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Systematics of Seagrasses (Zosteraceae) in Australia and New Zealand

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ABSTRACT. Previous taxonomic treatments of the family Zosteraceae in Australia / New Zealand have recognized *Heterozostera tasmanica* (monotypic) and four *Zostera* species all belonging to subgenus *Zosterella*: *Z. capricorni*, *Z. muelleri*, *Z. mucronata*, *Z. novazelandica*. *Zostera* has always been taxonomically problematic in Australia, where researchers have expressed difficulty with species recognition due to vague or inconsistent morphological characters. There also has been a lack of agreement on generic (notably the distinctness of *Heterozostera*) and subgeneric delimitation. Recent anatomical, developmental, and molecular studies urge a reevaluation of relationships in the family. To clarify the taxonomy of Zosteraceae, we investigated interspecific phylogenetic relationships focusing on Australian species of subgenus *Zosterella*. We examined material comprising all genera of Zosteraceae (*Heterozostera*, *Nanozostera*, *Phyllospadix*, *Zostera*), six / seven species of *Zostera* subgenus *Zosterella* (including all Australian / New Zealand species), and one of four species of *Zostera* subgenus *Zostera*. We conducted phylogenetic analyses of morphological data and DNA sequences from nuclear (ITS) and plastid (*trnK* intron, *rbcL*) genomes. Our results indicate two major clades (highly divergent at both morphological and molecular levels) and two subclades (with low morphological and molecular divergence) within Zosteraceae. Little morphological and molecular variation was observed among representatives within the clade of Australian / New Zealand members of subgenus *Zosterella*, and none provided cladistic support for taxa recognized formerly as separate species. We recommend that Zosteraceae comprise two genera (*Phyllospadix*, *Zostera*) with the latter subdivided into three subgenera (*Zostera*, *Zosterella*, *Heterozostera*). Furthermore, Australian / New Zealand representatives of *Zostera* subgenus *Zosterella* should be merged within a single species (*Z. capricorni*) to reflect the inability of morphological or molecular data to effectively delimit additional species in this group.

The marine, monocotyledonous Zosteraceae Dumort. are typically circumscribed to comprise three genera (*Heterozostera*, *Phyllospadix*, *Zostera*) with approximately 18 species (Phillips and Meñez 1988; Les et al. 1997). The family is distributed mainly in the temperate oceans of the northern and southern hemispheres, although the ranges of some species extend into tropical latitudes (Hartog 1970). Contemporary taxonomic treatments of Australian Zosteraceae have consistently recognized two genera: *Heterozostera* (Setch.) Hartog (monotypic as *Heterozostera tasmanica* (G.Martens ex Asch.) Hartog) and *Zostera* L., which includes *Zostera capricorni* Asch., *Z. mucronata* Hartog, and *Z. muelleri* Irmisch ex Asch. (Hartog 1970; Aston 1973; Robertson 1984; Phillips and Meñez 1988). Although the current taxonomy of Australian Zosteraceae is widely followed, the delimitation of taxa at both the generic and species levels is difficult and deserves further scrutiny. Robertson (1984) observed ''a broad spectrum of intergrades'' in Australian *Zostera* material and called for further work ''to elucidate the *Z. mucronata*-*Z. muelleri*-*Z. capricorni* complex.''

Setchell (1933) recognized *Zostera tasmanica* Asch. as distinct morphologically from other *Zostera* species and placed it within a monotypic section (*Heterozostera*). Hartog (1970) later elevated section *Heterozostera* to generic rank without elaboration, simply remarking that *Heterozostera* ''. . . can easily be confused with *Zostera* . . .'' but had a ''different way of branching'' and a distinctive rhizome vasculature. Incorporating these features into his key, Hartog distinguished *Heterozostera* as having a ligneous sympodial rhizome, 4–12 vascular bundles in the cortical layer, and erect, unbranched stems. In contrast, *Zostera* and *Phyllospadix* were described as having herbaceous monopodial rhizomes, two vascular bundles in the cortical layer, and a short, lateral branch at each node. Subsequent studies have questioned the taxonomic reliability of the primary key characters used by Hartog to distinguish *Zostera* and *Heterozostera* (Taylor 1981; Tomlinson 1982; Soros-Pottruff and Posluszny 1995). When developmental studies failed to disclose any significant differences between the genera, Soros-Pottruff and Posluszny (1995) agreed with Taylor (1981), who recommended the taxonomic reinstatement of *Zostera tasmanica* with retention of *Heterozostera* as a subgenus. The same conclusion was reached by Les et al. (1997) when a molecular phylogenetic study of subclass Alismatidae clearly placed *Heterozostera* within the genus *Zostera*. It is evident that the generic distinctness of *Heterozostera* requires further consideration.

The subgeneric divisions of *Zostera* are also in need of reevaluation. All Australian / New Zealand *Zostera* belong to subgenus *Zosterella* (Asch.) Ostenf., which Hartog (1970) distinguished by the presence of retinacules, open leaf sheaths and fiber bundles in the inner

layers of the rhizome outer cortex. Because further study of these features (Jacobs and Williams 1980; Tomlinson 1982) have questioned the validity of some key characters (see discussion), the taxonomic integrity of subgenera in *Zostera* remains unsettled. Furthermore, Tomlinson and Posluszny (2001) have recently recommended the elevation of subgenus *Zosterella* to generic status as *Nanozostera*. Clearly, the major taxonomic subdivisions within Zosteraceae must be reconsidered.

The field identification of *Zostera* species in Australia continues to be difficult using the characters described in taxonomic treatments (Sainty and Jacobs 1981). Taxonomic distinctions in *Zostera* have been attained primarily on the basis of leaf morphology (Hartog 1970), which Phillips (1972) has shown to be environmentally labile or differentiated ecotypically (Backman 1991), at least with respect to length and width characters. The extent of variation in *Zostera* is not adequately understood, as evidenced by recent discoveries of *Z. caulescens* having leaves an order of magnitude larger than previously reported for the species (Aioi et al. 1998). In particular, species recognition in *Zostera* relies strongly on leaf tip and retinacule morphology, which exhibit extensive variation within species (Phillips and Meñez 1988).

Furthermore, we have observed a number of instances where critical key characters are either inconsistent with descriptions or have been improperly applied in *Zostera* (see discussion). These observations lead us to propose that extensive variability in key features (such as leaf tip morphology, nerve numbers and retinacule morphology), have been inadequately considered in prior taxonomic keys and descriptions of Zosteraceae. We further suggest that historical taxonomic treatments of Zosteraceae have relied on morphological concepts that do not reflect accurately the variational patterns of characters used to distinguish species and perhaps higher taxonomic subdivisions in the family. Thus, reconsiderations of the status of *Heterozostera*, the circumscription of subgenus *Zosterella*, and species limits in *Zostera* are necessary before an acceptable taxonomy of these groups can be achieved.

In this study, we evaluate taxonomic questions in *Zostera* by supplementing morphological data with DNA sequence data to examine genetic variation in specimens of *Zostera* subgenus *Zosterella* collected widely in Australia. By incorporating molecular markers that are not subject to phenotypic plasticity, an independent test of species limits drawn from phenotypically labile morphological markers is provided. Uchiyama (1996) also studied relationships of *Zostera* using molecular data (RFLP analysis of 18S ribosomal DNA), but that study did not include Australian taxa. We have included all Zosteraceae taxa currently recognized in Australia and New Zealand and have incorporated molecular data from both nuclear and plastid compartments. By determining whether classical, morphologically defined *Zostera* species are supported by species limits indicated by molecular data, we hope to arrive at a taxonomic scheme for Australian Zosteraceae that is both objective and defensible.

Materials and Methods

Morphological Analyses. Morphological characters and states were compiled from pertinent publications including Aioi et al. (1998), Aston (1973), Flahault (1908), Hair et al. (1967), Harada (1956), Hartog (1970), Hartog et al. (1987), Jacobs and Williams (1980), Kuo and McComb (1998), Phillips and Meñez (1988), Robertson (1984), Setchell (1933), Soros-Pottruff and Posluszny (1994, 1995), Tomlinson (1982) and Yip (1988). We coded 16 vegetative and 15 reproductive characters for *Phyllospadix*, *Heterozostera tasmanica*, and 11 *Zostera* species (Tables 1, 2). Morphological data were analyzed using maximum parsimony (Swofford 1998) with all character states left unordered except for character #25, which was ordered to reflect a postulated reduction series in retinacule length. *Phyllospadix* was used as the outgroup for rooting trees. All searches were conducted using branch-and bound (furthest addition sequence, MulTrees). Bootstrap support was obtained from 500 replicates. Final trees were obtained using strict consensus and 50% majority rule consensus. The 50% majority rule consensus tree topology was used to examine character homoplasy and to provide estimates of branch lengths for identification of taxonomically significant characters. Missing data comprised 6% of morphological data cells and were treated as missing in all phylogenetic analyses.

Molecular Analyses. Specimens of *Zostera* and *Heterozostera* were collected by the authors from October, 1999–January, 2000. Field collected material was immediately processed in saturated NaCl-CTAB buffer as described by Rogstad (1992). Additional material (fresh or silica-gel dried) was provided by M. Waycott (James Cook University, Townsville, Queensland, Australia), Y. Kadono (Kobe University, Nada, Kobe, Japan), C. T. Philbrick (Western Connecticut State University, Danbury, Connecticut), G. Procaccini (Stazione Zoologica 'A. Dohrn', Naples, Italy), P. B. Tomlinson (Harvard University, Petersham, Massachusetts) and Anne-Maree Schwarz (NIWA, New Zealand). Our material comprised all genera of Zosteraceae (*Heterozostera*, *Nanozostera*, *Phyllospadix*, *Zostera*), six/seven species of *Zostera* subgenus *Zosterella* (including all Australian species), and one/four species of *Zostera* subgenus *Zostera* (Table 3). Thus, all taxonomic levels of the family were represented and all Australian taxa were included. We also examined six accessions of *Heterozostera tasmanica*, four accessions of *Zostera capricorni*, three accessions of *Z. novazelandica*, and 21 accessions of *Z. muelleri* (throughout its range in Australia) to evaluate geographical divergence.

