



Relationships among the confounding genera *Ammannia*, *Hionanthera*, *Nesaea* and *Rotala* (Lythraceae)

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The relationships and taxonomic limits of four morphologically closely similar herbaceous genera of Lythraceae have long been poorly understood. *Ammannia*, *Hionanthera*, *Nesaea* and *Rotala* are small-flowered herbs of aquatic to amphibious habitats in subtropical to tropical Africa and Asia, with a minor presence in the New World. The shared generalized vegetative and floral structures and an inadequate knowledge of features regarded as diagnostic of the genera have resulted in diverse taxonomic delineations and multiple species transfers among *Ammannia*, *Nesaea* and *Rotala*. In this study, vegetative, anatomical, floral, seed and pollen characters are compared, new chromosome numbers are reported for *Ammannia* and *Nesaea*, and phylogenetic relationships of the four genera are hypothesized based on datasets from nuclear rDNA internal transcribed spacer (ITS) and plastid *rbcL* and *trnL-trnF* regions. The results indicate that *Rotala*, together with the American genera *Heimia* and *Didiplis*, forms an early lineage of the family that is only distantly related to the other three genera. *Ammannia*, *Hionanthera* and *Nesaea* form a strongly supported clade in which *Ammannia* and *Nesaea* are paraphyletic and *Hionanthera* is sister to different species of *Nesaea* depending on the analysis. Total morphological and molecular evidence supports congeneric status for *Ammannia*, *Hionanthera* and *Nesaea* under the earliest name, *Ammannia*. © 2011 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2011, 166, 1–19.

ADDITIONAL KEYWORDS: Africa – aquatic plants – chromosome numbers – ITS, *rbcL*, *trnL-F* – pollen morphology – seed morphology.

INTRODUCTION

The relationships and taxonomic limits of four morphologically closely similar herbaceous genera of Lythraceae have long been poorly understood. *Ammannia* L., *Hionanthera* A.Fern. & M.A.Diniz, *Nesaea* Comm. ex Kunth and *Rotala* L. (Fig. 1) are small-flowered, glabrous, mostly amphibious or aquatic herbs of subtropical to tropical Africa, Asia and Australia with a minor presence in the New World. Their similar habit, floral and seed structure, highly plastic floral merosity and an inadequate knowledge of features considered to be diagnostic of

the genera have led to various generic and infrageneric delineations and a multiplicity of species transfers among *Ammannia*, *Nesaea* and *Rotala*.

Ammannia comprises c. 25 annual, predominantly African species. Some species are well defined, but others are highly variable and present numerous unresolved taxonomic problems (Graham, 1985; Immelman, 1991). Ephemeral but distinctive forms cause additional difficulties in identification (Verdcourt, 1994: 38). *Hionanthera*, the most recently described genus in the group, is a rare mono- or ditypic annual of the East African flora (Fernandes & Diniz, 1955), disjunctly distributed in Zimbabwe, Tanzania and Mozambique and known from perhaps fewer than a dozen collections. The vegetative habit suggests a close relationship to *Ammannia*, but *Hionanthera*

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Figure 1. A, *Ammannia coccinea*, Louisiana, USA. B, *Hionanthera mossambicensis*, Tanzania. C, *Nesaea longipes*, Texas, USA. D, *Rotala ramosior*, Missouri, USA. Photographs A, B, C by S. Graham; photograph D by R. H. Mohlenbrock@USDA-NRCS PLANTS Database/USDA SCS.

differs in having flowers with persistent petals and just two to five large, purple–black seeds per capsule. *Ammannia*, *Nesaea* and *Rotala* have caducous petals and c. 200 or more minute brown seeds per capsule. Fernandes & Diniz in the protologue of *Hionanthera* (1955: 91) positioned the genus between *Ammannia* and *Rotala*. Cook *et al.* (1974) included it as a synonym of *Ammannia* and later suggested it was ‘probably best united with *Ammannia*’ (Cook, 1996a: 122).

Nesaea is the most species rich of the four genera with between c. 55 (Verdcourt, 1994) and 70 (Immelman, 1991) species. The plants are erect to prostrate, herbaceous or suffrutescent, annual or perennial herbs of temporary or permanently wet places or, less often, are submerged. They occur primarily in Africa and Madagascar, although five species are endemic to Australia (Hewson, 1990) and three species are rare endemics of southern Texas and adjacent north-eastern Mexico. The North American species are narrowly adapted to alkaline soils in marshy grasslands (Graham, 1977). Some species of *Nesaea* are easily recognized, but others are confusingly similar. Taxonomic difficulties in both *Nesaea* and *Ammannia* can be attributed to one or more of several factors. They must, in part, be the result of selfing within local isolated populations and the consequent accumulation of small genetic and morphological differences. They may also be a result of occasional hybridization (Graham, 1978) or of the expressed effects of polyploidy and aneuploidy. Heterostyly is a factor in *Nesaea*, where c. 16 species are reported to be di- or trimorphic (Koehne, 1903; Immelman, 1991). Different floral morphs have been described as different species (e.g. *N. kuntzei* Koehne and *N. schinzii* Koehne, *vide* Immelman, 1991).

Rotala, with c. 49 species, is represented in Africa and Asia by approximately equal numbers of species. The African taxa are morphologically similar inbreeding plants, whereas species of southern Asia display greater morphological diversity. Cook (1979) considered southern Asia to be the probable centre of origin of the genus. *Rotala* is represented in the New World by two species: the terrestrial endemic *Rotala ramosior* (L.) Koehne and the aquatic, near-cosmopolitan tropical *R. mexicana* Schltdl. & Cham.

Ammannia, *Hionanthera*, *Nesaea* and *Rotala* co-occur in many parts of their ranges. Two or more species of one genus or a mix of the genera can grow intermingled in and around lagoons, swamps, temporary pools, flooded and drying rice fields and other similar moist sites (Cook, 1979; S. Graham, pers. observ.). Taxonomists have questioned the recognition of four genera because of their similar floral structure and shared ecological parameters. In the *Flora of Tropical East Africa*, Verdcourt (1994: 37) accepted *Hionanthera* and *Rotala*, but was unable to satisfac-

torily separate *Ammannia* and *Nesaea*, stating he ‘would query their distinctness.’ Immelman (1991), in a synopsis of 36 taxa of *Nesaea* and *Ammannia* in southern Africa, combined them into a single key, citing the difficulty in determining the state of capsule dehiscence, the major morphological character employed to separate them.

The first phylogenetic study to include the four genera was morphologically based (Graham, Crisci & Hoch, 1993). In the strict consensus of five most parsimonious trees, the genera formed a clade with three other wetland herbs: *Didiplis* Raf., *Lythrum* L. and *Peplis* L. The first molecular phylogeny of Lythraceae included *Ammannia*, *Nesaea* and *Rotala* and utilized combined data from the nuclear rDNA internal transcribed spacer (ITS) and plastid regions *rbcL*, *trnL-trnF* and *psaA-ycf3* (Graham *et al.*, 2005). In the *rbcL* and *trnL-trnF* analyses, in which two species of *Ammannia* and one of *Nesaea* were included, *Nesaea* was sister to one of the two *Ammannia* spp. in a three-member clade. This result left unanswered the question of whether *Ammannia* and *Nesaea* were congeneric until more species could be analysed. *Rotala* appeared as sister to the New World shrub *Heimia* Link in a lineage distant from *Ammannia* and *Nesaea*. Material of *Hionanthera* was not available for the study. In another phylogenetic analysis of the family, Morris (2007) found the endemic North American genus *Didiplis* to be sister to *Rotala*, and *Heimia* to be sister to both of these genera. Additional research on the florally much reduced aquatic *Didiplis* is currently in progress (J. A. Morris, Kent State University, Kent, OH, USA, pers. comm.).

Recently, new collections have been made that include *Hionanthera mossambicensis* A.Fern. & M.A.Diniz, four species of African *Ammannia* and five species of *Nesaea*. A more critical examination of the generic limits and relationships is now possible. Here, we briefly review the historical taxonomy of the genera; compare their morphology and evaluate the characters traditionally used to segregate them; construct molecular phylogenetic trees using ITS, *rbcL* and *trnL-trnF* sequences to further clarify relationships; and assess the molecular phylogenetic and morphological support for taxonomic recognition of the genera.

MATERIAL AND METHODS

MORPHOLOGICAL DATA AND CHROMOSOME NUMBERS

Morphological characters were compared by the first author following field collections in North America and Tanzania, observations of glasshouse-grown progeny of wild-collected seeds and examination of herbarium collections from DSM, MO and NHT

Table 1. Morphological comparison of *Ammannia*, *Hionanthera*, *Nesaea* and *Rotala*

Character	<i>Ammannia</i>	<i>Nesaea</i>	<i>Hionanthera</i>	<i>Rotala</i>
Stem aerenchyma	Yes	Yes	No	No
Inflorescence	Cymose	Cymose	Cymose	Racemose
Floral tube length (mm)	0.3–6	1–4.7	1–1.5	0.3–2
Floral merosity	4(5–8)-merous	(4–)6(–8)-merous	(3)4(5)-merous	(3–)4(–6)-merous
Epicalyx	None to short	None to long	Short	None to long
Heterostyly	Monomorphic	Mono-, di-, trimorphic	Monomorphic	Mono-, dimorphic
Petals	Caducous	Caducous	Persistent	Caducous
Stamen position	Haplostemonous	Diplo-, haplo- and obhaplostemonous	Haplostemonous	Haplostemonous
Pollen size (µm)	30–34 × 24–28	28–38 × 20–32	27–29 × 23–25	18–24 × 15–20
Pollen pseudocolpi	6 distinct	6 distinct	6 faint (to 0?)	6 faint (to 0?)
Pollen exine	Striate, interlaced	Striate, interlaced	Striate, parallel	Scabrate to verrucate
Locule number	(1–)4(5)	2–5	2	2–5
Ovule number per capsule	Numerous	2–5	Numerous	Numerous
Capsule wall	Smooth	Smooth	Smooth	Transversely striated
Dehiscence	Irregular	Circumscissile then irregular	Irregular	Septicidal (2–4 valves)
Seeds buoyant	Yes	Yes	No	Yes
Seed size (mm)	0.4 × 0.3	0.3–0.4 × 0.4–0.5	1–1.8 × 0.7–1.3	0.3–1 × 0.2–0.4
Seed shape	Obovoid–ovoid	Obovoid	Oblong	Obovoid–semi-ovoid
Seed colour	Brown, gold	Brown, gold, red–brown	Dark violet	Brown, gold, red

Key diagnostic characters of the genera as traditionally accepted are in bold type.

