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Delimitation of the Segregate Genera of *Maytenus* s. l. (Celastraceae) Based on Morphological and Molecular Characters

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Abstract*—Maytenus* s. l. (including *Gymnosporia*) is a morphologically diverse genus of about 300 species that is widely distributed in the tropics and subtropics of both the Old and New Worlds. Its delimitation has been extensively debated and despite the segregation of *Gymnosporia*, *Maytenus* s. s. remains a heterogeneous, polyphyletic group. To delimit natural segregate genera we increased taxon sampling and generated sequences from two nuclear gene regions (ITS and 26S rDNA) and two plastid loci (*matK* and *trnL–F*) to analyze together with morphological characters. Both *Moya* and *Tricerma* were found to be nested within the New World *Maytenus* and are recognized as synonyms of *Maytenus* s. s.. In contrast, the three New World species of *Gymnosporia* are recognized as a new genus that is closely related to *Gyminda*. ***Haydenia*** is erected for these three species: ***H. gentryi, H. haberiana***, and ***H. urbaniana***. One or more previously proposed or novel genera are required to accommodate the systematically difficult African *Maytenus*. *Putterlickia*, and most likely *Gloveria*, are nested within *Gymnosporia* and should be synonymized with that genus. New binomials are required for four Chinese and one Rapan species of *Gymnosporia* that have been previously treated only as *Maytenus*: ***Gymnosporia austroyunnanensis***, ***G. confertiflora***, ***G. dongfangensis***, ***G. guangxiensis***, and ***G. pertinax***. Austral-Pacific *Maytenus* are transferred to *Denhamia*, requiring eight new binomials: ***Denhamia bilocularis, D. cunninghamii, D. cupularis, D. disperma, D. fasciculiflora, D. ferdinandii, D. fournieri***, and ***D. silvestris***. Existing intrageneric classifications of *Gymnosporia* and *Maytenus* s. s. were not supported in their entirety. *Gymnosporia* is inferred to have had an African origin followed by dispersals to Madagascar, southeast Asia and the Austral-Pacific.

Keywords*—Denhamia*, *Gloveria*, *Gymnosporia*, *Moya*, *Putterlickia*, *Tricerma*.

Maytenus Molina s. l. [including *Gymnosporia* (Wight & Arn.) Hook. f.] is a large genus of about 300 species that is widely distributed in the tropics and subtropics of both the Old and New Worlds. The delimitation of *Maytenus* s. l. has been extensively debated, particularly with respect to distinctions between *Celastrus* L., *Gymnosporia*, and *Maytenus* s. s. (e.g. Baillon 1880; Loesener 1942; Ding Hou 1955, 1962; Sebsebe 1985). It is now well established that the 31 species of *Celastrus* and 99 species of *Gymnosporia* are distinct from *Maytenus* s. s. (Ding Hou 1955; Jordaan and van Wyk 1999, 2006; Simmons and Hedin 1999; Simmons et al. 2001a, 2001b, 2008).

Maytenus s. s. is a morphologically diverse genus of trees or shrubs with alternate leaves, axillary inflorescences of various types, bisexual or unisexual flowers on monoecious or dioecious plants, 4- or 5-merous flowers, intrastaminal disks, 2– or 3–locular ovaries, one or two erect (i.e. basal placentation) ovules per locule, loculicidally dehiscent capsules, and arils that partially or completely envelop the seeds (Loesener 1942; Simmons 2004a). The type species (*M. boaria*) and the center of diversity are in South America (Loesener 1942).

Despite the segregation of *Celastrus* and *Gymnosporia* from *Maytenus*, *Maytenus* s. s. remains a heterogeneous, polyphyletic group (Jordaan and van Wyk 1999, 2003; Simmons et al. 2001a, 2001b, 2008). In the previous study with the best taxon and character sampling for *Maytenus* yet performed, Simmons et al. (2008) resolved three divergent lineages of *Maytenus* s. s. First, the five sampled species of Austral-Pacific *Maytenus* were resolved as an unambiguously supported clade sister to *Denhamia* Meisn. as part of a larger clade with 12 other Austral-Pacific genera. Second, the three South American species of *Maytenus* were unambiguously resolved in a clade together with *Moya* Griseb. and *Tricerma* Liebm., with *Maytenus floribunda* Reissek unambiguously supported as more closely related to *Moya spinosa* Griseb. and *Tricerma texanum* (Lundell) Lundell than to the other two species of South American *Maytenus*. This clade of five species was resolved as sister to a clade of seven genera from Asia and the New World. Third, the three species of African *Maytenus* were resolved in a strongly supported clade with five other African genera. One of these three species was part of a polytomy with *Mystroxylon aethiopicum* (Thunb.) Loes. and *Robsonodendron eucleiforme* (Eckl. & Zeyh.) R. H. Archer.

Like *Maytenus* s. s., *Gymnosporia* may not be a natural (i.e. monophyletic) genus. Simmons et al. (2008) resolved two species of *Putterlickia* Endl. as a strongly supported clade that is nested within *Gymnosporia*. Jordaan and van Wyk (1999) cited a single fixed morphological difference between the two genera: usually 6–12 ovules per locule in *Putterlickia* vs. two ovules per locule in *Gymnosporia*. Yet Jordaan and van Wyk (1998b) noted that *Putterlickia* may have as few as three ovules per locule while Davison (1927) described *P. saxatilis* as having two ovules per locule.

Jordaan and van Wyk (1998a) recognized *Gloveria integrifolia* (L. f.) M. Jordaan as a monotypic genus distinct from *Gymnosporia* and *Putterlickia*. Jordaan and van Wyk (1998a, 1999) distinguished *Gloveria* from *Putterlickia* based on ovule number per locule (three to six in *Gloveria* vs. six to twelve in *Putterlickia*, number of nodes per thorn (two to five in *Gloveria* vs. one in *Putterlickia*), and leaf morphology. Robson (1965) had treated specimens that Jordaan and van Wyk (1998a) recognized as *Gloveria* as members of *Putterlickia pyracantha* (L.) Szyszyl. Given that both *Gloveria* and *Putterlickia* have bisexual flowers (instead of typically unisexual flowers in *Gymnosporia*; Jordaan and van Wyk 1999) and an overlapping distribution in ovule number that is putatively distinct from that in *Gymnosporia*, both *Gloveria* and *Putterlickia* are probably a single clade nested within *Gymnosporia*. This inference is also consistent with the two genera being restricted to southern Africa and Robson's (1965) treatment of *Putterlickia pyracantha* (i.e. both species being closely related to each other).

Gymnosporia had been considered to have a strictly Old World distribution until Lundell (1971) described *G. gentryi* Lundell and *G. magnifolia* (Loes.) Lundell [= *G. urbaniana*

(Loes.) Liesener; Hammel 1997]. Loesener (1942) had previously recognized *Maytenus magnifolia* Loes. as the only member of *Maytenus* section *Magnifolia*. Lundell (1971:313) justified treating these two species as *Gymnosporia* because they "… have inflorescences and capsules typical of *Gymnosporia*." In 1993, Liesner synonymized one of Lundell's two species with *Gymnosporia urbaniana*. Likewise, Hammel (1997) followed Lundell's (1971) treatment of New World *Gymnosporia* and added *G. haberiana* Hammel. Yet none of these three New World species of *Gymnosporia* have thorns and Jordaan and van Wyk (1999, 2003, 2006) restricted their delimitation of *Gymnosporia* to Old World species with thorns. Jordaan and Van Wyk's (1999, 2003) delimitation was corroborated by Simmons et al. (2008), wherein *G. haberiana* and *G. urbaniana* were well supported as a clade sister to *Gyminda*, and only distantly related to *Gymnosporia* s. s.

