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Phylogeny of Celastraceae tribe Euonymeae inferred from morphological characters and nuclear and plastid genes

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ABSTRACT

The phylogeny of Celastraceae tribe Euonymeae (~230 species in eight genera in both the Old and New Worlds) was inferred using morphological characters together with plastid (*matK*, *trnL-F*) and nuclear (ITS and 26S rDNA) genes. Tribe Euonymeae has been defined as those genera of Celastraceae with generally opposite leaves, isomerous carpels, loculicidally dehiscent capsules, and arillate seeds (except *Microtropis*). *Euonymus* is the most diverse (129 species) and widely cultivated genus in the tribe. We infer that tribe Euonymeae consists of at least six separate lineages within Celastraceae and that a revised natural classification of the family is needed. *Microtropis* and *Quetzalia* are inferred to be distinct sister groups that together are sister to *Zinowiewia*. The endangered *Monimopetalum chinense* is an isolated and early derived lineage of Celastraceae that represents an important component of phylogenetic diversity within the family. *Hedraianthera* is sister to *Brassiantha*, and we describe a second species (*Brassiantha hedraiantheroides* A.J. Ford) that represents the first reported occurrence of this genus in Australia. *Euonymus globularis*, from eastern Australia, is sister to *Menepetalum*, which is endemic to New Caledonia, and we erect a new genus (*Dinghoua* R.H. Archer) for it. The Madagascan species of *Euonymus* are sister to *Pleurostylia* and recognized as a distinct genus (*Astrocassine* ined.). *Glyptopetalum*, *Torralbasia*, and *Xylonymus* are all closely related to *Euonymus* sensu stricto and are questionably distinct from it. Current intrageneric classifications of *Euonymus* are not completely natural and require revision.

1. Introduction

In the most recent comprehensive classification of Celastraceae, Loesener (1942) recognized tribe Euonymeae as including *Euonymus* L., *Glyptopetalum* Thwaites, *Hedraianthera* F. Muell., *Microtropis* Wall. ex Meisn., *Monimopetalum* Rehder, *Otherodendron* Makino (= *Microtropis*; Merrill and Freeman, 1940), and *Torralbasia* Krug. and Urb. Loesener (1942) defined Euonymeae as those genera of Celastraceae with generally opposite leaves, isomerous carpels, loculicidally dehiscent capsules, and arillate seeds (except *Microtropis*).

Previous delimitations of Euonymeae were both narrower and broader. Loesener (1892) restricted Euonymeae to just four genera (*Euonymus*, *Glyptopetalum*, *Lophopetalum* Wight ex Arn., and *Microtropis*), whereas Bentham and Hooker (1862) included those four genera together with eight others that are currently recognized as members of Celastraceae (*Cassine* L., *Catha* Forssk. ex Scop., *Kokoona* Thwaites, *Lauridia* Eckl., and Zeyh., *Paxistima* Raf., *Pleurostylia* Wight and Arn., *Ptelidium* Thouars, and *Zinowiewia* Turcz.). *Quetzalia* Lundell was later recognized as a segregate genus for the New World species of *Microtropis* (Lundell, 1970), and *Xylonymus* Kalkman was added as a monotypic genus that Kalkman (in Ding Hou, 1962) suggested is closely related to *Euonymus*.

As currently defined, Euonymeae include eight genera and about 230 species that are broadly distributed in both the Old and New Worlds. Four genera are monotypic (*Hedraianthera* in Australia, *Monimopetalum* in China, *Torralbasia* in the Caribbean, and *Xylonymus* in New Guinea), whereas the most diverse extant genus is *Euonymus*, with 129 species (Ma, 2001). Although Blakelock (1951) recognized at least 176 species of *Euonymus*, Ma (2001) criticized Blakelock for over-splitting at both the species and variety levels. *Euonymus* is widespread, primarily in the Northern Hemisphere of the Old and New Worlds, with the center of diversity (~95 species) in East Asia (Ma, 2001).

Many species of *Euonymus* are economically important for both traditional medicine and horticulture. For example, Gaur et al. (1986) described how various parts of *Euonymus tingens* Wall. are used for medicines, a dye, fodder, and timber and have been incorporated into folk songs in the Garhwal Himalaya. Other species are

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variously used as sources of oil and/or gutta-percha, to kill lice, and for wood turning (Blakelock, 1951). About 42 species of *Euonymus* are cultivated in temperate Europe and North America as ornamental hedges, groundcover, and/or for their fall leaf, fruit, and aril color (Rehder, 1934; Bailey et al., 1976; Huxley et al., 1992). *Euonymus alatus* (Thunb.) Siebold ("burning bush," "winged spindle tree") and *Euonymus fortunei* (Turcz.) Hand.-Mazz. ("wintercreeper euonymus"] in particular are extensively cultivated.

Many authors since 1825 have recognized subgenera, sections, and/or series within *Euonymus* (reviewed by Blakelock (1951)), with the most recent comprehensive classifications provided by Blakelock (1951) and Ma (2001). Blakelock (1951) recognized two subgenera, seven sections, and 14 series. Note that one subgenus contains only a single section and three sections each contain only a single series. Ma (2001) recognized the same two subgenera as Blakelock (1951), but only five sections and no series. Subgenus *Kalonymus* R. Beck. contains a single section in both classifications. Ma's (2001) sections differ from Blakelock's (1951) in that Ma grouped together sections *Biloculares*, *Multiovulatus*, and *Stenocarpus* into section *Euonymus*. Savinov and Baikov (2007) proposed a revised classification of *Euonymus* that consists of the same two subgenera together with 12 sections and eight series, but they only treated 57 European and Asian species. They favored Blakelock's (1951) classification over that provided by Ma (2001; Savinov, 2007).

The three primary goals of this study are to infer intergeneric relationships among Euonymeae, test the monophyly of genera within Euonymeae, and test the intrageneric classifications of *Euonymus* proposed by Blakelock (1951) and Ma (2001). Of particular interest is whether *Euonymus*, *Glyptopetalum*, *Microtropis*, and *Quetzalia* are natural genera. Perrier de la Bâthie (1942) expressed doubts whether the Madagascan species of *Euonymus* are properly included within that genus, and Ma (2001) only tentatively assigned them to section *Euonymus*, pending study of additional specimens. Ding Hou (1975) suggested that the Australian *Euonymus globularis* Ding Hou may be distinct from *Euonymus*. Likewise, den Hartog and Baas (1978) recognized *E. globularis* as distinct from three other species of *Euonymus* examined based on its stomate type and undulating leaf anticlinal walls. Baillon (1880) treated *Glyptopetalum* as a section of *Euonymus* whereas Thwaites (1856), Bentham and Hooker (1862), Ding Hou (1963) and Savinov (2007) recognized the two genera as distinct but closely related. Finally, Lundell's (1970) segregation of *Quetzalia* from *Microtropis* is clearly delimited based on geographical distribution, but it may render one of the genera paraphyletic.

To address these goals, we used the taxon and character sampling from Simmons et al. (2008) as a basis with which to substantially increase our taxon sampling for seven of the eight Euonymeae genera. Only two (*Euonymus* and *Quetzalia*) of the eight Euonymeae genera have previously been sampled in molecular phylogenetic analyses. Just one or two species of *Euonymus* have been included in each of these studies, often serving as an exemplar for Celastraceae (e.g., Soltis et al., 2000). Sequence data were generated from two nuclear gene regions (26S rDNA and the internal transcribed spacers [ITS of rDNA]), and two plastid loci (maturase K [*matK*] and *trnL-F*). These data were analyzed together with morphological characters and 18S rDNA, *atpB*, *phyB*, and *rbcL* sequences generated in previous analyses.

2. Methods

2.1. Taxon sampling

A total of 129 taxa were sampled (Appendix A; 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 nine taxa for a total of 138 terminals included in the simultaneous analyses (Kluge, 1989; Nixon and Carpenter, 1996). Forty-nine new samples were included, including 28 from *Euonymus* (Appendix A). Sequences downloaded from GenBank were newly incorporated for *Euonymus americanus* (26S rDNA: EU002149, *atpB*: EU002160, *matK*: EU002170, and *rbcL*: EU002277) and *E. hamiltonianus* (18S rDNA: AB233630, *atpB*: AB233734, and *rbcL*: AB233942).

