American Journal of Botany 96(3): 686–706. 2009.

PHYLOGENY AND CLASSIFICATION OF THE SPECIES-RICH PANTROPICAL SHOWY GENUS *IXORA* (RUBIACEAE-IXOREAE) WITH INDICATIONS OF GEOGRAPHICAL MONOPHYLETIC UNITS AND HYBRIDS[1]

ARNAUD MOULY,[2,3] SYLVAIN G. RAZAFIMANDIMBISON,[3] ANBAR KHODABANDEH,[3] AND BIRGITTA BREMER[3]

[2]UMS 2700 CNRS—USM 0602 MNHN: Taxonomie et Collections, Département de Systématique et Évolution, Muséum national d'Histoire naturelle, 16 rue Buffon 75005 Paris, France; [3] Bergius Foundation, the Royal Swedish Academy of Sciences and Botany Department, Stockholm University, SE-106 91 Stockholm, Sweden

Species-rich genera often have various conflicting circumscriptions from independent regional flora treatments. Testing the monophyly of these groups of plants is an important step toward the establishment of a phylogenetic classification. The genus *Ixora* of the tribe Ixoreae in the subfamily Ixoroideae (coffee family or Rubiaceae) is a species-rich pantropical genus of ca. 500 species. Phylogenetic analyses of Ixoreae based on combined sequence data from one nuclear (nrETS) and two chloroplast (*rps16* and *trnT-F*) markers reveal the paraphyly of *Ixora* as presently delimited and also show that the tribe can be subdivided into three major clades: the Mascarene/neotropical/Malagasy/African clade, the Pacific clade, and the Asian clade. Given the lack of morphological synapomorphies supporting the different *Ixora* clades and the morphological consistency of the ingroup taxa, we propose a broad circumscription of *Ixora* including all its satellite genera: *Captaincookia*, *Doricera*, *Hitoa*, *Myonima*, *Sideroxyloides*, *Thouarsiora*, and *Versteegia*. The current infrageneric classification of *Ixora* is not supported. The different *Ixora* subclades represent geographical units. Nuclear and chloroplast tree topologies were partially incongruent, indicating at least four potential natural hybridization events. Other conflicting positions for the cultivated species are most likely due to anthropogenic hybridization.

Key words: hybridization; *Ixora*; molecular phylogenetics; nrETS; ornamentals; *rps16*; Rubiaceae; taxonomy; *trnT-F*.

The mainly pantropical Rubiaceae (coffee family) is the fourth largest flowering plant family, which comprises many species-rich genera with more than 200 species (e.g., *Psychotria* L. with ca. 1800 species [Govaerts et al., 2006]; *Ixora* L. with at least 500 species [De Block, 1998; Mouly and Hoang, 2007]; *Oldenlandia* L. and *Spermacoce* Gaertn. each with at least 250 species [Govaerts, et al., 2006]; *Pavetta* L. and *Tarenna* Gaertn., each with ca. 200 species [Mabberley, 1997]). Testing the monophyly of these large genera is an important step toward the establishment of a phylogenetic classification. On the other hand, this task has proven to be challenging for at least two reasons: (1) difficulty in obtaining a sufficiently representative sampling of their species for molecular investigations, and (2) lack of modern floras or taxonomic treatments.

[1] Manuscript received 10 July 2008; revision accepted 5 December 2008.

The authors thank Dr. P. De Block, F. Jacq, Dr. J.-F. Butaud, Dr. J.-Y. Meyer, and Dr. J. Munzinger for providing *Ixora* material for biomolecular studies; two anonymous reviewers for comments on the manuscript; the authorities of the North and South provinces of New Caledonia and the French Polynesian Government for access to the field and authorization to collect specimens and the tribal authorities who allowed us to work on their territories; the institutions of BR, G, K, L, MO, NOU, P, S, TAN, TEF, and UPS for granting access to herbarium collections and providing material for bio-molecular studies. Financial support was provided by the UMS CNRS 2700 – USM MNHN 0602 "Taxonomie et Collections", the Department "Systématique et Evolution", MNHN, Paris; the Missouri Botanical Garden through Dr. G. McPherson for support of fieldwork in New Caledonia; the European Commission's Research Infrastructure Action via the SYNTHESYS Project (EU) to A.M. to access BR and L; the Swedish Research Council and the Knut and Alice Wallenberg Foundation to B.B.

[4] Author for correspondence (e-mail: arnaud@bergianska.se)

doi:10.3732/ajb.0800235

The pantropical *Ixora* (Appendix S1, A–J, see Supplemental Data with online version of this article) is probably the second largest Rubiaceae genus after *Psychotria*, with the highest species diversity (at least 200 species) in tropical Asia (De Block, 1998; Puff et al., 2005). Approximately 60 species are restricted to the neotropics and mid-Atlantic islands (Andersson, 1992), 22 species on the Fiji Islands (Smith and Darwin, 1988), ca. 34 species in continental Africa (De Block, 1998), and probably as many in Madagascar (De Block et al., 2007). The genera *Pavetta* and *Ixora* were traditionally treated as a single genus since Linnaeus' (1753) descriptions and until the beginning of the 20th century (Schumann, 1891; Bremekamp, 1934, 1937b). However, *Ixora* can easily be distinguished from *Pavetta* by its salverform bifid stigmas and can additionally be characterized by a combination of the following characters: articulate petioles, hermaphroditic flowers, hypocrateriform corollas, two-carpellate ovaries containing a solitary ovule in each carpel locule, and drupaceous fruits (De Block, 1998; Lorence et al., 2007; Fig. 1, E3, E4, E7, E9). Andreasen and Bremer's (2000) study based on combined data from *rbcL* sequences and morphological data demonstrated that the two genera are not closely related. Accordingly, the tribe Pavetteae A.Rich. was restricted to *Pavetta* and its allied genera (e.g., *Tarenna* Gaertn., *Leptactina* Hook.f.); plus, the reinstatement of the tribe Ixoreae A.Gray (Gray, 1858) was necessary to accommodate *Ixora* and its satellite genera (see Table 1).

Several monotypic and small genera with apparent affinities to *Ixora* were described based on one or two autapomorphic characters (see Table 1). The neotropical genus *Sideroxyloides* Jacq. (Jacquin, 1763; Bentham, 1850) was established on the basis of its caulinary inflorescences. The Pacific *Hitoa* Nadeaud (Nadeaud, 1899; Fig. 1, E10) and the Malagasy *Thouarsiora* Homolle ex Arènes (Arènes, 1960), both monotypic genera,

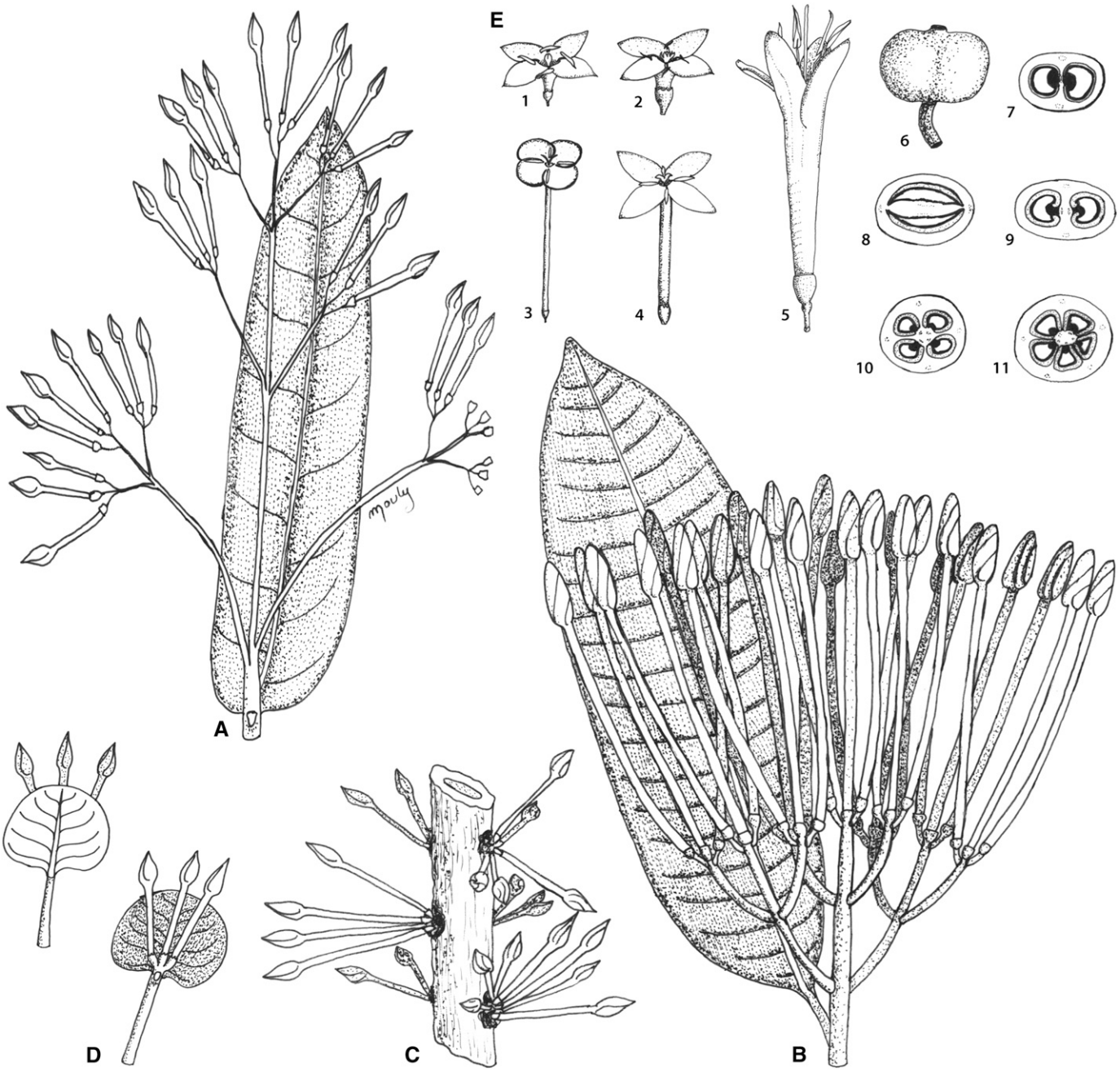


Fig. 1. Illustrations of morphological features in the tribe Ixoreae commonly used for *Ixora* satellite genera distinction and infrageneric classification within *Ixora*. (A) *I. yaouhensis*, inflorescence with subopposite and nonarticulate axes (typical of subgenus *Pavettoides*); (B) *I. crassipes*, inflorescence with opposite and articulate axes (typical of subgenus *Ixora*); (C) *I. cauliflora*, caulinary inflorescence; (D) *I. collina*, inflorescence triflorous, protected by foliaceous bracts (typical of section *Phylleilema*); (E) flowers (1–5) and fruit (6–11) details: 1, male, *Myonima obovata*; 2, female, *Myonima obovata*; 3, hermaphrodite, *I. chinensis*; 4, hermaphrodite, *I. coccinea*; 5, hermaphrodite, *Captaincookia* sp. 1 (typical of *Captaincookia*); 6, *Captaincookia margaretae*; 7, section, *I. coccinea;* 8, section, *Versteegia cauliflora*; 9, section, *I. francii*; 10, section, *Hitoa mooreensis*; 11, section, *Myonima obovata*.

were independently described based on their four-carpellate ovaries. The Mascarene genus *Myonima*, presently consisting of four species of shrubs, was established based on its short corolla tubes (shorter than lobes), 2–7-carpellate ovaries (Fig. 1 E10), and functional dioecious flowers (Verdcourt, 1989; Fig. 1, E1, E2). The genus was once placed among many others under synonymy of *Ixora* (Baillon, 1879), but no Rubiaceae systematists seem to have accepted this taxonomic decision. *Versteegia* Valeton was described to accommodate three New Guinean species with small caulinary flowers and fruits with disciform pyrenes (Valeton, 1911, 1927; Fig. 1, E8). The New Caledonian monotypic genus *Captaincookia* N.Hallé (Hallé, 1973) accommodates a species with large drooping, caulinary red flowers, infundibuliform corollas, and stigmatic arms permanently erected (Fig. 1, E5; online Appendix S1, K). A more recently described Mascarene genus, *Doricera* Verdc.

TABLE 1. Reproductive characters used as diagnostic elements for generic classification in the tribe Ixoreae.

Features	*Ixora*	*Captaincookia*	*Doricera*	*Hitoa*	*Myonima*	*Sideroxyloides*	*Thouarsiora*	*Versteegia*
Breeding system	monoecious	monoecious	dioecious	monoecious	dioecious	monoecious	monoecious	monoecious
Inflorescence position	terminal	caulinary	terminal	terminal	terminal	caulinary	terminal	caulinary
Flower orientation	erect	drooping	erect	erect	erect	erect	erect	erect
Corolla tube/lobe ratio	>1	>1	<1	>1	<1	>1	>1	>1
Ovary	2(−4)-carpellate	2-carpellate	3-carpellate	4-carpellate	2–7-carpellate	2-carpellate	4-carpellate	2-carpellate
Pyrenes shape	globose	globose	globose	globose	globose	globose	globose	flattened

(Verdcourt, 1983), consists of a sole species, which was originally described as *Pyrostria* Comm. ex Juss. in the tribe Vanguerieae due to its axillary inflorescences. Bridson and Robbrecht (1985) proposed a close relationship between *Doricera* and *Ixora*, and the placement of *Doricera* in Ixoreae was supported by Mouly et al. (2005, in press) and also recently by Davis et al. (2007).

Ixora has always been recognized as a well-circumscribed genus (Fosberg, 1937; De Block, 1998) based on flower morphology (e.g., hypocrateriform corolla, contorted bud, and salverform stigma). However, Andreasen and Bremer (2000), also endorsed by Mouly et al. (in press), indicated that the present circumscription of *Ixora* was not monophyletic. In the latter study, all sequenced representatives of *Captaincookia*, *Doricera*, *Myonima*, and *Versteegia* were nested within the seven sampled *Ixora* species (including the type species *I. coccinea* L.).

Bremekamp (1937a, b, 1938, 1940; Table 2) subdivided *Ixora* into three subgenera including only Malesian species: *Ixora*, *Pavettoides* Bremek., and *Sathrochlamys* Bremek, though these names are applicable to all species. Subgenus *Ixora* is, in fact, pantropical and is mostly characterized by a combination of the following features: inflorescence axes opposite and articulate (Fig. 1, B), flowers in distinct triads, pedicels of lateral flowers articulate, and bracts and bracteoles well developed (Bremekamp, 1937b). Subgenus *Pavettoides*, occurring from the Seychelles to India, tropical Asia, Micronesia, and northern Australia (Bremekamp, 1937b; Smith and Darwin, 1988; De Block, 1998), is recognized by its upper inflorescence axes usually subopposite (Fig. 1, A), nonarticulate flowers in less distinct triads, nonarticulate pedicels, and bracts and bracteoles weakly developed (Bremekamp, 1937b). Subgenus *Sathrochlamys* is restricted to the eastern part of the Malaysian archipelago, northern Borneo, and New Guinea (De Block, 1998) and presents the following characteristics: inflorescences never opposite nor articulate, flowers not in distinct triads, pedicels with nonarticulate, and bracts and bracteoles minute or absent (Bremekamp, 1937b). These subgeneric circumscriptions were not confirmed from Husain and Paul's (1989) palynological studies based on the Indo-Asian *Ixora* species. More recently, De Block (1998) observed that some of the character states used by Bremekamp (1937b) were dubious and not always consistent.

Within the subgenera, Bremekamp (1937a, b, 1938, 1940) recognized up to 20 sections, also based solely on the Malesian representatives of *Ixora*: sect. *Ixora* (ca. 90 species), sect. *Brachypus* Bremek. (20 species), sect. *Chlamydanthus* Bremek. (9 species), sect. *Erythrocalyx* Bremek. (1 species), sect. *Octobathrum* Bremek. (44 species), and sect. *Stenopus* Bremek. (1 species) in subgen. *Ixora*; sect. *Raphidanthus* Bremek. (12 species), sect. *Pavettopsis* Bremek. (38 species), sect. *Pogonanthus* Bremek. (10 species), and sect. *Amphorion* Bremek. (2 species) in subgen. *Pavettoides*; and sect. *Pseudobandhuca* Bremek. (1 species), sect. *Gymnocorymbus* Bremek. (1 species), sect. *Hypsophyllum* Bremek. (6 species), and sect. *Macropus* Bremek. (5 species) in subgen. *Sathrochlamys*. The characteristic features of these sections are generally based on inflorescence and flower organizations (De Block, 1998; Table 2). Bremekamp (1937b) postulated that the African *Ixora* belonged to sect. *Otobactrum*; the Indian and Continental Asian *Ixora* to sections *Ixora*, *Brachypus*, *Chlamydanthus*, *Otobactrum*, *Amphorion*, and *Pavettopsis*; and the Pacific representatives to sect. *Pavettopsis*. More sections were described in *Ixora*: sect. *Phylleilema* A.Gray (Gray, 1858) for Pacific taxa with 3(−10)-flowered inflorescences subtended by narrow foliaceous bracts (Fig. 1, D; online Appendix S1, B, J), sect. *Cremixora* Baill. for a single species in Madagascar (Baillon, 1880; online Appendix S1, E) with pendulous ovules, sect. *Micrixora* Hochr. (Hochreutiner, 1908) for species having small corollas, sect. *Microthamnus* (Homolle ex Arènes) Guédès (Guédès, 1986) for one-flowered Malagasy species, and sect. *Vitixora* Fosberg (Fosberg, 1942; online Appendix S1, D) for the Fijian species with congested subsessile inflorescences. The *Myonima* group, when placed under *Ixora* (Baillon, 1879; Table 2), formed its own section. The infrageneric classifications of *Ixora*, notably the ones proposed by Bremekamp (1937b), have seriously been questioned by many authors (Corner, 1941; Fosberg, 1942; Husain and Paul, 1989; De Block, 1998) but have never been tested using molecular-based phylogenies.

Sequence data from the *rps16* and *trnT-F* chloroplast regions have been used separately and/or in combination with data from the *rbcL* chloroplast coding gene in Mouly et al. (in press), for the circumscription of Ixoreae. Here, we report phylogenetic analyses using combined data from the nuclear ribosomal external transcribed spacer (nrETS), *rps16*, and *trnT-F* to reconstruct a robust phylogeny for many more species of Ixoreae. ETS, used in combination with nrITS, has recently been shown to be useful for assessing phylogenetic relationships in closely related genera of the tribe Naucleeae s.l. in the subfamily Cinchonoideae, Rubiaceae (Razafimandimbison et al., 2005). The resulting phylogeny from our combined data will be used to (1) rigorously test the monophyly of the present circumscription of *Ixora*, (2) present new generic limits within Ixoreae, if necessary, (3) and test the monophyly of the current infrageneric classifications of *Ixora*.

MATERIALS AND METHODS

Taxon sampling—We sequenced 106 specimens (Appendix 1) representing 93 Ixoreae taxa including: 79 *Ixora* species (including *Hitoa*, *Sideroxyloides*, and *Thouarsiora*), the type species of the monotypic *Captaincookia* and an undescribed species closely related to it (represented by two specimens from two different populations), the monotypic *Doricera*, four *Myonima* representatives, and two *Versteegia* species. *Cyclophyllum ixoroides* Guillaumin (Vanguerieae) was also included because it was suspected by Guillaumin (1930) to be closely related to *Ixora*.