Routine procedures for the extraction, amplification, and sequencing of ITS (ITS-1 and ITS-2 regions including the 5.8S rDNA gene), *trnK* 5′ and 3′ introns and *rbcL* followed those described in Padgett et al. (1999) and Les et al. (2002). All sequences were obtained using automated methods as described by Les et al. (2002). Due to low levels of *rbcL* nucleotide divergence, only one exemplar for each species was compared in the analysis of *rbcL* sequence data. Technical difficulties (degraded DNA, failed PCR reactions), prevented us from obtaining *trnK* intron sequences for three accessions (*Waycott 94018*; *Les 639*; *Les 641*). We did not sequence *trnK* in two accessions (*Waycott s.n.-2*; *Waycott s.n.-3*), which represented populational samples of *Waycott s.n.-1*. Sequences used in our analyses have been deposited in the GenBank database (Table 1).

Molecular data were analyzed using the PAUP* computer program (Swofford 1998). Ranges in pairwise sequence divergence values (uncorrected 'p' values) were compared among nine (ITS) and eight (*trnK*) Zosteraceae taxa. Gaps were treated as missing data in all analyses, but indel information for ITS and *trnK* was included as separate data partitions. For each gap, different indel

TABLE 1. Voucher specimens of Zosteraceae examined in molecular analyses. [a] Latitude and longitude are provided for Australian and New Zealand collections; [b] identical sequences within a taxon are referenced by the same accession number; [c] three accessions only; n.d. = no data.

Taxon	Geographical origin	[a] Latitude	longitude	Voucher; Genbank accession numbers[b] [ITS, 3′*trnK*, 5′*trnK*, *rbcL*[c]]
I) *Phyllospadix* Hook.				
1. *P. torreyi* S. Watson	California, USA	—	—	*Philbrick 2274* (WCSU); [AY077985, AY077965, AY077975]
II) *Heterozostera* (Setch.) Hartog				
1. a. *H. tasmanica* (G.Martens ex Asch.) Hartog	New South Wales, Australia	35°46′S	150°13′E	*Les 535* (CONN); [AY077987, AY077969, AY077979]
b. *H. tasmanica*	Western Australia	31°50′S	115°42′E	*Waycott 94007* (UWA); [AY077990, AY077967, AY077978]
c. *H. tasmanica*	Western Australia	31°60′S	115°45′E	*Waycott s.n.* (CONN); [AY077988, AY077968, AY077978]
d. *H. tasmanica*	Western Australia	34°57′S	115°42°E	*Waycott s.n.* (CONN); [AY077989, n.d., n.d.]
e. *H. tasmanica*	Western Australia	34°57′S	115°42′E	*Waycott s.n.* (CONN); [AY077989, n.d., n.d.]
f. *H. tasmanica*	Western Australia	34°57′S	115°42′E	*Waycott s.n.* (CONN); [AY077989, AY077968, AY077978]
III) *Zostera* L.				
1. a. *Z. capicorni* Asch.	Queensland, Australia	16°54′S	145°45′E	*Les 605 & Jacobs 8582* (CONN); [AY077995, AY077973, AY077983]
b. *Z. capricorni*	New South Wales, Australia	30°29′S	153°00′E	*Les 624 & Jacobs 8627* (CONN); [AY077996, AY077973, AY077983]
c. *Z. capricorni*	New South Wales, Australia	30°38′S	152°59′E	*Les 625 & Jacobs 8628* (CONN); [AY077996, AY077973, AY077983, AY077963]
d. *Z. capricorni*	New South Wales, Australia	32°40′S	152°10′E	*Les 626 & Jacobs 8629* (CONN); [AY077996, AY077973, AY077983]
2. *Z. japonica* Asch. & Graebn.	Hokkaido, Japan	—	—	*Iida s.n.* (CONN); [AY077991, AY077970, AY077980, AY077964]
3. *Z. marina* L.	Connecticut, USA	—	—	*Yarish s.n.* (CONN); [AY077986, AY077966, AY077976]
4. *Z. mucronata* Hartog	Western Australia	32°03′S	115°45′E	*Waycott 94018* (UWA); [AY077993, n.d., n.d.]
5. a. *Z. muelleri* Irmisch ex Asch.	New South Wales, Australia	34°35′S	150°54′E	*Les 528* (CONN); [AY077998, AY077974, AY077984]
b. *Z. muelleri*	New South Wales, Australia	34°40′S	150°51′E	*Les 529* (CONN); [AY077998, AY077974, AY077984, AY077962]
c. *Z. muelleri*	South Australia	34°40′S	138°26′E	*Bayer SA-99006 & Chandler* (CONN); [AY077997, AY077974, AY077984]
d. *Z. muelleri*	New South Wales, Australia	34°45′S	150°50′E	*Les 530* (CONN); [AY077998, AY077974, AY077984]
e. *Z. muelleri*	New South Wales, Australia	34°46′S	150°49′E	*Les 531* (CONN); [AY077998, AY077974, AY077984]
f. *Z. muelleri*	New South Wales, Australia	34°50′S	150°44′E	*Les 532* (CONN); [AY077998, AY077974, AY077984]
g. *Z. muelleri*	New South Wales, Australia	35°42′S	150°11′E	*Les 536* (CONN); [AY077998, AY077974, AY077984]
h. *Z. muelleri*	New South Wales, Australia	35°43′S	150°12′E	*Les 533* (CONN); [AY077998, AY077974, AY077984]
i. *Z. muelleri*	Victoria, Australia	38°36′S	146°55′E	*Les 634* (CONN); [AY077999, AY077974, AY077984]
j. *Z. muelleri*	Victoria, Australia	38°38′S	146°47′E	*Les 635* (CONN); [AY077999, AY077974, AY077984]
k. *Z. muelleri*	Victoria, Australia	38°38′S	146°39′E	*Les 636* (CONN); [AY077999, AY077974, AY077984]
l. *Z. muelleri*	Victoria, Australia	38°38′S	145°47′E	*Les 639* (CONN); [AY078002, AY077974, AY077984]
m. *Z. muelleri*	Victoria, Australia	38°41′S	146°14′E	*Les 638* (CONN); [AY078000, AY077974, AY077984]
n. *Z. muelleri*	Victoria, Australia	38°42′S	146°27′E	*Les 637* (CONN); [AY078001, AY077974, AY077984]
o. *Z. muelleri*	Tasmania, Australia	40°48′S	145°14′E	*Les 641* (CONN); [AY078003, AY077974, AY077984]
p. *Z. muelleri*	Tasmania, Australia	40°49′S	145°14′E	*Les 642* (CONN); [AY078003, AY077974, AY077984]
q. *Z. muelleri*	Tasmania, Australia	41°01′S	146°48′E	*Les s.n.* (CONN); [AY078003, AY077974, AY077984]
r. *Z. muelleri*	Tasmania, Australia	42°20′S	147°57′E	*Les 644* (CONN); [AY078004, AY077974, AY077984]
s. *Z. muelleri*	Tasmania, Australia	42°20′S	147°57′E	*Les 645* (CONN); [AY078005, AY077974, AY077984]
6. *Z. noltii* Hornem.	Naples, Italy	—	—	*Procaccini s.n.* (CONN); [AY077992, AY077971, AY077981]
7. a. *Z. novazelandica* Setch.	New Zealand	36°00′S	174°10′E	*Tomlinson s.n.* (CONN); [AY077994, AY077972, AY077982]
b. *Z. novazelandica*	New Zealand	37°07′S	175°53′E	*Schwarz s.n.* (CONN); [AY077994, AY077972, AY077982]
c. *Z. novazelandica*	New Zealand	37°07′S	175°53′E	*Schwarz s.n.* (CONN); [AY077994, AY077972, AY077982]

TABLE 2. Morphological characters and character states used in a cladistic analysis of the Zosteraceae (see text for references). [a] This distinction made by Hartog (1970); Tomlinson (1982) and Tillich (1995) report absence of primary root for all Zosteraceae.

Character	Character states
Vegetative morphology:	
1 Primary root[a]:	0 = present; 1 = absent
2 Root morphology:	0 = long, thin; 1 = short, thick
3 Rhizome growth form:	0 = linear; 1 = undulating
4 Rhizome internodes:	0 = elongated (>2 mm); 1 = congested (<2 mm)
5 Rhizome cortical cells:	0 = thick-walled; 1 = thin-walled
6 Rhizome cortical fiber bundles:	0 = present; 1 = absent
7 Rhizome cortical vascular bundles (maximum):	0 = >4; 1 = 4
8 Squamule # (maximum):	0 = 3–4; 1 = 2
9 Leaf sheath:	0 = open; 1 = fused
10 Leaf sheath persistence:	0 = decays into wooly fibers; 1 = deciduous; 2 = persistent
11 Leaf sheath margins (at maturity):	0 = overlap throughout; 1 = overlap only at base
12 Max. leaf length (cm):	0 = > 150; 1 = > 50 < 150; 2 = < 50
13 Leaf margin ''fin cells'':	0 = present; 1 = absent
14 Leaf blade texture:	0 = coriaceous; 1 = translucent
15 Max. # leaf blade nerves:	0 = > 7; 1 = 7
16 Mature leaf tip:	0 = notched; 1 = notched or truncate; 2 = truncate; 3 = mucronate
Reproductive morphology:	
17 Sexual condition:	0 = monoecious; 1 = dioecious
18 Reproductive shoot position:	0 = lateral; 1 = terminal
19 Peduncle:	0 = free; 1 = partly coalesced to axis
20 Spadix:	0 = emerging from spathal sheath; 1 = enclosed within spathal sheath
21 Retinacules:	0 = present at all male flowers; 1 = absent or only at lowest male flower
22 Retinacule texture:	0 = coriaceous; 1 = membranous
23 Retinacule nervature:	0 = 1-nerved; 1 = nerveless
24 Retinacule vasculature:	0 = present; 1 = absent
25 Retinacule length (maximum):	0 = long (14 mm); 1 = medium (3 mm); 2 = short (1¾ mm) [ordered]
26 Seed surface:	0 = ribbed (costate) longitudinally; 1 = ridged; 2 = striate; 3 = smooth
27 Fruit morphology:	0 = ovoid/ellipsoidal; 1 = crescent-shaped
28 Exocarp:	0 = scarious; 1 = soft, ephemeral
29 Fruit ''grappling apparatus'':	0 = absent; 1 = present
30 Chromosome number ($2n$ = ?):	0 = 12; 1 = 16–20; 2 = 24; 3 = 36
31 Mature pollen length (μm):	0 = 1500; 1 = 500

TABLE 3. Morphological data matrix for Zosteraceae taxa (characters and states from Table 2). ? = state unknown; — = state not applicable. [a] Three characters homoplasious in maximum parsimony analysis; CI = 1.0 for the remainder.

