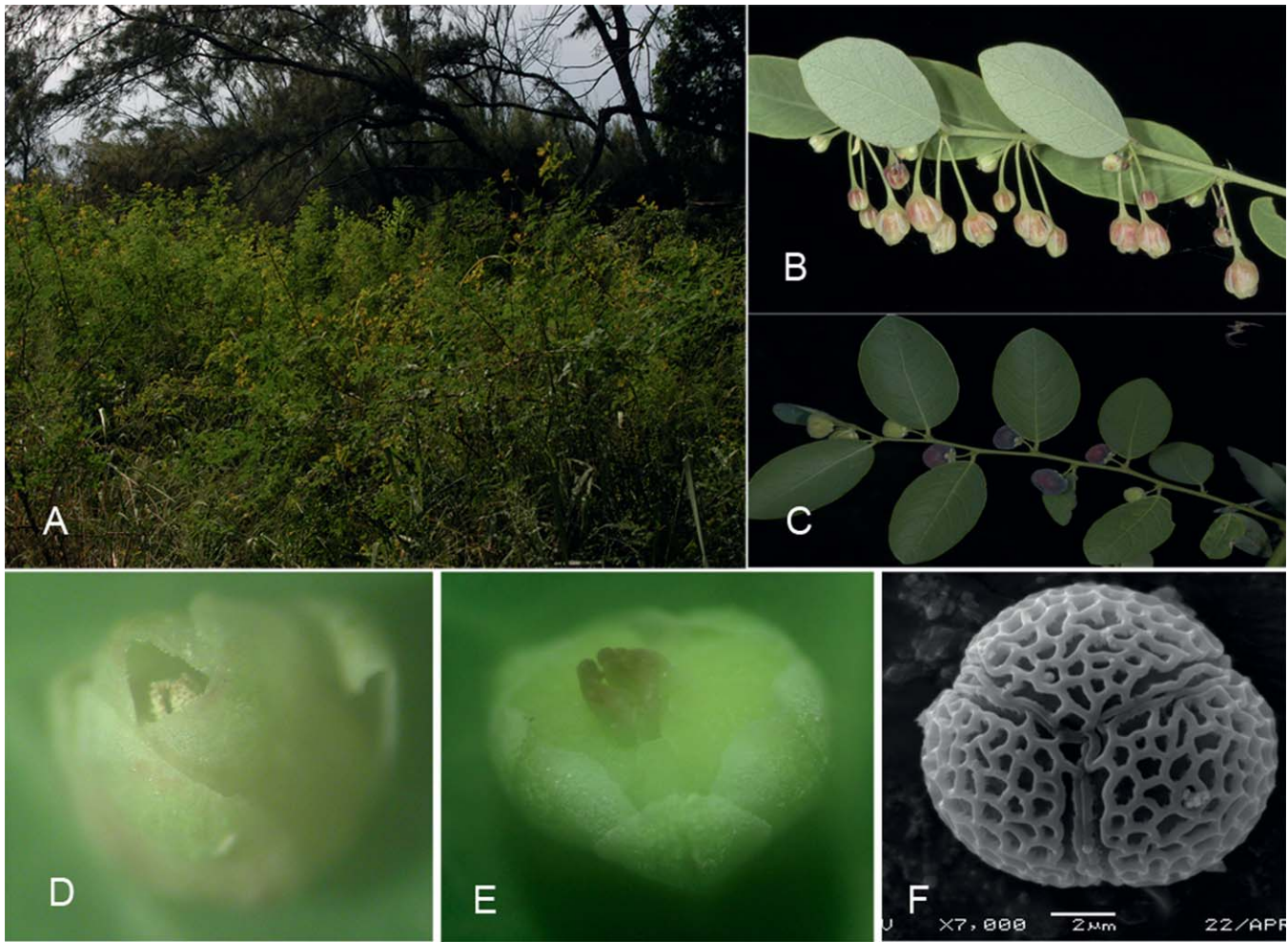


FIG. 3. *Phyllanthus reticulatus*. A. Habit in the natural habitat in Hainan, China. B. Inflorescence with pistillate and staminate flowers. C. Fruiting branchlet. D. Staminate flower. E. Pistillate flower with emerging styles. F. Scanning electron micrograph (SEM) of a pollen grain.

