SYSTEMATICS AND PHYLOGENY

Didymochlaenaceae: A new fern family of eupolypods I (Polypodiales)

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DOI http://dx.doi.org/10.12705/641.4

Abstract The pantropical monospecific fern genus *Didymochlaena* had long been placed in the family Dryopteridaceae before the advent of molecular phylogenetics. Recent molecular studies confirmed that it is a member of eupolypods I, but its familial placement has been controversial. It has been resolved using molecular data either as a member of Hypodematiaceae or as sister to the rest of eupolypods I. In the present study we performed phylogenetic analyses (MP, ML, BI) based on DNA data from five plastid loci (*atpA*, *rbcL*, *rps4-trnS*, *trnL*, *trnL-F*) of 88 accessions representing 79 species in 20 genera of all 9 families currently recognized in eupolypods I as ingroup and 5 species from 3 families of eupolypods II as outgroups. Our analyses resolved *Didymochlaena* as sister to the rest of eupolypods I with strong support. The results of an analysis of nuclear 18S data available from GenBank did not contradict those based on plastid data. This resolution is consistent with its sorus/indusium shape and a previous phylogenetic analysis based on both plastid and nuclear loci. Sorus shape, spore characters, and chromosome number previously used to place the genus in Dryopteridaceae are in fact not synapomorphies of the family. Our study clearly showed that the family Didymochlaenaceae should be recognized and we hereby validate this name previously proposed by Ching.

Keywords chromosome number; *Didymochlaena*; Didymochlaenaceae; Dryopteridaceae; eupolypods I; fern phylogeny; Hypodematiaceae; spore morphology

Supplementary Material Electronic Supplement (Figs. S1–S2) and alignment files are available in the Supplementary Data section of the online version of this article at http://www.ingentaconnect.com/iapt/tax

■ INTRODUCTION

The beautiful fern species *Didymochlaena truncatula* (Sw.) J.Sm. (Figs. 1–2) is commonly grown as a houseplant, and is sometimes known as the mahogany maidenhair or mahogany fern. Before the advent of molecular phylogenetics the monospecific genus *Didymochlaena* Desv. (Desvaux, 1811) had often been included in the families Aspleniaceae, Dennstaedtiaceae, Dryopteridaceae, Polypodiaceae, or its own family. For example, although assigning *Didymochlaena* in the broadly defined tribe Aspidieae (including Dryopteridaceae sensu Zhang & al., 2013, and many other families in current definitions) under Polypodiaceae, Smith (1841) noticed the peculiarity in the tribe of its elongated sori. Hooker & Baker (1874) also placed it under Aspidieae of the "sub-ord. Polypodiaceae". In view of its elliptic-oblong sori, dimidiate pinnules, spore morphology (though based on wrong information), etc. Ching (1940) treated it as a distinct family. Copeland (1947) and Sledge (1973) put it under the very broadly defined "Aspidiaceae" (nom. illeg. = Dryopteridaceae). Holttum (1947, 1949) placed the genus in Dryopteridoideae, one of 11 subfamilies he recognized under Dennstaedtiaceae. Tardieu-Blot (1958) also recognized it as a member of the broadly defined "Dennstaedtiacées" (Dennstaedtiaceae) but without subfamilial assignment. Crabbe & al. (1975) listed it as a genus of Tectarioideae, one of six subfamilies they accepted in Aspleniaceae. After the wide acceptance of Dryopteridaceae since Ching (1965), Pichi Sermolli (1977), Tryon & Tryon (1982), Kramer & al. (1990), and Tryon & Lugardon (1991) all treated *Didymochlaena* as a member of Dryopteridaceae, presumably mainly based on the chromosome number of $x = 41$ (Manton, 1954) and the three or more vascular bundles in the petiole of *Didymochlaena* (Kramer & al., 1990). These are assumed to be synapomorphies of Dryopteridaceae.

Molecular phylogenetic studies also produced controversial results concerning the relationships of *Didymochlaena*. Using plastid *rps4-trnS* and *trnL-F* data, Smith & Cranfill (2002) first related *Didymochlaena* with *Hypodematium* A.Rich., but these were the only two taxa of eupolypods I sampled in their study. Based on *rbcL* and *rps4* data, Schneider & al. (2004) resolved *Didymochlaena* and *Hypodematium* as sister to each other with low support, and they together were resolved as sister to the remainder of the seven eupolypods I from four families. Tsutsumi & Kato (2005, 2006) provided strong support for a sister relationship between *Hypodematium* and *Leucostegia* C.Presl, and also strong support for a sister relationship between these two together and the remaining eupolypods I, but *Didymochlaena* was not sampled in their analysis. Smith & al. (2006) tentatively included *Didymochlaena* (and *Hypodematium*

Received: 2 Oct 2014 | returned for (first) revision: 19 Nov 2014 | (last) revision received: 9 Dec 2014 | accepted: 12 Dec 2014 || publication date(s): online fast track, n/a; in print and online issues, 2 Mar 2015 ||