There are seven primary questions for this study. First, is *Denhamia* distinct from the Austral-Pacific *Maytenus* or should they be merged into a single genus as suggested by Ding Hou (1962) and Robson (1965)? Second, is *Moya* nested within, or distinct from, the New World *Maytenus*? If *Moya* is found to be nested within *Maytenus*, this would support Lourteig and O'Donell's (1955) transfer of *Moya* to *Maytenus* rather than Loesener's (1942) recognition of the two genera as distinct. Third, is *Tricerma* nested within, or distinct from, New World *Maytenus*? If *Tricerma* is found to be nested within *Maytenus*, this would support Lobreau-Callen's (1975) assertion based on pollen morphology that the genera are not distinct rather than Lundell's (1971) recognition of *Tricerma* as distinct from *Maytenus*. Fourth, are the African *Maytenus* a natural group distinct from the monotypic African genera *Mystroxylon* Eckl., *Pseudosalacia* Codd, and *Robsonodendron* R. H. Archer? Fifth, after increased sampling of *Gymnosporia* is *Putterlickia* still resolved as a monophyletic group nested within a paraphyletic *Gymnosporia*? If so, *Putterlickia* should be synonymized with *Gymnosporia* as suggested by Robson (1965). Sixth, with all three species of the New World *Gymnosporia* sampled and increased sampling of *Gyminda* and New World *Maytenus*, do the New World *Gymnosporia* form a natural group that merits recognition as a new genus? Seventh, are the most recent sectional classifications of *Maytenus* by Loesener (1942) and Carvalho-Okano (1992), and *Gymnosporia* by Jordaan and van Wyk (2006) congruent with our inferred phylogeny?

To address these study questions, we increased taxon sampling for the pertinent lineages from that used by Simmons et al. (2008). Sequences were generated from two nuclear gene regions [the internal transcribed spacers (ITS) of rDNA and 26S rDNA] and two plastid loci [maturase K (*matK*) and *trnL–F*]. These sequences were analyzed together with morphological characters representing variation in vegetative and floral morphology, leaf and seed anatomy, and pollen morphology.

Materials and Methods

***Taxon Sampling*—**Eighty-four taxa were sampled (Appendix 1; see also Simmons et al. 2001a, 2001b, 2008; Islam et al. 2006; Zhang and Simmons 2006; Coughenour et al. 2010 for vouchers and GenBank accession numbers for taxa and sequences sampled from those studies). Two accessions were sampled from some species for a total of 90 terminals included in the simultaneous analyses (Kluge 1989; Nixon and Carpenter 1996). The following new specimens were included: one *Gyminda*, six *Gymnosporia*, and 23 *Maytenus* (Appendix 1). No new specimens of Austral-Pacific *Denhamia* or *Maytenus* s. s. (other than *M. pertinax*, which is actually a member of *Gymnosporia*; see results) were obtained.

The identifications of three specimens included in Simmons et al. (2008) were changed based on annotations by Robert H. Archer. *R. H. Archer et al. 2935* was changed from *G. linearis* (L. f.) Loes. to *G. madagascariensis*, *R. H. Archer et al. 3028* was changed from *Maytenus undata* to *M. fasciculata* (Tul.) Loes., and *R. H. Archer et al. 3030* was changed from *Gymnosporia senegalensis* to *G. grossulariae* (Tul.) Loes. The corresponding GenBank records were updated in July 2010.

Preliminary parsimony tree searches based on the taxon sampling used by Simmons et al. (2008), which included members of Lepidobotryaceae and Parnassiaceae as outgroups, indicated that all new samples were resolved within the following numbered clades identified by Simmons et al. (2008): clade 2 (a New World/Asian clade that includes all New World *Gymnosporia* and *Maytenus*), 4 (an African clade that includes all African *Maytenus*), and 6 (an African clade that includes all *Putterlickia* and Old World *Gymnosporia*). All three clades were well supported (≥ 95% parsimony jackknife support [JK; Farris et al. 1996]; ≥ 89% likelihood bootstrap support [BS; Felsenstein 1985]) in the simultaneous analysis of Simmons et al. (2008). Therefore, to speed tree searches and help decrease alignment ambiguity caused by inclusion of divergent sequences while still maintaining dense taxon sampling within the relevant lineages to facilitate alignment (Simmons and Freudenstein 2003) and minimize the potential for long-branch attraction (Felsenstein 1978), our ingroup sampling was limited to these three clades. Analyses were performed for clade 6 separately from clades 2 and 4. The reason for combining clades 2 and 4 was so that they could reciprocally root each other. Although African *Maytenus* was well nested within clade 4, the New World *Maytenus* in clade 2 was sister to the other taxa in that clade.

***Morphological Characters*—**Morphological characters were derived from matrices previously published by Simmons and Hedin (1999), Simmons et al. (2001a, 2001b, 2008), and Islam et al. (2006). For the 84 taxa sampled in this study, 17 and 16 characters are parsimony informative in clades 2 and 4, and 6, respectively, representing variation in vegetative and floral morphology, leaf and seed anatomy, and pollen morphology (Appendix 2). Where possible, characters were scored using reductive coding rather than composite coding (Wilkinson 1995; Simmons and Freudenstein 2002). The codings for most morphological characters are described in detail by Simmons and Hedin (1999, p. 746–751). All morphological characters are included as part of the simultaneous-analysis data matrix that has been posted on TreeBASE (S10859).

***Molecular Methods*—**Total genomic DNA was extracted from herbarium specimens and fresh, silica gel- and sodium chloride/CTAB-preserved (Chase and Hills 1991; Rogstad 1992) leaves using the protocol described by Alexander et al. (2006). New sequences for two loci from the plastid genome (*matK* and *trnL–F*) and two gene regions from the nuclear genome (ITS and 26S rDNA) were generated for this project. All four gene regions were amplified with the following PCR protocol: an initial denaturation of 96° preceding 10 cycles denaturation (96° for 45 s), annealing (50–53° for 30 s), and extension (72° for 2 min), followed by 25 cycles of denaturation (96° for 20 s), annealing (50–53° for 30 s), and extension (72° for 2 min).

Most amplifications of the *matK* locus were split into two reactions, one using the primer combination trnK-710 (Johnson and Soltis 1995) and matK-R1 (Yokoyama et al. 2000) for the 5′ end, and the second reaction using matK-F1 (Yokoyama et al. 2000) and matK-8R (Steele and Vilgalys 1994) for the 3′ end. When one or both of the above combinations did not amplify or produced poor electropherogram reads, alternate primers were used. The primer matK-441R (Zhang et al. 2006) was used in place of matK-R1, and matK-F3 (Yokoyama et al. 2000) was used in place of matK-F1. When matK-441R was used, the combination of matK-F1 and matK-R1 was used to amplify the central region of the *matK* locus.

The *trnL* intron and the *trnL–F* intergenic spacer were amplified in one reaction using primers 'c' and 'f,' or in two reactions using the combinations 'c' and 'd' for the 5′ end and 'e' and 'f' for the 3′ end (Taberlet et al. 1991). The ITS region (ITS1–5.8S-ITS2) was amplified with the primer combination ITSA and ITSB (Blattner 1999). Most amplifications of 26S rDNA were performed using the primers 26S1 and 950rev, or in two reactions using 26S1 with 641rev for the 5′ end and 26S2 with 950rev for the 3′ end (Kuzoff et al. 1998). Amplified products were purified using Qiagen PCR purification kits. Purified PCR products were sequenced by Macrogen (Seoul, Korea) or the University of Chicago Cancer Research Center DNA Sequencing Facility using automated fluorescent sequencing with ABI DNA Analyzers. The same primers used for amplification were also used for sequencing. All new sequences generated in this study have been deposited in GenBank under accession numbers HQ267097 to HQ267221 (Appendix 1).

***Data Analysis*—**Preliminary nucleotide alignments were obtained independently for each gene region using the default alignment parameters

in MUSCLE ver. 3.6 (Edgar 2004). Manual adjustments to the MUSCLE alignments were performed in MacClade ver. 4.03 (Maddison and Maddison 2001) using the procedure outlined by Simmons (2004b) following Zurawski and Clegg (1987). We observed some ambiguously aligned regions where one or more sequences had a duplicate insertion (or the others had a deletion of one of two repeats) and the character-state distribution among the characters in the ambiguously-aligned region was identical for those sequences that have both repeats. Therefore, the character-state distribution among the positions in question would be identical for either of the alternative alignments. In these cases the ambiguously-aligned regions were kept in the analysis following Davis et al. (1998). A total of 79 ambiguously-aligned positions were excluded from the African-and-New World-*Maytenus* analysis (ITS: 57 positions from seven regions; *trnL–F*: 22 positions from one region). Two ambiguously-aligned positions were excluded from the *Gymnosporia* analysis (ITS: two positions from one region). Ambiguously-aligned nucleotides of individual sequences in regions that could not be unambiguously aligned with the remaining sequences were scored as ambiguous ("?").