Table 2. New chromosome number counts for *Ammannia*, *Nesaea* and *Rotala*

Taxon	Count	Voucher for new counts
<i>Ammannia auriculata</i> Willd.	$n = 16$	Tanzania: <i>Graham 1153</i>
<i>Ammannia baccifera</i> L.	$2n = 24, n = 12 + 1B$	Tanzania: <i>Graham 1154</i>
<i>Ammannia prieuriana</i> Guill. & Perr.	$2n = 32, n = 16$	Tanzania: <i>Graham 1157, 1158</i>
<i>Ammannia</i> sp. nov. (= <i>Ammannia</i> sp. A, Verdcourt, 1994)	$2n = 16, n = 8$	Tanzania: <i>Graham 1156</i>
<i>Nesaea longipes</i> A.Gray	$2n = 52, n = 26$	USA: Texas, <i>Graham 1161, 1162</i>
<i>Nesaea radicans</i> Guill. & Perr. var. <i>radicans</i>	$2n = 32, n = 16$	Tanzania: <i>Graham 1149</i>
<i>Nesaea schinzii</i> Koehne	$2n = 24, n = 12$	Tanzania: <i>Graham 1151</i>

The chromosome number of *Hionanthera* is unknown. Vouchers for new counts are deposited at MO.

(Holmgren & Holmgren, 1998). Pollen characters were obtained from Graham *et al.* (1985, 1987, 1990), and additional pollen samples were studied using single anther mounts in cotton blue/lactic acid viewed with a light microscope. Seed morphology was compared using light and scanning electron microscopy. New chromosome counts were obtained from field-collected flower buds fixed in 3:1 ethyl alcohol–glacial acetic acid, stored in 70% ethyl alcohol, hydrolysed in 1 M HCl for 10 min and stained for visualization in 2% acetocarmine solution. Comparative morphology and new chromosome number counts are summarized for the genera in Tables 1 and 2.

MOLECULAR METHODS

Sequences from ITS (consisting of ITS1–5.8S–ITS2) and plastid *rbcl* and *trnL–trnF* regions were utilized for phylogenetic analyses. The aligned ITS matrix consisted of 50 ingroup taxa, including representatives of 29 of the 31 genera of Lythraceae. The *rbcl* matrix included 23 genera and the *trnL–trnF* matrix included 28 genera. The outgroup taxa for all analyses were *Fuchsia* L. and *Ludwigia* L. from the sister family Onagraceae and *Combretum* Loefl. from Combretaceae (Sytsma *et al.*, 2004; Maurin *et al.*, 2010). Total genomic DNA was extracted from fresh leaves

or silica gel-dried leaf material using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) and a modified protocol to remove excessive secondary compounds and proteins. PCR products were purified using the QIAquick PCR purification kit (Qiagen) following the manufacturer's recommendations. Sequencing of PCR products was performed by Macrogen Inc. (Seoul, South Korea) using the same primers as in the PCRs. Consensus sequences were assembled and edited in Sequencher Σ (version 4.2; Gene Codes Corporation Inc., Ann Arbor, MI, USA) followed by manual adjustments in Se-Al (version 2.0a11; Rambaut, 2007). Ambiguous positions were coded with the relevant IUPAC codes and indels were treated as missing data. New sequences were aligned with published sequences from previous phylogenetic analyses of Lythraceae (Graham *et al.*, 2005). Voucher information and GenBank accession numbers for all taxa are listed in Appendix 1.

PHYLOGENETIC ANALYSIS

Phylogenetic analyses were performed under maximum parsimony (MP) in three different data partitions: (1) each individual matrix; (2) a matrix containing the concatenated plastid sequences; and (3) a combined dataset of ITS and the two plastid DNA matrices. Maximum likelihood (ML) analysis was employed for ITS and for the three-marker combined dataset. For ITS and plastid DNA partitions, parsimony analyses used PAUP* version 4.0b10 (Swofford, 2002) with a heuristic search strategy, excluding uninformative characters, with tree bisection–reconnection (TBR) branch swapping and MULTREES options. For each data partition, 1000 random addition replicates were performed, saving ten trees per replicate. The *rbcL* gene and *trnL-trnF* region were analysed individually and, because plastid loci are linked on a nonrecombinant chromosome inherited as a single unit and have similar rate distributions (Olmstead, Reeves & Yen, 1998), they were also combined for analysis. Finally, given the close congruence of the three datasets, as determined by inspection of the individual cladistic results, the datasets were combined and MP analysis was performed on the combined matrix. Clade support for MP reconstructions under PAUP* was estimated via nonparametric bootstrapping with 10 000 pseudoreplicates (Felsenstein, 1985) using a simple addition sequence and TBR, but saving only one tree per replicate.

The ML estimates were conducted in Garli version 1.0 (Zwickl, 2006; <http://www.garli.nescent.org/>) for the ITS and combined ITS and plastid sequence data using the default parameters and running 1×10^6 generations. Model parameters were not specified a

priori because Garli estimates the best-fit model during analysis. ML bootstrap support (BS) was also estimated using Garli with default topology and threshold settings and 100 replicates. Three independent analyses were run for each of the data partitions.

TAXONOMIC OVERVIEW

Linnaeus, in describing *Ammannia* (*Sp. Pl.* 1: 119. 1753), included three species in the genus: *A. baccifera* L., *A. latifolia* L. and *A. ramosior* L. [= *Rotala ramosior* (L.) Koehne]. Later, he described *Rotala* (Linnaeus, *Mantissa* 175. 1771), based on *R. verticillaris* L., a species from eastern India and Sri Lanka. *Nesaea* Comm. ex Kunth (in Humboldt *et al.*, *Nov Gen. Sp.* 6: 151. 1823; *nom. conserv.*) included species now recognized in *Heimia*, *Decodon* J.F.Gmel. and *Diplusodon* Pohl, as well as in *Nesaea* (Koehne, 1903; Graham, 1977).

Rotala has had a tortuous nomenclatural and taxonomic path; no less than 13 generic synonyms are attributed to it (Panigrahi, 1976). Hiern (1871) considered *Rotala* to be distinct from *Ammannia* and *Nesaea*, distinguishing it by means of valvate capsules and sessile, usually solitary flowers, characters still generally regarded as diagnostic of the genus. Koehne (1880) recognized the taxonomic utility of the microscopically fine, transversely striated capsule wall in *Rotala*, and was the first to employ the character to distinguish *Rotala* from *Ammannia*. The striated capsule is unique to *Rotala* in this family of capsular-fruited genera. In the monograph of Lythraceae, Koehne (1903) included ten genera and selected species from four other genera in the synonymy of *Rotala* and more narrowly defined *Ammannia*. *Hionanthera*, described much later than the others, remains free of taxonomic and nomenclatural changes at the generic level.

Many nomenclatural transfers of species have occurred among the genera in the absence of universally accepted generic limits. Five of the 11 species that Hiern (1871) included in *Ammannia* are now recognized in *Nesaea*. Koehne (1882) transferred ten species of *Ammannia* to *Nesaea*. In a world revision of *Rotala*, Cook (1979) transferred 45 species of *Ammannia* to *Rotala*. *Ammannia triflora* Benth. (*Fl. Austral.* 3: 297. 1867), subsequently recognized as *Nesaea lanceolata* var. *pubiflora* Koehne (*Bot. Jahrb. Syst.* 3: 325. 1882), is once again *Ammannia triflora* (Bean, 2008). The only change in *Hionanthera* has been the reduction from the four original species to two, with the possibility of a further reduction to one (Verdcourt, 1994).

The infrafamilial and generic classification of Lythraceae in use today is based on Koehne's monograph (1903). The classification divides the family into two tribes, separating *Nesaea* in tribe Nesaeae Koehne

from *Ammannia* and *Rotala* in tribe Lythreae Koehne. Nesaeae comprises genera said to have the septa of the ovary complete above the placenta and the placenta continuous with the style. In Lythreae, the septa are described as incomplete, ceasing below the apex of the placenta, and the placenta is not attached to the base of the style. The distinctions have since proven to be inaccurate following detailed anatomical studies (Tobe, Graham & Raven, 1998), and no longer provide a basis for separating *Nesaea* from *Ammannia* and *Rotala* at the tribal level.

MORPHOLOGY, ECOLOGY AND CHROMOSOME NUMBERS

HABIT AND HABITAT

Species of *Ammannia* are true annuals or short-lived perennials that become suffrutescent under extended moist conditions. *Hionanthera* is exclusively annual, germinating in February and usually dying within 3–4 months as the habitat dries. *Nesaea* and *Rotala* include both annuals and perennials. The four genera inhabit similar annually inundated areas, such as low swales, ditches, rice fields or other seasonally wet places. Populations vary from a few individuals to large numbers when the habitat is extensive. The presence of the annual species at any one locality over a period of years can be highly irregular, with large populations found in one year and no or few plants at the same site in the next. Flooded rice fields in Africa and Asia sustain large populations of *Ammannia* and *Rotala*, and rice culture has been the source of introductions of the genera into many parts of the world (e.g. Barrett & Seaman, 1980; Chiang, 1995; Cook, 1996b; Turki, 2007).