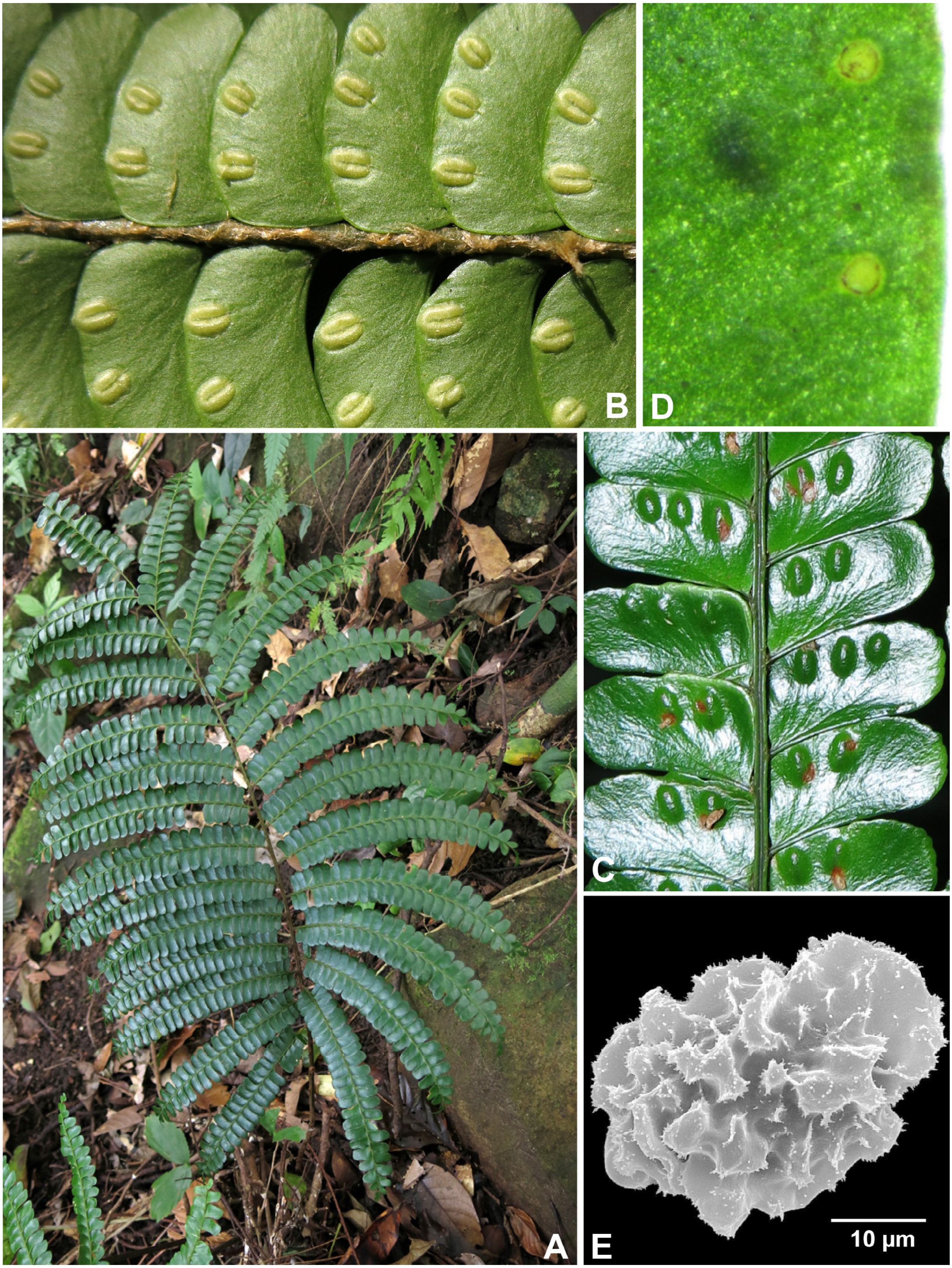

Fig. 1. Morphology of *Didymochlaena truncatula*. **A,** habit; **B,** portion of pinna showing elliptic-oblong sori; **C,** portion of pinna showing shape of pinnules and bumps caused by sunken sori on abaxial leaf surface; **D,** portion of pinnule margin showing clavate veinlet ends; **E,** spore. — D & E. courtesy of Robbin C. Moran.

and *Leucostegia*) in Dryopteridaceae pending further studies. Based on *atpA*, *atpB*, and *rbcL* data Schuettpelz & al. (2007) associated *Didymochlaena* with *Davallia* Sm. (Davalliaceae), *Dryopteris* Adans. (Dryopteridaceae), and *Nephrolepis* Schott (Nephrolepidaceae). Based on a larger taxon sampling and the same gene sampling, Schuettpelz & Pryer (2007) confirmed that *Didymochlaena* was a member of eupolypods I and resolved it as either sister to *Hypodematium* and *Leucostegia*, but without strong support. Using *atpB* and *rbcL* sequences, Liu & al. (2007b) concluded that *Didymochlaena*, *Hypodematium*, and *Leucostegia* should be excluded from Dryopteridaceae. Based on *matK* data, five plastid (*atpA*, *atpB*, *matK*, *rbcL*, *trnG-R*), and eight nuclear (18S) and plastid (*atpA*, *atpB*, *rbcL*, *rpoC1*, *rps4*, *trnH-psbA*, *trnL-trnF*) loci, respectively, Kuo & al. (2011), Rothfels & al. (2012a), and Lehtonen & al. (2012) found that *Didymochlaena* was sister to the rest of the 18, 8, and 7 species of eupolypods I sampled, respectively. Sampling six plastid loci (*atpA*, *atpB*, *rbcL*, *rps4-trnS*, *trnL-F*, *trnG-R*) Liu & al. (2013) appeared to have found that *Didymochlaena* was sister to *Hypodematium*+*Leucostegia* with strong support, though they did not include any eupolypods II in their taxon sampling.

The objectives of this study were: (1) to resolve the relationships of *Didymochlaena* based on multiple plastid loci and dense sampling of eupolypods I; (2) to evaluate previous morphological/cytological hypotheses about its relationships with Dryopteridaceae, Hypodematiaceae, and the rest of eupolypods I; and (3) to validly describe the family Didymochlaenaceae if it should be recognized.

