6

In time and with water . . . the systematics of alismatid monocotyledons

Donald H. Les and Nicholas P. Tippery

6.1 Introduction

In time and with water, *everything changes*.

(Leonardo da Vinci)

In a way, Leonardo da Vinci's succinct characterization of water also applies appositely to the aquatic subclass Alismatidae Takht. ('alismatids'). Indeed, the long evolutionary history of this remarkably diverse group of monocotyledons has resulted in numerous adaptations to facilitate an existence in water, and these represent a substantial departure from their terrestrial counterparts.

Arguably, alismatids represent the greatest adaptive radiation of freshwater plants on Earth. Here recognized to include 11 families, 57 genera and approximately 477 species (Table 6.1), the group contains a rich diversity of floral and vegetative habits, pollination systems (including the largest concentration of water-pollinated species) and also the only occurrences of marine plants in the angiosperms.

Formal studies of alismatid relationships began 40 years ago, initially by phenetic and cladistic analyses of nonmolecular data. The first macromolecular-based phylogeny of the subclass did not appear until 1993, when roughly half of the families and fewer than 20% of genera were evaluated using *rbcL* sequence data (Les et al., 1993). The most comprehensive phylogenetic survey of alismatids

Early Events in Monocot Evolution, ed. P. Wilkin and S. J. Mayo. Published by Cambridge University Press.

Table 6.1 Taxonomic synopsis of subclass Alismatidae showing families (bold font; number of genera indicated in brackets) and subordinate genera (approximate number of species in parentheses).

Alismataceae Vent. [17]
Albidella Pichon (1), *Alisma* L. (11), *Astonia* S.W.L. Jacobs (1), *Baldellia* Parl. (2), *Burnatia* Micheli (1), *Butomopsis* Kunth (1), *Caldesia* Parl. (4), *Damasonium* Mill. (4), *Echinodorus* Rich. and Engelm. ex A. Gray (28), *Helanthium* Engelm. ex Benth. and Hook.f. (3), *Hydrocleys* Rich. (5), *Limnocharis* Humb. and Bonpl. (2), *Limnophyton* Miq. (4), *Luronium* Raf. (1), *Ranalisma* Stapf (2), *Sagittaria* L. (40), *Wiesneria* Micheli (3)
Aponogetonaceae Planch. [1]
Aponogeton L.f. (50)
Butomaceae Mirb. [1]
Butomus L. (1)
Cymodoceaceae Vines [6]
Amphibolis C.Agardh (2), *Cymodocea* K.D.Koenig (4), *Halodule* Endl. (6), *Ruppia* L. (4), *Syringodium* Kütz. (2), *Thalassodendron* Hartog (2)
Hydrocharitaceae Juss. [17]
Apalanthe Planch. (1), *Appertiella* C.D.K. Cook and L. Triest (1), *Blyxa* Noranha ex Thouars (9), *Egeria* Planch. (3), *Elodea* Michx. (5), *Enhalus* Rich. (1), *Halophila* Thouars (10), *Hydrilla* Rich. (1), *Hydrocharis* L. (3), *Lagarosiphon* Harv. (9), *Limnobium* Rich. (2), *Najas* L. (39), *Nechamandra* Planch. (1), *Ottelia* Pers. (21), *Stratiotes* L. (1), *Thalassia* Banks ex K.D. Koenig (2), *Vallisneria* L. (18)
Juncaginaceae Rich. [3]
Cycnogeton Endl. (1), *Tetroncium* Willd. (1), *Triglochin* L. (15)
Maundiaceae Nakai [1]
Maundia F.Muell. (1)
Posidoniaceae Vines [1]
Posidonia K.D. Koenig (5)
Potamogetonaceae Bercht. and J. Presl [7]
Althenia F.Petit (1), *Groenlandia* Fourr. (1), *Lepilaena* Harv. (5), *Potamogeton* L. (100), *Pseudalthenia* (Graebn.) Nakai (1), *Stuckenia* Börner (3), *Zannichellia* L. (6)
Scheuchzeriaceae F. Rudolphi [1]
Scheuchzeria L. (1)
Zosteraceae Dumort. [2]
Phyllospadix Hook. (5), *Zostera* L. (9)

was published in 1997 and included all recognized families, 83% of genera and 15% of the species. Phylogenetic studies consistently have supported the monophyly of alismatids, as we will discuss.

In this chapter we provide an overview of phylogenetic research directed at the elucidation of relationships with and within the alismatids since these initial efforts, and summarize how various genetic loci have been used to evaluate high, intermediate and low taxonomic levels in the group. A main objective is to provide a comprehensive, comparative evaluation of existing systematic information and to identify those groups that would benefit most from additional study.

6.2 New data

Many of the phylogenetic trees presented in the discussion have been taken directly from pertinent published literature, but have been adapted and redrawn to summarize the results as succinctly as possible. In these instances a brief summary of the original methodology used to derive the trees is provided in the text and/or figure captions. Because these trees have been redrawn, we have not provided values of internal nodal support and refer the reader to the original publications for more specific details of these analyses.

In addition, we also include several phylogenetic trees from our own ongoing work, which are based on new, unpublished data and analyses. In these instances, a consistent analytical methodology was used, which is summarized briefly as follows. Nucleotide sequence data were obtained from specimens preserved in CTAB (Rogstad, 1992) or dried in silica. In addition to our own collections, supplementary materials were provided by J. Bogner (*Aponogeton*), C. Bove (*Najas*), C. B. Hellquist (*Elodea*), W. Iles (*Maundia*), S. W. L. Jacobs (various species), C. Kasselmann (*Aponogeton*), D. Padgett (*Vallisneria*), D. Perleberg (*Najas*), C. Phiri (*Vallisneria*) and H. W. E. van Bruggen (*Aponogeton*). Genomic DNA was extracted using a standard method (Doyle and Doyle, 1987) then amplified and sequenced for select gene regions following Les et al. (2008), using previously published primers (Baldwin 1992; Johnson and Soltis 1995; Bremer et al., 2002; Les et al., 2008, 2009; Tippery et al., 2008). Molecular data were analysed using both equally weighted maximum parsimony (MP) and maximum likelihood (ML) methods. Heuristic MP tree searches and bootstrap (BS) analyses were performed in PAUP* ver. 4.0b10 (Swofford, 2002) using the parameters given in Les et al. (2009). Single-tree and bootstrap (1000 replicates) ML analyses were conducted in GARLI ver. 0.97.r737 (Zwickl, 2006) on data partitioned among different gene regions using models recommended by jModelTest ver. 0.1.1 under the AIC criterion (Posada, 2008). The resulting trees are depicted as MP strict consensus trees with the captions indicating the specific ML evolutionary model

applied, nodal support values and the following tree statistics: CI (consistency index), CI_{exc} (consistency index excluding uninformative sites) and RI (retention index). These newly generated trees appear in Figs 6.2, 6.4, 6.11, 6.15, and 6.16. GenBank accession numbers for all sequences used in our analyses (including those newly generated) are provided in the Appendix.

6.3 Circumscription of Alismatidae

The spate of phylogenetic studies conducted over the past several decades has helped to clarify the circumscription of Alismatidae substantially. All pertinent molecular phylogenetic studies to date (e.g. Fig 6.1) consistently have resolved at least the core alismatid families (see Les et al., 1997) as a clade, thus confirming their monophyly. To this end, necessary taxonomic alterations have included the removal of two enigmatic families (Triuridaceae Gardner, Petrosaviaceae Hutch.) once placed traditionally with alismatids (Tomlinson, 1982), but since demonstrated to resolve outside the group phylogenetically (Cameron et al., 2003; Li and Zhou, 2007).

In recent taxonomic syntheses (e.g. APG I, 1998; APG II, 2003; APG III, 2009), alismatids were treated as a single order (Alismatales Dumort.), which, along with the more traditional families, also included Araceae Juss. and Tofieldiaceae Takht. In that interpretation, the Alismatales were monophyletic with Tofieldiaceae regarded as the sister group to the more traditional alismatid taxa and Araceae as the sister to that clade (Judd et al., 2008).

That circumscription has become accepted by many, due in large part to the results of several published studies which have rendered this same congruent topology (Fig 6.1A) from analyses of a variety of molecular data sets, including *rbc*L, *ndh*F, combined *mat*K + *rbc*L sequences and combined cpDNA (*atp*B + *rbc*L) + nuclear 18S rDNA sequences (Chase et al., 1993; Tamura et al., 2004; Givnish et al., 2006; Soltis et al., 2007).

However, that proposed circumscription of Alismatales is not without inconsistencies and there also exists a fair amount of conflicting data. A large amount of cpDNA sequence data (Fig 6.1B) resolves Araceae, not Tofieldiaceae, as the sister group of core alismatids, and some combined data (Fig 6.1F) resolve Araceae and Tofieldiaceae as their sister clade (Li and Zhou, 2009). More problematic are nuclear *PHYC* sequences (Duvall and Ervin, 2004) which place Tofieldiaceae completely outside of the group (Fig 6.1C) and some combined DNA data, which place Acoraceae Martinov as the sister group of core alismatids (Fig 6.1D, E). In fact, the most diverse data set (which includes chloroplast, mitochondrial and nuclear gene sequences) also is one that resolves Acoraceae as the sister group of alismatids (Fig 6.1D), an interesting result given that Acoraceae have become accepted widely as the sister group to all other monocotyledons.

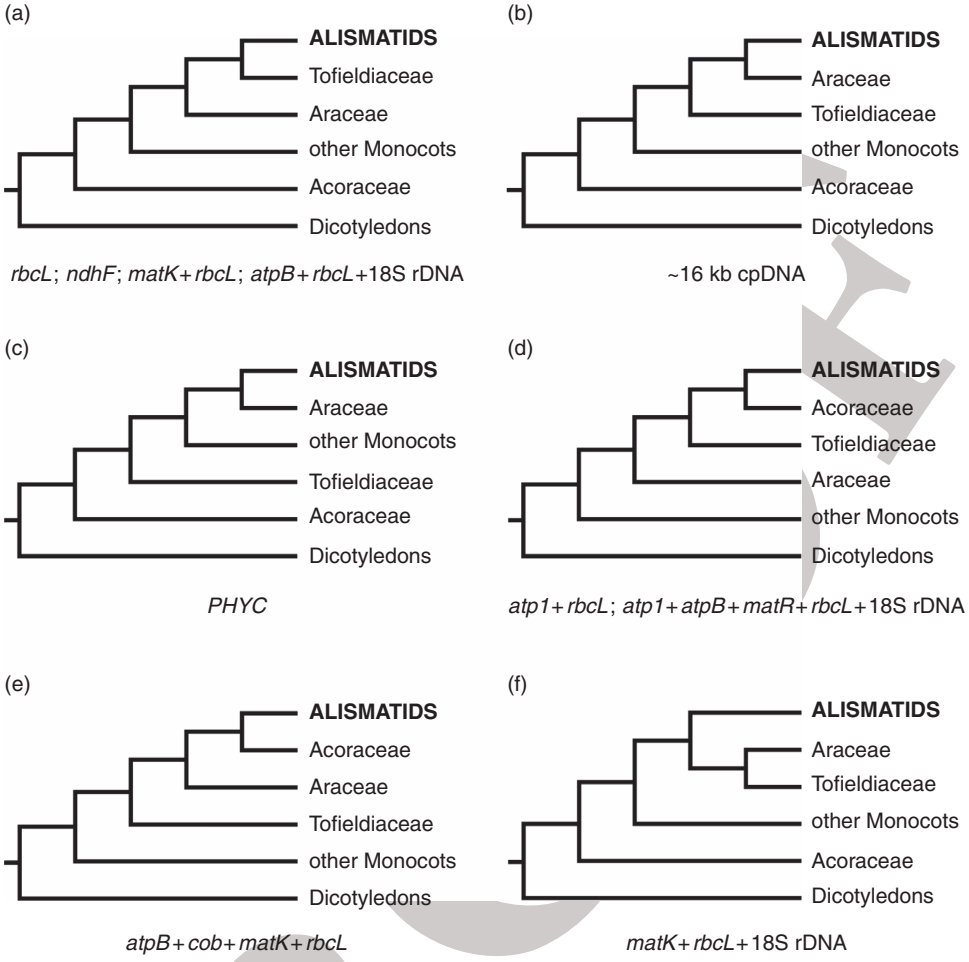

Fig 6.1 Generalized cladograms based on several studies incorporating various sources of DNA sequence data, showing different placements of alismatids among other monocotyledons. The trees shown are simplified substantially from the original studies cited; in most cases, the dicotyledons comprise a paraphyletic sister grade. A: Tofieldiaceae resolve as the sister group of alismatids in analyses of cpDNA loci including *rbc*L (MP combinable component consensus, Chase et al., 1993), *ndh*F (MP; Givnish et al., 2006), combined *mat*K and *rbc*L sequences (MP strict consensus; Tamura et al., 2004) and combined analyses (BI majority rule consensus) of cpDNA (*atp*B + *rbc*L) and nuclear 18S rDNA sequences (Soltis et al., 2007). B: Araceae resolve as the sister group of alismatid monocotyledons in analyses based on 16 kb of cpDNA sequence data; Tofieldiaceae represent the next sister group (ML; Graham et al. 2006). C: Araceae resolve as the sister group of alismatid monocotyledons in analysis of nuclear *PHYC* sequences (BI consensus); the majority of remaining monocots represents the next sister group (Duvall and Ervin, 2004). D: Acoraceae resolve as the sister group of alismatid monocotyledons (Tofieldiaceae represent the next sister group) in combined analyses (MP strict consensus) of one mtDNA

The focus of this chapter is not to pursue an extensive discussion on the problems associated with phylogenetic reconstruction at deep taxonomic levels. Yet, given these discrepancies, we advocate that a more conservative circumscription of alismatids be adopted, specifically one that is limited to those core families that consistently resolve as a clade. Accordingly, we follow here the circumscription of the group *sensu stricto* as advocated by Haynes and Les (2005), where Alismatidae are recognized at the level of subclass, and comprise two distinct clades that represent the orders Alismatales and Potamogetonales Dumort. (= Zosterales Nakai). This group essentially represents the same one as indicated by the analysis of Davis et al. (2004). The circumscription is similar to that indicated by the classification system of Thorne and Reveal (2007), but substitutes the rank of subclass (Alismatidae) for superorder (Alismatanae Takht.), in exclusion of Araceae and Tofieldiaceae.

The realization that extant alismatids comprise two distinct clades was first indicated by a phylogenetic analysis of single-gene (*rbc*L) sequence data for 11 taxa representing 9 of the accepted families (Les et al., 1993). Subsequently, that result was maintained in more comprehensive *rbc*L surveys, including representatives of 25 genera from 15 families (Les and Haynes, 1995), and then 69 species from 47 genera (Les et al., 1997). We now have evaluated an even larger *rbc*L data set consisting of 167 *rbc*L sequences representing 158 alismatid taxa and 9 outgroup (Arales Dumort.) taxa (Fig 6.2). This most comprehensive analysis of alismatid taxa to date is consistent with previous studies in resolving the same two major clades (designated as clade I and clade II), which are recognized here as the orders Alismatales and Potamogetonales. Nine subclades (A–I) also are evident and this notation is used in the remainder of the text to facilitate discussion.

In addition to the single-gene *rbc*L studies, other data sets also confirm the fundamental subdivision of the Alismatidae into two clades. Analyses of nearly 14 kb of cpDNA sequence data (Iles et al., 2009), as well as combined mtDNA (*atp1* + *cob*) and cpDNA (*rbc*L) sequence data (Petersen et al., 2006) all provide highly congruent phylogenetic topologies (Fig 6.3) that agree with the results of the various *rbc*L analyses. Furthermore, these two major clades are associated with fundamentally different derivations of floral structure, which have been termed

Caption for Fig 6.1 (*cont.*) locus (*atp*1) and one cpDNA locus (*rbc*L) (Davis et al. 2004) and in combined analyses of mitochondrial (*atp*1 + *atp*B + *mat*R), chloroplast (*rbc*L), and nuclear (18S rDNA) sequences (MP; Qiu et al., 2000). E: Acoraceae resolve as the sister group of alismatid monocotyledons in combined analyses (MP) of two mtDNA loci (*atp*1 + *cob*) and two cpDNA loci (*mat*K + *rbc*L); Araceae represent the next sister group (Petersen et al., 2006). F: An Araceae/Tolfieldiaceae clade resolves as the sister group of alismatid monocotyledons in combined analyses (MP strict consensus) of cpDNA (*mat*K + *rbc*L) and 18S rDNA sequences (Li and Zhou, 2007).

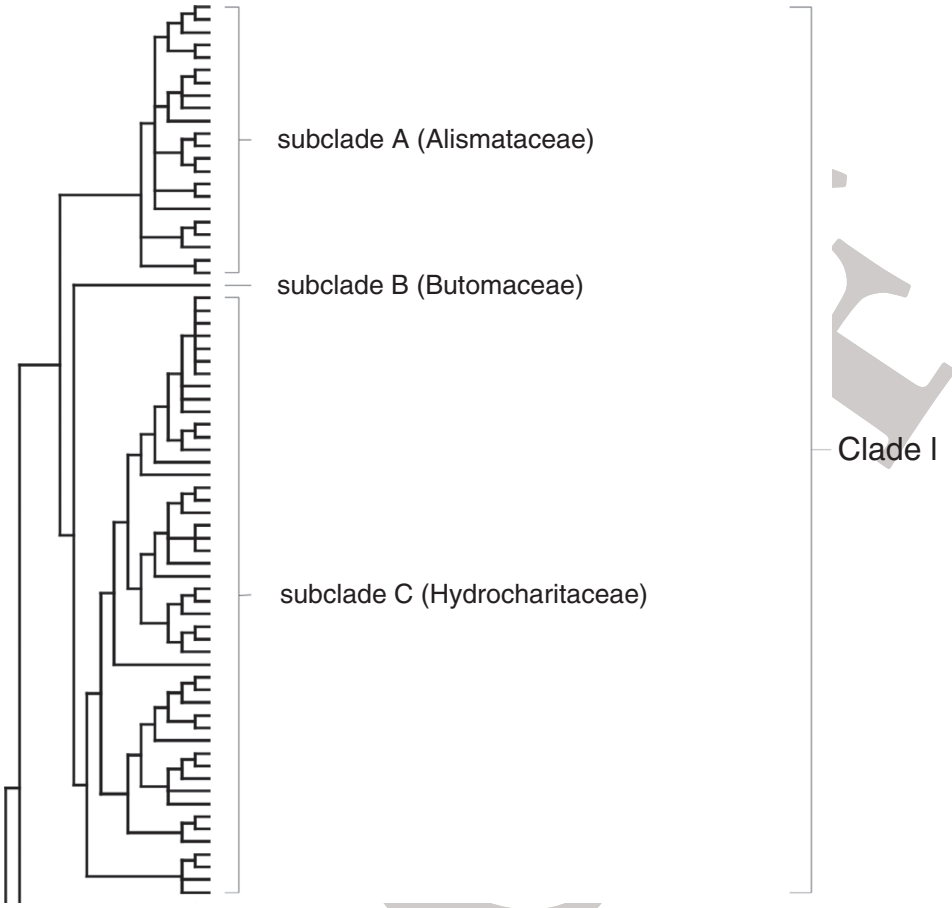

Fig 6.2 Phylogenetic analysis of Alismatidae using *rbc*L sequence data for 167 taxa (current study). The tree identifies two major clades (I, II), which represent the orders Alismatales and Potamogetonales, respectively. Nine subclades (A–I) have been identified in order to facilitate discussion throughout the text. The MP strict consensus tree is shown. Because of space constraints, nodal support values were excluded from this tree. However, expanded details of several subclades are shown in Figs 6.10, 6.16, 6.18 and 6.23, in which the values of MP and ML (GTR+I+G model) nodal BS support are indicated above and below branches respectively ('–' indicates <50% support). In addition, several text discussions provide specific support values for relevant portions of this tree that have not been shown in any of the additional figures. Tree statistics: CI = 0.36; CI_{exc} = 0.32; RI = 0.86; ln*L* = –12283.

