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PHYLOGENETIC ANALYSIS OF THE LYTHRACEAE BASED ON FOUR GENE REGIONS AND MORPHOLOGY

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The family limits of the Lythraceae and relationships among the ca. 31 genera remain poorly known in spite of previous phylogenetic studies. We use morphology and DNA sequences from the *rbc*L gene, the *trn*L-F region, and *psa*A-*ycf*3 intergenic spacer of the chloroplast and the internal transcribed spacer region of the nucleus to explore relationships for up to 27 genera of the Lythraceae *sensu stricto* and the monogeneric families Duabangaceae, Punicaceae, Sonneratiaceae, and Trapaceae. Maximum parsimony, maximum likelihood, and Bayesian likelihood approaches are employed. Morphology alone provided little phylogenetic resolution. Results from individual gene regions were relatively well resolved and largely congruent, whereas basal relationships were poorly supported. A combined gene analysis of 20 genera produced one fully resolved maximum parsimony tree that corresponded closely to the maximum likelihood and Bayesian trees in which a monophyletic Lythraceae includes *Duabanga*, *Punica*, *Sonneratia*, and *Trapa* as derived genera within the family. *Decodon* is sister to the rest of the family in the maximum parsimony and Bayesian trees, followed by *Lythrum* and *Peplis* at the node above and then by the rest of the family, which diverges into two superclades. In the maximum likelihood tree, the family diverges at the base into two superclades. Six crown clades are well supported but lack unique morphological synapomorphies. Neither superclades nor crown clades correspond to the present tribal and subtribal classification. The New World genera are embedded in four Old World clades. If the basal and near-basal positions for the Northern Hemisphere genera *Decodon*, *Lythrum*, and *Peplis* are confirmed, the origin of the family is more likely Laurasian than Gondwanan. The biogeography and early fossil record of the family indicate that the family originated by the late Cretaceous and had extensively diversified and radiated into Southeast Asia and North America by the lower Eocene.

Keywords: chloroplast *rbc*L, *trn*L-F, *psa*A-*ycf*3, *Duabanga*, nuclear ITS, Lythraceae, phylogeny, *Punica*, *Sonneratia*, *Trapa*.

Introduction

The Lythraceae are a moderate-sized family (Myrtales) that, when broadly delimited, include ca. 31 genera and 600 species occurring worldwide (fig. 1; Graham et al. 1993*a*). As traditionally circumscribed (Koehne 1903), the family comprises 28 genera and is easily recognized by a suite of characters: opposite entire leaves; a persistent, perigynous, campanulate to tubular floral tube with crinkled petals inserted at the rim; two whorls of stamens inserted deep in the tube; and a many-seeded capsular fruit. The genera are clearly delimited and are regarded as monophyletic with the exception of three possible congeneric pairs *Ammannia-Nesaea*, *Lythrum-Peplis* (Graham et al. 1993*a*), and *Ginoria-Haitia* (S. A. Graham, personal observation). Most genera are woody shrubs or small trees, but eight are herbs. The four largest genera, *Cuphea*, *Diplusodon*, *Lagerstroemia*, and *Nesaea*, account for three-fourths of all the species, whereas 17 genera (61% of the family) are mono- or ditypic. Of some economic importance are *Lagerstroemia*, the cultivated crape myrtle; *Lawsonia*, the source of henna dye; and *Cuphea*, a source of specialized seed oils and horticultural garden plants.

The Lythraceae are found today on all major continents except Antarctica, raising biogeographic questions about place and time of origin of the family and paths of radiation and generic diversification. The family is geographically divided between the Old World (18 genera) and the New World (13 genera), with the greatest concentration of genera in tropical America and Africa and poor representation in the northern latitudes. In the northern latitudes, *Lythrum* and *Peplis* occur across both hemispheres, and *Didiplis* and *Decodon* are restricted to eastern North America, although *Decodon* once ranged widely in Europe and Asia (Little and Stockey 2003). Cross-continental sister relationships have been hypothesized on morphological grounds for several genera: for example, between *Ginoria* in the Caribbean and *Tetrataxis* on Mauritius (Koehne 1886, 1903; Graham 2002); between *Didiplis* in the eastern United States and the European genera *Lythrum* and *Peplis* (Koehne 1903; Graham et al. 1993*a*); and between South American *Adenaria* and *Pehria* and the Afro-Asian genus *Woodfordia* (Graham 1995*b*). These projected relationships and the phylogenetic

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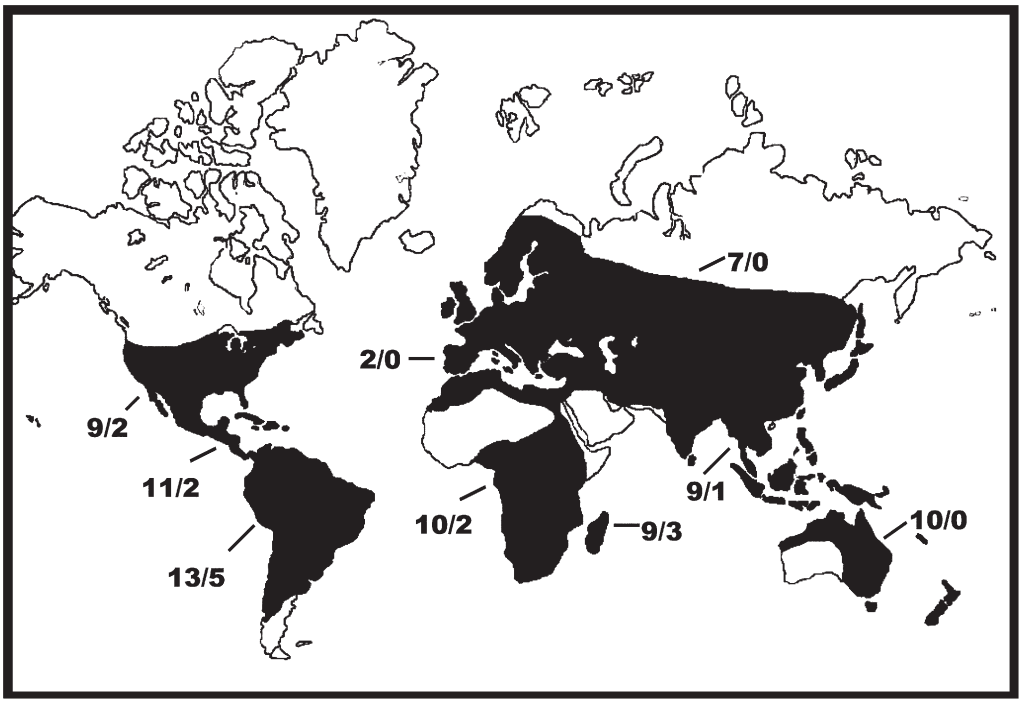

Fig. 1 Distribution of the Lythraceae, with the number of genera/number of endemic genera indicated for the following areas: United States and Canada; Mexico, Central America, and the Antilles; South America; western Europe; eastern Europe and northern Asia, including Japan; Africa; Madagascar and Mauritius; southeastern Asia and the Pacific Islands; Australia.

limits of the family based on morphological cladistic analysis (Johnson and Briggs 1984; Graham et al. 1993*a*) have yet to be fully tested by an independent molecular data source.

The family was monographed by Koehne (1881, 1903), who considered the semi-inferior and inferior ovary monogeneric families Duabangaceae, Punicaceae, and Sonneratiaceae outside the limits of a perigynous-flowered Lythraceae. Dahlgren and Thorne (1984) suggested that the three genera be treated as subfamilies of the Lythraceae, a suggestion supported by Johnson and Briggs (1984), who determined in a cladistic analysis of morphological characters for the order Myrtales that a monophyletic Lythraceae must include *Duabanga*, *Punica*, and *Sonneratia*. They also found the Onagraceae and Trapaceae to be sisters to the Lythraceae s.l., although their findings were weakly supported cladistically. Morphological cladistic analyses of an expanded Lythraceae produced two major but very weakly supported clades (Graham et al. 1993*a*). One comprised *Duabanga*, *Punica*, and *Sonneratia*, together with *Lagerstroemia* and *Lawsonia* of the traditional Lythraceae; the other clade included the remainder of the Lythraceae, with *Decodon-Pemphis* at the base of the clade and sister to the rest of the genera. No morphological synapomorphies uniquely defined the expanded family.

The enigmatic, monogeneric Trapaceae have long been considered either a separate family close to the Lythraceae and Onagraceae or within the Onagraceae (Cronquist 1981; Dahlgren and Thorne 1984). The exact relationship of the Trapaceae has remained uncertain because the morphology is too divergent to allow direct comparisons with other taxa (Miki 1959; Vasil'ev 1967; Tobe and Raven 1983). Conti et al. (1996, 1997) found *Trapa* to be sister to *Lythrum* in a chloroplast *rbc*L study of the Myrtales that included 10 members of the Onagraceae and seven genera of the Lythraceae but not *Sonneratia*. Huang and Shi (2002), comparing sequences of three gene regions for 16 genera of the Lythraceae, found *Trapa* to be sister to *Sonneratia*, a surprising relationship never previously suggested. They also found good molecular support for four clades of two to four genera but weak support at deeper nodes in the phylogeny. We evaluate these findings by comparison of other gene regions and additional sampling.

The current taxonomic classification of the Lythraceae (Koehne 1881) divides the narrowly circumscribed family into two tribes, each with two subtribes (table 1). The classification is highly artificial, with the tribes and subtribes delimited by few nonexclusive characters. Further, Tobe et al. (1998) found the defining feature of the tribes, the presence or absence of complete septal walls in the ovary, to be erroneous. Anatomical sections revealed incomplete septal walls at the apex of the placenta in all genera, negating the sole basis for the primary division of the family. A morphological cladistic analysis of the Lythraceae did not recover clades equivalent to the tribal or subtribal groupings or produce well-supported alternative relationships (Graham et al. 1993*a*).

In this study, we analyze sequence variation from chloroplast *rbc*L, the *trn*L-F intergenic spacer and *psa*A-*ycf*3 spacer regions, and nuclear rDNA internal transcribed spacer (ITS) for up to 27 genera of Lythraceae s.l. and Trapaceae, using maximum parsimony, maximum likelihood, and Bayesian

Table 1

Classification of the Lythraceae Based on Koehne (1903), Excluding Unnamed Series

Classification and genera	Defining characters
Subfamily Sonneratioideae:	
Sonneratia	
Subfamily Duabangoideae:	
Duabanga	
Subfamily Punicoideae:	
Punica	
Subfamily Lythroideae (Lythraceae *sensu stricto*):	
Tribe *Lythreae*:	Septum of the ovary interrupted or split above the placenta; the placenta is thus not continuous with the style
Subtribe *Lythrinae*:	
Rotala, *Ammannia*, [*Hionanthera*], *Peplis*, *Didiplis*, *Lythrum*, *Woodfordia*, *Cuphea*, *Pleurophora*	Seeds without a margin or, when margined, the flowers always zygomorphic
Subtribe *Diplusodontinae*:	
Galpinia, *Pemphis*, [*Capuronia*], *Diplusodon*, *Physocalymma*, *Lafoensia*	Seeds dorsally compressed and encircled by a wing; placenta of the mature fruit strongly depressed, basal; flowers always actinomorphic
Tribe *Nesaeeae*:	Septum of the ovary complete, the placenta thus continuous with the style; flowers always actinomorphic
Subtribe *Nesaeinae*:	
Crenea, *Nesaea*, *Heimia*, *Decodon*, *Pehria*, *Adenaria*, [*Koehneria*], [*Lourtella*], *Tetrataxis*, *Ginoria*, [*Haitia*]	Seed coat neither thickened nor winged
Subtribe *Lagerstroemiinae*:	
Lagerstroemia, *Lawsonia*	Seed coat either extended as a wing or the apex truly spongy

Note. Subfamilies were added by Dahlgren and Thorne (1984). Genera in brackets were described after 1903.

approaches. A morphological cladistic analysis of the expanded family is presented to allow comparison of morphological and molecular phylogenetic hypotheses. The evolution of selected morphological characters of prospective taxonomic utility is examined. Our main objectives are (1) to test molecular support for the hypothesis that a monophyletic Lythraceae includes the former satellite families Duabangaceae, Punicaceae, Sonneratiaceae, and Trapaceae; (2) to produce a generically more inclusive molecular phylogeny with stronger support of the lineages; (3) to compare phylogenetic results to the current taxonomic structure; and (4) to identify character states that describe major clades uncovered in the study. The biogeography and early fossil record of the family are briefly reviewed. The more inclusive phylogeny of the family generated by the enlarged data set is a step toward future reconstruction of the historical biogeography of this widespread family and a new classification that more closely reflects phylogenetic relationships.