Gap characters, whose inclusion often affects the inferred tree topology and increase branch-support values (Simmons et al. 2001c), were manually scored using modified complex indel coding (Simmons and Ochoterena 2000; Müller 2006). Only parsimony-informative complex-indel-coding gap characters were scored from unambiguously aligned regions. A total of 49 gap characters were scored (26S rDNA: 4; ITS: 25; *matK*: 2; *trnL–F*: 18) for inclusion in the African-and-New World-*Maytenus* parsimony analyses. Sixteen gap characters were scored (ITS: 8; *trnL–F*: 8) for inclusion in the *Gymnosporia* parsimony analyses.

As a means of data exploration, several alternative potential process partitions (Bull et al. 1993) of the characters were analyzed. Each of the four gene regions was analyzed independently from one another to resolve their respective gene trees. Putative coalescent genes (Hudson 1990; Doyle 1995) were then analyzed and their trees compared to check for well supported, contradictory signal that may have been caused by lineage sorting, introgression, and/or unrecognized paralogy (Doyle 1992). As such, gene trees for the adjacent rDNA gene regions and the plastid loci were analyzed independently to check for potential introgression of the plastid genome or rDNA (Doyle 1992; Wendel et al. 1995) or unrecognized paralogy problems with rDNA (Álvarez and Wendel 2003; Bailey et al. 2003). An analysis of all molecular characters was then performed, followed by a simultaneous analysis of all morphological and molecular characters (using parsimony only), which was conducted as the primary basis for phylogenetic inference.

Equally weighted parsimony tree searches were conducted for each data matrix using 2,000 random addition tree-bisection-reconnection (TBR) searches in PAUP* ver. 4.0b10 (Swofford 2001) with a maximum of ten trees held per replicate. Parsimony JK analyses were conducted using PAUP* with the removal probability set to approximately e^{-1} (36.7879%), and "jac" resampling emulated. Two-thousand JK replicates were performed with 100 random addition TBR searches (each with a maximum of ten trees held) per replicate.

jModeltest ver. 0.1.1 (Posada 2008) was used to select the best-fit likelihood model for each data matrix using the Akaike information criterion (Akaike 1974) without considering invariant-site models following Yang (2006). With one exception (*matK* for *Gymnosporia*), the models selected all incorporated the gamma distribution. The Q-matrices selected were all variants of SYM, TIM, TrN, TPM, TVM, or GTR.

Maximum likelihood (Felsenstein 1973) analyses of nucleotide characters from each of the molecular data matrices were performed as (fallible; Gaut and Lewis 1995; Siddall 1998; Sanderson and Kim 2000) tests for long-branch attraction. Likelihood analyses were conducted using RAxML ver. 7.03 (Stamatakis 2006). Given that RAxML only implements GTR Q-matrices for nucleotide characters, more restrictive variants of the GTR matrix were not used when selected by the AIC. Likewise, given that RAxML only implements the GTR model with the gamma distribution, this same model was applied to the *matK* matrix for *Gymnosporia*. Optimal likelihood trees were searched for using 1,000 independent searches starting from randomized parsimony trees with the GTRGAMMA model and four discrete rate categories. Likelihood BS analyses were conducted with 2,000 replicates with ten searches per replicate using the "–f i" option, which "refine[s] the final BS tree under GAMMA and a more exhaustive algorithm" (Stamatakis 2008:9).

Results

The simultaneous-analysis parsimony JK trees of all four gene regions and morphological characters are presented in Figs. 1–2 with BS values below each branch given for likelihood analysis of all gene regions. The parsimony JK trees with JK values above each branch and likelihood BS values below each branch for each of the four gene regions, the three remaining combined analyses and the parsimony JK morphology tree are presented in Figs. S2–S17 as online supplementary data. These trees were created using TreeGraph 2 (Stöver and Müller 2010). Data-matrix and tree statistics for the *Maytenus* and *Gymnosporia* analyses are presented in Tables 1 and 2, respectively. Gap and morphological characters were mapped onto the strict consensus of the most parsimonious trees from the simultaneous analyses using unambiguous optimization in MacClade to infer synapomorphies for selected clades.

Nucleotide-Frequency Heterogeneity—Only ITS was found to exhibit significant nucleotide-frequency heterogeneity for the parsimony-informative nucleotide characters among different terminals, as determined by the χ^2 test implemented in PAUP* (which ignores phylogenetic correlations). This occurred for both the African-and-New World-*Maytenus* as well as the *Gymnosporia* analyses. In both cases, no obvious outlier terminals were identified and the heterogeneity was no longer significant after inclusion of all variable nucleotide characters.

Incongruence—A single mutually well supported (≥ 70% JK and BS support) case of incongruence was observed in the parsimony and likelihood gene trees of individual gene regions. The parsimony-based *trnL–F* gene tree resolved *Cassine peragua* and *Maurocenia frangula* as sister groups with 74% JK (Fig. S14). This resolution was supported by two indel synapomorphies: a 4-bp insertion at positions 298–301 in the *trnL* intron and an indel (ambiguously optimized as an insertion or deletion because of multiple alleles at the poly-T microsatellite) at positions 319–323 in the *trnL–F* intergenic spacer, neither of which were included in the DNA-based likelihood analyses. Curiously, despite there not being a single synapomorphy for the clade, *Allocassine laurifolia* and *Cassine peragua* were resolved as sister groups with 96% BS in the likelihood-based *trnL–F* gene tree (Fig. S18). The likelihood-based *trnL–F* resolution was primarily derived from the *trnL–F* intergenic spacer, which by itself supported the clade of *Allocassine laurifolia* and *Cassine peragua* with 93% BS (Fig. S20). We consider this likelihood-based resolution to be an artifact and note that *Cassine peragua* and *Maurocenia frangula* are resolved as a paraphyletic group with < 50% BS on the plastid gene tree, as in the likelihood-based *matK* gene tree.

Three mutually well supported (≥ 70% JK support) cases of incongruence were identified when comparing gene-tree topologies. All three of these conflicts are between ITS and *trnL–F* gene trees. None of these conflicts were mutually highly supported, with 88% vs. 78% JK for the resolution of *Gyminda* (Figs. S3, S6), 71% vs. 84% JK for the resolution of *Celastrus rosthorianus* Loes. (Figs. S3, S6), and 79% vs. 80% JK for the resolution of *Gymnosporia wallichiana* (Spreng.) M. A. Lawson (Figs. S11, S14). Given that ITS and *trnL–F* are the two most rapidly evolving gene regions sampled and included regions that were difficult to align, rather than reflecting different histories between the plastid genome and nuclear rDNA, these may simply be artifacts of alignment and/or caused by multiple hits. This inference is reinforced by none of the conflicts having mutually high support and that each of the conflicts differs by whether two taxa are sister groups or paraphyletic. As such, we consider the simultaneous analyses (Figs. 1, 2) to provide the best estimates of the phylogeny.