'petaloid' (Alismatales) and 'tepaloid' (Potamogetonales) forms (Posluszny et al., 2000). Taken together, these data indicate that this circumscription of Alismatales and Potamogetonales does appear to designate cohesive and phylogenetically meaningful clades.

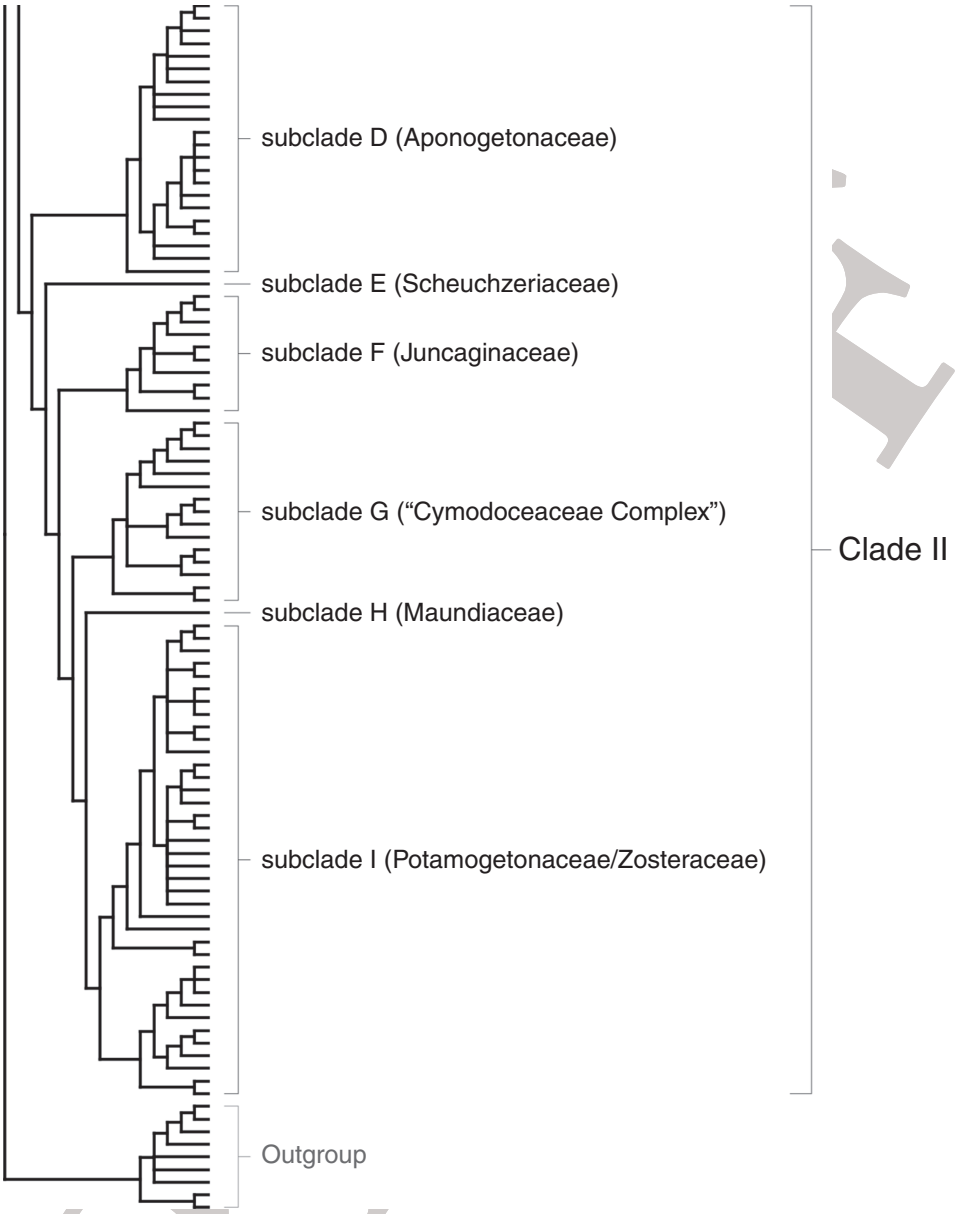

Fig 6.2 (*cont.*)

6.4 Alismatales (clade I)

The circumscription of Alismatales followed here includes three families (Alismataceae, Butomaceae and Hydrocharitaceae) and differs from the concept presented by Haynes and Les (2005) by transferring various genera included

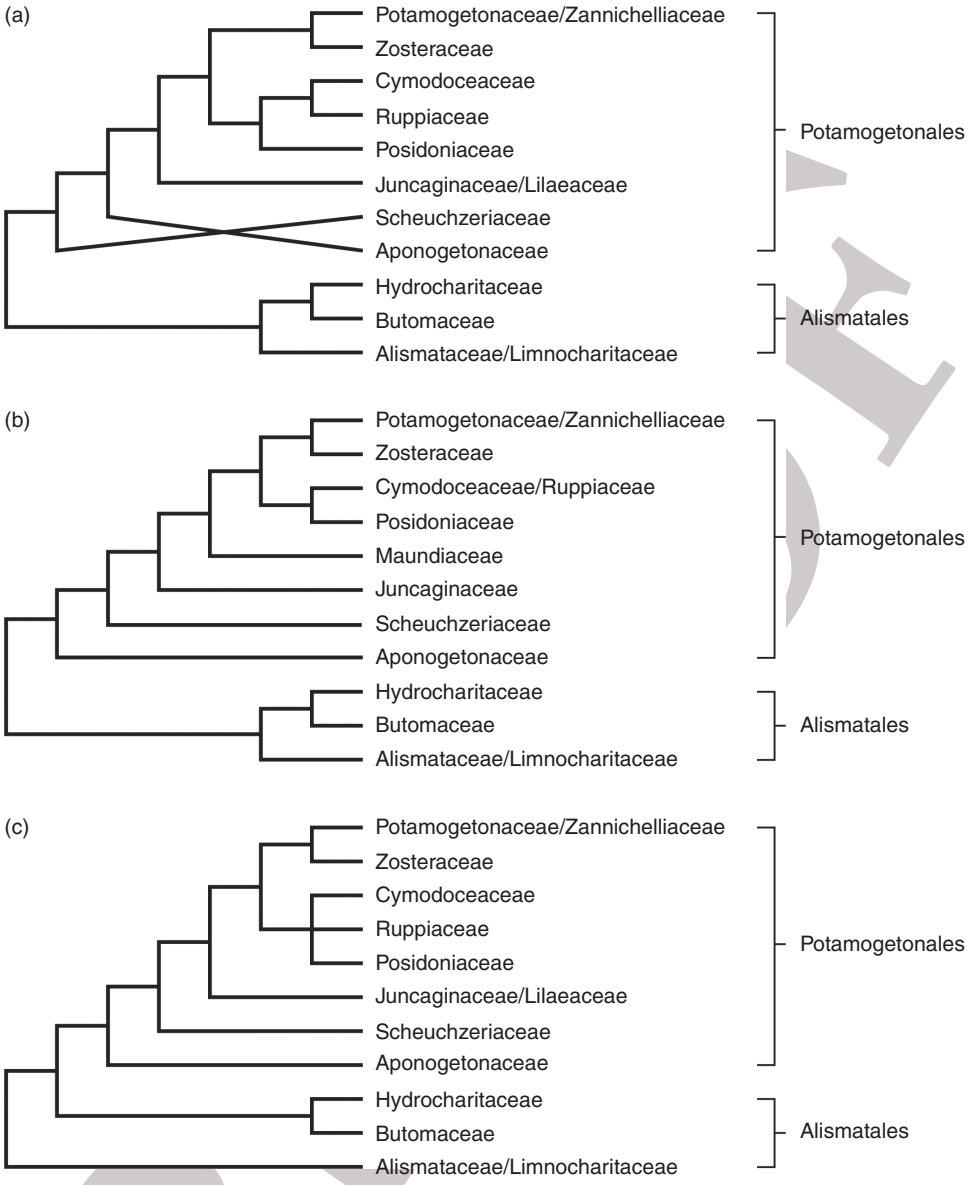

Fig 6.3 Phylogenetic relationships among alismatid families. A: Resolution of two distinct clades corresponding to Potamogetonales and Alismatales sensu stricto (s.s.) resulting from analysis of *rbc*L sequence data (weighted MP strict consensus; Les et al., 1997). B: Topology highly congruent with previous tree (with addition of Maundiaceae) resulting from parsimony/likelihood analyses of roughly 14 kb of DNA sequence data (ML; Iles et al., 2009). C: Relationships derived from combined analysis (MP) of two mitochondrial loci (*atp*1, *cob*) and chloroplast (*rbc*L) data differ slightly from previous analyses primarily by the resolution of Alismatales s.s. as a grade rather than a clade (Petersen et al., 2006). None of the conflicts observed among these trees is strongly supported.

formerly in Limnocharitaceae and Najadaceae to Alismataceae and Hydrocharitaceae, respectively.

The small family Limnocharitaceae Takht. was established less than 60 years ago. The three component genera (*Butomopsis*, *Hydrocleys* and *Limnocharis*) traditionally had been placed in Butomaceae, but were viewed also as having affinities with Alismataceae (Cronquist, 1981). In this sense, Limnocharitaceae were regarded as intermediate phylogenetically between Alismataceae and Butomaceae. However, in recent treatments, Limnocharitaceae were merged with Alismataceae essentially because 'Convincing evidence for the monophyly of Alismataceae s.s. is lacking . . .' (APG III, 2009).

The 47-genera *rbc*L sequence analysis by Les et al. (1997) was consistent with the latter conclusion by placing two of the three genera of Limnocharitaceae (*Hydrocleys*, *Limnocharis*) within a clade containing eight genera of Alismataceae; in that study, however, the two families were maintained as separate, awaiting more conclusive evidence necessitated by the low level of internal support obtained for critical branches. That study at least clarified that neither *Hydrocleys* nor *Limnocharis* was closely allied with Butomaceae, as some earlier authors had believed.

We since have been able to procure material of the third genus assigned to Limnocharitaceae (*Butomopsis*) and have revisited the issue of relationships by analyzing a combined dataset of *mat*K + *rbc*L + nrITS sequences (Fig 6.4). The results of this analysis yielded a strongly supported clade comprising the three former Limnocharitaceae genera, which was embedded within Alismataceae by both ML (not shown) and MP analyses (Fig 6.4). Although the precise placement of this clade amongst other Alismataceae was not strongly supported (BS = 62–66%), the result paralleled those obtained by the earlier *rbc*L analysis (Les et al., 1997) and even by the large cpDNA dataset analysis (Iles et al., 2009), which also provided only weak support. Nevertheless, because none of these analyses resolved Limnocharitaceae as a sister clade to Alismataceae, the consistent placement of the former as a clade within the latter leads us to recommend that the groups be merged.

A similar hesitancy has characterized taxonomic dispositions of the genus *Najas* in recent years. Once typifying the order Najadales Dumort., which included such families as Cymodoceaceae, Potamogetonaceae, Ruppiaceae Horan. and Zosteraceae (Cronquist, 1981), *Najas* (Najadaceae Juss.) actually was found to possess peculiar seed coat structures present elsewhere only among some members of Hydrocharitaceae (Shaffer-Fehre, 1991a, 1991b). Shortly afterwards, analyses of *rbc*L sequence data pointed similarly to the relationship of *Najas* and Hydrocharitaceae and also indicated its substantial taxonomic distance from other families with which it long had been associated (Les et al., 1993, 1997). Yet, the level of internal support for critical branches in the resulting phylogenies remained

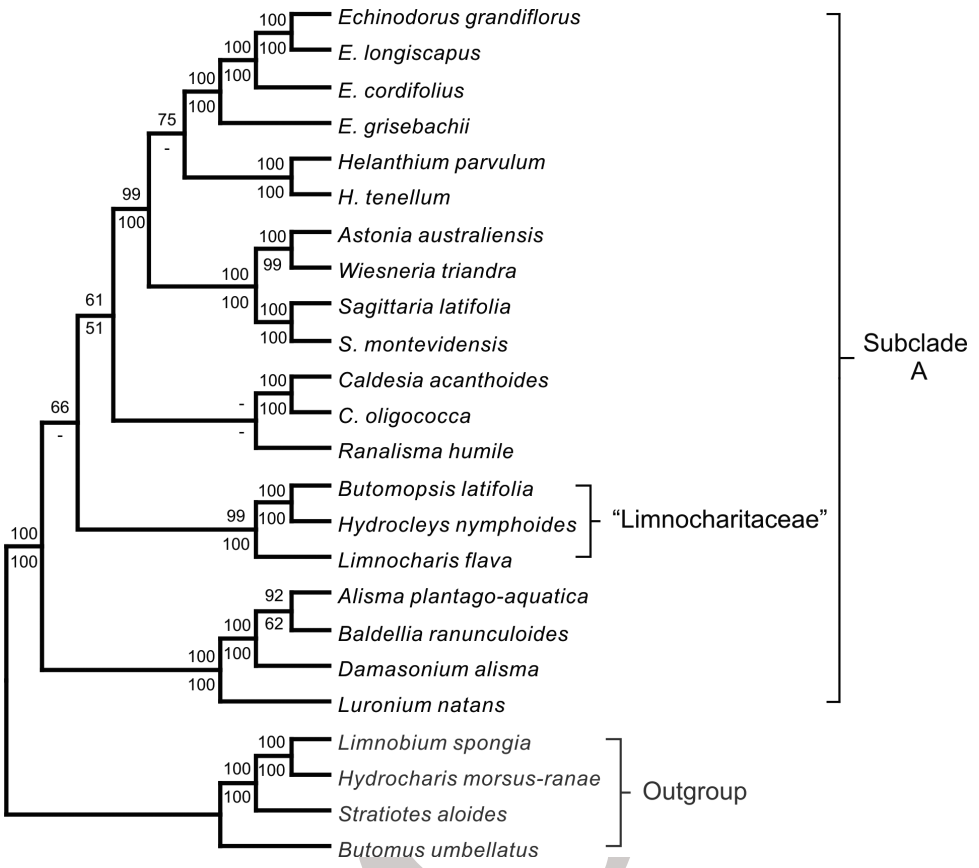

Fig 6.4 Phylogenetic relationships of Alismataceae and genera assigned formerly to Limnocharitaceae (*Butomopsis*, *Hydrocleys* and *Limnocharis*) as indicated by MP/ML analysis of combined *mat*K/*trn*K (ML: TVM+G model) and *rbc*L (ML: GTR+I+G model) data (current study). MP strict consensus tree is shown. Values of MP and ML nodal BS support are indicated above and below branches respectively ('–' indicates <50% support). Tree statistics: CI = 0.66; CI_{exc} = 0.60; RI = 0.70; ln*L* = –26078.

low, and the merger of Najadaceae and Hydrocharitaceae was deferred pending the acquisition of more persuasive evidence. Although not definitive, those studies did clarify that Najadaceae were not allied with Potamogetonaceae as formerly believed, but instead were placed phylogenetically either within Hydrocharitaceae or at least as its sister group.

More convincing evidence finally was obtained by a combined analysis of morphological and molecular (nrDNA, cpDNA) data (Les et al., 2006), which provided strong internal support for the inclusion of *Najas* within Hydrocharitaceae in a position with *Hydrilla* and among other members of subfamily Hydrilloideae Luerss. Confirmation of this general result came from analysis of *atp1* + *cob* +

*rbc*L sequences (Petersen et al. 2006), which also placed *Najas* in Hydrocharitaceae (Hydrilloideae) in proximity to *Hydrilla*. A similar position is indicated also in our 167-taxon *rbc*L analysis (Fig 6.2). The inclusion of *Najas* in Hydrocharitaceae is also strongly indicated in analyses of large amounts of cpDNA data (Iles et al., 2009).

Li and Zhou (2009) challenged the association of *Najas* in Hydrocharitaceae by the results of a combined *rbc*L-morphology analysis, despite admitting that the morphological characters used in their analysis were highly homoplasious. That analysis placed *Najas* as the sister group of Zosteraceae, but with low internal support. This association of *Najas* is not tenable. The inclusion of *Najas* in Hydrocharitaceae is supported by *mat*K (Tanaka et al., 1997), combined cpDNA, nrITS, morphology data (Les et al., 2006) and by the results of a 17-gene phylogeny (Iles et al., 2009). Furthermore, *rbc*L data alone (without morphological data) consistently have indicated the same association (Les et al., 1993, 1997).

None of these studies supports the placement of *Najas* with Zosteraceae, which is reminiscent of some results obtained by Les and Haynes (1995), who clearly demonstrated that homoplasious morphological data associated with Alismatidae can produce a misleading phylogenetic signal, which can significantly alter the placement of genera. It is evident that the association of Najadaceae and Zosteraceae obtained by Li and Zhou (2009) similarly is an artefact of homoplasious morphological data. Conversely, all of the molecular results, as well as some morphological analyses (Les and Haynes, 1995) resolve *Najas* within or as the sister to Hydrocharitaceae. Given the preponderance of data in support of this association, we have unhesitatingly accepted the transfer of *Najas* to Hydrocharitaceae.