M.P. Simmons 1776 (BH), which was treated as *Tripterygium wilfordii* Hook. f. in Simmons et al. (2008) following Ma et al. (1999), is now properly treated as *Tripterygium regelii* Sprague and Takeda based on Law et al.'s (2011) finding that the two species are phylogenetically distinct from each other. The corresponding GenBank records were updated in September 2010.

Preliminary parsimony tree searches based on the taxon sampling used by Simmons et al. (2008) indicated that all new samples were resolved as early derived members of the family (for *Microtropis*, *Monimopetalum*, and *Quetzalia*) or within clades 1 (an Austral-Pacific clade that includes *Brassiantha*, *Euonymus globularis*, and *Hedraianthera*), 2 (an Asian-New World clade that includes *Euonymus*, *Glyptopetalum*, and *Torralbasia*), and the sister group of the former Hippocrateaceae that includes the most recent common ancestor of *Elaeodendron* and *Polycardia* (*Astrocassine* was resolved within this clade). All four of these lineages were well-supported ($\geqslant$95% parsimony jackknife support [JK; Farris et al., 1996]; $\geqslant$86% likelihood bootstrap support [BS; Felsenstein, 1985]) as distinct from the other lineages sampled 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 four lineages. Lepidobotryaceae and Parnassiaceae were used as outgroups following Zhang and Simmons (2006).

2.2. Morphological characters

Morphological characters were derived from matrices previously published by Simmons and Hedin (1999), Simmons et al. (2001a, 2001b, 2008), Islam et al. (2006) and Coughenour et al. (2010). For the 129 taxa sampled in this study, 38 characters are parsimony informative, representing variation in vegetative and floral morphology, leaf and seed anatomy, and pollen morphology. To the degree 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, pp. 746–751). All morphological characters, including both character and character-state definitions, are included as part of the simultaneous-analysis data matrix that has been posted as online supplementary data on the journal's website.

2.3. 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 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 the Qiagen PCR Purification Kit. 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 HQ393636 to HQ393854 (Appendix A).

2.4. Data analysis

Preliminary nucleotide alignments were obtained independently for each gene region using MAFFT ver. 6.5 (Katoh and Toh, 2008a). Q-INS-i, which considers inferred secondary structure of rDNA (Katoh and Toh, 2008b), was used for alignments of 18S, ITS, and 26S rDNA. G-INS-i, the most accurate MAFFT algorithm for aligning loci other than rDNA, was used for all other loci. The 20PAM nucleotide scoring matrix was used for the more divergent ITS and *trnL-F* loci, whereas the 1PAM matrix was used for all other loci. The default gap opening penalty was applied (1.53) and the gap offset value was set to 0.1.

Manual adjustments to the MAFFT alignments were performed in MacClade ver. 4.03 (Maddison and Maddison, 2001) using the procedure outlined by Simmons (2004) 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 such that 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 636 ambiguously-aligned positions were excluded from the analyses (ITS: 373 positions from 12 regions; *trnL-F*: 263 positions from seven regions). Ambiguously-aligned nucleotides of individual sequences in regions that could not be unambiguously aligned with the remaining sequences were scored as ambiguous ("?").

Two inversions were inferred in the *trnL-F* intergenic spacer: a 42-bp inversion between positions 610–673 in *Canotia holacantha* and *Lophopetalum arnhemicum* Byrnes, and a 62-bp inversion between positions 603–686 in *Euonymus* aff. *carnosus*. These inversions were rotated in the alignment to reflect the ancestral orientation and a character was added to the analysis to reflect these inversions in an analogous manner to uninode coding (Simmons et al., 2000).

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). A total of 118 parsimony-informative complex-indel-coding gap characters were scored from unambiguously aligned regions (18S rDNA: 5; 26S rDNA: 8; ITS: 37; *matK*: 7; *trnL-F*: 61) for inclusion in the 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 eight gene regions was analyzed independently of 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 three adjacent rDNA gene regions and the four plastid loci were analyzed independently of one another 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. The simultaneous-analysis data matrix is posted as supplemental online data.

Equally weighted parsimony tree searches were conducted for each data matrix using 2000 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). The models selected all incorporated the gamma distribution. The Q-matrices selected were all variants of TIM, TPM, TVM, or GTR.

Maximum likelihood (Felsenstein, 1973) analyses of nucleotide characters from each of the molecular data matrices were performed as tests for long-branch attraction (Felsenstein, 1978), though they are fallible (Gaut and Lewis, 1995; Siddall, 1998; Sanderson and Kim, 2000). 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. Optimal likelihood trees were searched for using 1000 independent searches starting from randomized parsimony trees with the GTRGAMMA model and four discrete rate categories. Likelihood BS analyses were conducted with 2000 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, p. 9).

3. Results

A simplified version (wherein selected genera that are not the focus of this study are represented by single terminals) of the simultaneous-analysis parsimony strict consensus tree of all eight gene regions and morphological characters is presented in Fig. 1 with parsimony JK values $\geqslant$50% above each branch and likelihood BS values $\geqslant$50% below each branch for likelihood analysis of

nucleotide characters from all eight gene regions. The complete tree is presented in Fig. S1 as supplemental online data. Equivalent trees for each of the 12 partitioned analyses listed in Table 1 are presented in Figs. S2–S13. These trees were created using TreeGraph 2 (Stöver and Müller, 2010). Support values were mapped onto the parsimony strict consensus tree so as to help minimize frequency-within-replicates (Davis et al., 1998) and undersampling-within-replicates BS and JK artifacts (Simmons