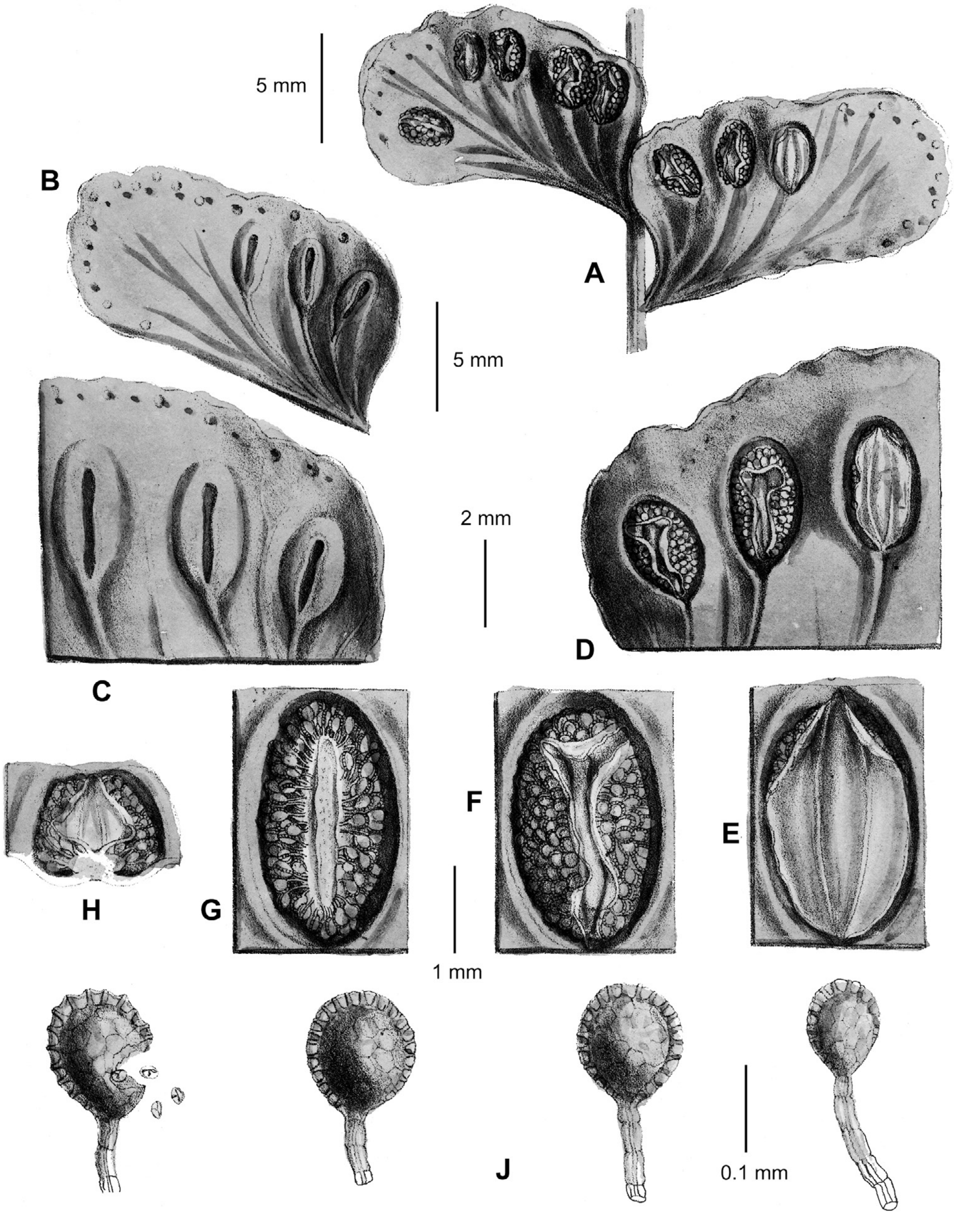

Fig. 2. Illustration of *Didymochlaena truncatula*. **A,** portion of pinna showing arrangement of pinnules; **B,** pinnule; **C–D,** portions of pinnules showing shape of sori; **E,** young sorus covered by indusium; **F,** mature sorus and shrunken indusium; **G,** mature sorus without indusium; **H,** cross section of sorus; **J,** development of sporangium. — Drawn by Francis Bauer; reproduced and modified from Hooker, 1842: pl. 120).

■ MATERIALS AND METHODS

Taxon sampling. — To resolve the phylogenetic position of *Didymochlaena*, representatives of all nine families included as members of eupolypods I by Liu & al. (2013) were sampled. One accession of *Didymochlaena truncatula* of wild origin from Ecuador (Zamora-Chinchipe) and three accessions of cultivated material, were included in our analysis. Other taxa of eupolypods I sampled included 16 species representing *Arthropteris* J.Sm., the only genus, of Arthropteridaceae sensu Liu & al. (2013); 2 species representing 2 genera, *Araiostegia* Copel. and *Davallia*, of Davalliaceae sensu Tsutsumi & Kato (2006); 6 species representing 3 genera, *Ctenitis* (C.Chr.) C.Chr., *Dryopteris*, and *Lastreopsis* Ching, of Dryopteridaceae sensu Liu & al. (2007b) and Zhang & al. (2013); 8 species representing *Hypodematium* and *Leucostegia*, the only 2 genera of Hypodematiaceae; 8 species representing 2 genera, *Cyclopeltis* J.Sm. and *Lomariopsis* Fée, of Lomariopsidaceae; 4 species representing *Nephrolepis*, the only genus of Nephrolepidaceae (Hennequin & al., 2010); 5 species representing *Oleandra* Cav., the only genus of Oleandraceae; 3 species representing 3 genera, *Arthromeris* (Moore) J.Sm., *Phlebodium* (R.Br.) J.Sm., and *Polypodium* L., of Polypodiaceae; and 26 species representing *Hypoderris* R.Br., *Pteridrys* C.Chr. & Ching, *Tectaria* Cav., and *Triplophyllum* Holttum, the only 4 genera of Tectariaceae sensu Moran & al. (2014). In total, our ingroup contained 88 accessions representing 79 species in 20 genera of the eupolypods I. Five species of eupolypods II were used as outgroups following previous studies that resolved eupolypods I and II as sister to each other (e.g., Schneider & al., 2004; Schuettpelz & Pryer, 2007; Lehtonen, 2011; Lehtonen & al., 2012; Rothfels & al., 2012a). These five outgroup species included two species of *Athyrium* Roth (Athyriaceae), one species each of *Acystopteris* Nakai and *Cystopteris* Bernh. (Cystopteridaceae), and one species of *Hemidictyum* C.Presl (Hemidictyaceae).

Voucher information and GenBank accession numbers for each sampled taxon are provided in Appendix 1.

Morphology. — Morphological data were obtained from observations of herbarium specimens and from the literature (e.g., Ching, 1940; Kramer & al., 1990). Herbarium investigations were carried out at MO and digital images provided by H were studied.

DNA extraction, amplification, and sequencing. — Total genomic DNA was extracted from silica-dried material or sometimes from herbarium specimens using the TIANGEN plant genomic DNA extraction kit (TIANGEN Biotech., Beijing, China) or DNeasy Plant Mini Kits (Qiagen, Hilden, Germany) following the manufacturers' protocols.

Five plastid regions (*atpA* gene, *rbcL* gene, *rps4-trnS* intergenic spacer, *trnL* intron, *trnL-F* intergenic spacer) were selected based on their use in earlier studies of Dryopteridaceae, other eupolypods I, eupolypods II, etc. (Li & Lu, 2006b; Rothfels & al., 2012a; Sessa & al., 2012a; Zhang & Zhang, 2012; Zhang & al., 2012; Liu & al., 2013; Ding & al., 2014; Moran & al., 2014). The *atpA* gene was amplified with primers ESATPF412F and ESTRNR46F (Schuettpelz & al., 2006). Most *rbcL* sequences were amplified with primers F1 (Fay & al., 1997) and 1379R originally designed by Zurawski & al. (1984) and modified by Wolf & al. (1999). For some herbarium specimens with degraded DNA, the internal primers 595F, 650R, and 819R of the *rbcL* gene (Zhang & al., 2014) were employed when amplification of the larger region was unsuccessful. The *rps4-trnS* intergenic spacer was amplified with primers TRNS (Souza-Chies & al., 1997) and an anonymous primer derived from Li & Lu (2006b). The *trnL* intron and *trnL-F* intergenic spacer were amplified together using primers FERN1 (Trewick & al., 2002) and F (Taberlet & al., 1991). The PCR conditions followed Zhang & al. (2001). Amplified fragments were purified with TIANquick Mini Purification Kits (TIANGEN). Purified PCR products were sequenced by Invitrogen (Shanghai, China).

To obtain nuclear evidence for the phylogeny of *Didymochlaena*, nuclear 18S gene was chosen based on earlier studies on a phylogeny of leptosporangiate fern (Pryer & al., 2004) and a phylogeny of Lindsaeaceae (Lehtonen & al., 2012). Nine species representing six families of eupolypods I and six species representing four families of eupolypods II were included in the analysis (see Appendix 1 and Electr. Suppl.: Fig. S1). Two species of Pteridaceae were used as outgroups.

Sequence alignment and phylogenetic analysis. — Sequencher v.4.1 (Gene Codes Corp., Ann Arbor, Michigan, U.S.A.) was used to assemble and edit complementary strands. Sequences obtained for each locus were aligned individually using Clustal X v.1.81 (Thompson & al., 1997) followed by manual adjustments using BioEdit v.7.0.9.0 (Hall, 1999). Partial regions of the *rps4-trnS* spacer and *trnL-F* spacer were excluded prior to analysis, because they were highly divergent and difficult to align.

Equally weighted maximum parsimony (MP) analyses were conducted for each locus and the simultaneous analysis (Kluge, 1989; Nixon & Carpenter, 1996) of all nucleotide characters using 1000 tree-bisection-reconnection (TBR) searches in PAUP* v.4.0b10 (Swofford, 2002) with MAXTREES set to increase without limit. Insertions and deletions were coded as missing data. Parsimony jackknife (MP JK) analyses (Farris & al., 1996) were conducted using PAUP* with the removal probability set to approximately 37%, and "jac" resampling emulated. Two thousand replicates were performed with 10 TBR searches per replicate and a maximum of 100 trees held per TBR search.

jModelTest v.0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) was used to select the best-fitting likelihood model for maximum likelihood (ML; Felsenstein, 1973) analyses. The Akaike information criterion (Akaike, 1974) was used to select among models instead of the hierarchical likelihood ratio test, following Pol (2004) and Posada & Buckley (2004). The best-fitting models and parameter values are provided in Table 1.