As a consequence of the preceding discussion, we have delimited the order Alismatales as comprising only the families Alismataceae, Butomaceae and Hydrocharitaceae, along with the dissolution of Limnocharitaceae and Najadaceae for the reasons given.

6.4.1 Alismataceae (subclade A)

We delimit Alismataceae as comprising 17 genera and 113 species (Table 6.1). A number of intergeneric phylogenetic studies have been conducted on the family, which have included analyses of one gene/two genera (Les et al., 1993), 4 genes/2 genera (Petersen et al., 2006), 1 gene/10 genera (Les et al., 1997), 17 genes/3 genera (Iles et al., 2009) and 3 genes/14 genera (Fig 6.4). The latter, which is newly reported here, represents the most comprehensive study to date with respect to taxon sampling because it surveys 14/17 (82%) of the recognized genera, excluding only *Albidella*, *Burnatia* and *Limnophyton*. The inclusion of the latter in Alismataceae was verified independently by Keener (2005), who demonstrated a close association of *Limnophyton* and *Wiesneria* using 5S-NTS sequence data.

Lehtonen and Myllys (2008) independently evaluated the position of *Albidella* using combined morphological and DNA sequence data. Although they advocated the removal of the genus from *Echinodorus*, their results showed major inconsistencies with morphology resolving *Albidella* as the sister to *Echinodorus*, *mat*K placing *Albidella* within *Echinodorus* and nuclear DNA placing it far removed from *Echinodorus* as the sister to all other Alismataceae. We also have experienced some difficulties evaluating the sequences reported for *Albidella* and recommend that the phylogenetic placement of this genus be re-evaluated with additional data.

It also is noteworthy that our combined data analysis of 14 Alismataceae genera (Fig 6.4) resolves *Helanthium* as the sister group to *Echinodorus* with weak support. Even if this result is accepted, we still advocate that these groups be retained as separate genera (rather than subgenera), because they are distinct morphologically (Lehtonen and Myllys, 2008) and are characterized by a substantial amount of genetic divergence in all molecular analyses.

Taken together, the results from all of these analyses indicate consistently that Alismataceae are monophyletic, at least in exclusion of *Burnatia*, which remains unsurveyed phylogenetically for any locus.

6.4.1.1 Infrageneric relationships

Five of seventeen genera in Alismataceae are monotypic, thus obviating the need for infrageneric phylogenetic evaluation (Table 6.1). Of the three bitypic genera (Table 6.1), where interspecific relationships similarly are uncomplicated, both species of *Ranalisma* (Fig 6.4) and *Baldellia* (Fig 6.6) have been analysed phylogenetically to confirm their monophyly; *Limnocharis laforestii* Duchass. ex Griseb. remains unsurveyed.

All three species of *Helanthium* have been evaluated thoroughly (Lehtonen, 2006, 2008; Lehtonen and Myllys, 2008), using a combination of *mat*K + nrITS + LEAFY + 5S-NTS sequences and morphological data (Fig 6.5). Additional population sampling (and perhaps also the use of higher-resolution genetic markers) will be required to satisfactorily resolve the taxonomic status of *H. bolivianum* (Rusby) Lehtonen & Myllys, which DNA sequences indicate to be paraphyletic. Some of the

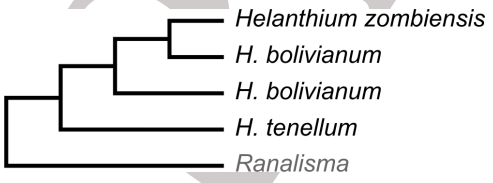

Fig 6.5 Relationships in *Helanthium* based on combined analysis (MP) of *mat*K + nrITS + LEAFY + 5S-NTS sequences (adapted from Lehtonen and Myllys, 2008).

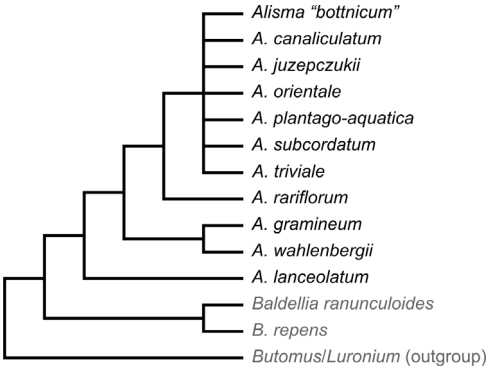

Fig 6.6 Interspecific relationships in *Alisma*/*Baldellia* as indicated by MP analysis of nrITS sequence data (redrawn from Jacobson and Hedrén, 2007).

smaller genera still in need of denser taxonomic coverage include *Caldesia*, *Damasonium*, *Hydrocleys*, *Limnophyton* and *Wiesneria*.

The three largest genera (*Alisma*, *Echinodorus and Sagittaria*) have been fairly well studied phylogenetically, at least enough to propose circumscriptions that arguably reflect monophyletic taxa. Jacobson and Hedrén (2007) evaluated all 11 species of *Alisma* (and both species of *Baldellia*) using nrITS/*trn*L sequences and RAPD data (Fig 6.6). In the case of *Alisma*, nrITS sequences provided low resolution for seven of the species; however, further resolution was achieved by evaluation of RAPD data (Jacobson and Hedrén, 2007). The *trn*L locus also was evaluated, but provided minimal information (due to the low number of parsimony-informative sites) and the phylogenetic signal reportedly conflicted with that obtained for the two nuclear data sets (Jacobson and Hedrén, 2007). It would be informative to obtain additional cpDNA data for *Alisma* to determine whether other chloroplast loci indicate similar discrepancies due possibly to factors such as hybridization.

Echinodorus has been studied quite thoroughly (Lehtonen, 2006, 2008; Lehtonen and Myllys, 2008) by evaluating morphological data for all 28 species and multiple DNA sequences (*mat*K + nrITS + LEAFY + 5S-NTS) for 23 of the 28 recognized species (Fig 6.7). These studies provide a reasonable overview of phylogenetic relationships in *Echinodorus* and support its distinction from *Helanthium*. Yet, despite this fair amount of systematic evaluation, several questions remain unsettled, including the appropriate taxonomic disposition of several putative new species, as well as a reconsideration of the taxonomy for several existing species (e.g. *E. grandiflorus*, *E. cordifolius*, *E. macrophyllus* (Kunth) Micheli), which resolve as poly- or paraphyletic (Fig 6.7).

Relationships among most (35 of 40) *Sagittaria* species have been evaluated using 5S-NTS data (Fig 6.8; Keener, 2005), which provides a reasonable hypothesis

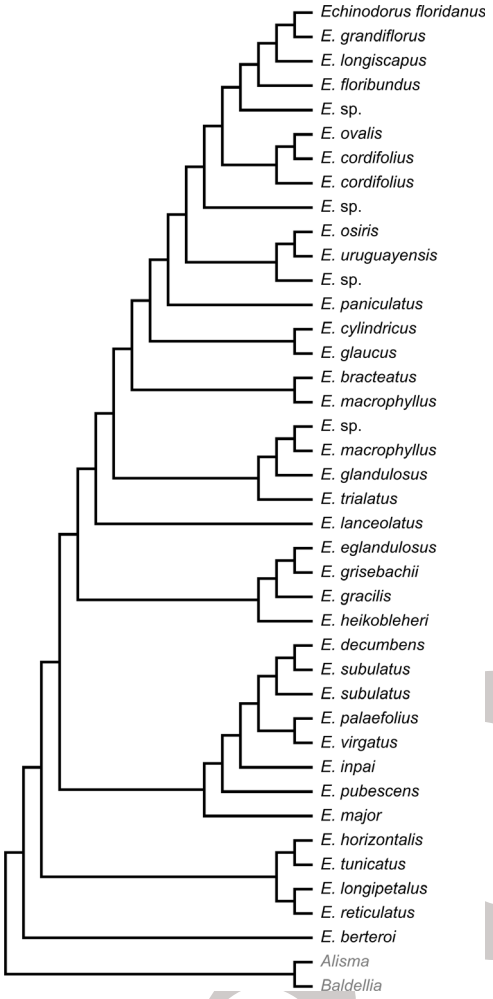

Fig 6.7 Phylogenetic relationships in *Echinodorus* indicated by MP analysis of combined morphological data and DNA (5S-NTS + LEAFY + *mat*K + nrITS) sequences (adapted from Lehtonen and Myllys, 2008).

of interspecific relationships in the genus. Here it would be desirable to analyse at least some cpDNA data in addition, to verify the species groups rendered by the nuclear sequence data analysis. Also, the taxonomic status of *Lophotocarpus* T.Durand has not been settled fully by the 5S-NTS data, which resolve several taxa assigned to the former *Lophotocarpus* (*S. montevidensis*) as a sister clade to the remainder of *Sagittaria* (Fig 6.8). Whether members of this clade differ materially from *Sagittaria* to warrant the re-establishment of *Lophotocarpus* should be given further consideration.

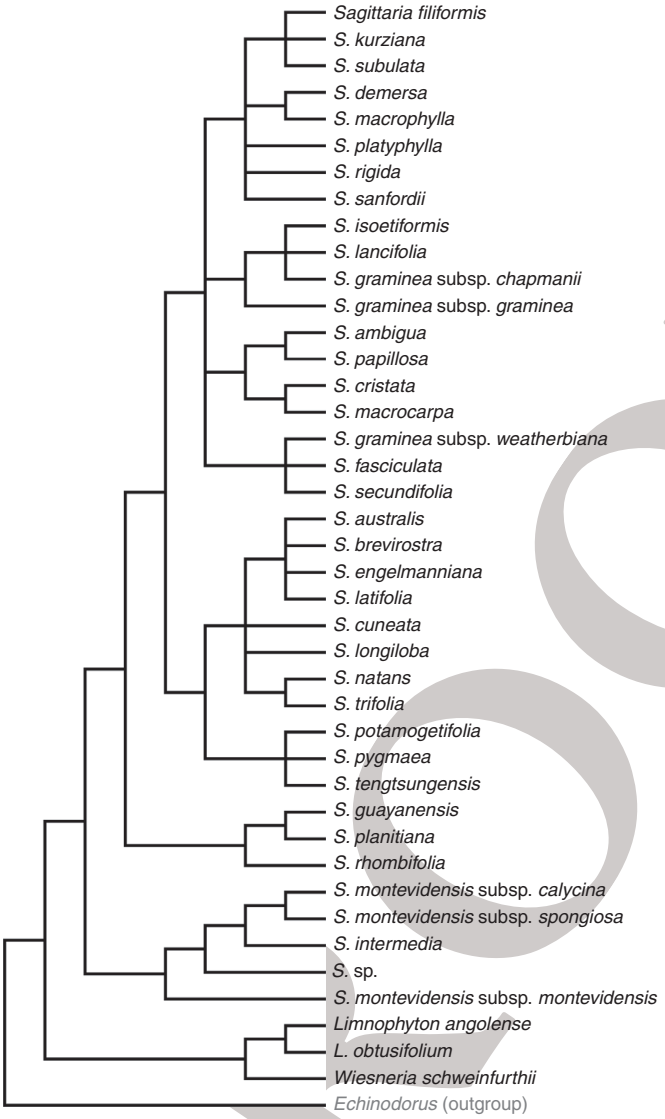

Fig 6.8 Interspecific relationships in *Sagittaria* based on analysis (MP strict consensus) of 5S-NTS data (adapted from Keener, 2005).

6.4.2 Butomaceae (subclade B)

Butomaceae solely comprise the monotypic *Butomus umbellatus*, which resolves in an isolated position between the families Alismataceae and Hydrocharitaceae, specifically as the sister group to the latter (Figs 6.2–6.4). This placement of the genus is supported in analyses incorporating up to14–16 kb of cpDNA data

(Graham et al., 2006; Iles et al., 2009) and also by results obtained from combined cpDNA/mtDNA (Petersen et al., 2006) and *atp*1 + *rbc*L (Davis et al., 2004) data analyses. *Butomus* itself consists of two distinct diploid and triploid cytotypes (Krahulcová and Jarolímová, 1993); however, most authors recognize only a single species in the genus (Cook, 1996).

6.4.3 Hydrocharitaceae (subclade C)

The 'hydrocharits' contain 17 genera and roughly 127 species (Table 6.1). Sixteen of the genera have been included in phylogenetic studies (Figs 6.2, 6.9, 6.10) with only *Appertiella* remaining unsurveyed by any type of molecular data analysis. A consistent result of these phylogenetic analyses is the inclusion of the three marine genera (*Enhalus*, *Halophila*, *and Thalassia*) within a single clade. These studies also place the genera within four natural groups, which have been designated as distinct subfamilies (*Anacharidoideae* Thomé, *Hydrilloideae*, *Hydrocharitoideae* Eaton, and *Stratiotoideae* Luerss.). These groups have been recovered consistently by different phylogenetic analyses incorporating not only single and multiple gene sequences (cpDNA, mtDNA and nrDNA), but morphological data as well (Fig 6.9). The most comprehensive taxon coverage has been achieved by analysis of *rbc*L data (Figs 6.2, 6.10), which includes 48 species, thereby providing an overview of interspecific relationships for nearly 40% of the family.

6.4.3.1 Infrageneric relationships

Six genera are monotypic (Table 6.1); however, at least one of these (*Hydrilla*) probably consists of at least one additional species (L. Benoit, pers. comm.), and one genus (*Appertiella*) has remained elusive in attempts to procure material for molecular studies. Both species in each of the bitypic genera (*Limnobium*, *Thalassia*) have been surveyed and phylogenetic analyses confirm that these genera are monophyletic (Figs 6.2, 6.10). Monophyly also is indicated by analyses of 2/3 of the species of *Hydrocharis*, and 3/5 of the species of *Elodea* (Figs 6.10, 6.11). *Elodea* requires more intensive study, especially given that our preliminary analysis of the genus (Fig 6.11) has verified the presence of natural interspecific hybrids between *E. canadensis* and *E. nuttallii*, thus corroborating earlier crossing studies that demonstrated the interfertility of these species (Les and Philbrick, 1993). Although *Elodea* itself is monophyletic, *Egeria* introduces additional problems, with the two surveyed species failing to resolve as a clade in analyses incorporating either cpDNA (Figs 6.2, 6.10) or nrITS data (Fig 6.11). These results raise the question of whether *Elodea* and *Egeria* might better be combined and treated taxonomically as a single genus. The elucidation of this question and clarification of generic limits for these genera eventually will require the inclusion of the remaining unsampled species of *Elodea* (*E. callitrichoides* Casp., *E. potamogeton* Espinosa) and *Egeria* (*E. heterostemon* S. Koehler and C.P. Bove) in phylogenetic analyses.

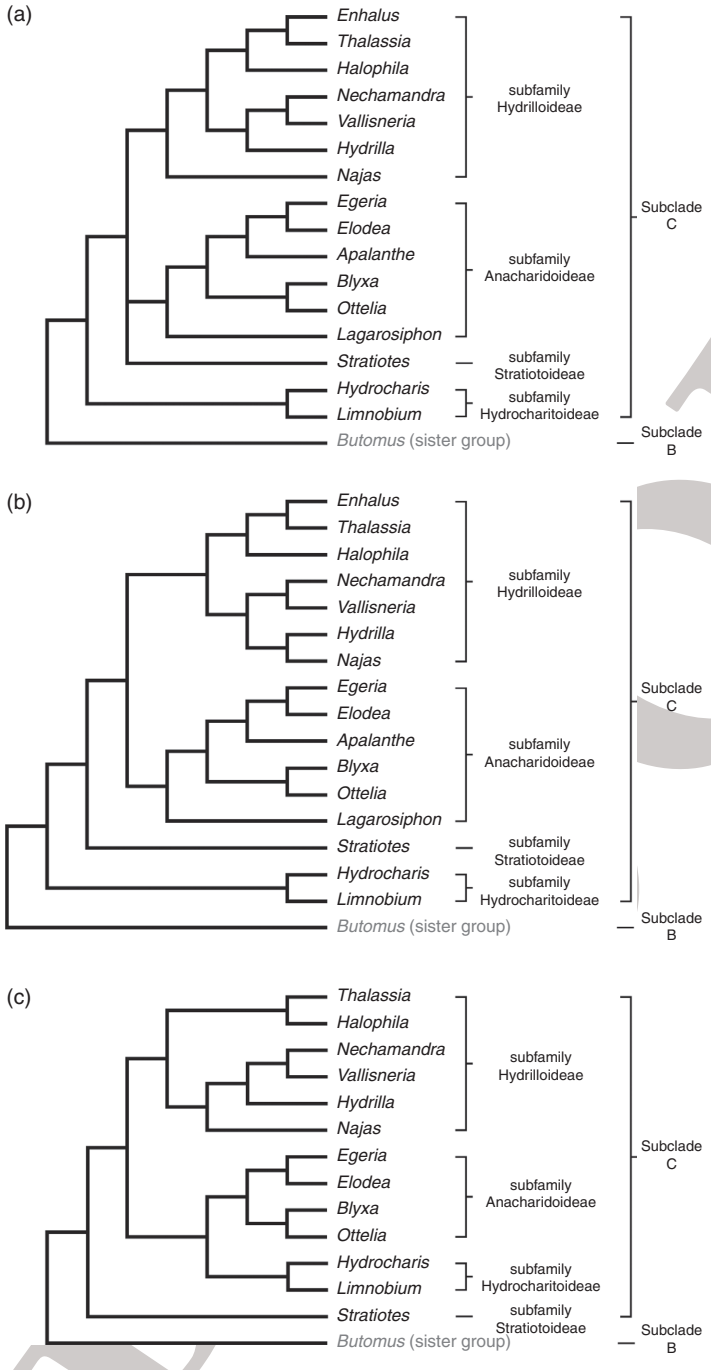

Fig 6.9 Similar intergeneric relationships in Hydrocharitaceae showing the consistent resolution of four groups, which have been designated as subfamilies. A: *rbc*L data (weighted MP strict consensus; Les et al. 1997). B: *mat*K + *rbc*L + *trn*K introns + nrDNA + morphology showing slightly different placement of *Najas* and resolution of *Stratiotes* (MP; Les et al., 2006). C: cladogram derived from *atp*1 + *cob* + *rbc*L data (MP; Petersen et al., 2006) closely resembles previous results, with the exception of slightly different positions of *Stratiotes* and subfamily Hydrocharitoideae.