	Characters																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16[a]	17	18[a]	19	20	21	22	23	24	25	26[a]	27	28	29	30	31
Phyllospadix	1	1	0	1	0	1	1	?	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	2	1	1	1	0
Heterozostera	?	0	1	0	1	0	0	1	0	1	0	2	1	1	1	0	0	1	1	1	0	1	1	1	1	2	0	0	0	3	?
Zostera subg. *Zostera*																															
Z. marina	1	0	0	0	1	0	1	0	1	1	—	1	1	1	0	2	0	1	1	1	1	—	—	—	—	0	0	0	0	0	1
Z. caulescens	1	0	0	0	1	0	1	1	1	1	—	0	1	1	0	2	0	1	1	1	1	—	—	—	—	2	0	0	0	0	1
Z. asiatica	1	0	0	0	1	0	1	1	1	1	—	1	1	1	0	1	0	1	1	1	1	—	—	—	—	3	0	0	0	0	1
Z. caespitosa	1	0	0	0	1	0	1	1	1	2	—	1	1	1	0	1	0	1	1	1	1	—	—	—	—	0	0	0	0	0	1
Zostera subg. *Zosterella*																															
Z. capensis	0	0	0	0	1	0	1	1	0	1	0	2	1	1	1	0	0	0	1	1	0	1	1	0	2	1	0	0	0	?	1
Z. capricorni	0	0	0	0	1	0	1	1	0	1	1	2	1	1	1	2	0	0	1	1	0	1	1	0	2	2	0	0	0	2	1
Z. japonica	0	0	0	0	1	0	1	1	0	1	0	2	1	1	1	0	0	0	1	1	0	1	1	0	2	2	0	0	0	0	1
Z. mucronata	0	0	0	0	1	0	1	?	0	1	1	2	1	1	1	3	0	0	1	1	0	1	1	0	2	1	0	0	0	?	1
Z. muelleri	0	0	0	0	1	0	1	1	0	1	1	2	1	1	1	0	0	0	1	1	0	1	1	0	2	1	0	0	0	2	1
Z. noltii	0	0	0	0	1	0	1	1	0	1	0	2	1	1	1	0	0	0	1	1	0	1	1	0	2	2	0	0	0	0	1
Z. novazelandica	0	0	0	0	1	0	1	1	0	1	1	2	1	1	1	1	0	0	1	1	0	1	1	0	2	1	0	0	0	2	1

variants were scored as discrete character states in the partitions. Indel data were analyzed separately for ITS, but not *trnK* sequences which had only three gaps in our alignment.

Molecular data were analyzed initially as six separate partitions: 1) ITS excluding gaps; 2) ITS gap data alone; 3) ITS including gap data; 4) *trnK* including gap data; 5) *rbcL* (no gaps present); 6) all molecular data combined (except *rbcL*). Analysis of the *rbcL* data was provided only for comparative purposes and these data were excluded from the combined molecular analyses. Trees for *rbcL* were computed using the branch and bound algorithm. Trees for ITS, *trnK* and combined analyses were obtained using heuristic searches (simple addition sequence, MulTrees, TBR). Because *trnK* data (sequence data and gaps) were invariant in all Australian / New Zealand accessions of *Zostera* subgenus *Zosterella* taxa surveyed, there were thousands of trees retained resulting in excessive analytical times. We resolved this problem by using only one exemplar sequence from the invariant group (*Les 528*) in the *trnK* analysis. In drawing the tree, the excluded taxa with identical sequences were added by depicting them in an unresolved ''comb'' along with the exemplar sequence. All sequences were used in the combined molecular analyses. Missing data comprised less than 1% of data cells used in all analyses except for the combined analysis where they comprised 8% due to the inclusion of five accessions missing the *trnK* intron region.

Strict consensus trees were output as tree files for tree description purposes. Consensus trees are shown with relative branch lengths as indicated for the described trees. Bootstrap support for clades was generated using 500 replicates and search parameters as described above. Due to excessive analysis times, bootstrap values for the combined molecular data tree were obtaining without implementing the MulTrees option during the heuristic search.

RESULTS

Morphological Analyses. Analyses using maximum parsimony recovered 68 trees at 45 steps with CI = 0.889, $CI_{(exc)}$ = 0.792, and RI = 0.857, indicating relatively low homoplasy. The strict consensus tree (Fig. 1a) was minimally resolved, but the recovered clades corresponded well to taxonomic subdivisions traditionally and recently applied to the genus. The 50% majority rule consensus tree (Fig. 1b) was better resolved but still showed relatively weak separation of species. Character analysis on this tree (using either ACCTRAN or DELTRAN) indicated only three homoplasious characters (#16, leaf tip morphology; #18, reproductive shoot position, and #26, seed surface morphology).

The genus *Phyllospadix* was clearly differentiated from all other Zosteraceae by 19 morphological character states (61.3% of total). *Zostera tasmanica* (=''*Heterozostera*'') associated with species of *Zostera* subgenus *Zosterella* in 75% of the equally parsimonious trees recovered. The majority rule consensus tree resolved *Z. noltii* and *Z. japonica* as a distinct group within subgenus *Zosterella*, but bootstrap support was weak for most species associations. Clades corresponding to previous taxonomic subdivisions were well supported morphologically, e.g. subgenus *Zosterella* (71% bootstrap support) and subgenus *Zostera* (96% bootstrap support). The former (= ''*Nanozostera*'') is supported by two characters (#18, reproductive shoot position; #25, max. retinacule length) and the latter (= ''*Zostera*'' sensu stricto) by three characters (#9, leaf sheath mor-

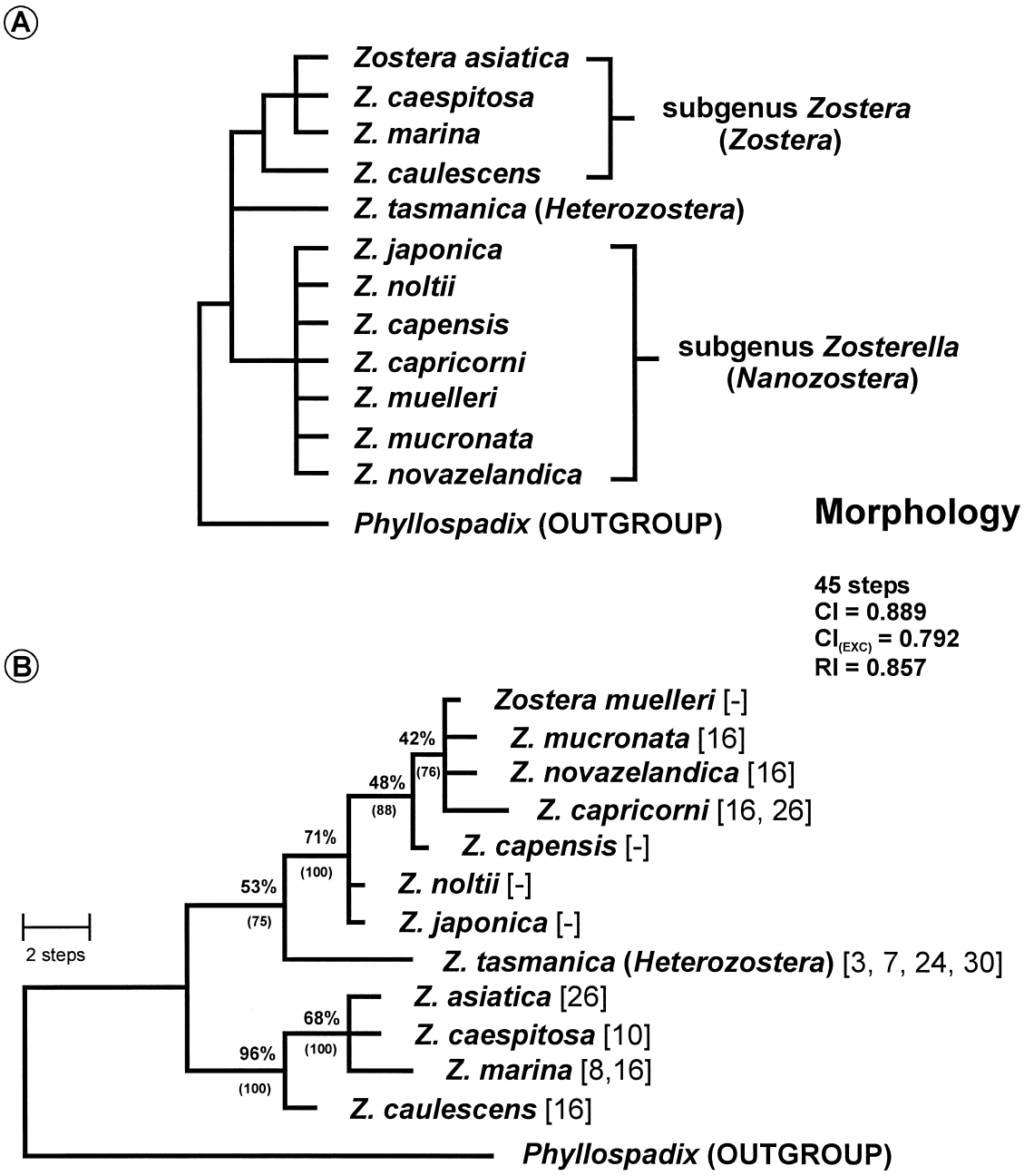

FIG. 1. Morphologically derived maximum parsimony cladograms for Zosteraceae. A) Strict consensus tree (branch lengths not proportional) showing poor resolution of most taxa. Previously proposed generic segregates are indicated in parentheses. B) Resolution of taxa as indicated by the 50% majority-rule consensus tree (converted into a phylogram showing proportional branch lengths). Bootstrap support (%) is indicated above branches; frequency of occurrence (%) in the majority rule tree is indicated in parentheses below branches. Numbers in square brackets indicate characters (apomorphies) from Tables 1–2 (- = no characters) that define the terminal taxa on the tree.

phology; #15, max. number of leaf blade nerves, #21, retinacule occurrence). *Zostera tasmanica* was well-defined morphologically by four, non-homoplasious characters (#3, rhizome growth form; #7, rhizome cortical vascular bundle number; #24, retinacule vasculature; and #30, chromosome number).