Material and Methods

Sampling and DNA Sequencing

Sources and GenBank accession numbers used in the molecular study are provided (table 2). Of the 165 sequences utilized, 93 are new to this study. All but four genera of the family are represented by at least one molecular sequence; missing are *Didiplis*, *Crenea*, *Haitia*, and *Hionanthera*. Total genomic DNA was extracted from 1.0 g fresh, liquid nitrogen–frozen, or silica gel–dried leaves. Most DNA was extracted with 6× CTAB following methods cited by Smith et al. (1991). The higher percentage of CTAB and precipitation with ethanol and sodium chloride reduced problematic DNA-polysaccharide complexing, which is extreme in many Lythraceae. Cleaner, more easily amplified DNA was obtained from liquid nitrogen–frozen ground leaves stored for one or more years, possibly because the products complexed with the DNA degraded over time. For later extractions, we used DNeasy kits (Qiagen, Valencia, CA), following manufacturer's directions.

Sequences of *rbc*L employed the primers, equipment, and procedures detailed by Conti et al. (1996). Polymerase chain reaction amplification and cycle sequencing followed Conti et al. (1996). Addition of DMSO enhanced results in the *rbc*L sequencing reactions. Sequences of *rbc*L generated by Y.-L. Huang and S.-H. Shi were obtained using the same primers used by Conti et al. (1996). Sequence length was 1428 bp, except that sequences by Huang and Shi terminated at position 1208. Sequences of the *trn*L-F chloroplast intergenic spacer region, consisting of partial sequences of the *trn*L gene, the intergenic spacer between the *trn*L and *trn*F, and partial *trn*F sequences, were generated by J. Hall, with additions by Huang using Taberlet et al.'s (1991) universal primers c, d, e, and f. The first 32 and final 45 base pairs were excluded from all analyses to eliminate ambiguous alignments and excessive missing data. Sequences of ITS (18s rRNA, ITS-1, 5.8s gene, and ITS-2) and the intergenic spacer between the chloroplast genes *psa*A and *ycf*3 were contributed by Huang and Shi (2002) with ITS additions by J. Hall and S. A. Graham. The amplification and sequencing primers are listed in Huang and Shi (2002). The *psa*A-*ycf*3 primers included the 5′ end of the *psa*A gene, the spacer, and the 3′ end

Table 2

Species, GenBank Accession Numbers, and Vouchers

Taxon and gene region	GenBank accession	Locality and voucher[a]
Adenaria floribunda Kunth:		
*rbc*L[c]	AY905403	Mexico; Breedlove 38133 (CAS)
*trn*L-F[c]	AY905451	Mexico; Breedlove 38133 (CAS)
Ammannia baccifera L.:		
*rbc*L[b]	AY036145	China; Tang 99010301 (SYS)
*trn*L-F[bc]	AY905452	China; Tang 99010301 (SYS)
ITS[bc]	AY905419	China; Tang 99010301 (SYS)
*psa*A[bc]	AY905434	China; Tang 99010301 (SYS)
Ammannia latifolia L.:		
*rbc*L[c]	AY905404	Puerto Rico; Liogier 10314 (MO)
*trn*L-F[c]	AY905453	Puerto Rico; Liogier 10314 (MO)
Baeckea ramosissima A. Cunn.:		
*rbc*L	AF489376	Conti 103 (WIS)
Capuronia madagascariensis Lour.:		
*rbc*L[bc]	AY905405	Madagascar; D'Arcy 15439 (MO)
*trn*L-F[bc]	AY905454	Madagascar; D'Arcy 15439 (MO)
ITS[bc]	AY905420	Madagascar; D'Arcy 15439 (MO)
*psa*A[bc]	AY905435	Madagascar; D'Arcy 15439 (MO)
Clarkia xantiana A. Gray:		
*rbc*L	L10225	USA; Gottlieb 7436 (DAV)
Combretum paniculatum Vent.:		
*trn*L[bc]	AY905455	Africa; J. Hall s.n. (WIS)
Combretum wallichii DC.:		
*rbc*L[b]	AY036151	China; Shi 990703005 (SYS)
ITS[b]	AF208731	China; Shi 990703005 (SYS)
*psa*A[b]	AY035743	China; Shi 990703005 (SYS)
Cuphea hookeriana Walp.:		
ITS	AF201691	China; Tang 99070501 (SYS)
*psa*A	AY035723	China; Tang 99070501 (SYS)
Cuphea lanceolata Ait.:		
*rbc*L[b]	AY036145	China; Tang 99010301 (SYS)
ITS[b]	AY035754	China; Tang 99010301 (SYS)
*psa*A[b]	AY035723	China; Shi 99090201 (SYS)
Cuphea llavea Lex.:		
*rbc*L	AF495773	Mexico; Graham 1024 (MO)
Cuphea utriculosa Koehne:		
*trn*L-F[bc]	AY905456	Mexico; Graham 1086 (MO)
Decodon verticillatus (L.) Ell.:		
*rbc*L[bc]	AY905406	USA; Graham 917 (MO)
*trn*L-F[bc]	AY905457	USA; Graham 917 (MO)
ITS[bc]	AY905421	USA; Graham 917 (MO)
*psa*A[bc]	AY905436	USA; Graham 917 (MO)
Diplusodon glabrescens DC.:		
ITS[c]	AY905422	Shi 871 (SYS)
*psa*A[c]	AY905442	Shi 871 (SYS)
Diplusodon paraisoensis Lour.:		
*trn*L-F[c]	AY905458	Brazil; Cavalcanti et al. 2210 (CEN)
Duabanga grandiflora (DC.) Walp.:		
*rbc*L[c]	AY905407	Thailand; Maxwell s.n. in 1991, no voucher
*trn*L-F	AF354179	China; Ge et al. s.n., no voucher
ITS[b]	AF163695	China; Huang 990401 (SYS)
*psa*A[b]	AY035737	China; Huang 990401 (SYS)
Duabanga moluccana Blume:		
*rbc*L[bc]	AY905408	Sarawak; Chai s.n. in 1990 (MO)
*trn*L-F[bc]	AY905459	Sarawak; Chai s.n. in 1990 (MO)
Epilobium angustifolium L.:		
*rbc*L	L10217	USA; Sytsma 5012 (WIS)
Francoa sonchifolia Cav.:		
*rbc*L	L11184	USA; Soltis & Soltis 2479 (WIS)

Table 2

(***Continued***)

Taxon and gene region	GenBank accession	Locality and voucher[a]
Fuchsia cyrtandroides J.W. Moore:		
*rbc*L[b]	L10220	Venezuela; Berry 4618 (MO)
*trn*L-F[bc]	AY905460	Venezuela; Berry 4618 (MO)
Fuchsia hybrid:		
ITS[b]	AY035748	China; Jian 20010207 (SYS)
*psa*A[b]	AY035748	China; Jian 20010207 (SYS)
Galpinia transvaalica N.E. Brown:		
*rbc*L[bc]	AY905409	Africa; Balsinhas 3263 (MO)
*trn*L-F[bc]	AY905461	Africa; Balsinhas 3263 (MO)
ITS[bc]	AY905423	Africa; Balsinhas 3263 (MO)
*psa*A[bc]	AY905443	Africa; Balsinhas 3263 (MO)
Geranium cinereum Cav.:		
*rbc*L	L14695	USA; R. Prince s.n. (IND)
Ginoria americana Jacq.:		
ITS[bc]	AY078421	Cuba; Graham 1127 (MO)
*psa*A[bc]	AY905437	Cuba; Graham 1127 (MO)
Ginoria glabra Griseb.:		
*trn*L-F[bc]	AY905462	Cuba; Fairchild Garden s.n. (MO)
Ginoria nudiflora (Hemsl.) Koehne:		
*rbc*L[bc]	AY078418	Mexico; Gutierrez 3098 (MO)
Heimia salicifolia Link:		
*rbc*L[bc]	AY905410	Paraguay; Pérez 1070 (MO)
*trn*L-F[bc]	AY905463	Paraguay; Pérez 1070 (MO)
Heimia montana (Griseb.) Lillo:		
*trn*L-F[c]	AY905464	Bolivia; Solomon 10509 (MO)
Heimia myrtifolia Cham. & Schtldl.:		
ITS[b]	AF201693	China; Tang 99070502 (SYS)
*psa*A[b]	AY035735	China; Tang 99070502 (SYS)
Koehneria madagascariensis S.A. Graham:		
*trn*L-F[c]	AY905465	Madagascar; D'Arcy & Rakotozfy 15317 (MO)
ITS[c]	AY905424	Madagascar; D'Arcy & Rakotozfy 15317 (MO)
*psa*A[c]	AY905444	Madagascar; D'Arcy & Rakotozfy 15317 (MO)
Lafoensia acuminata (Ruiz & Pav.) DC.:		
*rbc*L[bc]	AY905411	Ecuador; Neil 8930 (MO)
*trn*L-F[bc]	AY905466	Ecuador; Neil 8930 (MO)
ITS[bc]	AY905425	Ecuador; Neil 8930 (MO)
*psa*A[bc]	AY905438	Ecuador; Neil 8930 (MO)
Lagerstroemia indica L.:		
*rbc*L[c]	AY905412	USA; Cult., no voucher
*trn*L-F[c]	AY905467	USA; Cult., no voucher
Lagerstroemia speciosa (L.) Pers.:		
*rbc*L[b]	AY036149	China; Shi 99060103 (SYS)
*trn*L-F[bc]	AY905468	China; Shi 99060103 (SYS)
ITS[b]	AF163696	China; Shi 99060103 (SYS)
*psa*A[b]	AY035737	China; Shi 99060103 (SYS)
Lagerstroemia villosa S. Kurz:		
*trn*L-F[c]	AY905469	China; Shi 2000-01037 (SYS)
ITS	AY035755	China; Shi 2000-01037 (SYS)
*psa*A	AY035739	China; Shi 2000-01037 (SYS)
Lawsonia inermis L.:		
*rbc*L[bc]	AY905413	Bahamas; Correll 45915 (TEX)
*trn*L-F[bc]	AY905470	Bahamas; Correll 45915 (TEX)
ITS[bc]	AY905426	Shi 494 (SYS)
*psa*A[bc]	AY905439	Shi 494 (SYS)
Lopezia langmaniae Miranda:		
*trn*L-F[c]	AY905471	Sytsma s.n. (WIS)
Lourtella resinosa S.A. Graham et al.:		
ITS[c]	AY905427	Bolivia; Graham 1116 (MO)
*psa*A[c]	AY905445	Bolivia; Graham 1116 (MO)

Table 2

(***Continued***)

Taxon and gene region	GenBank accession	Locality and voucher[a]
Ludwigia hyssopifolia (G. Don f.) Exell:		
*rbc*L[b]	AY036152	China; Yuan 2000-72401 (SYS)
*trn*L-F[bc]	AY905472	China; Yuan 2000-72401 (SYS)
ITS[b]	AY035747	China; Yuan 2000-72401 (SYS)
*psa*A[b]	AY035745	China; Yuan 2000-72401 (SYS)
Ludwigia peploides (Kunth) Raven:		
*rbc*L	L10222	USA; Sytsma 5010 (WIS)
*trn*L-F[c]	AY905473	USA; Sytsma 5010 (WIS)
Lythrum hyssopifolia L.:		
*rbc*L[b]	L10218	USA; Baldwin 500 (DAV)
ITS[bc]	AY905428	Canada: Johnson s.n. in 1998 (MO)
*psa*A[bc]	AY905440	Canada: Johnson s.n. in 1998 (MO)
Lythrum maritimum Kunth:		
*trn*L-F[bc]	AY905474	Hawaii; Graham 1098 (MO)
Lythrum salicaria L.:		
*rbc*L	AF421496	China; Tsang 27844 (IBSC)
ITS	AY035749	China; Lei 005 (SYS)
*psa*A	AY035727	China; Lei 005 (SYS)
Mouriri cyphocarpa Standl.:		
*rbc*L	U26327	Nepokroeff & Hammel 724 (WIS)
Myrcianthes fragrans (Sw.) McVaugh:		
*rbc*L	U26328	Conti 108 (WIS)
Nesaea aspera (Guill. & Perr.) Koehne:		
*rbc*L[bc]	AY905414	Africa; Drummond 11446 (MO)
*trn*L-F[bc]	AY905475	Africa; Drummond 11446 (MO)
ITS[bc]	AY905429	Africa; Drummond 11446 (MO)
*psa*A[bc]	AY905441	Africa; Drummond 11446 (MO)
Oenothera elata Kunth:		
*rbc*L	NC002693	Not available
Pehria compacta (Rusby) Sprague:		
*trn*L-F[c]	AY905476	Venezuela; Berry s.n. in 1979 (MO)
ITS[c]	AY905430	Venezuela; Berry s.n. in 1979 (MO)
*psa*A[c]	AY905446	Venezuela; Berry s.n. in 1979 (MO)
Pemphis acidula Forst. 1:		
*rbc*L[b]	AY036138	China; Liao 1150 (A)
ITS[b]	AY035762	China; Liao 1150 (A)
*psa*A[b]	AY035725	China; Liao 1150 (A)
Pemphis acidula Forst. 2:		
*trn*L-F[bc]	AY905477	Marshall Islands; Vandervelde s.n. in 2000, no voucher
Peplis portula L. 1:		
*rbc*L[bc]	AY036139	Portugal; Montezuma s.n. (MO)
*trn*L-F[c]	AY905478	Portugal; Montezuma s.n. (MO)
ITS[b]	AY035751	Portugal; Montezuma s.n. (MO)
*psa*A[b]	AY035726	Portugal; Montezuma s.n. (MO)
Peplis portula L. 2:		
*rbc*L[c]	AY905415	Portugal; Montezuma s.n. (MO)
Physocalymma scaberrimum Pohl 1:		
*trn*L-F[c]	AY905479	Brazil; Cavalcanti et al. 2512 (CEN)
ITS[c]	AY905431	Brazil; Cavalcanti et al. 2512 (CEN)
*psa*A[c]	AY905447	Brazil; Cavalcanti et al. 2512 (CEN)
Physocalymma scaberrimum Pohl 2:		
*trn*L-F[c]	AY905480	Shi 861 (SYS)
ITS[c]	AY905432	Shi 861 (SYS)
*psa*A[c]	AY905448	Shi 861 (SYS)
Pleurophora anomala (St.-Hil.) Koehne:		
*rbc*L[c]	AY905416	Brazil; Roath s.n. (MO)
*trn*L-F[c]	AY905481	Brazil; Cavalcanti et al. 368 (MO)
Pleurophora pungens Don:		
ITS	AF268395	China; Huang s.n., no voucher