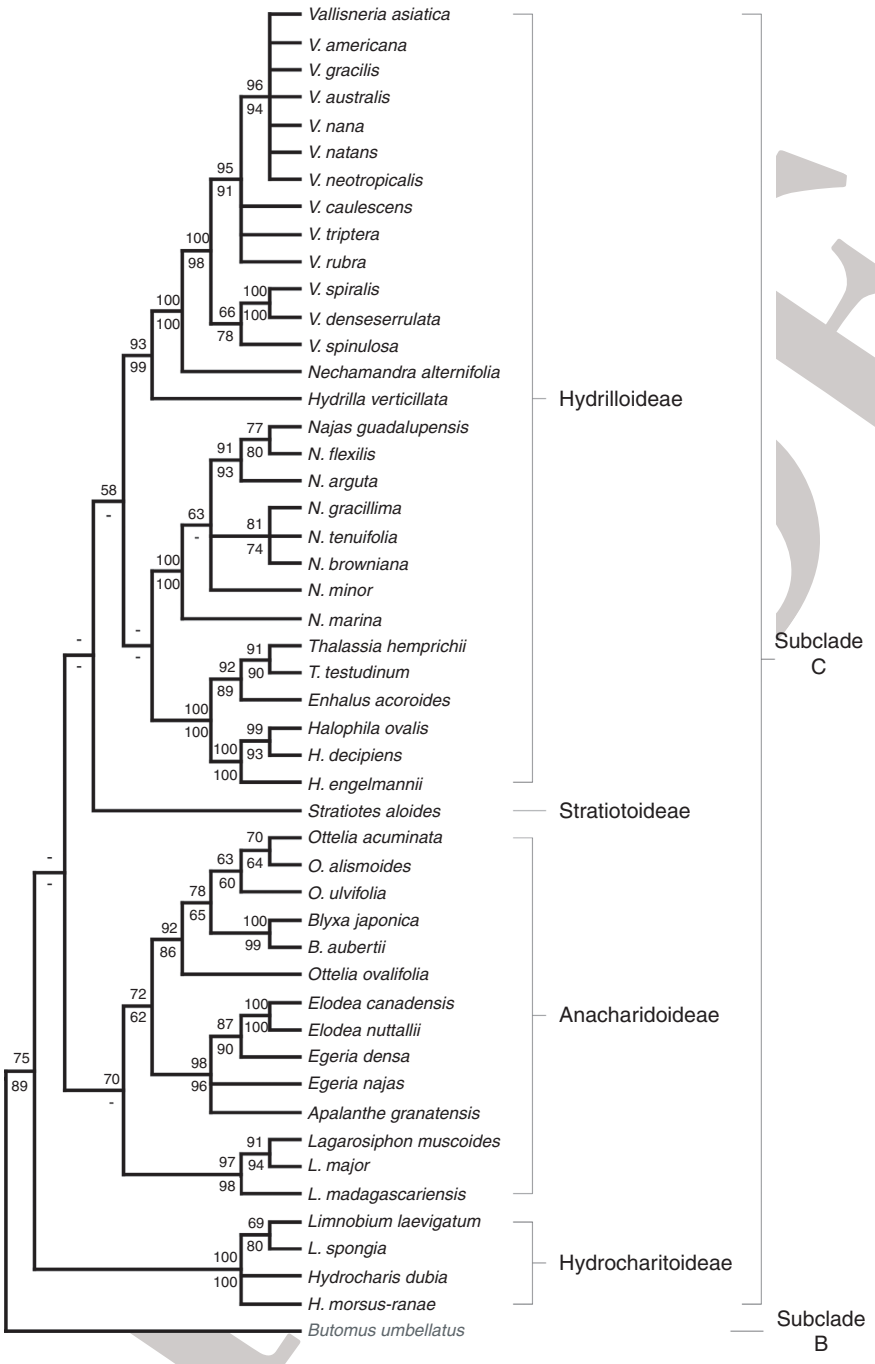

Fig 6.10 Expanded version of 'subclade C' (see Fig 6.2) showing details of relationships in Hydrocharitaceae as indicated by the results of a 167-taxon *rbc*L analysis.

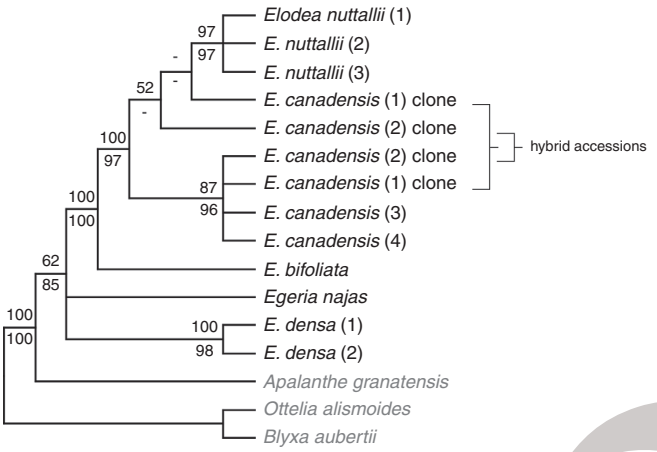

Fig 6.11 Phylogenetic relationships of *Apalanthe*, *Egeria* and *Elodea* as indicated by MP/ML analysis (ML: TrN+G model) of nrITS data (current study). MP strict consensus tree is shown. The association of several cloned nrITS alleles indicates the existence of interspecific hybrids involving *E. canadensis* and *E. nuttallii*. Nodal BS support values are indicated above (MP) and below (ML) branches respectively ('–' indicates <50% support). Tree statistics: CI = 0.85; CI_{exc} = 0.73; RI = 0.77; ln*L* = –3617.

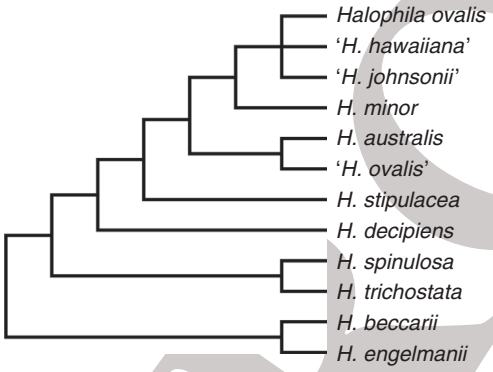

Fig 6.12 Phylogenetic relationships in *Halophila* as indicated by MP analysis of nrITS data (adapted from Waycott et al., 2002).

Several of the larger hydrocharit genera have been studied fairly comprehensively. Up to 14 species have been recognized in *Halophila* by some authors (e.g. Green and Short, 2003), but the actual number remains uncertain. Waycott et al. (2002) analysed nrITS sequences for 11 *Halophila* species (Fig 6.12), and concluded that two species (*H. hawaiiana* Doty and B.C.Stone, *H. johnsonii* N.J.Eiseman) could not be distinguished from *H. ovalis*. They also found that specimens identified as *H. ovalis* fell into two clades, with one set of accessions

resolving with *H. australis* Doty and B.C.Stone (Fig 6.12). The misplaced '*H. ovalis*' material could indicate a cryptic species (Waycott et al. 2002); thus the true number of species in *Halophila* probably is 13. This comprehensive study of *Halophila* confirmed that the genus is monophyletic and also indicated that a phylogenetic reduction series exists, which proceeds from larger, more complex-leaved species to more diminutive, simpler forms. However, it would be useful to obtain additional data for *Halophila* (such as cpDNA sequences) to provide a means of comparison with the nrITS data (Waycott et al., 2006).

Najas is a genus of 39 species worldwide (Cook, 1996). As already discussed, *Najas* recently has been transferred to Hydrocharitaceae as a result of the placement of the genus indicated by numerous phylogenetic studies. A comprehensive, formal phylogenetic analysis of the genus itself has not been conducted to date; however, *Najas* presently is the focus of systematic investigations by the senior author. One investigation based on analysis of combined nrITS and cpDNA sequence data (*mat*K + *rbc*L + *trn*K) for eight *Najas* species emphasized North American taxa (Fig 6.13), but provided support for the maintenance of two subgenera (*Caulinia* (Willd.) A.Braun, *Najas* L.) and also demonstrated the distinctness of sections *Americanae* Magnus and *Euvaginatae* Magnus (Les et al., 2010). Additional phylogenetic studies currently are underway by the senior author and now include roughly half of the species; eventually the completion of this work should yield a much-improved phylogenetic understanding of the genus. These molecular studies also have indicated for the first time the occurrence of interspecific hybridization in *Najas*, which had long gone undetected due to the high degree of morphological similarity between the hybrids and their maternal parents (Les et al., 2010).

Vallisneria (18 species) also is well-studied phylogenetically as a result of analyses incorporating various molecular loci (nrITS, *rbc*L, *trn*K 5′ intron sequences) and morphological data (Les et al. 2008). The study by Les et al.

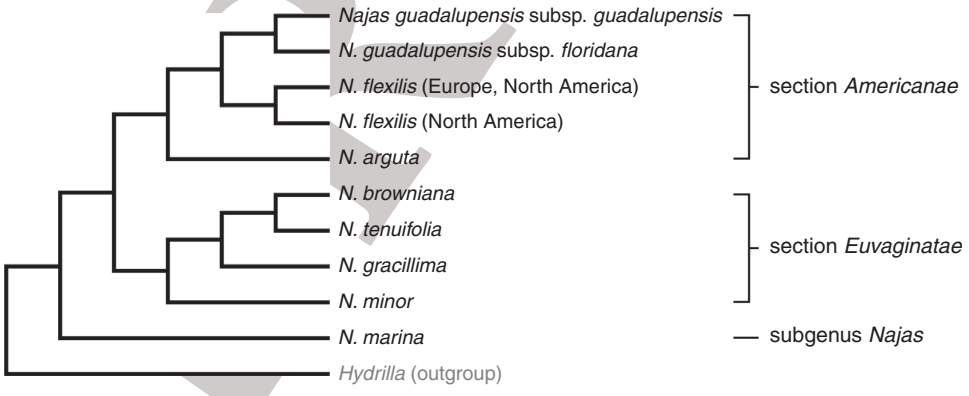

Fig 6.13 Systematic relationships in the genus *Najas*, inferred from phylogenetic analysis (MP strict consensus) of combined DNA sequence data (adapted from Les et al., 2010).

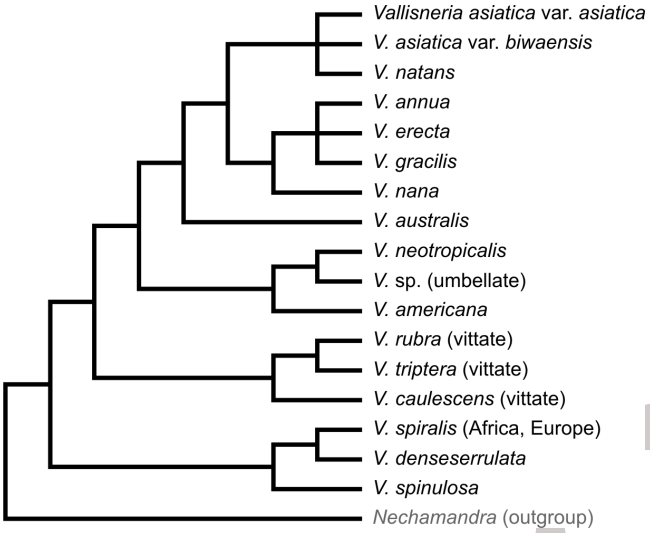

Fig 6.14 Interspecific relationships in *Vallisneria* as supported by phylogenetic analysis (MP strict consensus) of combined molecular data (redrawn from Les et al., 2008). This analysis excluded only *V. aethiopica* (Africa), which might be closely related to *V. spiralis* (see text). The vittate (caulescent) ingroup species are indicated; the remainder comprises rosulate taxa.

(2008) summarized phylogenetic relationships for 17 of the 18 species recognized worldwide (Fig 6.14) and excluded only the African *V. aethiopica* Fenzl. We since have acquired and analysed material attributed to *V. aethiopica*, but found it to be indistinguishable genetically from *V. spiralis* (Les and Tippery unpublished). However, the status of the morphologically distinctive *V. aethiopica* requires further evaluation.

Phylogenetic analyses of both molecular and morphological data (Les et al. 2008) placed the former genus *Maidenia* Domin (now *Vallisneria rubra*) within a clade of several leafy-stemmed (i.e. 'vittate') *Vallisneria* taxa, which was embedded among the rosulate species in all analyses using molecular data (Fig 6.14). That study also proposed that several characters formerly thought to be fairly informative taxonomically were highly homoplasious, probably as the result of selection to maintain the efficiency of the pollination system (Les et al. 2008).

6.4.3.2 Poorly-studied taxa

Blyxa, *Lagarosiphon* and *Ottelia* represent the three hydrocharit genera most in need of more comprehensive study. Three of the nine *Lagarosiphon* species available for phylogenetic estimation resolve as a clade (Figs 6.2, 6.10); however, additional species sampling is necessary to further corroborate the monophyly of

the genus. Only 7 of the 30 or so species in *Blyxa* and *Ottelia* have been analysed simultaneously, and the preliminary results are insufficient to demonstrate that these genera resolve as distinct clades (Figs 6.2, 6.10). An extensive systematic study of both genera is encouraged.

6.5 Potamogetonales (clade II)

Results of recent phylogenetic analyses have warranted a number of alterations to the taxonomy of this order, such as the removal of Najadaceae and its transfer to Alismatales as already discussed. The taxonomic concept of Potamogetonales presented here includes six families (Aponogetonaceae, Juncaginaceae, Maundiaceae, Potamogetonaceae, Scheuchzeriaceae, Zosteraceae) in addition to the 'Cymodoceaceae complex', which also includes Posidoniaceae and Ruppiaceae (tentatively). Although Zannichelliaceae Chevall. also have been recognized as distinct in many past treatments, we have decided to merge this family with Potamogetonaceae for reasons given below.

The disposition of the monotypic *Maundia triglochinoides* (Maundiaceae), which typically has been included in Juncaginaceae (Cook, 1996), warrants additional explanation. This genus was not included in the *rbc*L analysis of Alismatidae by Les et al. (1997), who consequently could not evaluate its phylogenetic position. Iles et al. (2009) procured material of *Maundia* and included it in an analysis of key alismatid families for which approximately 14 kb of cpDNA data were evaluated. Their analysis distinguished *Maundia* from Juncaginaceae as an isolated sister group to the clade containing the Cymodoceaceae complex, Potamogetonaceae, Zannichelliaceae and Zosteraceae; however, Juncaginaceae (four genera) was represented only by *Triglochin* in that analysis. These results inspired some authors (APG III, 2009) to consider the transfer of *Maundia* (monotypic) to a distinct family (Maundiaceae); however, a further evaluation of the question was urged. A recent analysis of *rbc*L, *mat*K and *atp*1 data for various monocots including *Cycnogeton*, *Lilaea* Bonpl. (monotypic), *Maundia*, *Tetroncium* (monotypic) and *Triglochin*, also failed to resolve *Maundia* with other Juncaginaceae, thereby strengthening arguments to remove it from that family (von Mering and Kadereit, 2010). The same study also advocated the merger of *Lilaea* with *Triglochin*, leaving Juncaginaceae with only three genera (*Cycnogeton*, *Tetroncium*, *and* *Triglochin*).

Our 167-taxon *rbc*L analysis (Fig 6.2) also resolved *Maundia* as distinct from Juncaginaceae, which was represented in our analysis by *Tetroncium*, *Triglochin*/*Lilaea* (7 species), and *Cycnogeton* (2 species). In this analysis (Fig 6.2), *Maundia* resolved as the sister group to the Potamogetonaceae/Zosteraceae clade, but only with weak internal support (<53% ML or MP BS support for critical nodes; values not shown on tree). To provide additional insight, we conducted an analysis of

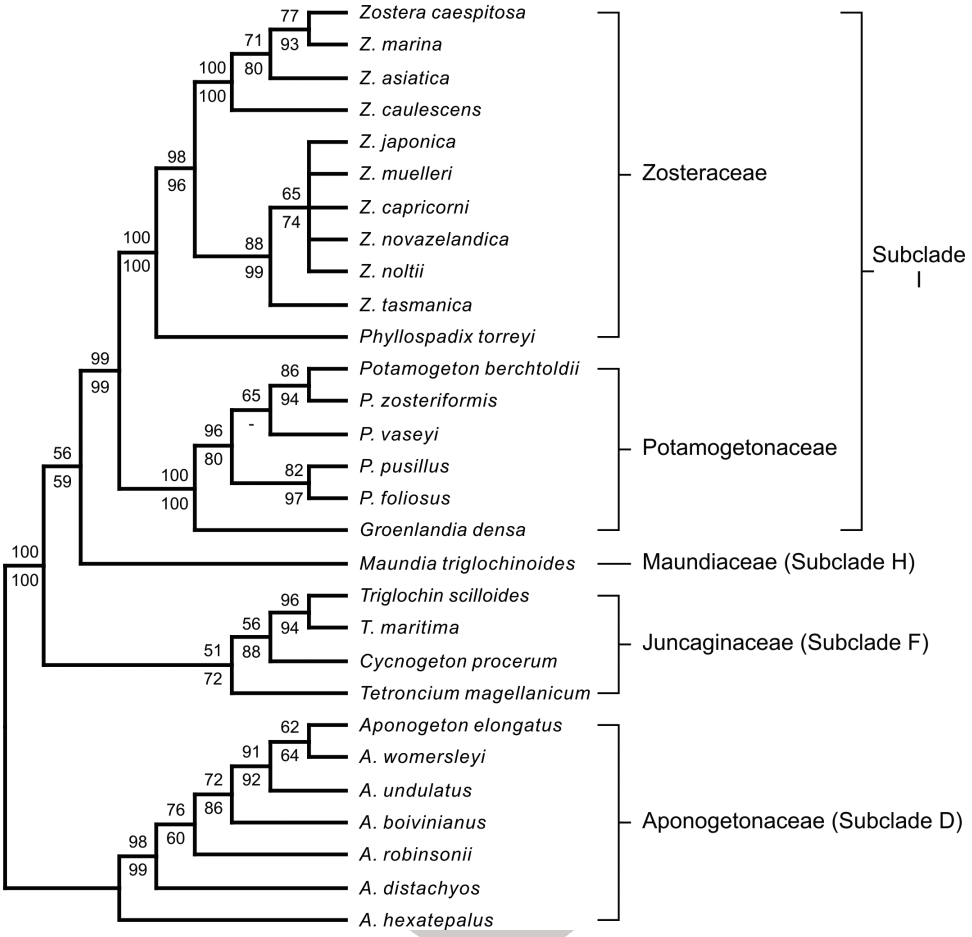

Fig 6.15 Phylogenetic placement of *Maundia* (Maundiaceae) as determined by analysis of *trn*K 5′ intron sequence data (current study). The MP strict consensus tree situates *Maundia* as the sister group of the Potamogetonaceae/Zosteraceae clade, but only with weak internal support. BS support values are indicated above (MP) and below (ML; TVM+G model) nodes respectively ('–' indicates <50% support). Tree statistics: CI = 0.78; CI_{exc} = 0.72; RI = 0.91; ln*L* = –3523.

trnK 5′ intron sequence data, which included *Maundia* and representatives of all three genera of Juncaginaceae (*Cycnogeton*, *Tetroncium* and *Triglochin* [including *Lilaea*]). That analysis (Fig 6.15) further supported the results obtained by Iles et al. (2009), von Mering and Kadereit (2010), and our own 167-taxon analysis by resolving *Maundia* outside of Juncaginaceae as the sister group to the Potamogetonaceae/Zosteraceae clade (but again with fairly weak support). The lack of strong support for the position of *Maundia* in all of these analyses is somewhat

disconcerting; however, it does seem apparent that the genus at least does not associate closely with members of Juncaginaceae. For this reason it seems appropriate to accept the taxonomic recognition of *Maundia* as a distinct, monotypic family, as advocated by von Mering and Kadereit (2010).