Several specimens of *Ixora* included in the analyses could not be identified to species mainly due to the lack of modern treatments of *Ixora* for many flora regions. *Ixora* sp. 5, *I. finlaysiana* Wall. ex G.Don, *I. casei* Hance, *I. chinensis*

TABLE 2. Synthesis by De Block (1998) of the infrageneric classification of the genus *Ixora* and characteristics and distributions based on literature and completed in this study.

Subgenera	Sections	Morphological characteristics of sections	Distribution of sections
Ixora	*Ixora* syn.: *Megalixora* Hochr. (1908) syn.: *Ixorastrum* Bremek. (1937) Type: *I. coccinea* L.	Inflorescences sessile or moderately pedunculate, erect; calyx tubes short but distinct; corollas white, red, orange or yellon; tubes much longer than lobes; stamens much shorter than corolla lobes; anthers with short cells; styles glabrous or pubescent, shortly exserted	Sri Lanka, India, Burma, Laos, Cambodia, Vietnam, Malay Peninsula, Indonesia, New Guinea, and Caroline Islands
Ixora	*Brachypus* Bremek. (1937) Type: *I. cuneifolia* Roxb.	Inflorescences subsessile to moderately or rarely long pedunculate, erect or drooping; bracts and bracteoles narrow; calyx tubes sometimes distinct; corollas white or reddish; stamens as long as corolla lobes; anthers with long cells; styles glabrous, exserted portion ± as long as corolla lobes	India, Burma, Laos, Cambodia, Vietnam, and Malay Peninsula
Ixora	*Chlamydanthus* Bremek. (1937) Type: *I. umbellata* Valeton ex Koord. & Valeton	Inflorescences subsessile or shortly pedunculate, erect; central flowers in ultimate triads sessile and ebracteolate; bracts and bracteoles well developed; bracteoles usually longer than ovaries; corollas white or ochre-yellow; tubes 3–6 times longer than the lobes; stamens usually shorter than corolla lobes; anthers with long cells; styles glabrous, exserted portion somewhat shorter than corolla lobes	India, Burma, Laos, Cambodia, Vietnam, and Malay Peninsula
Ixora	*Cremixora* Baill. (1880) Type: *I. cremixora* Drake	Ovaries with descendant ovules	Madagascar and Comoro Islands
Ixora	*Erythrocalyx* Bremek. (1937) Type: *I. curtisii* Ridl.	Inflorescences subsessile, subcapitate, pauciflorous; bracts and bracteoles linear, large, red; terminal flowers solitary and ebracteolate; calyx tubes reduced, lobes linear, red, (almost) as long as the corolla tube: corollas white, lobes and tube short; stamens much shorter than corolla lobes, anthers with moderately long cells; styles densely pubescent, shortly exserted	Indonesia
Ixora	*Micrixora* Hochr. (1908) Types: *I. drakei* Hochr., *I. humblotii* Drake, *I. microphylla* Drake, *I. pudica* Baker	Flowers small; corolla tube <1.5 cm	Madagascar and Seychelles
Ixora	*Myonima* Baill. (1879) Type: Non designatus	Corollas small; tubes shorter than lobes; ovaries two- to plurilocular	Mascarene Islands
Ixora	*Otobactrum* Bremek. (1937) Type: *I. palludosa* (Bl.) Kurz	Inflorescences long pedunculate, multiflorous; bracts and bracteoles narrowly triangular to filiform; corollas white, rarely reddish or yellowish; stamens somewhat shorter than corolla lobes or of equal length, anthers with long cells; styles glabrous, exserted portion ± as long as corolla lobes	Tropical Asia and New Guinea, and Occidental Africa
Ixora	*Stenopus* Bremek. (1937) Type: *I. filipes* Valeton	Inflorescences distinctly pedunculate, consisting of 1–7 flowers, at the end of axillary short shoots arising on the green part of the branches; lower bracts narrowly triangular, the others wide; bracteoles ovate; corollas white; stamens shorter than corolla lobes, anthers with long cells; styles glabrous	Philippines and Celebes
Pavettoides Bremek.	*Amphorion* Bremek. (1937) Type: *I. brunnescens* Kurz	Inflorescences shortly or moderately pedunculate, subpaniculate; flowers small; corolla tubes with bearded throat	Tropical Asia and Indonesia
Pavettoides Bremek.	*Pavettopsis* Bremek. (1937) Type: *I. blumei* Zoll. & Mor.	Inflorescences subsessiles or shortly pedunculate, trichotomously or pentachotomously corymbose; corolla tubes glabrous or pubescent inside but never bearded; stamens equalling corolla lobes in length; styles glabrous or pilose in their middle part	Seychelles, Sri Lanka, Andaman, Nicobar Islands, India, Malay Peninsula, New Guinea, Melanesia, and Micronesia
Pavettoides Bremek.	*Pogonanthus* Bremek. (1937) Type: *I. timorensis* Decne.	Inflorescences subsessile or shortly pedunculate; corolla tubes bearded at throat or pubescent in its upper half inside; styles near the middle usually densely pilose or rarely glabrous	Indonesia, Celebes, Muloccas, New Guinea, and Australia
Pavettoides Bremek.	*Raphidanthus* Bremek. (1937) Type: *I. capillaris* Bremek.	Inflorescences long or very long pedunculate; corolla tubes slender or very slender, never bearded, much longer than corolla lobes; stamens frequently much shorter than corolla lobes; styles glabrous	Indonesia
Pavettoides Bremek.	*Vitixora* Fosberg (1942) Types: *I. amplexicaulis* Gillepsie, *I. coronata* A.C.Sm., *I. pelagica* Seem., *I. somosomaensis* Gillepsie	Leaves shortly petiolate to subsessile; inflorescences multiflorous, strongly congested head-like cymes, sessile between a terminal pair of leaves (not modified); bracts linear and filiform crowded among the flowers; calyx lobes linear or narrowly triangular	Fiji and New Caledonia
Sathrochlamys Bremek.	*Gymnocorymbus* Bremek. (1937) Type: *I. paradoxalis* Bremek.	Inflorescences pedunculate; bracts and bracteoles absent; styles glabrous	Philippines and Moluccas
Sathrochlamys Bremek.	*Hypsophyllum* Bremek. (1937) Type: *I. dolichothyrsa* Bremek.	Leaves (sub)sessile, base rounded to cordate or subauriculate; inflorescences long pedunculate; bracts filiform and setaceous; calyx lobes and tube equal in length; corollas white or ochre-yellow; styles pubescent in their middle part	New Guinea
Sathrochlamys Bremek.	*Macropus* Bremek. (1937) Type: *I. whitii* S.Moore	Leaves petiolate, base acute; inflorescences long pedunculate; basal bracts setaceous, others minute; bracteoles absent; calyces cupuliform; styles pubescent in their middle part	New Guinea, Philippines, and Moluccas

TABLE 2. Continued.

Subgenera	Sections	Morphological characteristics of sections	Distribution of sections
Sathrochlamys Bremek.	*Pseudobandhuca* Bremek. (1937) Type: *I. philippinensis* Merr.	Inflorescences shortly or moderately pedunculate, supported by a pair of sessile leaves not reduced in size; bracts and bracteoles small but distinct; corollas white or pink; styles glabrous	Philippines, Moluccas, Celebes, and Borneo
	Microthamnus (Homolle ex Arènes) Guédès (1986) Type: *I. reducta* Drake ex Guédès	Inflorescences terminal on main and lateral branches, uniflorous, sessile but with modified inflorescence-supporting leaves present; calyces truncate or with small triangular lobes	Madagascar and New Caledonia
	Phylleilema A.Gray (1858) Types: *I. amplifolia* A.Gray, *I. fragrans* (Hook. & Arn.) A.Gray, *I. samoensis* A.Gray, *I. vitiensis* A.Gray	Inflorescences pauciflorous (often consisting of three flowers), sessile but modified inflorescence-supporting leaves present, the latter obtusely triangular to ± orbicular, with cordate bases.	From Marquesas as far westward as New Caledonia

Lam., and *I. pavetta* Andr. were all represented in the study by individuals cultivated in botanical gardens (Appendix 1); therefore, they are possibly of hybrid origins. Following Mouly et al. (in press), three suitable outgroup taxa from the tribes Aleisanthieae and Greeneeae (Mouly et al., in press), and Vanguerieae were chosen to root the trees (Appendix 1).

DNA extraction, amplification, sequencing, and alignment (Appendix 1)—Total DNA was extracted from dried material preserved in silica gel (Chase and Hillis, 1991) or herbarium specimens following the mini-prep procedure of Saghai-Maroof et al. (1984), as modified by Doyle and Doyle (1987). Extracted DNA was cleaned with a Qia-Quick PCR purification kit (Qiagen, Solna, Sweden). PCR reactions were as follows: 27.25 μL H_2O, 5 μL of PCR buffer, 5 μL of $MgCl_2$, 5 μL of 0.1 M tetramethylammonium chloride (TMACl), 4 μL of dNTP, 0.25 μL *Taq* polymerase, 0.5 μL of each primer, and 0.5 μL of 1% of bovine serum albumin. PCR amplifications, performed in a Eppendorf Mastercycler gradient, started with an initial melting phase of 2 min at 95°C; followed by 35–37 cycles of 30 s at 95°C, 1 min at 50–55°C, and 2 min at 72°C; and ended with a final extension phase of 7 min at 72°C. In all PCR runs, one reaction was run with water instead of DNA as a negative control to check for contamination. The *rps16* intron was amplified with the primer pair rpsF/rpsR2 (Oxelman et al., 1997). For half of the species, we repeatedly failed to obtain amplification in one reaction because of a problematic poly A/T at the 3′ end of the intron (Shaw et al., 2005). However, amplification was successful with the internal primer pair rpsF2/rpsR3 (Bremer et al., 2002), but the sequences were 50–70 bp shorter. The entire *trnT-F* region (including the *trnL* intron) was amplified in two parts. The *trnT-trnL* segment was amplified with primer pair A1/I (Razafimandimbison and Bremer, 2002; Bremer et al., 2002). The second segment, *trnL-trnF*, was amplified with primers C/F (Taberlet et al., 1991). For *trnL-F*, sequencing reactions were performed using the two external primers C/F and two internal primers D/E (Taberlet et al., 1991) to produce a complete sequence of the entire *trnT-F* region, with at least partial overlaps. The segment of the noncoding region ETS was amplified and sequenced with primer pair 18S-E/H 5′-CTTGTAGGGTTGGTTGGA-3′ (Baldwin and Markos, 1998; H was designed by H. Lantz, Uppsala University, Sweden, unpublished).

For 13 specimens, direct sequencing of purified PCR products consistently produced multiple sequence signals for ETS, indicating the presence of infra-individual polymorphism. Therefore, the PCR products of these species were cloned using a TOPO TA cloning kit (Invitrogen, Paisley, UK). This kit used unpurified PCR-amplified DNA, the TOPO vector, and a vial of One Shot chemically competent *Escherichia coli* according to the manufacturer's instructions. Two to four white colonies from the cloning reaction of the concerned species were screened and amplified with the M13F and M13R competent primers that were included in the TOPO TA cloning kit. Their respective purified PCR products were sequenced with the 18S-E/H.

All sequencing reactions of the markers were performed with a Big Dye Terminator v3.1 Cycle Sequencing kit and Bid Dye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Life Technologies, Carlsbad, California, USA) and subsequently analyzed using a 3100 Genetic Analyzer (Applied Biosystems).

The *rps16*, *trnT-F*, and ETS sequences were assembled using the Staden Package version 1.6.0 beta (Staden, 1996) and the program Sequencher 3.1.1 (Gene Codes Corp., Ann Arbor, Michigan, USA) and edited manually. All sequences were aligned manually following similarity criterion (e.g., Simmons, 2004) with the program Se-Al version 1.0al (Rambaut, 1996) (matrices are in Appendices S1–S6, see Supplemental Data with the online version of this article). Unambiguously aligned insertions and deletions (indels) were then coded as binary characters using 0 and 1 symbols for terminals with sequence and those that have a gap, respectively.

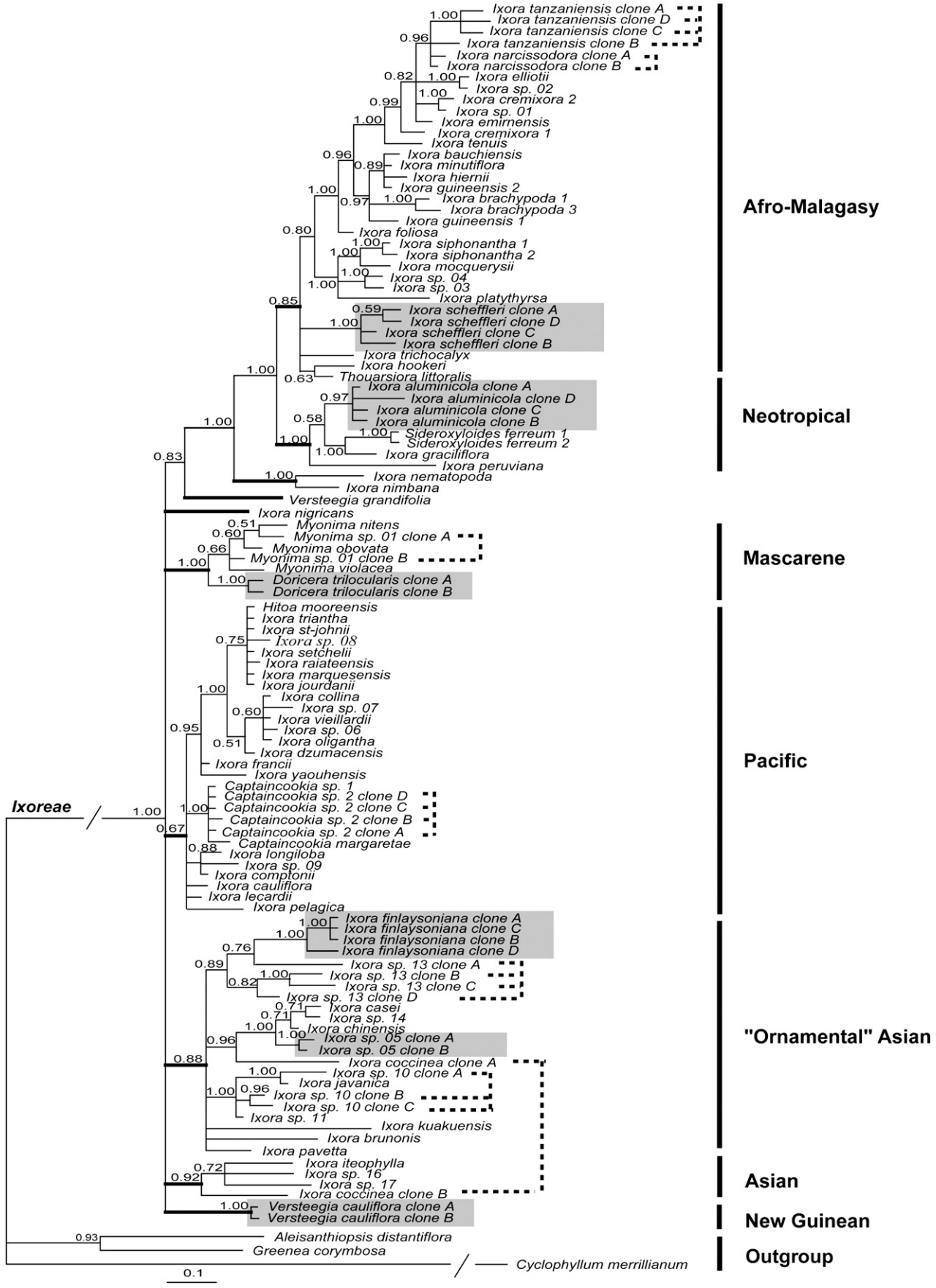
Phylogenetic analyses—Separate and combined analyses of the *rps16*, *trnT-F*, and ETS matrices (online Appendices S2–5) were performed using Bayesian Markov chain Monte Carlo (MCMC) inference (Yang and Rannala, 1997) as implemented in the program MrBayes version 3.0B (Huelsenbeck and Ronquist, 2001) for partitioned data sets, and maximum parsimony (MP) methodology, as implemented in the program PAUP* version 4.0b8 (Swofford, 2002). The Bayesian approach evaluates the posterior probability (PP) of a tree given the character matrix, i.e., the probability that the tree is correct. Bootstrap support (BS) was calculated for MP analyses.

Analysis settings—For each partition, the program MrModeltest 2.0 (Nylander, 2004) was used to choose the model of nucleotide substitution that best fits the data, following Akaike's information criterion (Akaike, 1974). The selected models were general time reversible (GTR) with among-site substitution rate heterogeneity described by a gamma distribution (Yang, 1994) for *rps16* and the *trnT-L* segment as well as the ribosomal ETS (GTR + G), GTR with a fraction invariant site constraint for the *trnL-F* part (GTR + I). Partitioned Bayesian analyses were conducted to account for the combination of molecular data, according to the nucleotide evolution models selected before, and a no-common-mechanism model (Tuffley and Steel, 1997) for standard binary characters of gap coding. All analyses were performed with four independent Markov chains run for 3×10^6 Metropolis-coupled MCMC generations, with tree sampling every 10^3 generations, and burn-in after 500 sampled trees (as detected by plotting the log likelihood scores against generation number). The analyses were run three times using different random starting trees to evaluate the convergence of the likelihood values and posterior clades probabilities (Huelsenbeck et al., 2002). Saved trees from the three independent runs were pooled to build the consensus tree. Groups characterized by a posterior probability over 95% were regarded as strongly supported.

Phylogenetic analyses using paralogous sequences may be misinterpreted if the orthology of the nuclear alleles is erroneously assumed. Orthologous markers derive from the same locus, whereas paralogous markers derive from different loci that originated by a DNA region duplication event (Fitch, 1970). Considering that cloned ETS *Ixora* sequences did not always form exclusive lineages in the preliminary analyses, we selected representatives for combined nr- and cpDNA analyses as follows: one randomly selected ETS clonal sequence for those forming exclusive lineages and one randomly selected per lineage for those not forming exclusives lineages. For each selected ETS clonal sequence, the correspondent cpDNA sequences were duplicated according to the number of ETS

→

Fig. 2. Ixoreae phylogram of the Bayesian analysis majority rule consensus tree illustrating the position of the clone sequences for each cloned taxon of the ETS sequence analysis. Names of cloned individuals end in "clone" and a letter; clones from the same individual grouping in the same clade are shaded in gray; dashed lines connect clones from the same individuals when they fall into separate clades.