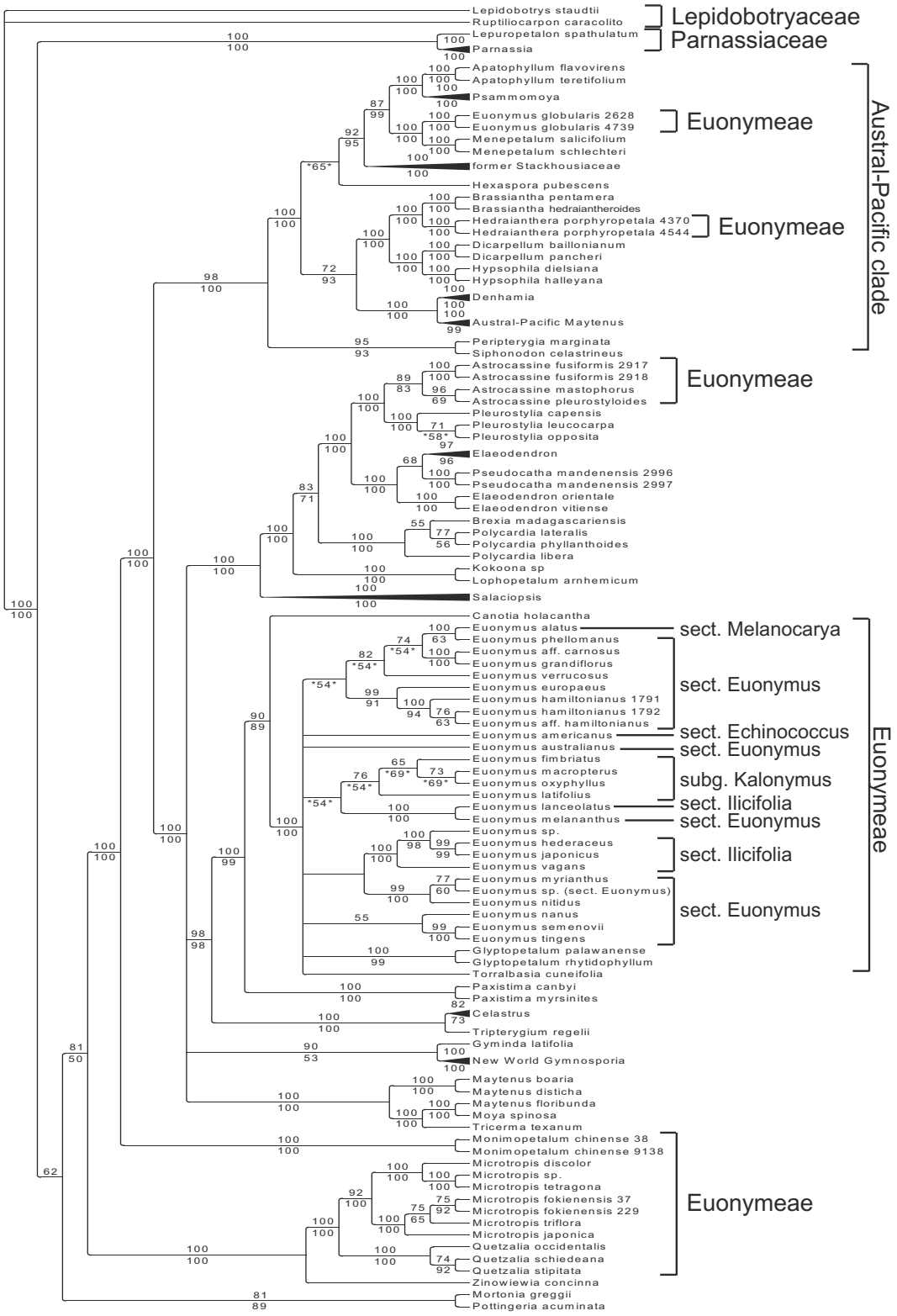

Fig. 1. Simplified simultaneous-analysis parsimony strict consensus tree with parsimony JK values above each branch and likelihood BS values for all nucleotide characters below each branch. Clades that were contradicted by ⩾50% BS or JK support are indicated by *XX* with BS/JK support for the contradictory clade with the highest support listed. The *Euonymus* subgenera and sections recognized by Ma (2001) are shown; all sections noted belong to *Euonymus* subgenus *Euonymus*.

Table 1
Data-matrix and tree statistics for each of the 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
18S rDNA	29	2486	136	31.1	340	20	15/16	81.3/78.8	0.62	0.84
26S rDNA	132	983	220	9.9	1059	960	68/71	85.3/81.5	0.34	0.81
ITS rDNA (no Stack.)	124	659	324	19.2	2217	1260	70/74	89.4/87.6	0.31	0.75
rDNA (18S, ITS, 26S)	138	4128	681	58.9	3665	90	83/99	91.5/88.5	0.39	0.77
phyB	20	1123	185	6.1	644	4	13/14	92.8/86.1	0.63	0.64
atpB	30	1506	207	5.0	546	3	21/25	85.9/82.4	0.65	0.85
matK	130	1409	546	13.6	1667	17,850	86/93	87.5/86.4	0.57	0.86
rbcL	31	1428	163	3.2	527	6	24/17	76.3/75.5	0.56	0.73
trnL-F	134	1633	500	42.4	1540	18,870	89/103	85.2/83.0	0.63	0.90
Plastid (*atpB* → *trnL-F*)	135	5976	1426	51.9	4310	16,730	110/113	89.4/89.1	0.60	0.87
Morphology only	129	56	38	25.2	174	10	13	74.3	0.36	0.81
All molecular	138	11,227	2292	59.5	8716	599	114/116	93.1/90.4	0.47	0.82
Simultaneous parsimony	138	11,283	2330	59.3	8968	187	120	92.7	0.47	0.81

and Freudenstein, 2011). Data-matrix and tree statistics for all 13 analyses are presented in Table 1. 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 as described below.

3.1. Nucleotide-frequency heterogeneity

None of the eight gene regions was found to exhibit significant nucleotide-frequency heterogeneity when considering all nucleotide characters among different terminals based on the chi-square test implemented in PAUP* (which ignores phylogenetic correlations). The same held when the tests were restricted to the parsimony informative characters for *phyB* and all four plastid loci. In contrast, all three rDNA gene regions were found to exhibit significant nucleotide-frequency among the parsimony informative characters, as was previously reported by Simmons et al. (2008). For both 18S and 26S rDNA, the heterogeneity was no longer significant after restricting the test to the parsimony informative characters among the taxa of Celastraceae, whereas the heterogeneity remained significant for ITS. We were unable to identify any lineages within Celastraceae that were clear outliers in ITS nucleotide frequencies relative to all other members of the family.

3.2. Incongruence

No mutually well-supported (⩾70% JK and BS support) incongruent clades were resolved in any of the parsimony and likelihood analyses for the same data matrix. In cases of weakly supported conflict between the likelihood-based combined analysis of all sequence characters and the parsimony-based simultaneous analysis of all characters, we favor the parsimony-based resolution because more data are included (morphological and gap characters) and likelihood is susceptible to serious topological artifacts caused by non-random distributions of missing data among the heterogeneously evolving loci (with respect to rates of change) sampled in the combined analysis (Lemmon et al., 2009).

As reported by Simmons et al. (2008), the three genera of the former Stackhousiaceae (*Macgregoria*, *Stackhousia*, and *Tripterococcus*) are resolved outside the Austral-Pacific clade (as sister to *Monimopetalum*) in preliminary ITS analyses. This ITS gene-tree resolution conflicts with the 18S rDNA, *atpB*, *matK*, *rbcL*, and *trnL-F* gene trees. None of these three genera was sampled for *phyB*, and the 26S rDNA gene tree is ambiguous (Fig. S3). Following Lecointre and Deleporte's (2005) approach to excluding phylogenetically misleading data and Simmons et al. (2008), these Stackhousiaceae ITS sequences were excluded from all further analyses.

As reported by Simmons et al. (2008), the *phyB* gene tree (Fig. S6) conflicts with all other gene trees (except for 26S rDNA, which is ambiguous) by resolving *Tripterygium* as more closely related to *Paxistima* than it is to *Celastrus*. This well-supported conflict with the rDNA and plastid gene regions suggest that at least one portion of the *phyB* gene tree is not tracking the phylogenetic tree, perhaps due to introgression or sampling of unrecognized paralogs (Doyle, 1992; Simmons et al., 2008).

Four cases of mutually well-supported incongruence were observed when comparing the plastid and rDNA trees. In the rDNA tree (Fig. S5), the Austral-Pacific clade (not including *Peripterygia* and *Siphonodon*) is resolved as more closely related to the large clade that includes *Astrocassine* and *Kokoona* (74% JK/79% BS), whereas the Austral-Pacific clade including *Peripterygia* and *Siphonodon* is supported in the plastid tree (Fig. S11) with 100% JK and BS. This plastid-based resolution is nearly as well-supported (99% JK/100% BS) in the simultaneous analysis (Fig. 1). The second case of incongruence is whether *Celastrus rosthorianus* and *Celastrus scandens* are a monophyletic group (plastid; 94% JK/93% BS) or a paraphyletic group (rDNA; 80% JK/64% BS). The third case of incongruence is whether *Astrocassine mastophorus* and *Astrocassine pleurostyloides* are a monophyletic group (rDNA; 99% JK/89% BS) or a paraphyletic group (plastid; 98% JK/99% BS). This extremely well-supported case of incongruence within *Astrocassine*, which is endemic to Madagascar, suggests introgression of either the plastid genome or rDNA and we are unable to distinguish between these alternatives with the current data. ITS sequences of *A. mastophorus* and *A. pleurostyloides* as well as the *matK* sequence of *A. pleurostyloides* were independently sequenced by two co-authors as a test of a lab artifact, but all three sequences were validated. The fourth case of incongruence, which was previously investigated by Zhang and Simmons (2006) and Simmons et al. (2008), is whether *Pottingeria* is more closely related to *Mortonia* (plastid; 96% JK/96% BS) or Parnassiaceae (rDNA; 76% JK/87% BS). Although *Pottingeria* is clearly an early derived member of the Celastrales, its closest relatives remain ambiguous, in part due to fragmentary DNA sequences obtained from old collections (1930 and 1938; Zhang and Simmons, 2006).