6.5.1 Aponogetonaceae (subclade D)

Aponogetonaceae consistently resolve as the sister group to all other members of Potamogetonales in various relevant analyses (Figs. 6.2, 6.3). Only one notable exception occurs, wherein the phylogenetic position of the family is exchanged with Scheuchzeriaceae (Fig 6.3A). In that anomalous analysis, *rbc*L data were analysed by MP using step-matrix weighting (Les et al., 1995). However, in other analyses (including our 167-taxon MP/ML *rbc*L analysis), the former position is retained (Figs. 6.2, 6.3) and is accepted here to represent the most reasonable phylogenetic placement of Aponogetonaceae within the subclass.

6.5.1.1 Infrageneric relationships

Les et al. (2005) evaluated phylogenetic relationships of 21 Aponogetonaceae taxa, focusing on the Australian species. In that study, which incorporated morphological and molecular (*mat*K, nrITS, *trn*K 5′ intron) sequence data, the genus was structured phylogenetically as four major clades, each recognized taxonomically as a section. That analysis resolved the Australian *Aponogeton hexatepalus* (section *Viridis* Les, M.Moody and S.W.L.Jacobs) as sister to the remainder of the genus, which mainly comprised clades with strong geographical affinities, such as section *Flavida* Les, M.Moody and S.W.L.Jacobs (Australia), section *Aponogeton* L.f. subsection *Aponogeton* (Asia) and section *Aponogeton* subsection *Polystachys* A.Camus (Madagascar). Numerous instances of hybridization and polyploidy present technical difficulties that must be considered when evaluating phylogenetic relationships in *Aponogeton* (Les et al., 2005).

Since the study by Les et al. (2005), we have acquired a total of 29 species (roughly 60% of the estimated 50 species worldwide), which has allowed us to expand our original survey of *Aponogeton*. In this analysis, relationships were re-evaluated using combined nrITS and *trn*K 5′ intron sequence data. The resulting phylogenetic relationships (Fig 6.16) were similar to those reported by Les et al. (2005) with several noteworthy differences. The Australian taxa remained in two groups, with *A. hexatepalus* as the sister to the rest of the genus. The newly included *A. womersleyi* (New Guinea) resolved within the major Australian clade as considered by Les et al. (2005); however, it was found to be nearly identical to *A. cuneatus*, which was described recently by Jacobs et al. (2006). Because *A. womersleyi* has never been reported in Australia, the taxonomic status of *A. cuneatus* will require further evaluation to determine whether these species are distinct. Our expanded taxon analysis is consistent with the original study by

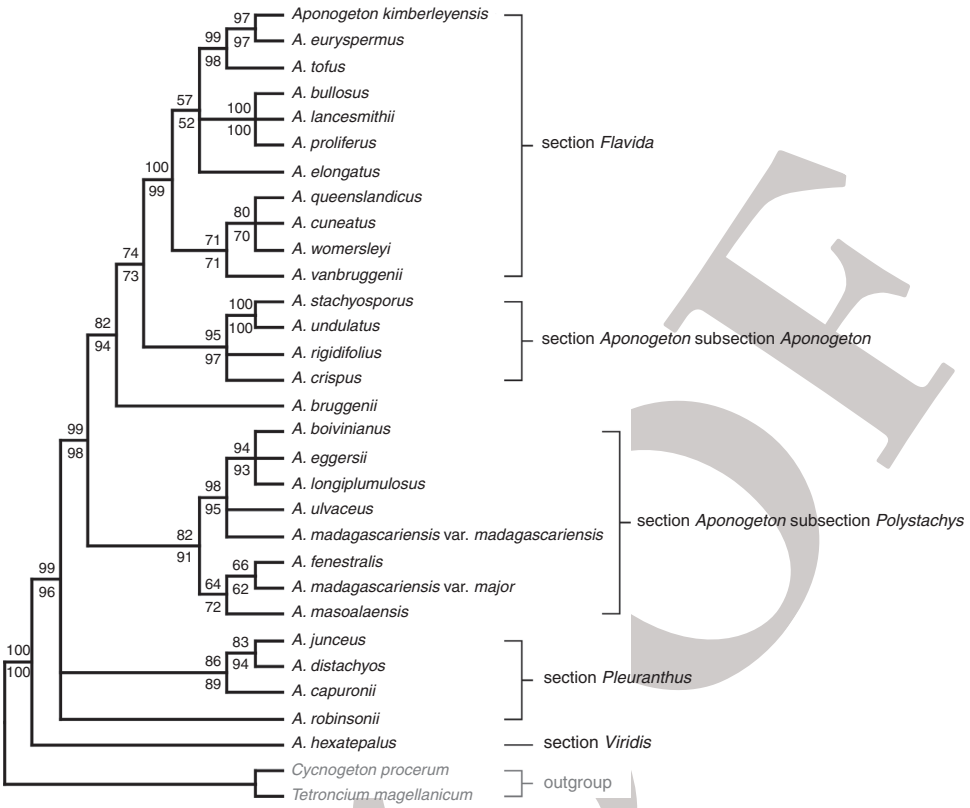

Fig 6.16 Interspecific relationships in *Aponogeton* (Aponogetonaceae) reconstructed by MP/ML analysis of combined nrITS (ML: TIM3+G model) + *trn*K 5′ intron (ML: TPM1uf+I model) sequence data (current study). MP strict consensus tree is shown. Values indicating the degree of internal (BS) support are indicated above (MP) and below (ML) nodes respectively ('–' indicates <50% support). Tree statistics: CI = 0.79; CI_{exc} = 0.67; RI = 0.79; ln*L* = –7025.

indicating an Asian clade (i.e. *Aponogeton* subsection *Aponogeton*) as sister to the major Australian clade (section *Flavida*); however, an additional Asian (Indian) species (*A. bruggenii*) resolved as the sister group to the Australian/Asian clade (Fig 6.16). A major Malagasy clade (*Aponogeton* subsection *Polystachys*; now represented by at least eight taxa) similarly is resolved; however, one Madagascar species (*A. capuronii*) was situated as the sister group to a clade of species from continental Africa. Although the continued inclusion of taxa undoubtedly will further clarify phytogeographical affinities of *Aponogeton* species, these preliminary studies indicate that most Australian and Malagasy species were derived from Asian progenitors, and that continental African species probably were derived from Madagascar.

Aponogeton eventually will require further taxonomic refinements. The monophyly of section *Pleuranthus* could not be confirmed due to our inability to resolve the position of *A. robinsonii* with certainty. Although section *Aponogeton* is paraphyletic as circumscribed, its two major subsections do resolve as distinct subclades, and most other recognized sections and subsections of the genus resolve as clades in phylogenetic analyses (Fig 6.16).

6.5.2 Scheuchzeriaceae (subclade E)

This monotypic family of bog-plants (*Scheuchzeria palustris*) resolves consistently as the sister group to Potamogetonales in exclusion of Aponogetonaceae (Figs 6.2–6.3; however, see discussion under Aponogetonaceae). Old and New World populations differ somewhat by fruit and stigma/style characters (Fernald, 1923); however, these morphological differences generally are treated taxonomically at the subspecific level. A genetic survey of this species on a worldwide basis could shed additional light on patterns of divergence in this widespread taxon.

6.5.3 Juncaginaceae (subclade F)

Following the circumscription proposed by von Mering and Kadereit (2010), Juncaginaceae contain three genera: *Cycnogeton* (8 species), *Tetroncium* (monotypic) and *Triglochin* (~20 species). To summarize intergeneric relationships we have conducted an updated evaluation of *rbc*L data for a third of the taxa, which includes *Tetroncium*, *Cycnogeton* (2 species) and *Triglochin* (7 species). The resulting topology (Figs 6.2, 6.17) resolves each genus as a clade and is consistent with results of several previously published analyses (Les et al., 1997; Petersen et al., 2006; von Mering and Kadereit, 2010), as well as our analysis of *trn*K 5′ intron sequence data (Fig 6.15). In all instances *Triglochin* and *Cycnogeton* are sister clades, with *Tetroncium* sister to the remainder of the family.

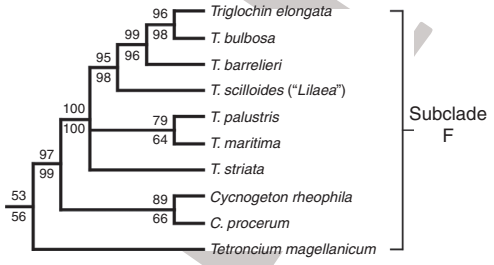

Fig 6.17 Expanded version of 'subclade F' (see Fig 6.2) showing details of relationships in Juncaginaceae as indicated by the results of a 167-taxon *rbc*L analysis.

6.5.3.1 Infrageneric relationships

Analysis of *rbc*L data (Figs 6.2, 6.17) provides the most inclusive molecular phylogenetic survey of Juncaginaceae available to date. It is apparent from this analysis that the taxon once segregated as the genus *Lilaea* (*T. scilloides*) clearly nests within *Triglochin*, as reported previously by von Mering and Kadereit (2010), and there is no compelling reason to accept this taxon as anything other than a highly specialized member of *Triglochin*. The inclusion of additional *Cycnogeton* and *Triglochin* species will be necessary before any more definitive evaluation of interspecific relationships can be made in these genera. Additional work in this area currently is underway by von Mering, who now has acquired molecular data for at least 15 *Triglochin* species (personal communication).

6.5.4 'Cymodoceaceae complex' (subclade G)

Les et al. (1997) designated as the 'Cymodoceaceae complex' a group comprising the families Cymodoceaceae, Posidoniaceae, and Ruppiaceae. These three families had not been associated together in treatments prior to that study, where they resolved as a weakly supported clade (40% MP bootstrap) by analysis of *rbc*L sequence data. The same clade was recovered in our 167-taxon analysis, but with higher internal support (Fig 6.18). This clade also received a moderate level of support (77% MP BS) in the *rbc*L analysis by von Mering and Kadereit (2010). In addition to these single-gene analyses, further corroborative evidence supporting the phylogenetic integrity of the Cymodoceaceae complex has been provided by Iles et al. (2009), whose analysis of large amounts of cpDNA sequence data resolved the same group as a clade with high internal support (ML BS >95%). A combined analysis of *cob*, *atp*1 and *rbc*L sequences (Petersen et al., 2006) also yielded this same clade. The high degree of consistency obtained among these various molecular analyses, and the high level of internal support obtained in at least some of the analyses provides substantial evidence to indicate that these three families associate as a natural clade.

Li and Zhou (2009) challenged the inclusion of *Ruppia* within the Cymodoceaceae complex based upon their combined analysis of *rbc*L sequences and morphological data. In that analysis, *Ruppia* associated with Zannichelliaceae and Potamogetonaceae rather than with Cymodoceaceae and Posidoniaceae. However, several aspects of their study render that result untenable. First, the internal support for the proposed association remained weak (64% MP BS; <50% for all the other critical nodes). The addition of morphological data also influenced the degree of internal support for some of their 'strongly supported' groups (such as Potamogetonaceae and Zosteraceae), which was lower than values obtained for those same groups in analyses using *rbc*L data only (Les et al., 1997). Moreover, all pertinent analyses of *rbc*L data alone have resolved the Cymodoceaceae complex

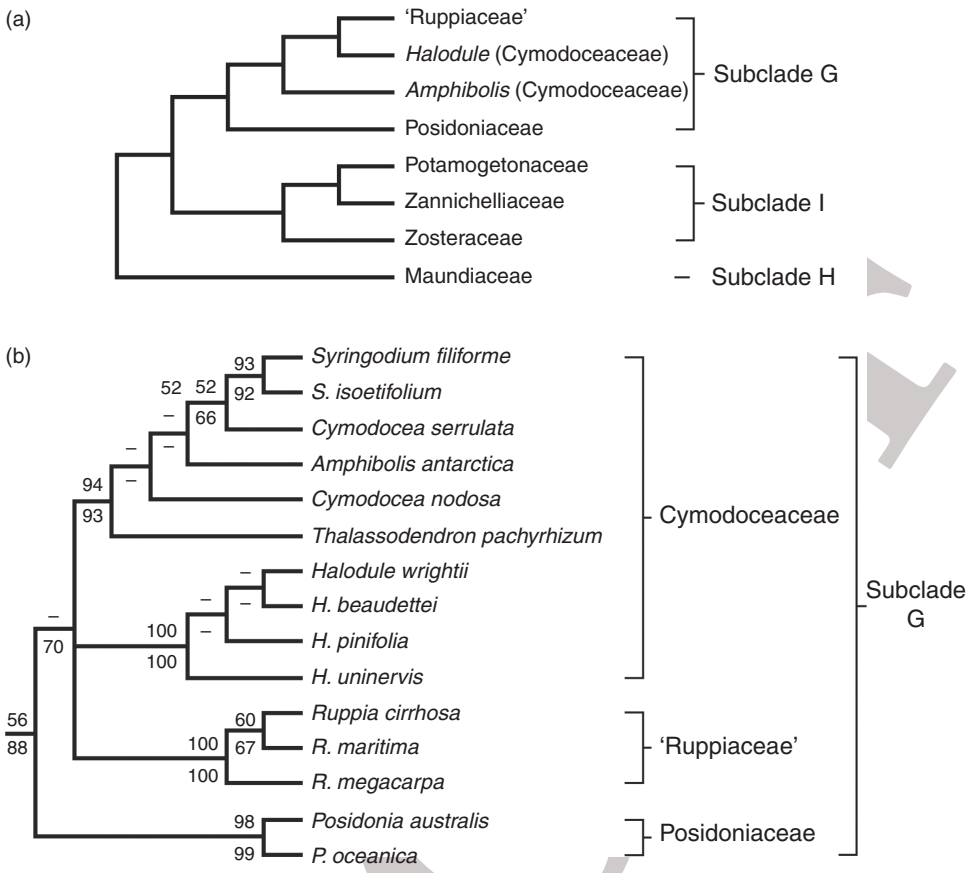

Fig 6.18 Intergeneric relationships in the 'Cymodoceaceae complex'. A: Results from ML analysis of approximately 14 kb of cpDNA sequence data support the Cymodoceaceae 'complex' as a clade with >95% BS support (adapted from Iles et al., 2009). Posidoniaceae are sister to the remainder of the complex with the same high level of support. B: Expanded version of 'subclade G' (see Fig 6.2) shows similar relationships as indicated by the results of a 167-taxon *rbc*L analysis (current study). The failure of Ruppiaceae to resolve as a clade distinct from Cymodoceaceae in such studies provides ample justification to merge these families.

as a clade, indicating that the morphological data indeed were responsible for influencing the different placement of Ruppiaceae in the one instance reported by Li and Zhou (2009). This result is not surprising given that several studies (Les and Haynes, 1995; Les et al., 1997) have demonstrated the misleading effect of homoplasious morphological data on phylogenetic reconstruction in alismatids.

Li and Zhou (2009) acknowledged that morphological data for the alismatids were highly homoplasious and discussed in some detail the probable convergence of a number of characters included in their analysis. They also called for the need

to acquire additional DNA sequence data besides *rbc*L, which are now available. In this case a large amount of cpDNA sequence data (Iles et al., 2009) and combined cpDNA/mtDNA sequences (Petersen et al., 2006) have corroborated the results indicated by the initial *rbc*L analysis of Les et al. (1997) and never have returned results that would support those obtained by Li and Zhou (2009). As a consequence, we continue to recognize the Cymodoceaceae complex as a meaningful clade based on the best available current evidence.

What is less certain is whether the Cymodoceaceae complex should be recognized taxonomically as a single family rather than in its present disposition as comprising three families. In evaluating *rbc*L data (Fig 6.18; Les et al., 1997; von Mering and Kadereit, 2010) or multiple cpDNA sequences (Iles et al. 2009), *Posidonia* consistently resolves as a sister clade to the remaining genera; however, the distinction of Ruppiaceae and Cymodoceaceae is not evident either in those analyses or by analysis of combined cpDNA/mtDNA sequence data (Petersen et al., 2006), which all are equivocal in distinguishing Ruppiaceae and Cymodoceaceae as separate clades. Given these circumstances it seems reasonable and appropriate to merge Ruppiaceae with Cymodoceaceae (which has nomenclatural priority) as we have done here.