Ixora tanzaniensis clone A
Ixora tanzaniensis clone D
Ixora tanzaniensis clone C
Ixora tanzaniensis clone B
Ixora narcissodora clone A
Ixora narcissodora clone B
Ixora elliotii
Ixora sp. 02
Ixora cremixora 2
Ixora sp. 01
Ixora emirnensis
Ixora cremixora 1
Ixora tenuis
Ixora bauchiensis
Ixora minutiflora
Ixora hiernii
Ixora guineensis 2
Ixora brachypoda 1
Ixora brachypoda 3
Ixora guineensis 1
Ixora foliosa
Ixora siphonantha 1
Ixora siphonantha 2
Ixora mocquerysii
Ixora sp. 04
Ixora sp. 03
Ixora platythyrsa
Ixora scheffleri clone A
Ixora scheffleri clone D
Ixora scheffleri clone C
Ixora scheffleri clone B
Ixora trichocalyx
Ixora hookeri
Thouarsiora littoralis
Ixora aluminicola clone A
Ixora aluminicola clone D
Ixora aluminicola clone C
Ixora aluminicola clone B
Sideroxyloides ferreum 1
Sideroxyloides ferreum 2
Ixora graciliflora
Ixora peruviana
Ixora nematopoda
Ixora nimbana
Versteegia grandifolia
Ixora nigricans
Myonima nitens
Myonima sp. 01 clone A
Myonima obovata
Myonima sp. 01 clone B
Myonima violacea
Doricera trilocularis clone A
Doricera trilocularis clone B
Hitoa mooreensis
Ixora triantha
Ixora st-johnii
Ixora sp. 08
Ixora setchelii
Ixora raiateensis
Ixora marquesensis
Ixora jourdanii
Ixora collina
Ixora sp. 07
Ixora vieillardii
Ixora sp. 06
Ixora oligantha
Ixora dzumacensis
Ixora francii
Ixora yaouhensis
Captaincookia sp. 1
Captaincookia sp. 2 clone D
Captaincookia sp. 2 clone C
Captaincookia sp. 2 clone B
Captaincookia sp. 2 clone A
Captaincookia margaretae
Ixora longiloba
Ixora sp. 09
Ixora comptonii
Ixora cauliflora
Ixora lecardii
Ixora pelagica
Ixora finlaysoniana clone A
Ixora finlaysoniana clone C
Ixora finlaysoniana clone B
Ixora finlaysoniana clone D
Ixora sp. 13 clone A
Ixora sp. 13 clone B
Ixora sp. 13 clone C
Ixora sp. 13 clone D
Ixora casei
Ixora sp. 14
Ixora chinensis
Ixora sp. 05 clone A
Ixora sp. 05 clone B
Ixora coccinea clone A
Ixora sp. 10 clone A
Ixora javanica
Ixora sp. 10 clone B
Ixora sp. 10 clone C
Ixora sp. 11
Ixora kuakuensis
Ixora brunonis
Ixora pavetta
Ixora iteophylla
Ixora sp. 16
Ixora sp. 17
Ixora coccinea clone B
Versteegia cauliflora clone A
Versteegia cauliflora clone B
Aleisanthiopsis distantiflora
Greenea corymbosa
Cyclophyllum merrillianum
Ixoreae
0.1
Afro-Malagasy
Neotropical
Mascarene
Pacific
"Ornamental" Asian
Asian
New Guinean
Outgroup

copies used in the combined nr- and cpDNA matrices. The duplication of cpDNA information can affect the support of inferred clades, but this effect was not significant in the present case (e.g., the subclade containing *Ixora coccinea* obtained PP = 0.66 with duplication of cpDNA information, cf. Fig. 4, and PP = 0.64 without duplication; results of the latter analysis not shown)

Separate nuclear and chloroplast data—We initially performed separate analyses of the ETS (online Appendix S2), *rps16*, and *trnT-F* data (online Appendix S3). The ETS matrix included the cloned ETS sequences of several species. Parsimony bootstrapping (Felsenstein, 1985; NNI, Multrees off, 10^4 replicates) and jackkniffing (Farris et al., 1996; NNI, Multrees off, 10^4replicates) of the *rps16* and *trnT-F* analyses under PAUP* (Swofford, 2002), yielded largely unresolved trees; as result, we conducted a combined *rps16*/*trnT-F* chloroplast (cp) analysis, considering the cp-regions are not subject to intergenomic recombination. Visual inspection of the trees shows that some *Ixora* species had strongly supported conflicting positions between nuclear and chloroplast data.

Combined nuclear and chloroplast data—Interpretation of gene trees may result in false species-tree hypotheses (Alvarez and Wendel, 2003; Bailey et al., 2003; Ochieng et al., 2007), notably when different gene trees show conflicting relationships (Mort et al., 2007). Previous knowledge regarding incongruent data sets in Rubiaceae (Bremer et al., 1999; Andreasen and Bremer, 2000) has led to combining data to obtain a more supported hypothesis and maximize congruence among all characters sampled (Nixon and Carpenter, 1996; Razafimandimbison and Bremer, 2002). Combination of nr- and cpDNA has been shown to strengthen the signal masked by homoplasy, exhibiting secondary or additive cpDNA signal (Nixon and Carpenter, 1996; Wenzel and Siddall, 1999), which provides an increase of resolution and support (Mort et al., 2007). Combination of chloroplast markers has been used for Ixoreae with *rps16*, *trnT-F*, and *rbcL* markers (Mouly et al., in press), and combination of chloroplast and nuclear regions for several Rubiaceae groups as for the close relative of Ixoreae, tribe Vanguerieae (Razafimandimbison et al., in press). Combination of data sets has been extensively used to reconstruct phylogenies to increase the number of informative characters, notably in case of taxa showing low substitution rates for investigated markers (Soltis et al., 1998). Soltis et al. (1998: 297–348, Fig. 11.1: 301) also pointed out that in case of incongruent topologies for separate data analyses, combining these conflicting data is possible by pruning taxa. On the basis of the conflicting positions between nr- and cpDNA tree topologies, we conducted two types of combined analyses: one excluding all taxa with conflicting positions and the other excluding the putative hybrid species collected from botanical gardens but including only the nuclear data of the species with detected conflicting positions possibly due to natural hybridization (*I. brunonis* Wall. ex G.Don, *I. kuakuensis* S.Moore, *I. nematopoda* K.Schum., *I. nimbana* Schnell, *I. triantha* Volkens, and *Versteegia grandifolia* Valeton). The combined matrices included one to several clone sequences, according to the different paralogous lineages found (Fig. 1). Consequently, the restricted combined data set analyses comprised 91 terminal units representing 85 specimens (online Appendix S4), while the complete combined data sets analyzed included 98 terminal units representing 91 specimens (online Appendix S5).

Character optimizations—Currently, several reproductive characters (e.g., inflorescence, ovary, flower sex) are used in combination for generic recognition within in Ixoreae (Table 1). Information about the taxonomic characters (e.g., seed numbers: two or numerous, position of inflorescences: terminal or caulinary, breeding systems: hermaphroditism or functional dioecy) were observed on herbarium material (BR, G, K, L, MO, NOU, NY, P, PAP, TAN) and in the field or compiled from the literature. To illustrate the character state evolution, we optimized them onto the Bayesian majority consensus tree from the combined nuclear–chloroplast analyses manually using Fitch's (1971) incremental character optimization.

Ixora infrageneric classification mapping—No infrageneric treatment has ever been provided for the entire genus *Ixora*; however, regional works have presented classification tools along with morphological characteristics (Gray, 1858; Baillon, 1879, 1880; Hochreutiner, 1908; Bremekamp, 1937a, b, 1938, 1940; Fosberg, 1942; Guédès, 1986). The available infrageneric classification, summarized in Table 2 (updated and completed from De Block, 1998), was thought applicable to the entire genus. To assess the limits of the *Ixora* infrageneric classification (Table 2), we assigned to ingroup taxa names of subgenera and sections, according to morphological characteristics of each group given by previous authorities (Table 2; Appendix 2). These names were then mapped on the tree topology of the complete combined markers analysis to estimate their validity. Observed patterns did not suffer any effect from the potential for hybridization of the genus, considering hybridization did not influence significantly the main *Ixora* subclades circumscriptions (observed from both separated and combined data sets).

RESULTS

Phylogenetic analyses—In this section, we provide (1) the sequence characteristics and the outputs of the separate nuclear (Fig. 2) and chloroplast (Fig. 3) analyses, with an emphasis on the detailed results of the ETS analysis including the cloned sequences; (2) an illustration of conflicting positions between these data sets (Figs. 2–4); and (3) the characteristics and relationships information for both the restricted and complete sampling analyses of the combined datasets (Figs. 5, 6).

Sequence characteristics (Figs. 2, 3; Tables 3, 4)—Ambiguously aligned sites and indels were excluded from the data sets before analyses (Table 3). These excluded data represented 15.1% of the *rps16* nucleotide matrix, 14.0% of the *trnT-F* matrix, 9.5% of the ETS matrices, and 10.3% of the combined data for both combined analyses. The number of parsimony informative characters (PIC) was very low within the complete combined data set (314 bp), representing ca. 10% of the aligned sequences.

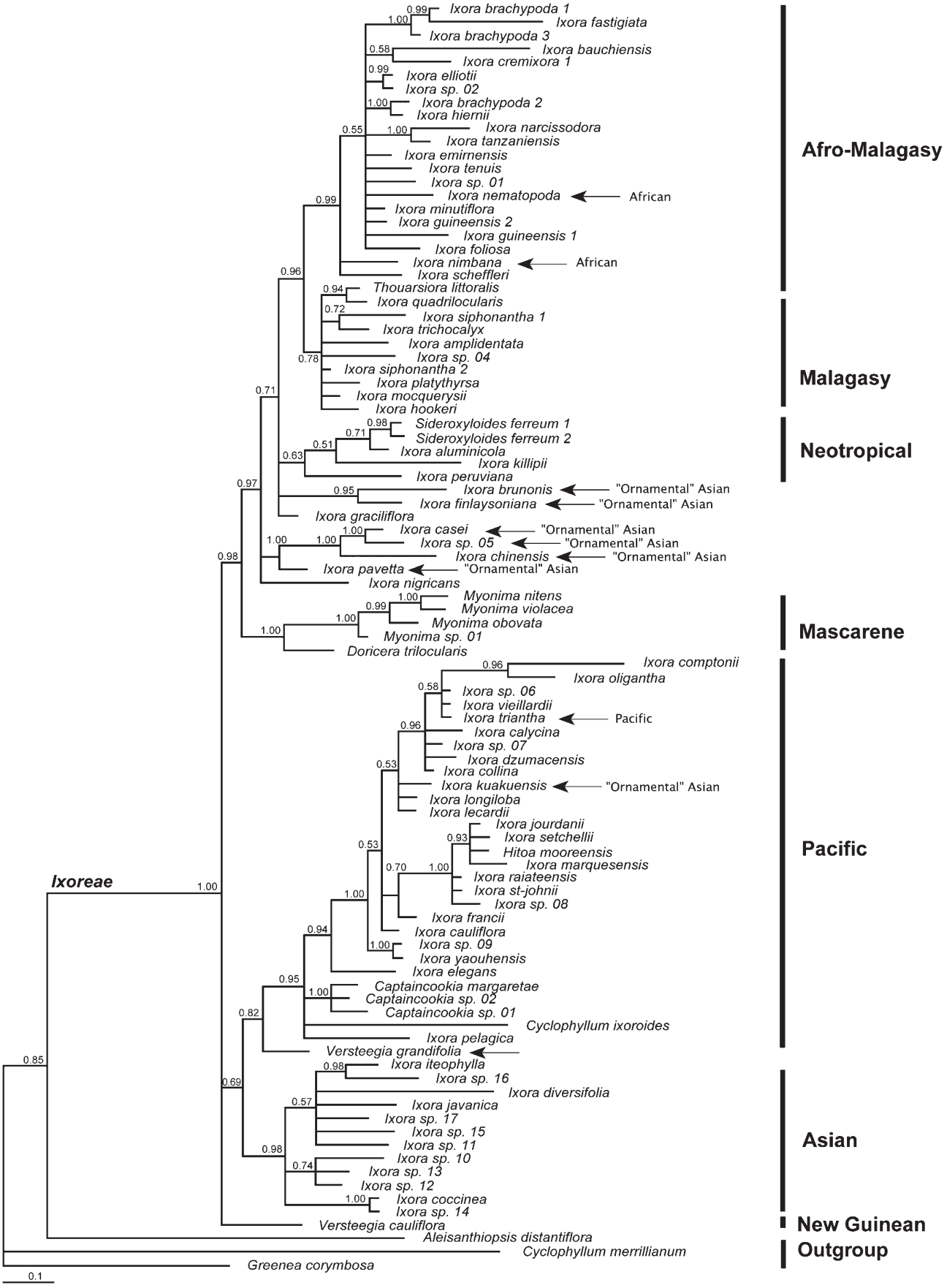
The *trnT-F* matrix contained the longest sequences, and most of the PIC were localized in the noncoding *trnT-L* segment. However, many substitutions and most of the indels, which were resolved as unambiguous synapomorphies for many clades, were from within the *trnL* intron. The ETS, comprising about half of the PIC of the complete combined data set, was useful to resolve relationships within the ingroup lineage and to detect incongruence between the nuclear and chloroplast data.

The ETS matrix included all sampled cloned sequences of the 13 polymorphic taxa. The pairwise sequence divergences of these cloned sequences varied from 0 to 4.5%, with the exception of *Ixora coccinea* with 7.9% (see Table 4). Only six of the 13 polymorphic specimens had their sampled ETS cloned sequences forming exclusive lineages (Fig. 2; Table 4): *Ixora scheffleri* K.Schum. & K.Krause (PP = 1.00), *I. aluminicola* Steyerm. (PP = 0.97), *I. finlaysoniana* (PP = 1.00), *Ixora* sp.5 (PP = 1.00), *Doricera trilocularis* (Balf.f) Verdc. (PP = 1.00), and *Versteegia cauliflora* (K.Schum. & Lauterb.) Valeton (PP = 1.00).

Separate nuclear and chloroplast analyses—The nuclear and chloroplast trees (Figs. 2, 3) both resolved the presently circumscribed *Ixora* as paraphyletic and retained the following clades: the Mascarene *Myonima-Doricera* clade (nr-tree, PP = 1.00; cp-tree, PP = 1.00); the Afro-Malagasy-American *Ixora* clade (nr-tree, PP = 0.71; cp-tree PP = 1.00); the Pacific clade (nr-tree, PP = 0.67; cp-tree, PP = 0.95), and *Versteegia cauliflora* was left unresolved and isolated in a large polytomy. Nev-

→

Fig. 3. Ixoreae phylogram of the Bayesian analysis majority rule consensus tree of the chloroplast (combined *rps16* and *trnT-F*) sequence analysis; posterior probability is indicated for each node. Arrows indicate species with conflicting positions according to nuclear data (Fig. 2); bold lines indicate the main groups named in the figure.


Ixora brachypoda 1
Ixora fastigiata
Ixora brachypoda 3
Ixora bauchiensis
Ixora cremixora 1
Ixora elliotii
Ixora sp. 02
Ixora brachypoda 2
Ixora hiernii
Ixora narcissodora
Ixora tanzaniensis
Ixora emirnensis
Ixora tenuis
Ixora sp. 01
Ixora nematopoda
African
Ixora minutiflora
Ixora guineensis 2
Ixora guineensis 1
Ixora foliosa
Ixora nimbana
African
Ixora scheffleri
Thouarsiora littoralis
Ixora quadrilocularis
Ixora siphonantha 1
Ixora trichocalyx
Ixora amplidentata
Ixora sp. 04
Ixora siphonantha 2
Ixora platythyrsa
Ixora mocquerysii
Ixora hookeri
Sideroxyloides ferreum 1
Sideroxyloides ferreum 2
Ixora aluminicola
Ixora killipii
Ixora peruviana
Ixora brunonis
"Ornamental" Asian
Ixora finlaysoniana
"Ornamental" Asian
Ixora graciliflora
Ixora casei
"Ornamental" Asian
Ixora sp. 05
"Ornamental" Asian
Ixora chinensis
"Ornamental" Asian
Ixora pavetta
"Ornamental" Asian
Ixora nigricans
Myonima nitens
Myonima violacea
Myonima obovata
Myonima sp. 01
Doricera trilocularis
Ixora comptonii
Ixora oligantha
Ixora sp. 06
Ixora vieillardii
Ixora triantha
Pacific
Ixora calycina
Ixora sp. 07
Ixora dzumacensis
Ixora collina
Ixora kuakuensis
"Ornamental" Asian
Ixora longiloba
Ixora lecardii
Ixora jourdanii
Ixora setchellii
Hitoa mooreensis
Ixora marquesensis
Ixora raiateensis
Ixora st-johnii
Ixora sp. 08
Ixora francii
Ixora cauliflora
Ixora sp. 09
Ixora yaouhensis
Ixora elegans
Captaincookia margaretae
Captaincookia sp. 02
Captaincookia sp. 01
Cyclophyllum ixoroides
Ixora pelagica
Versteegia grandifolia
Ixora iteophylla
Ixora sp. 16
Ixora diversifolia
Ixora javanica
Ixora sp. 17
Ixora sp. 15
Ixora sp. 11
Ixora sp. 10
Ixora sp. 13
Ixora sp. 12
Ixora coccinea
Ixora sp. 14
Versteegia cauliflora
Aleisanthiopsis distantiflora
Cyclophyllum merrillianum
Greenea corymbosa
Ixoreae
0.1
Afro-Malagasy
Malagasy
Neotropical
Mascarene
Pacific
Asian
New Guinean
Outgroup

ertheless, there were some topological conflicts between the nr- and cp-trees. The cp-tree (Fig. 3) resolved 12 of the 18 Asian *Ixora* species in a strongly supported clade; two *Ixora* species (*I. brunonis*, *I. finlaysoniana*) formed a highly supported clade nested but unresolved in the Afro-Malagasy-American *Ixora* clade. The remaining four *Ixora* species (*I. casei*, *Ixora* sp.5, *I. chinensis*, and *I. pavetta*) constituted a poorly supported clade left unresolved at the base of the Afro-Malagasy-American *Ixora* clade (Fig. 3). In contrast, the large Asian *Ixora* clade collapsed in the nr-tree (Fig. 2) and instead split into two groups: one forming 13 *Ixora* species, including the aforementioned six *Ixora* species, called the "ornamental" Asian group; and the other containing *I. iteophylla* Bremek., *Ixora* sp. 16 and 17, and *I. coccinea* L. clone B, called Asian clade. The cp-tree (Fig. 3) placed *I. kuakuensis* in the Pacific *Ixora* clade, while the nr-tree (Fig. 2) resolved it in the "ornamental" Asian *Ixora* group. The two ETS cloned sequences of *I. coccinea* did not form a clade: one nested in the Asian *Ixora* subclade and the other together with several "ornamental" Asian *Ixora*. Furthermore, *I. nematopoda* and *I. nimbana* formed a strongly supported clade (PP = 1.00) sister to the Afro-Malagasy-neotropical clade in the nr-tree (Fig. 2), whereas they did not form a clade and were embedded within the Afro-Malagasy clade in the cp-tree (Fig. 3). *Versteegia grandifolia* was sister to the Afro-Malagasy-neotropical clade (PP = 0.83) in the nr-tree (Fig. 2) but sister to the Pacific clade (PP = 0.82) in the cp-tree (Fig. 3).