Four species (*Z. capensis*, *Z. japonica*, *Z. muelleri*, *Z. noltii*) lacked defining character states (autapomorphies) entirely. Three species (*Z. caulescens*, *Z. mucronata*, *Z. novazelandica*) were differentiated only by states of character #16 (leaf tip morphology), which was also one of two defining characters for *Z. capricorni* and *Z.*

TABLE 4. Observed range in percent (%) nucleotide divergence (*trnK*—upper half; ITS—lower half) derived from uncorrected distance ('p') for pairwise comparisons of nine Zosteraceae taxa recognized in previous taxonomic treatments. CAP = *Z. capricorni*; JAP = *Z. japonica*; MAR = *Z. marina*; MUC = *Z. mucronata*; MUE = *Z. muelleri*; NOL = *Z. noltii*; NOV = *Z. novazelandica*; PHY = *Phyllospadix*; TAS = *Z. tasmanica*. N.D. = no data.

	NOV	MUE	MUC	CAP	NOL	JAP	TAS	MAR	PHY
NOV	—	0.0	N.D.	0.0	0.6	0.3	0.6–1.0	5.9	6.9
MUE	0.0–0.5	—	N.D.	0.0	0.6	0.3	0.6–1.0	5.9	6.6–6.9
MUC	0.5	0.5–1.1	—	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CAP	0.2–0.3	0.0–1.0	0.8–1.0	—	0.6	0.3	0.6–1.0	5.9	6.6–6.9
NOL	2.2	2.0–2.7	2.9	2.2–2.5	—	0.2	1.1–1.4	6.0	7.3
JAP	2.2	2.0–2.7	2.9	2.2–2.5	1.7	—	0.9–1.2	6.2	7.3
TAS	5.4–5.8	5.5–6.3	5.9–6.3	5.4–6.3	5.7–6.0	5.8–6.1	—	6.0–6.2	6.7–8.0
MAR	16.1	16.1–16.6	16.8	16.3–16.6	16.3	16.3	16.0–16.4	—	7.4
PHY	26.0	25.8–26.5	26.7	26.0–26.3	25.2	25.7	21.8–23.9	25.6	—

marina. Single characters defined *Z. asiatica* (#26, seed surface morphology) and *Z. caespitosa* (#10, leaf sheath). Of these 11 species, only two non-homoplasious characters (#10, leaf sheath; #8, squamule number) served as defining synapomorphies (for *Z. caespitosa* and *Z. marina* respectively).

Molecular Analyses. ITS DATA. Of the 640 ITS/5.8S sequence characters included in the analysis, 417 (65.2%) were invariant; of the 223 variable characters, 66 (29.6%) were parsimony informative. Pairwise comparisons of sequence divergence ('p' distance) values ranged from 0–1.1% (among all Australian/New Zealand subgenus *Zosterella* taxa) to 25.6% (*Phyllospadix* vs. *Zostera*) (Table 4). Maximum parsimony analysis of the ITS sequence data (excluding gaps) yielded 2,300 trees of 265 steps (CI = 0.97; $CI_{(exc)}$ = 0.90; RI = 0.97). Gaps in ITS provided 29 characters ranging from 2–5 states. Parsimony analysis of the gap data alone resulted in a single tree (41 steps), which was devoid of homoplasy (CI = 1.00; $CI_{(exc)}$ = 1.00; RI = 1.00; Fig. 2a). Gaps were identical in all 25 accessions of Australian/New Zealand *Zostera* (excluding *Z. tasmanica*). Combined ITS sequence/gap data generated 669 characters. Of these, 417 (62.3%) were invariant; of the 252 variable characters, 79 (31.4%) were parsimony informative. Maximum parsimony analysis of the ITS sequence/gap data yielded 2,300 trees at 306 steps (CI = 0.97; $CI_{(exc)}$ = 0.92; RI = 0.97; Fig. 2b).

Because of polyploidy in the Australian Zosteraceae, we carefully examined the nuclear ITS sequences for polymorphic sites. Polymorphisms were detected only at six/223 (2.7%) of the variable sites. In most cases, they occurred between populations of the same presumptive species; however, some were shared between presumptive species (between *Z. muelleri* and both *Z. capricorni* and *Z. novazelandica*). Unique polymorphisms were observed once in *Z. mucronata* (*Waycott 94–018*) and once in *Z. muelleri* (*Bayer SA-99006 & Chandler*) (see discussion).

RBC*L* DATA. The *rbcL* data provided 1,182 characters of which 1,142 (96.6%) were constant; of the 40 variable characters, 8 (20.0%) were informative phylogenetically. Parsimony analysis produced a single tree of 41 steps with CI = 0.98; $CI_{(exc)}$ = 0.89 (excluding autapomorphies); RI = 0.89 (Fig. 3). Bootstrap support ranged from 63–96% for the three internal nodes resolved. The *rbcL* topology was congruent with those obtained in all other analyses.

TRN*K* INTRON DATA. Sequence and indel data from the two introns of the plastid *trnK* gene generated 929 characters of which 839 (90.3%) were constant; of the 90 variable characters, 27 (30%) were informative. Parsimony analysis generated a single tree of 103 steps with CI = 0.97; $CI_{(exc)}$ = 0.91 (excluding autapomorphies); RI = 0.93 (Fig. 4a). Bootstrap support ranged from 63–100% for nodes resolved in the analysis. The topology of this tree was congruent with those generated from morphological, *rbcL* and ITS data (Figs. 1–3).

COMBINED ITS/TRN*K* INTRON DATA. The combined molecular data set comprised 1,598 characters where 1,256 (78.6%) were constant; of the 342 variable characters, 106 (31.0%) were parsimony informative. Maximum parsimony analysis of these data generated 2,301 equally minimal length trees of 410 steps (CI = 0.97; $CI_{(exc)}$ = 0.91; RI = 0.97; Fig. 4b). Bootstrap support for nodes ranged from 61–100% (Fig. 4).

Although cladograms generated from all of the different data sets differed by their relative degree of resolution, there was only one minor conflict between ITS and *trnK* data whereby the positions of *Z. tasmanica* accessions from New South Wales and Western Australia were exchanged (Figs. 2b, 4a). We concluded that this high degree of congruence adequately warranted the combination of ITS and *trnK* data. We did not combine the highly conservative *rbcL* data which was sequenced only in exemplar accessions of each taxon where it was invariant in *Z. capricorni*, *Z. mucronata*, *Z. muelleri*, and *Z. novazelandica*. Also, we did not combine the morphological data, which was compiled from descriptions of taxa rather than from the same specimens used in the molecular analyses (which were mainly