Table 2

(***Continued***)

Taxon and gene region	GenBank accession	Locality and voucher[a]
Punica granatum L. 1:		
*rbc*L[b]	L10223	USA; Cult., Conti 1001 (WIS)
*trn*L[bc]	AY905482	USA; Cult., Conti 1001 (WIS)
Punica granatum L. 2:		
*trn*L[c]	AY905483	China; Hao 20000318 (SYS)
ITS[b]	AY035760	China; Hao 20000318 (SYS)
*psa*A[b]	AY035724	China; Hao 20000318 (SYS)
Punica granatum L. 3:		
ITS	AY035761	China; Wang 00-041602 (SYS)
*psa*A	AY035742	China; Wang 00-041602 (SYS)
Quisqualis indica L.:		
*rbc*L	L01948	USA; W. Anderson s.n. (MICH)
Quisqualis indica L.:		
ITS	AF160470	China; Ye 99031301 (SYS)
*psa*A	AY035744	China; Ye 99031301 (SYS)
Rotala indica (Willd.) Koehne:		
*rbc*L[b]	AY036148	China; Tang 99070503 (SYS)
*trn*L-F[bc]	AY905484	China; Tang 99070503 (SYS)
ITS[b]	AY035758	China; Tang 99070503 (SYS)
*psa*A[b]	AY035736	China; Tang 99070503 (SYS)
Rotala ramosior (L.) Koehne:		
*rbc*L[c]	AY905417	Mexico; Graham 1028 (MO)
*trn*L-F[c]	AY905485	Mexico; Graham 1028 (MO)
Rotala rotundifolia (L.) Koehne:		
*trn*L-F[c]	AY905486	China; Gong 2000-52303 (SYS)
Sonneratia alba J. Smith:		
ITS	AF163701	China; Chen 990604 (SYS)
*psa*A	AY035741	China; Chen 990604 (SYS)
Sonneratia apetala Buch.-Ham.:		
ITS[b]	AF163697	China; Qiu 974312 (SYS)
*psa*A[b]	AY035740	China; Qiu 974312 (SYS)
Sonneratia caseolaris (L.) Engl.:		
*rbc*L[b]	AY036143	China; Huang 990435 (SYS)
Sonneratia ovata Backer 1:		
*trn*L-F[bc]	AY905487	Sarawak; P. Chai s.n. in 1990, no voucher
Sonneratia ovata Backer 2:		
ITS	AF163702	China; Chang 9711912 (SYS)
Sonneratia sp.:		
ITS[c]	AY905433	Shi s.n., no voucher
*psa*A[c]	AY905449	Shi s.n., no voucher
Terminalia catappa L.:		
*rbc*L	U26338	USA; Conti 1003 (WIS)
Terminalia hainanensis:		
*trn*L-F[c]	AY905488	China; Cult., Ye 381 (SYS)
Tetrataxis salicifolia (Tul.) Baker:		
ITS	AY078423	Mauritius; Lorence 1231 (MO)
*psa*A[c]	AY905450	Mauritius; Lorence 1231 (MO)
Tibouchina urvilleana (DC.) Cogn.:		
*rbc*L	U26339	USA; Cult., Goldblatt s.n., no voucher
Trapa maximowiezii 1:		
*rbc*L[b]	AY036141	China; Wang 2000-041601 (SYS)
*trn*L[c]	AY905489	China; Wang 2000-041601 (SYS)
ITS[b]	AY035757	China; Wang 2000-041601 (SYS)
*psa*A[b]	AY035729	China; Wang 2000-041601 (SYS)
Trapa maximowiezii 2:		
*trn*L-F[c]	AY905490	China; Zhang 2000-1010 (SYS)
ITS	AY035756	China; Zhang 2000-1010 (SYS)
*psa*A	AY035730	China; Zhang 2000-1010 (SYS)

Table 2

(*Continued*)

Taxon and gene region	GenBank accession	Locality and voucher[a]
Trapa natans L.:		
*trn*L-F[bc]	AY905491	Japan; Graham 1102 (MO)
Woodfordia fruticosa (L.) S. Kurz 1:		
*rbc*L[bc]	AY905418	Nepal; USDA-PI 19882 (MO)
*trn*L-F[bc]	AY905492	Nepal; USDA-PI 19882 (MO)
Woodfordia fruticosa (L.) S. Kurz 2:		
ITS[b]	AF201692	China; Tang 99070504 (SYS)
*psa*A[b]	AY035722	China; Tang 99070504 (SYS)

[a] A = Arnold Arboretum; CAS = California Academy of Science; CEN = CENARGEN, Brasilia, Brazil; DAV = University of California, Davis; IBSC = South China Institute of Botany, Guangzhou, China; IND = Indiana University; MICH = University of Michigan; MO = Missouri Botanical Garden; SYS = Zhongshan University, Guangzhou, China; TEX = University of Texas; WIS = University of Wisconsin.

[b] Sequences employed in the combined data set.

[c] New sequences generated for this study.

of the *ycf*3 gene. The sequencing of *trn*L-F, ITS, and *psa*A-*ycf*3 regions used the Applied Biosystems Model 377 automated DNA sequencing system (PE Biosystems). Sequences were initially aligned in Sequencher, version 4.1.2 (Gene Codes), Se-Al (Rambaut 1996), or ClustalX, version 1.8 (Thompson et al. 1997). Final manual adjustments were made in MacClade 4.0 using color-coded bases (Maddison and Maddison 2000). Unambiguous shared indels were coded for use as presence/absence characters in the maximum parsimony analyses, adding 15 characters to the *trn*L-F data set and 13 characters to the ITS data set. Some regions of ITS-I and ITS-2 were too divergent to be aligned with certainty, resulting in the exclusion of five regions totaling 136 characters in some analyses.

Molecular Phylogenetic Analyses

Minimally, representatives from the Combretaceae and Onagraceae were chosen as outgroups. Combretaceae is sister to all other Myrtales (Sytsma et al. 2004), and Onagraceae is the sister family to the Lythraceae based on morphological and molecular evidence (Johnson and Briggs 1984; Conti et al. 1997; Sytsma et al. 2004).

For the slowly evolving *rbc*L gene, additional outgroups were utilized to provide a strong test of the monophyly of the family and to verify its position among other families of the Myrtales, particularly with respect to the Onagraceae and the Combretaceae. For the *rbc*L analyses, Memecylaceae, Melastomataceae, and Myrtaceae were included from the Myrtales, and Geraniaceae and Saxifragaceae were added as ultimate outgroups based on their phylogenetic position relative to Myrtales in angiosperm molecular phylogenies (Chase et al. 1993; Soltis et al. 2000). Trees were rooted with a monophyletic Geraniaceae and Saxifragaceae. The necessity of a sufficiently large outgroup to achieve an accurate phylogeny was emphasized by the fact that the monophyly of the family was only established once the outgroup in the *rbc*L analysis consisted of the four more distant rosid families. Broad phylogenetic analyses also have been required in other angiosperm groups, such as in the Malpighiales, to accurately place taxa (Soltis et al. 2000).

We utilized three approaches to obtain phylogenetic hypotheses based on the DNA data: maximum parsimony (MP), maximum likelihood (ML), and Bayesian phylogenetic inference (MB, using MrBayes 3.0; Huelsenbeck and Ronquist 2001; Huelsenbeck et al. 2002). MP analyses were performed in PAUP* 4.0b10 (Swofford 2002) using heuristic search algorithms with random addition and 1000 replicates and holding 10 trees to search for islands of equally most parsimonious trees. ACCTRAN character optimization, tree bisection reconnection (TBR) branch swapping, and the options MulTrees (keeping equally most parsimonious trees) and "steepest descent off" were selected. Characters were unordered and equally weighted. Because gene phylogenies may differ as a result of differing rates of evolution in different parts of the genome (Bull et al. 1993), the multiple data sets were first analyzed individually and then combined. Bootstrap analysis with 100 replicates (Felsenstein 1985) or parsimony jackknife analysis with 1000 replicates (Farris et al. 1996) identified clades with 50% or greater support. We used MacClade 4.0 (Maddison and Maddison 2000) to construct constraint trees and later to examine the evolution of selected morphological characters on trees.

An appropriate model of nucleotide substitution for use in setting ML parameters for each molecular marker and the combined molecular data set was chosen using a starting MP tree in Modeltest 3.06 (Posada and Crandall 1998; software available at http:darwin.uvigo.es/software/modeltest.html), which selected the best among 56 ML models of DNA substitution. The parameters utilized in the ML and MB analyses were generated from base pair sequences omitting indels. Among 56 models tested before ML and MB analyses, the *rbc*L, ITS, *psa*A-*ycf*3, and combined data sets best fitted the general time reversible model (GTR + I + G); *trn*L-F best fitted the transversional model (TVM + G). Heuristic searches

were performed using random addition of sequences and five replicates (10 replicates in the combined four-gene data set), holding one tree at each step, and with TBR branch swapping to completion.

Bayesian analyses utilized uniform prior probabilities, the general time reversible + gamma (GTR + Γ) model setting, and a random starting tree. An estimate of stationarity was determined by a trial run of 50,000 generations. Stationarity in actual runs was reached at ca. 100,000 generations with the *rbc*L data; ca. 60,000 generations for *trn*L-F, ITS, and *psa*A-*ycf*3 data; and ca. 20,000 generations for the combined data set. Four Markov chains were run simultaneously for 500,000 generations, swapping chains several times during the run, and a tree was saved every 100 generations. We sampled 4000 trees from the total of 5000 saved, discarding the first 1000 trees obtained (the "burn-in") to insure that the consensus was built from trees produced after the area of highest posterior probability (i.e., the more or less stable likelihood value of the trees) had been reached.

After analyses of the four independent data sets, an incongruence length difference test (ILD) (Farris et al. 1994) was implemented in PAUP*. The ILD is a statistical test of the null hypothesis that the individual character sets are drawn randomly from a single population representing a single phylogeny. Although increasing *P* values (decreasing significance levels) generated by ILD have been considered indicative of improved phylogenetic accuracy, the test is best treated as one of several methods for assessing combinability of data rather than being used to indicate degree of phylogenetic accuracy (Hipp et al. 2004). If data partitions are not strongly incongruent, their combination has been considered an acceptable means of increasing the number of characters and reducing the sampling error that estimates the phylogeny (Sytsma 1990; Johnson and Soltis 1998). Because the ambiguity caused by multiple most parsimonious results of the individual data sets decreases with additional data, support increases for more clades (Bull et al. 1993; Barker and Lutzoni 2002). One hundred data partition replicates were run (heuristic search, simple addition, TBR branch-swapping). The high probability we obtained ($P = 0.85$) indicated that the four data sets were substantially congruent and could be analyzed in combination. Sequences for the 20 Lythraceae genera held in common across the four gene data sets were combined for analysis using the same procedures applied to the individual data sets.

After all analyses, we used the Shimodaira-Hasegawa nonparametric test of topologies as implemented in PAUP* to test whether the likelihood values for the topologies being compared were significantly different (Shimodaira and Hasegawa 1999; Goldman et al. 2000; Shimodaira 2002). If not significant, each topology would be considered an equally likely phylogenetic hypothesis. The test was run with full optimization and 1000 bootstrap replicates.