3.3. Simultaneous analysis

All of the following systematic inferences are based on the simultaneous-analysis parsimony tree, though we do not make any conclusions regarding the four above mentioned cases of mutually well-supported incongruence that were observed when comparing the plastid and rDNA trees. Tribe Euonymeae consists of at least six separate lineages, each of which is more closely related to members of other taxa in Celastraceae than they are to each other (Fig. 1). First, as an early derived lineage of Celastraceae, the ⩾five species sampled for *Microtropis* are a strongly supported (92% JK/100% BS) clade sister to the unambiguously supported (100% JK/100% BS) clade of the three species sampled for *Quetzalia*. Synapomorphies for the clade of *Quetzalia* include a 1-bp deletion at position 244 in the *trnL-F* spacer. The unambiguously supported (100% JK/100% BS) clade of *Microtropis* + *Quetzalia* is unambiguously supported (100% JK/100% BS) as sister to *Zinowiewia* from subfamily Tripterygioideae. Synapomorphies for the clade of *Microtropis* + *Quetzalia* include a 5-bp deletion between positions 574–588 of the *trnL* intron, a 5-bp insertion at positions 247–251 of the *trnL-F* spacer, a 9-bp deletion at positions 229–237 of the *trnL-F* spacer, and capsular fruit dehiscence by laterally splitting along one side. Second, the two accessions of *Microtropis chinense* are unambiguously supported (100% JK/100% BS) as an early derived exclusive lineage that is unambiguously supported (100% JK/100% BS for successive clades) as sister to all genera of Celastraceae sampled except for *Microtropis*, *Mortonia*, *Pottingeria*, *Quetzalia*, and *Zinowiewia*.

Third, the two accessions of *Hedraianthera porphyropetala* are unambiguously supported (100% JK/100% BS) as an exclusive lineage that is unambiguously supported (100% JK/100% BS) as sister to the unambiguously supported (100% JK/100% BS) clade of the two species of *Brassiantha* (including one new species described below). Synapomorphies for the clade of *Brassiantha* + *Hedraianthera* include an 11-bp insertion at positions 593–603 in the *trnL* intron. Synapomorphies for the clade of *Brassiantha* include a 1-bp insertion at position 755 in ITS and a discontinuous floral disc. Fourth, the two accessions of *Euonymus globularis* are an exclusive lineage that is sister to the clade of the two species of *Menepetalum* (tribe Celastreae) sampled. All three of those clades are unambiguously supported (100% JK/100% BS). Synapomorphies for the clade of *Euonymus globularis* + *Menepetalum* include a 1-bp deletion at position 477 in the *trnL* intron. Both *E. globularis* and *Hedraianthera porphyropetala* are nested within the Austral-Pacific clade reported by Simmons et al. (2008).

Fifth, the three species of *Astrocassine* (*Euonymus* from Madagascar) sampled are well-supported (89% JK/83% BS) as a clade that is unambiguously supported (100% JK/100% BS) as sister to the unambiguously supported (100% JK/100% BS) clade of the three species *Pleurostylia* (tribe Cassineae) sampled. Sixth, all remaining members of *Euonymus* sampled, together with *Glyptopetalum* and *Torralbasia*, are unambiguously supported (100% JK/100% BS) as a clade that is well-supported (90% JK/89% BS) as sister to *Canotia*. Synapomorphies for the clade of *Euonymus* + *Glyptopetalum* + *Torralbasia* include a 5-bp insertion at positions 74–78 in the *trnL-F* spacer, a 5-bp insertion at positions 515–519 in the *trnL-F* spacer, a 7-bp insertion at positions 546–552 in the *trnL-F* spacer, a 6-bp insertion at positions 41–46 in *matK*, and a 1-bp insertion at position 538 in 26S rDNA. As part of the polytomy (Fig. 1) that includes *Euonymus* and *Torralbasia*, the two species of *Glyptopetalum* sampled are highly (100% JK/99% BS) supported as a clade. Synapomorphies for the clade of *Glyptopetalum* include a 1-bp deletion at position 247 in ITS and a branched raphe.

Within *Euonymus*, the large polytomy precludes thorough testing of Blakelock's (1951), Ma's (2001) and Savinov and Baikov's (2007) intrageneric classifications, but the species sampled from subgenus *Kalonymus* are weakly supported (76% JK/contradicted by a clade with 54% BS) as a clade separate from all species sampled from subgenus *Euonymus*. Within subgenus *Euonymus*, the three of the four species sampled of section *Ilicifolia* Nakai are unambiguously supported (100% JK/100% BS) as a clade distinct from the other sections of subgenus *Euonymus* that were sampled, though the fourth species (*Euonymus lanceolatus*) was unambiguously supported (100% JK/100% BS) as sister to *Euonymus melananthus* of Ma's (2001) section *Euonymus*. The one species of section *Melanocarya* (Turcz.) Nakai that was sampled is unambiguously supported in the parsimony analysis (82%, 74%, and 100% JK on successive clades; but weakly supported in the likelihood analysis with 63% BS) as nested within Ma's (2001) section *Euonymus*. Blakelock's (1951) series *Lophicarpi* (Loes.) Blakelock, *Myrianthi* Blakelock, and *Pseudovyenomi* (Nakai) Blakelock within section *Biloculares* Rouy and Foucaud were all resolved as para- or polyphyletic groups. None of these sections was recognized by Ma (2001).

4. Discussion

4.1. Intergeneric relationships

As previously inferred by Simmons et al. (2001a,b, 2008), Loesener's (1942) subfamilies and tribes of Celastraceae sensu stricto do not represent natural groups. Like Simmons et al.'s (2008) finding that Loesener's (1942) tribe Celastreae consists of multiple independent lineages, we infer that tribe Euonymeae consists of at least six separate lineages. Clearly a natural classification of the Celastraceae is needed, and this study, which includes six genera that had not yet been included in any molecular phylogenetic analysis (*Astrocassine* ined., *Dinghoua*, *Glyptopetalum*, *Hedraianthera*, *Monimopetalum*, and *Torralbasia*) represents a step in that direction.

The strong support for *Microtropis* (five of ~66 species sampled) and *Quetzalia* (three of 11 species sampled) being monophyletic genera is consistent with Lundell's (1970) recognition of *Quetzalia* (Central America, Mexico) as distinct from *Microtropis* (SE Asia, Malesia), and we see no need to reduce *Quetzalia* to *Microtropis*. Loesener (1898) suggested that *Microtropis* and *Quetzalia* may represent a polyphyletic group given their disjunct distribution in the Old and New Worlds, but we infer the genera to be a clade with unambiguous support, as asserted by Sprague (1909).

In his original description of *Monimopetalum chinense*, Rehder (1926) asserted that the species is closely related to *Euonymus* despite his noting differences in several vegetative and reproductive characters. Our inferred phylogeny provides unambiguous support for *Monimopetalum chinense* as being a phylogenetically isolated and early derived lineage of Celastraceae, and therefore represents an important component of phylogenetic diversity (Nixon and Wheeler, 1992) within the family. Therefore, conservation of this endangered species (Xie, 1998; Xie et al., 2005) is particularly important.

This resolution of *Monimopetalum* helps provide strong evidence (together with the geographic distributions of other early derived members of the Celastraceae) for a Laurasian origin of the family (with Parnassiaceae, *Mortonia*, *Pottingeria*, *Microtropis*, *Quetzalia*, and *Zinowiewia* also having distributions consistent with a Laurasian origin), as proposed by Raven and Axelrod (1974) and Gentry (1982).

Hedraianthera porphyropetala, from eastern Australia, is nested as a derived member of the Austral-Pacific clade that is unambiguously supported as sister to *Brassiantha* (Fig. 1). This resolution supports Ding Hou's (1964, 1969) assertion that the two genera are closely related to each other based on their numerous shared reproductive character states, including their unusual apically

hollow pistil, which is also present in *Siphonodon*. *Brassiantha* was formerly a monotypic genus from New Guinea but is herein newly described from northeastern Queensland, Australia with a second species (*B. hedraiantheroides* A.J. Ford; see species description below). Smith and Bailey (1941) did not mention *Hedraianthera* when describing *Brassiantha*. Although the new species of *Brassiantha* described below has a slightly different disk morphology relative to *Brassiantha pentamera* A.C. Sm., we see no need to describe a new monotypic genus. There are far too many similarities in gross morphology between the species (Table 2) for them to be generically separated.