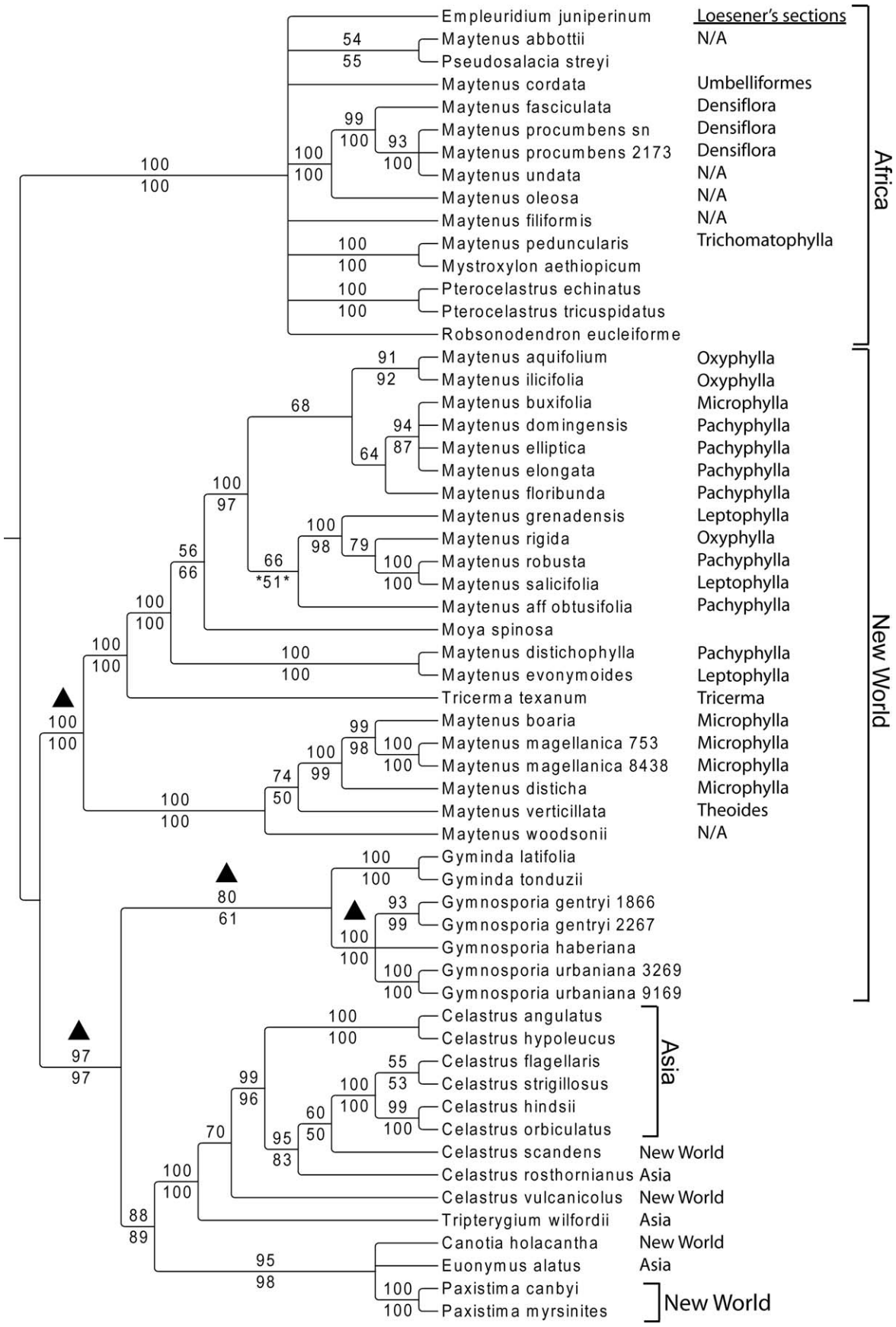

FIG. 1. Simultaneous analysis of morphological and molecular-characters parsimony JK tree of the American and African *Maytenus* clades with parsimony JK values ≥ 50% above each branch, and likelihood BS values ≥ 50% below each branch. The clade in the parsimony JK tree that was contradicted by a clade in the likelihood BS tree is indicated by *51*, with BS support for the highest contradictory likelihood clade listed. Branches separating New World *Gymnosporia* from New World *Maytenus* are indicated by solid triangles. Sections of *Maytenus* are from Loesener (1942).

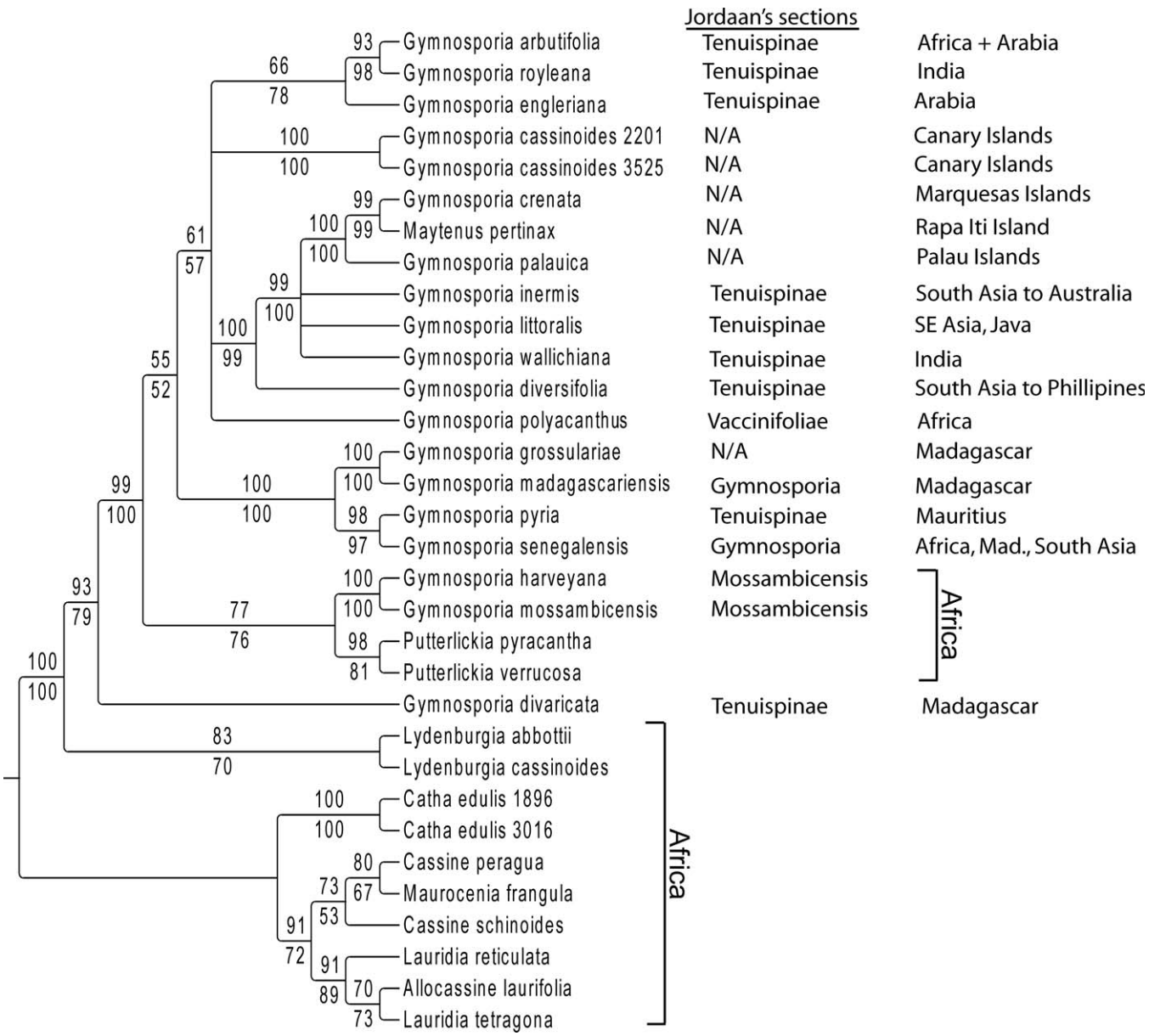

FIG. 2. Simultaneous analysis of morphological and molecular characters parsimony JK tree of the *Gymnosporia* clade with parsimony JK values ≥ 50% above each branch, and likelihood BS values ≥ 50% below each branch. Sections of *Gymnosporia* are from Jordaan and van Wyk (2006).

Simultaneous Analyses—Among the 58 clades resolved in the African-and-New World *Maytenus* simultaneous analysis, the following noteworthy clades were supported (Fig. 1). New World *Maytenus* was unambiguously supported (100% JK / 100% BS) as a monophyletic group together with *Moya* and *Tricerma*. Synapomorphies for this clade include a 1-bp deletion at position 546 in the *trnL* intron as well as having fasciculate inflorescences (albeit with reversals). *Tricerma* was unambiguously supported (100% JK / 100% BS) as nested within one of two main clades of New World *Maytenus*. *Moya* was unambiguously supported (100% JK / 100% BS and 56% JK / 66% BS on successive branches) as further nested within this same clade of New World *Maytenus*.