Ammannia, *Hionanthera* and *Nesaea* (A/H/N) are typically erect to decumbent, terrestrial or amphibious herbs, whereas plants of *Rotala* are more often amphibious or aquatic with floating or submerged vegetative stems and submerged or emergent inflorescences. When terrestrial, the stems in *Rotala* are often creeping. About eight species of *Rotala* are cultivated for the aquatic plant trade. One native of South-East Asia, *R. rotundifolia* (Roxb.) Koehne, has become invasive in canals in southern Florida (Jacono & Vandiver, 2007). *Ammannia gracilis* Guill. & Perr., *A. senegalensis* Lam., *Nesaea pedicellata* Hiern. and *N. crassicaulis* Koehne are also cultivated as aquarium plants.

Some of the morphological similarities shared among the genera are also found in unrelated amphibious and aquatic members of other families. It is not uncommon to find these genera misidentified in herbaria as *Ludwigia* (Onagraceae) or *Elatine* L. or *Bergia* L. (Elatinaceae). Convergent adaptation to

ecologically specialized habitats with seasonally fluctuating water levels is postulated as the explanation for such shared similarities (Cook, 1979: 10).

STEM AND LEAF COMPARISONS

Ammannia and *Nesaea* are capable of producing extensive spongy aerenchymatous phellem on submerged stems (Schrenk, 1889). The tissue has not been reported from *Hionanthera* or *Rotala*, although it is known from several other genera of Lythraceae (Lempe, Stevens & Peterson, 2001), from Onagraceae and from other members of Myrtales (Little & Stockey, 2003).

With respect to wood anatomy, Baas & Zweypfening (1979) found that *Ammannia* and *Nesaea* and other herbaceous or semi-woody genera of Lythraceae (*Rotala* and *Hionanthera* were not surveyed) share an unspecialized juvenilistic pattern typified by scanty or no paratracheal parenchyma and uniseriate rays of mostly erect cells. *Ammannia* and *Nesaea* further share an incidental specialization, the absence of septate fibres in the stem, a condition occurring also in two distantly related genera, *Diplusodon* Pohl and *Pemphis* J.R.Forst. & G.Forst. Anatomical characters of the stem are of limited value in determining the relationships among all the herbaceous members of Lythraceae (Baas & Zweypfening, 1979). An anatomical survey by Panigrahi (1976, 1982, 1988) described variation at the species level in some *Ammannia*, *Hionanthera*, *Nesaea* and *Rotala*; the significance of the variation is difficult to assess at the generic level.

Leaves in all the genera are simple, entire margined and primarily decussate, with subalternate or whorled leaves present in a few species of *Nesaea* and *Rotala*, but rarely in *Ammannia* (Koehne, 1903). Phyllotaxy in *Hionanthera* is exclusively decussate. Leaf bases in all genera range from attenuate to cordate or auriculate. In North America, the attenuate to truncate leaf bases of *R. ramosior* and *R. mexicana* distinguish them from the New World species of *Ammannia* which have a cordate or auriculate leaf base. Several African–Asian species of *Rotala*, however, have cordate-based leaves; thus differences in leaf shape only separate *Ammannia* from *Rotala* in the New World. In *Hionanthera*, the leaf base is strongly auriculate and clasping. Leaves of the four genera have anomocytic stomata and lack true subsidiary cells, a pattern common in Lythraceae (Esser, 1987; S. Graham, pers. observ.). No significant differences in the cuticle and stomatal patterns of leaves of the four genera were found by Panigrahi (1980).

INFLORESCENCES AND FLOWERS

Inflorescences are typically clustered to tightly clustered cymes in *Ammannia* (infrequently with solitary

flowers), tightly clustered cymes in *Hionanthera* and lax to tightly clustered cymes or solitary axillary flowers on long pedicels in *Nesaea* (Fig. 2G). Flowers are racemose or solitary in *Rotala*, never in cymes (Cook, 1979). A few species of *Rotala* form globose racemes or terminal short spikes. Floral tubes are small in all genera, from scarcely 1 mm to generally not exceeding 6 mm in length. They are campanulate, urceolate, turbinate or subglobose, and often visibly ribbed externally by the vascular system.

Floral shapes and sizes overlap considerably, so that, on the whole, the genera are indistinguishable by these means. In *Hionanthera*, uniquely among the genera, petals are persistent well into capsule maturity, at which time most of the petal tissue dries and falls away. In Lythraceae, persistent petals are otherwise only known from *Cuphea* P.Browne section *Pseudocircaea* Koehne. They differ from those in *Hionanthera* by deflexing into the floral tube as the flower ages and are permanently retained. Petals of *Ammannia*, *Nesaea* and *Rotala* are caducous. Anatomically, floral tubes in *Hionanthera* include a dense crystal cell layer in which each cell is approximately half-filled by a single druse (S. Graham, pers. observ.). This has not been reported in the other genera, although not all species have been surveyed. All genera produce densely staining nectariferous tissue at the gynoecium/floral tube junction, a nectary position shared with seven other genera of the family (Tobe *et al.*, 1998). Panigrahi & Panigrahi (1977) surveyed the system of vascular traces to the flowers in exemplars of the genera, and found that the origin and subsequent development of floral traces in relation to leaf traces varied and overlapped among the genera.

Floral merism is highly plastic in this group, a flexibility that extends to the family generally (Dahlgren & Thorne, 1984; Tobe *et al.*, 1998). Variation in the number of floral parts occurs on flowers of the same and of different plants, and among different organs in a single flower. The state of floral merism is determined by the most frequent number of sepals and petals, rather than by the more variable numbers of stamens and carpels. In *Ammannia*, flowers are predominantly tetramerous, less often penta- to octomerous. In *Nesaea*, the mode is hexamerous with variation from tetra- to octomerous, although flowers of section *Salicariastrum* Koehne are consistently tetramerous. Flowers in *Hionanthera* are tri- to pentamerous, tetramerous being the mode in the few specimens available for study. *Rotala* is usually tetramerous; Cook (1979) recorded just four species that sometimes had trimerous flowers and six species that were sometimes pentamerous. Appendages appearing externally on the floral tube immediately below the sinus between the sepals collectively compose the epicalyx. They occur in some species of

all the genera and vary by species from scarcely developed to prominent.

Staminal position in the floral tube also varies among and within the genera, especially in *Nesaea*. Stamens emerge from near the base of the ovary to approximately mid-level in the floral tube, and differences are not significant at the generic level. When mapped on the most recent molecular phylogenetic trees for Lythraceae (Graham *et al.*, 2005), the plesiomorphic staminal arrangement in Lythraceae is diplostemonous, the stamens forming two whorls in the floral tube, one in front of the sepals and the other in front of the petals. In *Nesaea*, the condition is mostly diplostemonous, although a few species are haplostemonous (stamens in front of the sepals only) or obhaplostemonous (stamens in front of the petals only). *Ammannia*, *Hionanthera* and *Rotala* are haplostemonous, except in hexa- to octomerous flowers of *Ammannia*.

Heterostyly has developed within *Nesaea* and *Rotala*, but is unknown in *Ammannia* and *Hionanthera*. In the primarily monomorphic *Nesaea*, Koehne (1903) described six possibly trimorphic species and ten dimorphic species. Immelman (1991) verified two trimorphic and two dimorphic species of *Nesaea* in southern Africa. *Rotala* has four dimorphic species, the remainder being monomorphic (Cook, 1979).

The most important morphological features used to separate the genera are derived from the ovary and the capsular fruit, namely the structure of the capsule wall, the form of the septa and placenta, the number of locules and the type of dehiscence. Anatomical serial sections through the gynoecium have shown the ovarian septa of all genera of Lythraceae to be incomplete to a greater or lesser degree at the apex of the ovary. In *Nesaea*, some species appear to have septa extending fully to the top of the ovary and others not. However, even when the septa appear complete, anatomical sections demonstrate they are not united at the ovary apex (e.g. Tobe *et al.*, 1998: fig. 24). With respect to the continuity of the placenta with the style, or lack of continuity, a distinction also used by Koehne to separate tribe Nesaeae from Lythraeae, the connection appears to vary with the age, expansion and final shape of the placenta (S. Graham, unpubl. data). In many species of *Nesaea* and in some *Ammannia* and *Rotala*, the axile placenta is elongate, ovoid or somewhat compressed or lobed at capsule maturity and a connection to the style persists. In others, especially in *Ammannia*, the placenta enlarges significantly with maturity, becoming globose. With enlargement, the connection to the apex of the ovary is broken and the placenta appears as free central. The ovary and placenta in *Hionanthera* are distinctly different from those of the other genera. The placenta is thin, flattened and extends together with partial septa to only