Euonymus globularis, from Queensland, Australia, is a derived member of the Austral-Pacific clade that is only distantly related to *Euonymus* sensu stricto (Fig. 1). In his original description of the species, Ding Hou (1975) noted that the ovule number per carpel for this species (eight) is rare within *Euonymus* and that *E. globularis* has an aril form (“fleshy, narrow and slightly puberulous aril attached to [the seed] base and side up to about half of its length”; Ding Hou, 1975, p. 273) that is unique relative to all other species in the genus. Furthermore, Ding Hou (1975, p. 274) recognized pollen and fruit similarities between *E. globularis* and *Brassiantha* (fruit only) as well *Hedraianthera*, which are two other members of the Austral-Pacific clade, and suggested that, “It may be possible that [*E. globularis*] should be placed in a group of its own.” *Euonymus globularis* is unambiguously supported as sister to the New Caledonian endemic genus *Menepetalum*, from which it differs by its perfect flowers and 5-locular ovaries with 8 ovules per carpel (imperfect flowers and 3-locular ovaries with 2[−6] ovules per carpel in *Menepetalum*). The new genus is formally described below.

Like *Euonymus globularis*, the three species of Madagascan *Euonymus* sampled are only distantly related to *Euonymus* sensu stricto (Fig. 1) and will be formally recognized as the genus *Astrocassine* in a forthcoming publication by R.H.A. Perrier de la Bâthie (1942) questioned the inclusion of the Madagascan species within *Euonymus*, Blakelock (1951) treated them as incertae sedis, and Ma (2001) only provisionally placed them within *Euonymus* section *Euonymus* pending availability of additional specimens. Apart from the little known *Euonymus congolensis* R. Wilczek from Congo, Gabon and Zaire (Ma, 2001), there are no other putative species of *Euonymus* in Madagascar or Africa outside of north Africa, and recognition of *Astrocassine* as distinct from *Euonymus* eliminates this disjunction in the distribution of *Euonymus*. Fruit of the new genus has been misinterpreted by previous authors based on incomplete material (Loesener, 1942; Perrier de la Bâthie, 1942; Ma, 2001). The fruits are conspicuously large, woody, entirely indehiscent and in various shapes from round to fusiform, and the seeds lack arils; these character states are the main differences with *Euonymus*, which has dehiscent fruits with usually colorful arillate seeds.

Both *Glyptopetalum* and *Torralbasia* are closely related to *Euonymus* as previously asserted for both *Glyptopetalum* (Thwaites, 1856; Bentham and Hooker, 1862; Baillon, 1880; Ding Hou, 1963; Savinov, 2007) and *Torralbasia* (Urban, 1904). Baillon (1880) treated *Glyptopetalum* as a section of *Euonymus*, but others, including Ding Hou (1963), who provided the best overview of *Glyptopetalum* and noted three reproductive differences with *Euonymus*, recognized *Glyptopetalum* as a distinct genus. The two (of ~20) species of *Glyptopetalum* are a strongly supported clade in our simultaneous analysis, yet part of a large polytomy together with *Euonymus* and *Torralbasia* (Fig. 1), which precludes inference of whether *Euonymus* and *Glyptopetalum* are reciprocally monophyletic genera. But based on the three reproductive differences noted by Ding Hou (1963), we recommend that *Glyptopetalum* continue to be recognized as distinct from *Euonymus*.

We were unable to access any specimens of *Xylonymus* that were promising for DNA isolation, which is not surprising given that we know of only a single collection (the type) from New Guinea. Nonetheless, we did include the morphological characters for *Xylonymus* in a second simultaneous analysis for which 263 most parsimonious trees were found with *Xylonymus* resolved as part of the large polytomy within the *Euonymus* + *Glyptopetalum* + *Torralbasia* clade (data not shown). A close relationship between *Euonymus*, *Glyptopetalum*, and *Xylonymus* was also inferred by Simmons and Hedin (1999). Both of these studies support Kalkman’s (Ding Hou, 1962) assertion that *Xylonymus* is closely related to *Euonymus* and contradict Ding Hou’s (1969) assertion that *Sarawakodendron* is closely related to *Xylonymus*.

4.2. Alternative classifications of Euonymus

The large polytomy within the clade of *Euonymus* + *Glyptopetalum* + *Torralbasia* precludes thorough testing of Blakelock’s (1951), Ma’s (2001) and Savinov and Baikov’s (2007) intrageneric classifications of *Euonymus*. All three authorities recognized subgenus *Kalonymus* as distinct from subgenus *Euonymus*, and that distinction is weakly supported here by the parsimony-based simultaneous analysis (Fig. 1). In addition to the polytomy, we lack sufficient taxon sampling to test the monophyly of all of Blakelock’s (1951), Ma’s (2001) or Savinov and Baikov’s (2007) sections

Table 2
Morphological comparison between *Hedraianthera porphyropetala*, *Brassiantha hedraiantheroides*, and *B. pentamera*.

Character	*H. porphyropetala*	*B. hedraiantheroides*	*B. pentamera*[a]
Inflorescence	Raceme or occasionally monochasial	Paniculate, cymes	Paniculate of racemes, rarely cymes
Calyx (at anthesis)	Spreading	Erect	Erect
Corolla	Imbricate	Valvate	Valvate
Corolla in bud	Globose	Ellipsoid	Globose
Corolla longevity	Deciduous	Persistent	Persistent (?)
Petal length	3–5 mm	2–2.5 mm	1.75–2.5 mm
Pedicel articulation	Present, near apex	Present or absent; if present at very base	Present, well above base but below middle
Filament length	<0.5 mm	0.9–1.2 mm	<0.5 mm
Filament insertion	Under outer margin of disc at sinuses	Inside disc at sinuses	Inside disc at sinuses
Aril indumentum	Pubescent	Glabrous	Glabrous
Aril color	White (brown tips)	Red–orange	Orange
Aril form	Confined to one edge and has a worm-like or hinge-like appearance	Covers whole seed, except for a small opening on one side near apex	Covers whole seed, except for a small opening on one side near apex
Fruit color	Green	Green	Red
Fruit surface	Hard leathery	Bony	Bony
Seed length	7–12 mm	5–7.2 mm	12–15 mm
Seed width	3.5–5 mm	4–4.5 mm	7–10 mm

[a] Mostly taken from Ding Hou (1964).

within subgenus *Euonymus*. Ma's (2001) sections within subgenus *Euonymus* differ from Blakelock's (1951) in that Ma grouped together sections *Biloculares*, *Multiovulatus* Loes., and *Stenocarpus* Blakelock into section *Euonymus*. We only sampled two species from section *Multiovulatus* (*E.* aff. *carnosa* and *Euonymus grandiflorus*), which are unambiguously supported (100% JK/100% BS) as a clade that is nested within a clade that includes members of section *Biloculares* (in the parsimony analysis only; Fig. 1) and did not sample any species within section *Stenocarpus*.

Despite our limited resolution and taxon sampling, we conclude that there is need for a further revised classification of subgenus *Euonymus* given that there is at least one well-supported clade that contradicts the monophyly of one or more of the sections recognized by Blakelock (1951), Ma (2001) and Savinov and Baikov (2007). Both Blakelock (1951) and Savinov and Baikov (2007) treated *Euonymus phellomanus* as a member of section *Biloculares*, yet we infer that *E. phellomanus* is more closely related to *E. alatus* from their section *Melanocarya* than it is to the other species of section *Biloculares* that we sampled. Ma (2001) treated *E. lanceolatus* as a member of section *Ilicifolia* (Blakelock (1951) treated this species as incertae sedis), but this treatment is contradicted by an unambiguously supported clade (100% JK/100% BS) uniting *E. lanceolatus* with *E. melananthus* from section *Euonymus* (Fig. 1).

4.3. Formal description of Dinghoua

Dinghoua R.H. Archer genus novum *Menepetalo* Loes. affinis, sed inflorescentia solitaria, brevi, ad 10 mm longa, bracteis decussates, condensatis, persistentibus, floribus hermaphroditis, ovario 5-loculare, ovulis in quoque loculo 8, biseriatis, et a *Euonymo* L. arillo angustato, leviter puberulo, differt.—TYPE: *Dinghoua globularis* (Ding Hou) R.H. Archer.

Dinghoua globularis (Ding Hou) R.H. Archer, comb. nov., *Euonymus globularis* Ding Hou Blumea 22: 271 (1975); Jessup in Flora Australia 22: 175 (1984).—TYPE: AUSTRALIA. Queensland: Cook District, Shipton's Flat, *L.J. Brass 20224* (holotype: L; isotypes BRI, K!SING!).