For each locus and the simultaneous analysis, ML tree searches and ML bootstrapping (ML BS) were conducted using the web server RAxML-HPC2 on TG v.7.2.8 (Stamatakis & al., 2008; Miller & al., 2010) with 5000 rapid bootstrap analyses followed by a search for the best-scoring tree in a single run (Stamatakis & al., 2008).

Bayesian inference (BI) was conducted using MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck,

2003) on Cipres (Miller & al., 2010). Four Markov chain Monte Carlo chains were run, each beginning with a random tree and sampling one tree every 1000 of 10 million generations. Convergence among generations was checked using Tracer v.1.4 (Rambaut & Drummond, 2007) and the first 25% was discarded as burn-in to ensure that stationarity in log-likelihood had been reached. The remaining trees were used to calculate a 50% majority-rule consensus topology.

■ RESULTS

The dataset characteristics and tree statistics for the analyses are presented in Table 2. Comparisons of tree topologies from the MP JK analyses of the individual markers identified no well-supported conflicts (JK ≥ 70%; Mason-Gamer & Kellogg, 1996; Zhang & Simmons, 2006; Zhang & al., 2012; Zhang & al., 2014). Thus, the five plastid datasets were concatenated. The topology of the ML, MP, and BI trees based on the combined dataset (Fig. 3) were mostly identical to those based on each individual marker, but with generally increased support values.

The monophyly of eupolypods I was strongly supported in ML, MP, and BI analyses based on plastid data, which helped confirm the eupolypod I ferns as a natural group (Fig. 3). All but one (Tectariaceae) families as well as *Didymochlaena* were recovered as monophyletic groups with strong support. The familial relationships within eupolypods I were well resolved with strong support values. The overall relationships were consistent with previous studies (e.g., Lehtonen, 2011; Liu & al., 2013). *Didymochlaena* was resolved as sister to the rest of eupolypods I, followed by Hypodematiaceae, Dryopteridaceae, the Lomariopsidaceae+Nephrolepidaceae clade, the Arthropteridaceae+Tectariaceae clade, and the Oleandraceae +(Davalliaceae+Polypodiaceae) clade.

The best tree from the ML analysis of nuclear 18S data resolved *Didymochlaena* as a member of the eupolypods I clade (ML BS = 95%, MP JK = 86%) and being embedded within Dryopteridaceae, but with no significant support (<50% ML BS & MP JK; Electr. Suppl.: Fig. S1).

■ DISCUSSION

Our study is the first to include multiple samples of *Didymochlaena*. Our analysis showed that *Didymochlaena* is monophyletic and in fact there is very little intraspecific variation among the four samples included (Fig. 3). Based on the largest sampling so far of eupolypods I and sequence data from five plastid loci, our study resolved *Didymochlaena* as sister to the

Table 1. Best-fitting models and parameter values for separate and simultaneous plastid datasets in this study.

	AIC selected	Base frequencies				Substitution model (rate matrix)								
Region	model	A	C	G	T	A–C	A–G	A–T	C–G	C–T	G–T	Ti/Tv	I	G
atpA gene	GTR+I+G	0.3007	0.1946	0.2081	0.2966	1.5950	9.0007	0.3177	0.8158	17.6157	1.0000	–	–	0.3020
rbcL gene	GTR+I+G	0.2710	0.2113	0.2477	0.2701	1.5035	7.6960	0.8849	0.9820	11.7343	1.0000	–	0	0.2180
rps4-trnS spacer	GTR+G	0.3167	0.1762	0.1851	0.3220	1.1480	3.2923	0.4524	0.5946	3.5496	1.0000	–	0	0.9120
trnL intron	GTR+G	0.3246	0.1762	0.2085	0.2907	0.8977	3.0000	0.2121	0.4549	3.7664	1.0000	–	0	1.0250
trnL-F spacer	HKY+I+G	0.2967	0.2077	0.1837	0.3120	–	–	–	–	–	–	1.4795	0	1.2830
trnL intron+*trnL-F* spacer	GTR+G	0.3219	0.1806	0.1926	0.3048	1.0216	2.4402	0.3356	0.6280	3.0361	1.0000	–	0	1.1540
simultaneous	GTR+G	0.2967	0.1932	0.2130	0.2971	1.2652	4.3398	0.5270	0.6860	6.5504	1.0000	–	0	0.3790

G, gamma distribution shape parameter (Yang, 1994); GTR, general-time-reversible model (Tavaré, 1986); I, proportion of invariable sites; Ti/Tv, transition/transversion ratio.

Table 2. Data matrices and tree statistics for each of the analyses.

Matrix	Number of accessions	Number of characters	Number of parsimony-informative characters (%)*	Most parsimonious tree length	Number of MP JK/ML BS clades with support ≥50%	Average MP JK/ML BS support [%]	Consistency index	Retention index
atpA gene	54	1759	498 (28.3)	1639	43/42	76/90	0.4753	0.7230
rbcL gene	93	1318	371 (28.1)	1383	73/71	89/87	0.4013	0.7968
rps4-trnS spacer	43	1138	393 (34.5)	1246	36/31	90/92	0.6364	0.7372
trnL intron	21	665	198 (29.8)	580	17/16	95/94	0.6741	0.7415
trnL-F spacer	55	532	237 (44.5)	981	42/41	84/86	0.5240	0.7067
trnL intron+*trnL-F* spacer	56	1197	439 (36.7)	1586	44/43	88/90	0.5744	0.7130
simultaneous	93	5412	1701 (31.4)	5979	78/75	93/94	0.5089	0.7414

* Inclusive of outgroups. — BS, bootstrap; JK, jackknife; ML, maximum likelihood; MP, maximum parsimony.

rest of eupolypods I with strong support (99% ML BS, 96% MP JK, 1.0 BI PP). This resolution is consistent with the findings by Kuo & al. (2011; based on *matK*, *rbcL*, and *atpA* data) and Rothfels & al. (2012a; based on data from five plastid loci) where the sister relationships between *Didymochlaena* and the rest of 18 and 8 eupolypods I sampled, respectively, were weakly supported. Our resolution of *Didymochlaena* is also in accordance with results of earlier studies by Schuettpelz & al. (2007; based on *atpA*, *atpB*, and *rbcL* data), which found that *Didymochlaena* was sister to the remaining three eupolypods I sampled. Notably, a phylogenetic analysis by Lehtonen & al. (2012) based on plastid and nuclear data strongly (89% JK) supported *Didymochlaena* as sister to a clade containing members of eupolypods I only, e.g., *Dryopteris*, *Rumohra* Raddi (Dryopteridaceae), *Davallia* (Davalliaceae), *Nephrolepis* (Nephrolepidaceae), *Polypodium* (Polypodiaceae), and *Tectaria* (Tectariaceae).