Combined nuclear–chloroplast analyses—The restricted combined ETS/*rps16*/*trnT-F* data set contained 91 terminal units, and the complete combined data set consisted of 98 terminal units. Each matrix comprised 2770 characters, of which 44 (1.7%) were coded indels. These matrices, respectively, contained 324 and 356 (11.7% and 12.9%) PIC. The relationships did not differ significantly between the two combined analyses or between parsimony (figures available from A. Mouly) and model-based analyses, with the exception of the unplaced Mascarene subclade of *Ixora* in the parsimony topologies. Several main clades were more strongly supported in the restricted combined analysis (Fig. 5) than in the complete combined analysis (Fig. 6) for both parsimony and model-based reconstructions. We only present the results from the model-based analyses to simplify the comprehensive results. Both the posterior probabilities values from the restricted and complete analyses are given (PP of Fig. 5; PP of Fig. 6, respectively) for comparable nodes present in both inferred consensus tree topologies. The ingroup taxa were resolved in two lineages, consisting of an Afro-Indian Ocean-neotropical clade (PP = 0.85; PP = 0.64) and an Asian-Pacific clade (PP = 0.50; collapsed). The first ingroup lineage was fully resolved in five well-supported main clades: Afro-Malagasy *Ixora* clade (PP = 0.99; PP = 1.00), Malagasy clade (PP = 0.97; PP = 0.99), neotropical clade (PP = 1.00; PP = 0.94), *I. nigricans*, and Mascarene clade (PP = 1.00; PP = 1.00). The Mascarene clade was sister to the remaining Afro-Indian Ocean-neotropical clade. *Ixora nigricans* R.Br. ex Wight & Arn. was the first lineage to branch off, followed by the neotropical and Malagasy clades; the Malagasy clade was resolved as sister to the Afro-Malagasy clade. The second ingroup lineage consisted of poorly to moderately supported relationships (PP = 0.83; PP = 0.56) of two large sister clades: a well-supported Pacific clade including Pacific *Captaincookia* and 23 *Ixora* species (PP = 0.99; PP = 1.00) and a moderately supported Asian clade (PP = 0.92 in Fig. 5) including the genus type, *Ixora coccinea*. The inclusion of taxa with numerous missing data in the complete combined analysis considerably decreased the support for the Asian clade (PP = 0.66 in Fig. 6). *Versteegia cauliflora* was resolved in the restricted combined analysis as sister to the Asian-Pacific association, with very low support (PP = 0.50 in Fig. 5), but was basal in Ixoreae in a trichotomy with the two main clades in the complete combined analysis (Fig. 6). All sampled *Ixora* species from French Polynesia formed a well-supported clade sister to a mostly and strongly supported New Caledonian *Ixora* clade. In addition, all sampled African, Malagasy, and neotropical *Ixora* species were more closely related to the sequenced Mascarene *Doricera* and *Myonima* species than they were to the sampled Asian and Pacific *Ixora* species. The type species *I. coccinea* and the sampled Pacific *Ixora* were more closely related to *Captaincookia* and *Versteegia grandifolia* than they were to the sequenced African, Malagasy, and neotropical *Ixora* species. Furthermore, *Thoursiora littoralis* Homolle ex Arènes was nested in the strongly supported Malagasy clade (PP = 0.97; PP = 0.99), while *Ixora ferrea* (Jacq.) Benth. (= *Sideroxyloides*) was embedded within the strongly supported neotropical clade (PP = 1.00; PP = 0.94). Within the Mascarene clade, the sampled *Myonima* representatives formed a monophyletic group (PP = 1.00; PP = 1.00), which was in turn sister to *Doricera*. *Ixora mooreensis* (Nadeaud) Fosberg (= *Hitoa*) was nested in the strongly supported (PP = 1.00; PP = 1.00) French Polynesian *Ixora* clade in the supported Pacific *Ixora* clade (PP = 0.99; PP = 1.00). The three specimens of *Captaincookia* also formed a highly supported monophyletic group (PP = 1.00; PP = 1.00) near the base of the Pacific clade.

Three taxa represented by two or three specimens each did not form exclusive lineages: *I. brachypoda*, *I. cremixora*, and *I. guineensis* (Figs. 5, 6; results also observed from separate nr- and cpDNA data analyses in Figs. 2, 3).

Character optimizations—The character state optimization outputs did not differ between the nrDNA, cpDNA, and combined data sets, respectively, or between the parsimony and model-based analyses (results not shown). On the tree presented in Fig. 6, the optimized reproductive characters currently used for generic recognition within Ixoreae (Table 1) are shown to be homoplastic. The caulinary inflorescences that mainly distinguished *Versteegia*, *Captaincookia*, and *Sideroxyloides* from *Ixora* with terminal inflorescences corresponded to at least six convergent evolution events. The number of carpels per ovary was also shown to be homoplastic, with the two-carpellate ovaries inferred as plesiomorphic and >2-carpellate ovaries appearing independently three times within the ingroup. A reversal from a >2- to 2-carpellate ovary was inferred within the Mascarenes subclade (Fig. 6) for *Myonima violacea*. On the other hand, functional dioecy (Fig. 1, E1, E2) evolved just once within Ixoreae (the Mascarene clade), and hermaphroditism is plesiomorphic.

Ixora infrageneric classification mapping—According to the assignment of our sampled species to the infrageneric classification of *Ixora* (Appendix 2) mapped on the combined nr- and cp-tree topology (Fig. 7), the two tested subgenera *Ixora* and *Pavettoides* were not monophyletic. Section *Myonima* was monophyletic, and sections *Amphorion*, *Brachypus*, and *Microthamnus* were each represented by a single species. Sections *Cremixora*, *Ixora*, *Micrixora*, *Otobactrum*, *Pavettopsis*, and *Raphidanthus* were not resolved as natural lineages.

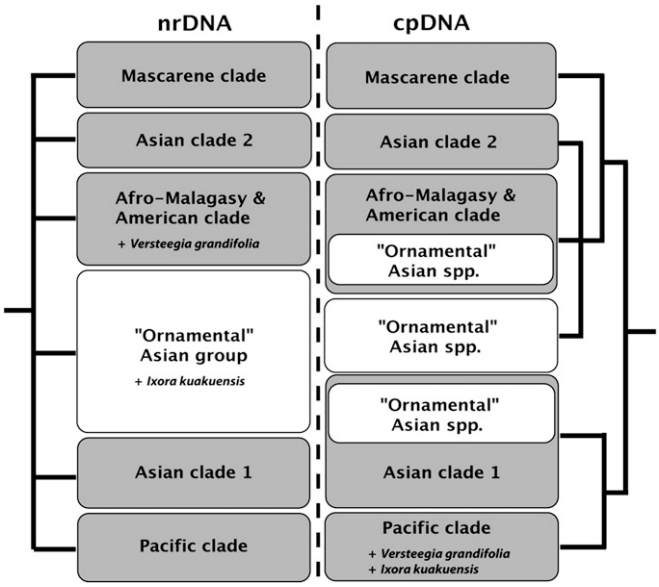


Fig. 4. Schematic comparison of nrDNA (ETS tree of Fig. 2) and cpDNA (combined *rps16* and *trnT-F* of Fig. 3) tree topologies, summarizing species with conflicting positions between data sets.

TABLE 3. Information for phylogenetic analyses of (A) separate (*rps16*, *trnT-F*, ETS) and (B) combined chloroplast (*rps16* + *trnT-F*) or combined (*rps16* + *trnT-F* + ETS) data sets.

Phylogenetic information	*rps16*	*trnT-F*	ETS
A) Separate analyses			
No. of sequences investigated	92	92	112
No. of new sequences	80	80	112
Range of sequence lengths (bp)	485–645	531–1584	281–411
Length of aligned matrices (bp)	762	1863	458
No. of ambiguous sites and gaps excluded	115	260	47
No. of PIC	53	95	166
No. of coded indel events	1	17	3
Range of G : C within ingroup (mean)	26.8–32.9% (31.5%)	24.6–35.5% (30.6%)	53–60.5% (57.4%)
Length of MP trees	201	327	623
Consistency index	0.83	0.83	0.59
Retention index	0.88	0.90	0.83
Retention index corrected	0.73	0.75	0.49

Phylogenetic information	Chloroplast	Restricted combined [a]	Complete combined [a]
B) Combined analyses			
No. of sequences investigated	95	91	98
Length of aligned matrices (bp)	2625	3083	3083
No. ambiguous sites and gaps excluded	365	421	421
No. PIC	148	266	275
No. indel events	18	21	21
Length of MP trees	542	995	1052
Consistency index	0.81	0.71	0.69
Retention index	0.88	0.84	0.83
Retention index corrected	0.71	0.60	0.57

[a] In restricted combined analysis, data set was restricted to samples for which a potential hybridization was not suspected. For complete combined analysis, all sample specimens were included, except potential anthropogenic hybrids and potential natural hybrids chloroplast information.

Notes: bp = base pairs, MP = most parsimonious, parsimony informative characters = PIC.

DISCUSSION

Following the recent resurrection (Andreasen and Bremer, 2000) and recircumscription (Mouly et al., in press) of tribe Ixoreae, the current study constitutes the first phylogeny of the species-rich genus *Ixora* based on a large sampling of *Ixora* and its allies. The present discussion focuses on (1) the composition of the nuclear data set, (2) incongruencies between the nuclear and chloroplast data sets, (3) the circumscriptions and preliminary biogeographical elements of *Ixora* and its infra-generic classification, and (4) taxonomic implications of the phylogenetic results.

ETS data set information (Fig. 2; Table 4)—The ETS region is part of the 18S-26S nrDNA and has a role in the maturation of rRNAs. The clones obtained for each polymorphic species are generally poorly differentiated (ranging from 0 to ca. 4.5%; mean < 2%) within a species, with the exception of *I. coccinea* with ca. 8% divergence. Despite the low differentiation of paralogs (Table 4), only half of the polymorphic species (*Doricera trilocularis*, *Ixora aluminicola*, *I. finlaysoniana*, *I. scheffleri*, *Ixora* sp. 5, and *Versteegia cauliflora*) form separate groups (Fig. 2; Table 4). The sampled clones of *I. tanzaniensis* Bridson, *I. narcissodora* K.Schum. and *Ixora* sp. 10, *Ixora* sp. 13, and *Myonima* sp.1 do not form separate lineages (Fig. 2; Table 4). The observed levels of intraindividual polymorphisms can be explained by different rates of concerted evolution of the ETS region among the species in question. In other words, the presence of divergent ETS paralogs in 13 Ixoreae species indicates that mutation rates are faster than that of concerted evolution; as a result, concerted evolution has not been fast enough to nullify the differences between the paralogs (see also Razafimandimbison et al., 2004). Nonconcerted evolution has been reported for nrITS (e.g., Buckler et al., 1997; Alvarez and Wendel, 2003; Bailey et al., 2003; Harpke and Peterson, 2006; Zheng et al., 2008) but is less documented for the ETS region. Divergent ITS and ETS pseudogenes (Razafimandimbison et al., 2004, 2005, respectively) have also been reported from the tribe Naucleeae s.l. in subfamily Cinchonoideae (Rubiaceae). The percentage of GC content in the ingroup ETS sequences ranges from 53 to 60.5% (mean 57.4%) compared to that reported from the genus *Neonauclea* Merr. in Naucleeae s.sl. (61.4–62%; Razafimandimbison et al., 2005). The large range of GC content in *Ixora* ETS sequences can be an indicator of the presence of putative nrDNA pseudogenes (e.g., Bailey et al., 2003), characterized by lower GC contents compared to their functional counterparts in the ETS matrix (e.g., Ochieng et al., 2007). For example, the two sampled ETS clonal sequences of *I. coccinea* may belong to putative nonfunctional nrDNA regions (clones A and B with GC contents of 56 and 57.5%, respectively), but no definitive conclusion can be drawn from the ETS only, according to the nrDNA pseudogene indicators listed in Bailey et al. (2003). On the other hand, each case of individual polymorphism studied here (*I. coccinea* excepted) represents shallow paralogy that does not interfere significantly in the phylogenetic reconstruction process (Bailey et al., 2003).

Plausible causes of the topological conflicts in Ixora and data sets combination—Incongruence between nr- and cp-trees (Figs. 2–4) are evident in our study, as many species have

TABLE 4. ETS clonal sequence differences within a specimen, by clone pair; and the phylogenetic status of clones of a specimen within the tree topologies inferred from nuclear data (Fig. 2) and combined data (Fig. 6).

Polymorphic taxa	Genetic distances among ETS clones (%)	ETS clonal relation
Captaincookia sp. 02	0–0.24	unresolved
Doricera trilocularis	0.48	clustered
Ixora aluminicola	0.24–1.43	clustered
Ixora coccinea	7.87	not clustered
Ixora finlaysoniana	0–1.19	clustered
Ixora narcissodora	0.24	unresolved
Ixora scheffleri	0.48–1.67	clustered
Ixora sp. 05	0.24	clustered
Ixora sp. 10	0.72–3.1	unresolved
Ixora sp. 13	1.06–4.33	not clustered
Ixora tanzaniensis	0.95–2.15	not clustered
Myonima sp. 01	1.19	not clustered
Versteegia cauliflora	0	clustered

highly supported conflicting positions: *I. nimbana* and *I. nematopoda* in Africa; *I. brunonis*, *I. casei*, *I. chinensis*, *I. finlaysoniana*, *I. pavetta*, and *Ixora* sp. 5 in Asia (as the "ornamental" group in Fig. 4); *I. kuakuensis*, *I. triantha*, and *Versteegia grandifolia* in Pacific Islands. Three main biological processes are known to produce such effects: paralogy, incomplete lineage sorting, and hybridization (Sang and Zhong, 2000; Takahashi et al., 2001; Funk and Omland, 2003; Hudson and Turelli, 2003; Maddison and Knowles, 2006; Baum, 2007).

No intraindividual polymorphism is observed in the sampled noncultivated *Ixora* species; this observation would also favor the hybridization hypothesis because polymorphic alleles would be expected to be more randomly distributed under a scenario of incomplete lineage sorting (Soltis et al., 1998: 273–277). The hypothesis of paralogy is unlikely to explain the observed incongruencies in the results because the ETS cloned sequences from the same individuals of *I. scheffleri*, *I. aluminicola*, *I. finlaysiana*, *Ixora* sp. 5, *Doricera trilocularis*, and *Vesteegia cauliflora* respectively, form separate clades (Fig. 2). Neither the sampled ETS cloned sequences of *Ixora* sp. 10 nor those of *Ixora* sp.13 nor those of *I. coccinea* form a monophyletic group; however, these species are still placed in the two Asian and "ornamental" Asian subclades (Fig. 2).

Interestingly, five of the six sampled Asian *Ixora* species with conflicting positions in nr- and cp-trees (Figs. 2, 3) are commonly cultivated *Ixora* (see Appendix 1). Some of these cultivated specimens are likely to have hybrid origins, because plant breeders have successfully produced many cultivars of *I. casei*, *I. coccinea*, and *I. chinensis* (Fosberg and Sachet, 1989a, b; Staples and Herbst, 2005). The fact that the sampled *I. casei*, *I. chinensis*, *I. pavetta*, and *Ixora* sp. 5 form a clade (Fig. 3) may indicate that their maternal parents, which belong to the large neotropical-Afro-Malagasy-Mascarene *Ixora* lineage, are closely related. The current study clearly demonstrates how important it is for phylogeneticists to know the origin of specimens they include in their studies, notably for cultivated ones. Not considering this origin may lead to misinterpretations of the results (see Fig. 4). For example, phylogeneticists may end up performing age estimates for what appears to them to be a case of natural hybridization. Another case of an unnatural hybridization has been observed by Razafimandimbison et al. (unpublished data) during ITS investigations of three cultivated individuals of *Mussaenda philippica* A.Rich.

A well-supported sister-group relation (Fig. 3) between the noncultivated individual of *I. brunonis* from Thailand and the cultivated individual of *I. finlaysoniana* does not seem to support an artificial hybrid origin of the latter, because no artificial hybrids have been reported from the latter species; instead, it appears to support the occurrence of natural hybridizations (Arnold, 1997) between two *Ixora* species, which currently have allopatric distributions but may have grown sympatrically in the past. A larger sampling of the Asian *Ixora* species is needed to retest the monophyly of the Asian group. If the placement of the noncultivated *I. nigricans* as sister to the neotropical-Afro-Malagasy lineage is correct (Figs. 3, 5), this latter clade could have had an Asian origin. The conflicting positions of the Micronesian species *I. triantha* within the Pacific clade and that of the Asian *I. kuakuensis* in the "ornamental" Asian (Figs. 2, 4) and in the Pacific clades (Figs. 2, 3) could represent two independent cases of natural hybridizations.

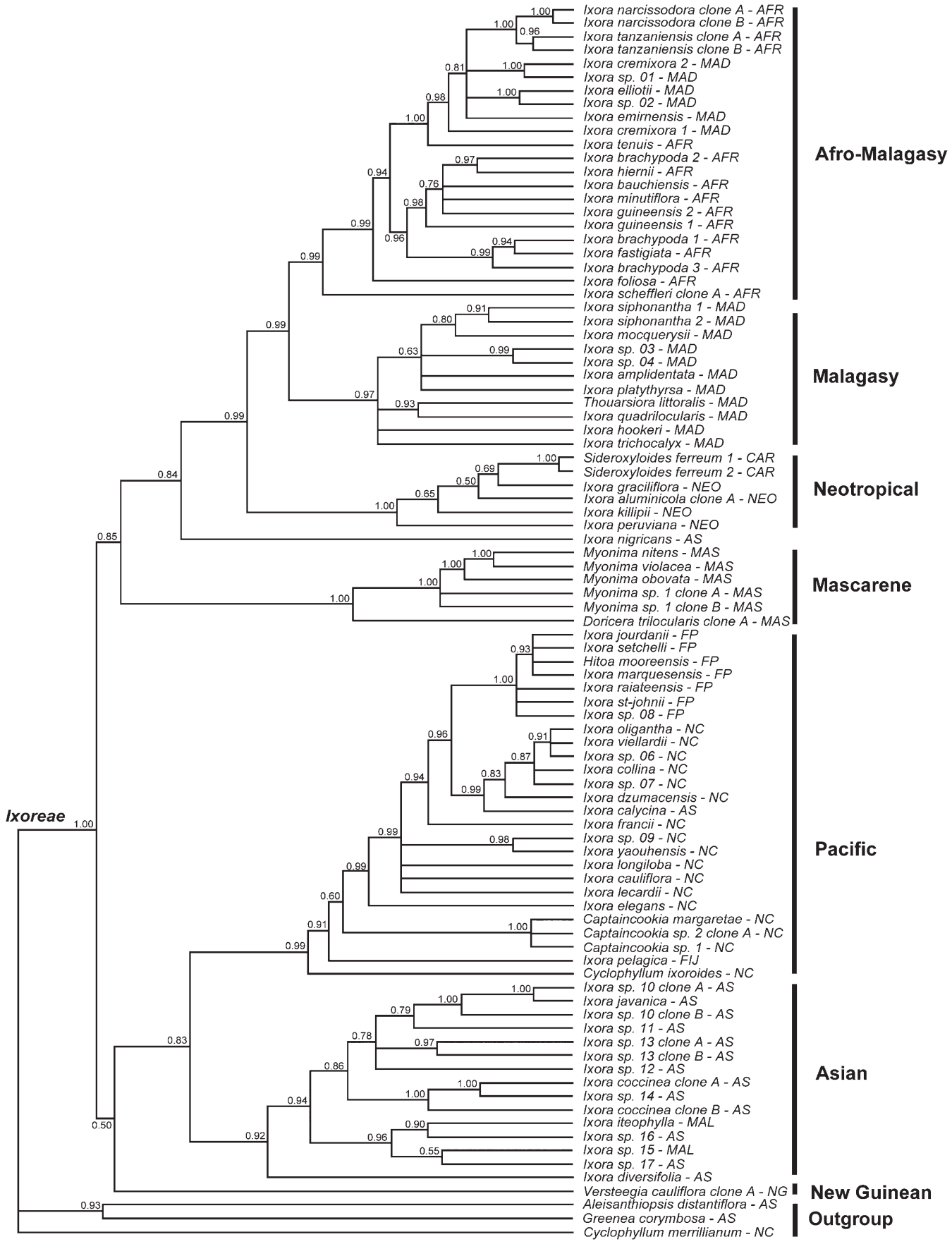
Interspecific hybridization is considered common among plants (Soltis et al., 1998; Hegarty and Hiscock, 2005; Pan et al. 2007), but phylogenetic methods produce only divergently branching hypotheses and thus cannot give a reliable interpretation of the tree topology if an analysis includes hybrids (McDade, 1990), except by using reticulation models. If the hypothesis of hybridization put forward here is true, then relations within *Ixora* should be inferred from nuclear or chloroplast data alone and gene-tree topologies discussed separately. On the other hand, an inclusion of specimens from probable anthropogenic crossing origin would very much affect the phylogenetic reconstruction and the tree topology. For evolutionary assessments, it is important to solely combine and analyze nuclear and chloroplast data sets if taxa with highly supported conflicting positions due to hybridization are excluded.