6.5.4.1 Cymodoceaceae

We regard Cymodoceaceae as a seagrass clade comprising six genera: *Amphibolis*, *Cymodocea*, *Halodule*, *Ruppia*, *Syringodium* and *Thalassodendron*. The genera *Amphibolis*, *Syringodium* and *Thalassodendron* each contain only two species, but both species have been analysed phylogenetically only in *Syringodium* (Fig 6.18) as a test of monophyly. At least for *rbc*L data (which represents the densest taxonomic coverage of any systematic evaluation for this group to date), it is difficult to distinguish *Amphibolis*, *Cymodocea*, *Syringodium* and *Thalassodendron* as distinct. Rather, the species of these genera analysed thus far appear to be quite closely related (by exhibiting relatively low pairwise levels of genetic divergence) and associate together in one clade (Fig 6.18). These observations could justify the merger of these species into a single genus, of which *Cymodocea* would have nomenclatural priority. However, before such a nomenclatural modification is adopted, it would be advisable to include the four species (*Amphibolis griffithii* (J.M.Black) Hartog, *Cymodocea angustata* Ostenf., *C. rotundata* Asch. and Schweinf., *Thalassodendron ciliatum* (Forssk.) Hartog), which have not yet been evaluated in phylogenetic analyses of this group, as well as to reconsider the morphological characters that have been used to define these genera in the past. It is noteworthy in this respect, that *Syringodium isoetifolium*, *Amphibolis antarctica*, *A. griffithii* and *Thalassodendron ciliatum* all were named originally as species of *Cymodocea*.

In contrast, *Halodule* and *Ruppia* appear to be monophyletic, with each genus resolving as a distinct, strongly supported clade within the family (Fig 6.18).

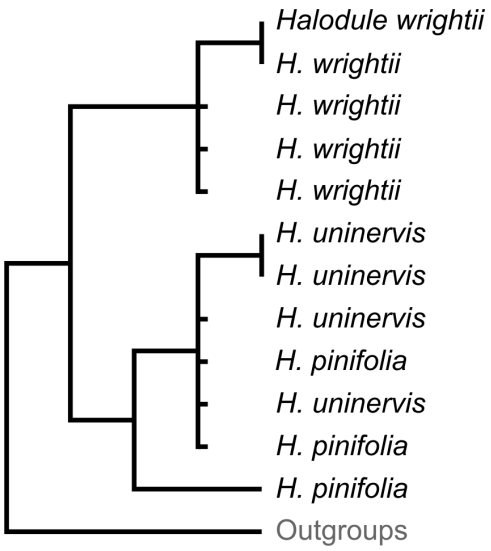

Fig 6.19 Interspecific relationships in *Halodule* as indicated by MP analysis of nrITS sequence data (adapted from Waycott et al., 2006).

Relationships within *Halodule* have been investigated in some detail by Waycott et al. (2006), who analysed nrITS and *trn*L sequence data for multiple populations representing three of the six currently recognized species. The results of those analyses were inconclusive, but indicated that there may only be as few as two defensible species in the genus (Fig 6.19). At the least, that study found no justification to support the continued taxonomic segregation of *H. pinifolia* from *H. uninervis*. Additional populations of *Halodule* need to be investigated and additional genetic loci included in its phylogenetic evaluation so that a comprehensive assessment of relationships can be made for the genus.

Cook (1996) estimated that *Ruppia* contained from 2–10 species. A recent phylogenetic study of *Ruppia* by Ito et al. (2010) indicated that there are at least four distinct species, with several potentially additional taxa indicated within the *R. maritima* 'complex' (Fig 6.20). Polyploidy has complicated efforts to evaluate relationships in the *Ruppia maritima* complex, which includes diploid, triploid and tetraploid cytotypes (Ito et al., 2010). Consequently, molecular analyses of this group should take into account the possibility of potentially paralogous loci, which could present interpretational difficulties.

6.5.4.2 Posidoniaceae

Phylogenetic relationships in *Posidonia* (the sole genus of Posidoniaceae) have been investigated using combined nrITS and *trn*L sequence data together with morphological analyses (Waycott et al., 2006). Those analyses (Fig 6.21) have

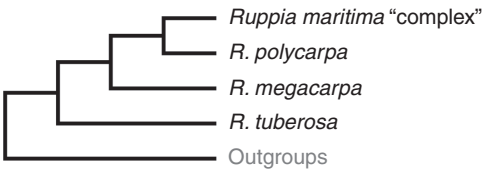

Fig 6.20 Interspecific relationships in *Ruppia* as indicated by MP analysis of *PHYB* data (simplified from Ito et al., 2010). In that same study, cladograms constructed from combined cpDNA data (*mat*K + *rbc*L + *rpo*B + *rpo*C1) showed a similar topology but with the positions of *R. megacarpa* and *R. tuberosa* reversed.

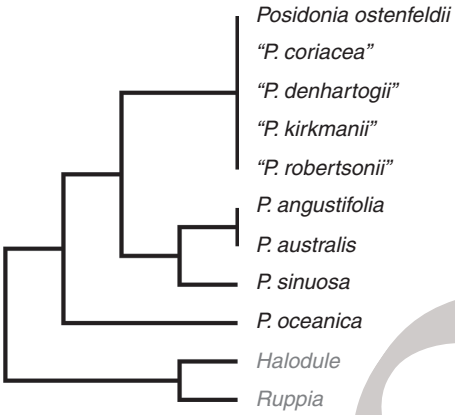

Fig 6.21 Phylogenetic relationships in *Posidonia* as indicated by MP analysis of combined nrITS and *trn*L sequence data (adapted from Waycott et al., 2006).

confirmed the monophyly of the genus and support the recognition of five distinct species within it. They also warranted the merger of four taxa (*P. coriacea* M.L.Cambridge and J.Kuo, *P. denhartogii* J.Kuo and M.L.Cambridge, *P. kirkmanii* J.Kuo and M.L.Cambridge, and *P. robertsonii* J.Kuo and M.L.Cambridge) with *P. ostenfeldii* Hartog, from which they appear to be indistinguishable both genetically and morphologically (Waycott et al., 2006). The morphological and genetic data consistently indicate that the Mediterranean *Posidonia oceanica* is the sister species to all of the remaining (Australian) taxa (Waycott et al., 2006).

6.5.5 Maundiaceae (subclade H)

As described above, the phylogenetic placement of Maundiaceae is somewhat problematic due to the lack of pertinent internal support generated in some molecular data analyses. In the *rbc*L analysis performed by von Mering and Kadereit (2010), *Maundia* associated as the sister group to the

Potamogetonaceae/Zosteraceae clade, but with weak (<70%) internal (MP BS) support. In fact, no critical branch (those delimiting the family from Juncaginaceae) received more than 71% support. Our 167-taxon *rbc*L analysis (Fig 6.2) resolved *Maundia* in the same position, but with even less support (ML/MP BS values no higher than 57% at critical nodes). A similar placement (and similarly low support vales of <59%) was obtained in our *trn*K 5′ intron analysis (Fig 6.15); however, that analysis unfortunately did not include any representatives of the Cymodoceaceae complex.

The only analysis providing reasonable internal support for the position of *Maundia* was by Iles et al. (2009), who analysed roughly 14 kb of cpDNA sequence data. In that study *Maundia* resolved in a similar position (Fig 6.3B), specifically as the sister group to a clade that included the Cymodoceaceae complex, Potamogetonaceae and Zosteraceae, and with a high level of internal support (ML bootstrap >95%). Consequently, we have accepted the well-supported topology from the study by Iles et al. (2009) as representing the most reasonable hypothesis among the two alternative placements of the family indicated by existing molecular phylogenetic analyses. In any event the alternative placements of Maundiaceae do not differ substantially with respect to other alismatid taxa.

6.5.6 Potamogetonaceae/Zosteraceae (subclade I)

This group was among the best supported clades (100% MP BS) recovered in the original *rbc*L analysis by Les et al. (1997). Understandably, the same group (subclade I) also was resolved with high support (99–100% ML/MP BS) in our 167-taxon *rbc*L analysis (Fig 6.2) and by the large cpDNA data analysis (Iles et al., 2009) and combined *cob*, *atp*1, *rbc*L sequence analysis (Petersen et al., 2006). This consistent, well-supported phylogenetic topology provides strong evidence that Potamogetonaceae (including Zannichelliaceae) and Zosteraceae represent a cohesive, sister group relationship.

6.5.6.1 Potamogetonaceae

Although the latter two studies (Iles et al., 2009; Petersen et al., 2006) resolved Zannichelliaceae as the sister clade to Potamogetonaceae, the minimal taxon sampling in both cases was insufficient to determine with certainty whether either family was monophyletic. Evidence to the contrary appeared in the *rbc*L study by Les et al. (1997), where two genera of Zannichelliaceae (*Lepilaena*, *Zannichellia*) resolved as a clade (with 100% MP BS support) among other Potamogetonaceae. Comparable support (99–100% ML/MP BS) for the same result was maintained in our 167-taxon *rbc*L analysis (Fig 6.2), which included nearly a quarter of the estimated 100 *Potamogeton* species. Furthermore, cladograms based on combined *trn*L, *psb*A-*trn*H and 5S-NTS sequence data (Fig 6.22) also grouped Zannichelliaceae with Potamogetonaceae (Lindqvist et al. 2006).

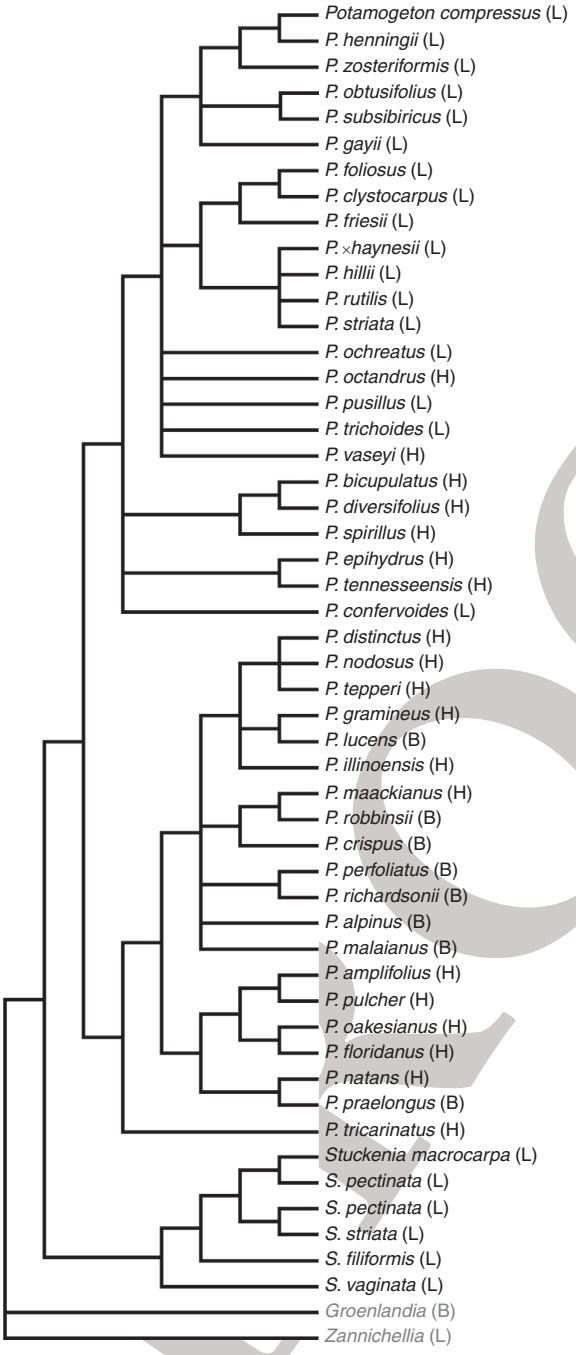

Fig 6.22 Phylogenetic relationships in Potamogetonaceae as indicated by analysis (jackknife majority rule consensus) of combined nuclear (5S-NTS) and cpDNA (*trn*L + *psb*A-*trn*H) sequence data (redrawn from Lindqvist et al., 2006). Basic morphological types are indicated as H (heterophyllous), B (broad-leaved homophyllous) or L (linear-leaved homophyllous).

Superficially, Zannichelliaceae are distinctive morphologically, and non-molecular data never have indicated the phyletic proximity of the family with Potamogetonaceae (Les and Haynes, 1995). However, Potamogetonaceae are extremely diverse morphologically and possess some characters (e.g. fruit morphology) that do not differ fundamentally from Zannichelliaceae. Despite the high degree of reduction that characterizes this taxon, there also are certain features (such as the presence of unusual, coiled cotyledons in *Groenlandia* and *Zannichellia*) that potentially are synapomorphic (Haynes et al., 1998).

Philosophically, there is no compelling reason why aquatic plants should be classified into numerous, depauperate families, especially when there is strong evidence indicating very close relationships among them. In such instances, the recognition of larger, more diverse families would be more in line with taxonomic concepts applied to the majority of terrestrial families, which often contain diverse assemblages of genera. For these reasons, we have elected to merge Zannichelliaceae with Potamogetonaceae. In this circumscription, Potamogetonaceae are recognized as including seven genera: *Althenia*, *Groenlandia*, *Lepilaena*, *Potamogeton*, *Pseudalthenia*, *Stuckenia* and *Zannichellia*. Phylogenetic relationships have been evaluated among five of these genera (excluding *Althenia*, *Pseudalthenia*) using *rbc*L sequence data (Fig 6.2; Les et al., 1997) and among four of the genera (further excluding *Lepilaena*) using combined *trn*L, *psb*A-*trn*H and 5S-NTS sequence data (Lindqvist et al., 2006).

In all cases, *Potamogeton* (in exclusion of *Stuckenia*) resolves as monophyletic. The distinctness of these genera is evidenced by the substantial level of genetic divergence indicated in every pertinent molecular phylogenetic analysis conducted to date (e.g. Fig 6.2; Les et al., 1997; Lindqvist et al., 2006). Some opposition to segregating *Potamogeton* and *Stuckenia* has been expressed by Zhang et al. (2008), who conducted a phylogenetic analysis of *Potamogeton* using *trn*T–*trn*F spacer sequence data; yet, their own results also clearly resolve these groups as distinct, strongly supported clades. Zhang et al. (2008) based their scepticism on one anomalous accession of *S. filiformis* (Pers.) Börner, which resolved within *Potamogeton* (with *P. gramineus*). However, two other *S. filiformis* accessions resolved appropriately within *Stuckenia*, as also did the accession of this species analysed by Lindqvist et al. (2006). Zhang et al. (2008) suggested that the anomalous placement of the single *S. filiformis* accession with *P. gramineus* could indicate hybridization. However, given the extensive level of genetic divergence between these genera and the lack of any reported morphological indications of hybridity in that accession, this situation requires further evaluation, especially to exclude contamination as a possible explanation.

Thus far, each surveyed genus (*Groenlandia*, *Lepilaena*, *Potamogeton*, *Stuckenia* and *Zannichellia*) is distinct genetically; however, their inter-relationships have not been resolved adequately. A complete picture of intergeneric relationships in

Potamogetonaceae will not be possible until species from the unsurveyed genera *Althenia* and *Pseudalthenia* are included in phylogenetic analyses, along with additional data from other genetic loci.

6.5.6.2 Infrageneric relationships

Infrageneric relationships have been poorly studied in *Lepilaena* and *Zannichellia*, and these genera require additional systematic evaluation. *Althenia*, *Groenlandia* and *Pseudalthenia* arguably are monotypic (Cook, 1996).

Lindqvist et al. (2006) evaluated roughly half of the estimated 100 species of *Potamogeton* using a combination of nuclear and cpDNA molecular markers. Their resulting phylogenetic tree divided the genus into two clades, which roughly represent morphological groups corresponding to narrow-leaved (upper clade) and broader-leaved (lower clade) taxa (Fig 6.22). This result parallels those reported earlier by Iida et al. (2004), who also recovered two major clades of linear-leaved and broad-leaved taxa (based on noncoding cpDNA sequences) in their study of Japanese *Potamogeton*.

The occurrence of heterophyllous species in each of the major clades recovered by Lindqvist et al. (2006) also is consistent with earlier hypotheses (based on patterns of secondary metabolites), suggesting that *Potamogeton* species are heterophyllous ancestrally and that linear-leaved and broad-leaved homophyllous species are multiply derived from heterophyllous ancestors (Les and Sheridan, 1990).

The phylogenetic overviews of *Potamogeton* by Iida et al. (2004) and Lindqvist et al. (2006) have presented useful starting points for evaluating relationships in this large and complex genus. However, a comprehensive overview of inter-relationships in the genus will not be achieved until additional taxa are evaluated and complications related to polyploidy and hybridization have been dealt with effectively. Now that preliminary estimates of relationships in *Potamogeton* are available, we recommend that intensive studies proceed following a clade-by-clade approach, which incorporates increased taxon sampling (including multiple populations of species), additional genetic loci, and evaluation of hybridization and paralogy. A number of striking anomalies, such as the association of the morphologically disparate *P. praelongus* and *P. natans* (Iida et al., 2004; Lindqvist et al., 2006) also need to be better reconciled. Even analyses of relatively small portions of the genus have disclosed substantial taxonomic discrepancies (Les et al., 2009) and it is apparent that a large amount of work still is necessary before a satisfactory phylogenetic picture of this genus emerges.

6.5.6.3 Zosteraceae

Only two sister genera (*Phyllospadix*, *Zostera*) currently are recognized in this family due to the merger of the former genus *Heterozostera* (Setch.) Hartog which was found to nest phylogenetically within *Zostera* (Les et al., 2002; Tanaka et al.,

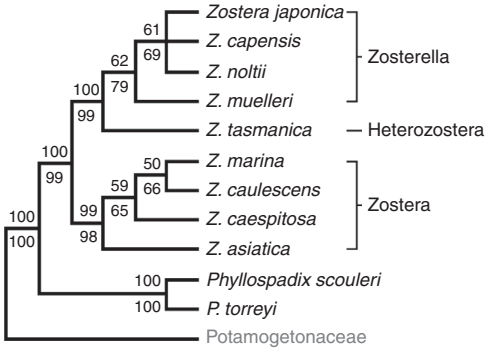

Fig 6.23 Relationships in Zosteraceae as resolved by analysis of *rbc*L data (see 'subclade I', Fig 6.2). Subgenera of *Zostera* are delimited by brackets.

2003). The monophyly of both genera has been confirmed by phylogenetic analysis of morphological data (Les et al., 2002), as well as by phylogenies generated using molecular datasets (Les et al., 2002; Tanaka et al., 2003).

Zostera is subdivided into three subgenera: subgenus *Zosterella* (Asch.) Ostenf., which consistently resolves as the sister clade to the former genus *Heterozostera* (now recognized as the monotypic subgenus *Heterozostera* Setch.); in turn, subgenus *Zostera* L. represents the sister group to the subgeneric *Heterozostera*/*Zosterella* clade (Fig 6.23). All currently recognized *Zostera* species now have been analysed phylogenetically if one excludes several new taxa named by Kuo (2005), and *Z. pacifica* S.Watson (Coyer et al., 2008), which are weakly defined morphologically and perhaps warrant recognition as infraspecific taxa instead of distinct species.