Nuclear data have rarely been used for phylogenetic inference in ferns, often because of lack of molecular markers with appropriate variability. Where nuclear markers have been used,

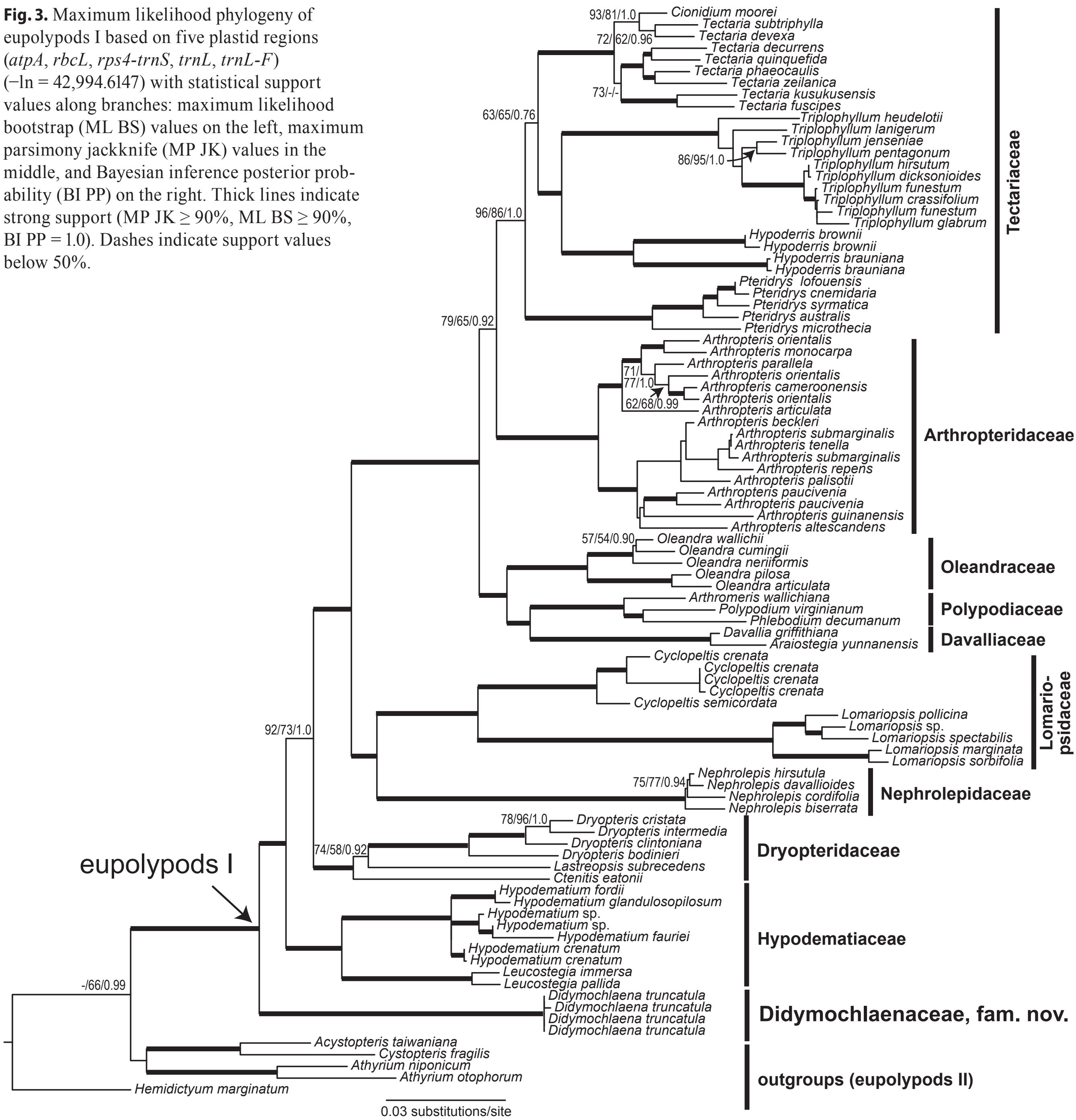

Fig. 3. Maximum likelihood phylogeny of eupolypods I based on five plastid regions (*atpA*, *rbcL*, *rps4-trnS*, *trnL*, *trnL-F*) (−ln = 42,994.6147) with statistical support values along branches: maximum likelihood bootstrap (ML BS) values on the left, maximum parsimony jackknife (MP JK) values in the middle, and Bayesian inference posterior probability (BI PP) on the right. Thick lines indicate strong support (MP JK ≥ 90%, ML BS ≥ 90%, BI PP = 1.0). Dashes indicate support values below 50%.

this has largely been limited to studies addressing intrageneric or interspecific relationships, e.g., combined ITS, *gapCp*, and *pgiC* data for studying the reticulate evolution of some species of *Asplenium* L. (Schneider & al., 2013), *pgiC* data for reconstructing the phylogeny of *Dryopteris* (Juslén & al., 2011; Sessa & al., 2012b), and *gapCp* for confirming allopolyploidy in Cystopteridaceae (Rothfels & al., 2014). These markers have proved either too variable to be useful at higher levels or otherwise problematic in different clades e.g., due to multiple copies in polyploids (Rothfels & al., 2014). Neither these, nor equivalent more variable nuclear markers, are currently available for accessions representing the breadth of fern taxa analysed here, although analysis of such data is clearly warranted. At interfamilial level the only nuclear marker for which there is currently somewhat representative data is the 18S gene. However, this has shown only limited resolving power (Pryer & al., 2004). Our result based on nuclear 18S data is hence far from decisive: although it is consistent with the plastid data in placing *Didymochlaena* within eupolypods I, with regard to relationships within that (sparsely sampled) clade, we can only conclude that it does not contradict the plastid tree (Electr. Suppl.: Fig. S1).

The resolution of *Didymochlaena* as sister to the rest of eupolypods I appears conflicting with the findings by Schneider & al. (2004; based on *rbcL* and *rps4* data) and Schuettpelz & Pryer (2007; based on *atpA*, *atpB*, and *rbcL* data) who resolved *Didymochlaena* as sister either to *Hypodematium* (support value unknown) or to the *Hypodematium*+ *Leucostegia* clade (54% SB support). Liu & al. (2013) found the same resolution of *Didymochlaena* as did Schuettpelz & Pryer (2007) with *Didymochlaena* being sister to the *Hypodematium*+ *Leucostegia* clade (strong support) and in consequence they included *Didymochlaena* in Hypodematiaceae. However, Liu & al. (2013) did not sample any eupolypods II in their study and their sampling of eupolypods I was relatively sparse. We added five eupolypods II (*Asplenium trichomanes* L., Aspleniaceae; *Athyrium otophorum* (Miq.) Koidz., *Deparia lancea* (Thunb.) Fraser-Jenk., and *Diplazium dilatatum* Blume, Athyriaceae; *Woodwardia unigemmata* (Makino) Nakai, Blechnaceae) to Liu & al.'s alignment available online (Liu & al., 2013, Supplementary Data) and conducted an ML analysis. This new analysis resolved *Didymochlaena* as sister to the rest of eupolypods I with 99% BS support (Electr. Suppl.: Fig. S2), consistent with our results (Fig. 3).