Within the African clade, *Maytenus peduncularis* and *Mystroxylon aethiopicum* were unambiguously supported (100% JK / 100% BS) as sister groups. Four of the six species of African *Maytenus* (*M. fasciculata, M. oleosa, M. procumbens,* and *M. undata*) were unambiguously supported (100% JK / 100% BS) as a clade. Synapomorphies for this clade include an 11-bp insertion and a 9-bp deletion in the *trnL* intron at positions 158–168 and 241–249, respectively. Otherwise, the African clade was largely unresolved with an eight-way polytomy at the base. *Maytenus abbottii*, which was not sampled for *matK*, was identified as a wildcard taxon (Nixon and Wheeler 1991) contributing to this polytomy. After exclusion of *Maytenus abbottii*, two additional clades were resolved: (*Maytenus, Mystroxylon,* and *Robsonodendron*) with 64% JK support and (*Maytenus cordata, M. filiformis, M. peduncularis,* and *Mystroxylon aethiopicum*) with 67% JK support (Fig. S1).

New World *Gymnosporia* was unambiguously supported as distinct from New World *Maytenus* by four successive clades (indicated by solid triangles on Fig. 1). New World *Gymnosporia* was well supported as sister to *Gyminda* (80% JK / 61% BS), and unambiguously distinct from it (clades of both *Gyminda* and New World *Gymnosporia* received 100% JK / 100% BS). Both *G. gentryi* and *G. urbaniana*, for which two

TABLE 1. Data-matrix and tree statistics for each of the African-and-American-*Maytenus*-clades analyses. "CI" = ensemble consistency index (Kluge and Farris 1969) on the most parsimonious tree(s) for the parsimony-informative characters. "RI" = ensemble retention index (Farris 1989).

Matrix	# terminals	# characters analyzed	# of parsimony informative characters	% missing / inapplicable	Most parsimonious tree length	# of most parsimonious trees	# of jackknife / bootstrap clades ≥ 50%	Average jackknife / bootstrap support (%)	CI	RI
26S rDNA	54	952	87	9.6	267	16,311	28 / 26	78.9 / 79.6	0.51	0.85
ITS rDNA	56	757	309	13.0	1,237	372	42 / 40	88.1 / 90.5	0.45	0.78
rDNA (ITS, 26S)	58	1,709	396	15.8	1,526	73	48 / 43	84.9 / 88.1	0.46	0.79
matK	50	1,351	121	8.2	314	17,719	23 / 27	81.7 / 80.4	0.75	0.92
trnL–F	53	1,151	122	22.3	302	19,550	27 / 33	83.4 / 79.3	0.78	0.95
plastid (*matK, trnL–F*)	55	2,502	243	20.5	623	16,230	31 / 33	87.5 / 84.7	0.75	0.93
morphology only	54	56	17	24.9	60	180	3	71.3	0.44	0.80
all molecular	58	4,211	639	21.1	2,166	148	42 / 43	91.3 / 89.7	0.52	0.83
simultaneous parsimony	58	4,267	656	21.1	2,253	18	44	90.2	0.51	0.82

specimens were sampled for each species, were highly supported as exclusive lineages.

Among the 32 clades resolved in the Old World-*Gymnosporia* simultaneous analysis, the following noteworthy clades were supported (Fig. 2). *Gymnosporia*, including *Putterlickia*, was highly supported as a clade (93% JK / 79% BS). *Putterlickia* was highly supported as a clade (98% JK / 81% BS) that is highly supported as nested within *Gymnosporia* (99% JK / 100% BS and 77% JK / 76% BS on successive branches). Synapomorphies for the clade of (*Gymnosporia harveyana, G. mossambicensis, Putterlickia pyracantha,* and *P. verrucosa*) include having seeds completely enveloped by arils, which also occurs in the two remaining species of *Putterlickia* (Jordaan and van Wyk 1998b). Synapomorphies for the clade of the two *Putterlickia* species include an increase in ovule number relative to *Gymnosporia*.

Discussion

Loesener (1942) delimited 15 sections of *Maytenus* s. s., of which nine were sampled in Fig. 1. Of the five sections for which two or more species were sampled, only *Densiflora* Loes. (from Africa) was supported as a monophyletic group. Although Loesener's (1942) sections were generally not supported, seven of his eight sections that included New World species were restricted to the New World, which is largely consistent with our revised delimitation of *Maytenus* as a strictly New World genus.

***New World Maytenus*—**Carvalho-Okano (1992) revised Loesener's (1942) New World sections of *Maytenus* by continuing to recognize *M.* section *Oxyphylla* (with the addition of *M. macrophylla* Mart.) while placing five other sections (*Coriifolia, Leptophylla*, *Microphylla*, *Pachyphylla*, and *Theoides*) within *Maytenus* section *Maytenus* Carvalho-Okano. We reject this treatment because *Maytenus* section *Oxyphylla* is highly supported as a polyphyletic group nested within *Maytenus* section *Maytenus* (Fig. 1).

A new section of *Maytenus* could be created for the clade consisting of *M. boaria, M. disticha, M. magellanica, M. verticillata*, and *M. woodsonii*, given that this clade is unambiguously supported as sister to all other species of *Maytenus* s. s. sampled. Unfortunately, we were unable to identify any fixed morphological differences between the species in these two clades. As such, we are unable to extend the current results to the numerous species of *Maytenus* that we did not sample (i.e. we cannot confidently assign those species that we did not sample to one of these two clades) or provide diagnostic morphological differences for the two potential sections.

***Moya and Tricerma*—**Although we only sampled a single species for each of these two genera, they are unambiguously supported as nested within the New World *Maytenus* (Fig. 1). This resolution is consistent with Lourteig and O'Donell's (1955) transfer of *Moya* to *Maytenus* [contra Loesener (1942)] as well as Lobreau-Callen's (1975) assertion that *Tricerma* is not distinct from *Maytenus* based on pollen morphology [contra Lundell (1971)]. Therefore, we treat both *Moya* and *Tricerma* as synonyms of *Maytenus*. No new binomials are required for either case of synonymy.

***African Maytenus*—**The African species of *Maytenus* are the most difficult group treated here with respect to both

TABLE 2. Data-matrix and tree statistics for each of the *Gymnosporia*-clade analyses. "CI" = ensemble consistency index on the most parsimonious tree(s) for the parsimony-informative characters. "RI" = ensemble retention index.

Matrix	# terminals	# characters analyzed	# of parsimony informative characters	% missing / inapplicable	Most parsimonious tree length	# of most parsimonious trees	# of jackknife / bootstrap clades ≥ 50%	Average jackknife / bootstrap support (%)	CI	RI
26S rDNA	31	943	34	12.1	83	17,268	10 / 11	70.3 / 75.7	0.59	0.77
ITS rDNA	31	719	145	5.1	400	9	26 / 27	86.0 / 86.6	0.59	0.77
rDNA (ITS, 26S)	32	1,662	179	11.9	491	91	24 / 27	82.7 / 84.3	0.58	0.76
matK	32	1,313	13	8.6	47	2,958	6 / 12	69.0 / 68.0	0.76	0.93
trnL–F	30	1,061	37	10.2	77	11,568	9 / 16	78.3 / 74.6	0.86	0.94
plastid (*matK, trnL–F*)	32	2,374	50	11.8	126	5,302	14 / 17	74.0 / 71.1	0.82	0.93
morphology only	30	56	16	27.9	25	19,020	4	64.5	0.70	0.90
all molecular	32	4,036	229	11.9	626	108	24 / 28	86.4 / 82.7	0.61	0.79
simultaneous parsimony	32	4,092	246	12.1	664	18	25	89.0	0.60	0.79

species delimitation (R. H. Archer, pers. comm. 2010) as well as assignment to genus. *Maytenus peduncularis* is unambiguously supported as sister to the monotypic *Mystroxylon* (Fig. 1), and *Mystroxylon* could be expanded to include *Maytenus peduncularis* (though we do not propose this taxonomic change here). Both species are morphologically similar other than their fruit and seed characters (R. H. Archer, pers. comm. 2010).