is needed to stabilize the current usage of the name *P. reticulatus*. We therefore here lectotypify the name *Phyllanthus reticulatus* Poirlet in Lam., Encycl. 5: 298. 1804, which is based on the mixed gathering P00381823 (P-LA) collected by an unknown collector at an unknown place presumed to be in Asia, with the central sprig of that sheet, labeled 2 in our Fig. 1A.

In the molecular phylogeny (Fig. 2), *P. reticulatus* accessions from India, the Philippines, Hainan, Taiwan, and Jamaica form a clade (84% ML bootstrap support) that is sister to a clade (74% ML bootstrap support) comprising accessions from Sri Lanka, Nepal, and China (mainland and Hainan). The latter accessions match the syntypes of *P. microcarpus* (Benth.) Müll. Arg. collected near Hong Kong. African accessions identified as *P. reticulatus*, form a group with a Madagascan species (*P. casticum*) and one from the Comoros (*P. pervoilleanus*). Morphologically, these accessions match a syntype of *P. polyspermus* collected in Ghana (Fig. 1B), and we have therefore used this name for these accessions in Fig. 2.

In China and Vietnam, *Phyllanthus microcarpus* and its sister species *P. reticulatus* differ in the following characters: *Phyllanthus reticulatus* is a coastal or lowland forest species, while *P. microcarpus* usually occurs between 500 and 1,200 m elevation. *Phyllanthus reticulatus* is a shrub up to 5 m tall (Fig. 3A), while *P. microcarpus* is a scrambling shrub (Fig. 4A), reaching up to 18 m high. *Phyllanthus reticulatus* has axile

flower fascicles often composed of one female and two to four male flowers (Fig. 3B), while *P. microcarpus* branchlets produce female flowers (Fig. 4F) until the fruits are mature, at which point male flowers start developing (Fig. 4G & H). Once the mature fruits have been shed, the branchlet becomes male (Fig. 4H). The male flowers of *P. reticulatus* (Fig. 3B) have pink sepals (Fig. 3D), while those of *P. microcarpus* have green sepals (Fig. 4B & C). *Phyllanthus reticulatus* styles emerge from the flowers (Fig. 3E), while those of *P. microcarpus* are much shorter and do not visibly emerge (Fig. 4D). Both species have coarsely reticulate pollen (Fig. 3F, 4E). Excellent images of the pollen of a Philippine *P. reticulatus* are included in Chen et al. (2009). *Phyllanthus microcarpus* occurs in China, Taiwan, Laos, Vietnam, Thailand, India, and Sri Lanka. In China, it occurs in the provinces Fujian, Hainan, Guangdong, Hong Kong, Guangxi, Jiangxi, Yunnan, and Sichuan. The exact ranges of the two species on the Indian subcontinent cannot be resolved with the present sampling, and local floras and checklists do not help because it is unclear whether they refer to a single species or a mix of *P. microcarpus* and *P. reticulatus*.

The Chinese language version of the *Flora of China* (Li 1994) accepted two varieties of *P. reticulatus*, var. *reticulatus* and var. *glaber* Müll. Arg., with the specimens here treated as *P. microcarpus* assigned to var. *glaber*. Varietas *glaber* was originally described from Sri Lanka, but the name has also been applied