Morphological Phylogenetic Analyses

An initial MP analysis was run for all Lythraceae s.l. genera, employing a data matrix of 35 taxa and 31 morphological characters (tables 3 and 4). The Combretaceae, represented by *Combretum*, and *Fuchsia* and *Ludwigia*, representing the two basal lineages of the sister family Onagraceae, acted as outgroups. The matrix was revised from an earlier 31-taxa × 27-character matrix that employed different outgroups (Graham et al. 1993*a*). The new 35-member matrix subsequently was pruned to the 23 taxa represented in the combined gene data set, and MP analyses were performed separately and in combination with the molecular data as a means of increasing resolution and branch support (Gontcharov et al. 2004). The heuristic searches employed random addition (RA) sequences, 1000 replicates holding 10 trees at each step, and TBR and Multrees settings, with the steepest descent option off. Characters were unordered and equally weighted. The full 35-taxa heuristic search was terminated after 200 RA sequences because of the large number of trees generated. Bootstrap values were calculated from 100 replicates and RA sequences with 1000 replicates holding one tree and with 10,000 fast addition jackknife resamplings in the 35-member matrix.

Results

Comparative statistics for the MP analyses of the four gene regions and morphology are summarized in table 5. Results of parsimony and likelihood analyses of both chloroplast and nuclear genome sequences were congruent in finding a monophyletic Lythraceae that includes *Duabanga*, *Punica*, *Sonneratia*, and *Trapa* nested together with genera of Lythraceae *sensu stricto*.

The earliest branching events in the family are unclear. Either (1) *Decodon* is sister to the rest of the family, followed by *Lythrum-Peplis* at the node above (ITS: ML and MB; *psa*A-*ycf*3: MP; combined analysis: MP and MB), (2) the base of the family diverges as two superclades (*trn*L-F: ML; combined analysis: ML), or (3) the base is polytomous (*rbc*L: MP and MB; *trn*L-F: MP and MB; *psa*A-*ycf*3: ML and MB; morphology).

Six crown clades were recovered in all analyses, minimally including (1) *Ammannia* + *Nesaea-Ginoria* + *Lawsonia*, (2) *Duabanga* + *Lagerstroemia-Sonneratia* + *Trapa*, (3) *Lafoensia-Punica-Pemphis*, (4) *Cuphea-Woodfordia*, (5) *Lythrum-Peplis*, and (6) *Heimia-Rotala*. The greater number of taxa in the *trn*L-F, ITS, and *psa*A-*ycf*3 data sets compared with the more limited *rbc*L data set expanded clade 3 to include *Capuronia* and *Galpinia* and clade 4 to include *Adenaria*, *Pehria*, *Koehneria*, and *Pleurophora* (*trn*L-F only). The relationships of *Physocalymma* and *Diplusodon* differed depending on the analysis. *Lourtella*, represented only by an ITS sequence, was sister to *Diplusodon*.

Analysis of rbc*L*

The *rbc*L gene provided the lowest percentage of informative characters in any individual gene analysis conducted, even though five more distant families, Memecylaceae, Melastomataceae, Myrtaceae, Saxifragaceae, and Geraniaceae, were part of the data set (table 5). The three most parsimonious trees generated in MP found a monophyletic Lythraceae that included Sonneratiaceae, Duabangaceae, Punicaceae, and Trapaceae but with ≤50% bootstrap support (fig. 2*A*). The MP tree differed from the ML and MB trees in the position of *Capuronia*, *Galpinia*, *Cuphea*, and *Woodfordia*. The MP relationships of these genera based on *rbc*L are not supported by any other gene tree, whereas their positions in the ML

Table 3

Character Coding for Morphological Analysis

Character	Codes
1. Vascular tracheids	0 = absent; 1 = present
2. Spiral thickenings in vessels	0 = absent; 1 = present
3. Septate wood fibers	0 = present; 1 = absent
4. Fiber dimorphism (thin, short fibers and normal fibers)	0 = absent; 1 = present
5. Crystalliferous fibers	0 = absent; 1 = present
6. Leaf structure	0 = dorsiventral; 1 = isobilateral
7. Leaf sclereids	0 = absent; 1 = present
8. Leaf apical glands	0 = absent; 1 = present
9. Resin-secreting multicellular trichomes	0 = absent; 1 = present
10. Stipules	0 = flanking base of leaf petiole; 1 = in leaf axil; 2 = absent
11. Prophylls ("bracteoles")	0 = absent; 1 = present
12. Inflorescence	0 = anthotelic (monotelic); 1 = blastotelic (polytelic)
13. Merosity	0 = 5; 1 = 4; 2 = 6; 3 = 7 or more
14. Heterostyly	0 = absent; 1 = present
15. Calyx lobe length	0 = half or more of total floral tube length; 1 = less than half of total floral tube length
16. Petals	0 = present; 1 = absent
17. Stamen : calyx lobe ratio	0 = 2 : 1; 1 = 1 : 1; 2 =≥3 : 1
18. Carpel : calyx lobe ratio	0 = 1 : 1 to 1 : 2; 1 =>1 : 1; 2 = less than 1 : 2
19. Stamen whorls	0 = two whorls; 1 = one whorl opposite calyx lobes; 2 = one whorl opposite petals
20. Pollen shape	0 = prolate to prolate-spheroidal; 1 = oblate to oblate-spheroidal
21. Pollen pseudocolpi	0 = absent; 1 = 3 pseudocolpi; 2 = 6 pseudocolpi
22. Pollen exine sculpture	0 = psilate-scabrate to verrucate; 1 = striate; 2 = onagraceous (global to rodlike elements and/or viscin threads)
23. Nectary position	0 = floral tube/ovary junction; 1 = on floral tube; 2 = on gynoecium; 3 = absent
24. Ovary position	0 = inferior to semi-inferior; 1 = superior
25. Ovule outer integument	0 = nonmultiplicative; 1 = multiplicative
26. Embryo sac	0 = eight-nucleate; 1 = four-nucleate
27. Cotyledons	0 = convoluted (folded or spiraled); 1 = straight
28. Fruit	0 = capsule; 1 = berry; 2 = drupaceous
29. Seed compression	0 = not compressed; 1 = slightly to strongly compressed
30. Seed wing	0 = absent; 1 = present, unilateral; 2 = present, encircling seed body
31. Seed internal epidermal trichomes	0 = absent; 1 = present, straight; 2 = present, spirally twisted

and MB trees are consistent with results in other gene and the morphological analyses. Deep branches in MP are short under ACCTRAN optimization, except for a long branch to *Heimia-Rotala*. In contrast, several terminal branches are long, especially for *Capuronia* (65 changes, including nine additional bases present between bases 461 and 501).

Analysis of trn*L-F*

The *trn*L-F data set includes four genera not sequenced for *rbc*L: *Pehria*, *Koehneria*, *Physocalymma*, and *Diplusodon*. Length of the *trn*L-F sequences varied from 906 bases in *Lagerstroemia villosa* S. Kurz to 1097 in *Trapa natans* L. *Lagerstroemia villosa* was exceptional among all taxa in having two large gaps of 64 base pairs each at positions 602–666 and 811–875 in the alignment. The deep branches of the family were unresolved in MP and in MB (fig. 3*A*, 3*C*), whereas in the ML tree (fig. 3*B*) a basal, poorly supported dichotomy resulted in two major clades of 12 and 14 genera. *Pehria* and *Koehneria* joined the *Adenaria-Pleurophora* clade. The Brazilian/Bolivian cerrado genus *Diplusodon* was sister to the European herb *Peplis* in this analysis, but in the ITS and *psa*A-*ycf*3 analyses it was intuitively more plausibly associated with the other woody South American genera *Physocalymma* and *Lourtella*. The MP and MB trees are identical in structure, except that the *Heimia-Rotala* lineage is sister to the *Duabanga-Trapa* clade in the MB tree but remains part of a large polytomy in the MP tree.

Analysis of ITS

ITS sequences were highly variable and presented areas in ITS I and ITS 2 that were difficult to align. The ITS data set included *Tetrataxis* and *Lourtella*, genera not sequenced for *rbc*L or *trn*L-F. Sequence lengths varied from 552 bases in *Lythrum hyssopifolia* L. and *Rotala indica* (Willd.) Koehne to 630 bases in *Sonneratia apetala* Buch-Ham. The percentage of informative ITS characters was more than double that of the other gene sequences (table 5).

Two analyses were made, one employing all sequence data and the other excluding the following five areas, totaling 136 bases, that were difficult to align: bases 67–77, 179–238, 471–518, 547–555, and 617–624 in the aligned sequences. ITS without the difficult alignments produced two highly resolved trees under MP analysis that differed only in the placement of *Heimia-Rotala* as sister to clade *Lythrum-Peplis*

Table 4

Morphological Data Matrix

Taxa	12345	1 67890	11111 12345	11112 67890	22222 12345	222233 678901
Outgroups:						
Combretum	10001	00002	01a01	00000	1a301	002000
Ludwigia	00000	00000	11a00	00001	02000	110000
Fuchsia	00000	00000	01101	00001	0200?	111100
Ingroups:						
Adenaria	00000	00000	11111	00200	00111	010100
Ammannia	00100	00001	11101	00000	21010	010101
Capuronia	00001	10100	11201	01220	0011?	010100
Crenea	00000	0000?	11100	02000	20310	010101
Cuphea	00000	00010	11201	00201	0a010	010102
Decodon	00000	00000	11011	00000	00210	010000
Didiplis	00100	?0001	01101	11211	01010	010101
Diplusodon	00100	00001	11201	00200	00311	010121
Duabanga	00100	a1000	10b00	0b000	00000	010000
Galpinia	00001	00100	10201	01220	00210	010120
Ginoria	0000a	00000	11b00	00000	20310	010101
Haitia	00000	00000	11201	02000	20310	010101
Heimia	11000	00000	11201	00000	00310	010101
Hionanthera	00100	?0001	11101	01010	2101?	010101
Koehneria	00000	00000	11201	02000	10011	010100
Lafoensia	00000	00101	11301	00200	20111	010122
Lagerstroemia	000a1	00000	10201	02000	20310	000010
Lawsonia	00011	00000	10100	00010	20310	010000
Lourtella	00001	00001	11101	00200	0011?	010100
Lythrum	00000	00001	112a1	0020a	11010	010101
Nesaea	01100	00001	11ba1	00000	21010	010101
Pehria	00001	00000	11101	00200	00111	010102
Pemphis	00100	10000	01211	00200	20111	010000
Peplis	00100	?0001	11201	11211	11310	010101
Physocalymma	0001a	00001	11301	02200	00311	010120
Pleurophora	10000	00010	11201	012ca	00010	010102
Punica	10011	00100	10001	02100	00001	001000
Rotala	00100	00001	111a1	01010	20010	010101
Sonneratia	00000	11101	10100	02100	10000	001100
Tetrataxis	00000	00000	11100	11020	00310	010100
Trapa	?0?0?	00001	01100	01011	00001	012000
Woodfordia	10000	00000	11201	00200	00010	010102

Note. See table 3 for codes. ? = missing data; a = 0 and 1; b = 1 and 2; c = 0 and 2.

or sister to clade *Ammannia-Lawsonia* (fig. 4*A*). The full-sequence data resulted in seven most parsimonious, less well-resolved trees (not illustrated). As in the other analyses, support for the deep internal branches was ≤50% in MP and ML analyses (fig. 4*A*, 4*B*), but posterior probabilities using Bayesian analysis were high (fig. 4*C*).

The MP consensus phylogeny of the two trees placed *Pleurophora* at the base of the family as sister to the rest of the family (fig. 4*A*), a position not supported by any other analysis. In the consensus of the seven trees generated by the full sequences including the areas of difficult alignment, *Pleurophora* was nested within the family as sister to a poorly resolved clade of 12 genera. When we removed *Pleurophora* from the MP analyses (not illustrated), *Decodon* became sister to the rest of the family. The sister relationship of the monospecific genus *Lourtella* of Peru and Bolivia to the Brazilian *Diplusodon* is also found in analysis of the *psa*A-*ycf*3 sequences, the only other data set that includes these genera.

Analysis of psa*A*-ycf*3*

This chloroplast spacer region is highly conserved; informative characters are 21% of total characters, a figure only slightly higher than that found in the *rbc*L data set (18% informative characters), which included five more distant rosids in the outgroup. The length of the region varied from 739 bases (*Peplis portula* L.) to 786 bases (*Physocalymma scaberrimum* Pohl). Most changes in the *psa*A-*ycf*3 region are autapomorphies on terminal branches. The *psa*A-*ycf*3 phylogenies are not illustrated.

In the *psa*A-*ycf*3 MP consensus, *Decodon* was sister to the rest of the family. The remainder of the genera were represented by a polytomy of 13 branches at the next higher node. Several sister relationships recovered from other gene regions were

Table 5

Comparative Statistics for the Maximum Parsimony Analyses of the Four Gene Regions and Morphology

	*rbc*L	*trn*L-F	ITS	*psa*A-*ycf*3	4 genes	4 genes + morphology	Morphology
Total sequences	44	43	38	37	23	23	...
Lythraceae genera	22	26	27	26	20	20	32
No. of characters	1428	1182[a]	715[b]	960	4285	4316	31
Informative characters	251	264	312	199	743	773	31
Percentage informative	18	22	54	21	17	18	100
Trees retained	3	4726	2	748	1	1	5974
Tree length	1023	712	1600	545	3401	3528	133
CI	0.59	0.78	0.46	0.74	0.63	0.62	0.33
RI	0.60	0.78	0.59	0.73	0.47	0.47	0.56
RC	0.36	0.61	0.27	0.55	0.30	0.29	0.19

Note. CI = Consistency Index; RI = Retention Index; RC = Rescaled CI.