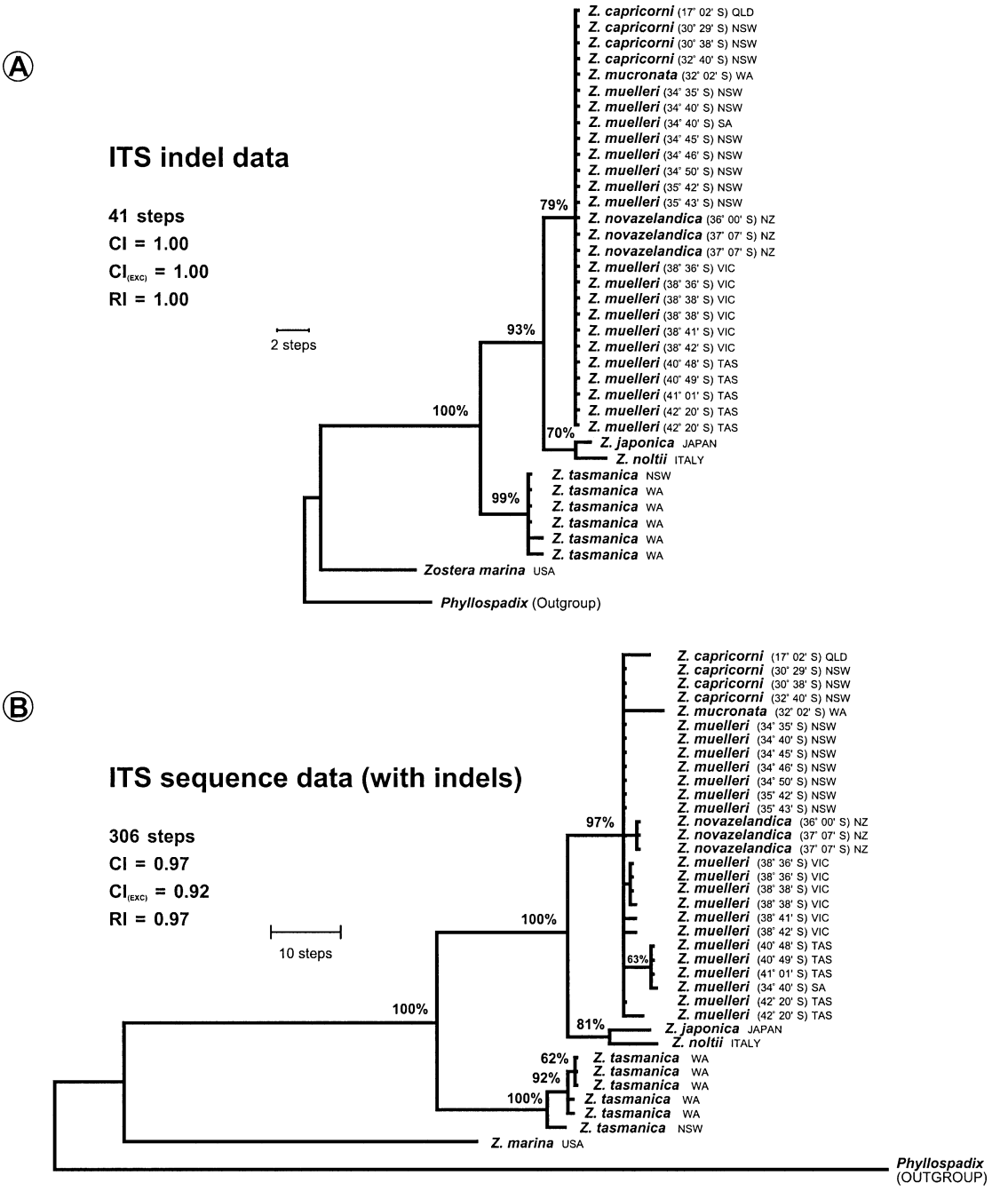

FIG. 2. Maximum parsimony cladograms for Zosteraceae derived from ITS/5.8S DNA sequence data. Latitudes for terminal clade taxa provided for comparative purposes. Australian states of origin are abbreviated as: NSW = New South Wales, QLD = Queensland, SA = South Australia, TAS = Tasmania, VIC = Victoria, WA = Western Australia. NZ = New Zealand. USA = United States. A) Single most-parsimonious phylogram (branch lengths proportional) resulting from phylogenetic analysis of indel data (sequence data excluded) with scale provided. Bootstrap support (%) for nodes is shown above branches. B) Strict consensus tree (converted into phylogram; relative branch lengths indicated by scale) based upon analysis of ITS/5.8S DNA sequence data (indel data included) showing similar resolution. Bootstrap support (%) for nodes is shown above branches.

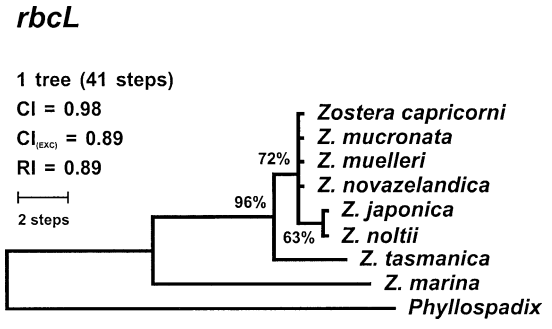

FIG. 3. Single most-parsimonious phylogram (relative branch lengths indicated by scale) resulting from phylogenetic analysis of *rbcL* data of nine Zosteraceae taxa. Bootstrap support (%) is shown at nodes.

vegetative and lacked many of the characters considered). Nevertheless, cladograms from both the morphology (Fig. 1) and *rbcL* (Fig. 3) data were entirely compatible with those of the other data sets.

DISCUSSION

For over 30 years, Hartog (1970) has been the primary source for seagrass identification and many authors have faithfully adopted his taxonomy, keys and descriptions, usually with few modifications. However, Hartog's (1970) taxonomic treatment of Zosteraceae (as Potamogetonaceae subfamily *Zosteroideae*) contains numerous ambiguities and discrepancies in relevant keys and descriptions, which have made his taxonomic scheme difficult to apply. Here we wish to thoroughly reconsider that treatment with respect to the taxonomy of Zosteraceae, particularly as it bears upon the delimitation of Australian taxa.

Heterozostera, Nanozostera or Zostera? The generic distinction of *Zostera* and *Heterozostera* has become increasingly unsettled due to uncertainty in the reliability of key taxonomic characters. Taxonomists have found it difficult to separate the morphologically similar *Zostera* and *Heterozostera* (Aston 1973; Jacobs and Williams 1980). Aston (1973) and Phillips and Meñez (1988) followed Hartog (1970) who distinguished between monopodial (former) vs. sympodial (latter) rhizomes to separate the genera. However, Tomlinson (1982) and Soros-Pottruff and Posluszny (1995) showed that this often cited feature (sympodial, unbranched rhizome) is erroneous and should not be used to distinguish the genera. Tomlinson (1982) rejected this feature taxonomically, observing that rhizomes in *Heterozostera* can appear either sympodial or monopodial. Through decisive developmental studies, Soros-Pottruff and Posluszny (1995) demonstrated that the *Heterozostera* rhizome is clearly monopodial (as in all other Zosteraceae), but possesses an undulating growth pattern that mimics sympodial growth. Robertson (1984) followed Tomlinson's (1982) recommendations and considered both *Heterozostera* and *Zostera* as having monopodial, herbaceous rhizomes. Instead, she relied on differences in cortical vascular bundle number (employed as the secondary key character by Hartog) and retinacule shape to separate the genera. However, Yip (1988) showed overlap in the number of cortical bundles in *Zostera* (2–4) and *Heterozostera* (2–12). From our experience, the number of cortical bundles recorded depends on where the section of the internode is taken; apparently, leaf traces separate sooner after the node in *Heterozostera* than in *Zostera*. The above studies effectively nullify the original key morphological differences used by Hartog (1970) to distinguish *Zostera* and *Heterozostera*, therefore the generic distinctness of *Heterozostera*, at least as circumscribed by Hartog, cannot be accepted.

Although Soros-Pottruff and Posluszny (1995) settled the question of rhizome construction in *Heterozostera* and *Zostera* (both monopodial), their clarification provided a new distinction between the taxa, i.e., an undulating growth pattern which, in the family, is apparently unique to *Heterozostera*. Soros-Pottruff and Posluszny (1995) also included the presence of wiry, erect stems, a tendency toward increased cortical vascular bundles, and lack of vascularization in retinacules as additional features that separate this taxon from other *Zostera* species. Three of these characters (#3, 7, 24, Table 2) emerged as defining apomorphies in our formal cladistic analysis (Fig. 1b). Retinacule morphology, which is lanceolate in *Heterozostera* and triangular/suborbicular in *Zostera* (Roberts 1984) can be added as another character. According to Hartog (1970), retinacules are elongate ($2^1/_2$-14 mm) in *Phyllospadix*, moderately long (2,3 mm) in *Heterozostera* and either short ($^1/_2$-$1^3/_4$ mm) or absent in *Zostera*. Differences in retinacule length appear to be more approximate than exact. Retinacule length in ''*Zostera americana*'' (= *Z. japonica*) is given as $^3/_4$-$1^3/_4$ mm in the Latin diagnosis, but as $^3/_4$-$1^1/_4$ mm in the accompanying English description (Hartog 1970). Retinacule length in *Z. capricorni* (1–$1^1/_3$ mm) is somewhat shorter than in ''*Z. novazelandica*'' (1–$1^3/_4$ mm; Hartog 1970) which is usually regarded as synonymous with *Z. capricorni* (Phillips and Meñez 1988). Regardless, the longer ($\geq$ 2 mm) retinacules of *Heterozostera* separate it from *Zostera* ($<1^3/_4$ mm) without overlap.

Although some distinctions between *Heterozostera* and *Zostera* are flawed, a modified character set (e.g., above), can effectively separate these taxa taxonomically. Furthermore, *Heterozostera* is hexaploid (Kuo and McComb 1998; Kuo 2001), a ploidy level unique in the family. Thus, the principal issue is not whether *Heterozostera* is distinct taxonomically, but rather which taxonomic rank is most appropriate given the observed differences. Purely in a taxonomic sense, the question of distinctness must consider whether undulating rhi-