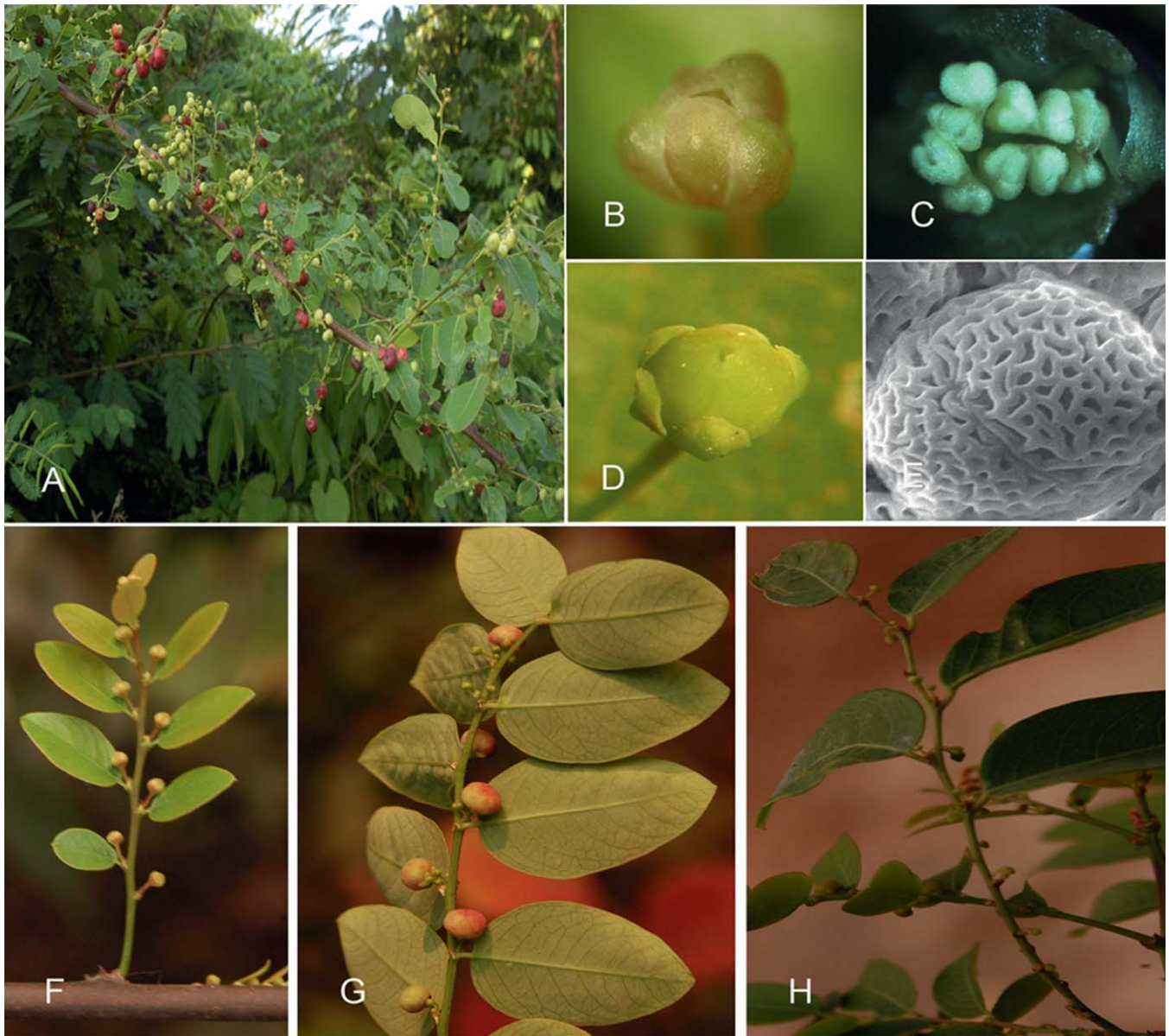


FIG. 4. *Phyllanthus microcarpus*. A. Habit in the natural habitat in Guangdong, China. B. Staminate flower. C. Stamens with dehiscing pollen sacs. D. Pistillate flower with minute styles. E. Pollen under SEM. F. Branchlet with pistillate flowers. G. Branchlet with mature fruits and staminate flower buds. H. Branchlet with new staminate flowers after the fruits drop.