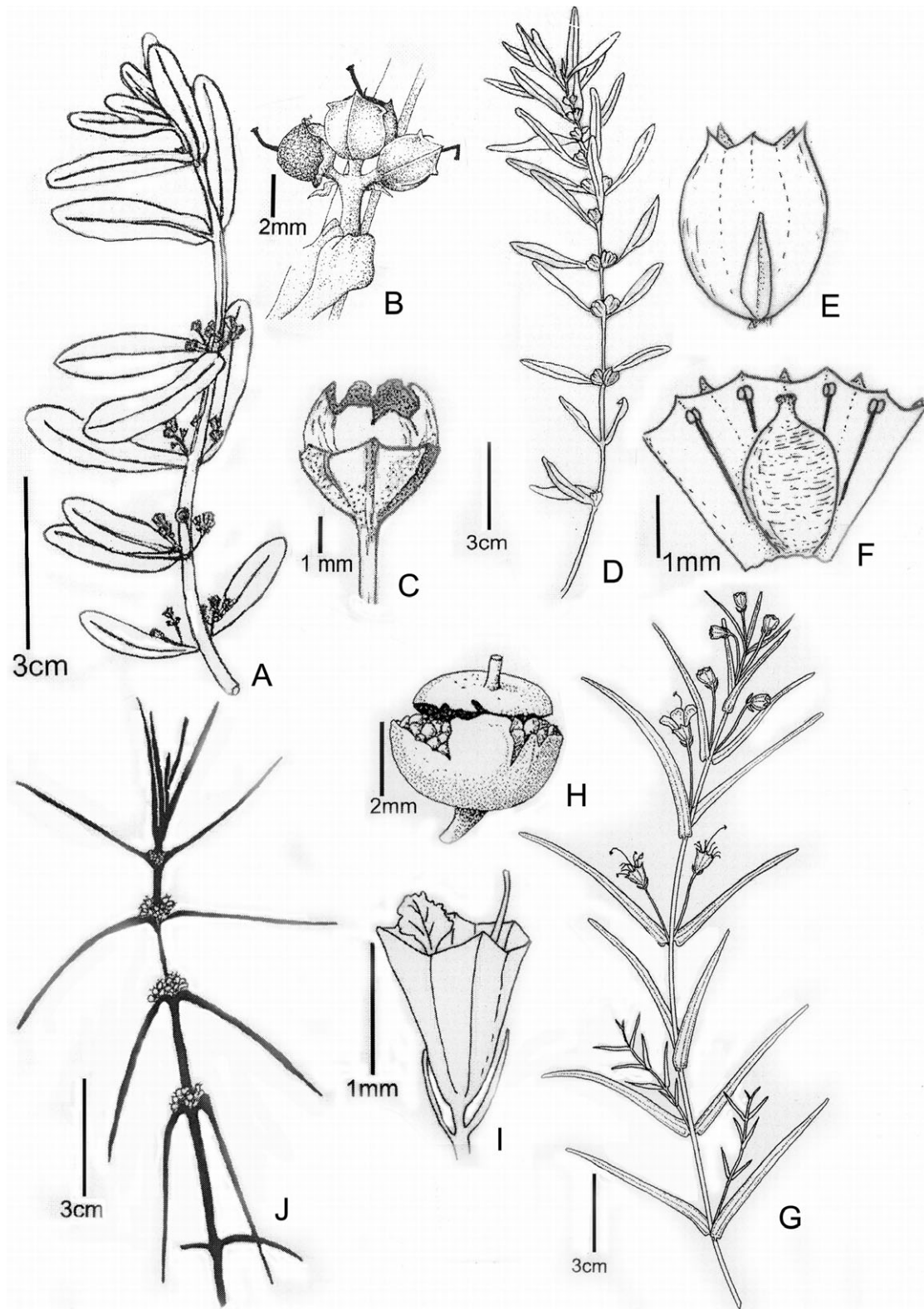


Figure 2. A, *Ammannia senegalensis*, habit. B, *A. coccinea*, floral cyme. C, *A. senegalensis*, capsule with irregular dehiscence. D, *Rotala ramosior*, habit. E, F, *R. ramosior*, flower. G, *Nesaea longipes*, habit. H, *N. longipes*, capsule with circumscissile and irregular dehiscence. I, *Hionanthera mossambicensis*, flower, three petals and stamens missing. J, *Hionanthera mossambicensis*, habit. A, C, modified from Koehne 1903.

half the length of the bilocular ovary or less. The few large seeds are attached near the base of this short placenta and fill the open space in the ovary above the abbreviated septa and placenta.

All genera produce a thin, dry, nearly transparent capsule wall. *Rotala*, uniquely in the family, has a transversely striated wall, the result of strong lignification of the narrow, elongate cells constituting the inner layer of the thin, bilayered pericarp (Leeuwen, 1974; Panigrahi, 1976). The striations are best seen at 10× or greater magnification. Capsule dehiscence in *Rotala* is septicial with two to four valves. One exception may be *R. hexandra* Koehne, described as breaking irregularly at maturity (Cook, 1996b).

Ammannia has a smooth-walled, (one) two to four (five)-locular capsule with dehiscence accomplished by irregular splitting of the capsule (Fig. 2C). The capsule of *Hionanthera* is also smooth and splits irregularly as in *Ammannia*; there is no indication of initial circular dehiscence (Fernandes & Diniz, 1955; S. Graham, pers. observ.). *Nesaea* is said to initially dehisce in circumscissile fashion at the capsule apex, and then split irregularly below (Koehne, 1903: Fig. 2H). Hiern (1871), however, found capsules of *Nesaea* (in species still classified as *Nesaea* today) to dehisce in one of three ways: septifragally by four or five valves; by slits at the apex; or by a short, i.e. circumscissile, lid followed by longitudinal irregular splitting. Immelman (1991: 36) concluded that 'with some specimens it may not be possible to establish the type of capsule dehiscence.' Verdcourt (1994) also found the capsule character difficult to assess and considered that both the more or less circumscissile capsules and those bursting irregularly were present in *Ammannia* and *Nesaea*. Further, he found *N. burttii* Verdc. to have an exceptional, incompletely five-valvate capsule. In spite of the examination of many species from herbarium specimens for this study, we have not been able to conclusively establish the extent of variation in capsule structure and dehiscence, although it appears that, in all A/H/N (some valvate exceptions in *Nesaea*), capsules ultimately irregularly split as seeds reach full maturity.

SEED AND POLLEN MORPHOLOGY

Seeds in the four genera are bilateral and convex-concave from anatropous ovules. *Ammannia*, *Nesaea* and *Rotala* produce numerous, small, semi-ovoid to obovoid, golden to dark brown seeds, 0.3–1.0 × 0.2–0.5 mm (Fig. 3A–D). The average number of seeds per capsule in *Ammannia* in North America is 250 (Graham, 1985); an equally high or higher number of seeds (c. 300) is produced in capsules of *Nesaea* and *Rotala* (S. Graham, unpubl. data). *Hionanthera*, in contrast, produces two to four, rarely five, larger,

oblong, purple–black seeds, 1–1.8 × 0.7–1.3 mm (Fig. 3E). The concave, adaxial, raphal side of the seeds of *Ammannia*, *Nesaea* and *Rotala* is covered by a two- or three-layered aerenchymatous float (Fig. 3B). No evidence of an aerenchymatous float was found in rehydrated, hand-sectioned seeds of *H. mossambicensis*. In *Hionanthera*, cells of the mesotesta are filled by a large solitary druse in each cell. All genera have highly unusual invaginated seed trichomes in the cells of the convex, abaxial exotesta (Fig. 3D). The trichomes evert on wetting and are erect, sparsely tubercled and mucilaginous (Panigrahi, 1986; Graham, 1995).

Pollen in the genera is prolate to prolate-spheroidal in shape, tricolporate with straight colpi, circular apertures and six pseudocolpi (Graham *et al.*, 1985, 1987, 1990). The pseudocolpi are distinct in *Ammannia* and *Nesaea* and faint (perhaps sometimes absent) in *Hionanthera* and *Rotala*. The exine sculpture in *Ammannia*, *Nesaea* and *Hionanthera* is finely striate with the striae interlaced in *Ammannia* and *Nesaea* (Fig. 4A, C). The striae in *Hionanthera* are parallel to the colpi near the apertures and oriented at approximately right angles in the mesocolpal region, but can be disrupted near the apertures (Fig. 4B). Pollen of *Rotala* is distinctly different, smaller than the others, with a scabrate to verrucate, not striate, exine (Fig. 4D).

CHROMOSOME NUMBERS

Chromosome numbers have been reported for ten species of *Ammannia* (Graham & Cavalcanti, 2001). Haploid numbers include 8, 9, 12, 13, 14, 15, 16, 17, 18, 20, 24 and 33; three species each have two reported numbers. The basic number for the genus is uncertain as a result of extensive aneuploidy and polyploidy, although it is probably eight, the basic number of the family. The highest numbers are reported from the endemic American species *A. latifolia* L., $n = 24$, and *A. coccinea* Rottb., $n = 33$. In *Nesaea*, chromosome numbers are known for six species and include haploid numbers of 5, 12, 15, 16, 23, *c.* 25 and 26. Previous numbers of $n = 5$, 15, 23 and *c.* 25 suggested that the basic number might be five, but new counts (Table 2) add haploid numbers of 12, 16 and 26. The diversity of numbers obscures the basic number. In *Rotala*, three species have recorded chromosome numbers: *R. indica* (Willd.) Koehne, $n = 16$; *R. ramosior*, $n = 8, 16$; and *R. rotundifolia*, $n = 15, 16$; the basic number of the genus is eight. *Hionanthera* chromosomes have not been counted. The wide array of chromosome numbers suggests that the plants are actively speciating. Both autogamy, which tends to generate aneuploids (Comai, 2005), and polyploidy, through hybridization or unreduced