[a] Excluding the first 32 and last 45 characters of the *trn*L-F data set and including 15 indels.

[b] Including all ITS characters plus 13 indels.

present, but all were weakly supported, at ≤75% parsimony jackknife support, except the sister relationships of *Nesaea* and *Ammannia* at 100%, *Tetrataxis* and *Ginoria* at 98%, and *Duabanga* and *Lagerstroemia* at 75%. In the ML topology, the Lythraceae diverged as two major clades of 12 and 13 taxa. The MB consensus was less resolved as a polytomy of nine branches from the basal node, with *Decodon* as one branch. The clades or sister relationships recognized in the *psa*A-*ycf*3 MB phylogeny at 75% or greater clade credibility values were *Ammannia-Nesaea-Rotala* + *Ginoria-Tetrataxis*, *Capuronia-Galpinia-Lafoensia* + *Punica* + *Pemphis*, *Lythrum-Peplis*, *Diplusodon-Lourtella*, *Koehneria-Woodfordia* + *Pehria* + *Cuphea*, and *Duabanga-Lagerstroemia* + *Sonneratia-Trapa*.

Combined Analysis of Four DNA Regions

Data sets were available across the four regions for 20 genera of the Lythraceae and three outgroup genera from the Combretaceae and Onagraceae. MP produced one fully resolved tree (fig. 5*A*), in which *Decodon*, on a long branch, is basal in the family, followed next by a long but well-supported branch to *Lythrum-Peplis* and then by divergence of the rest of the family as two major clades, superclades I and II. Six crown clades of two to five genera are robustly supported (fig. 5*A*–5*C*, clades I–VI). *Cuphea* and *Woodfordia* (clade IV) are recognized as a clade apart from clade III, because other genera included in individual gene analyses expand clade IV to include *Pleurophora*, *Koehneria*, *Pehria*, and *Adenaria*. ML and Bayesian trees (fig. 5*B*, 5*C*) closely correspond to the MP tree in recognizing (1) an expanded Lythraceae, (2) an early dichotomy in the family resulting in superclades I and II (table 7), and (3) the six major clades previously identified in analyses of individual gene regions.

Incongruencies among the three trees are due to ambiguous phylogenetic placements of *Decodon* + *Lythrum-Peplis* and clade II, the *Duabanga-Trapa* clade (fig. 5*A*–5*C*). The parsimony cost of accepting the ML topology over the MP is 13 steps (MP tree length = 3399 steps vs. ML tree length constrained under identical PAUP analysis = 3412 steps). The MB consensus topology (fig. 5*C*) can be derived from the MP tree, by making clade II sister to clade I, at a much lower parsimony cost of four steps (MB tree length constrained under identical PAUP analysis = 3403 steps). With this move, *Heimia-Rotala* (clade V) becomes part of the nine-member superclade II. Results of a Templeton test of the significance of difference in tree lengths between the MP and ML trees ($P = 0.1784$) and between the MP and MB trees ($P = 0.6698$) were insignificant at the 5% probability level. Results of the Shimodaira-Hasegawa test (table 6) found no significant difference in likelihood value between the MP and ML or MB topologies.

Morphological and Combined Morphological/Molecular Results

The consensus tree of 5974 MP trees produced from the matrix of 35 taxa × 31 unweighted morphological characters was a large polytomy with minimal resolution and failed to exclude the Onagraceae from the Lythraceae (fig. 5*D*; table 5). The morphological data for the 20 Lythraceae genera were subsequently added to the combined gene data in an MP analysis, and as expected, the strong molecular phylogenetic signal resulted in a single tree identical to the MP combined gene tree (fig. 5*A*).

Discussion

Support for two superclades and for internal branches to the six crown clades recovered in the study varies depending on the gene region and the type of analysis; however, the highest values are attained in the Bayesian consensus in each instance. The discrepancy between lower bootstrap support and higher Bayesian posterior probabilities observed in the present molecular analyses has been generally observed (Suzuki et al. 2002; Cummings et al. 2003). The reasons for the difference are complex but are partially explained by the differing parameters employed in ML bootstrapping versus Bayesian analysis (Erixon et al. 2003; Cummings et al. 2003). Although the likelihoods of the MP, ML, and MB trees are not different statistically (table 6), structurally they imply different early histories that will require further analyses incorporating all genera and additional sequences from appropriately evolving genes.

The position of *Decodon* as sister to the rest of the family is notable because *Decodon* has an extensive and early fossil record (see "Historical Biogeographical Considerations") and

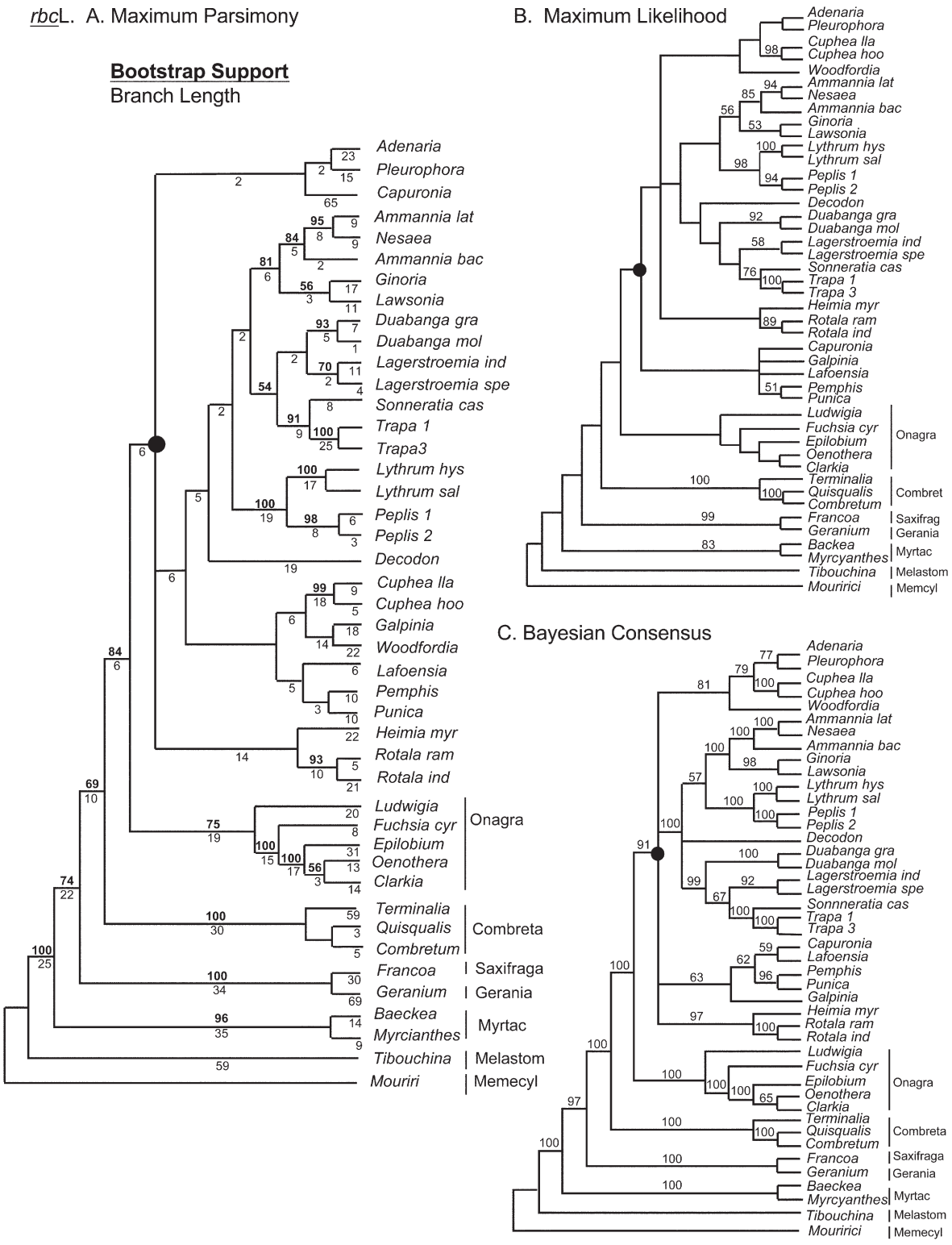

Fig. 2 Topologies generated by *rbc*L data. *A*, Maximum parsimony (MP) strict consensus tree of three trees; Consistency Index = 0.59, Retention Index = 0.60. *B*, Maximum likelihood strict consensus of two trees. C, Bayesian 50% majority-rule consensus of 4000 trees. The base of the Lythraceae is indicated by a black dot; bootstrap values (≥50%) are above the branches in *A* and *B*; clade credibility values (true posterior probabilities) are above the branches in *C*; branch lengths for one of the most parsimonious MP trees are below the branches in *A*.

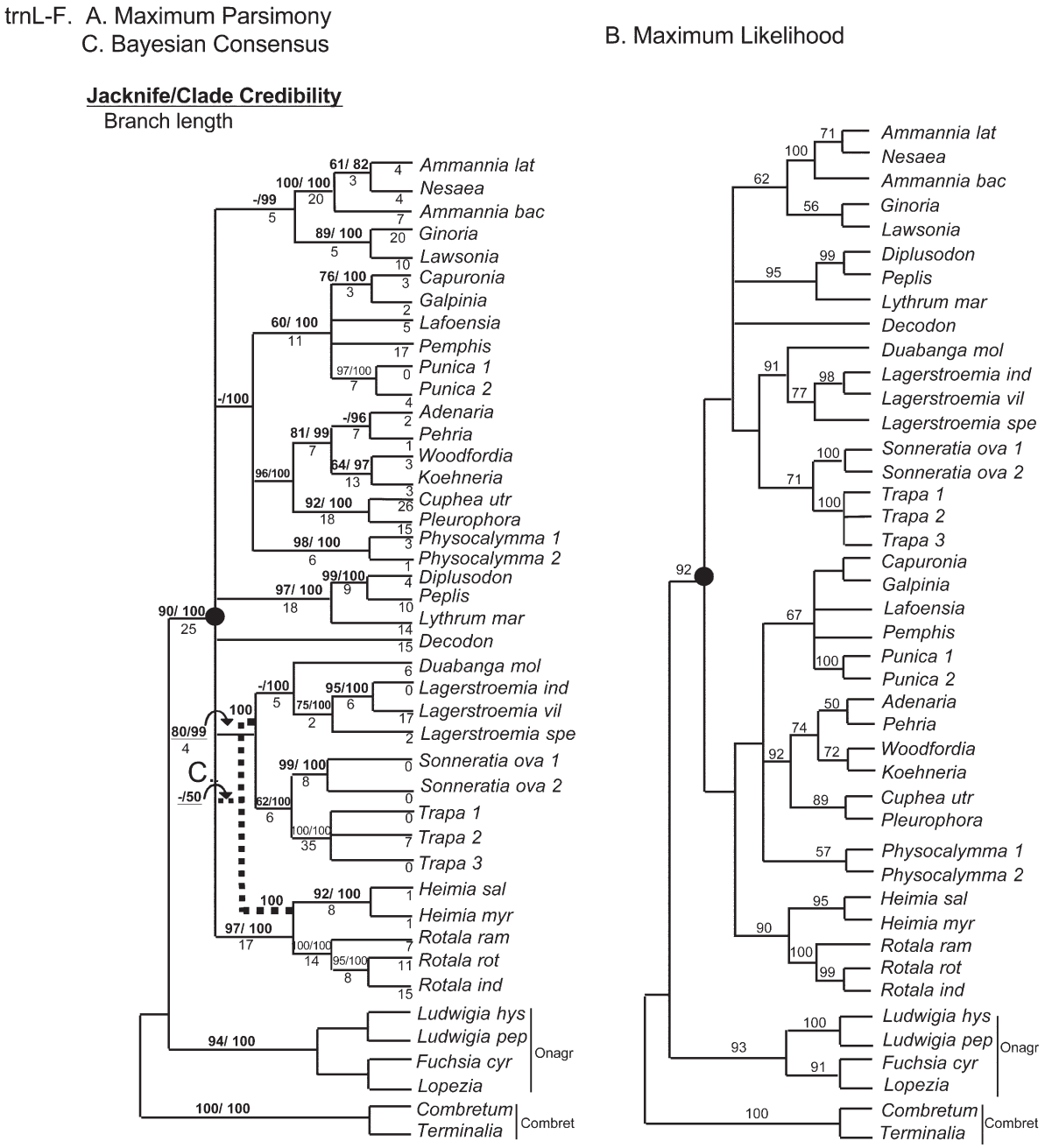

Fig. 3 Topologies generated by *trn*L-F data. *A*, Maximum parsimony (MP) strict consensus of 4726 trees from the data set including 15 indels; Consistency Index = 0.78, Retention Index = 0.78. *B*, Maximum likelihood strict consensus tree of two trees. *C*, Bayesian 50% majority-rule consensus of 4000 trees. The Bayesian topology is identical to the MP consensus tree (*A*) except for the sister relationship between clades *Duabanga-Trapa* and *Heimia-Rotala* (indicated by dashed lines). The base of the Lythraceae is indicated by a black dot; bootstrap values (≥50%) are above the branches in *A* and *B*; clade credibility values (true posterior probabilities) are to the right of the bootstrap values in *C*; branch lengths for one of the most parsimonious MP trees are below the branches in *A*.

because it is one of only three Lythraceae genera with a tristylous breeding system. If *Decodon* is sister to the rest and is followed at the next higher node by the mostly heterostylous *Lythrum* (fig. 5*A*, 5*C*), heterostyly would be plesiomorphic for the family, although homoplastic because heterostyly also occurs in *Adenaria* and *Pemphis*, as well as in some species of *Rotala* and *Nesaea*.