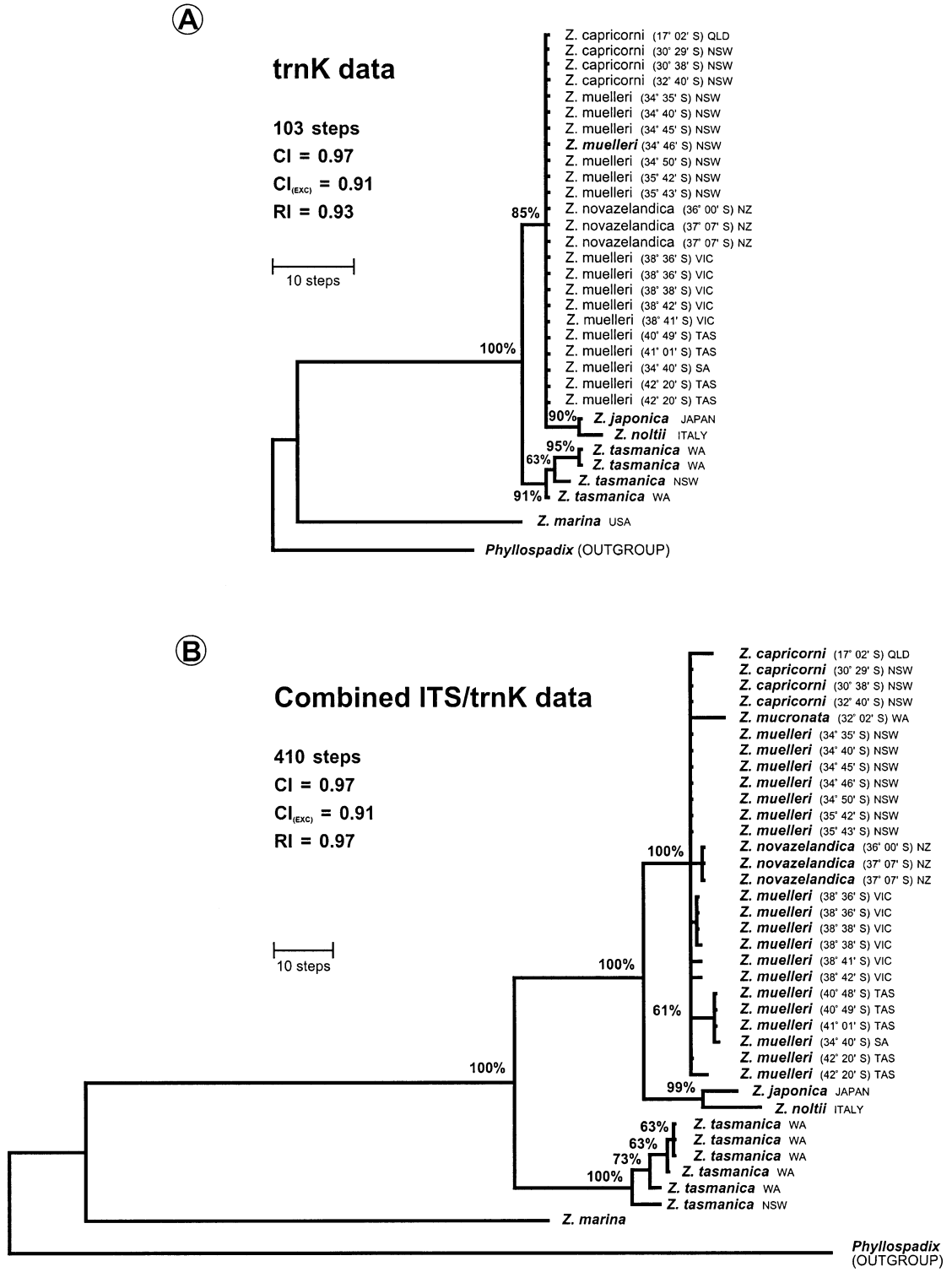

FIG. 4. Maximum parsimony cladograms (strict consensus trees converted into phylograms; relative branch lengths indicated by scales) for Zosteraceae derived from *trnK* and combined ITS/*trnK* data. Bootstrap support (%) for nodes is shown above branches. Abbreviations are as in Fig. 2. A) Cladogram based on analysis of *trnK* data. B) Cladogram based on analysis of combined ITS/*trnK* data.

zomes, additional vascular bundles, and long, unvascularized retinacules are ''adequate'' to separate *Heterozostera* and *Zostera* at the generic level. Obviously, no one answer can be defended unequivocally, as any interpretation is subject to individual opinion.

Admittedly, the circumscription of ranks (genera, sections, species) is always somewhat subjective; however, greater objectivity can be achieved by adopting phylogenetic criteria (i.e., taxa should represent monophyletic groups; Judd et al. 1999). Arguments concerning the type of characters, or number of characters necessary to delimit a particular rank (e.g., genus) are less compelling than those that either demonstrate or refute the monophyly of a taxon. Although several morphological characters once used to distinguish *Heterozostera* are inappropriate, there remain unique, defining features that can delimit the taxon at some rank. A more critical question is whether recognition of *Heterozostera* at generic rank is defensible phylogenetically.

Alone, morphological data cannot effectively answer this question because of low resolution. The unresolved position of *Heterozostera* in the strict consensus tree (Fig. 1a) is compatible with interpretations that either combine it with *Zostera*, or retain it as a separate genus. If the topology of the majority rule consensus tree (Fig. 1b) is used as a guideline, then *Heterozostera* must either be combined with *Zostera*, or four separate genera of Zosteraceae must be recognized (see Fig. 1a) to avoid paraphyletic taxa.

The latter approach (Tomlinson and Posluszny 2001) seems unnecessarily excessive. Tomlinson and Posluszny (2001) proposed a new genus ''*Nanozostera*'' to accommodate species in *Zostera* subgenus *Zosterella*. They provided no new data, but essentially echoed results of Soros-Pottruff and Posluszny (1995) as the basis of their generic segregation. Because neither study performed a phylogenetic analysis, the conclusions were reached purely on the basis of perceived morphological incongruities. However, our cladistic analyses indicate that none of the genera recognized by Tomlinson and Posluszny is well-defined morphologically, especially when compared to *Phyllospadix*. ''*Nanozostera*'' is defined by only two synapomorphies, ''*Zostera*'' (sensu stricto) by three synapomorphies, and ''*Heterozostera*'' by four synapomorphies (see Results). In perspective, *Z. noltii* and *Z. japonica* are differentiated from other members of subgenus *Zosterella* also by two synapomorphies, yet have never been considered as a separate genus. This level of differentiation is miniscule when compared to *Phyllospadix*, which is separated from these taxa by 19 morphological apomorphies (Fig. 1b). Comparatively, the low level of morphological differentiation would argue for the maintenance of only a single genus (*Zostera*) in addition to *Phyllospadix*.

Les et al. (1997) tested the phylogenetic integrity of *Heterozostera* by performing a cladistic analysis of *rbcL* sequence data for *Phyllospadix*, *Heterozostera*, and *Zostera* (including species from both putative subgenera). That study firmly placed *Heterozostera* within a clade comprising *Zostera* species. Phylogenetic analysis of *rbcL* data did not show *Heterozostera* to be distinct from *Zostera* and its recognition as a genus necessarily rendered *Zostera* as paraphyletic. Our expanded *rbcL* analysis (which included additional taxa) produced the same result (Fig. 3). However, organismal phylogenies are more credible when inferred using multiple, congruent data sets (Page and Holmes 1998). Ideally, congruence should be observed among molecular data sets from different genomic compartments (e.g., nuclear and plastid genomes) to avoid possible confounding factors (e.g., paralogy, hybridization, etc.), which can create discrepancies between gene trees and species trees (Soltis et al. 1998).

Here we incorporated additional molecular data sets to infer the phylogenetic position of *Heterozostera* and other Zosteraceae. We analyzed DNA sequence data from the nuclear ITS/5.8S region of nrDNA and from the *trnK* 3′,5′ introns as well as the *rbcL* gene of cpDNA. We expanded our study to include all Australian and New Zealand Zosteraceae taxa, several of which have been sampled from a wide range of geographical localities. Our results show that both nuclear and chloroplast encoded sequences provide congruent phylogenetic trees for the Zosteraceae (Figs. 2–4) and that the tree resulting from the combined molecular data (Fig. 4b) is congruent with trees derived from morphological data (Fig. 1). Internal support is high for major clades in the combined molecular analysis. From these results, we conclude that molecular data produce a robust and reasonable estimate of phylogenetic relationships for the taxa of Zosteraceae studied, which is suitable for directing taxonomic decisions in this group.

Phylogenetic results using molecular data closely parallel those obtained from morphology. With respect to DNA sequence divergence, taxa representing *Zostera* subg. *Zosterella* (''*Nanozostera*'') differ only slightly from *Z. tasmanica* (''*Heterozostera*'') (< 6.3%, ITS; <1.4%, *trnK*); but differ 3–5 times as much from *Z. marina* (16.8%, ITS; 6.2%, *trnK*) or *Phyllospadix* (26.7%, ITS; 7.3%, *trnK*) (Table 4). Because they are so weakly differentiated at both the morphological and molecular levels, it seems superfluous to recognize either ''*Nanozostera*'' or ''*Heterozostera*'' as separate genera. Doing so would unnecessarily disrupt nomenclature in use for more than a century while providing no useful improvement to the classification.

Clayton (1972) emphasized that a trend to recognize new genera in some families is slowly overtaking recognition of new species, a practice that blurs the distinction between these ranks. He attributed this factor

to an emphasis on recognition of differences rather than similarities among taxa. This trend is wholly evident in Zosteraceae, where excessive generic subdivision seems illogical, especially with so few species. Molecular data provide an appropriate means of demonstrating similarity among Zosteraceae taxa, which should be considered along with the few morphological differences proposed in prior taxonomic treatments.

Phylogenetic analyses of Zosteraceae resolve the same four clades from a variety of data, either singly or in combination (Figs. 1–4). Although each clade could be recognized as a distinct genus cladistically, doing so would create several highly similar and weakly differentiated genera. We emphatically recommend retaining only two genera in Zosteraceae, namely *Zostera* and *Phyllospadix*, which depict the major phylogenetic lineages within this family. These genera are well differentiated at both the morphological and molecular levels. The three subclades within *Zostera* should continue to be recognized as subgenera (see below), namely as subg. *Zostera*, subg. *Heterozostera* and subg. *Zosterella* (Fig. 1a). In any case, prior taxonomic treatments recognizing *Heterozostera* and *Zostera* (incl. subgenera *Zostera*, *Zosterella*) as genera cannot be supported as a result of our phylogenetic analyses.

Subgeneric Subdivisions in Zostera? Traditionally, two subgenera have been recognized in *Zostera*: subg. *Zostera* (fiber bundles in outermost layers of the outer cortex, fused sheaths, terminal reproductive shoots, retinacules absent or only subtending lowest male flower) and subg. *Zosterella* (fiber bundles in innermost layers of the outer cortex, open sheaths, lateral reproductive shoots, retinacules accompanying each stamen)(Hartog 1970). All Australian *Zostera* have traditionally been placed within subg. *Zosterella* (Hartog 1970).

Jacobs and Williams (1980) found no difference in fiber bundle distribution (Hartog's primary key character) between either *Z. capricorni* or *Z. muelleri* (subg. *Zosterella*) and *Z. marina* (subg. *Zostera*). Tomlinson (1982) arrived at the same conclusion after examining a large sample of material. However, other differences exist. Retinacules occur only occasionally in subgenus *Zostera* (Hartog 1970), and the subgenera can further be delimited by the fused or open condition of the leaf sheath (Jacobs and Williams 1980). Similar to the case of ''*Heterozostera*'', segregation of subgenera in *Zostera* is more reliably achieved by characters other than the principal key characters proposed in prior treatments. According to Hartog's (1970) descriptions, subgenus *Zostera* has terminal reproductive shoots (lateral in subg. *Zosterella*) and seeds lacking a primary root (primary root developed in subg. *Zosterella*). However, the latter feature apparently represents another misconception, given that primary roots never form in Zosteraceae (Tillich 1995).

Our morphological cladistic analysis (Fig. 1) confirmed the utility of several characters used historically to delimit subgenera in *Zostera*. Cladistically, subg. *Zosterella* is defined by reproductive shoot position and retinacule length, whereas subg. *Zostera* is supported by leaf sheath morphology, number of leaf blade nerves, and lack of retinacules. *Zostera* subg. *Heterozostera* is supported by its undulating rhizome, number of rhizome cortical vascular bundles, retinacule vasculature and chromosome number.