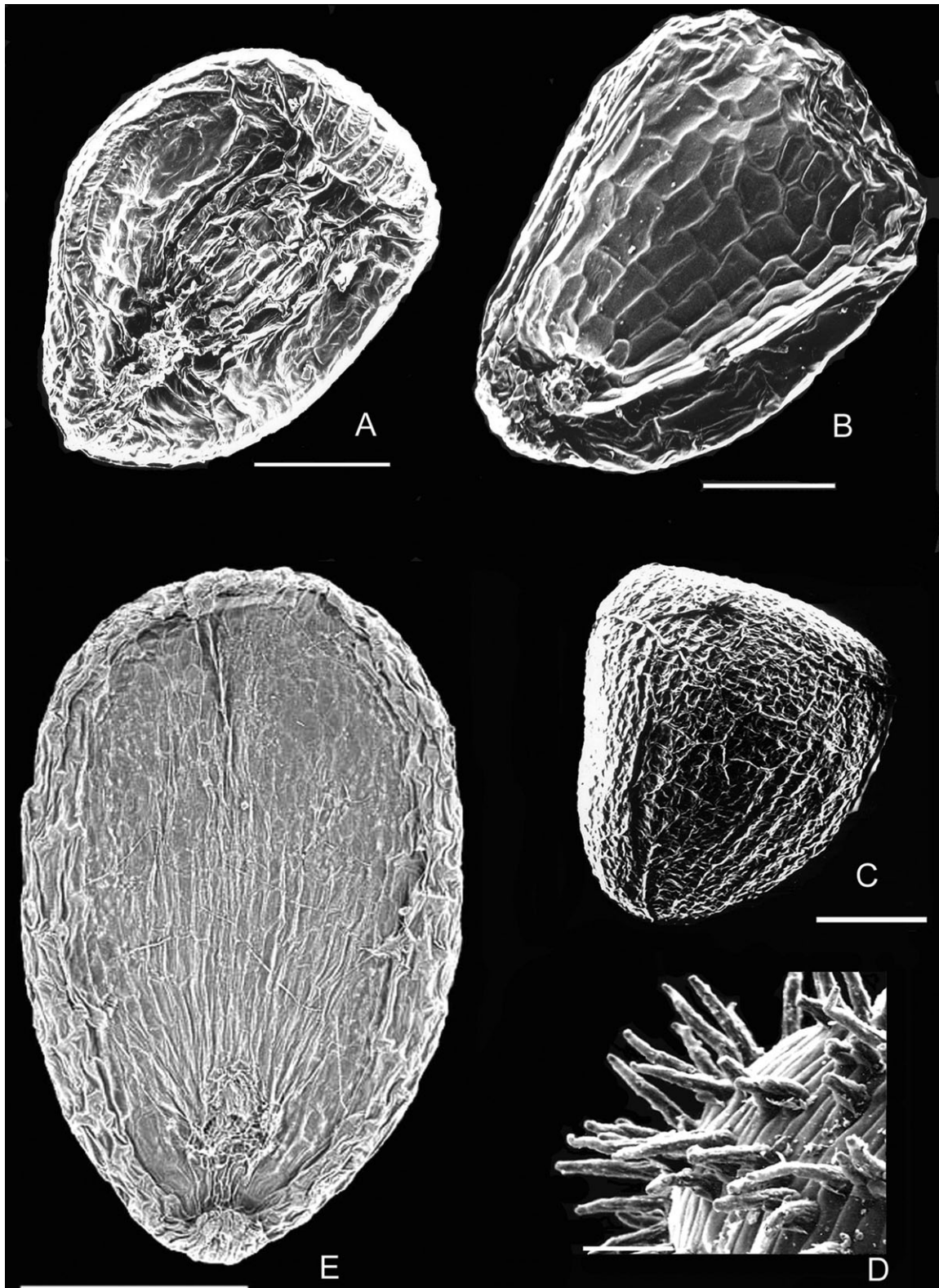


Figure 3. Seeds. A, *Nesaea erecta* Guill. & Perr., adaxial view, float aerenchyma collapsed; bar, 100 μm . B, *Ammannia coccinea*, adaxial view, float aerenchyma inflated; bar, 100 μm . C, *Rotala ramosior*, abaxial view, dry seed; bar, 100 μm . D, *R. ramosior*, seed exotestal trichomes, wetted seed; bar, 50 μm . E, *Hionanthera mossambicensis*, adaxial view; bar, 500 μm .

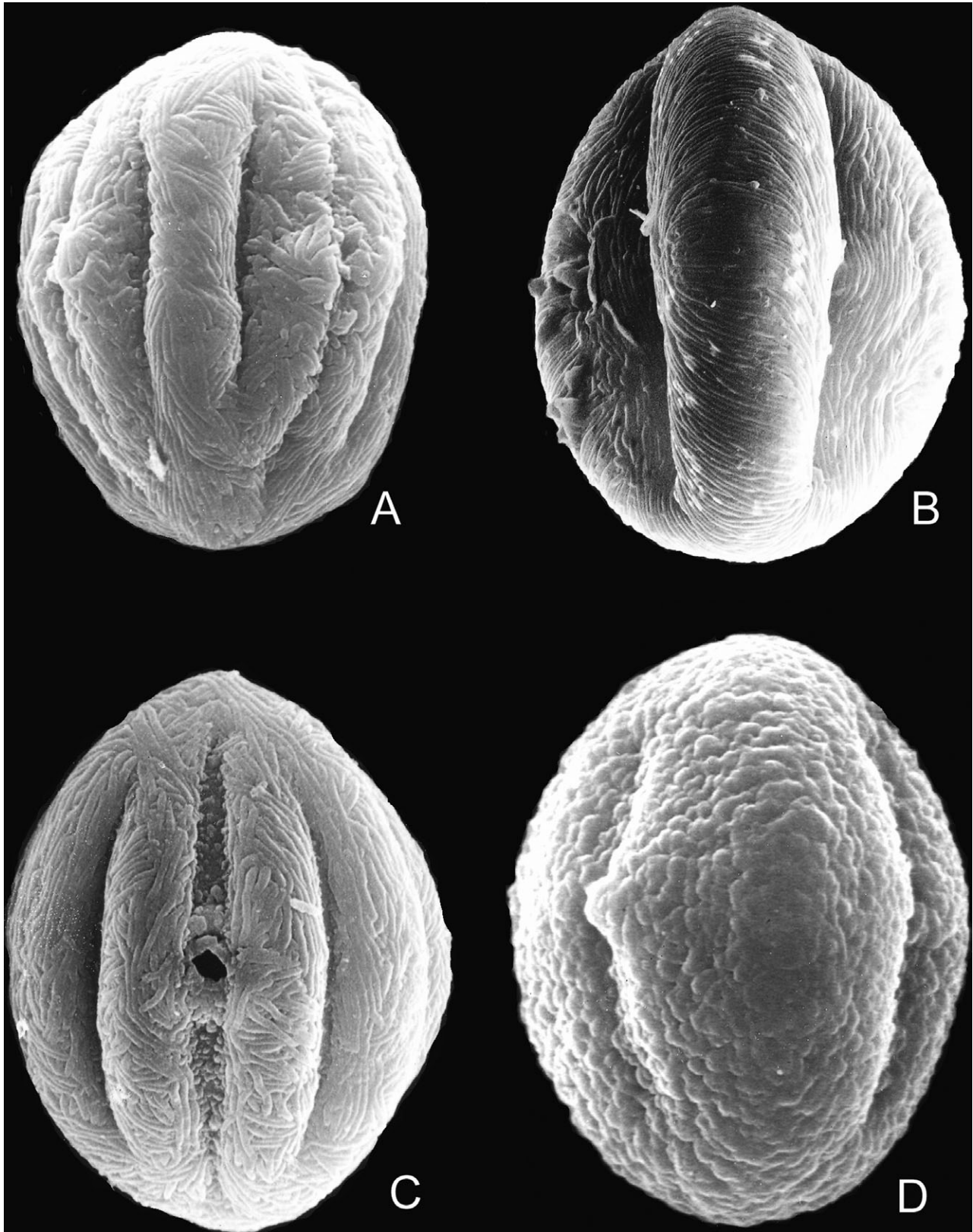


Figure 4. Pollen, equatorial view. A, *Ammannia multiflora* Roxb., actual size $33 \times 27 \mu\text{m}$. B, *Hionanthera mossambicensis*, actual size $37 \times 29 \mu\text{m}$. C, *Nesaea radicans*, actual size $37 \times 31 \mu\text{m}$. D, *Rotala ramosior*, actual size $22 \times 17 \mu\text{m}$.

gametes, result in changes in chromosome numbers and, ultimately, to morphological changes and species formation. In *Ammannia* (Graham, 1978), the allopolyploid *A. coccinea*, $n = 33$, is of putative hybrid origin from *A. auriculata* Willd., $n = 16$ and *A. robusta* Heer, $n = 17$. Given the diversity and range of shared numbers, chromosome numbers do not aid in the delimitation of the genera.

RESULTS

MORPHOLOGICAL CHARACTER COMPARISONS

The characters described above are summarized in Table 1. Comparisons indicate that *Rotala* is morphologically distanced from the others by at least five features, including racemose inflorescences, transversely striated capsules, septicidal capsule dehiscence and smaller pollen with a scabrate to verrucate exine. The seeds of *Rotala*, on the other hand, appear to be identical to those of *Ammannia* and *Nesaea* in size, shape, number and possession of an aerenchymatous float. A/H/N share cymose inflorescences, smooth capsule walls, irregular capsule dehiscence and larger pollen with a striated exine. *Nesaea*, as currently defined, is morphologically the most variable, having a greater range of flower size, greater diversity in staminal arrangements, with up to three floral morphs, and an apparent diversity in capsular dehiscence that may not always be initiated by a circumscissile opening. Apart from the uncertain status of capsular dehiscence in *Nesaea*, no synapomorphies separate *Ammannia* and *Nesaea*. *Hionanthera* has accumulated a number of autapomorphies: persistent petals; an ovary with an abbreviated placenta and septa and two to five ovules; seeds that are oblong, dark violet, lack float tissue, have a crystal-filled seed coat and are *c.* three times longer than those of the other genera; and pollen with parallel, not interlaced, striae.

MOLECULAR RELATIONSHIPS

The ITS dataset contains the largest number of taxa in the molecular study, representing 29 of the 31 genera in Lythraceae. Phylogenetic trees reconstructed from MP (not shown) and ML analyses (Fig. 5) of the ITS data partition are closely congruent. An early diverging lineage, comprising *Rotala*, *Didiplis* and *Heimia*, forms a sister clade to the rest of the family with high or moderate BS, depending on the optimality criterion (MP/ML BS, 94%/72%). The sister relationship of *Rotala* to *Didiplis* is only moderately supported (BS, 72%) in the ITS ML tree, although it was strongly supported in the family study by Morris (2007) based on plastid *trnK-matK*, *trnL-trnF* and *rbcL* data. In Graham *et al.* (2005),

MP, ML and Bayesian analyses consistently recovered a *Rotala-Heimia* sister relationship, but the position of the clade in the family was unstable, switching between the two superclades or appearing as an independent lineage depending on the dataset. In all present molecular analyses, the clade in which *Rotala* occurs is sister to the rest of Lythraceae and distant from A/H/N.