The uncertainty of the phylogenetic relationship of the Trapaceae to the Lythraceae and Onagraceae, a result of its highly autapomorphic morphology and embryology, is resolved at the molecular level. MP and ML analyses of all molecular data sets agree that the Asian herb *Trapa* is a derived member of the Lythraceae, most closely related to the three Southeast Asian tree genera of the family, *Sonneratia*,

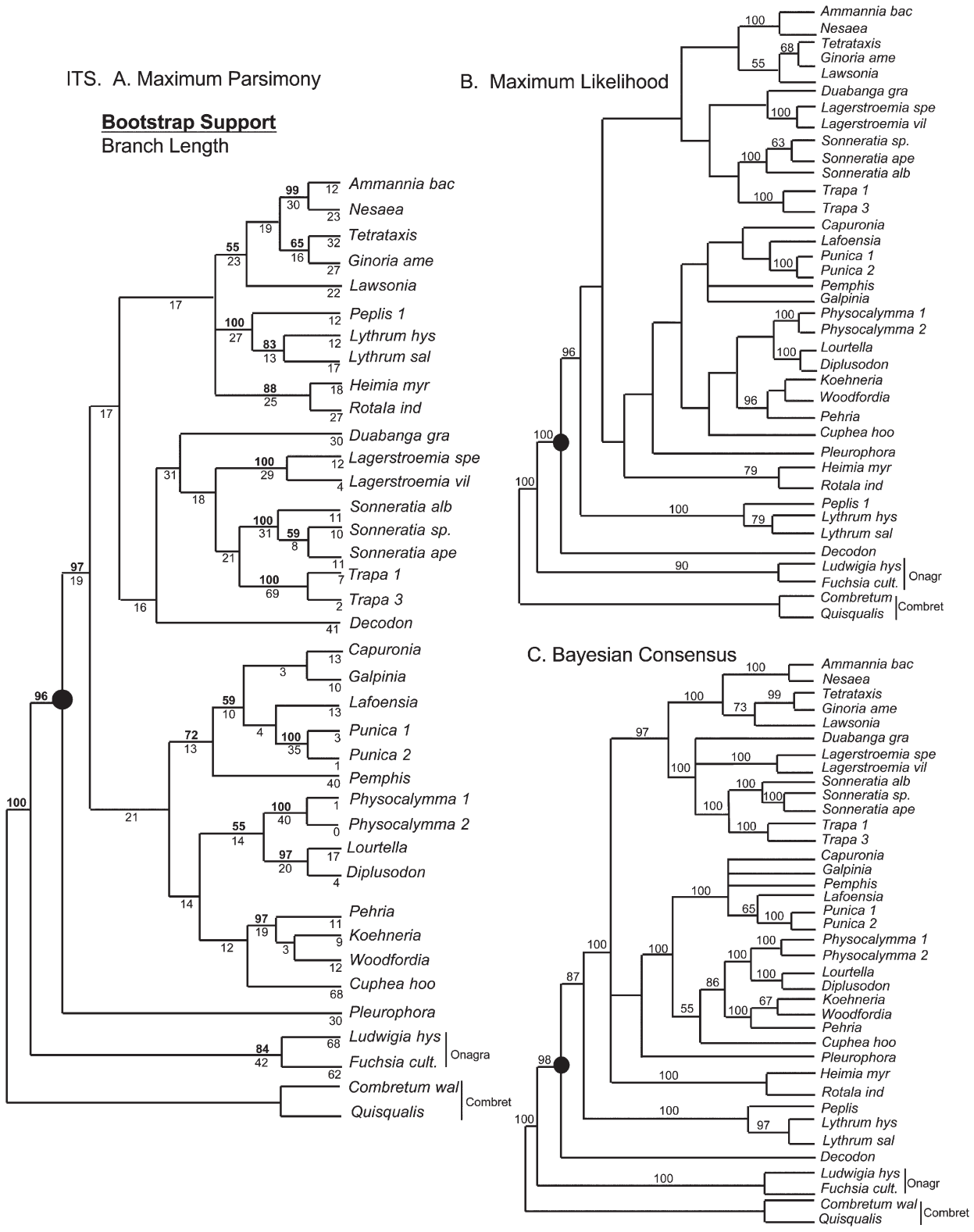

Fig. 4 Topologies generated by ITS data. *A*, Maximum parsimony (MP) strict consensus tree of two trees, from the data set excluding 136 characters in regions difficult to align and including 13 indels; Consistency Index = 0.46, Retention Index = 0.59. *B*, Maximum likelihood tree. *C*, Bayesian 50% majority-rule consensus of 4000 trees. The base of the Lythraceae is indicated by a black dot; bootstrap values (≥50%) are above the branches in *A* and *B*; clade credibility values (true posterior probabilities) are above the branches in *C*; branch lengths for one of the most parsimonious MP trees below the branches in *A*.

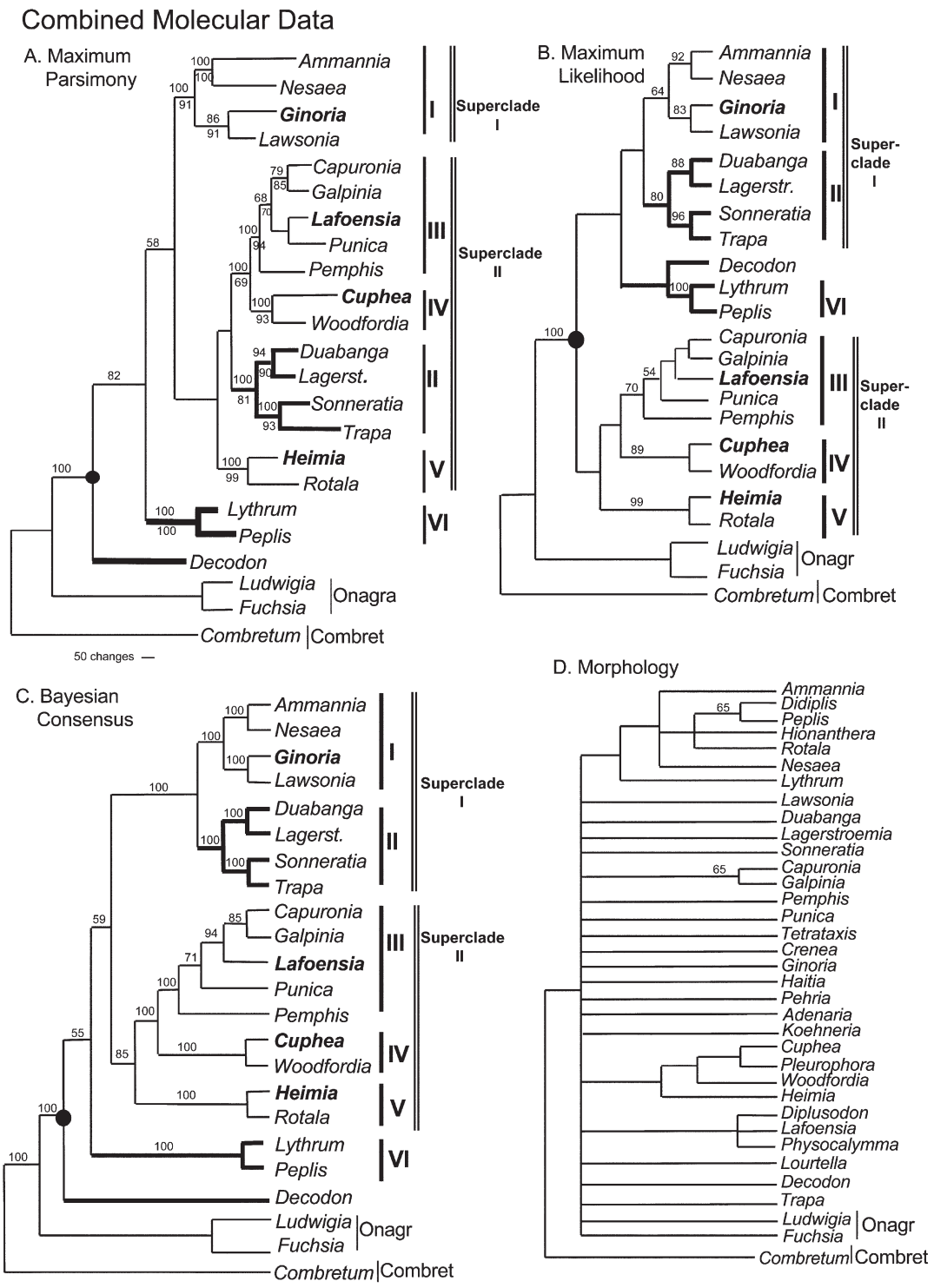

Fig. 5 Hypotheses of relationships within the Lythraceae based on the combined data set of four gene regions and morphology. *A*, Phylogram of the single maximum parsimony (MP) tree of the combined molecular data; Consistency Index (CI) = 0.63, Retention Index (RI) = 0.47; bootstrap values (≥50%) are above the branches. The same MP tree was derived from the combined molecular/morphological data; CI = 0.62, RI = 0.47; bootstrap values (≥50%) are below the branches. *B*, Maximum likelihood tree of the combined molecular data. *C*, Bayesian 50% majority-rule consensus of 4000 trees from the combined molecular data. *D*, MP strict consensus tree of 5974 trees, based on unweighted morphological data; CI = 0.33, RI = 0.56. Bootstrap values (≥50%) are above the branches in *B* and *D*; clade credibility values (true posterior probabilities) are above the branches in *C*. The base of the Lythraceae is indicated by a black dot. Taxa whose relationships change depending on the analysis performed are indicated by heavy branches. New World genera are in boldface. The composition of superclades I and II and clades 1–6 are indicated to the right of the trees in *A*–*C*.

Table 6

Results of the Shimodaira-Hasegawa Test

Tree	ln-*L*	ln-*L* difference	*P*
Maximum parsimony	22,730.04662	16.27517	0.144
Maximum likelihood	22,713.77144	Best	
Bayesian analysis	22,720.85036	7.07892	0.411

Note. The test compares the significance of difference among likelihoods of the alternative phylogenetic hypotheses generated by maximum parsimony, maximum likelihood, and Bayesian methods.

Duabanga, and *Lagerstroemia*, and sister to *Sonneratia*. The sister relationship of *Trapa* to *Lythrum* recovered in an ordinal-level study based on *rbc*L sequences (Conti et al. 1996) is explained by the absence of *Sonneratia* in the study and by the few representatives of the Lythraceae that were included. Given the unequivocal molecular evidence of relationships, the monogeneric Trapaceae should now be regarded as exceptionally specialized members of the Lythraceae.

The Punicaceae, like the Trapaceae, were generally considered closely related to, but distinct from, the Lythraceae. Morphologically, the monogeneric Punicaceae differs from the Lythraceae by a multistaminate leathery floral tube with an inferior ovary and berry-like fruits with sarcotestal seeds (Cronquist 1981; Takhtajan 1987). Molecular results of this study consistently place *Punica* in a clade with *Pemphis* and *Lafoensia*, which are usually joined by *Capuronia* and *Galpinia*. The position of *Punica* in the clade varies with the data set explored, being sister to *Pemphis* (*rbc*L), sister to *Lafoensia* (ITS, *psa*A-*ycf*3, and MP of combined data), in a grade between *Pemphis* and *Lafoensia* (ML and MB of combined data), or part of a polytomy of these genera (*trn*L-F). Flowers of *Punica* and *Lafoensia* are similar, having large, thickened, leathery floral tubes with numerous petals and stamens, and they are specialized over the smaller, simple, six-merous, slightly coriaceous flowers of *Pemphis*. Members of the clade are distributed from southern Eurasia to East Africa and eastward on islands of the Indian-Pacific Ocean, except *Lafoensia*, which is restricted to the forests and cerrados of South America.