Morphologically, *Didymochlaena* is distinct from the rest of eupolypods I in a number of characters. Smith (1841) noticed that its elongated sori are peculiar in Aspidieae sensu Smith (1841). In fact, its elliptic-oblong sori (Figs. 1B, 2A–G) are different from those of any other eupolypods I, but similar to those of some Aspleniaceae or Athyriaceae of eupolypods II. Its dimidiate pinnules are reminiscent of those of *Lindsaea* Dryand. ex Sm. (Lindsaeaceae, the basalmost family in Polypodiales; Lehtonen & al., 2012) as noted by Ching (1940). Its pinnule shape and arrangement (Fig. 1A, C) are also similar to those of *Asplenium*.

Tryon & Lugardon (1991) placed *Didymochlaena* in Dryopteridaceae based on three characters of *Didymochlaena*: (1) the elongate indusia; (2) the chromosome number of $x = 41$ (Manton, 1954; Smith & Foster, 1984; Walker, 1985); and (3) the tuberculate folds of the spores. As discussed above, elliptic-oblong sori/indusia are not found in Dryopteridaceae in their current circumscription (e.g., Zhang & al., 2013). In fact in other eupolypods I only some species of *Tectaria* (Tectariaceae) and some of Polypodiaceae have sori spread out along veins or leaf margins. Thus the elongate sori/indusia are not a synapomorphy of Dryopteridaceae. The chromosome number $x = 41$ is dominant in eupolypods I except Davalliaceae, Polypodiaceae, and some Tectariaceae (Smith & al., 2006). A few species of *Hypodematium* (Hypodematiaceae) also have $x = 40$ in addition to the dominant $x = 41$ in *Hypodematium*, but $x = 41$ in *Hypodematium* is plesiomorphic, whereas $x = 40$ is apomorphic (Wang & al., 2014). The distribution of the chromosome number $x = 41$ in eupolypods I, in conjunction with the phylogeny of eupolypods I resolved in our analysis (Fig. 3), suggests that the chromosome number $x = 41$ is most likely a synapomorphy of the entire eupolypods I (evolved from $x = 41$ to different numbers in Davalliaceae, Hypodematiaceae, Polypodiaceae, and some Tectariaceae). It is not a synapomorphy of Dryopteridaceae. Sundue & Rothfels (2014) even concluded that $x = 41$ is pleisiomorphic for the entire eupolypod clade (both I and II). Thick perispores with tuberculate folds are not a synapomorphy of Dryopteridaceae either, since this character state or its modification (wings) exists in *Arthropteris* (Arthropteridaceae), Dryopteridaceae, *Cyclopeltis* and *Lomariopsis* (Lomariopsidaceae), Hypodematiaceae, *Nephrolepis* (Nephrolepidaceae), Oleandraceae, and Tectariaceae, while Davalliaceae and Polypodiaceae have thin perispores with a plain, papillate, verrucate, or granulate surface (Tryon & Lugardon, 1991). It seems plausible that, similar to the chromosome number $x = 41$, the folds/wings on perispores are possibly a synapomorphy of eupolypods I, too (evolved from thick folds/wings to thin verrucae, granulae, etc. in Davalliaceae and Polypodiaceae). Therefore, none of the three characters Tryon & Lugardon (1991) used to place *Didymochlaena* in Dryopteridaceae is a synapomorphy of Dryopteridaceae. A cladistic analysis based on morphological characters for the whole eupolypods I clade in the future would address this issue more clearly.

The hypothesis by Ching (1940: 254) that *Didymochlaena* was somewhat related to *Sphaerostephanos* J.Sm. (Thelypteridaceae) based on "the type of spores and the peculiar soral character" of *Didymochlaena* is not supported by our study because Thelypteridaceae are clearly members of eupolypods II (Rothfels & al., 2012a).

Given the well-supported sister relationship between *Didymochlaena* and the rest of eupolypods I (Fig. 3; Electr. Suppl.: Fig. S1), the striking morphology of *Didymochlaena* (Figs. 1–2), and the fact that a number of families within eupolypods I are generally recognized (e.g., Smith & al., 2006; Schuettpelz & Pryer, 2007; Lin & al., 2013; Liu & al., 2013), it is compelling to validate the family Didymochlaenaceae earlier proposed by Ching (1940) who provided no Latin description and thus failed to validly published this family (*ICN* Art. 39.1, McNeill & al., 2012). Another motivating factor for recognizing

Didymochlaenaceae is the deep isolation of *Didymochlaena* from any other extant group of ferns, and the fact that lumping *Didymochlaena* with any other group (while maintaining monophyletic families) would require that all eupolypods I be treated as a single family, including such distinct and long-recognized taxa as Dryopteridaceae and Polypodiaceae. This general philosophy (emphasizing the value of recognizing distinct deeply isolated lineages and the problems with lumping distinct taxa that have long histories of recognition) has been followed before in other classifications, and is articulated in, for example, the Smith & al. (2006) classification of ferns, the Rothfels & al. (2012b) classification of the eupolypods II clade of ferns, and the APG classifications (e.g., APG, 2009).

■ TAXONOMIC TREATMENTS

Didymochlaenaceae Ching ex Li Bing Zhang & Liang Zhang, **fam. nov.** – Type: *Didymochlaena* Desv.

Diagnosis. – Didymochlaenaceae are similar to Dryopteridaceae in having rhizomes and petioles with scales, petioles with three or more vascular bundles arranged in a semi-circle, perispores with tuberculate folds, and a chromosome number of $x = 41$, but Didymochlaenaceae have elongate sori, while Dryopteridaceae have rounded sori.

Terrestrial ferns with short, erect, subarborescent, dictyostelic rhizome bearing long, narrow and small, subentire, hair-like scales and ± persistent petiole bases. Leaves tufted, large, monomorphic, firmly herbaceous, long-petiolate. Petiole stramineous, at least the base ± persistently scaly, adaxially grooved, not articulated, with three or more vascular bundles arranged in a semi-circle. Lamina bipinnate (Fig. 1A), oblong-ovate, anadromous or isodromous; neither upper nor lower pinnae reduced, apical pinna conform. Rachis and costa with very narrow scales, adaxially grooved. Pinnules dimidiate, somewhat rectangular, subsessile, at least the basal ones articulated at the base, apex rounded, basal margins thickened. Upper pinnules abruptly reduced and confluent. Midrib near the posterior margin or diagonal, weakly developed, not grooved. Lateral veins free, forked, with clavate ends before reaching subentire to crenate margins (Figs. 1D, 2A–C). Sori elliptic-oblong (Figs. 1B, 2A–G), terminating a vein, indusiate, often somewhat sunken in blade, forming bumps on adaxial side (Fig. 1C); margins of fertile leaves often sterile. Indusia elongate, centrally attached along a line, opening on either side. Receptacle elongate, bearing sporangia on both sides and around its distal extremity. Sporangia long-stalked (Fig. 2J). Spores monolete, ellipsoidal to globose, tuberculate and echinate on perispore (Fig. 1E). Chromosome number $x = 41$ (Manton, 1954; Smith & Foster, 1984; Walker, 1985).