to African (Müller 1866; Govaerts et al. 2000) and Jamaican (Müller 1866) plants. The English language version of the *Flora of China* (Li and Gilbert 2009) and the *Flora of Taiwan* (Hsieh et al. 1993: 497, plate 259) both treat *P. reticulatus* s. l., that is, including *P. microcarpus*. By contrast, Chantharanonthai's (2005, 2007) treatments of the genus for Thailand accepts two separate species, although the elevational ranges provided differ from our observations (500–1,150 m for *P. reticulatus*, 150–525 m for *P. microcarpus*). The *Flora of Taiwan* (l. c.) uses *P. multiflorus* Willd. as the name for *P. reticulatus*, without accounting for the names *P. reticulatus* and *P. microcarpus*. While Willdenow's (1805) name indeed refers to *P. reticulatus*, it is a junior homonym of *P. multiflorus* Poir. (1804) from Madagascar and therefore cannot be used.

African "*P. reticulatus*" plants from Tanzania, Namibia, and Kenya that we sequenced all clustered with other African species (Fig. 2), not Asian *P. reticulatus*. The oldest available

name for African plants formerly identified as *P. reticulatus* is *P. polyspermus* Schumach. (Schumacher, 1827), based on *Thonning 51* from Ghana (Fig. 1B). This collection was annotated as *P. reticulatus* var. *glaber* by Hutchinson, a name accepted by Li (1994) for Chinese *P. microcarpus*, which however differs from the African plants (Fig. 2). The name *P. polyspermus* has not been used for over a century; Müller (1866) considered it a synonym of *P. reticulatus*, and subsequent authors followed this assessment.

Jamaican *P. reticulatus* was described as *P. jamaicensis* (Grisebach 1859). About 100 yr later, Webster (1957) realized that the plants represented *P. reticulatus*, suggesting anthropogenic introduction and stressing that "The only striking peculiarity of the West Indian specimens of *P. reticulatus* appears to be their infertility." (l. c., p. 60). The molecular phylogeny (Fig. 2) supports Webster's assessment, and observations made in Jamaica show that *P. reticulatus* indeed fails to set fruit there

(A. Kawakita, University of Tokyo, personal communication, March 2010). This fits with the introduced species lacking its obligate pollinators, *Epiccephala* moths (Kawakita and Kato 2009). The spread of *P. reticulatus* in Jamaica most likely is due to vegetative growth, and the plants are also planted as an ornamental in private gardens (A. Kawakita personal communication), which probably helps the species' persistence. The oldest collections of *P. reticulatus* on Jamaica are by J. Macfadyen (1800–1850; Fawcett 1897; Morris 1906), and by N. Wilson (appointed island botanist in 1846). Both men were in steady contact with colleagues working at the Royal Botanic Gardens at Kew, and introduced many plants from India and Indochina, including mangosteen, Tonquin bean, mangoes, and *Pinus excelsa* Wall. ex Lamb. from the Himalayas (Fawcett 1897; Morris 1906). It is therefore possible that *P. reticulatus* was introduced from India to Jamaica around 1830–1850.

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#### APPENDIX 1. GenBank accession numbers and vouchers of plant material from which DNA was extracted for sequencing.

- Phyllanthus reticulatus* S. L. Luo. G. Yao and S. X. Luo 075 (M), GU477722; G. Yao and S. X. Luo 081 (M), GU477723; S. X. Luo 326 (IBSC), GU477721; A. Godbole 45197 (MO), GU477724; A. Kawakita 303 (KYO), HM106987; U. F. Ferrers et al. 698 (NHU), HM106988. *Phyllanthus microcarpus* M. Suzuki et al. 9455084 (MO), GU477718; S. S. Renner 2842 (M), GU477719; S. X. Luo 320 (IBSC), GU477720. *Phyllanthus polyspermus* S. R. Semsei 3029 (M), GU477715; H. Mpanga & Singbad 100 (MO), GU477716; B. de Winter & Leistner 5762 (M), GU477717. *Phyllanthus clarkei* S. X. Luo 425 (IBSC), HM106989. *Phyllanthus glaucus* S. X. Luo 446 (IBSC), HM106990. *Phyllanthus* sp. G. Yao 128 (IBSC), HM106991.