The A/H/N clade is strongly supported (MP/ML BS, 94%/88%) and sister to clade *Lawsonia + Ginoria-Tetrataxis* in one of the two superclades (Graham *et al.*, 2005) of the family. *Ammannia* is nested within *Nesaea*, and *Hionanthera* is sister to the widespread African/Madagascan species *N. radicans* Guill. & Perr. Within the A/H/N clade, branch lengths are short and there is little or no support defining relationships among most taxa.

The individual plastid *rbcL* and *trnL-trnF* parsimony analyses (not shown) produced very similar results to the ITS ML tree, with two exceptions: in the *rbcL* strict consensus tree, 11 genera (including *Rotala*, but not the other three genera) formed a polytomy at the base of the family; and, in the *trnL-trnF* strict consensus, the A/H/N clade was unresolved internally. The combined plastid data produced an MP strict consensus tree from 27 minimum length trees that recovered the same relationships as the ITS analysis, but with overall weaker support. The *Rotala-Heimia* clade was sister to the rest of the family, but with low support (BS < 50%). Support for the A/H/N clade (BS, 85%) and for the *Ginoria-Lawsonia-A/H/N* clade (BS, 64%) was stronger. *Ammannia baccifera* L. was sister to the rest of the A/H/N clade.

A combined analysis of sequences for all taxa represented by the three DNA regions (comprising 22 genera and 11 A/H/N species) was justified by the close congruence of the individual phylogenetic trees. Each region yielded nearly identical topologies in phylogenetic analysis, and there was no conflict between the nuclear and plastid regions with respect to the questions being investigated. The analyses of the three combined markers resulted in identical, single, minimum length MP and ML trees. The same superclades found in the ITS ML analysis (Fig. 5) occurred in the combined ML analysis (Fig. 6). Nine genera form Superclade I (MP/ML BS, 76%/96%) in which clade *Ginoria-Nesaea* is sister to four South-East Asian genera (*Duabanga + Lagerstroemia-Sonneratia + Trapa*). *Ginoria-Lawsonia* is sister (MP/ML BS, 97%/100%) to the fully supported A/H/N lineage (MP/ML BS, 100%/100%). The internal branches of the A/H/N clade are short and moderately to poorly supported, reflecting the close morphological similarities and consequent difficulties in taxonomic separation of the genera. Support values could potentially be improved with further sampling, but, given

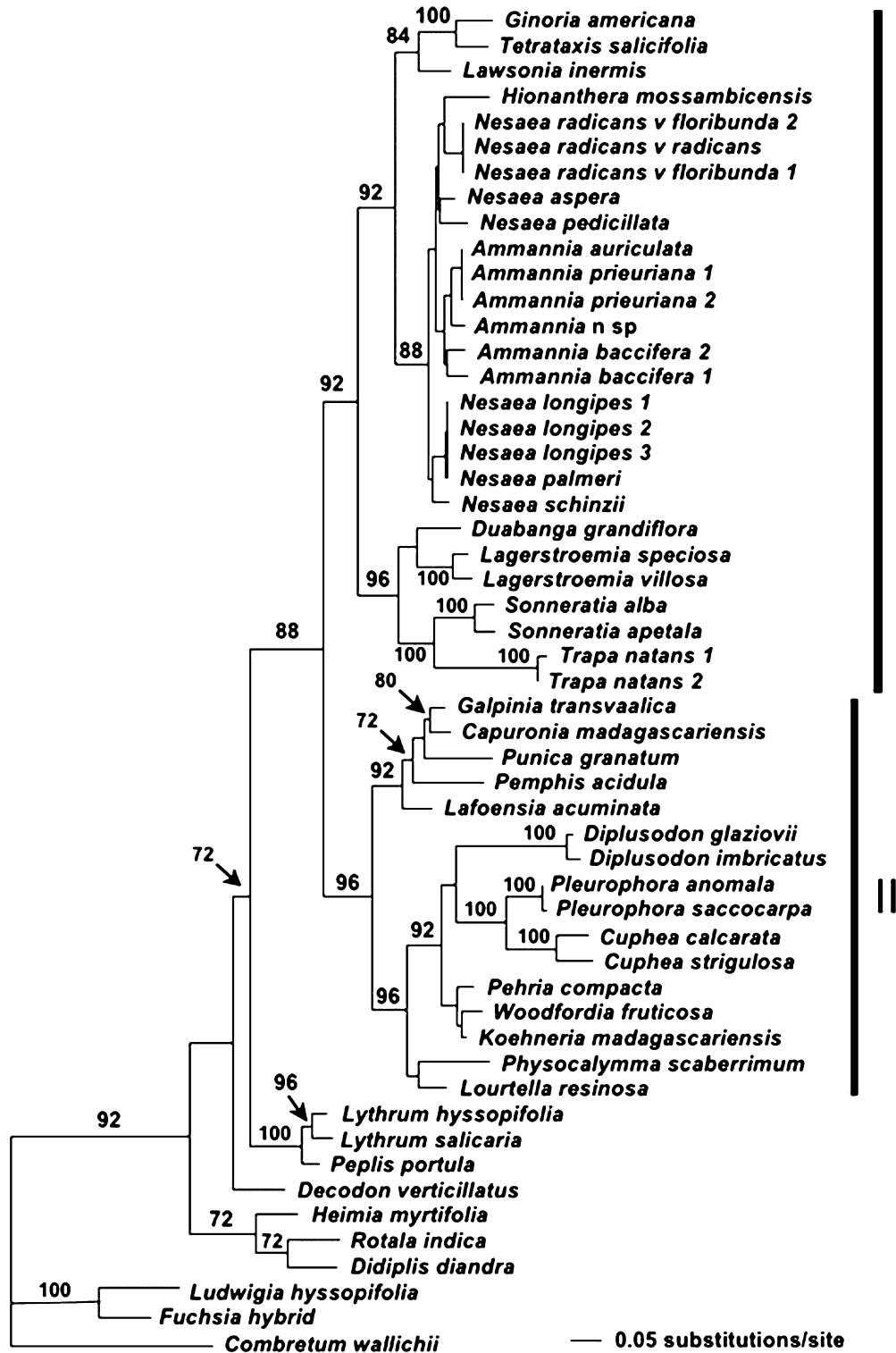


Figure 5. Maximum likelihood (ML) tree for Lythraceae generated from nuclear rDNA internal transcribed spacer (ITS). Numbers on the branches represent ML bootstrap proportions > 70%. Numbers following taxon names refer to different populations of the same species (identified in Appendix 1). *Ludwigia* and *Fuchsia* (Onagraceae) and *Combretum* (Combretaceae) represent the outgroup. The two large lineages identified by vertical bars correspond to Lythraceae Superclades I and II of Graham *et al.* (2005), except that, here, *Heimia* and *Rotala* are excluded from Superclade II. *Didiplis* was not sampled in the earlier study.

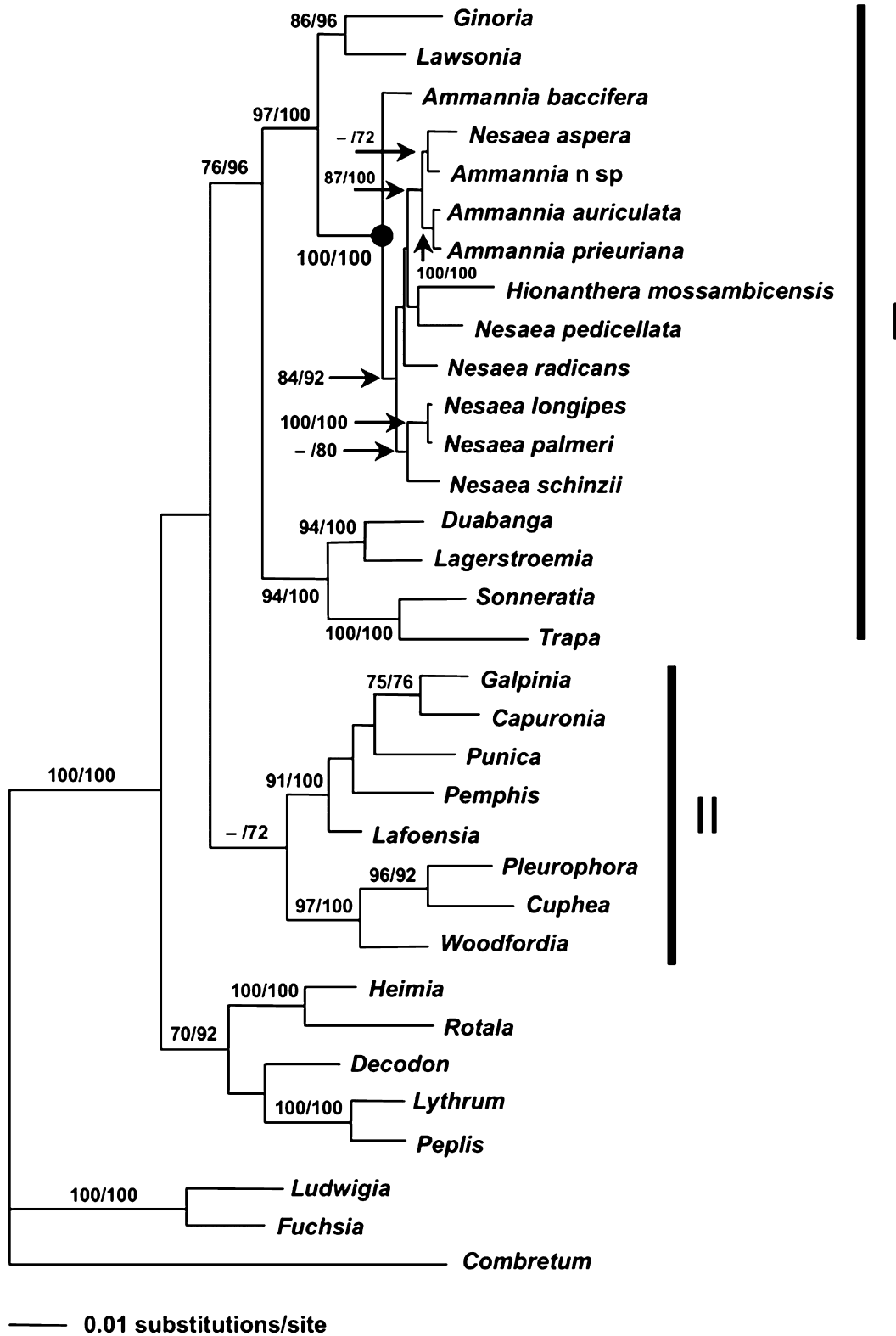


Figure 6. Maximum likelihood tree for Lythraceae generated from combined sequence data of nuclear rDNA internal transcribed spacer (ITS) and the chloroplast *rbcL* and *trnL-trnF* regions. Numbers on the branches represent bootstrap proportions > 70% (left, maximum parsimony; right, maximum likelihood). The filled circle indicates the *Ammannia*–*Hionanthera*–*Nesaea* (A/H/N) clade.