A monogeneric family containing only one (*Didymochlaena truncatula* (Sw.) J.Sm.) or more species with nearly pantropical distribution, but not in Australia.

Both Hooker (1842: pl. 120) and Ching (1940) described the spores of *Didymochlaena* as trilete. Ching (1940) further reported that the spores have no perispores. These statements are incorrect (see our Fig. 1E).

■ ACKNOWLEDGMENTS

We thank Robbin Moran for allowing us to use two images of *Didymochlaena truncatula*; Na-ngern Prathomrak for sharing photos of this species; Aino Juslén, Ari Taponen, and Henry Väre for helping with image of a voucher; Ngan Thi Lu for participicting the fieldwork in finding *D. truncatula*; and three anonymous reviewers and Michael D. Pirie for valuable suggestions and comments.

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Appendix 1. List of taxa sampled with information related to taxonomy, GenBank accession numbers, references (numbers without reference were newly generated), and voucher information.

Adiantum raddianum C.Presl, *Wolf & Anderson-Wong 244* (UTC), U.S.A. (Hawaii): 18S U18621 (Wolf, 1995). ***Araiostegia yunnanensis*** (Christ) Copel., *Wu 2458* (KUN), Laos: *rbcL* JX103718 (Kim & al., 2013), *rps4-trnS* JX103760 (Kim & al., 2013). ***Asplenium nidus*** L., *Lehtonen 716* (TUR), unknown: 18S HQ157257 (Lehtonen & al., 2012). ***Athyrium filix-femina*** (L.) Roth, *Christenhusz 4279* (TUR), unknown: 18S HQ157254 (Lehtonen & al., 2012). ***A. niponicum*** (Mett.) Hance, unknown, Japan: *rbcL* JF832057 (Rothfels & al., 2012a), *atpA* JF832097 (Rothfels & al.,2012a); *Wang 013* (WNU), China (Chongqing): *trnL* intron & *trnL-F* spacer AF515256 (Wang & al., 2002); *Lu FH25* (PYU), China: *rps4-trnS* JN168077 (Li & al., 2011). ***A. otophorum*** (Miq.) Koidz., *Smith s.n.* (UC), cult.: *rbcL* EF463305 (Schuettpelz & Pryer, 2007), *atpA* EF463563 (Schuettpelz & Pryer, 2007); unknown, cult.: *trnL* intron & *trnL-F* spacer EU329087 (Adjie & al., 2008); *Lu X8* (PYU), China: *rps4-trnS* JN168076 (Li & al., 2011). ***Acystopteris taiwaniana*** (Tagawa) Á.Löve & D.Löve, *Schuettpelz & al. 1127A* (DUKE), China (Taiwan): *rbcL* JF832052 (Rothfels & al., 2012a), *atpA* JF832091 (Rothfels & al., 2012a). ***Arthromeris wallichiana*** (Spreng.) Ching, *Schneider s.n.* (E), cult.: *rbcL* EF463243 (Schuettpelz & Pryer, 2007), *atpA* EF463799 (Schuettpelz & Pryer, 2007). ***Arthropteris altescandens*** J.Sm., *Solbrig 3825* (NY), Chile: *rbcL* KF667636 (Moran & al., 2014), *trnL-F* spacer KF667606 (Moran & al., 2014), *rps4-trnS* KF667550 (Moran & al., 2014). ***A. articulata*** (Brack.) C.Chr., *Leon Perri*e *FJ11-50* (WELT), Fiji: *rbcL* KC977367 (Liu & al., 2013), *atpA* KC977329 (Liu & al., 2013), *trnL* intron & *trnL-F* spacer KC977411 (Liu & al., 2013), *rps4-trnS* KC977437 (Liu & al., 2013). ***A. beckleri*** Mett., *Taylor 4761* (NY), Chile: *rbcL* KF667637 (Moran & al., 2014), *trnL-F* spacer KF667607 (Moran & al., 2014). ***A. cameroonensis*** Alston, *Carvalho 3771* (NY), Equatorial Guinea: *rbcL* KF667638 (Moran & al., 2014), *rps4-trnS* KF667551 (Moran & al., 2014). ***A. guinanensis*** H.G.Zhou & Y.Y.Huang, *Jiang JRH1506* (IBK), China (Guangxi): *rbcL* KC977370 (Liu & al., 2013), *atpA* KC977324 (Liu & al., 2013), *trnL* intron & *trnL-F* spacer KC977405 (Liu & al., 2013), *rps4-trnS* KC977446 (Liu & al., 2013). ***A. monocarpa*** (Cordem.) C.Chr., *Kukkonen 12551* (H), Ethiopia: *rbcL* KF887155 (Wang & al., 2014), *atpA* KF897993 (Wang & al., 2014), *trnL-F* spacer KF897941 (Wang & al., 2014). ***A. orientalis*** (J.F.Gmel.) Posth., *Hennequin S156* (BM), Réunion: *rbcL* KC977378 (Liu & al., 2013), *trnL* intron & *trnL-F* spacer KC977420 (Liu & al., 2013), *rps4-trnS* KC977435 (Liu & al., 2013). ***A. orientalis*** (J.F.Gmel.) Posth., *Robinson 6213* (NY), Zambia: *rbcL* KF667639 (Moran & al., 2014), *trnL-F* spacer KF667608 (Moran & al., 2014), *rps4-trnS*KF667552 (Moran & al., 2014). ***A. orientalis*** (J.F.Gmel.) Posth., *Smith s.n.* (UC), Madagascar: *rbcL* KJ196903 (Ding & al., 2014), *atpA* KJ196684 (Ding & al., 2014). ***A. palisotii*** (Desv.) Alston, *Patrick J. Acock PA1033* (BM), Australia: *rbcL* KC977379 (Liu & al., 2013); *s.coll., s.n.* (SZG), China (Guangxi): *atpA* KC977325 (Liu & al., 2013); *Dong 3607* (IBSC), China (Yunnan): *trnL-F* spacer KF561671 (Ding & al., 2014); *Crawford 11126* (V), Masatierra: *rps4-trnS* KC977433 (Liu & al., 2013). ***A. parallela*** C.Chr., *Schuettpelz 524* (GOET), cult.: *rbcL* EF463266 (Schuettpelz & Pryer, 2007), *atpA* EF463862 (Schuettpelz & Pryer, 2007), *trnL-F* spacer KC977425 (Liu & al., 2013), *rps4-trnS* KC977453 (Liu & al., 2013). ***A. paucivenia*** (C.Chr.) H.M.Liu & al., *Rakotondrainibe 6585* (P), Madagascar: *rbcL* KJ 196902 (Ding & al., 2014), *trnL-F* spacer KJ196681 (Ding & al., 2014). ***A. paucivenia*** (C.Chr.) H.M.Liu & al., *Rakotondrainibe 6586* (P), Madagascar: *rbcL* EF463268 (Schuettpelz & Pryer, 2007), *atpA* EF463864 (Schuettpelz & Pryer, 2007), *trnL-F* spacer KC977426 (Liu & al., 2013), *rps4-trnS* KC977454 (Liu & al., 2013). ***A. repens*** (Brack.) C.Chr., *Leno Perrie FJ11-191* (WELT), Fiji: *rbcL* KC977368 (Liu & al., 2013), *atpA* KC977330 (Liu & al., 2013), *trnL* intron & *trnL-F* spacer KC977412 (Liu & al., 2013), *rps4-trnS* KC977438 (Liu & al., 2013). ***A. submarginalis*** Domin, *Acock PA1031* (BM), Australia (Queensland): *rbcL* KC977380 (Liu & al., 2013), *atpA* KC977338 (Liu & al., 2013), *trnL* intron & *trnL-F* spacer KC977422 (Liu & al., 2013), *rps4-trnS* KC977451 (Liu & al., 2013). ***A. submarginalis*** Domin, *Acock PA1028* (BM), Australia (Queensland): *rbcL* KC977381 (Liu & al., 2013), *atpA* KC977339 (Liu & al., 2013). ***A. tenella*** (G.Forst.) J.Sm., *Leon Perrie 6429* (BM), New Zealand: *rbcL* KC977363 (Liu & al., 2013), *trnL* intron & *trnL-F* spacer KC977424 (Liu & al., 2013), *rps4-trnS* KF011547 (Liu & al., 2013). ***Blechnum brasiliense*** Desv., *Wolf 297* (UTC), unknown: 18S AF313570 (Pryer & al., 2001). ***B. occidentale*** L., unknown: 18S U18622 (Wolf, P.G., 1995). ***Ctenitis eatonii*** (Baker) Ching, *Jiang H2036* (IBK), China (Guangxi): *rbcL* KF709483 (Liu & al., 2014), *atpA* KF709456 (Liu & al., 2014). ***Cyclopeltis crenata*** (Fée) C.Chr., *Dong 1021* (IBSC), China (Hainan): *rbcL* DQ054517 (Li & Lu, 2006a); KBCC, Cecellia Koo Botanic Conservation Center, Taiwan K016940, cult. originally from Thailand: *atpA* JF304016 (Kuo & al., 2011); *Li 568* (TAIF), cult. originally from Taiwan: *trnL-F* spacer EU216747 (Li & al., 2009). ***C. crenata*** (Fée) C.Chr., *Jin 5130* (KUN), China: *rbcL* DQ508766 (Lu & Li, unpub.), *trnL* intron & *trnL-F* spacer DQ51 4488 (Lu & Li, unpub.). ***C. crenata*** (Fée) C.Chr., *Liu 5524*, China: *rbcL* HM748134 (Hennequin & al., 2010), *rps4-trnS* HM748161 (Hennequin & al., 2010). ***C. crenata*** (Fée) C.Chr., *Zhang 2312* (PE), China: *rbcL* EF463119 (Liu & al., unpub.), *rps4-trnS* EF540718 (Liu & al., unpub.). ***C. semicordata*** (Sw.) J.Sm., *Barrington 2129* (VT), Costa Rica: *rbcL* EF463234 (Schuettpelz & Pryer, 2007), *atpA* EF463775 (Schuettpelz & Pryer, 2007). ***Cystopteris fragilis*** (L.) Bernh., *Larsson 21* (UPS), Sweden (Uppsala): *rbcL* JF832062 (Rothfels & al., 2012a), *atpA* JF832108 (Rothfels & al., 2012a); *De Groot 912* (EB), unknown: *trnL* intron & *trnL-F* spacer HQ676522 (De Groot & al., 2011); *Lu XZ88* (PYU), China (Xizang): *rps4-trnS* JN168082 (Li & al., 2011). ***Davallia griffithiana*** Hook., *Schuettpelz 506* (DUKE), cult.: *rbcL* EF463165 (Schuettpelz & Pryer, 2007), *atpA* EF463649 (Schuettpelz & Pryer, 2007). ***D. solida*** var. ***fejeensis*** (Hook.) Noot., *Qiu 01055* (MICH), unknown: 18S DQ629432 (Qiu & al., 2006). ***Didymochlaena truncatula*** (Sw.) J.Sm., *Christenhusz 5861* (H), unknown: *rbcL* KJ716405 (Lehtonen, unpub.), 18S HQ157248 (Lehtonen & al., 2012). ***D. truncatula*** (Sw.) J.Sm., *Cranfill s.n.* (UC), cult.: *rbcL* AF425105 (Smith & Cranfill, 2002), *rps4-trnS* AF425161 (Smith & Cranfill, 2002). ***D. truncatula***(Sw.) J.Sm., Cecellia Koo Botanic Conservation Center, Taiwan K017011, cult.: *rbcL* JF303975 (Kuo & al., 2011). ***D. truncatula*** (Sw.) J.Sm., RBGE, cult.: *rbcL* DQ508769 (Lu & Li, unpub.), *trnL* intron & *trnL-F* spacer DQ514491 (Lu & Li, unpub.); *Schuettpelz 267* (DUKE), Ecuador (Zamora-Chinchipe): *atpA* JF832112 (Rothfels & al., 2012a). ***Dryopteris bodinieri*** (Christ) C.Chr., *Liang Zhang 1378* (CDBI), China (Sichuan): *rbcL* KP271073, *trnL*