Maytenus fasciculata, M. oleosa, M. procumbens, and *M. undata* are unambiguously supported as a clade. The three remaining species of African *Maytenus* that we sampled (*M. abbottii, M. cordata,* and *M. filiformis*) were not resolved as a clade, even after the removal of the wildcard *M. abbottii* (Fig. S1). Some or all of these African *Maytenus* species may be placed in their own genus, perhaps resurrecting Ecklon and Zeyher's (1834–1835) *Scytophyllum* to accommodate some or all of them (R. H. Archer, pers. comm. 2010). Robert H. Archer (pers. comm. 2011) will propose new combinations for the African *Maytenus* species in a future manuscript.

Austral-Pacific Maytenus—Although no additional relevant taxa were included in this study, we use the topology of Simmons et al. (2008 Fig. 1) as the basis for our taxonomic treatment of the Austral-Pacific *Maytenus*. In that study, the following three relationships were unambiguously supported (100% JK / 100% BS): all five species of *Denhamia* sampled were resolved as a clade, all five species of Austral-Pacific *Maytenus* sampled were resolved as a clade, and *Denhamia* was sister to the Austral-Pacific *Maytenus*.

Recognition of the Austral-Pacific *Maytenus* as distinct from other lineages of *Maytenus* may be loosely traced back to Loesener (1942), wherein he assigned two Australian species [*M. bilocularis* (F. Muell.) Loes. and *M. disperma* (F. Muell.) Loes.] to their own subgenus (*Maytenus* subgenus *Pseudocelastrus*), separate from all other species of *Maytenus* (in *Maytenus* subgenus *Maytenus*). Loesener assigned a third Austral-Pacific species [*M. cunninghamii* (Hook.) Loes.] to its own monotypic section within *Maytenus* subgenus *Maytenus*, and a fourth species [*M. fournieri* (Pancher & Sebert) Loes.] into *Maytenus* section *Laxifolia* with 10 other Old World species [three of which have since been synonymized with *M. fournieri* by Müller (1996)]. The remaining four species of Austral-Pacific *Maytenus* were described in 1962–1984, after Loesener's death.

Because we lack any known morphological characters that distinguish all seven species of *Denhamia* (Jessup 1984) from all eight species (and one subspecies) of Austral-Pacific *Maytenus*, we simply transfer those eight species of *Maytenus* to *Denhamia* rather than proposing a new genus for them. We do not include *Maytenus rapakir* Loes. in *Denhamia* because Jordaan and van Wyk (2003) noted that this species probably belongs to *Gymnosporia*.

New World Gymnosporia—The New World *Gymnosporia* are unambiguously supported as a clade distinct from both the New World *Maytenus* and *Gyminda* (Fig. 1). Although we were unable to identify any morphological synapomorphies for the New World *Gymnosporia*, they are clearly morphologically distinct from *Gyminda*, for which Simmons et al. (2008) identified four morphological synapomorphies. Based on our sampling of all three species of New World *Gymnosporia*, their unambiguous support as a clade distinct from other genera, and their morphological distinctiveness from *Gyminda*, we transfer these species to a new genus, *Haydenia*, in honor of W. John Hayden (1951-).

Old World Gymnosporia—Jordaan and van Wyk (2006) recognized eight sections of *Gymnosporia*, of which four are monotypic. Four of the eight sections were sampled here (Fig. 2). Only one of the three sections for which two or more species were sampled was resolved as monophyletic (*Gymnosporia* section *Mossambicensis*), while the other two sections were resolved as (ambiguously) paraphyletic groups. Based on our tree topology, *Gymnosporia* section *Tenuispinae* appears to be delimited based on plesiomorphic character states given that the other sections are nested within it, though it may be natural if it is more narrowly defined.

Putterlickia is highly supported as a clade nested within *Gymnosporia* (Fig. 2), as previously inferred by Simmons et al. (2008) with less sampling of *Gymnosporia*. As noted in the introduction, only a single questionably fixed character-state difference (ovule number) has been reported between all species of *Gymnosporia* and all species of *Putterlickia*. Although all four species of *Putterlickia* are probably a clade (that we expect includes *Gloveria*; see below), recognition of the genus makes *Gymnosporia* paraphyletic.

Despite multiple attempts using four separate specimens, we were unable to successfully isolate and amplify DNA from *Gloveria*. As such, we did not include *Gloveria* in this study (an exploratory study in which *Gloveria* was included but only coded for morphological characters resulted in an unresolved tree in large part because of the high amount of missing morphological data for some species). Nonetheless, *Gloveria* and *Putterlickia* have nearly adjacent native ranges (Jordaan and van Wyk 1998a), both have an increase in ovule number per locule, and both have the putative synapomorphy of functionally bisexual flowers (in contrast to the generally unisexual flowers of *Gymnosporia*; Jordaan and van Wyk 1998b). Likewise, arils that ± completely envelop seeds are a probable synapomorphy for this clade. *Gloveria* is morphologically distinct from *Putterlickia* in having more than one node on each thorn (Jordaan and van Wyk 1999), but this is clearly an autapomorphy for *Gloveria integrifolia* (L. f.) Jordaan. Finally, Robson (1965) had previously treated the species currently recognized as *Gloveria integrifolia* as a synonym of *Putterlickia pyracantha*.

Based on the three probable morphological synapomorphies cited above that unite *Gloveria* and *Putterlickia*, we consider both genera to be a clade nested within a paraphyletic *Gymnosporia*. Therefore, we believe that all five species should be transferred to *Gymnosporia*. *Gloveria integrifolia* already has a name within *Gymnosporia* [*G. integrifolia* (L. f.) Glover], but new binomials are needed for three of the four species of *Putterlickia* [not including *Gymnosporia saxatilis* (Burch.) Davison]. Although *Celastrus* section *Gymnosporia* was published in 1834, it was not raised to the genus level until 1862, after *Putterlickia* was published in 1840. Therefore, according to article 11.2 of the International Code of Botanical Nomenclature (McNeill et al. 2007), *Gymnosporia* must first be conserved against *Putterlickia* before new binomials may be proposed for those three species.

Based on the resolution of *Gymnosporia* in Fig. 2 in the context of the native ranges of the species sampled, we infer an African origin of the genus that was followed by at least two successful dispersals into Madagascar and one dispersal from Madagascar to Mauritius. Another lineage of *Gymnosporia* dispersed into South Asia, which then later dispersed into Pacific Ocean islands. We infer that an ancestor of *G. cassinoides*, which is endemic to the Canary Islands, dispersed there from Africa.

Taxonomic Treatment

Nine new combinations for Austral-Pacific *Maytenus*, here transferred to *Denhamia*, are as follows.

Denhamia bilocularis (F. Muell.) M. P. Simmons, comb. nov. *Maytenus bilocularis* (F. Muell.) Loes., Nat. Pflanzenfam., ed. 2, 20b: 135. 1942. *Celastrus bilocularis* F. Muell., Trans. & Proc. Philos. Inst. Victoria 3: 31. 1859.

Denhamia cunninghamii (Hook.) M. P. Simmons, comb. nov. *Maytenus cunninghamii* (Hook.) Loes., Nat. Pflanzenfam., ed. 2, 20b: 136. 1942. *Celastrus cunninghamii* (Hook.) F. Muell., Trans. & Proc. Philos. Inst. Victoria 3: 30. 1859. *Catha cunninghamii* Hook., in T. Mitchell, J. Exped. Trop. Austral. 387. 1848.

Denhamia cupularis (Ding Hou) M. P. Simmons, comb. nov. *Maytenus cupularis* Ding Hou, Fl. Males., Ser. 1, Spermat. 6: 243. 1963.

Denhamia disperma (F. Muell.) M. P. Simmons, comb. nov. *Maytenus disperma* (F. Muell.) Loes., Nat. Pflanzenfam., ed. 2, 20b: 135. 1942. *Celastrus dispermus* F. Muell., Trans. & Proc. Philos. Inst. Victoria 3: 31. 1859.

Denhamia fasciculiflora (Jessup) M. P. Simmons, comb. nov. *Maytenus fasciculiflora* Jessup, Fl. Australia 22: 223. 1984.

Denhamia ferdinandii (Jessup) M. P. Simmons, comb. nov. *Maytenus ferdinandi* Jessup, Fl. Australia 22: 223. 1984.