Table 3. Comparative statistics for the maximum parsimony analyses of the three DNA regions

Dataset	No. of taxa	Aligned length	No. of informative characters	% Informative characters	No. of trees	Tree length	CI	RI
ITS	53	888	470	52.9	5	2461	0.43	0.67
Plastid <i>trnL-trnF</i>	46	1492	211	14.1	15 000	453	0.63	0.76
Plastid <i>rbcL</i>	40	1428	155	10.8	70	452	0.46	0.64
Plastid regions combined	33	2912	463	15.9	27	994	0.63	0.85
Three regions combined	33	3747	684	18.3	1	2568	0.50	0.59

CI, consistency index; ITS, internal transcribed spacer; RI, retention index.

the close relationships of the three genera, branch lengths would not be expected to change significantly. As in the combined plastid analysis, *Ammannia baccifera* is sister to the rest of the A/H/N species.

Hionanthera is embedded within the A/H/N clade on a long terminal branch and is weakly supported as sister to *N. pedicellata* Hiern. (MP/ML BS, 61%/56%), rather than to *N. radicans* as in the ITS phylogeny. The American species *N. longipes* A.Gray and *N. palmeri* S.A.Graham are scarcely differentiated molecularly, and are sister to the widespread African perennial *N. schinzii* Koehne with poor to moderate support (MP/ML BS, 56%/80%). In the combined analysis, clades *Rotala-Heimia* and *Decodon+Lythrum-Peplis* form a moderately to strongly supported lineage (MP/ML BS, 70%/92%). However, relationships between this lineage and the superclades remain unsettled because of the poor support (MP/ML BS < 50%/52%) for the sister relationship of the superclades. Significantly, the molecular results show that *Rotala* has clearly evolved independently of A/H/N and that A/H/N constitutes a monophyletic assemblage.

MORPHOLOGICAL CHARACTER EVOLUTION

Previous morphologically based reconstructions of 31 characters for Lythraceae demonstrated that the evolution of morphological features has been complex with high levels of homoplasy. An analysis of morphology alone provided little phylogenetic signal (Graham *et al.*, 2005: Fig. 5). Because the backbone structure of the family lacks strong support, the evolutionary direction of many characters is uncertain, and many of the morphological characters have been gained and lost more than once in the family. We re-examined morphological changes on the combined three-gene ML tree using the character matrix for the family (Graham *et al.*, 2005: Table 3) with attention to the evolutionary direction of morphological traits of the four genera. Some selected traits are here mapped on a portion of the phylogeny to illustrate the characters gained or already present on the branch to

Superclade I and prominent evolutionary changes leading to the A/H/N clade (Fig. 7; homoplasious changes in these character states elsewhere in the family are not shown). On the branch to Superclade I, floral merosity changes from basically hexamerous to basically tetramerous and the sepals lengthen to form half or more of the total length of the flower. Divergence at the next higher node involves numerous changes on the *Duabanga-Trapa* branch (examples of five changes are shown), whereas the branch to the sister clade is supported by a single synapomorphy: six-pseudocolpate pollen. Pseudocolpi are postulated to serve a harmomegathic function, expanding or contracting the pollen grain in relation to surrounding increasing or decreasing humidity (Muller, 1981), and their presence in this clade may be an adaptation to changing humidity levels in the flooding vs. drying habitats of these genera. No synapomorphies define the *Ginoria-Lawsonia* clade.

The ultimate branch to the A/H/N clade is supported by the acquisition of the herbaceous habit, reduction of sepal length from *c.* one-half to one-quarter to one-sixth of the total flower length, striation of the pollen exine and the development of an aerenchymatous float on the convex side of the seed (reversed in *Hionanthera*), a feature increasing buoyancy for seed dispersal in an aquatic environment. As the herbaceous habit was gained, septate woody fibres of the stem were lost, in keeping with the change to a juvenilistic anatomy typical of herbaceous genera of the family (Baas & Zweypfenning, 1979). All individual changes on the A/H/N branch have also evolved independently elsewhere in the family on one or more occasions.

With respect to the traditional reliance on morphological characters in determining relationships, the independently derived *Rotala* exhibits several of the same character states as the A/H/N clade: herbaceous habit; small, basically tetramerous flowers that are deeply campanulate with short sepals; nearly identical light-weight, boat-shaped seeds with floats; and pollen with six pseudocolpi. The molecular evidence reveals

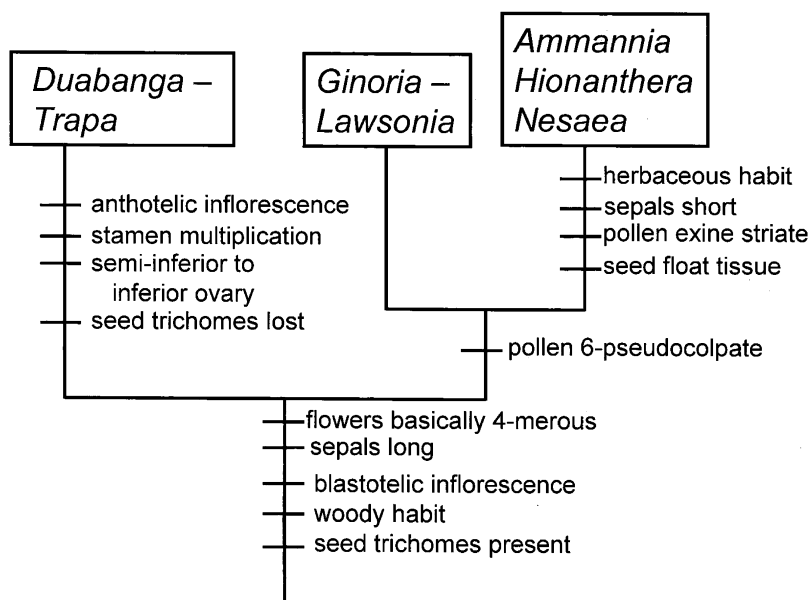


Figure 7. Selected morphological traits gained on branches leading to the *Ammannia*–*Nesaea*–*Hionanthera* clade in Superclade I of Lythraceae.

an instance in which plants have independently converged on closely similar seed and pollen adaptations for reproductive success in varying wet environments.

DISCUSSION

Prior to this study, the only explicit hypothesis of relationships among the genera *Ammannia*, *Nesaea* and *Rotala* was that proposed by Koehne (1885a: 7, 32; Koehne, 1885b: 276) who provided a type of nearest-neighbour diagram (Koehne, 1885a: 32) illustrating *Nesaea* as the ‘primaeval’ genus central to all subsequent evolution in the family, and suggesting that *Ammannia* and *Rotala* independently diverged directly from *Nesaea*. The results of the present molecular analyses put this hypothesis to rest. *Rotala*, in spite of its long confusion with *Ammannia*, is only distantly related to the A/H/N lineage. The probable centre of origin of *Rotala* in Asia and that of A/H/N in Africa is further evidence of a separate, but highly convergent, evolution of the two lineages. *Rotala* can be distinguished morphologically from A/H/N taxa by the combination of an inflorescence of racemose or rarely solitary flowers, a striated capsule wall, and septicidally dehiscent, two- to four-valvate capsules.

The totality of evidence demonstrates that A/H/N constitute a monophyletic assemblage that is derived within one of the two superclades of the family. *Ammannia* and *Nesaea* are paraphyletic as currently defined. Morphological features accepted as diagnostic of the two genera are either identical or overlap (cf. Table 1), lending support to the inference from the

molecular results that the genera are congeneric. The extent of variation in capsule dehiscence in *Nesaea* is not fully known, but does not change the conclusion that the preponderance of evidence, both morphological and molecular, best supports the treatment of *Ammannia* and *Nesaea* as a single genus.

The numerous morphological autapomorphies of *Hionanthera* that have arisen on the terminal branch to the genus make it easily identifiable taxonomically, in contrast with the difficulties in distinguishing *Ammannia* from *Nesaea*. However, given the full phylogenetic molecular support for the A/H/N clade, we choose to unite A/H/N as a single monophyletic genus under the earliest name, *Ammannia*.

As recognized here, *Ammannia* is a genus of herbaceous plants, primarily diversified in Africa, occupying temporarily or permanently wet terrestrial, amphibious or aquatic habitats, and defined by the following unique suite of features: herbaceous habit; leaves simple, decussate; inflorescence cymose; flowers perigynous, monomorphic or heteromorphic, basically tetramerous but flexibly tri- to hexamerous; sepals *c.* one-quarter to one-sixth of the total floral length; stamens equal to the number of sepals to double the number; pollen tricolpate, six-pseudocolpate with a striate exine; ovary superior, locules (one) two to five; style simple; stigma capitate; capsules thin-walled, smooth, primarily irregularly dehiscent, with or without an initial circumscissile opening; and seeds bilateral with exotestal invaginated simple trichomes. The nomenclatural changes necessitated by the unification of A/H/N require the

transfer of approximately 100 names to *Ammannia*. Consequently, the nomenclatural issues will be dealt with in a separate publication.