Illustrations: Ding Hou (1975: 272), Hyland et al. (2003, 2010).

4.4. Formal description of Brassiantha hedraiantheroides

Brassiantha hedraiantheroides A.J. Ford, sp. nov.—TYPE: AUSTRALIA. Queensland: Cook District, Cultivated Tolga (ex-Browns Creek, Yarrabah), 11 November 1998, *A. Ford 2122* (holotype: BRI!; isotypes: CNS! (plus spirit), CS!, K!, L!, MO!, NSW!, PRE!, Z!).

Hedraianthera sp. (Mossman [town]; *V.K. Moriarty 2557*) (Hyland et al., 2003, 2010).

Hedraianthera sp. (Mossman) (Cooper, 2004: 116).

Hedraianthera sp. (Mossman; *V.K. Moriarty 2557*) (Jessup, 2007, p. 44).

Illustrations: Hyland et al. (2003, 2010), Cooper (2004, p. 116) as *Hedraianthera* sp. (Mossman).

Brassiantha hedraiantheroides A.J. Ford species nova affinis *Brassianthae pentamera* A.C. Smith a qua differt filamentis longioribus (0.9–1–2 mm longis, neque brevioribus quam 0.5 mm) et capsulis viridibus (neque rubris).

Bushy tall shrubs or poorly formed multistemmed trees 2–8 m tall, stem diameters to 10 cm dbh. Trunk buttresses absent. Bark creamish, slightly spongy and slightly corky. Wood yellowish, roots orange. Branchlets glabrous, slightly laterally compressed (elliptic in TS), becoming ± terete with age, green at first and aging to red-brown and finally creamy-brown with longitudinal striations (lenticels) on older twigs; terminal bud glabrous. Stipules minute, 0.1–0.2 mm long, ovate, brownish, persistent, becoming slightly corky with age. Leaves petiolate, distichous, simple. Petioles 4–7 mm long, glabrous, yellowish when fresh and becoming slightly corky and creamish on older and senescing leaves, adaxially channeled. Leaf blades (3.7–) 4.5–9.1 cm long, 1.6–3.4 cm wide, elliptic (rarely obovate-elliptic), discolorous, leathery, base cuneate, apex acute with a minute mucro, venation brochidodromous, margin flat, entire; glabrous on both surfaces, shiny above and very dull below, midvein raised above and below when fresh (when dry midvein is raised below and slightly raised above but only in lower half, upper half of midvein is flat); lateral primary veins 4–6 on each side of the midvein, secondary veins obscure, tertiary veins not discernible. Inflorescence axillary (rarely ramiflorous), a simple dichasial cyme (rarely fasciculate) or more often a 2–3 (rarely 4) armed paniculate dichasial cyme. Primary peduncle on paniculate dichasial cymes ± absent or to 1.2 mm long or for simple dichasial cymes primary peduncle 7–9 mm long and then terminated in a solitary flower, terete, glabrous, green. Secondary peduncle 12–22 mm long, terminated by a solitary flower. Tertiary peduncles 1–3 mm long. Bracts 0.4–0.7 mm long, glabrous, ovate, apex shortly acuminate, persistent. Pedicels filiform 0.3–0.4 mm diameter, glabrous, terete, 7–8 mm long; articulation present or absent, if present at very base of pedicel. Flowers 5-merous, bisexual, hypogynous, 4–4.5 mm diameter, not fragrant. Calyx lobes 5, imbricate, green, erect, 0.6 mm long and 0.8 mm wide, glabrous, crescent-like or broadly semi-circular, apex rounded, margin with irregular minute teeth. Corolla valvate, ellipsoid in bud. Petals 5, entire, pale pink or cream to white (rarely pink–red), 2.3–2.5 mm long, 1.2–1.3 mm wide, apex acute, reflexed, elliptic, abaxial surface glabrous, adaxial surface minutely papillose between five longitudinal veins, persisting beyond anthesis. Stamens 5, alternating with petals; filaments 0.9–1.2 mm long, inserted inside and between sinuses of fleshy disc; anthers pale pink, dehiscing apically (transversely) through slits but oblique prior to dehiscence, c. 0.2 mm long, c. 0.3 mm wide, basifixed, not versatile (fixed), base of anther sac swollen (tissue sterile), connective extension absent. Anthers congested around ovary apex with erect filaments at anthesis, but filaments reflexed between petals after anthesis. Pollen not conspicuously aggregated. Disc consists of five fleshy glands that are discontinuous; glands c. 0.3 mm long, c. 0.4 mm wide, glabrous, columnar, apex obtuse-truncate, swollen at base and thinner near apex. Ovary green, glabrous, pyriform, 1.1–1.3 mm long, apex slightly flared, truncate, hollow in upper half; style absent, stigmatic area in a slight crater at carpel summit (5 indistinct lines visible in crater?); 5-locular; 2–4 ovules per placenta, axile, vertically arranged. Septa walls complete. Fruit a capsule, loculicidally dehiscent, bony, subglobose to depressed globular, 19–31 mm long, 19–40 mm wide, often slightly 5-lobed, columella present, 5-valved, glabrous on both surfaces, green when ripe, adaxial surface blood red or red–purple, calyx persistent. Aril attached to one edge (raphe) of seed and arises from conspicuous hilum-like structure, membranous throughout and occasionally with folds at apex, red–orange, completely enclosing seed or sparingly open on one side. Raphe unbranched. Seeds 2–4 per locule (12–18 per fruit), 5–7.2 mm long, 4–4.5 mm wide, 2.5–4 mm thick, usually more or less 3-faced, obovoid to ellipsoidal; testa membranous, firm, pale brown, smooth; endosperm corneous, white; embryo 4–5.6 mm long, creamy green, straight; cotyledons 2.5–3.6 mm long, 1.5–1.9 mm wide, elliptic, abaxial surface very shallowly convex, adaxial surface flat; radicle 1.5–2 mm long, c. 1 mm diameter, cylindrical, straight. Germination epigeal (phanerocotylar); cotyledons distinctly petiolate, elliptic, 19–28 mm long and 11–18 mm wide, apex obtuse, base cuneate-obtuse, penniveined, midvein raised on adaxial surface, venation prominent on adaxial surface and visible on abaxial surface; hypocotyl terete, small wings formed from decurrent cotyledon bases and extends down hypocotyl as raised ridges; first leaves and subsequent leaves with entire margins.

Phenology. Flowering August to December (including observations from cultivated specimens); fruiting March to May (cultivated specimens extend to July and August).

Distribution. Northeastern Queensland (often referred to as the 'Wet Tropics'), where it occurs in lowland to upland rainforest and sclerophyll forests with a rainforest understory on clay soils derived from metasediments; sea level to 560 m. Most collections come from secondary or disturbed rainforests with *B. hedraiantheroides* currently known from three distinct populations (from north to south):

1. Mossman–Julatten area.
2. Cairns–Yarrabah area.
3. Moresby–El Arish area.

Brassiantha hedraiantheroides has been collected in sclerophyll forests and rainforests containing the following dominant canopy species: *Acacia celsa* Tindale, *Acacia mangium* Willd., *Alstonia muelleriana* Domin, *Beilschmiedia bancroftii* (F.M. Bailey) C.T. White, *Corymbia intermedia* (R.T. Baker) K.D. Hill and L.A.S. Johnson, *Elaeocarpus bancroftii* F. Muell. and F.M. Bailey, *Elaeocarpus grandis* F. Muell., *Litsea leefeana* (F. Muell.) Merr., *Lophostemon suaveolens* (Sol. ex Gaertn.) Peter G. Wilson and J.T. Waterh. and *Toechima erythrocarpum* (F. Muell.) Radlk.

Conservation Status. *Brassiantha hedraiantheroides* is currently not recognized with any formal conservation status. However, under the Nature Conservation Act 1992 of the Queensland Government (see www.derm.qld.gov.au/register/p01273aa.doc), *B. hedraiantheroides* satisfies Near Threatened (Category D., less than 3000 mature individuals and is known from less than 10 locations/populations).