Denhamia fournieri (Pancher & Sebert) M. P. Simmons, comb. nov. *Maytenus fournieri* (Pancher & Sebert) Loes., Nat. Pflanzenfam., ed. 2, 20b: 137. 1942. *Gymnosporia fournieri* (Pancher & Sebert) Loes., Bot. Jahrb. Syst. 39: 163. 1906. *Celastrus fournieri* Pancher & Sebert, Rev. Marit. et Colon 41: 209. 1874.

Denhamia fournieri (Pancher & Sebert) M. P. Simmons subsp. **drakeana** (Loes.) M. P. Simmons, comb. nov. *Maytenus fournieri* (Pancher & Sebert) Loes. subsp. drakeana (Loes.) I. H. Müller, Fl. Nouv.-Calédonie 20: 73. 1996. *Gymnosporia drakeana* Loes., Bot. Jahrb. Syst. 39: 160. 1906.

Denhamia silvestris (Lander & L. A. S. Johnson) M. P. Simmons, comb. nov. *Maytenus silvestris* Lander & L. A. S. Johnson, Contr. New South Wales Natl. Herb. 4: 373. 1973.

New binomials are required for four Chinese species of *Gymnosporia* that have been previously treated only as *Maytenus*. Three of these species were treated by Ma et al. (2008) as part of *Maytenus* despite their recognition of *Gymnosporia* as a distinct genus. None of these four species were treated by Jordaan and van Wyk (2006). All four species were confirmed as having thorns as reported by Sha et al. (1981), Ma et al. (2008), and/or Qin et al. (2008). Two species recognized by Ma et al. (2008) as *Maytenus* already have binomials within *Gymnosporia*.

The only doubtful species of *Maytenus* s. s. in China is *M. inflata* S. J. Pei & Y. H. Li. This species is not described (or illustrated) as having thorns in either Pei and Li (1981) or Ma et al. (2008). But given that Pei and Li (1981) based their species description for *M. inflata* by comparing it to *Gymnosporia wallichiana* Spreng., together with the geographical distribution of *M. inflata*, this species is most likely a *Gymnosporia*. Nonetheless, given the ambiguity, we do not transfer the species to *Gymnosporia* at this time.

Gymnosporia austroyunnanensis (S. J. Pei & Y. H. Li) M. P. Simmons, comb. nov. *Maytenus austroyunnanensis* S. J. Pei & Y. H. Li, Acta Bot. Yunnan. 3: 245. 1981.

Gymnosporia confertiflora (J. Y. Luo & X. X. Chen) M. P. Simmons, comb. nov. *Maytenus confertiflora* J. Y. Luo & X. X. Chen, Acta Phytotax. Sin. 19: 233. 1981.

Gymnosporia dongfangensis (F. W. Xing & X. S. Qin) M. P. Simmons, comb. nov. *Maytenus dongfangensis* F. W. Xing & X. S. Qin, Bot. J. Linn. Soc. 158: 534. 2008.

Gymnosporia guangxiensis (C. Y. Cheng & W. L. Sha) M. P. Simmons, comb. nov. *Maytenus guangxiensis* C. Y. Cheng & W. L. Sha, Acta Phytotax. Sin. 19: 232. 1981.

A new combination is needed for *Maytenus pertinax*, which is clearly a derived member of *Gymnosporia* (Fig. 2). But future detailed investigation of herbarium specimens may reveal that this taxon is conspecific with *Gymnosporia vitiensis* Seem. (R. H. Archer, pers. comm. 2010).

Gymnosporia pertinax (N. Hallé & J. Florence) M. P. Simmons, comb. nov., *Maytenus pertinax* N. Hallé & J. Florence, Dir. Centres d'Expér. Nucl., Service Mixte Contrôle Biol., Rapa 138. 1986 publ. 1987.

A new genus, as well as new species combinations, are needed for the three New World species of *Gymnosporia*, which are unambiguously supported as a clade sister to *Gyminda* (Fig. 1). The Latin diagnosis is based on Hammel's (1997, p. 149) comparison of New World *Gymnosporia* with New World *Maytenus*. The new genus is named in honor of W. John Hayden (1951-), who began M. P. S. on his research career in systematic botany.

Haydenia M. P. Simmons genus novum—TYPE: *Haydenia urbaniana* (Loes.) M. P. Simmons.

Maytenus Molina affine, sed integrifoliis virescentibus in sicco, inflorescentiis cymosa, large laxa, 4-meris floribus, fructibus frequenter 2–4 seminibus praeditis differt.

Haydenia gentryi (Lundell) M. P. Simmons, comb. nov., *Gymnosporia gentryi* Lundell, *Phytologia* 57: 313. 1985.

Haydenia haberiana (Hammel) M. P. Simmons, comb. nov., *Gymnosporia haberiana* Hammel, *Novon* 7: 147. 1997.

Haydenia urbaniana (Loes.) M. P. Simmons, comb. nov., *Gymnosporia urbaniana* (Loes.) Liesner, Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1254. 1993. *Rhacoma urbaniana* Loes., Repert. Spec. Nov. Regni Veg. 1: 162. 1905.

Gymnosporia magnifolia (Loes.) Lundell, *Phytologia* 57: 314. 1985. *Maytenus magnifolia* Loes., Vernh. Bot. Vereins Prov. Brandenburg 48: 176. 1906, publ. 190).

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Appendix 1. List of taxa sampled with taxonomic authorities, voucher information and GenBank accession numbers for new sequences (26S rDNA, ITS rDNA, *matK*, *trnL* intron, *trnL–F* spacer) generated for this study.

Gyminda tonduzii Loes.—*C. C. Clevinger 110*, Mexico (TEX); HQ267200, HQ267170, HQ267102, HQ267147, HQ267124. ***Gymnosporia crenata*** Seem.—*D. H. Lorence 8956*, Marquesas Islands (PTBG); HQ267195, HQ267165, HQ267097, HQ267142, HQ267119. ***Gymnosporia gentryi*** Lundell—*F. Hurtado et al. 1866,* Ecuador (MO); HQ267201, HQ267171, —, —, —. ***Gymnosporia gentryi*** Lundell—*F. Hurtado & A. Alvarado 2267,* Ecuador (MO); HQ267202, HQ267172, —, —, —. ***Gymnosporia littoralis*** (Backer) Jordaan—*T. Flynn 5472,* cult. Hawaii (PTBG); HQ267196, HQ267166, HQ267098, HQ267143, HQ267120. ***Gymnosporia palauica*** Loes.—*D. H. Lorence 9665,* Palau (PTBG); HQ267197, HQ267167, HQ267099, HQ267144, HQ267121. ***Gymnosporia wallichiana*** (Spreng.) M. A. Lawson—*D. H. Lorence 9936,* cult. Hawaii (PTBG); HQ267198, HQ267168, HQ267100, HQ267145, HQ267122. ***Maytenus abbottii*** A. E.van Wyk—*A. T. D. Abbott 8902,* South Africa (PRE); HQ267203, HQ267173, —, HQ267148, HQ267125. ***Maytenus aquifolium*** Mart.—*J. A. Lombardi 6831,* Brazil (HRCB); HQ267204, HQ267174, HQ267103, HQ267149, HQ267126. ***Maytenus buxifolia*** Griseb.—Fairchild Tropical Garden Acc. #FTBG-63293A; HQ267205, HQ267175, —, HQ267150, —. ***Maytenus distichophylla*** Cuatrec.—*J. A. Lombardi 7212,* Brazil (HRCB); HQ267206, HQ267176, HQ267104, HQ267151, HQ267127. ***Maytenus domingensis*** Krug. & Urb.— *T. Chase et al. 09-17*, Dominican Republic (CS); HQ267207, HQ267177, HQ267105, —, —. ***Maytenus elliptica*** Krug & Urb.—Fairchild Tropical Garden Acc. #FTBG-91503B; HQ267208, HQ267178, HQ267106, HQ267152, HQ267128. ***Maytenus elongata*** Britton—*C. M. Taylor & J. Bithorn 11823,* Puerto Rico (MO); HQ267209, HQ267179, HQ267107, HQ267153, —. ***Maytenus evonymoides*** Reissek—*J. A. Lombardi 6874,* Brazil (HRCB); HQ267210, HQ267180, HQ267108, HQ267154, HQ267129. ***Maytenus filiformis*** ined.—*A. T. D. Abbott 8901,* South Africa (PRE); HQ267211, HQ267181, HQ267109, HQ267155, HQ267130. ***Maytenus grenadensis*** Urb.—*D. Jules et al. 09-51*, Grenada (CS); —, HQ267182, —, —, —. ***Maytenus ilicifolia*** Mart. ex Reissek—*A. Jardim & A. Cadden 2162*, Bolivia (F); HQ267212, HQ267183, HQ267110, HQ267156, HQ267131. ***Maytenus magellanica*** Hook. f.—*N. Goodall 753*, Argentina (US); HQ267213, HQ267184, HQ267111, HQ267157, HQ267132. ***Maytenus magellanica*** Hook. f.—*O. Zollner 8438*, Chile (MO); HQ267214, HQ267185, —,HQ267158, HQ267133. ***Maytenus* aff.** *obtusifolia* Mart.—*J. A. Lombardi 7213,* Brazil (HRCB); —,HQ267186, —, —, HQ267134. ***Maytenus oleosa*** A. E. van Wyk & R. H. Archer—*A. T. D. Abbott 8903,* South Africa (PRE); HQ267215, HQ267187, HQ267112, HQ267159, HQ267135. ***Maytenus peduncularis*** (Sond.) Loes.—*A. T. D. Abbott 8905,* South Africa (PRE); HQ267216, HQ267188, HQ267113, HQ267160, HQ267136. ***Maytenus pertinax*** N. Hallé & J. Florence—*T. J. Motley & R. Fenstemacher 2716,* French Polynesia, Rapa (PTBG); HQ267199, HQ267169, HQ267101, HQ267146, HQ267123. ***Maytenus procumbens*** (L. f.) Loes.—*B. Albanese s. n.*, cult. Florida, U. S. A. (PRE); —,HQ267189, HQ267114, HQ267161, HQ267137. ***Maytenus rigida*** Mart.—*J. A. Lombardi 7219,* Brazil (HRCB); HQ267217, HQ267190, —, —, HQ267138. ***Maytenus robusta*** Reissek—*J. A. Lombardi 6902,* Brazil (HRCB); HQ267218, HQ267191, HQ267115, HQ267162, HQ267139. ***Maytenus salicifolia*** Reissek—*J. A. Lombardi 6666,* Brazil (HRCB); HQ267219, HQ267192, HQ267116, HQ267163, HQ267140. ***Maytenus verticillata*** DC.—*M. Merello et al. 1100,* Peru (MO); HQ267220, HQ267193, HQ267117, —, —. ***Maytenus woodsonii*** Lundell—*R. L. Wilbur 26083,* Costa Rica (F); HQ267221, HQ267194, HQ267118, HQ267164, HQ267141.