Species Concept in Zostera. *Zostera* taxonomy has always followed a morphological species concept. Ascherson and Graebner (1907) delimited genera and subgenera in *Zostera* using reproductive characters, and determined species differences mainly by vegetative characters. In their key for subg. *Zosterella*, species distinctions emphasized differences in leaf tip morphology. Leaf tip morphology was also emphasized by Setchell (1933) in his enumeration of morphological characters used to differentiate *Zostera* species. Setchell (1933, p. 812) remarked that: ''. . . the tips of the mature true foliage leaves are characteristic of the individual species'', but added that: ''The differences are not readily expressed in words. . . ''

Leaf tip morphology figured prominently in Hartog's (1970) keys to *Zostera* species, particularly for subg. *Zosterella* where leaf tip shape appears as the primary key character in six of the eight couplets. However, application of this character is difficult in the keys. Hartog's (1970) key separates *Z. capensis* (leaf-tip obtuse, deeply cleft) from *Z. capricorni*, *Z. muelleri* and *Z. novazelandica* (leaf-tip truncate, sometimes obtuse, not cleft). However, immediately following under the latter lead is *Z. muelleri*, which is keyed by having a leaf tip that is ''obtuse or truncate, deeply notched'', a combination of features thus indistinguishable from those of *Z. capensis*. Our inability to unambiguously distinguish between ''deeply cleft'' and ''deeply notched'' makes it impossible to separate *Z. capensis* and *Z. muelleri* by their leaf-tip morphology using this key. Hartog also used leaf tip shape as the key character for separating *Z. capricorni* (leaf-tip truncate) from *Z. novazelandica* (leaf-tip truncate or slightly emarginate) but clearly the character states overlap making it impossible to identify plants with truncate leaf tips. In his species key for *Zostera* subg. *Zostera*, Hartog (1970) separated *Z. caulescens* (obtuse to mucronate leaf tips) from *Z. asiatica* (truncate to emarginate leaf tips). Yet in his accompanying descriptions, the leaf tip of *Z. caulescens* is characterized as ''broadly obtuse,'' which overlaps with that of *Z. asiatica* described as ''obtuse to truncate, often emarginated.''

Leaf tip morphology is no more successful in separating non-Australian species in *Zostera* subg. *Zosterella*. Hartog (1970) used leaf-tip morphology as the primary key character to distinguish *Z. noltii* (emarginate)

from *Z. japonica* and *Z. americana* (obtuse). However, this distinction is contradicted in the next couplet where *Z. japonica* is said to possess ''slightly emarginate'' leaf tips and *Z. americana* to have ''notched'' leaf tips. The failure of leaf tip morphology to separate these three species is further evidenced by the fact that Hartog's ''*Z. americana*'' (putatively separable from *Z. japonica* by leaf tip morphology) was later considered identical to *Z. noltii* (Phillips and Shaw 1976), but eventually was determined to represent introduced plants of *Z. japonica* (Bigley and Barreca 1982; Harrison and Bigley 1982).

The number of intermediate nerves occurring between the midvein and two main lateral nerves of the leaf blade has also been used to delimit *Zostera* species. Ascherson and Graebner (1907) distinguished *Z. capricorni* (two intermediate nerves) from *Z. noltii*, *Z. muelleri*, and *Z. tasmanica* (intermediate nerves lacking). Setchell (1933) regarded the number of intermediate leaf nerves to be ''of diagnostic importance'' when taken ''in connection with other characters.'' Variation in nerve number comprises the first couplet in Hartog's (1970) key to species of subg. *Zosterella*. However, this character is also difficult to apply. Hartog's key to subg. *Zosterella* begins with an overlap in the primary key character (nerve number), which is described as ''3–5'' in *Z. capricorni* and ''3'' in remaining species. Hartog's description of *Z. capricorni* is contradictory, simply stating ''nerves 5''. Furthermore, *Z. capricorni* keys out again under the group of species having only three nerves. Hartog's (1970) key to species in subg. *Zostera* has similar difficulties. There, the numbers of leaf nerves (7–11 in *Z. caulescens*; 9–13 in *Z. asiatica*) not only overlap, but disagree with values cited in each of the species descriptions (5–9 in *Z. caulescens*; 7–11 in *Z. asiatica*). In this instance, the number of nerves listed in the description of *Z. asiatica* exactly matches a key character for *Z. caulescens*.

We have found it difficult to determine differences in the number of leaf nerves in specimens assigned to various *Zostera* taxa. Several longitudinal nerves are evident under magnification in most *Zostera* specimens, but the nerves differ by their degree of exsertion and coloration. Specimens of ''*Z. muelleri*'' often have smooth lamina surfaces where none of the veins are exserted, but in which three veins are darkly colored, thus rendering a tri-nerved appearance. In some specimens of ''*Z. muelleri*'', there is no distinct coloration to the veins, and some lateral veins are slightly exserted, giving leaves a multi-nerved appearance. Specimens of ''*Z. capricorni*'' generally lack pigmentation in veins and the lateral veins tend to be more strongly exserted, thus rendering a multi-nerved appearance. However, these categorizations are highly subjective, and often differ among leaves on the same specimen. Thus, leaf nerve number is ambiguous and should be reconsidered as a taxonomic character.

Setchell (1927) questioned the segregation of species within wide ranging taxa such as *Z. marina*, because of insufficient knowledge regarding the range of morphological variation and its relationship to ecological variation. He evaluated geographical variation in *Z. marina* and a broader leaved segregate known as ''*Z. latifolia*.'' By comparing morphological variation in specimens from the east and west coasts of North America, Setchell (1927) hypothesized that ''*Z. latifolia*'' was an environmentally induced ''ecad'' of *Z. marina* relating to temperature differences between east vs. west coast habitats. Setchell attributed quantitative differences (length, width of leaves, # veins, # of seed coat ribs, etc.) to temperature induced differences in growth and development. However, in absence of appropriate experiments, he left open the possibility that these forms may represent genetically differentiated ecotypes.

Phillips (1972, 1980) concluded from reciprocal transplant studies that leaf morphology in *Zostera marina* was phenotypically plastic with respect to length and width characters often used to differentiate taxa. Ultimately, Phillips' experiments led him to conclude: ''The use of characteristics, such as the shape of the leaf tip ... to separate species of *Zostera* should be discouraged'' (Phillips and Meñez 1988, p. 30). It is noteworthy that keys to *Zostera* species in Phillips and Meñez (1988) entirely exclude the use of leaf nerve number and incorporate leaf tip morphology only with reservation. Backman (1991) carried out detailed common garden studies of *Z. marina* populations on the west coast of North America, demonstrating the presence of genetically distinct ecotypes (recognized taxonomically by him as varieties) that differed considerably in their leaf morphology. The range of interspecific morphological variation in *Z. marina* is extensive, with leaves ranging from 1.5–20 mm in width (Backman 1991).

Although experimental investigations have not yet included Australian *Zostera* species, comparable levels of morphological variability would be predicted. Conacher et al. (1994) observed wide morphological variability within populations of *Z. capricorni* from Queensland, Australia, with small, medium, and large plants occupying different regions of the littoral zone. It would be informative to conduct similar common garden experiments with *Z. capricorni* as those performed by Backman (1991) for *Z. marina*.

Ecotypic and environmentally labile intraspecific variability in width, nerve number, and tip morphology, limits the taxonomic utility of these characters in *Zostera*. Presently there is no experimental evidence demonstrating consistent interspecific differences in these characters throughout a range of environmental

conditions. Until such evidence may be obtained, their use as taxonomic markers is not recommended.

Morphological cladistic analysis (Fig. 1b) weakly separates *Z. capricorni*, *Z. muelleri*, *Z. mucronata*, and *Z. novazelandica*, indicating that these taxa are distinguishable only by their leaf tip morphology (character #16; truncate, mucronate or notched) and seed surface morphology (character #26; striate or ridged). Both characters were identified as homoplasious in our analysis, and their practical taxonomic implementation would be virtually impossible due to overlap and polymorphism of states (Fig. 2). We have found no other morphological characters that aid in the distinction of these taxa.

Hartog (1970) incorporated a secondary key character that distinguished *Z. capricorni* as having rhizomes with two ''groups'' of roots per node, compared to other species with ''2 (sometimes 1–4)'' roots per node. It is unclear whether species with more than two roots per node would possess them in two ''groups'' or not and the character is not clarified by the descriptions. Phillips and Meñez (1988) also described *Z. capricorni* as having ''2 groups of roots'' per node; however, their illustration of this species (their Fig. 12) clearly depicts a plant with only two roots per node. Soros-Pottruff and Posluszny (1995) determined *Z. capricorni* to have two roots per node. Robertson (1984) described typical and estuarine forms of Z. muelleri which differed by having two roots per node or two groups of 2–6 roots per node respectively, thus indicating intraspecific variability in this character. We conclude from these examples that *Z. capricorni* cannot be distinguished by root number from other species in subgenus *Zosterella*.

Comparisons of retinacule features in this group also fail taxonomically as they are said to be ''obliquely triangular'' in *Z. capricorni* and ''oblique, broadly triangular'' in *Z. novazelandica* (Hartog 1970). Understandably, Phillips and Meñez (1988) merged *Z. novazelandica* with *Z. capricorni*, concluding that descriptions in Hartog (1970) showed ''no differences.''

Some taxonomic decisions have been based on features perceived as unique. ''*Zostera mucronata*'' was considered distinct entirely because of its mucronate leaf tip; the species was described prior to acquisition of fertile material (Hartog 1970). Yet, mucronate leaf tips occur in other Zosteraceae such as *Z. marina* where they can be ''often slightly mucronate'' (Hartog 1970). Flahault (1908) showed that a transformation from mucronate to indented leaf apices occurs as juvenile leaves mature in *Zostera noltii* (as *Z. nana*). Furthermore, Robertson (1984) observed that: ''many populations in South Australia and Victoria are now known with some leaves bearing a more or less well-developed central mucro and other leaves notched'' adding that ''It is not always possible to distinguish between *Z. mucronata* and *Z. muelleri* with these intergrades.''