The possible congeneric status of *Ammannia* and *Nesaea* indicated by absence of significant morphological differences (Verdcourt 1994) is confirmed by the nesting of *Nesaea aspera* within the *Ammannia* clade in *rbc*L and *trn*L-F analyses (fig. 2; fig. 3*A*, 3*B*). The close relationship of *Ammannia* and *Rotala*, previously recognized as a single genus and still often confused taxonomically (Koehne 1903; Cook 1979), is negated by the molecular evidence. In the morphological analysis, *Rotala* was part of a clade consisting of the aquatic or marsh-inhabiting genera of the family (i.e., *Ammannia*, *Nesaea*, *Lythrum*, *Peplis*, *Didiplis*, and *Hionanthera*), genera that mostly share the herbaceous habit and simple floral structure of *Rotala*. The position is in contrast to the strongly supported relationship between *Rotala* and the American shrub genus *Heimia* recovered in all the molecular analyses. Morphological adaptations of the herbaceous *Rotala* to life in wet habitats appear to have obscured its still genetically discernible sister relationship to *Heimia* (fig. 5*A*–5*C*; clade V).

The relationship of *Lythrum* to *Peplis* (clade VI) is one of sister genera in the analyses where two species of *Lythrum* were compared with *Peplis* (*rbc*L, ITS, and *psa*A-*ycf*3). The possibility that they are congeneric, as Webb (1967) concluded, requires more extensive sampling of *Lythrum*, especially of the diminutive Eurasian species; this research is now in progress (J. Morris, Kent State University, personal communication). The sister relationship of *Tetrataxis*, a rare monospecific genus endemic to Mauritius, to the Caribbean genus *Ginoria* was first predicted by Koehne (1886) and is supported by the ITS and *psa*A-*ycf*3 sequence data. The six-member clade *Adenaria-Cuphea*, in the *trn*L-F analyses, was previously recognized in a morphological cladistic analysis (Graham et al. 1993*a*); the six genera are united by the unique synapomorphy of resin-secreting trichomes.

The tentative relationship of *Diplusodon-Lourtella-Physocalymma* may lead to recognition of a seventh clade. Additional sequencing is needed to validate the relationship because only ITS sequences were available for comparison of the three genera. In all analyses, the relationship of *Physocalymma* is with members of superclade II, either as sister to *Diplusodon-Lourtella* or as part of an unresolved polytomy.

The conflicting tree topologies in this study in large part result from the absence of information about the earliest branching events in the history of the family. The parsimony and likelihood hypotheses are in substantial agreement as to terminal relationships, but the early divergence of the lineages remains to be firmly established. The numerous short internal branches that contrast with long terminal branches raise the possibility that incorrect relationships may be recognized as a result of long-branch attraction. Addition of missing genera and greater sampling within the genera can be expected to help rectify the problem in future investigations.

Morphological Character Changes, Chromosomes, and Classification

All morphological characters, when mapped to the internal branches of trees from the combined analyses, are homoplastic. The evolution of several characters of taxonomic importance in the Lythraceae s.l. has been exceptionally labile and complex. The presence of an inferior to semi-inferior ovary (the floral tubes epigynous or hemiepigynous) was influential in recognition of *Duabanga*, *Punica*, and *Sonneratia* as independent families (Cronquist 1981; Johnson and Briggs 1984; Takhtajan 1987) or as subfamilies of the Lythraceae (Thorne 1992). Molecular analyses indicate that the superior ovary (perigynous floral tube) is plesiomorphic for the Lythraceae, having evolved on the branch leading to the family from an ancestor with an inferior ovary in an epigynous flower. Most genera of the Lythraceae have a superior ovary, with the

staminal filaments emerging near the base of the ovary. Semiinferior to inferior ovaries are secondarily derived twice in the family, once on the branch to clade *Duabanga-Trapa*, with a reversal in *Lagerstroemia*, and independently in *Punica*. In tandem with the change from a superior to an inferior ovary, stamen insertion moved from near the base of the floral tube in perigynous flowers to the upper levels or rim of the tube in epigynous flowers. Stamens inserted at or near the rim, as in *Duabanga* and *Sonneratia,* represent a derived condition, rather than an ancestral one as earlier postulated (Dahlgren and Thorne 1984).

Inflorescence form in the Lythraceae is of two types (fig. 5*A*). The anthotelic condition (inflorescence terminating in a flower) that in the earlier morphological analysis of Graham et al. (1993*a*) defined a clade composed of *Duabanga*, *Sonneratia*, *Punica*, *Lagerstroemia*, and *Lawsonia* in the present analyses is distributed homoplastically in both superclades, no matter whether the clade *Duabanga-Trapa* is a member of superclade I or II. All genera with anthotelic inflorescences are included in the combined gene analysis, allowing the determination that anthotely is derived from the primitive blastotelic condition (inflorescence terminating in a vegetative meristem) minimally four times within the family and reversed in *Trapa* (figs. 5*A*, 6*A*).

Floral merosity (fig. 6*B*) is divided almost equally between four- and six-merous genera of the Lythraceae but retains flexibility to varying degrees within most genera. Merosity is exceptionally plastic within *Ammannia*, *Ginoria*, and *Rotala*. In the outgroups, the Combretaceae are both four- and five-merous and the Onagraceae are primitively four-merous (Hoch et al. 1993; Levin et al. 2003; P. Hoch, personal communication). In the expanded Lythraceae, only *Decodon* is nearly always five-merous. *Punica* is basically five-merous, but floral parts are multiplicative, obscuring the primary condition (Leins 1988). Dahlgren and Thorne (1984) suggested that the Lythraceae were primitively four-merous because carpels commonly number two to four. If *Decodon* proves sister to the rest of the family and the Lythraceae are derived from an earlier five-merous Combretaceae lineage, then five-mery is primitive for the Lythraceae. In this case, four- and six-merous lineages must have become established early in the history of the family because, to a large extent, they circumscribe the two superclades (fig. 6*B*).

Other characters of taxonomic value and phylogenetic interest discovered since the family classification was proposed by Koehne (1903) were investigated as possible sources of synapomorphies for defining clades. These include characters of the pollen, seed coat, and chromosome number.

Pollen of the Lythraceae is the most diverse of any family of the Myrtales (Patel et al. 1984), and in seven of the 14 families of the order it features the presence of pseudocolpi (subsidiary colpi or heterocolpi), i.e., aperture-like thinnings in the exine that, unlike the true colpi, do not function in pollen tube emission. In the Lythraceae, pseudocolpi are present or incipiently present in 15 genera. Six-pseudocolpate pollen grains occur in *Ammannia*, *Crenea*, *Ginoria*, *Haitia*, *Hionanthera*, *Lafoensia*, *Lagerstroemia*, *Lawsonia*, *Nesaea*, *Pemphis*, and *Rotala*. Three-pseudocolpate pollen grains occur in *Koehneria*, *Lythrum*, and *Peplis* and indistinctly in some *Sonneratia* (fig. 6*C*; *Crenea*, *Haitia*, *Hionanthera*, and *Koehneria* are not represented in the combined gene analyses). In the Combretaceae, all but three genera have three-pseudocolpate pollen, whereas pseudocolpi are absent in the Onagraceae (Patel et al. 1984). The MP and MB results of combined data indicate that both three-pseudocolpate and six-pseudocolpate grains arose from the nonpseudocolpate condition multiple times across the family. Three-pseudocolpate grains are derived early, in *Lythrum-Peplis,* and six-pseudocolpate grains appear later on terminal branches, except in clade I, where the six-pseudocolpate state is synapomorphic for the clade (minus *Tetrataxis*; see below). In the combined gene phylogenies (missing four genera with pseudocolpate pollen that are not taken into account here), three pseudocolpi arose minimally twice and six pseudocolpi minimally five times (fig. 6*C*). There was also one reversal from six to zero pseudocolpi (not illustrated) in clade I in *Tetrataxis*, the sister genus to *Ginoria* (based on ITS; fig. 4). Muller (1981) proposed a harmomegathic function for pseudocolpi, suggesting that the evolution of pseudocolpi was related to radiation of lineages into wetter or drier habitats. However, in the Lythraceae there is no distinction in habitat between genera with pseudocolpate versus nonpseudocolpate pollen.

Seed coats in approximately one-half the genera of the Lythraceae are unique among all angiosperms in having inverted exotestal trichomes formed by protrusion of a membranous finger-like process into the cell lumens of the outermost seed coat cell layer (Graham 1995*a*). The trichomes remain invisible until the seeds become wet, at which time they evaginate through a lidlike opening in the outer cell wall, collectively forming a hairy, ultimately mucilaginous, coating. The extensive, absorptive mucilaginous surface is thought to hasten the softening of the seed coat for germination, act to decrease dessication during germination, or possibly serve other functions (Werker 1997).

Seventeen genera of Lythraceae have seed trichomes; in 12 genera they are short, variously ornamented, and erect on emergence, and in five they are long and spirally twisted. The morphological cladistic analysis of Graham et al. (1993*a*) found that the straight and spiral forms each originated once, with spiral trichomes uniquely synapomorphic for a five-member clade (*Cuphea-Pleurophora-Woodfordia-Pehria-Lafoensia*). The molecular evidence provides a different, surprisingly homoplastic evolutionary history for this unique character in light of its chemical and structural complexity (Stubbs and Slabas 1982). In the combined gene data analyses, straight seed trichomes are an early acquisition, evolving before the divergence of the superclades. They characterize clades I (excepting *Lawsonia*), V, and VI (figs. 5*C*, 6*D*). Straight seed trichomes are lost from clades II and III. The more complex spiral trichomes are not derived from straight trichomes but arose independently twice from the trichomeless state, in *Lafoensia* of clade III and in clade IV.

Chromosome numbers in the Lythraceae are based on x=8 (Graham and Cavalcanti 2001). *Decodon* is a paleopolyploid with a secondary basic number of 16. More than half of the genera in the family, particularly those in superclade I, have basic numbers that are polyploid. The basic number for clade I is uncertain because a range of aneuploid numbers exists. *Ammannia*, *Nesaea*, and *Lawsonia* are possibly x=8, whereas the sister genera *Ginoria* and *Tetrataxis* are

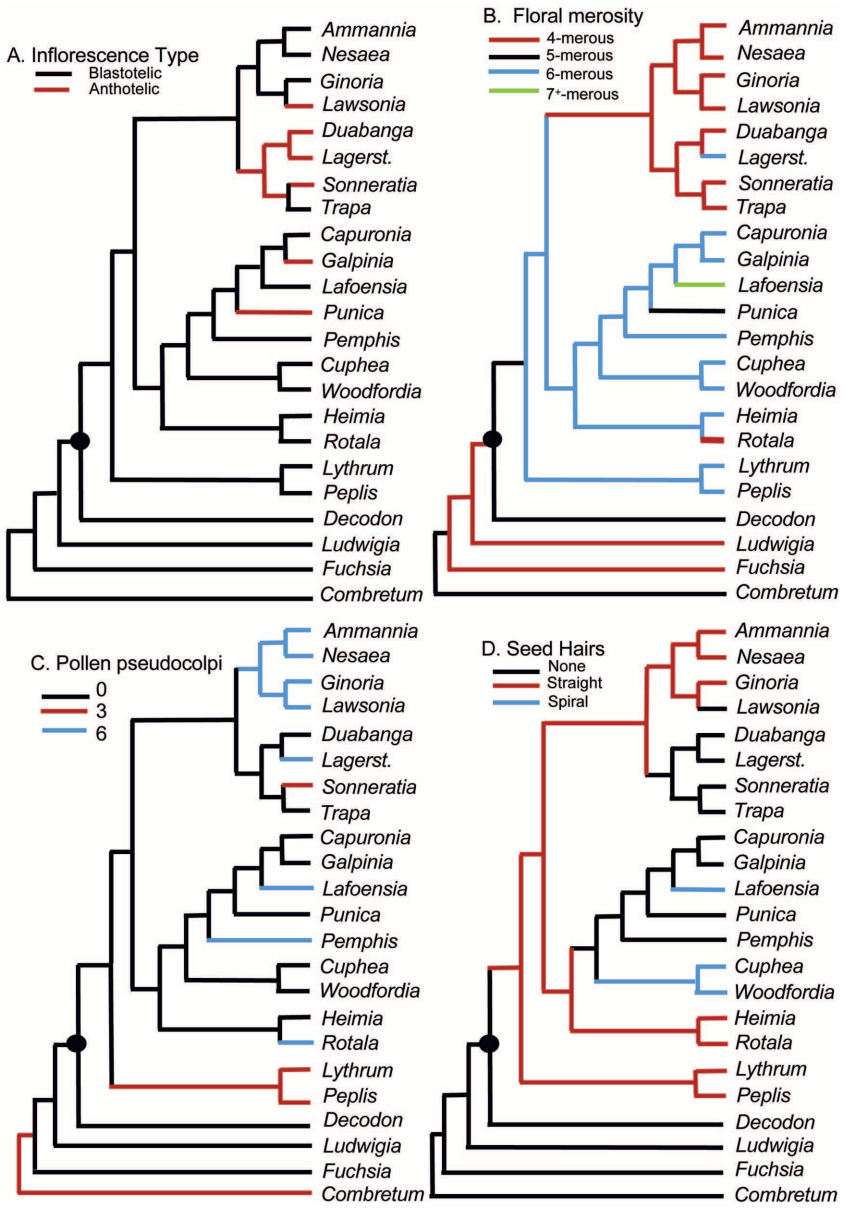

Fig. 6 Selected morphological characters mapped on the Bayesian tree from the combined molecular data set of 20 genera of Lythraceae (fig. 5*C*). The base of the Lythraceae is indicated by a black dot. *A*, Inflorescence type; *B*, floral merosity; *C*, number of pollen pseudocolpi; *D*, type of seed trichomes present in the epidermal cells of the seed coat.

paleopolyploids with x=28, a number exceeded in the family only by the morphologically related but molecularly unknown *Crenea*, with x=32. The basic number for clade II is 12, the same as that proposed for the order (Raven 1975). Given certain derivation of this clade from within the Lythraceae, x=12 is synapomorphic for these taxa rather than plesiomorphic as previously indicated (Graham et al. 1993*b*). Clade III is consistently x=8. Clade IV has x=8 in *Cuphea* and *Woodfordia*, whereas in the New World genera of the clade (fig. 3; *trn*L-F), *Pleurophora* shows an aneuploid decrease to x=7 and *Adenaria* and *Pehria* a tetraploid increase to x=16. In the Old World, *Koehneria* is a hexaploid with x=24. In clade V, *Heimia* and *Rotala* have x=8. In clade VI, *Lythrum* and *Peplis* have x=5, a number presumably derived through aneuploid loss from 8.