In the case of *Ixora*, phylogenetic information appeared to benefit from the data set combination, considering the more resolved and supported phylogenetic hypothesis obtained in combination of data sets (Fig. 5), compared to separate genomes analyses (Figs. 2, 3). The current study concurs with Barber et al. (2007), who favor using a multiple marker approach from both nuclear and plastid genomes for assessing infrageneric relationships and controlling the occurrence of supported incongruencies, notably in the case of large genera like *Ixora*. Conflicts due to paralogy and/or hybridization observed from our data set do not appear to significantly affect the main subclades circumscriptions or their relations.

New circumscriptions of Ixora—The present analyses (Figs. 5, 6) demonstrate that *Ixora* as currently circumscribed is paraphyletic and that *Versteegia* is polyphyletic. According to the combined nr- and cpDNA analyses (Figs. 5, 6), the sequenced African, Malagasy, and neotropical *Ixora* seem more closely related to the Mascarene *Doricera* and *Myonima* than they are to the Pacific and Asian *Ixora* (including the genus type *I. coccinea*). Plus, *Captaincookia* is nested within the highly supported

→

Fig. 5. Ixoreae cladogram of the Bayesian analysis majority rule consensus tree for the restricted sampling (excluding putative hybrids) of combined data sets. The native area of species follows the taxon: AFR, Africa; AS, Asia; CAR, Caribbean; FIJ, Fiji; FP, French Polynesia; MAD, Madagascar; MAL, Malesia; MAS, Mascarenes; MIC, Micronesia; NC, New Caledonia; NEO, neotropics; NG, New Guinea.


Ixoreae
Ixora narcissodora clone A - AFR
Ixora narcissodora clone B - AFR
Ixora tanzaniensis clone A - AFR
Ixora tanzaniensis clone B - AFR
Ixora cremixora 2 - MAD
Ixora sp. 01 - MAD
Ixora elliotii - MAD
Ixora sp. 02 - MAD
Ixora emirnensis - MAD
Ixora cremixora 1 - MAD
Ixora tenuis - AFR
Ixora brachypoda 2 - AFR
Ixora hiernii - AFR
Ixora bauchiensis - AFR
Ixora minutiflora - AFR
Ixora guineensis 2 - AFR
Ixora guineensis 1 - AFR
Ixora brachypoda 1 - AFR
Ixora fastigiata - AFR
Ixora brachypoda 3 - AFR
Ixora foliosa - AFR
Ixora scheffleri clone A - AFR
Afro-Malagasy
Ixora siphonantha 1 - MAD
Ixora siphonantha 2 - MAD
Ixora mocquerysii - MAD
Ixora sp. 03 - MAD
Ixora sp. 04 - MAD
Ixora amplidentata - MAD
Ixora platythyrsa - MAD
Thouarsiora littoralis - MAD
Ixora quadrilocularis - MAD
Ixora hookeri - MAD
Ixora trichocalyx - MAD
Malagasy
Sideroxyloides ferreum 1 - CAR
Sideroxyloides ferreum 2 - CAR
Ixora graciliflora - NEO
Ixora aluminicola clone A - NEO
Ixora killipii - NEO
Ixora peruviana - NEO
Neotropical
Ixora nigricans - AS
Myonima nitens - MAS
Myonima violacea - MAS
Myonima obovata - MAS
Myonima sp. 1 clone A - MAS
Myonima sp. 1 clone B - MAS
Doricera trilocularis clone A - MAS
Mascarene
Ixora jourdanii - FP
Ixora setchelli - FP
Hitoa mooreensis - FP
Ixora marquesensis - FP
Ixora raiateensis - FP
Ixora st-johnii - FP
Ixora sp. 08 - FP
Ixora oligantha - NC
Ixora viellardii - NC
Ixora sp. 06 - NC
Ixora collina - NC
Ixora sp. 07 - NC
Ixora dzumacensis - NC
Ixora calycina - AS
Ixora francii - NC
Ixora sp. 09 - NC
Ixora yaouhensis - NC
Ixora longiloba - NC
Ixora cauliflora - NC
Ixora lecardii - NC
Ixora elegans - NC
Captaincookia margaretae - NC
Captaincookia sp. 2 clone A - NC
Captaincookia sp. 1 - NC
Ixora pelagica - FIJ
Cyclophyllum ixoroides - NC
Pacific
Ixora sp. 10 clone A - AS
Ixora javanica - AS
Ixora sp. 10 clone B - AS
Ixora sp. 11 - AS
Ixora sp. 13 clone A - AS
Ixora sp. 13 clone B - AS
Ixora sp. 12 - AS
Ixora coccinea clone A - AS
Ixora sp. 14 - AS
Ixora coccinea clone B - AS
Ixora iteophylla - MAL
Ixora sp. 16 - AS
Ixora sp. 15 - MAL
Ixora sp. 17 - AS
Ixora diversifolia - AS
Asian
Versteegia cauliflora clone A - NG
New Guinean
Aleisanthiopsis distantiflora - AS
Greenea corymbosa - AS
Cyclophyllum merrillianum - NC
Outgroup

Pacific *Ixora* clade; *Versteegia cauliflora* is sister to the moderately supported Asian-Pacific *Ixora* clade (PP = 0.83). Our molecular phylogenetic results support the inclusion of *Hitoa*, *Sideroxyloides*, and *Thouarsiora* in *Ixora as* previously suggested by Fosberg (1937), Bentham (1850), Guédès (1986), respectively, based on morphological data (see Fig. 1, E10, for *Hitoa*). Based on the evidence presented here, *Ixora* is in need of a new circumscription.

Splitting up *Ixora* into several small genera is conceivable and necessitates the reinstatement of the existing generic names (e.g., *Hitoa*, *Sideroxyloides*, *Thouarsiora*). On the other hand, we find no obvious features for distinguishing or characterizing the major clades identified by our combined tree (Fig. 5). The recognition of several genera would also imply a restriction of the name *Ixora* to a single group of Asian species and would thus require hundreds of new combinations. We favor a broad circumscription of *Ixora*, which comprises *Captaincookia*, *Doricera*, *Hitoa*, *Myonima*, *Sideroxyloides*, *Thouarsiora*, and *Versteegia*, because this would constitute a relatively homogenous genus and only requires a maximum of 11 new combinations. Our results corroborate Baillon's (1879) decision to merge *Myonima* in *Ixora* because he argued that plurilocular ovaries (Fig. 1, E11) did not constitute a strong character for generic recognition for *Myonima*. Also, the sexual differentiation of flowers in *Myonima* and *Doricera* (Fig. 1, E1, E2; Fig. 6) does not seem to be sufficient for generic separation. Indeed, the Seychellean species *Ixora pudica* Baker (not included here), supposed to be related to Melanesian and Malesian species (Bremekamp, 1934; Friedmann, 1994), also has unisexual flowers (Friedmann, 1994). Caulinary inflorescences (Fig. 1, C; online Appendix S1, C, K), used among other characters for segregating *Captaincookia*, *Sideroxyloides*, and *Versteegia* from *Ixora*, have evolved several times in Ixoreae (Fig. 6), also in several *Ixora* s. s. (Fig. 1, C–E), of which *I. cauliflora* and *I. kuakuensis* are included in our study (Fig. 6). *Captaincookia* mainly differs from *Ixora* by drooping flowers, infundibuliform corollas (Fig. 1, E5), and permanently erected stigma lobes, but its fruits (Fig. 1, E6) resemble those of the Pacific *Ixora* representatives. The placement of *Versteegia* within Ixoreae is still unresolved because *V. cauliflora* is either sister to all other Ixoreae (Fig. 5) or unresolved at the base of the tribe (Fig. 6), and the position of *V. grandifolia* is unclear in our study (cf. basal nodes support in Fig. 6). Only the flattened convex pyrene (Valeton, 1911; Fig. 1, E8) differentiates these two latter species from other *Ixora* (with globose pyrenes; Fig. 1, E7, E9), a characteristic not shared by *V. solomonensis* Ridsd. (not included in our analyses) with globose pyrenes (Ridsdale et al., 1972). *Versteegia* is a morphologically poorly consistent genus, not supported as monophyletic by our study (Figs. 2, 6), and cannot be maintained at generic level. The species *Cyclophyllum ixoroides* is confirmed as a member of this broadly delimited *Ixora*, but this placement does not necessitate a reassessment of the genus *Cyclophyllum* in Vanguerieae (Mouly et al., 2007; Mouly and Achille, 2007; Razafimandimbison et al., in press). This broad circumscription of *Ixora* renders the tribe Ixoreae sensu Mouly et al. (in press) monogeneric.

Relations and geographic distributions of the major lineages of Ixora—The main diversity of *Ixora* is recorded from tropical Asia (i.e., India, South East Asia, and Malesia; Bremekamp, 1937b; De Block, 1998). The occurrence of the sister tribe Aleisanthieae in Malesia and the next closely related tribe Greeneeae in the tropical Asia strongly indicates a tropical Asian origin of *Ixora* and Ixoreae (Mouly et al., in press). The position of tropical Asian *Ixora* species both at the base of the *Ixora* tree and in the two main *Ixora* clades further supports this hypothesis (Fig. 6). Proper analyses to assess the historical biogeography of the newly circumscribed *Ixora* would benefit from a better geographical coverage of the species sampling. Consequently, the biogeography and the molecular dating of *Ixora* will be addressed in a separate study.

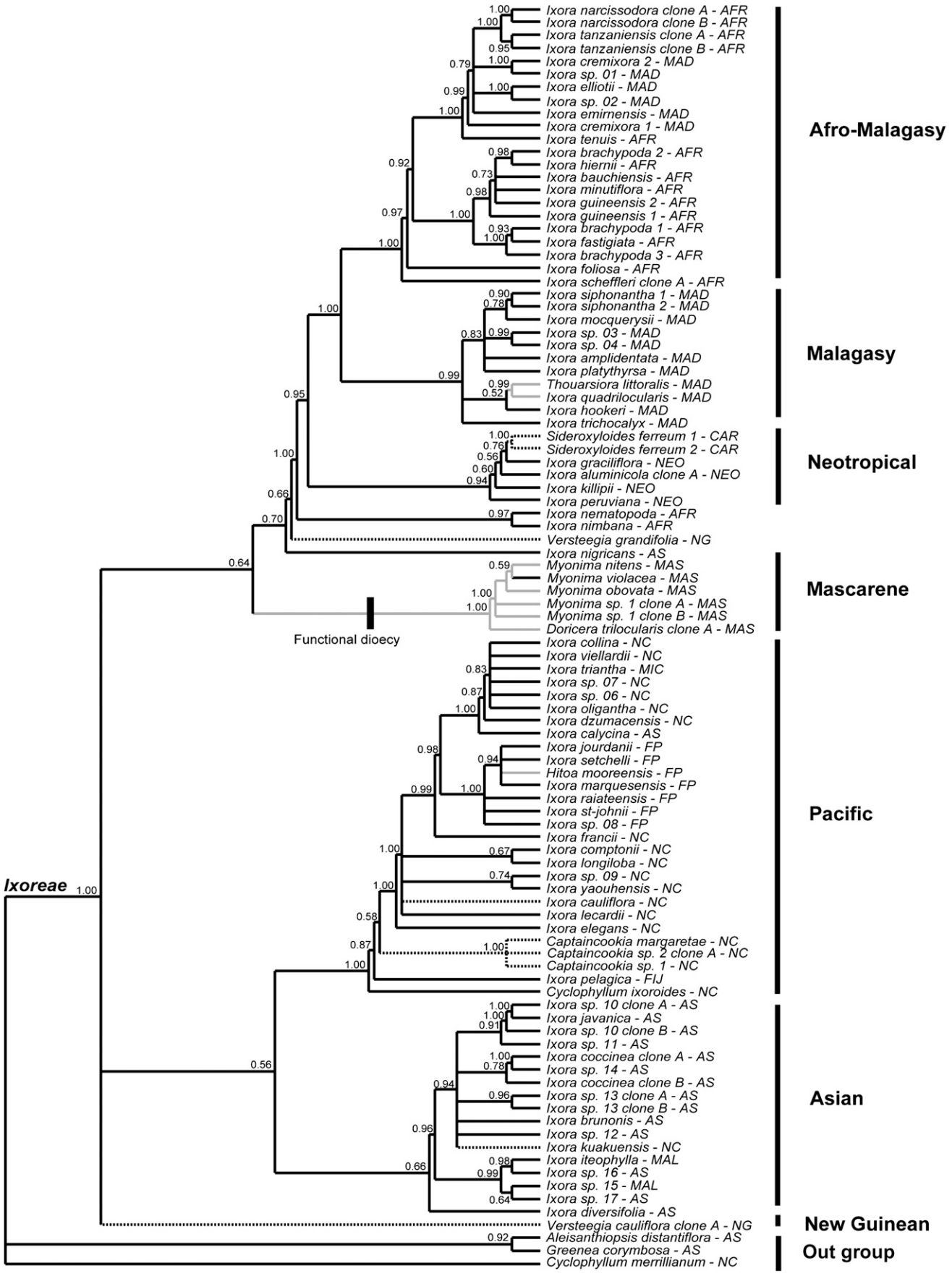
The present analyses (Fig. 5) identify monophyletic groups in *Ixora*, which largely correspond to the following tropical regions: tropical Asia, Pacific Islands, the neotropics, mainland Africa (including Madagascar), and the Mascarenes. The Malagasy and African *Ixora* clade appears surprisingly more closely related to neotropical *Ixora* than to the neighboring Mascarene Islands taxa. The sampled *Ixora* species are resolved in two large lineages: the Asian-Pacific and Afro-Malagasy-neotropical-Mascarene lineages. Within the tropical Asian-Pacific clade, our results support the monophyly of the sampled Pacific *Ixora* species, which are sister to a large tropical Asian clade. On the other hand, the monophyly of the tropical Asian *Ixora* is not supported, as the noncultivated Asian *I. calycina* and *I. nigricans*, respectively, are nested within the Pacific and Afro-Malagasy-neotropical-Mascarene lineages (Fig. 5). Six *Ixora* species (*I. brunonis*, *I. casei*, *I. chinensis*, *I. finlaysoniana*, and *I. pavetta*) are also nested in the latter lineage in the cp-tree (Fig. 3) but embedded in the Asian clade in the nr-tree (Fig. 2); we argue that the placement of these six species is likely to be a reflection of their hybrid origins due to recent artificial and/or natural crossings.

Within the Afro-Malagasy-neotropical-Mascarene lineage, the Mascarene clade is sister to the *Ixora nigricans*-neotropical-Afro-Malagasy clade (Fig. 6). The neotropical clade is sister to the Afro-Malagasy clade, and a strongly supported Malagasy clade is in turn sister to an Afro-Malagasy group. These two Malagasy groups indicate two independent colonization events of *Ixora* in Madagascar, of which one seems to have been via a single long-dispersal event from either tropical Asia (if the position of *I. nigricans* in the combined tree is correct) or Mascarene (if it turns out that tropical Asian *Ixora* descended from a single common ancestor) and the other via Eastern Africa.

Ixora infrageneric classification (Fig. 7)—The monophyly of the current subgeneric classifications (Bremekamp, 1937b; De Block, 1998; Table 2) of *Ixora* is tested for the first time here, by mapping the subgenera and sections of *Ixora* onto the combined nr- and cpDNA tree. It demonstrates that the combi-

→

Fig. 6. Ixoreae cladogram of the Bayesian analysis majority rule consensus tree for the large nuclear and chloroplast combined data sets, species with conflicting positions in Figs. 2 and 3 represented only by their ETS sequences, but excluding cultivated species. The native area of species is abbreviated after the taxon: AFR, Africa; AS, Asia; CAR, Caribbean; FIJ, Fiji; FP, French Polynesia; MAD, Madagascar; MAL, Malesia; MAS, Mascarenes; MIC, Micronesia; NC, New Caledonia; NEO, neotropics; NG, New Guinea. Optimization of characters on the tree topology; number of carpels per ovary: black lines = 2, gray lines = >2; position of inflorescences: solid lines = terminal, dashed lines = caulinary. The black rectangle shows the sole change to functional dioecy.


Ixora narcissodora clone A - AFR
Ixora narcissodora clone B - AFR
Ixora tanzaniensis clone A - AFR
Ixora tanzaniensis clone B - AFR
Ixora cremixora 2 - MAD
Ixora sp. 01 - MAD
Ixora elliotii - MAD
Ixora sp. 02 - MAD
Ixora emirnensis - MAD
Ixora cremixora 1 - MAD
Ixora tenuis - AFR
Ixora brachypoda 2 - AFR
Ixora hiernii - AFR
Ixora bauchiensis - AFR
Ixora minutiflora - AFR
Ixora guineensis 2 - AFR
Ixora guineensis 1 - AFR
Ixora brachypoda 1 - AFR
Ixora fastigiata - AFR
Ixora brachypoda 3 - AFR
Ixora foliosa - AFR
Ixora scheffleri clone A - AFR
Afro-Malagasy
Ixora siphonantha 1 - MAD
Ixora siphonantha 2 - MAD
Ixora mocquerysii - MAD
Ixora sp. 03 - MAD
Ixora sp. 04 - MAD
Ixora amplidentata - MAD
Ixora platythyrsa - MAD
Thouarsiora littoralis - MAD
Ixora quadrilocularis - MAD
Ixora hookeri - MAD
Ixora trichocalyx - MAD
Malagasy
Sideroxyloides ferreum 1 - CAR
Sideroxyloides ferreum 2 - CAR
Ixora graciliflora - NEO
Ixora aluminicola clone A - NEO
Ixora killipii - NEO
Ixora peruviana - NEO
Neotropical
Ixora nematopoda - AFR
Ixora nimbana - AFR
Versteegia grandifolia - NG
Ixora nigricans - AS
Myonima nitens - MAS
Myonima violacea - MAS
Myonima obovata - MAS
Myonima sp. 1 clone A - MAS
Myonima sp. 1 clone B - MAS
Doricera trilocularis clone A - MAS
Mascarene
Functional dioecy
Ixora collina - NC
Ixora viellardii - NC
Ixora triantha - MIC
Ixora sp. 07 - NC
Ixora sp. 06 - NC
Ixora oligantha - NC
Ixora dzumacensis - NC
Ixora calycina - AS
Ixora jourdanii - FP
Ixora setchelli - FP
Hitoa mooreensis - FP
Ixora marquesensis - FP
Ixora raiateensis - FP
Ixora st-johnii - FP
Ixora sp. 08 - FP
Ixora francii - NC
Ixora comptonii - NC
Ixora longiloba - NC
Ixora sp. 09 - NC
Ixora yaouhensis - NC
Ixora cauliflora - NC
Ixora lecardii - NC
Ixora elegans - NC
Captaincookia margaretae - NC
Captaincookia sp. 2 clone A - NC
Captaincookia sp. 1 - NC
Ixora pelagica - FIJ
Cyclophyllum ixoroides - NC
Pacific
Ixora sp. 10 clone A - AS
Ixora javanica - AS
Ixora sp. 10 clone B - AS
Ixora sp. 11 - AS
Ixora coccinea clone A - AS
Ixora sp. 14 - AS
Ixora coccinea clone B - AS
Ixora sp. 13 clone A - AS
Ixora sp. 13 clone B - AS
Ixora brunonis - AS
Ixora sp. 12 - AS
Ixora kuakuensis - NC
Ixora iteophylla - MAL
Ixora sp. 16 - AS
Ixora sp. 15 - MAL
Ixora sp. 17 - AS
Ixora diversifolia - AS
Asian
Versteegia cauliflora clone A - NG
New Guinean
Aleisanthiopsis distantiflora - AS
Greenea corymbosa - AS
Cyclophyllum merrillianum - NC
Out group
Ixoreae

nations of character states previously used for circumscribing infrageneric groups (Table 2) largely represent unnatural groups, because most of these are not monophyletic. For example, sampled species of the subgenus *Ixora*, characterized by opposite and articulate inflorescence branchlets (Fig. 1 B), are resolved in two distinct clades of *Ixora* (Fig. 7). Subgenus *Pavettoides*, which mostly belongs to the Pacific clade and is characterized by subopposite and not articulate branchlets (Fig. 1, A), is also paraphyletic (see Fig. 7). In contrast with Bremekamp (1937b) who thought that inflorescence structure was important for infrageneric classification, our results show that the paraphyly of tested subgenera (Fig. 1, A, B) renders it not diagnostic at the considered level. We are unable to test here the monophyly of the subgenus *Sathrochlamys* (few species from Papua New Guinea placed in four sections; Bremekamp, 1937b) because no recent material is available for DNA studies, but subgenus *Sathrochlamys* has been considered as poorly defined from a morphological ground (De Block, 1998).