***Representative Specimens Examined*: AUSTRALIA. Queensland**: **Cook District**, off Clacherty road, tributary of Devil Creek, Julatten, October 2006, *Ford AF4864* (BRI, CNS); The Pinnacle 2 km S of c. 13 km SSE of Mossman, December 1978, *Moriarty 2557* (BRI, CNS); Kingfisher Park, Julatten, October 1998, *Holmes 67* (CNS); SFR 42, Rex Range, W of Mowbray Falls, February 1985, *Dansie 20181* (CNS); Hills Creek, Cairns, May 1992, *Lyons 116* (CNS); 2 km SSW of Mt Murray Prior, Murray Prior Range, December 1990, *Lyons 85* (BRI, CNS); Cairns Hill Creek, May 1992, *Lyons* 115 (BRI, CNS); Rowles property, Basilisk Range, February 1998, *Cooper 75 and Jensen* (CNS); S of Innisfail, between Moresby and Warrubullen, Scenic Reserve 436, October 2000, *Lyons 204* (BRI, CNS).

Brassiantha hedraiantheroides is most similar to *Hedraianthera porphyropetala* from Eastern Australia and *B. pentamera* from New Guinea. A comparison of the three species is made in Table 2. Based on current records *B. hedraiantheroides* and *H. porphyropetala* do not co-occur, although their distributions do overlap. In the Yarrabah area (near Cairns) both species occur within one kilometer of each other, albeit in different habitats. The chief differences (and similarities) between the three species are with the following features: corolla aestivation, filament insertion relative to the disc, filament length, fruit color, fruit surface, aril form, aril indumentum, aril color and columella shape. Our simultaneous phylogenetic analysis unambiguously supports *B. hedraiantheroides* as sister to *B. pentamera*, which, together, are sister to *H. porphyropetala*.

Jessup (1984, p. 168) mentioned atypical specimens of *H. porphyropetala* from North Queensland having "staminal filaments 1–1.2 mm long and the raceme axis to 3 cm long." These specimens are now recognized as *B. hedraiantheroides.*

The fruit texture of *H. porphyropetala* requires some clarification. Bailey (1899) stated that the fruit of *Hedraianthera* is "bony," which Jessup (1984) and Hyland et al. (2003) perpetuated, although it is acknowledged by Jessup (pers. comm. 2010) that this original interpretation was almost certainly in reference to immature fruit. Mature fruit is hard leathery and certainly not bony as previously noted in the literature.

Aril morphology is a key distinguishing feature between *Brassiantha* and *Hedraianthera*. It has been long noted that the aril of *Hedraianthera* is unusual (Table 2; Ding Hou, 1964). But surprisingly it has not been recorded that this unique aril is also pubescent, a fact that also escaped the attention of van Wyk (1984) in his discussion of pubescent arils in Celastraceae.

Sterile dry specimens of *H. porphyropetala*, *B. pentamera* and *B. hedraiantheroides* are easily distinguished by midvein and venation features. The midvein of *H. porphyropetala* and *B. pentamera* is strongly raised adaxially, whereas the midvein of *B. hedraiantheroides* is slightly raised adaxially but only in the lower half with the upper half of the leaf blade having a flat midvein. Lateral primary veins of *B. pentamera* and *B. hedraiantheroides* are flat above and not strongly raised like in *H. porphyropetala*.

The specific epithet reflects the similarity of this species to the monotypic *Hedraianthera*, a related genus in Celastraceae (from the Greek *hedraios*, sessile, and *anthera*, anther, referring to the apparently sessile anthers), and the suffix *–oides*, like or resembling. In addition, this new species was previously known as *Hedraianthera* sp. (Mossman), and thus the chosen epithet commemorates this historical fact.

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Appendix A

List of taxa sampled with taxonomic authorities, voucher information and GenBank accession numbers for new sequences generated for this study.

Astrocassine fusiformis R.H. Archer ined.—*R.H. Archer et al. 2917*, Madagascar (CS); 26S rDNA HQ393652, ITS rDNA HQ393693, *matK* HQ393814, *trnL-F* spacer HQ393786. ***Astrocassine fusiformis*** R.H. Archer ined.—*R.H. Archer et al. 2918*, Madagascar (CS); 26S rDNA HQ393653, ITS rDNA HQ393694, *matK* HQ393815. ***Astrocassine mastophorus*** R.H. Archer ined.—*R.H. Archer et al. 2913*, Madagascar (CS); 26S rDNA HQ393651, ITS rDNA HQ393695, *matK* HQ393816, *trnL* intron HQ393741, *trnL-F* spacer HQ393787. ***Astrocassine pleurostylioides*** (Loes.) R.H. Archer ined.—*R.H. Archer et al. 2924*, Madagascar (CS); 26S rDNA HQ393650, ITS rDNA HQ393696, *matK* HQ393817, *trnL* intron HQ393742, *trnL-F* spacer HQ393785. ***Brassiantha hedraiantheroides*** A.J. Ford—*A. Ford 2122*, Australia (BRI); 26S rDNA HQ393647, ITS rDNA HQ393692, *matK* HQ393818, *trnL* intron HQ393739, *trnL-F* spacer HQ393781. ***E. americanus*** L.—*W. Hess et al. 9499*, Kentucky, USA (F); ITS rDNA HQ393722, *trnL* intron HQ393763, *trnL-F* spacer HQ393792. ***Euonymus australianus*** F. Muell.—*A. Ford 4496*, Australia (BRI); 26S rDNA HQ393664, ITS rDNA HQ393715, *matK* HQ393819, *trnL* intron HQ393751, *trnL-F* spacer HQ393802. ***Euonymus* aff. *carnosus*** Hemsl.—*M.W. Chase 36534*, cult. United Kingdom (K); 26S rDNA HQ393662, ITS rDNA HQ393712, *matK* HQ393820, *trnL* intron HQ393744, *trnL-F* spacer HQ393784. ***Euonymus europaeus*** L.—*M.P. Simmons 1779*, cult.