The taxonomy of Zosteraceae has been complicated because morphology is highly modified by ambient environmental conditions. Setchell (1927) noted a correlation between leaf width and water temperature in *Zostera marina*. Phillips (1972, 1980) demonstrated changes in leaf morphology associated with water depth. Ostenfeld (1908) and Van Goor (1919) found that *Zostera marina* produced narrower leaves on sand than on muddy substrates. Environmental heterogeneity may be responsible for some of the morphological variation observed in Australian Zosteraceae. ''*Zostera capricorni*'' is the only taxon that occurs in both temperate and tropical portions of eastern Australia, regions that are characterized by different habitats. In addition to differences in annual water temperature, it is notable that seagrass habitats along Australia's tropical east coast tend to be muddy because the Great Barrier Reef reduces wave energy, allowing fine erosional sediment to accumulate on the coast. It is also muddy where there has been intense human activity (clearing and erosion) thus corresponding to areas most accessible to botanical collectors. Seagrass habitats of the southern coast are mostly sandy (Robertson 1984). Given the results of Ostenfeld (1908) and Van Goor (1919) for *Z. marina*, it is possible that habitat differences could be responsible, at least in part, for some morphological differences associated with narrower leaved ''*Z. muelleri*'' phenotypes (temperate, southern coast) and broader-leaved ''*Z. capricorni*'' phenotypes (more commonly tropical, NE coast). Common garden experiments would be informative in this regard.

Molecular data cast further doubt on the distinctness of species within the Australian and New Zealand members of subg. *Zosterella*. No cpDNA variation was detected among accessions of *Z. capricorni*, *Z. muelleri*, and *Z. novazelandica*, which possessed identical *rbcL* and *trnK* intron sequences (*trnK* sequences were not obtained for *Z. mucronata*). In contrast, *trnK* introns differed between accessions of these three taxa and both *Z. noltii* and *Z. japonica* (subg. *Zosterella*) as well as between *Z. noltii* and *Z. japonica* (Fig. 4a). Nuclear ITS/5.8S rDNA sequences produced similar results. ITS sequences from all four species (including *Z. mucronata*) possessed identical gaps, but their indel organization differed from both *Z. noltii* and *Z. japonica*, which in turn also differed from each other (Fig. 2a). Minor DNA sequence variation occurred within this group, but mainly represented geographical discontinuities, as accessions of all four species were resolved within a single clade (Fig. 2b). Our one accession of *Z. mucronata* differed slightly from *Z. capricorni*, *Z. muelleri*, and *Z. novazelandica* accessions ($p = 0.49–1.1\%$), but this level of nucleotide divergence compares to that

among various *Z. muelleri* accessions (p = 0.0–0.98%). Furthermore, the *Z. mucronata* accession was the only material of this group collected from Western Australia, thus separated from other accessions geographically by more than 23° longitude. Similarly, the northernmost accession of *Z. capricorni* also differed by several nucleotide substitutions, yet other accessions of *Z. capricorni* possessed ITS sequences identical to many *Z. muelleri* accessions. Geographical divergence in ITS was also observed for several Tasmanian and Victorian populations of *Z. muelleri* (Fig. 2b). Our accessions of *Z. novazelandica* differed by a few ITS substitutions (p = 0–0.49%), which probably reflects their geographical location more than 20° longitude east of other accessions sampled. Interestingly, two collections of *Z. novazelandica* (*Schwarz, s.n.*; Table 1) were purposely sent to us for analysis because they were ''morphometrically dissimilar in appearance in the field'' (A.-M. Schwarz, pers. comm.). Nevertheless, despite their morphological variability, these two accessions had ITS and *trnK* sequences identical to each other and also to the other specimen of *Z. novazelandica* that we examined (*Tomlinson, s.n.*; Table 1).

Molecular data do not support the distinctness of *Z. capricorni*, *Z. mucronata*, *Z. muelleri*, and *Z. novazelandica* as discrete species, but indicate that some isolation by distance has occurred. Given that these taxa are separable morphologically essentially only by their leaftip morphology (and even then with difficulty), we highly recommend their taxonomic merger as a single species, which, by priority, should be called *Z. capricorni*. These results indicate that past taxonomic difficulties in *Zostera* subg. *Zosterella* are due to recognition of unnatural ''species'' that actually represent morphological variants of a single widespread species.

Despite the existence of considerable morphological variation, the similarity in reproductive features of different *Zostera tasmanica* specimens led Hartog (1970) to conclude that all material represented a single species. Our molecular results (Figs. 2, 4) confirm the similarity of *Z. tasmanica* accessions, but some geographical molecular divergence was observed among accessions of *Z. tasmanica* collected from different regions of Australia. Notably, eastern and western Australian populations of *Z. tasmanica* exhibited slight molecular divergence in both nuclear (ITS: 1.03–1.19%) and chloroplast encoded (*trnK*: 0.44–0.49%) DNA sequences (Figs. 2b; 4a). This low level of molecular divergence also probably reflects relatively prolonged geographical isolation of eastern and western populations of *Z. tasmanica* rather than evidence of a speciation event. As yet, there is insufficient evidence to warrant recognition of any of these populations as distinct taxonomically. However, a further study of eastern vs. western Australian populations of *Z. tasmanica* could be undertaken to determine whether the observed molecular divergence is correlated with reliable morphological markers.

Finally, the influence of polyploidy must be considered. The lowest reported chromosome number in Zosteraceae is 2n=12 (the presumed diploid level) which is reported for all species of subg. *Zostera* and for *Z. japonica* and *Z. noltii* in subg. *Zosterella* (Tables 2,3). Southern hemisphere species (remainder of subg. *Zosterella*) have 2n=24 and *Z. tasmanica* is 2n=36 (Kuo 2001). *Phyllospadix* (2n=16–20; Tables 2,3) is apparently an aneuploid derivative of this series. Chromosomal data indicate that Australian/New Zealand Zosteraceae represent a polyploid series derived from ancestral diploids in *Zostera*. The hexaploid *Z. tasmanica* is likely derived from within *Zostera* which contains both diploid and tetraploid taxa, an interpretation consistent with all phylogenetic analyses of Zosteraceae (Figs. 1–4).

Wide morphological variability as observed in Australian Zosteraceae is not unexpected in polyploids. As Stebbins (1950) observed, polyploidy is a ''complicating force'' that produces ''innumerable variations on old themes, but not originating any major new departures.'' In some cases, polyploid complexes consisting of morphologically distinct diploids can give rise to a series of auto- and allotetraploids that span the range of morphological features of the diploid parents (Bayer 1999). The morphological distinctness of the diploids can be obscured by the presence of the autopolyploids, and the entire complex therefore can form a morphological continuum (Bayer 1999). Additional even-polyploid levels, such as hexaploids, develop in mature polyploid complexes, and these tend to be reproductively isolated from other ploidy levels (Bayer 1999). Because members of these ploidy levels are usually sexual, individuals of divergent genetic makeup can cross with ease, forming large morphological continua within each ploidy level (Bayer 1999). Although the precise nature of polyploidy in *Zostera* has not been clarified, the existence of polyploidy may explain the range of morphological variability observed within Australian Zosteraceae.

Polymorphic ITS sites observed occasionally in Australian section *Zosterella* could result from gene flow or polyploidy. If the former explanation is correct, then ITS polymorphisms indicate the lack of effective isolating barriers and the occurrence of genetic recombination both within and between populations regarded previously as distinct species. On the other hand, these polymorphisms could be ancestral remnants of an initial polyploid event that have homogenized differentially among populations by gene flow. The largest number of polymorphic sites occurred in *Z. capricorni* from northern Queensland (*Les 605, Jacobs 8582*), which was fairly remote geographically from other populations studied. If ITS polymorphisms resulted from

TABLE 5. Taxonomic scheme proposed from results of present study (* = species recognized tentatively, but not evaluated in present study).

Family: Zosteraceae
1. Genus: *Phyllospadix* Hook. (species relationships not addressed in present study)
2. Genus: *Zostera* L.
   a. Subgenus: *Zostera*
      1. *Z. asiatica* Miki*
      2. *Z. caespitosa* Miki*
      3. *Z. caulescens* Miki*
      4. *Z. marina* L.
   b. Subgenus: *Heterozostera* Setch.
      1. *Z. tasmanica* G. Martens ex Asch.
   c. Subgenus: *Zosterella* (Asch.) Ostenf.
      1. *Z. capensis* Setch.*
      2. *Z. capricorni* Asch. (= *Z. mucronata* Hartog; = *Z. muelleri* Irmisch ex Asch.; = *Z. novazelandica* Setch.)
      3. *Z. japonica* Asch. & Graebn.
      4. *Z. noltii* Hornem.

polyploidy, then their widespread occurrence within and between populations of section *Zosterella* taxa is further evidence that no effective barrier to recombination exists, thus supporting our contention that these populations belong to a single, variable, widespread species.

Polyploidy imposes effective reproductive isolation (chromosomal) that may be critical for maintaining hydrophile species where other isolating mechanisms (e.g., genetic incompatibility) are weak (Philbrick and Les 1996). The relationship between polyploidy and speciation rates in aquatic plants has been pointed out previously (Les and Philbrick 1993). Our results indicate that substantial divergence exists between *Zostera* taxa of different ploidy levels, thus the clear distinction of *Z. noltii* and *Z. japonica* (diploids) from *Z. capricorni* (tetraploid) and *Z. tasmanica* (hexaploid), and also the distinction between all *Zostera* (x=6) and *Phyllospadix* (x=4–5).

Our systematic studies warrant a revised taxonomy of Zosteraceae which better reflects phylogenetic results obtained from a variety of molecular and nonmolecular data (Table 5). We recognize two genera, *Zostera* and *Phyllospadix*. *Zostera* is circumscribed as consisting of three subgenera, *Zostera*, *Heterozostera*, and *Zosterella*, with four, one, and four species, respectively. Eventually we hope to obtain molecular data for *Z. capensis*, *Z. asiatica*, *Z. caespitosa*, and *Z. caulescens* to further supplement this work and welcome receipt of material of any of these taxa.

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