At the section level, the mapping determined both natural and unnatural taxonomic concepts (Fig. 7). The sole clearly supported section is *Myonima* sensu Baillon (1879), although the author never clearly characterized or defined this section (Fig. 7). Within *Ixora* subgen. *Ixora*, six of the eight described sections are represented in the study (Fig. 6). Three are polyphyletic, namely, sections *Ixora*, *Otobactrum* (Bremekamp, 1937b), and *Cremixora* (Baillon, 1880). *Cremixora*, a monotypic section described by Baillon (1880), is probably polyphyletic here because the different specimens of *I. cremixora* included in our analyses (Figs. 5, 6) do not form exclusive lineages. Its diagnostic character, pendulous ovules, is also present in *Cyclophyllum ixoroides*, representing convergent evolution. Section *Micrixora* (Hochreutiner, 1908), described for species with flowers bearing minute corollas and here represented by the neotropical *I. aluminicola* and *I. peruviana*, is paraphyletic. Section *Chlamydanthus* cannot be tested here because it is represented by a single species, *I. finlaysoniana* (Bremekamp, 1937b), excluded from the combined analyses because of its conflicting positions. Within subgenus *Pavettoides*, four of the six recognized sections are tested (Fig. 7). Sections *Pavettopsis*, *Raphidanthus* (Bremekamp, 1937b) and *Vitixora* (Fosberg, 1942; Smith and Darwin, 1988) are polyphyletic. Section *Phylleilema* (Gray, 1858; Fosberg, 1937; Smith and Darwin, 1988; De Block, 1998), characterized by 3–10-florous subsessile inflorescences embedded within rounded foliaceous bracts (Fig. 1, D), is paraphyletic. Section *Microthamnus* was suspected by Guédès (1986) to form a natural lineage of species with solitary flowers embedded in a calycule. Considering the geographical units obtained here, this section solely represented by the New Caledonian *I. dzumacensis*, may not be supported if the other representatives, from Madagascar (e.g., *I. reducta* Drake ex Guédès), are demonstrated in the future to belong to the Afro-Malagasy clade.

According to the mapping of infrageneric names (Fig. 7, Table 2, Appendix 2), many sections are not monophyletic. Most of these infrageneric groups were described to accommodate the Malesian species (Bremekamp, 1937b, 1938), which are poorly represented in our study from lack of available material. Several sections or type species of sections are not sampled here, and consequently, no classification can be proposed for the infrageneric clades. It can still be concluded that several infrageneric taxa of *Ixora* do not constitute natural groups and that the characters combinations used to recognize them (e.g., length of the inflorescence peduncle, number of flowers per inflorescence, size and hairiness of the corolla, shape of the bracts and bracteoles) should be newly evaluated in the light of phylogenetic relationships and evolution in *Ixora*. An appropriate study of *Ixora* infrageneric classification would clearly benefit from a complete taxonomic revision of the genus, an investigation of morphology and anatomy of numerous species, and the inclusion of subgenera and types of sections, especially the Malesian ones, in phylogenetic reconstruction.

Note on widely distributed and morphologically variable Ixora species—At the species level, the Malagasy-Comorian *I. cremixora* discussed earlier (*Ixora infrageneric classification* section) and the African *I. brachypoda* and *I. guineensis*, represented in the study by two or three specimens each, do not form exclusive lineages (Figs. 5, 6). Interestingly, these species are largely widespread *Ixora*. Widely distributed species are known to present an important morphological variability, and the circumscription of *I. guineensis* has already been questioned based on morphological ground (De Block, 1998: 169). Considering so, and according to our molecular phylogenetic results, we suggest that these species need to be more carefully investigated using both molecular and morphological data.

Taxonomic synopsis—The enlargement of the genus *Ixora* to include *Captaincookia*, *Doricera*, *Myonima* and *Versteegia* species necessitates taxonomic changes. An updated circumscription of *Ixora* is here provided, with a list of synonyms and the consequent combinations, including the combination of species previously described for *Cyclophyllum*.

***Ixora* L.**, Sp. Pl.: 110 (1753).—Type: *I. coccinea* L. (lectotype designated by Hitchcock and Green, 1929).

Schetti Adans., Fam. Pl. 2: 146 (1763), nom. illeg.

Sideroxyloides Jacq., Strip. Amer.: 19, t. 175 (1763).—Type: *S. ferrum* Jacq.

Patabea Aubl., Fl. Guiane Fr.: 110, t. 43 (1775).—Type: *P. coccinea* Aubl.

Siderodendrum Schreb., Gen. Pl. ed. 8: 71 (1789), nom. illeg.—Type: *S. floribundum* Schreb.

Myonima Comm. ex A.Juss., Gen. Pl.: 206 (1789).—Type: non designatus.

Bemsetia Rafin., Sylv. Tellur.: 12 (1838).—Type: *B. paniculata* Rafin.

Pancheria Montrouz., Mém. Acad. Roy. Sci. Lyon, Sect. Sci. 10: 223 (1860), nom. rej.; Beauvisage, Gen. Montrouz.: 58–60 (1901); Guillaumin and Beauvisage, Species Montrouz.: 22 (1914).—Type: *P. collina* Montrouz.

Charpentiera Vieillard, Bull. Soc. Linn. Normandie 9: 346 (1865); Beauvisage, Gen. Montrouz.: 58 (1901), non Gaudich. (1826), nom. illeg.—Type: *C. bracteata* Vieill.

Hitoa Nadeaud, Journ. Bot. (Morot) 13: 2 (1899); K.Krause, in Engler and Prantl, Nat. Pfl. Fam. Nachtr. 3: 329 (1908).—Type: *H. mooreensis* Nadeaud.

Versteegia Valeton, Nova Guinea 8: 483 (1911), syn. nov.—Type: non designatus.

Becheria Ridl., J. Straits Branch Roy. Asiat. Soc. 61: 20 (1912).—Type: *B. parviflora* Ridl.

Thouarsiora Homolle ex Arènes, Not. Syst., Paris, 14: 19 (1961).—Type: *T. littoralis* Homolle ex Arènes.

Captaincookia N.Hallé, Adansonia, n.s., 13: 197 (1973), syn. nov.—Type: *C. margaretae* N.Hallé.

Doricera Verdc., Kew Bull. 37: 554 (1983), syn. nov.—Type: *D. trilocularis* (Balf.f.) Verdc.

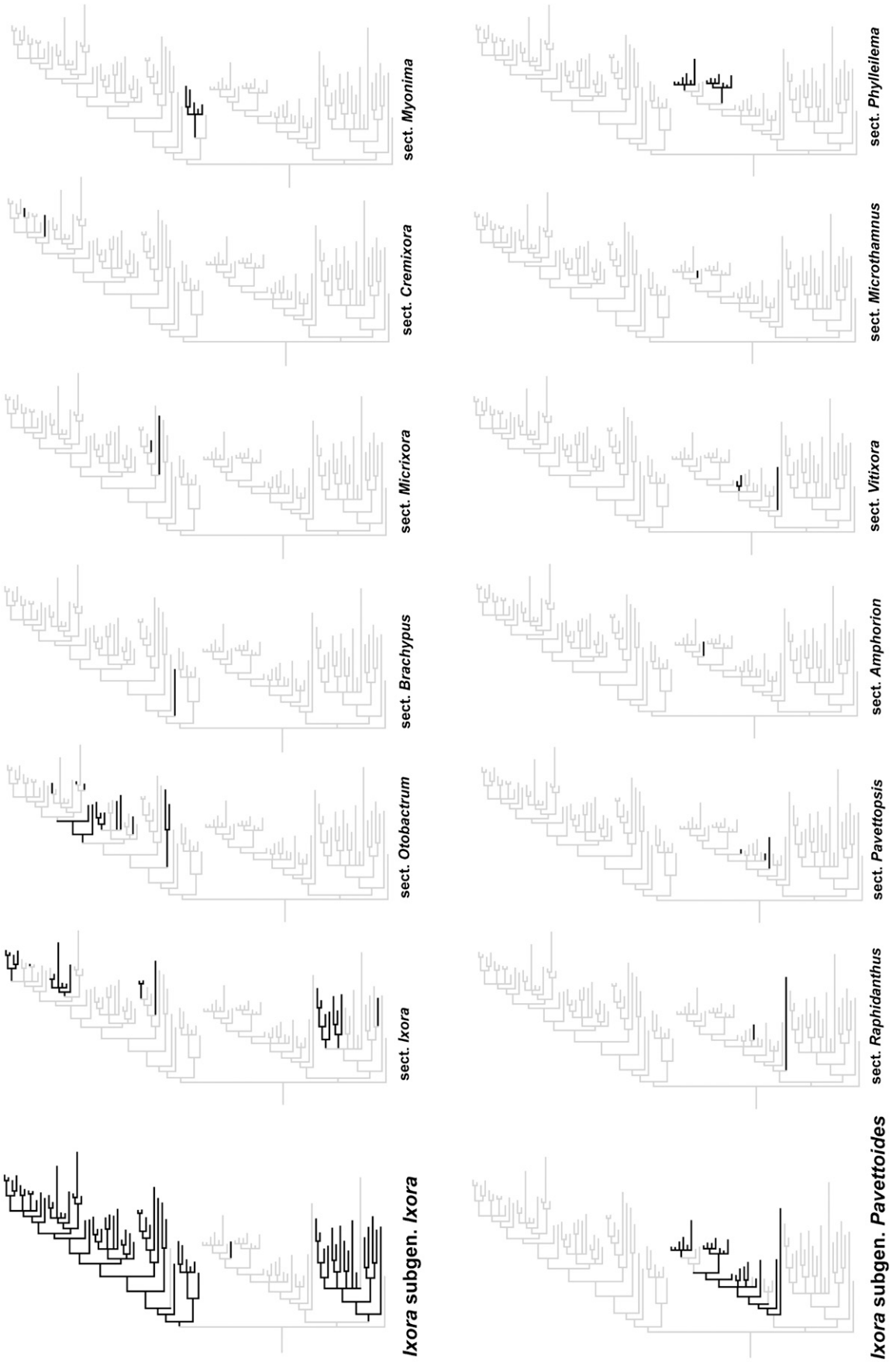


Fig. 7. *Ixora* phylogenetic trees illustrating the different subgenera or sections earlier proposed within *Ixora* (Appendix 2). The topology used for each case represents the phylogram of the ingroup from the complete combined data set (Fig. 6); branches indicated in bold belong to representatives of the infrageneric taxon indicated below the considered tree.

Tsiangia But, H.H.Hsue & P.T.Li, Blumea 31: 311 (1986).—Type: *T. hongkongensis* (Seem.) But, H.H.Hsue & P.T.Li.

1. *Ixora borboniae* Mouly & B.Bremer. nom. nov. Type: La Réunion, *Commerson & Sonnerat s.n.* (Holotype P-LA). *Myonima obovata* Lam., Illust. 1: 288 (1792), sine nomen; non *I. obovata* (E.Mey.) Kuntze, Rev. Gen. Pl.: 287 (1891); *Myonima myrtifolia* Lam., Illust. 1: 288 (1792), sine nomen; non *Ixora myrtifolia* A.C.Sm., Bull. Bishop Mus., Honolulu, 141: 142 (1936). *Note:* The specific epithet *borboniae* is chosen with the reference to a name already used for the species as "*? Myonima borboniae*" Raeusch., nom. nud.

2. *Ixora ixoroides* (Guillaumin) Mouly & B.Bremer. comb. nov. Basionym: *Cyclophyllum ixoroides* Guillaumin, Arch. Bot. Caen, Mém. 5: 22 (1930). Type: Nouvelle-Calédonie, Balade, *Vieillard 730* (Holotype P; Isotypes P). *Note:* This species was placed in the genus *Cyclophyllum* because of its ovule morphology, despite its extreme resemblance to *Ixora* species, as noted by Guillaumin (1930). The clear placement of the species in *Ixora* has been verified by our molecular data.

3. *Ixora margaretae* (N.Hallé) Mouly & B.Bremer. comb. nov. Basionym: *Captaincookia margaretae* N.Hallé, Adansonia, sér. 2, 13: 197 (1973). Type: Nouvelle Caledonie, près de Pouembout, forêt basse, fl. & fr., 2 Nov 1971, *MacKee 24542* (Holotype, P; Isotype, L, NOU, P).

4. *Ixora minor* (Valeton) Mouly & B.Bremer. comb. nov. Basionym: *Versteegia minor* Valeton, in Engler, Bot. Jahrb. 61: 67 (1927), in clavi. Type: Beaufort river, Nova Guinea Neerlandica meridionalis, 80 m, fl. 14 Nov 1912, *Pulle 347* (Lectotype BO, hic designatus; Isolectotype L). *Note:* The species was described in a key, without citation of material. After examination of the few specimens available for the species in L, this collection seemed the most representative of the original specimens.

5. *Ixora nitens* (Poir.) Mouly & B.Bremer. comb. nov. Basionym: *Myrtus nitens* Poir. in Lam., Encycl. Suppl. 4: 51 (1816). *Myonima nitens* (Poir.) Verdc., Kew Bull. 37: 558 (1983). Type: *Coll. unknown. s.n.* (Holotype Herb. Desf., FI; Photo K).

6. *Ixora novoguineensis* Mouly & B.Bremer. nom. nov. Replaced name: *Psychotria ? cauliflora* Laut. & K.Schum., Fl. Schutzgeb. Südsee: 574 (1901); *Versteegia cauliflora* (Laut. & K.Schum.) Valeton, Nova Guinea 8: 483, table 73 (1911), non *Ixora cauliflora* Montrouz, Mém. Acad. Lyon, 10: 224 (1860). Type: New Guinea, River Gogol, fr., 8 Nov 1890, *Lauterbach 910* (Syntype B); New Guinea, Bismarck-Ebene, 100 m, fr., 8 Jul 1896, *Lauterbach 2482* (Syntype B); Bismarck-Gebirge, 30 Jun 1899, *Rodatz & Klink 163* (Syntype B). *Note:* The species needs a new name because the binomial is already occupied. The species epithet was chosen to indicate the islands where the species grows. It is not yet possible to designate a lectotype because we have seen no original material. It is probable that these specimens were destroyed in B, and no duplicate has been seen yet in the other herbaria visited.

7. *Ixora ridsdalei* Mouly & B.Bremer. nom. nov. Replaced name: *Versteegia puberula* Ridsdale, Blumea 20: 340 (1972), non *Ixora puberula* (Hiern) Kuntze, Rev. Gen. Pl. 1: 287 (1891). Type: W. New Guinea, McCluer, Anahasi near Babo, alt. ca 50 m, 15 May1941, *Aet (exp. Lundquist) 107* (Holotype BO; Isotype L). *Note:* The new name is dedicated to the author of the species.

8. *Ixora solomonensis* (Ridsdale) Mouly & B.Bremer, comb. nov. Basionym: *Versteegia solomonensis* Ridsdale, Blumea 20: 340 (1972). Type: New Georgia Group, Baga Island, Solomon Islands, fl. 11 Jan 1963, *B.S.I.P. (leg. Whitmore) 1364* (Holotype L).

9. *Ixora trilocularis* (Balf.f.) Mouly & B.Bremer. comb. nov. Basionym: *Pyrostria trilocularis* Balf.f., J. Linn. Soc. Bot. 16: 14 (1877). *Doricera trilocularis* (Balf.f.) Verdc., Kew Bull. 37: 555 (1983). Type: Rodrigues, Aug/Dec 1874, fr., *Balfour s.n.* (Lectotype K, hic designatus; Isolectotypes E, P). *Note:* The lectotype was chosen among the original material observed from K.

10. *Ixora valetoniana* Mouly & B.Bremer. nom. nov. Replaced name: *Versteegia grandifolia* Valeton, Nova Guinea 8: 483, table 73 (1911), non *Ixora grandifolia* Zoll. & Mor., Syst. Verz.: 65 (1846). Type: New Guinea, Ort, 25 Mar 1908, *Branderhorst 320* (Syntype BO); Nova Guinea Neerlandica meridionalis, *Versteeg 1039* (Syntype BO). *Note:* The new name is dedicated to the author of the original description of the species. Because no original material has been seen, it was not possible to lectotypify the species.

11. *Ixora vaughanii* (Verdc.) Mouly & B.Bremer. comb. nov. Basionym: *Myonima vaughanii* Verdc., Kew Bull. 37: 558 (1983). Type: Mauritius, *Commerson 354* (Holotype P; Isotype L, P).

Complementary note: the fourth *Myonima* species does not need a new name because *Ixora parviflora* Lam. is the first available name for the species *Myonima violacea* (Lam.) Verdc., non *Ixora violacea* Lour. The illegitimate name *I. littoralis* (Homolle ex Arènes) Guédès non *I. littoralis* Merr., is not corrected here because it is included in the treatment of the revision of *Ixora* of Madagascar (De Block, in press).

Conclusion—Phylogenetic analyses of a large sample of taxa of Ixoreae for many molecular markers led to interesting results and perspectives for the ornamental, species-rich genus *Ixora*. The separate analyses of nuclear and chloroplast regions were partially incongruent. At least four natural hybridization events were detected, one or more in Asia, one in Africa, and two in Oceania. Other conflicting positions are most likely due to the inclusion of six specimens of cultivated species suspected to have artificial hybrid origins. The combined data sets provided good resolution and usually strong support for main clades, but considering the hypothesis of hybridizations, one might take into account only tree topologies inferred from nuclear sequences or chloroplast sequences separately. At the generic level, however, the hybridization effect on relationships is low, and both nuclear and chloroplast data sets, separately or combined, concurred to recognize the species-rich genus *Ixora*, as presently circumscribed, to be poly- or paraphyletic. According to our results, both *Captaincookia* and *Myonima* species formed natural lineages within *Ixora*, and the monotypic *Doricera* is sister group to the *Myonima* subclade. The genus *Versteegia* was polyphyletic because *V. cauliflora* was unresolved at the first divergence of Ixoreae, while the second species *V. grandifolia* was included in the Asian-Pacific clade. The results confirmed the previous inclusion of *Hitoa*, *Sideroxyloides* and *Thouarsiora* in *Ixora*. *Cyclophyllum ixoroides*, presumed closely related to *Ixora* based on morphology, was also placed within *Ixora*. Considering the few distinctive characters between the lineages, we propose a broad circumscription of *Ixora* including all other Ixoreae representatives: *Captaincookia*, *Doricera*, *Hitoa*, *Myonima*, *Sideroxyloides*, *Thouarsiora*, and *Versteegia*. The genus enlargement necessitates only 11 combinations or new names in *Ixora*. Within the newly circumscribed genus, several *Ixora* infrageneric taxa are shown to be poly- or paraphyletic. However, these latter interpretations may be more affected by the detected hybridization events and need to be assessed carefully for more nuclear data in the future. Molecular phylogenetic studies like the present one are probably the best approach for tackling species-rich, problematic genera and to clarify their circumscriptions. Such phylogenetic studies, including a wide sample of taxa, can be a good start for later global taxonomic treatments.