Although the phylogenetic relationships of *Zostera* now are fairly well understood and can be evaluated quite comprehensively using available *mat*K, nrITS and *rbc*L data (Les et al., 2002; Tanaka et al., 2003), *Z. capensis* remains somewhat problematic. Morphological data place *Z. capensis* within subgenus *Zosterella* (Hartog, 1970; Les et al., 2002). However, the *mat*K sequences reported by Tanaka et al. (2003) for *Z. capensis* and *Z. tasmanica* are identical, placing the former species anomalously within subgenus *Heterozostera*. Furthermore, the result from *mat*K conflicts with that we obtained using data from a different chloroplast locus (*rbc*L), which resolved *Z. capensis* in agreement with morphology (Figs 6.2, 6.23). Moreover, although the *rbc*L sequence was obtained directly from material collected in the native (African Cape) range of *Z. capensis* (Forest et al., 2007), it closely matches or is identical to *rbc*L sequences reported for *Z. japonica* (Appendix), another member of subgenus *Zosterella*.

Both *Z. capensis* and *Z. japonica* are extremely similar morphologically (Hartog, 1970), and differ primarily by whether the seed striae are distinct (*Z. capensis*) or

indistinct (*Z. japonica*). These observations raise the possibility of whether *Z. capensis* might represent an early introduction of *Z. japonica* to Africa, rather than a novel species, but this question deserves further evaluation. In any event, *mat*K and *rbc*L are both chloroplast genes, which share the same evolutionary history. Consequently, the different phylogenetic relationships indicated must be incorrect for either one of the sequences or for both sequences. Here, it is more likely that the *rbc*L sequence yields the correct phylogeny, as the result agrees with the morphological distinctions that characterize subgenus *Zosterella*. The summary phylogeny of *Zostera* provided by *rbc*L data (Fig 6.23) reflects this interpretation.

Despite its small size (five species), phylogenetic relationships within *Phyllospadix* have not yet been evaluated using either morphological or molecular data. Comparable molecular data (*rbc*L sequences in this case) exist for only two species (*P. scouleri*, *P. torreyi*), which have been included in the *rbc*L analysis summarized in Fig 6.23. Only one sequence (*mat*K) is available for a third species (*P. iwatensis* Makino), which precluded its inclusion in that assessment. No molecular data have yet been reported for either *P. japonicus* Makino or *P. serrulatus* Rupr. ex Asch.

6.6 Summary

Ongoing phylogenetic studies of alismatids consistently demonstrate that the group is monophyletic; however, it remains uncertain whether their immediate sister group is Acoraceae, Araceae or Tofieldiaceae. Phylogenetic analyses also corroborate that alismatids comprise two major clades, which we recognize taxonomically as: Alismatales (petaloid taxa) and Potamogetonales (tepaloid taxa).

Alismatids comprise 11distinct and phylogenetically defensible families. There has been no compelling evidence from any recent study that justifies the continued segregation of several families, namely Limnocharitaceae, Ruppiaceae and Zannichelliaceae. We recommend that these families be merged with Alismataceae, Cymodoceaceae and Potamogetonaceae, respectively. A consistent pattern of interfamilial relationships in Alismatidae is recovered in phylogenetic analyses. The associations of Potamogetonaceae with Zosteraceae, Posidoniaceae with Cymodoceaceae (including Ruppiaceae) and Butomaceae with Hydrocharitaceae are indicated repeatedly. The placement of *Najas* (Najadaceae) within Hydrocharitaceae now has been firmly established by analyses of a large amount of molecular data. Four subclades are resolved consistently within Hydrocharitaceae, which correspond to previously proposed subfamilies. The monotypic Maundiaceae clearly are distinct from Juncaginaceae; however, the precise relationship of the family to Cymodoceaceae remains uncertain. The dissolution of *Lilaea* and its merger with *Triglochin* is well-supported by various phylogenetic studies.

Intergeneric relationships have been elucidated quite well for most families. However, the precise relationship of some genera (e.g. *Albidella*) remains unsettled or has not been evaluated sufficiently (e.g. *Althenia*, *Lepilaena*, *Pseudalthenia* and *Zannichellia*).

Interspecific relationships have been clarified considerably in many major alismatid genera, such as *Alisma*, *Echinodorus*, *Halophila*, *Vallisneria* and *Zostera*. Although good progress also has been made in elucidating relationships in some of the larger genera, including *Aponogeton*, *Najas*, *Potamogeton* and *Sagittaria*, additional taxon sampling and data are needed for more comprehensive systematic coverage. Several large genera (*Blyxa*, *Lagarosiphon*, *Ottelia*) remain very poorly understood systematically.

6.7 Acknowledgements

This material is based upon work supported by the National Science Foundation under Grant No. DEB-0841658. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

6.8 References

APG I (Angiosperm Phylogeny Group I). (1998). An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Gardens*, **85**, 531–553.

APG II (Angiosperm Phylogeny Group II). (2003). An update of the Angiosperm Phylogeny Group classification for the orders of flowering plants: APG II. *Botanical Journal of the Linnean Society*, **141**, 399–436.

APG III (Angiosperm Phylogeny Group III). (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*, **161**, 105–121.

Baldwin, B. G. (1992). Phylogenetic utility of the internal transcribed spacers of ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution*, **1**, 3–16.

Bremer, B., Bremer, K., Heidari, N. et al. (2002). Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Molecular Phylogenetics and Evolution*, **24**, 273–300.

Cameron, K. M., Chase, M. W. and Rudall, P. J. (2003). Recircumscription of the monocotyledonous family Petrosaviaceae to include *Japonolirion*. *Brittonia*, **55**, 214–225.

Chase, M. W., Soltis, D. E., Olmstead, R. G. et al. (1993). Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. *Annals of the Missouri Botanical Garden*, **80**, 528–580.

Cook, C. D. K. (1996). *Aquatic Plant Book*, 2nd edn. Amsterdam: SPB Academic Publishing bv.

Coyer J. A., Miller, K. A., Engle, J. M. et al. (2008). Eelgrass meadows in the California Channel Islands and adjacent coast reveal a mosaic of two species, evidence for introgression and variable clonality. *Annals of Botany*, **101**, 73–87.

Cronquist, A. (1981).*An Integrated System of Classification of Flowering Plants*. New York: Columbia University Press.

Davis, J. I., Stevenson, D. W., Petersen, G. et al. (2004). A phylogeny of the monocots, as inferred from *rbc*L and *atp*A sequence variation, and a comparison of methods for calculating jackknife and bootstrap values. *Systematic Botany*, **29**, 467–510.

Doyle, J. J. and Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, **19**, 11–15.

Duvall, M. R. and Ervin, A. B. (2004). 18S gene trees are positively misleading for monocot/dicot phylogenetics. *Molecular Phylogenetics and Evolution*, **30**, 97–106.

Fernald, M. L. (1923). *The American variety of Scheuchzeria palustris. Rhodora*, **25**, 177–179.

Forest, F., Grenyer, R., Rouget, M. et al. (2007). Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature*, **445**, 757–760.

Givnish, T. J., Pires, J. C., Graham, S. W. **et al.** (2006). Phylogeny of the monocotyledons based on the highly informative plastid gene *ndhF*: evidence for widespread concerted convergence. In *Monocots: Comparative Biology And Evolution (Excluding Poales)*, ed. J. T. Columbus, E. A. Friar, C. W. Hamilton, et al. Claremont, CA: Rancho Santa Ana Botanic Garden, pp. 28–51.

Graham, S. W., Zgurski, J. M., McPherson, M. A. **et al.** (2006). Robust inference of monocot deep phylogeny using an expanded multigene plastid data set. *Aliso*, **22**, 3–20.

Green, E. P. and Short, F. T. (2003). *World Atlas Of Seagrasses*. Berkeley, CA: University of California Press.

Hartog, C. D en. (1970). *The Sea-grasses Of The World*. Amsterdam: North-Holland Publishing Co.

Haynes, R. R. and Les, D. H. (2005). Alismatales. In *Nature Encyclopedia of Life Sciences*. London: Nature Publishing Group.

Haynes, R. R., Les, D. H. and Holm-Nielsen, L. B. (1998). Zannichelliaceae. In *The Families And Genera Of Vascular Plants, Volume IV, Flowering Plants: Monocotyledons, Alismatanae And Commelinanae (Except Gramineae)*, ed. K. Kubitzki. Berlin: Springer-Verlag, pp. 470–474.

Iida, S., Kosugeb, K. and Kadono, Y. (2004). Molecular phylogeny of Japanese *Potamogeton* species in light of noncoding chloroplast sequences. *Aquatic Botany*, **80**, 115–127.

Iles, W., Smith, S. Y. and Graham, S. W. (2009). Robust resolution of the backbone of Alismatales phylogeny [abstract]. Vancouver: Botany 2009.

Ito, Y., Ohi-Toma, T., Murata, J. and Tanaka, N. (2010). Hybridization and polyploidy of an aquatic plant, *Ruppia* (Ruppiaceae), inferred from plastid and nuclear DNA phylogenies. *American Journal of Botany*, **97**, 1156–1167.

Jacobs, S. W. L., Les, D. H., Moody, M. L. and Hellquist, C. B. (2006). Two new species of *Aponogeton* (Aponogetonaceae), and a key to species from Australia. *Telopea*, **11**, 129–134.

Jacobson, A. and Hedrén, M. (2007). Phylogenetic relationships in *Alisma*

(Alismataceae) based on RAPDs, and sequence data from ITS and *trnL*. *Plant Systematics and Evolution*, **265**, 27–44.

Johnson, L. A. and Soltis, D. E. (1995). Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *matK* sequences. *Annals of the Missouri Botanical Garden*, **82**, 149–175.

Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. F. and Donoghue, M. J. 2008. *Plant Systematics. A Phylogenetic Approach*, 3rd ed. Sunderland, MA: Sinauer.

Keener, B. R. 2005. *Molecular systematics and revision of the aquatic monocot genus Sagittaria (Alismataceae)*. Ph.D. dissertation, Tuscaloosa, AL: The University of Alabama.

Krahulcová, A. and Jarolímová, V. (1993). Ecology of two cytotypes of *Butomus umbellatus* I. Karyology and breeding. *Folia Geobotanica et Phytotaxonomica*, **28**, 385–411.

Kuo, J. (2005). A revision of the genus *Heterozostera* (Zosteraceae). *Aquatic Botany*, **81**, 97–140.

Lehtonen, S. (2006). Phylogenetics of *Echinodorus* (Alismataceae) based on morphological data. *Botanical Journal of the Linnean Society*, **150**, 291–305.

Lehtonen, S. (2008). An integrative approach to species delimitation in *Echinodorus* (Alismataceae) and the description of two new species. *Kew Bulletin*, **63**, 525–563.

Lehtonen, S. and Myllys, L. (2008). Cladistic analysis of *Echinodorus* (Alismataceae): simultaneous analysis of molecular and morphological data. *Cladistics*, **24**, 218–239.

Les, D. H. and Haynes, R. R. (1995). Systematics of subclass Alismatidae: a synthesis of approaches. In *Monocotyledons: Systematics And Evolution*, ed. P. J. Rudall, P. J. Cribb, D. F. Cutler, and C. J. Humphries. Kew: Royal Botanic Gardens, pp. 353–377.

Les, D. H. and Philbrick, C. T. (1993). Studies of hybridization and chromosome number variation in aquatic angiosperms: evolutionary implications. *Aquatic Botany*, **44**, 181–228.

Les, D. H. and Sheridan, D. J. (1990). Biochemical heterophylly and flavonoid evolution in North American *Potamogeton* (Potamogetonaceae). *American Journal of Botany*, **77**, 453–465. http://www.eeb.uconn.edu/people/les/Manuscript_Files/AJB_77%28453%29%5B1990%5D.pdf

Les, D. H., Garvin, D. K. and Wimpee, C. F. (1993). Phylogenetic studies in the monocot subclass Alismatidae: evidence for a reappraisal of the aquatic order Najadales. *Molecular Phylogenetics and Evolution*, **2**, 304–314.

Les, D. H., Cleland, M. A. and Waycott M. (1997). Phylogenetic studies in Alismatidae, II: evolution of marine angiosperms ('seagrasses') and hydrophily. *Systematic Botany*, **22**, 443–463. http://www.eeb.uconn.edu/people/les/Manuscript_Files/Syst_Bot_22%28443%29.pdf

Les, D. H., Moody, M. L., Jacobs, S. W. L. and Bayer, R. J. (2002). Systematics of seagrasses (Zosteraceae) in Australia and New Zealand. *Systematic Botany*, **27**, 468–484.

Les, D. H., Moody, M. L., Jacobs, S. W. L. (2005). Phylogeny and systematics of *Aponogeton* (Aponogetonaceae): the Australian species. *Systematic Botany*, **30**, 503–519.

Les, D. H., Moody, M. L. and Soros, C. (2006). A reappraisal of phylogenetic relationships in the monocotyledon family Hydrocharitaceae. In *Monocots: Comparative Biology and Evolution*, ed.

J. T. Columbus, E. A. Friar, C. W. Hamilton et al. Claremont, California: Rancho Santa Ana Botanic Garden, pp. 211–230.

Les, D. H., Jacobs, S. W. L., Tippery, N. P. **et al.** (2008). Systematics of *Vallisneria* (Hydrocharitaceae). *Systematic Botany*, **33**, 49–65.

Les, D. H., Murray, N. M. and Tippery, N. P. (2009). Systematics of two imperiled pondweeds (*Potamogeton vaseyi, P. gemmiparus*) and taxonomic ramifications for subsection *Pusilli* (Potamogetonaceae). *Systematic Botany*, **34**, 643–651.

Les, D. H., Sheldon, S. P. and Tippery, N. P. (2010). Hybridization in hydrophiles: natural interspecific hybrids in *Najas* L. (Hydrocharitaceae). *Systematic Botany*, **35**, 736–744.

Li, X.-X. and Zhou, Z.-K. (2007). The higher-level phylogeny of monocots based on *mat*K, *rbc*L and 18S rDNA sequences. *Acta Phytotaxonomica Sinica*, **45**, 113–133.

Li, X. and Zhou, Z. (2009). Phylogenetic studies of the core Alismatales inferred from morphology and *rbc*L sequences. *Progress in Natural Science*, **19**, 931–945.

Lindqvist, C., De Laet, J., Haynes, R. R. et al. (2006). Molecular phylogenetics of an aquatic plant lineage, Potamogetonaceae. *Cladistics*, **22**, 568–588.

Petersen, G., Seberg, O., Davis, J. I. and Stevenson, D. W. (2006). RNA editing and phylogenetic reconstruction in two monocot mitochondrial genes. *Taxon*, **55**, 871–886.

Posada, D. (2008). jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.

Posluszny, U., Charlton, W. A. and Les, D. H. (2000). Modularity in Helobial flowers. In *Systematics and Evolution of Monocots*, ed. K. L. Wilson and D. Morrison. Victoria: CSIRO Publishing, pp. 63–74.

Qiu, Y.-L., Lee, J., Bernasconi-Quadroni, F. et al. (2000). Phylogeny of basal angiosperms: analyses of five genes from three genomes. *International Journal of Plant Science*, **161**(S6), S3–S27.

Rogstad, S. H. (1992). Saturated NaCl-CTAB solution as a means of field preservation of leaves for DNA analyses. *Taxon*, **41**, 701–708.

Shaffer-Fehre, M. (1991a). The endotegmen tuberculae: an account of little-known structures from the seed coat of the Hydrocharitoideae (Hydrocharitaceae) and *Najas* (Najadaceae). *Botanical Journal of the Linnean Society*, **107**, 169–188.

Shaffer-Fehre, M. (1991b). The position of *Najas* within the subclass Alismatidae (Monocotyledones) in the light of new evidence from seed coat structures in the Hydrocharitoideae (Hydrocharitales). *Botanical Journal of the Linnean Society*, **107**, 189–209.

Soltis, D. E., Gitzendanner, M. A. and Soltis, P. S. (2007). A 567-taxon data set for angiosperms: the challenges posed by Bayesian analyses of large data sets. *International Journal of Plant Sciences*, **168**, 137–157.

Swofford, D. L. (2002). *PAUP*. Phylogenetic analysis using parsimony (*and other methods)*, ver. 4. Sunderland, MA: Sinauer Associates.

Tamura, M. N., Yamashita, J., Fuse, S. and Haraguchi, M. (2004). Molecular phylogeny of monocotyledons inferred from combined analysis of plastid *mat*K and *rbc*L gene sequences. *Journal of Plant Research*, **117**, 109–120.

Tanaka, N., Setoguchi, H. and Murata, J. (1997). Phylogeny of the family Hydrocharitaceae inferred from *rbc*L and *mat*K gene sequence data. *Journal of Plant Research*, **110**, 329–337.

Tanaka, N., Kuo, J., Omori, Y., Nakaoka, M. and Aioi, K. (2003). Phylogenetic relationships in the genera *Zostera* and *Heterozostera* (Zosteraceae) based on *mat*K sequence data. *Journal of Plant Research*, **116**, 273–279.

Thorne, R. F. and Reveal, J. (2007). An updated classification of the class Magnoliopsida ('Angiospermae'). *Botanical Review*, **73**, 67–182.

Tippery, N. P., Les, D. H., Padgett, D. J. and Jacobs, S. W. L. (2008). Generic circumscription in Menyanthaceae: A phylogenetic evaluation. *Systematic Botany*, **33**, 598–612.

Tomlinson, P. B. (1982). *Anatomy Of The Monocotyledons VII. Helobiae (Alismatidae) (Including The Seagrasses)*. Oxford: Clarendon Press.

von Mering, S. and Kadereit, J. W. (2010). Phylogeny, systematics, and recircumscription of Juncaginaceae – a cosmopolitan wetland family. In *Diversity, Phylogeny, and Evolution in the Monocotyledons*, ed. O. Seberg, G. Petersen, A. S. Barfod, and J. I. Davis. Aarhus: Aarhus University Press, pp. 55–79.

Waycott, M., Freshwater, D. W., York, R. A., Calladine, A., and Kenworthy, W. J. (2002). Evolutionary trends in the seagrass genus *Halophila* (Thouars): Insights from molecular phylogeny. *Bulletin of Marine Science*, **71**, 1299–1308.