Ithaca, New York (BH); 26S rDNA HQ393674, ITS rDNA HQ393713, *matK* HQ393821, *trnL* intron HQ393747, *trnL-F* spacer HQ393795. ***Euonymus fimbriatus*** Wall.—*M.W. Chase 36535*, cult. United Kingdom (K); 26S rDNA HQ393670, ITS rDNA HQ393710, *matK* HQ393822, *trnL* intron HQ393743, *trnL-F* spacer HQ393808. ***Euonymus fortunei*** (Turcz.) Hand.-Mazz.—*M.P. Simmons 1913*, cult. Illinois, USA (CS); ITS rDNA HQ393699, *matK* HQ393828, *trnL* intron HQ393764, *trnL-F* spacer HQ393813. ***Euonymus globularis*** Ding Hou—*A. Ford 4739*, Australia (BRI); ITS rDNA HQ393697, *matK* HQ393825, *trnL* intron HQ393736, *trnL-F* spacer HQ393779. ***Euonymus globularis*** Ding Hou—*G. Sandowsky 2628*, Australia (BRI); 26S rDNA HQ393654, ITS rDNA HQ393698, *matK* HQ393824, *trnL* intron HQ393737, *trnL-F* spacer HQ393780. ***Euonymus grandiflorus*** Wall.—*M.P. Simmons 1789*, cult. New York Botanical Garden (BH); 26S rDNA HQ393666, ITS rDNA HQ393711, *trnL* intron HQ393745, *trnL-F* spacer HQ393789. ***Euonymus hamiltonianus*** Wall.—*M.P. Simmons 1791*, cult. New York, USA (BH); 26S rDNA HQ393676, ITS rDNA HQ393724, *matK* HQ393826, *trnL* intron HQ393748, *trnL-F* spacer HQ393798. ***Euonymus hamiltonianus*** Wall.—*M.P. Simmons 1792*, cult. New York, USA (BH); 26S rDNA HQ393675, ITS rDNA HQ393708, *matK* HQ393827, *trnL* intron HQ393749, *trnL-F* spacer HQ393797. ***Euonymus*** aff. ***hamiltonianus*** Wall.—*M.P. Simmons 1923*, cult. Missouri, USA (CS); 26S rDNA HQ393677, ITS rDNA HQ393723, *matK* HQ393838, *trnL* intron HQ393750, *trnL-F* spacer HQ393796. ***Euonymus japonicus*** Thunb.—*L. Tembrock 08-019*, cult. Colorado, USA (CS); 26S rDNA HQ393680, ITS rDNA HQ393700, *matK* HQ393829, *trnL* intron HQ393765, *trnL-F* spacer HQ393811. ***Euonymus lanceolatus*** Yatabe—*A. Whittemore 08-016*, cult. Washington, D.C., USA (NA); 26S rDNA HQ393673, ITS rDNA HQ393701, *trnL* intron HQ393760, *trnL-F* spacer HQ393800. ***Euonymus latifolius*** (L.) Mill.—*L. Tembrock 08-023*, cult. Colorado, USA (CS); 26S rDNA HQ393669, ITS rDNA HQ393707, *matK* HQ393830, *trnL* intron HQ393767, *trnL-F* spacer HQ393806. ***Euonymus macropterus*** Rupr.—*M.W. Chase 36536*, cult. United Kingdom (K); 26S rDNA HQ393671, *matK* HQ393831, *trnL* intron HQ393769, *trnL-F* spacer HQ393807. ***Euonymus melananthus*** Franch. and Sav.—*A. Whittemore 08-019*, cult. Washington, D.C., USA (NA); 26S rDNA HQ393672, ITS rDNA HQ393702, *matK* HQ393832, *trnL* intron HQ393761, *trnL-F* spacer HQ393801. ***Euonymus myrianthus*** Hemsl.—*M.P. Simmons and C. Sayre 1926*, cult. British Columbia, Canada (CS); 26S rDNA HQ393667, ITS rDNA HQ393721, *matK* HQ393833, *trnL* intron HQ393758, *trnL-F* spacer HQ393804. ***Euonymus nanus*** M.Bieb.—*M.W. Chase 36537*, cult. United Kingdom (K); 26S rDNA HQ393659, ITS rDNA HQ393709, *matK* HQ393834, *trnL* intron HQ393746, *trnL-F* spacer HQ393788. ***Euonymus nitidus*** Benth.—*A. Whittemore 08-017*, cult. Washington, D.C., USA (NA); 26S rDNA HQ393668, *matK* HQ393835, *trnL* intron HQ393757, *trnL-F* spacer HQ393803. ***Euonymus oxyphyllus*** Miq.—*L. Tembrock 08-021*, cult. Colorado, USA (CS); 26S rDNA HQ393658, ITS rDNA HQ393704, *matK* HQ393836, *trnL* intron HQ393768, *trnL-F* spacer HQ393810. ***Euonymus phellomanus*** Loes. ex Diels—*L. Tembrock 08-022*, cult. Colorado, USA (CS); 26S rDNA HQ393665, ITS rDNA HQ393718, *matK* HQ393837, *trnL* intron HQ393755, *trnL-F* spacer HQ393791. ***Euonymus semenovii*** Regel and Herder.—*M.W. Chase 36538*, cult. United Kingdom (K); 26S rDNA HQ393660, ITS rDNA HQ393719, *matK* HQ393839, *trnL* intron HQ393753, *trnL-F* spacer HQ393794. ***Euonymus* sp.**—*M.W. Chase 36547*, cult. United Kingdom (K); 26S rDNA HQ393679, ITS rDNA HQ393703, *matK* HQ393823, *trnL* intron HQ393762, *trnL-F* spacer HQ393812. ***Euonymus* sp. (section *Euonymus*)**—*M.W. Chase 36549*, cult. United Kingdom (K); ITS rDNA HQ393714, *matK* HQ393843, *trnL* intron HQ393759, *trnL-F* spacer HQ393805. ***Euonymus tingens*** Wall.—*M.W. Chase 36539*, cult. United Kingdom (K); 26S rDNA HQ393661, ITS rDNA HQ393716, *matK* HQ393840, *trnL* intron HQ393754, *trnL-F* spacer HQ393790. ***Euonymus vagans*** Wall.—*M.W. Chase 36548*, cult. United Kingdom (K); 26S rDNA HQ393678, ITS rDNA HQ393720, *matK* HQ393841, *trnL* intron HQ393766, *trnL-F* spacer HQ393809. ***Euonymus verrucosus*** Scop.—*M.P. Simmons and C. Sayre 1925*, cult. British Columbia, Canada (CS); 26S rDNA HQ393663, ITS rDNA HQ393717, *matK* HQ393842, *trnL* intron HQ393752, *trnL-F* spacer HQ393799. ***Glyptopetalum palawanense*** Merr.—*A.C. Podzorski SMHI 808*, Philippines (K); 26S rDNA HQ393656, ITS rDNA HQ393705. ***Glyptopetalum rhytidophyllum*** (Chun and F.C. How) C.Y. Cheng—*CKF Team CKF257*, Vietnam (KUN); 26S rDNA HQ393657, ITS rDNA HQ393706, *matK* HQ393844, *trnL* intron HQ393756, *trnL-F* spacer HQ393793. ***Hedraianthera porphyropetala*** F. Muell.—*A. Ford 4370*, Australia (BRI); 26S rDNA HQ393648, ITS rDNA HQ393689, *matK* HQ393846, *trnL* intron HQ393738, *trnL-F* spacer HQ393783. ***Hedraianthera porphyropetala*** F. Muell.—*A. Ford 4544*, Australia (BRI); 26S rDNA HQ393649, ITS rDNA HQ393690, *matK* HQ393845, *trnL* intron HQ393740, *trnL-F* spacer HQ393782. ***Microtropis* sp.**—*Y.M. Shui et al. 81585*, China (KUN); 26S rDNA HQ393644, ITS rDNA HQ393686, *matK* HQ393849, *trnL* intron HQ393734, *trnL-F* spacer HQ393778. ***Microtropis discolor*** (Wall.) Wall.—*GBOWS1466*, China (KUN); 26S rDNA HQ393642, ITS rDNA HQ393684, *matK* HQ393847, *trnL* intron HQ393726, *trnL-F* spacer HQ393776. ***Microtropis fokienensis*** Dunn—*Mu Xianyun 37*, China (BJFC); 26S rDNA HQ393639, ITS rDNA HQ393683, *trnL* intron HQ393730, *trnL-F* spacer HQ393774. ***Microtropis fokienensis*** Dunn—*Xiong Weizhong 229*, China (MO); 26S rDNA HQ393638, ITS rDNA HQ393682, *matK* HQ393848, *trnL* intron HQ393729, *trnL-F* spacer HQ393773. ***Microtropis japonica*** (Franch. and Sav.) Hallier f.—*F. Konta 24106*, Japan (MO); 26S rDNA HQ393636, *trnL* intron HQ393731, *trnL-F* spacer HQ393772. ***Microtropis tetragona*** Merr. and Freeman—Dulong Jiang Investigation Team 1194, China (CAL); 26S rDNA HQ393643, ITS rDNA HQ393685, *matK* HQ393850, *trnL* intron HQ393733, *trnL-F* spacer HQ393777. ***Microtropis triflora*** Merr. and Freeman—*H. He 09*, Chongqing, China (CTC); 26S rDNA HQ393637, ITS rDNA HQ393681, *matK* HQ393851, *trnL* intron HQ393732, *trnL-F* spacer HQ393775. ***Monimopetalum chinense*** Rehder—*D.W. Liu 9138*, China (MO); 26S rDNA HQ393645. ***Monimopetalum chinense*** Rehder—*Mu Xianyun 38*, China (BJFC); 26S rDNA HQ393646, ITS rDNA HQ393691, *matK* HQ393852, *trnL* intron HQ393735. ***Quetzalia schiedeana*** (Loes.) Lundell—*D.E. Breedlove and F. Almeda 59456*, Mexico (CAL); 26S rDNA HQ393640, ITS rDNA HQ393688, *matK* HQ393853, *trnL* intron HQ393727, *trnL-F* spacer HQ393770. ***Quetzalia stipitata*** (Lundell) Lundell—*R. Hernandez et al. 6408*, Mexico (MO); 26S rDNA HQ393641, ITS rDNA HQ393687, *matK* HQ393854, *trnL* intron HQ393728, *trnL-F* spacer HQ393771. ***Torralbasia cuneifolia*** (C. Wright ex A. Gray) Krug and Urb. —*R. Garcia et al. 6092*, Dominican Republic (F); 26S rDNA HQ393655, ITS rDNA HQ393725.

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.08.022.

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