LITERATURE CITED

AKAIKE, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.

ALVAREZ, I., AND J. F. WENDEL. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417–434.

ANDERSSON, L. 1992. A provisional checklist of neotropical Rubiaceae. *Scripta Botanica Belgica* 1: 1–199.

ANDREASEN, K., AND B. BREMER. 2000. Combined phylogenetic analysis in the Rubiaceae-Ixoroideae: Morphology, nuclear and chloroplast DNA data. *American Journal of Botany* 87: 1731–1748.

ARÈNES, J. 1960. A propos de quelques genres Malgaches de Rubiacées. *Notulae Systematicae (Paris)* 16: 6–19.

ARNOLD, M. L. 1997. Natural hybridization and evolution. Oxford University Press, Oxford, UK.

BAILEY, C. D., T. G. CARR, S. A. HARRIS, AND C. E. HUGHES. 2003. Characterization of angiosperm nrDNA polymorphism, paralogy, and pseudogenes. *Molecular Phylogenetics and Evolution* 29: 435–455.

BAILLON, H. 1879. Sur les limites du genre *Ixora. Adansonia* 12: 213–219.

BAILLON, H. 1880. Sur le *Cremixora*, nouveau type de Rubiacées. *Bulletin Mensuel de la Société Linnéene de Paris* 1: 265.

BALDWIN, B. G., AND S. MARKOS. 1998. Phylogenetic utility of external transcribed spacer (ETS) of 18S-26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* 10: 449–463.

BARBER, J. C., C. F. COURTNEY, J. FRANCISCO-ORTEGA, A. SANTOS-GUERRA, AND K. JANSEN. 2007. Hybridization in Macaronesian *Sideritis* (Lamiaceae): Evidence from incongruence of multiple independent nuclear and chloroplast sequence data sets. *Taxon* 56: 74–88.

BAUM, D. A. 2007. Concordance trees, concordance factors, and the exploration of reticulate genealogy. *Taxon* 56: 417–426.

BENTHAM, G. 1850. Plantae regnellianae—Rubiaceae. *Linnaea* 23: 443–466.

BREMEKAMP, C. E. B. 1934. A monograph of the genus *Pavetta* L. *Repertorium Species Novae Regni Vegetabile (Fedde's Repertorium)* 37: 1–208.

BREMEKAMP, C. E. B. 1937a. The *Ixora* species of Burma and the Adaman Islands. *Journal of Botany, London* 75: 108–111, 169–175, 260–266, 295–298, 318–326.

BREMEKAMP, C. E. B. 1937b. The Malesian species of the genus *Ixora* (Rub.). *Bulletin du Jardin Botanique de Buitenzorg*, série 3, 14: 197–367.

BREMEKAMP, C. E. B. 1938. The *Ixora* species of Burma and the Adaman Islands. Additions and emendations. *Journal of Botany, London* 76: 330–336.

BREMEKAMP, C. E. B. 1940. The Malesian species of the genus *Ixora* (Rub.). Additions and emendations. *Bulletin du Jardin Botanique de Buitenzorg*, série 3, 16: 276–299.

BREMER, B., K. BREMER, N. HEIDARI, P. ERIXON, R. G. OLMSTEAD, A. A. ANDERBERG, M. KÄLLERJÖ, AND E. BARKHORDARIAN. 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Molecular Phylogenetics and Evolution* 24: 274–301.

BREMER, B., R. K. JANSEN, B. OXELMAN, M. BACKLUND, H. LANTZ, AND K.-J. KIM. 1999. More characters or more taxa for a robust phylogeny in a case study from the coffee family (Rubiaceae). *Systematic Biology* 48: 413–435.

BRIDSON, D. M., AND E. ROBBRECHT. 1985. Further notes on the tribe Pavetteae (Rubiaceae). *Bulletin du Jardin Botanique National Belge* 55: 83–115.

BUCKLER, E. S. I., A. IPPOLITO, AND T. P. HOLTSFORD. 1997. The evolution of ribosomal DNA: Divergent paralogues and phylogenetic implications. *Genetics* 145: 821–832.

CHASE, M. W., AND H. HILLIS. 1991. Silica gel: An ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215–220.

CORNER, E. J. H. 1941. Notes on the systematy and distribution of Malayan phanerogams. IV. *Ixora. Garden's Bulletin*, *Straits Settlements* 11: 177–235.

DAVIS, A. P., M. CHESTER, O. MAURIN, AND M. F. FAY. 2007. Searching for the relatives of *Coffea* (Rubiaceae, Ixoroideae): The circumscription and phylogeny of Coffeeae based on plastid sequence data and morphology. *American Journal of Botany* 94: 313–329.

DE BLOCK, P. 1998. The African species of *Ixora* (Rubiaceae-Pavetteae). *Opera Botanica Belgica* 9: 1–218.

DE BLOCK, P. In press. The Madagascan species of *Ixora* (Rubiaceae). *Opera Botanica Belgica* 16.

DE BLOCK, P., J. TOSH, A. MOULY, AND S. DESSEIN. 2007. The diversity of Madagascan Ixoras revealed, with comments on their origin and their radiation on the islands. XVIIIth AETFAT Congress, 2007. AETFAT, Yaoundé, Cameroun: 23 (Abstract).

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

FARRIS, J. S., V. A. ALBERT, M. KÄLLERSJÖ, D. LIPSCOMB, AND A. G. KLUGE. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladisitcs* 12: 99–124.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.

FITCH, W. M. 1970. Distinguishing homologous from analogous proteins. *Systematic Zoology* 19: 99–113.

FITCH, W. M. 1971. Toward defining the course of evolution: Minimal change for a specific tree topology. *Systematic Zoology* 20: 406–416.

FOSBERG, F. R. 1937. Some Rubiaceae of southeastern Polynesia: *Ixora. Occasional Papers of the Bernice P. Bishop Museum* 13: 265–275.

FOSBERG, F. R. 1942. *Ixora* L. *In* A. C. Smith [ed.], Fijian plants studies, 2. *Sargentia* 1, 124. Arnold Arboretum of Harvard University, Jamaica Plain, Massachussets, USA.

FOSBERG, F. R., AND M.-H. SACHET. 1989a. Lectotypification of *Ixora coccinea* L. (Rubiaceae). *Taxon* 38: 486–489.

FOSBERG, F. R., AND M.-H. SACHET. 1989b. Three cultivated Ixoras. *Baileya* 23: 74–85.

FRIEDMANN, F. 1994. Flore des Seychelles. Dicotylédones. Editions de l'ORSTOM, Institut Français de Recherche Scientifique pour le Développement en Coopération, Paris, France.

FUNK, D. J., AND K. E. OMLAND. 2003. Species-level paraphyly and polyphyly: Frequency, causes and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology Evolution and Systematics* 34: 397–423.

GOVAERTS, R., M. RUHSAM, L. ANDERSSON, E. ROBBRECHT, D. BRIDSON, A. DAVIS, I. SCHANZER, AND B. SONKÉ. 2006. World checklist of Rubiaceae. Board of Trustees of the Royal Botanic Gardens, Kew. Website http://www.kew.org/wcsp/rubiaceae [accessed 11 November 2006].

GRAY, A. 1858. Notes upon some Rubieceae, collected in the United States South Sea exploring expedition under Captain Wilkes, with characters of new species. 3. Ixoreae. *Proceedings of the American Academy of Arts*, *Science* 4: 39–40.

GUÉDÈS, M. 1986. Ixoras malgaches nouveaux à fleurs solitaires. *Phytologia* 60: 243–250.

GUILLAUMIN, A. 1930. Matériaux pour la flore de la Nouvelle-Calédonie. XXVII. Révision des Rubiacées. *Archives de Botaniques, Caen*, 3. *Mémoire* 5: 1–48.

HALLÉ, N. 1973. *Captaincookia*, genre nouveau monotypique néocalédonien de Rubiaceae—Ixoreae. *Adansonia*, série 2, 13: 195–202.

HARPKE, D., AND A. PETERSON. 2006. Non-concerted evolution in *Mammillaria* (Cactaceae). *Molecular Phylogenetics and Evolution* 41: 579–593.

HEGARTY, M. J., AND S. J. HISCOCK. 2005. Hybrid speciation in plants: New insights from molecular studies. *New Phytologist* 165: 411–423.

HITCHCOCK, A. S., AND M. L. GREEN. 1929. Standard-species of Linnean genera of Phanerogamae (1753–1754). *In* T. A. Sprague, J. Rambottom, A. J. Wilmott, and E. M. Wakefield [eds.], International Botanical Congress, Cambridge (England), 1930: Nomencalture: Proposals by British Botanists, 111–199. HMSO, London, UK.

HOCHREUTINER, B. P. G. 1908. *Ixora*. Révisions des Ixoras de Madagascar et îles voisines. *Annales du Conservatoire et du Jardin Botanique de Genève* 11, 12: 100–111.

HUDSON, P. R., AND M. TURELLI. 2003. Stochasticity overrules the "three-times rule" genetic drift, genetic draft, and coalescence times for nuclear loci versus mitochondrial DNA. *Evolution* 57: 182–190.

HUELSENBECK, J. P., B. LARGET, R. E. MILLER, AND F. RONQUIST. 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. *Systematic Biology* 51: 673–688.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics (Oxford, England)* 17: 754–755.

HUSAIN, T., AND S. R. PAUL. 1989. Taxonomic studies on Indian species of the genus *Ixora* L. (Rubiaceae). *Journal of Economic Taxonomy and Botany*, additional series 6, 1–205.

JACQUIN, N. J. 1763. Selectarum stirpium americanarum historia. A. Blumauer, Vienna, Austria.

LINNAEUS, C. VON. 1753. Species plantarum. L. Salvius, Stockholm, Sweden.

LORENCE, D. H., W. R. WAGNER, A. MOULY, AND J. FLORENCE. 2007. Revision of *Ixora* (Rubieace) from the Marquesas Islands (French Polynesia). *Botanical Journal of the Linnean Society* 155: 581–597.

MABBERLEY, D. J. 1997. The plant-book: A portable dictionary of the higher plants, 2nd ed. Cambridge University Press, Cambridge, UK.

MADDISON, W. P., AND L. L. KNOWLES. 2006. Inferring phylogeny despite incomplete lineage sorting. *Systematic Biology* 55: 21–30.

MCDADE, L. 1990. Hybrids and phylogenetic systematics. I. Patterns of character expression in hybrids and their implications for cladistic analysis. *Evolution* 44: 1685–1700.

MORT, M. E., J. K. ARCHIBALD, C. P. RANDLE, N. D. LEVSEN, T. R. O'LEARY, K. TOPALOV, C. M. WIEGAND, AND D. J. CRAWFORD. 2007. Inferring phylogeny at low taxonomic levels: Utility of rapidly evolving cpDNA and nuclear ITS loci. *American Journal of Botany* 94: 173–183.

MOULY, A., AND F. ACHILLE. 2007. The enigmatic *Rhopalobrachium fragrans* transferred to *Cyclophyllum* (Rubiaceae). *Systematic Botany* 32: 883–887.

MOULY, A., B. BREMER, J. FLORENCE, AND J. JÉRÉMIE. 2005. Phylogeny and classification of Ixoreae (Rubiaceae: Ixoroideae). XVII International Botanical Congress, Vienna, Austria, Abstracts: 438 (Abstract).

MOULY, A., AND N. HOANG. 2007. Une espèce nouvelle d'*Ixora* (Rubiaceae) cauliflore de Nouvelle-Calédonie. *Adansonia*, série 3, 29: 123–128.

MOULY, A., S. G. RAZAFIMANDIMBISON, F. ACHILLE, T. HAEVERMANS, AND B. BREMER. 2007. Phylogenetic placement of *Rhopalobrachium fragrans* (Rubiaceae): Evidence from molecular (*rps*16 and *trn*T-F) and morphological data. *Systematic Botany* 32: 872–882.

MOULY, A., S. G. RAZAFIMANBIMBISON, J. FLORENCE, J. JÉRÉMIE, AND B. BREMER. In press. Paraphyly of *Ixora* and new tribal delimitation of Ixoreae (Rubiaceae): Inference from combined chloroplast (*rps*16, *rbc*L, and *trn*T-F) sequence data. *Annals of the Missouri Botanical Garden*.

NADEAUD, J. 1899. Plantes nouvelles des îles de la Société. *Journal of Botany (Morot)* 13: 1–8.

NIXON, K. C., AND J. M. CARPENTER. 1996. On simultaneous analysis. *Cladistics* 12: 221–241.

NYLANDER, J. A. A. 2004. MrModeltest 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.

OCHIENG, J. W., R. J. HENRY, P. R. BAVERSTOCK, D. A. STEANE, AND M. SHEPERD. 2007. Nuclear ribosomal pseudogenes resolve a corroborated monophyly of eucalypt genus *Corymbia* despite misleading hypotheses at functional ITS paralogs. *Molecular Phylogenetics and Evolution* 44: 752–764.

OXELMAN, B., M. LIDÉN, AND D. BERGLUND. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution* 206: 393–410.

PAN, J., D. ZHANG, AND T. SANG. 2007. Molecular phylogenetic evidence for the origin of a diploid hybrid of *Paeonia* (Paeoniaceae). *American Journal of Botany* 94: 400–408.

PUFF, C., K. CHAYAMARIT, AND V. CHAMCHUMROON. 2005. Rubiaceae of Thailand a pictorial guide to indigenous and cultivated genera. The Forest Herbarium, National Park, Wildlife and Plant Conservation Department, Bangkok, Thailand.

RAMBAUT, A. 1996. Se-Al, version 1.dl. Sequence alignment program. Website ftp://evolve.zo.ox.ac.uk/packages/Se-Al-All0a1.hqx.

RAZAFIMANDIMBISON, S. G., AND B. BREMER. 2002. Phylogeny and classification of Naucleeae s.l. (Rubiaceae) inferred from molecular (ITS, *rbcL* and *trnT-F*) and morphological data. *American Journal of Botany* 89: 1027–1041.

RAZAFIMANDIMBISON, S. G., E. A. KELLOGG, AND B. BREMER. 2004. Recent origin and phylogenetic utility of divergent ITS putative pseudogenes: A case study from Naucleeae (Rubiaceae). *Systematic Biology* 53: 177–192.

RAZAFIMANDIMBISON, S. G., H. LANTZ, A. MOULY, AND B. BREMER. In press. Evolutionary trends, major lineages, and new generic limits in the dioecious group of the tribe Vanguerieae (Rubiaceae): Insights into the evolution of functional dioecy. *Annals of the Missouri Botanical Garden*.

RAZAFIMANDIMBISON, S. G., J. MOOG, H. LANTZ, U. MASCHWITZ, AND B. BREMER. 2005. Re-assessment of monophyly, evolution of myrmecophytism, and rapid radiation in *Neonauclea* s.s. (Rubiaceae). *Molecular Phylogenetics and Evolution* 34: 334–354.

RIDSDALE, C. E., R. C. VAN DEN BRINK BAKHUIZEN, AND J. KOEK NOORMAN. 1972. Notes on New Guinean Rubiaceae. *Versteegia* and *Maschalodesme*. *Blumea* 20: 339–348.

SAGHAI-MAROOF, K., M. SOLIMAN, R. A. JORGENSEN, AND R. W. ALLARD. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences, USA* 81: 8014–8018.

SANG, T., AND Y. ZHONG. 2000. Testing hydridization hypotheses based on incongruent gene trees. *Systematic Biology* 49: 422–434.

SCHUMANN, K. 1891. Rubiaceae. *In* A. Engler and K. Prantl [eds.], Die natürlichen Pflanzenfamilien, vol. 4, 1–156. Engelmann, Liepzig, Germany.

SHAW, J., E. B. LICKEY, J. T. BECK, S. B. FARMER, W. LIU, J. MILLER, K. C. SIRIPUN, et al. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142–166.

SIMMONS, M. P. 2004. Independence of alignment and tree search. *Molecular Phylogenetics and Evolution* 31: 874–879.

SMITH, A. C., AND S. P. DARWIN. 1988. Family 168. Rubiaceae. *In* A. C. Smith [ed.], Flora vitiensis nova, vol. 4, 143–362. Pacific Tropical Botanical Garden, Lawai, Kawai, Hawaii, USA.

SOLTIS, D. E., P. S. SOLTIS, AND J. J. DOYLE. 1998. Molecular systematics of plants II. DNA sequencing. Kluwer, Boston, Massachusetts, USA.

STADEN, R. 1996. The Staden sequence analysis package. *Molecular Biotechnology* 5: 233–241.

STAPLES, G. W., AND D. R. HERBST. 2005. A tropical garden flora. Plants cultivated in the Hawaiian Islands and other tropical plants. Bishop Museum Press, Honolulu, Hawaii, USA.

SWOFFORD, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b8. Sinauer, Sunderland, Massachussets, USA.

TABERLET, P. L., L. GIELLY, G. PATOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

TAKAHASHI, K., Y. TERAI, M. NISHIDA, AND N. OKADA. 2001. Phylogenetic relationships and ancient incomplete lineage sorting among cichlid fishes in lake Tanganyika as revealed by analysis of the insertion of retroposons. *Molecular Biology and Evolution* 18: 2057–2066.

TUFFLEY, C., AND M. STEEL. 1997. Links between maximum likelihood and maximum parsimony under a simple model of site substitution. *Bulletin of Mathematical Biology* 59: 581–607.

VALETON, T. 1911. Rubiaceae. *In* H. A. Lorenz [ed.], Résultats de l'expédition scientifique néerlandaise à la Nouvelle-Guinée en 1907 et 1909, vol. 8, Botanique, 437–519 + illustr. Librairie et Imprimerie Ci-Devant E.J. Brill, Leiden, Netherlands.

VALETON, T. 1927. *Versteegia* Val. *In* T. Valeton, Die Rubiaceae von Papuasien. II. *Botanische Jahrbücher* 61: 67–68.

VERDCOURT, B. 1983. Notes on Mascarene Rubiaceae. *Kew Bulletin* 37: 521–574.

VERDCOURT, B. 1989. 108. Rubiacées. *In* J. Bosser, T. Cadet, J. Guého, and W. Marais, Flore des Mascareignes, La Réunion, Maurice, Rodrigues, 1–135. Sugar Industry Research Institute, Port Louis, Mauritius.

WENZEL, J. W., AND M. E. SIDDALL. 1999. Noise. *Cladistics* 15: 51–64.

YANG, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. *Journal of Molecular Evolution* 39: 306–314.

YANG, Z., AND B. RANNALA. 1997. Bayesian phylogenetic inference using DNA sequences: A Markov chain Monte Carlo method. *Molecular Biology and Evolution* 14: 717–724.