Waycott, M., Procaccini, G., Les, D. H. and Reusch, T. B. H. (2006). Seagrass evolution, ecology and conservation: a genetic perspective. In *Seagrasses: Biology, Ecology And Conservation*, ed. A. W. D. Larkum, R. J. Orth, and C. M. Duarte. Dordrecht: Springer-Verlag, pp. 25–50.

Zhang, T., Wang, Q., Li, W., Cheng, Y. and Wang, J. (2008). Analysis of phylogenetic relationships of *Potamogeton* species in China based on chloroplast *trnT-trnF* sequences. *Aquatic Botany*, **89**, 34–42.

Zwickl, D. J. (2006). *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. thesis*. Austin, TX: University of Texas.

6.9 Appendix

Voucher and GenBank accession number information for sequences newly analysed in this study. Paragraphs separate groups discussed in the text (see Fig 6.2). Order of information is as follows: taxon, authority, voucher information (only for sequences newly reported here), GenBank accession numbers (ITS, *mat*K/*trn*K, *rbc*L). Asterisks (*) indicate sequences newly reported; dashes (–) indicate sequences not applicable to our study; slashes (/) indicate discontinuous sequences that were joined in our analyses; numbers in brackets ([]) indicate additional published sequences for the same or related taxa that were not used in our study.

A. Alismataceae. *Alisma plantago-aquatica* L. (DQ339085, AF542573, L08759); *Astonia australiensis* (Aston) S.W.L.Jacobs, *Jacobs 9670* (NSW), (AY335952, HQ456456*/HQ456457*, HQ456499*); *Baldellia ranunculoides* (L.) Parlatore,

Charlton s.n. (MANCH), (HQ456394*, HQ456458*, U80677 [DQ859163]); *Butomopsis latifolia* Kunth, *Jacobs 9257* (NSW), (HQ456395*, HQ456459*, HQ456500*); *Caldesia acanthocarpa* (F.Muell.) Buchenau, *Jacobs 8209* (NSW), (HQ456396*, HQ456460*, HQ456501*); *C. oligococca* (F.Muell.) Buchenau, *Jacobs 8203* (NSW), (HQ456397*, HQ456461*, HQ456502* [AY277799]); *Damasonium alisma* Mill., *Charlton s.n.* (MANCH), (HQ456398*, HQ456462*, U80678); *Echinodorus amazonicus* Rataj, *Charlton s.n.* (MANCH), (–, –, HQ456503*); *E. cordifolius* (L.) Griseb. (EF088079, EF088127, DQ859164); *E. grandiflorus* (Cham. and Schltdl.) Micheli (EF088070, EF088118, U80679); *E. grisebachii* Small (EF088046, EF088095, –); *E. longiscapus* Arechav. (EF088068, EF088116, –); *E. osiris* Rataj (–, –, DQ859165); *Helanthium parvulum* (Engelm.) Small, *Moody 381* (CONN), (HQ456399*, HQ456463*, HQ456504*); *H. tenellum* (Mart.) Britton, *Charlton s.n.* (MANCH), (EF088056, EF088105, HQ456505*); *Hydrocleys nymphoides* (Willd.) Buchenau, *Les s.n.* (CONN), (HQ456423*, HQ456470*, U80716 [AB004900]); *Limnocharis flava* (L.) Buchenau, *Les s.n.* (CONN), (HQ456400*, HQ456464*, U80717 [AB088807]); *Luronium natans* (L.) Raf., *Charlton s.n.* (MANCH), (HQ456401*, HQ456465*, U80680); *Ranalisma humile* (Kuntze) Hutch., *Charlton s.n.* (MANCH), (HQ456402*, HQ456466*, U80681); *R. rostratum* Stapf (AY395986, AY952415, AY952438); *Sagittaria lancifolia* L., *Les s.n.* (CONN), (–, –, HQ456506*); *S. latifolia* Willd., *Les s.n.* (MANCH), (HQ456403*, HQ456467*, L08767); *S. montevidensis* Cham. and Schltdl., *Les s.n.* (CONN), (HQ456404*, HQ456468*, HQ456507*); *Wiesneria triandra* Micheli, *Cook s.n.* (Z), (AY335953, HQ535983*, U80682).

B. Butomaceae. *Butomus umbellatus* L., *Les 499* (CONN), (AY870346, HQ456469*, U80685) [AY149345]).

C. Hydrocharitaceae. *Apalanthe granatensis* (Humb. and Bonpl.) C.D.K.Cook and Urmi-König (AY870362, –, U80693); *Blyxa aubertii* L.C.Richard (AY870359, –, U80694 [AB088810 – *B. echinosperma* (C.B.Clarke) Hook.f.]); *B. japonica* Maxim. ex Asch. and Gürke (–, –, AB004886); *Egeria densa* Planch. (AY330707/AY870360, –, U80695 [AB004887]); *E. najas* Planch. (AY330708, –, DQ859166); *Elodea bifoliata* St.John, *Les 801* (CONN), (HQ456405*, –, –); *E. canadensis* Michx., (1) *Les 803* (CONN), (HQ456406-HQ456413*, –, –); (2) *Les 813* (CONN), (HQ456414-HQ456421*, –, –); (3) *Les 814* (CONN), (HQ456422*, –, –); (4) (AY330704, –, DQ859167); *E. nuttallii* (Planch.) H.St.John (AY330706/AY870361/EF526382, –, U80696 [AB004888]); *Enhalus acoroides* (L.f.) Rich. ex Steud. (–, –, U80697 [AB004889]); *Halophila decipiens* Ostenf. (–, –, U80698); *H. engelmannii* Asch. (–, –, U80699); *H. ovalis* (R.Br.) Hook.f. (–, –, AB004890 [DQ859168]); *Hydrilla verticillata* (L.f.) Casp. (–, –, U80700 [AB004891/GU135149/GU135242]); *Hydrocharis dubia* (Blume) Backer (–, –, AB004892); *H. morsus-ranae* L. (AY335962, AY870375/AY874445, U80701); *Lagarosiphon madagascariensis* Casp. (–, –, AB004893); *L. major* (Ridley) Moss (–, –, U80703); *L. muscoides* Harv.

(–, –, U80702); *Limnobium laevigatum* (Humb. and Bonpl. ex Willd.) Heine (–, –, AB004894); *L. spongia* (Bosc.) Steud., *Cook s.n.* (Z), (AY335963, HQ456471*, U80704); *Najas arguta* Kunth (–, –, HM240485); *N. browniana* Rendle (–, –, HM240486); *N. flexilis* (Willd.) Rostk. and W.L.E.Schmidt (–, –, HM240489); *N. gracillima* (A.Braun ex Engelm.) Magnus (–, –, HM240490); *N. guadalupensis* (Spreng.) Magnus (–, –, DQ859169 [DQ859170]); *N. marina* L. (–, –, U80705); *N. minor* All. (–, –, HM240506); *N. tenuifolia* R.Br. (–, –, HM240507); *Nechamandra alternifolia* (Roxburgh ex Wight) Thwaites (–, –, U80706 [AB506768]); *Ottelia acuminata* (Gagnep.) Dandy (–, –, AY952435); *O. alismoides* (L.) Pers. (AY870358, –, U80707 [AB004895]); *O. ovalifolia* Rich. (–, –, DQ859171); *O. ulvifolia* Walp. (–, –, U80708); *Stratiotes aloides* L., *Les s.n.* (CONN), (AY870357, HQ456472*, U80709 [AB004896]); *Thalassia hemprichii* (Ehrenb.) Asch. (–, –, U80710 [AB004897]); *T. testudinum* Banks ex K.Koenig (–, –, U80711); *Vallisneria americana* Michx. (–, –, EF143005); *V. asiatica* Miki (–, –, AB004898 [EF143007/EF155532]); *V. australis* S.W.L.Jacobs and Les (–, –, EF143008); *V. caulescens* F.M.Bailey and F.Muell. (–, –, EF143009); *V. denseserrulata* Makino (–, –, EF143010); *V. gracilis* F.M.Bailey (–, –, EF143012 [EF143006 – *V. annua* S.W.L. Jacbos and K.A.Frank]); *V. nana* R.Br. (–, –, EF143013); *V. natans* (Lour.) Hara (–, –, EF143014); *V. neotropicalis* Marie-Vict. (–, –, EF143015); *V. rubra* (Rendle) Les and S.W.L.Jacobs (–, –, EF143004); *V. spinulosa* S.Z.Yan (–, –, EF143017); *V. spiralis* L. (–, –, U80712 [DQ859177/EF143018]); *V. triptera* S.W.L.Jacobs and K.A.Frank (–, –, EF143019).

D. Aponogetonaceae. *Aponogeton boivinianus* Baill. ex Jum., *van Bruggen s.n.* (CONN), (HQ456425*, HQ456475*, HQ456508*); *A. bruggenii* S.R.Yadav and R.S. Govekar, *Herr s.n.* (CONN), (HQ456426*, HQ456476*, –); *A. bullosus* H.Bruggen, *Jacobs 8572 and Les 595* (CONN, NSW), (AY926318, AY926279/AY926344, HQ456509*); *A. capuronii* H.Bruggen, *van Bruggen s.n.* (CONN), (HQ456427*, HQ456477*, HQ456510*); *A. crispus* Thunb. (AY926288, AY926263/AY926328, DQ859162); *A. cuneatus* S.W.L.Jacobs (AY926291, AY926264/AY926329, –); *A. decaryi* Jum., *Eggers s.n.* (CONN), (–, –, HQ456511*); *A. distachyos* L.f. (AY926320, AY926281/AY926346, U80684); *A. eggersii* Bogner and H.Bruggen, *Schöpfel s.n.* (CONN), (HQ456437*, HQ456483*, –); *A. elongatus* F.Muell. ex Benth. (AY926296, AY926266/AY926331, U68091 [U80683]); *A. euryspermus* Hellq. and S.W.L.Jacobs, *Jacobs 8839* (NSW), (AY926310, AY926275/AY926340, HQ456512*); *A. fenestralis* (Pers.) Hook.f., *Kasselmann s.n.* (CONN), (–, HQ456487*, AB088808); *A. hexatepalus* H.Bruggen, *Sainty 434337* (NSW), (AY926321, AY926282/AY926347, HQ456513*); *A. junceus* Lehm. ex Schltr., *Viljoen s.n.* (CONN), (HQ456441*, HQ456486*, HQ456514*); *A. kimberleyensis* Hellq. and S.W.L.Jacobs (AY926309, AY926274/AY926339, –); *A. lancesmithii* Hellq. and S.W.L.Jacobs, *Jacobs 8567 and Les 590* (CONN, NSW), (AY926316, AY926277/AY926342, HQ456515*); *A. longiplumulosus* H.Bruggen, *Jacobs 8534 and Les 560* (CONN, NSW), (AY926284,

AY926260/AY926325, HQ456516*); *A. madagascariensis* (Mirb.) H.Bruggen, *Jacobs 8536 and Les 562* (CONN, NSW), (AY926285, AY926261/AY926326, HQ456517*); *A. madagascariensis* var. *major* (Baum) H.Bruggen, *Kasselmann s.n.* (CONN), (HQ456444*, HQ456488*, –); *A. masoalaensis* Bogner, *Bogner 2087* (M), (HQ456445*, HQ456489*, –); *A. proliferus* Hellq. and S.W.L.Jacobs, *Jacobs 8523 and Les 549* (CONN, NSW), (AY926315, AY926276/AY926341, HQ456518*); *A. queenslandicus* H.Bruggen, *Jacobs 8524 and Les 550* (CONN, NSW), (AY926293, AY926265/AY926330, HQ456519*); *A. rigidifolius* H.Bruggen, *Jacobs 8529 and Les 555* (CONN, NSW), (AY926287, AY926262/AY926327, HQ456520*); *A. robinsonii* A.Camus, *Bogner 2905* (M), (AY926319, AY926280/AY926345, HQ456521*); *A. stachyosporus* de Wit, *Jacobs 8538 and Les 564* (CONN, NSW), (AY926304, AY926272/AY926337, HQ456522*); *A. tofus* S.W.L.Jacobs (AY926301, AY926270/AY926335, –); *A. ulvaceus* Baker, *Eggers s.n.* (M), (AY926283, AY926259/AY926324, HQ456523*); *A. undulatus* Roxb., *van Bruggen s.n.* (CONN), (HQ456451*, HQ456492*, HQ456524*); *A. vanbruggenii* Hellq. and S.W.L.Jacobs, *Jacobs 8542 and Les 568* (CONN, NSW), (AY926300, AY926269/AY926334, HQ456525*); *A. womersleyi* H.Bruggen, *Bleher s.n.* (M), (HQ456452*, HQ456493*, –).

E. Scheuchzeriaceae. *Scheuchzeria palustris* L. (–, –, U03728).

F. Juncaginaceae. *Cycnogeton procerum* Buchenau (AY926323, AY926349, U80713); *Tetroncium magellanicum* L. (AY926322, AY926348, GQ452337); *Triglochin barrelieri* Loisel. (–, –, GQ452331); *T. bulbosa* L. (–, –, AM234996); *T. elongata* Buchenau (–, –, GQ452332); *T. maritima* L., *Les s.n.* (CONN), (HQ456455*, HQ456495*, U80714 [AB088811/GQ452333]); *T. palustris* L. (–, –, DQ859176 [GQ452334]); *T. rheophila* Aston (–, –, GQ452335); *T. scilloides* (Poir.) Mering and Kadereit, *Philbrick 3031* (WCSU), (HQ456453*, HQ456494*, U80715); *T. striata* Ruiz and Pav. (–, –, GQ452336).

G. Cymodoceaceae 'complex'. Cymodoceaceae. *Amphibolis antarctica* (Labill.) Asch. (–, –, U80686); *Cymodocea nodosa* (Ucria) Asch. (–, –, U80688); *C. serrulata* (R.Br.) Asch. and Magnus (–, –, U80687); *Halodule beaudettei* (Hartog) Hartog (–, –, U80689); *H. pinifolia* (Miki) Hartog (–, –, U80690); *H. uninervis* (Forssk.) Asch. (–, AY952424, AY952436); *H. wrightii* Asch. (–, –, AY787476/AY787477); *Syringodium filiforme* Kütz (–, –, U03727); *S. isoetifolium* (Asch.) Dandy (–, –, U80691); *Thalassodendron pachyrhizum* Hartog (–, –, U80692). Posidoniaceae. *Posidonia australis* Hook.f. (–, –, U80718); *P. oceanica* (L.) Delile (–, –, U80719). Ruppiaceae. *Ruppia cirrhosa* (Pentagna) Grande (–, –, DQ859175); *R. maritima* L. (–, –, U03729); *R. megacarpa* R.Mason (–, –, U80728).

H. Maundiaceae. *Maundia triglochinoides* F.Muell., *Stanberg and Sainty LS 80* (NSW), (HQ456454*, HQ456496*, GQ452330).

I. Potamogetonaceae/Zosteraceae. Potamogetonaceae. *Groenlandia densa* (L.) Fourr., *Philbrick 4585* (WCSU), (–, HQ456497*, U80720 [AB196954]); *Lepilaena australis* J.Drum. ex Harv. (–, –, U80729); *Potamogeton alpinus* Balb.

(–, –, AB196845); *P. amplifolius* Tuck. (–, –, L08765); *P. berchtoldii* Fieber (–, GQ247475, –); *P. compressus* L. (–, –, AB196846); *P. confervoides* Rchb. (–, –, U80721); *P. crispus* L. (–, –, AB196847 [U80722]); *P. cristatus* Regel and Maack (–, –, AB196939); *P. dentatus* Hagstrem (–, –, AB196940 [AB332412]); *P. distinctus* A.Benn. (–, –, AB196941 [AB004901/AB088809]); *P. foliosus* Raf. (–, GQ247493, –); *P. fryeri* A.Benn. (–, –, AB196942); *P. gramineus* L. (–, –, AB196943 [U80723/EF174581]); *P. lucens* L. (–, –, DQ859173); *P. maackianus* A.Benn. (–, –, AB196944 [AB506769]); *P. malaianus* Miq. (–, –, AB196945 [EU741053/EU741054]); *P. natans* L. (–, –, AB196946 [DQ859174]); *P. obtusifolius* Mert. and W.D.J.Koch (–, –, AB196947); *P. octandrus* Poir. (–, –, AB196948); *P. oxyphyllus* Miq. (–, –, AB196949); *P. perfoliatus* L. (–, –, AB196951 [U80724/EU741051]); *P. praelongus* Wulfen (–, –, AB196952); *P. pusillus* L. (–, GQ247502, AB196950 [AB250148]); *P. richardsonii* (A.Benn.) Rydb. (–, –, U03730); *P. robbinsii* Oakes (–, –, U80725); *P. vaseyi* J.W.Robbins ex A.Gray (–, GQ247506, –); *P. zosteriformis* Fernald, *Les 494* (CONN), (GQ247438, HQ456498*, U80726); *Stuckenia pectinata* (L.) Börner (–, –, U80727 [AB196953]); *Zannichellia palustris* L. (–, –, U03725 [AB196955]). Zosteraceae. *Phyllospadix scouleri* Hook. (–, –, DQ859172); *P. torreyi* S.Watson (–, AY077975, U80731); *Zostera asiatica* Miki (–, AB125360, AB125352); *Z. caespitosa* Miki (–, AB125359, AB125351); *Z. capensis* Setch. (–, –, AM235166); *Z. capricorni* Asch. (–, AY077983, –); *Z. caulescens* Miki (–, AB125358, AB125350); *Z. japonica* Asch. and Graebn. (–, AB125361, AB125353 [AY077964]); *Z. marina* L. (–, AB125357, AB125348 [U80734/AB125349]); *Z. muelleri* Irmisch ex Asch. (–, AY077984, AY077962); *Z. noltii* Hornemann (–, AY077981, U80733); *Z. novazelandica* Setch. (–, AY077982, –); *Z. tasmanica* Martens ex Asch. (–, AY077979, U80730).

OUTGROUP. Araceae. *Anchomanes difformis* Engl. (–, –, L10254); *Ariopsis peltata* J.Graham (–, –, L10255); *Gymnostachys anceps* R.Br. (–, –, M91629); *Lasia spinosa* Thwaites (–, –, L10250); *Montrichardia arborescens* Schott (–, –, L10248); *Pistia stratiotes* L. (–, –, M96963); *Symplocarpus foetidus* (L.) Salisb. (–, –, L10247); *Xanthosoma sagittifolium* (L.) Schott (–, –, L10246). Lemnaceae. *Lemna minuta* Kunth (–, –, M91630)