Recent phylogenetic analyses in Onagraceae indicate that the basic number is also 8, with an early shift to x=11 (Levin et al. 2003). In Combretaceae, the basic number appears to be 12 for the *Combretoideae*, although genera have x=7 and 11–13 (Raven 1975). The basic chromosome number for the other subfamily, *Strephonematoideae*, is unreported. The wide range of numbers within the Combretaceae, Lythraceae, and Onagraceae indicates that chromosome number changes, both aneuploid reductions and ploidal chromosome number increases, have played a major role in the diversification and expansion of this major lineage of the Myrtales.

The present taxonomic classification of the Lythraceae (Koehne 1903) is not congruent with the relationships uncovered in the molecular analyses of Huang and Shi (2002) or this study. Both the tribes and the subtribes within each tribe are paraphyletic. The key characters employed in the taxonomic classification appear in both superclades, and alternatively, the superclades and six crown clades generated by the molecular-based analyses lack unique synapomorphies (table 7). Although generic relationships suggested by Koehne (1886, 1903) are confirmed for *Lythrum-Peplis*, *Cuphea-Woodfordia*, and *Ginoria-Tetrataxis*, several sister genera in our results are taxonomically widely separated in different subtribes of a tribe (e.g., *Ginoria* and *Lawsonia*), in different tribes (e.g., *Heimia-Rotala* and *Ammannia-Nesaea*), or in different families (e.g., *Lagerstroemia* and *Duabanga*). The rank of subfamily for *Duabanga*, *Punica*, and *Sonneratia* (Dahlgren and Thorne 1984; Graham et al. 1998) must be discarded in light of current knowledge, and the Trapaceae should be subsumed into the Lythraceae. The Lythraceae are best treated without recognition of formal infrafamilial taxonomic ranks until the composition and definition of the superclades and clades are more fully characterized by morphological and molecular evidence representing all the genera.

Additional floral characters, such as stigmatic surface characters and differences in ovary wall, capsule, and seed anatomy that support lineages in the sister family Onagraceae (Levin et al. 2003), may offer synapomorphies of phylogenetic and taxonomic value in the Lythraceae once they are sufficiently known. The addition of more gene regions, generation of molecular sequences for the remaining unstudied genera, and more inclusive morphological characterization are needed in order to provide a robust basis for a new taxonomic classification of the family.

Historical Biogeographical Considerations

Analyses clearly demonstrate an Old World origin for the family. New World genera are not monophyletic but have evolved within Old World clades I, III, IV, and V (fig. 5*A*–5*C*; table 7). In clade I, *Ammannia* and *Nesaea* are Afro-Asian, *Tetrataxis* is endemic to Mauritius, *Lawsonia* is Eurasian, and *Ginoria* is centered in the Greater Antilles. The members of clade II are distributed from East Africa to Southeast Asia. Genera of clade III are from East Africa, Madagascar, and the Indo-Pacific, except *Lafoensia* from South America. Clade IV is South American but includes *Woodfordia* from Afro-Asia and *Koehneria*, an endemic of Madagascar. Clade V pairs the American genus *Heimia* and the primarily Afro-Asian *Rotala*. Clade VI comprises Old World northern latitude genera *Lythrum* and *Peplis*. *Decodon,* restricted to eastern North America today, was nearly cosmopolitan at northern latitudes during the Tertiary.

The oldest fossil remains attributed to a broadly delimited Lythraceae are from the Deccan intertrappean beds of western India, with an age of ca. 66–63.7 Ma, i.e., late Cretaceous or early Paleocene (Shukla 1950; Verma 1950; Widdowson et al. 2000). They are partial leaf impressions (*Lagerstroemia*?) and wood (*Sonneratia*?) with associated fruits (*Enigmocarpon*) and flowers (*Sahnianthus*) considered to have lythraceous affinities but not attributable to any modern taxon in the family. Distinctive *Sonneratia/Florschuetzia* pollen grains of Late Paleocene age are described from France (Gruas-Cavagnetto et al. 1988), and two fossil genera of fruits assignable at the family level are recorded from the Eocene London Clay flora (Reid and Chandler 1933; Collinson 1983).

The oldest known seeds of *Decodon* are from the lower Eocene beds in England (Chandler 1960), and seeds and other plant parts of *Decodon* and a *Lawsonia*-related seed type are present by the Middle Eocene in British Columbia (Cevallos-Ferriz and Stockey 1988; Little et al. 2004). If *Decodon* is accepted as sister to the rest of the family, followed in grade by *Lythrum* and *Peplis*, the Lythraceae may well have originated in Laurasia rather than in Gondwana, as was earlier proposed (Johnson and Briggs 1984). A northern latitude origin for the Lythraceae is comparable to recent findings that the northern Tethys margin was the place of origin of another major lineage of the Myrtales, the Melastomataceae (Renner and Meyer 2001; Renner et al. 2001).

Putative fossils of *Trapa* are also widely distributed geographically and stratigraphically in the northern latitudes, although primarily from the Miocene to present (Mai 1985; Zetter and Ferguson 2001). Those older than the Miocene include fossils attributed to, but definitely not related to, modern *Trapa* (Stockey and Rothwell 1997). The fossil record of *Trapa* requires comprehensive reevaluation before it is utilized in phylogenetic dating of the Lythraceae.

The Combretaceae and Onagraceae, reported from fossils of the Late Cretaceous, support the presence of the entire Combretaceae-Lythraceae-Onagraceae lineage by Late Cretaceous (Chmura 1973; Pares Regali et al. 1974*a*, 1974*b*; Friis et al. 1992; Takahashi et al. 1999; Martin 2003). Several other lineages within the order Myrtales have been dated (Melastomataceae: Renner and Meyer 2001; Renner et al. 2001; Crypteroniaceae: Conti et al. 2002; Myrtaceae, Vochysiaceae, and relatives: Sytsma et al. 2004). Sytsma et al. (2004) indicate the separation of the Lythraceae-Onagraceae lineage from the rest of the order by the end of the Albian (99 Ma), early in the history of the Myrtales. The divergence date of the Lythraceae and the Onagraceae, estimated at 93 Ma, in the Late Cretaceous, is considerably earlier than age of 66–63.7 Ma attributed to the oldest fossils of the Lythraceae.

The presence of putative fossil Lythraceae on the European, Asian, and African continents by the lower Eocene, together with the many short internal branches composing the two superclades, indicates that once established, early lythracean stock expanded and diversified rapidly. Initial radiation of the family must have occurred at a time when climates were warmer than today and when intercontinental dispersal was more easily accomplished, possibly before ca. 90 Ma (Crowley and North 1991; Huber et al. 2000). The highest number of Lythraceae genera and the highest number of endemic genera today occur in South America and in Africa/Madagascar/Mauritius (fig. 1).

Molecular data have significantly widened our understanding of the Lythraceae and its history and have contributed to recognition of relationships not apparent through traditional morphological approaches. The crown clades recovered using parsimony and likelihood approaches on sequences from

Table 7

Crown Clades and Selected Morphological Characters Supporting the Lineages of the Bayesian Consensus Tree Generated from Combined Molecular Data (Fig. 5*C*)

Clade and genera	Characters
Superclade I	Flowers basically four-merous (except six-merous in *Lagerstroemia*). Calyx lobes long, constituting half the length of the floral tube or more (exceptions: *Ammannia, Nesaea, Lagerstroemia*); carpels as many as the calyx lobes or half as many (exceptions: *Sonneratia* with more carpels than calyx lobes, *Lagerstroemia* with half as many or less than half as many)
Clade I: *Ammannia*, *Nesaea*, ***Ginoria***, *Tetrataxis*, *Lawsonia*	Pollen with 6 pseudocolpi; nectary absent (present in *Ammannia* and *Nesaea*); seed coat trichomes present, straight; possible basic chromosome number = 8
Clade 2: *Duabanga*, *Lagerstroemia*, *Sonneratia*, *Trapa*	Anthotelic inflorescences (blastotelic in *Trapa*); stamen number three or more times the number of calyx lobes (stamens equal to number of calyx lobes in *Trapa*); ovary semi-inferior to inferior (superior in *Lagerstroemia*); seed trichomes absent; probable basic chromosome number = 12
Superclade II	Flowers basically six-merous (four-merous in *Adenaria*, *Pehria*, and *Rotala*, five-merous in *Punica*, seven-merous or more in *Lafoensia*); calyx lobes short, less than half the length of the floral tube; carpels less than half as many as calyx lobes (except *Heimia*, *Koehneria* and *Rotala* with half as many carpels as calyx lobes and *Punica* with more carpels than calyx lobes)
Clade III: *Capuronia*, *Galpinia*, ***Lafoensia***, *Punica*, *Pemphis*	Leaf apical glands present (absent in *Pemphis*); pollen without pseudocolpi (six pseudocolpi in *Lafoensia* and *Pemphis*); ovary superior (inferior in *Punica*), mature placenta basal; ovule outer integument multiplicative (not in *Galpinia*, unknown in *Capuronia*); seed trichomes absent (present and spiral in *Lafoensia*); basic chromosome number = 8
Clade IV: ***Adenaria***, ***Pehria***, ***Cuphea***, ***Pleurophora***, *Koehneria*, *Woodfordia*	Resin-secreting trichomes present (in *Cuphea* and *Pleurophora;* multicellular nonsecreting trichomes in *Adenaria*, *Pehria*, *Koehneria*, and *Woodfordia* possibly homologous); stamens double the number of calyx lobes (equal number in *Pleurophora*, triple in *Koehneria*); bilocular ovary with one locule reduced (in *Cuphea* and *Pleurophora*); seed trichomes spiral (trichomes absent in *Adenaria* and *Koehneria*); basic chromosome number = 8
Clade V: ***Heimia***, *Rotala*	Calyx lobes short, less than half the length of the floral tube; carpels as many as the calyx lobes or half as many; seeds trichomes present, straight; basic chromosome number = 8
Genera outside the superclades: Clade VI: *Lythrum*, *Peplis*	Calyx lobes short, less than half the length of the floral tube; carpels less than half as many as the calyx lobes (i.e., 2 carpels : 6 calyx lobes); pollen with three pseudocolpi; seed trichomes present, straight; basic chromosome number = 5
Sister to all other Lythraceae: *Decodon*	Blastotelic inflorescence; flowers five-merous, tristylic; calyx lobes less than half the total length of the floral tube; ovary superior; pollen nonpseudcolpate; seed trichomes absent; basic chromosome number = 8

Note. All supporting characters are homoplastic. Gene analyses differed slightly in the genera sampled, so clade associations are a summary of terminal relationships from all analyses. Not shown: *Diplusodon-Lourtella* + *Physocalymma* restricted to the ITS and *psa*A-*ycf*3 data sets as a weakly supported sister clade to clade IV; and *Crenea*, *Didiplis*, *Haitia*, and *Hionanthera*, for which molecular data were unavailable. Genera endemic to the New World are in boldface.

chloroplast and nuclear genomes improve substantially on morphologically based hypotheses. A more complete understanding of the deepest branching events in the history of the family and the subsequent geographical radiation of the lineages remains to be acquired through greater sampling within the family, especially by the gathering of molecular data for the rare genera not included in this study and by careful reevaluation of selected fossils necessary for dating the phylogeny.

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