ZHENG, X., D. CAI, L. YAO, AND Y. TENG. 2008. Non-concerted ITS evolution, early origin and phylogenetic utility of ITS pseudogenes in *Pyrus*. *Molecular Phylogenetics and Evolution* 48: 892–903.

APPENDIX 1. Accession information for specimens included in the molecular analyses; accession numbers of new sequences are underlined; markers noted NA were not available for the study.

Taxon; locality; *voucher specimen* (herbarium code); GenBank accession numbers: ETS (clones); *rps16*; *trnT-F*.

Aleisanthiopsis distantiflora (Merr.) Tange; Indonesia; *Kessler et al. 41* (P); FJ150425; EU817434; EU817453.

Captaincookia margaretae N.Hallé; New Caledonia; *Mouly A. & Innocente E. 222* (P); FJ150426; EU817436; EU817456. ***Captaincookia* sp. 1**; New Caledonia; *Munzinger J. & Gateblé G. 2148* (NOU); FJ150427; FJ150613; FJ150537. ***Captaincookia* sp. 2**; New Caledonia; *Munzinger J. (leg. Létocart) 2182* (NOU); FJ150428, FJ150429, FJ150430, FJ150431; FJ150614; FJ150538.

Cyclophyllum ixoroides Guillaumin; New Caledonia; *McKee H.S. 33549* (P); NA; FJ150615; FJ150539. ***Cyclophyllum merrillianum*** Guillaumin; New Caledonia; *Mouly A. 150* (P); FJ150432; FJ150616; FJ150540.

Doricera trilocularis (Balf.f.) Verdc.; Rodrigues; *Lesouef 31* (TAN); FJ150433, FJ150434; EU817437; EU817457.

Greenea corymbosa (Jack) K.Schum.; Thailand; *Beusekom et al. 752* (P); FJ150435; EU817438; EU817458.

Hitoa mooreensis Nadeaud; Society Is.; *Florence s.n.* (P); FJ150478; EU817441; EU817462.

Ixora aluminicola Steyerm.; French Guyana; *Prévost 4160* (P); FJ150436, FJ150437, FJ150438, FJ150439; FJ150617; FJ150541. ***Ixora amplidentata*** De Block ined.; Madagascar; *Andrianantoanina et al. 292* (P); NA; FJ150618; FJ150542. ***Ixora bauchiensis*** Hutch. & Dalziel; Cameroun; *Fotius 3047* (P); FJ150440; FJ150619; FJ150543. ***Ixora brachypoda*** DC. 1; Gabon; *Bradley A.F. et al. 1022* (MO); FJ150441; EU817442; EU817463. ***Ixora brachypoda*** DC. 2; Togo; *Hakki et al. 297* (P); NA; FJ150620; FJ150544. ***Ixora brachypoda*** DC. 3; Gabon; *Walters et al. 1437* (MO); FJ150442; FJ150621; FJ150545. ***Ixora brunonis*** Wall. ex G.Don; Thailand; *Larsen K. et al. 43463* (P); FJ150443; EU817446; EU817470. ***Ixora calycina*** Thwaites; Sri Lanka; *Tirvengadum et al. 18* (P); NA; FJ150622; FJ150546. ***Ixora casei*** Hance; Cultivated Tahiti, French Polynesia; *Mouly A. & Florence J. 348* (P); FJ150444; FJ150623; FJ150547. ***Ixora cauliflora*** Montrouz.; New Caledonia; *Mouly A. & Innocente E. 267* (P); FJ150445; FJ150624; FJ150548. ***Ixora chinensis*** Lam.; Cultivated Uppsala, Sweden; no voucher; FJ150446; FJ150625; FJ150549. ***Ixora coccinea*** L.; Cultivated Uppsala, Sweden; *Bremer B. 2719* (UPS); FJ150447, FJ150448; EF205641; EU817464. ***Ixora collina*** (Montrouz.) Beauvis.; New Caledonia; *Mouly A. & Innocente E.* 236 (P); FJ150449; FJ150626; FJ150550. ***Ixora comptonii*** S.Moore; New Caledonia; *Munzinger J. 1606* (NOU); FJ150450; FJ150627; FJ150551. ***Ixora cremixora*** Drake 1; Madagascar; *Leeuwenberg 13879* (P); FJ150451; FJ150628; FJ150552. ***Ixora cremixora*** Drake 2; Madagascar; *Kårehed J. et al. 219* (UPS); FJ150452; NA; NA. ***Ixora diversifolia*** R.Br. ex Kurz; Thailand; *Charoenphol et al. 3719* (P); NA; FJ150629; FJ150553. ***Ixora dzumacensis*** Guillaumin; New Caledonia; *Mouly A. et al. 275* (P); FJ150453; FJ150630; FJ150554. ***Ixora elegans*** Gillepsie; Fiji; *Smith A.C. 9535* (P); NA; FJ150631; FJ150555. ***Ixora elliotii*** De Block ined.; Madagascar; *Dumetz 1175* (P); FJ150454; FJ150632; FJ150556. ***Ixora emirnensis*** Baker; Madagascar; *Lowry P.P II et al. 6018* (MO); FJ150455; FJ150633; FJ150557. ***Ixora fastigiata*** (R.G.Good) Bremek.; de Foresta HF1213 (P); NA, NA, FJ150558. ***Ixora finlaysoniana*** Wall. & G.Don.; Cultivated Tanzania; *Luke G. 9042* (S); FJ150458, FJ150459, FJ150460, FJ150461; EF205643; EU817466. ***Ixora foliosa*** Hiern; Cameroun; *Onana et al. 566* (P); FJ150462; FJ150635; FJ150560. ***Ixora francii*** Schltr. & K.Krause; New Caledonia; *Mouly A. & McPherson G. 126* (P); FJ150463; FJ150636; FJ150561. ***Ixora graciliflora*** Benth.; French Guyana; *De Granville J.J. 1459* (P); FJ150464; FJ150637; NA. ***Ixora guineensis*** Benth. 1; Ghana; *Gereau R.E. et al. 5601* (MO); FJ150465; EU817443; EU817467. ***Ixora guineensis*** Benth. 2; Cameroun; *Cheek 7075* (P); FJ150466; FJ150638; NA. ***Ixora hiernii*** Scott-Elliot; Sierra Leone; *Adam 23101* (P); FJ150467; FJ150639; FJ150562. ***Ixora hookeri*** (Oudem.) Bremek.; Cultivated Tahiti, French Polynesia; *Mouly A. & Florence J. 342* (P); FJ150468; EU817444; EU817468. ***Ixora iteophylla*** Bremek.; Malaysia; *Schaller et al. 3932* (P); FJ150469; FJ150640; FJ150563. ***Ixora javanica*** (Blume) DC.; Laos; *Munzinger J. 119* (P); FJ150519; NA; FJ150602. ***Ixora jourdanii*** Mouly & J.Florence; Marquesas Is.; *Mouly A. 513* (P); FJ150470; FJ150641; FJ150564. ***Ixora killipii*** Standl.; Colombia; *Andersson L. et al. 2160* (GB); NA; AF201001; AF152659. ***Ixora kuakuensis*** S.Moore; New Caledonia; *Munzinger J. 2180* (NOU); FJ150471; FJ150642; FJ150565. ***Ixora lecardii*** Guillaumin; New Caledonia; *Mouly A. et al. 282* (P); FJ150472; FJ150643; FJ150566. ***Ixora longiloba*** Guillaumin; New Caledonia; *Mouly A. 165* (P); FJ150474; FJ150644; FJ150567. ***Ixora marquesensis*** F.Br.; Marquesas Is.; *Mouly A. 504* (P); FJ150475; FJ150645; FJ150568. ***Ixora minutiflora*** Hiern; Gabon; *Hallé F. et al. 4778* (P); FJ150476; FJ150646; FJ150569. ***Ixora mocquerysii*** Aug.DC.; Madagascar; *Malcomber S. 2805* (MO); FJ150477; FJ150647; FJ150570. ***Ixora narcissodora*** K.Schum.; Kenya; *Luke G. 8324* (UPS); FJ150479, FJ150480; FJ150648; FJ150571. ***Ixora nematopoda*** K.Schum.; Cameroun; *Nemba et al. 332* (P); FJ150481; FJ150649; FJ150572. ***Ixora nigricans*** R.Br. ex Wight & Arn.; Thailand; *Larsen K. et al. 43037* (P); FJ150482; FJ150650; FJ150573. ***Ixora nimbana*** Schnell; Liberia; *Adam 26253* (P); FJ150483; FJ150651; FJ150574. ***Ixora oligantha*** Schltr. & K.Krause; New Caledonia; *Mouly A. et al. 296* (P); FJ150484; FJ150652; FJ150575. ***Ixora pavetta*** Andr.; Cultivated Uppsala, Sweden; *FTG P 1738* (UPS); FJ150485; FJ150653; FJ150576. ***Ixora pelagica*** Seem.; Fiji; *Smith A.C. 9288* (P); FJ150486; FJ150654; FJ150577. ***Ixora peruviana*** (Spruce ex K.Schum.) Standl.; Peru; *Plowman et al. 11541* (P); FJ150487; FJ150655; FJ150578. ***Ixora platythyrsa*** Baker; Madagascar; *Antilahimena 94* (P); FJ150488; NA; FJ150579. ***Ixora quadrilocularis*** Capuron ex De Block, ined.; Madagascar; *Capuron R. 23980SF* (P); NA; FJ150656; NA. ***Ixora raiateensis*** J.W.Moore; Society Is.; *Mouly A. et al. 400* (P); FJ150489; FJ150657; FJ150580. ***Ixora scheffleri*** K.Schum. & K.Krause; Tanzania; *Luke G. 9162* (UPS); FJ150490, FJ150491, FJ150492, FJ150493; FJ150658; FJ150581. ***Ixora setchellii*** Fosberg; Society Is.; *Mouly A. et al. 352* (P); FJ150494; FJ150659; FJ150582. ***Ixora siphonantha*** Oliv. 1; Madagascar; *Carlson 45* (P); FJ150495; FJ150660; FJ150583. ***Ixora siphonantha*** Oliv. 2; Madagascar; *Rabenantoandro et al. 944* (MO); FJ150496; FJ150661; FJ150584. ***Ixora* sp. 01**; Madagascar; *Kårehed J. et al. 234* (UPS); FJ150497; FJ150662; FJ150585. ***Ixora* sp. 02**; Madagascar; *Razafimandimbison S.G. et al. 519* (UPS); FJ150498; FJ150663; FJ150586. ***Ixora* sp. 03**; Madagascar; *Andrianjafy et al. 30* (MO); FJ150499; NA; NA. ***Ixora* sp. 04**; Madagascar; *RRH 5* (P); FJ150500; FJ150664; FJ150587. ***Ixora* sp. 05**; Cultivated Stockholm, Sweden; *Mouly s.n.* (P); FJ150501, FJ150502; FJ150665; FJ150588. ***Ixora* sp. 06**; New Caledonia; *Mouly A. & Innocente E. 215* (P); FJ150503; FJ150666; FJ150589. ***Ixora* sp. 07**; New Caledonia; *Mouly A. & Innocente E. 272* (P); FJ150504; FJ150667; FJ150590. ***Ixora* sp. 08**; Society Is.; *Florence J. 3936* (P); FJ150505; FJ150668; FJ150591. ***Ixora* sp. 09**; New Caledonia; *Dagostini G. 859* (NOU); FJ150506; FJ150669; FJ150592. ***Ixora* sp. 10**; Asia; *Martin 1314* (P); FJ150507, FJ150508, FJ150509; FJ150670; FJ150593. ***Ixora* sp. 11**; Thailand; *Geesink et al. 7226* (P); FJ150510; FJ150671; FJ150594. ***Ixora* sp. 12**; Thailand; *Vidal 5771* (P); NA; FJ150672; FJ150595. ***Ixora* sp. 13**; Thailand; *Vidal 5758B* (P); FJ150511, FJ150512, FJ150513, FJ150514; FJ150673; FJ150596. ***Ixora* sp. 14**; Cultivated Costa Rica; *Nissen 89BIO01344 DN89* (UPS); FJ150515; FJ150674; FJ150597. ***Ixora* sp. 15**; Vietnam; *Poilane 103* (P); NA; FJ150675; FJ150598. ***Ixora* sp. 16**; Brunei; *Malcomber S. et al. 2980* (MO); FJ150516; FJ150676; FJ150599. ***Ixora* sp. 17**; Thailand; *Larsen K. et al. 86KL14* (UPS); FJ150517; FJ150677; FJ150600. ***Ixora st-johnii*** Fosb.; Society Is.; *Mouly A. 450* (P); FJ150518; FJ150678; FJ150601. ***Ixora tanzaniensis*** Bridson; Tanzania; *Luke G. 9304* (UPS); FJ150520, FJ150521, FJ150522, FJ150523; EU817447; EU817471. ***Ixora tenuis*** De Block; Ghana; *Hall et al. 46573* (P); FJ150524; FJ150679; FJ150603. ***Ixora triantha*** Volkens; Mariannas Is.; *Stone 16019* (P); FJ150525; FJ150680; FJ150604. ***Ixora trichocalyx*** Hochr.; Madagascar; *Schatz et al. 3515* (P); FJ150526; FJ150681; FJ150605. ***Ixora vieillardii*** Guillaumin; New Caledonia; *Mouly A. et al. 322* (P); FJ150527; FJ150682; FJ150606. ***Ixora yaouhensis*** Schltr.; New Caledonia; *Mouly A. 153* (P); FJ150528; FJ150683; FJ150607.

Myonima nitens (Poir.) Verdc.; Mascarene Is.; *Friedmann F. 2631* (P); FJ150529; FJ150684; FJ150608. ***Myonima obovata*** Lam.; Mascarene Is.; *Friedmann F. 3049* (P); FJ150530; FJ150685; FJ150609. ***Myonima* sp.**

01; Mascarene Is.; *Andrianbololonera S. 47* (TAN); FJ150531, FJ150532; FJ150686; FJ150610. ***Myonima violacea*** (Lam.) Verdc.; Mascarene Is.; *Lorence D.L. 1526* (P); FJ150533; EU817449; EU817473.

Sideroxyloides ferreum Jacq. 1; Carribean Is.; *Taylor C. 11693* (UPS); FJ150456; EF205642; EU817465. ***Sideroxyloides ferreum*** Jacq. 2; Puerto Rico.; *Axelrod et al. 1515* (P); FJ150457; FJ150634; FJ150559.

Thouarsiora littoralis Homolle ex Arènes; Madagascar; *McPherson G. & Rabenantoandro J. 18287* (MO); FJ150473; EU817445; EU817469.

Versteegia cauliflora (K.Schum & Lauterb.) Valeton; Cultivated Bogor, Indonesia; *Drodz & Molem s.n*. (UPS); FJ150534, FJ150535; EU817451; EU817476. ***Versteegia grandifolia*** Valeton; Cultivated Bogor, Indonesia; *Ridsdale s.n.* (UPS); FJ150536; FJ150687; FJ150611.

APPENDIX 2. A priori assignation of ingroup species to infrageneric groups of *Ixora* based on literature and morphological limits presented in Table 2, to test infrageneric classifications proposed in the past for *Ixora* by optimization on the phylogram of the tree topology presented in Fig. 6 (see Fig. 7).

Infrageneric group: species list.

Subgenus *Ixora*:

Section *Brachypus*: *Ixora nigricans* R.Br. ex Wight & Arn., *Ixora tenuis* De Block.

Section *Chlamydanthus*: *Ixora finlaysoniana* Wall & G.Don.

Section *Cremixora*: *Ixora cremixora* Drake 1, *Ixora cremixora* Drake 2.

Section *Ixora*: *Ixora bauchiensis* Hutch. & Dalziel, *Ixora brunonis* Wall. ex G.Don, *Ixora casei* Hance, *Ixora chinensis* Lam., *Ixora coccinea* L., *Ixora elliotii* De Block ined., *Ixora emirnensis* Baker, *Ixora graciliflora* Benth., *Ixora foliosa* Hiern, *Ixora guineensis* Benth. 1, *Ixora guineensis* Benth. 2, *Ixora hiernii* Scott-Elliot, *Ixora iteophylla* Bremek., *Ixora javanica* (Blume) DC., *Ixora killipii* Standl., *Ixora minutiflora* Hiern, *Ixora narcissodora* K.Schum., *Ixora pavetta* Andr., *Ixora* sp. 01, *Ixora* sp. 02, *Ixora* sp. 05, *Ixora* sp. 10, *Ixora* sp. 11, *Ixora* sp. 14, *Ixora* sp. 15, *Ixora* sp. 16, *Ixora* sp. 17, *Ixora tanzaniensis* Bridson, *Ixora trichocalyx* Hochr.

Section *Myonima*: *Myonima nitens* (Poir.) Verdc., *Myonima obovata* Lam., *Myonima violacea* (Lam.) Verdc., *Myonima* sp. 1.

Section *Micrixora*: *Ixora aluminicola* Steyerm., *Ixora peruviana* (Spruce ex K.Schum.) Standl.

Section *Otobactrum*: *Ixora amplidentata* De Block ined., *Ixora brachypoda* DC. 1, *Ixora brachypoda* DC. 2, *Ixora brachypoda* DC. 3, *Ixora fastigiata* (R.G.Good) Bremek., *Ixora hookeri* (Oudem.) Bremek., *Ixora mocquerysii* Aug.DC., *Ixora nematopoda* K.Schum., *Ixora nimbana* Schnell, *Ixora platythyrsa* Baker, *Ixora scheffleri* K.Schum. & K.Krause, *Ixora siphonantha* Oliv. 1, *Ixora siphonantha* Oliv. 2, *Ixora* sp. 03, *Ixora* sp. 04, *Ixora* sp. 12, *Ixora* sp. 13.

Subgenus *Pavettoides*:

Section *Amphorion*: *Ixora calycina* Thwaites.

Section *Pavettopsis*: *Ixora elegans* Gillepsie, *Ixora francii* Schltr. & K.Krause, *Ixora lecardii* Guillaumin, *Ixora raiateensis* J.W.Moore, *Ixora* sp. 08, *Ixora* sp. 09, *Ixora vieillardii* Guillaumin, *Ixora yaouhensis* Schltr.

Section *Raphidanthus*: *Ixora diversifolia* R.Br. ex Kurz, *Ixora triantha* Volkens.

Section *Vitixora*: *Ixora comptonii* S.Moore, *Ixora longiloba* Guillaumin, *Ixora pelagica* Seem.

Not assigned to subgeneric group:

Section *Microthamnus*: *Ixora dzumacensis* Guillaumin.

Section *Phylleilema*: *Ixora collina* (Montrouz.) Beauvis., *Ixora jourdanii* Mouly & J.Florence, *Ixora marquesensis* F.Br., *Ixora oligantha* Schltr. & K.Krause, *Ixora setchellii* Fosberg, *Ixora* sp. 06, *Ixora* sp. 07, *Ixora st-johnii* Fosb.

Not assigned to section: *Cyclophyllum ixoroides* Guillaumin, *Captaincookia margaretae* N.Hallé, *Captaincookia* sp. 1, *Captaincookia* sp. 2, *Doricera trilocularis* (Balf.f.) Verdc., *Hitoa mooreensis* Nadeaud, *Ixora cauliflora* Montrouz., *Ixora kuakuensis* S.Moore, *Ixora quadrilocularis* Capuron ex De Block ined., *Sideroxyloides ferreum* Jacq. 1, *Sideroxyloides ferreum* Jacq. 2, *Thouarsiora littoralis* Homolle ex Arènes, *Versteegia cauliflora* (K.Schum & Lauterb.) Valeton, *Versteegia grandifolia* Valeton.