Appendix 2. List of morphological characters included in the simultaneous analyses.

1) thorn presence: 0 = absent, 1 = present, 2) stem apices: 0 = terminating bluntly, 1 = terminating in sharp points; 3) presence of glands on stems: 0 = absent, 1 = present; 4) phyllotaxy on vegetative shoots: 0 = alternate, 1 = opposite or whorled; 5) phyllotaxy on plants with alternate leaves: 0 = strictly alternate, 1 = alternate on vegetative shoots opposite on thorns or flowering shoots; 6) phyllotaxy on plants with opposite leaves: 0 = strictly opposite, 1 = opposite or whorled; 7) leaf form: 0 = planar, 1 = sessile delta-shaped scales, 2 = cataphylls, 3 = needle-like; 8) presence of upper pulvinus on leaves: 0 = absent, 1 = present; 9) leaf pubescence: 0 = without stellate hairs, 1 = with stellate hairs; 10) leaf venation: 0 = pinnate, 1 = acrodromous; 11) leaf position: 0 = more or less evenly spaced, 1 = fascicled on short branches; 12) inflorescence position: 0 = axillary, 1 = at least some inflorescences terminal, 2 = epiphyllous or rarely axillary 'leaf-opposed', 3 = cauline; 13) inflorescence type: 0 = cymose, 1 = thyrsoid to racemose, 2 = umbel, 3 = fasciculate, 4 = flowers solitary; 14) flower sexuality: 0 = unisexual, 1 = bisexual; 15) unisexual-flowered plants: 0 = dioecious, 1 = monoecious; 16) perianth merosity: 0 = 4-merous, 1 = 5-merous, 2 = 3-merous; 17) petal margin: 0 = entire, ciliate, or irregularly toothed, 1 = regularly toothed; 18) petal connation: 0 = free, 1 = free at base, connate above; 19) disk presence: 0 = absent, 1 = present; 20) disk division: 0 = continuous, 1 = discontinuous; 21) disk shape: 0 = cupular or columnar, 1 = annular, flat, or margins upturned; 22) disk pubescence: 0 = glabrous, 1 = conspicuously puberulent; 23) androgynophore presence: 0 = absent, 1 = present; 24) stamen plus staminode number: 0 = three or generally three; 1 = same as petal number, 2 = two, 3 = more than petal number; 25) fertile stamen length: 0 = all of a single length, 1 = of two different lengths; 26) staminode presence in same flower with functional stamens: 0 = absent, 1 = present; 27) numerous stamen arrangement: 0 = unicyclic, 1 = bicyclic; 28) numerous stamen number: 0 = twice the number of petals,

1 = more than twice the number of petals, 2 = less than twice the number of petals; 29) filament insertion relative to disk: 0 = at outer disk margin, 1 = on top of disk, 2 = inside inner edge of disk; 30) anther dehiscence plane: 0 = longitudinal, 1 = oblique, 2 = transverse; 31) anther attachment: 0 = basifixed, 1 = dorsifixed; 32) anther versatility: 0 = fixed, 1 = versatile; 33) connective extension shape: 0 = absent or apiculate, 1 = bilobed, 2 = large ornamented extension, 3 = gland-tipped; 34) pollen aggregation: 0 = monads, 1 = tetrads or polyads; 35) pollen annulus presence: 0 = absent, 1 = present; 36) ovary pubescence: 0 = glabrous, 1 = completely pilose, 2 = strigose, 3 = tomentose, 4 = dense hirsute, 5 = puberulent, 6 = stellate; 37) shape of ovary apex: 0 = contiguous, 1 = apical hollow; 38) style position on fruit: 0 = absent or terminal, 1 = lateral; 39) carpel number: 0 = one, 1 = two, 2 = three, 3 = perianth merosity, 4 = twice perianth merosity, 5 = four, when not equal perianth merosity, 6 = five, when not equal perianth merosity; 40) ovary septa walls: 0 = complete, 1 = incomplete, 2 = absent; 41) ovule number per locule: 0 = one, 1 = two or four, 2 = variable and more than four; 42) placentation: 0 = axile, 1 = parietal; 43) axile ovule attachment: 0 = basal to axile, 1 = erect to horizontal, 2 = pendulous; 44) fruit type: 0 = dehiscent, 1 = indehiscent, 2 = cocci; 45) indehiscent fruit type: 0 = drupe, 1 = berry, 2 = samara, 3 = nut or achene; 46) fruit wing form: 0 = at apex, 1 = at side, 2 = along each locule; 47) capsular fruit shape: 0 = lobed but not parted or flattened among locules, 1 = strongly parted among locules, 2 = flattened along each locule but not parted; 48) mericarp connation: 0 = separate, 1 = connate; 49) capsular fruit dehiscence: 0 = loculicidal, 1 = one side laterally split, 2 = septicidal, 3 = loculicidal and septicidal; 50) aril presence: 0 = present, 1 = absent; 51) aril position on seed: 0 = entirely enveloping seed, 1 = partly enveloping seed; 52) aril form: fleshy or thin membranous, 1 = mucilagenous pulp, 2 = wing surrounding seed with medial or basal attachment of funiculus, 3 = basal wing with vasculature of the funiculus attached above wing; 53) non-arillate basal seed wing presence: 0 = absent, 1 = present; 54) basal non-arillate seed wing form: 0 = membranous, papyraceous, or thin coriaceous, 1 = narrow stipe; 55) raphe branching: 0 = unbranched, 1 = branched; 56) endosperm presence: 0 = present, 1 = absent.