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APPENDIX 1

GENBANK ACCESSIONS USED IN THIS STUDY

GenBank numbers are cited in the following sequence: (ITS/*rbcL*/*trnL*–*trnF*). New sequences reported for the first time in this study are in bold type and sequences not available are indicated by ----. Citations of herbaria follow the acronyms of the *Index Herbariorum*, <http://www.sweetgum.nybg.org>. Vouchers are deposited in MO unless otherwise cited.

Ammannia auriculata Willd. Tanzania, *Graham 1153*; **HQ878334/HQ878352/HQ878361**. *Ammannia baccifera* L. (1): China, *Tang 99010301* (SYS); AY905419/AY036145/AY905452. (2): Tanzania, *Graham 1154*; **HQ878335**/----/**HQ878362**. *Ammannia latifolia* L. Puerto Rico, *Liogier 10314*; ----/AY905404/AY905453. *Ammannia* sp. nov. Tanzania, *Graham 1156*; **HQ878336/HQ878353/HQ878363**. *Ammannia prieuriana* Guill. & Perr. (1): Tanzania, *Graham 1157*; **HQ878337**/----/**HQ878364**. *Ammannia prieuriana* Guill. & Perr. (2): Tanzania, *Graham 1158*; **HQ878338/HQ878354**/----. *Capuronia madagascariensis* Lourt. Madagascar, *D'Arcy 15439*, AY905420/AY905405/AY905454. *Combretum paniculatum* Vent. Africa, *J. Hall s.n.* (WIS); ----/----/AY05455. *Combretum wallichii* DC. China, *Shi 990703005* (SYS); AF208731/AY036151/----. *Cuphea calcarata* Benth. Mexico, *Graham 145* (MICH); HM099526/----/HM099545. *Cuphea strigulosa* Kunth: Brazil, *Cavalcanti et al. 2324*; AY910702/----/HM099630. *Cuphea utriculosa* Koehne: Mexico, *Graham 1086*; ----/----/AY905456. *Decodon verticillatus* (L.) Ell. USA, *Graham 917*; AY905421/AY905406/AY905457. *Didiplis diandra* (DC.) Wood: USA, *Graham 1146*; **HQ878339**/----/----. *Diplusodon glaziovii* Koehne: Brazil, *Ingles s.n.* (CEN); **HQ878340**/----/----. *Diplusodon imbricatus* Pohl: Brazil, *Ingles s.n.* (CEN); **HQ878341**/----/----. *Diplusodon paraisoensis* Lour. Brazil: *Cavalcanti et al. 2210* (CEN); ----/----/AY905458. *Duabanga grandiflora* (DC.) Walp. China: *Huang 990401* (SYS); AF163695/----/----. *Duabanga grandiflora* (DC.) Walp. Thailand, *Maxwell s.n. in 1991*, no voucher; ----/AY905407/----. *Duabanga grandiflora* (DC.) Walp. China, *Ge et al. s.n.*, no voucher; ----/----/AF354179. *Fuchsia cyrtandroides* J.W.Moore: Venezuela, *Berry 4618*; ----/L10220/AY905460. *Fuchsia* hybrid: China, *Jian 20010207* (SYS); AY035748/----/----. *Galpinia transvaalica* N.E.Brown: Africa, *Balsinhas 3263*; AY905423/AY905409/AY905461. *Ginoria americana* Jacq. Cuba, *Graham 1127*; AY078421/----/----.

- Ginoria americana* Jacq. (orig. ID *G. glabra* Griseb.): Cuba, Fairchild Garden s.n.; ----/----/AY905462. *Ginoria nudiflora* (Hemsl.) Koehne: Mexico, Gutiérrez 3098; ----/AY078418. *Heimia myrtifolia* Cham. & Schltldl. China, Tang 99070502 (SYS); AF201693/----/----. *Heimia salicifolia* Link: Paraguay, Pérez 1070; ----/AY905410/AY905463. *Hionanthera mossambicensis* A.Fern. & Diniz: Tanzania, Graham 1155; **HQ878342/HQ878355/HQ878365**. *Koehneria madagascariensis* S.A.Graham: Madagascar, D'Arcy & Rakotozfy 15317; AY905424/----/AY905465. *Lafoensia acuminata* (Ruiz & Pav.) DC: Ecuador, Neil 8930; AY905425/905411/905466. *Lagerstroemia indica* L. USA, cultivated, no voucher; ----/AY905412/AY905467. *Lagerstroemia speciosa* (L.) Pers. China, Shi 99060103 (SYS); AF163696/AY036149/AY905468. *Lagerstroemia villosa* S.Kurz: China, Shi 2000-01037(SYS); AY035755/----/AY905469. *Lawsonia inermis* L. (1): China, Shi 494 (SYS); AY905426/----/----. *Lawsonia inermis* L. (2): Bahamas, Correll 45915 (TEX); ----/AY905413/AY905470. *Lourtelia resinosa* S.A.Graham, Baas & Tobe: Bolivia, Graham 1116; AY905427/----/----. *Ludwigia hyssopifolia* (G.Don f.) Exell: China, Yuan 2000-72401 (SYS); AY035747/AY036152/----. *Ludwigia peploides* (Kunth) Raven: USA, Sytsma 5010 (WIS); ----/----/AY905473. *Lythrum hyssopifolia* L. Canada, Johnson s.n. in 1998; AY905428/----/----. *Lythrum hyssopifolia* L. USA, Baldwin 500 (DAV); ----/L10218/----. *Lythrum maritimum* Kunth: Hawaii, Graham 1098; ----/----/AY905474. *Lythrum salicaria* L. China, Lei 005 (SYS); AY035749/----/----. *Lythrum salicaria* L. China, Tsang 27844 (IBSC); ----/AF421596/----. *Nesaea aspera* (Guill. & Perr.) Koehne: Africa, Drummond 11446; AY905429/AY905414/AY905475. *Nesaea longipes* A.Gray (1): USA, Graham 1161; **HQ878343/HQ878356/HQ878366**. *Nesaea longipes* A.Gray (2): USA, Graham 1162; **HQ878344**/----/----. *Nesaea longipes* A.Gray (3): USA, Graham 1163; **HQ878345**/----/----. *Nesaea palmeri* S.A.Graham: Mexico, Mora O. s.n. (UNAM); **HQ878346/HQ878357/HQ878367**. *Nesaea pedicellata* Hiern: Tanzania, Graham 1164; **HQ878347/HQ878358/HQ878368**. *Nesaea radicans* Guill. & Perr. var. *radicans*: Tanzania, Graham 1149; **HQ878348**/----/----. *Nesaea radicans* var. *floribunda* (Sond.) A.Fern. (1): Tanzania, Graham 1152; **HQ878349**/----/----. *Nesaea radicans* var. *floribunda* (Sond.) A.Fern. (2): Tanzania, Graham 1159; **HQ878350/HQ878359/HQ878369**. *Nesaea schinzii* Koehne: Tanzania, Graham 1151; **HQ878351/HQ878360/HQ878370**. *Pehria compacta* (Rusby) Sprague: Venezuela, Berry s.n. in 1979; AY905430/----/AY905476. *Pemphis acidula* Forst. (1): China, Liao 1150 (A); AY035762/AY036138/----. *Pemphis acidula* Forst. (2): Marshall Islands, VanderVelde s.n. in 2000, no voucher; ----/----/AY905477. *Peplis portula* L. Portugal, Montezuma s.n.; AY035751/AY036139/AY905478. *Physocalymma scaberrimum* Pohl: Brazil, Cavalcanti et al. 2512 (CEN); AY905431/----/----. *Pleurophora anomala* (St.-Hil.) Koehne: Brazil, Roath s.n.; ----/AY905416/----. *Pleurophora anomala* (St.-Hil.) Koehne: Brazil, Cavalcanti et al. 368; ----/----/AY905481. *Pleurophora pungens* Don: China, Huang s.n., no voucher; AF268395/----/----. *Punica granatum* L. (1): USA, cultivated, Conti 1001 (WIS); ----/L10223/AY905482. *Punica granatum* L. (2): China, Hao 200000318 (SYS); AY35760/----/AY035761. *Rotala indica* (Willd.) Koehne: China, Tang 99070503 (SYS); AY035758/AY036148/AY905484. *Rotala ramosior* (L.) Koehne: Mexico, Graham 1028; ----/AY90517/AY905485. *Sonneratia alba* J.Smith: China, Chen 990604 (SYS); AF163701/----/----. *Sonneratia apetala* Buch.-Ham. China, Qiu 974312 (SYS); AF163697/----/----. *Sonneratia caseolaris* (L.) Engl. China, Huang 990435 (SYS); ----/AY036143/----. *Sonneratia ovata* Backer: China, Chang 9711912 (SYS); AF163702/----/----. *Tetrataxis salicifolia* (Tul.) Baker: Mauritius: Lorence 1231; AY078423/----/----. *Trapa natans* L. (submitted as *T. maximowiczii* Korsh.) (1): China, Wang 2000-041601 (SYS); AY035757/AY036141/----. *Trapa natans* L. (submitted as *T. maximowiczii*). (2): China, Zhang 2000-1010 (SYS); AY035756/----/----. *Trapa natans* L. (3): Japan: Graham 1102; ----/----/AY905491. *Woodfordia fruticosa* (L.) S.Kurz (1): Nepal, USDA-PI 19882; ----/AY905418/AY905492. *Woodfordia fruticosa* (L.) S.Kurz (2): China, Tang 99070504 (SYS); AF201692/----/----.