Appendix 1. Continued.

intron & *trnL-F* spacer KP271092. ***D. clintoniana*** (D.C.Eaton) Dowell, *Henry s.n.* (OAC 96815), *Canada*: *rbcL* KF186502 (Henry & Newmaster, unpub.), *atpA* KF1 86535 (Henry & Newmaster, unpub.). ***D. cristata*** (L.) A.Gray, *Henry s.n.* (OAC 96818*)*, Canada: *rbcL* KF186503 (Henry & Newmaster, unpub.), *atpA* KF186536 (Henry & Newmaster, unpub.). ***D. filix-mas*** (L.) Schott, unknown: 18S HQ680976 (Lehtonen & al., 2012). ***D. intermedia*** (Muhl. ex Willd.) A.Gray, *Henry s.n.* (OAC 96834), Canada: *rbcL* KF186510 (Henry & Newmaster, unpub.), *atpA* KF186543 (Henry & Newmaster, unpub.). ***D. wallichiana*** (Spreng.) Hyl., *Qiu 98087* (Z), unknown: 18S DQ629428 (Qiu & al., 2006). ***Hemidictyum marginatum*** (L.) C.Presl, *Christenhusz 2476* (CAY), French Guiana: *rbcL* EF463318 (Schuettpelz & Pryer, 2007), *atpA* EF463922 (Schuettpelz & Pryer, 2007). ***Hypodematium crenatum*** (Forssk.) Kuhn & Decken, *Schneider s.n.* (GOET), cult.: *rbcL* EF463205 (Schuettpelz & Pryer, 2007), *atpA* EF463705 (Schuettpelz & Pryer, 2007). ***H. crenatum*** (Forssk.) Kuhn & Decken, *Yatskievych 09-072* (MO), Nepal (Kaski): *rbcL* JF832072 (Rothfels & al., 2012a), *atpA* JF832128 (Rothfels & al., 2012a). ***H. crenatum*** subsp. ***fauriei*** (Kodama) K.Iwats., (TNS 768177), Japan: *rbcL* AB575183 (Ebihara & al., 2010). ***H. fordii*** (Baker) Ching, (TNS 763905), Japan: *rbcL* AB575184 (Ebihara & al., 2010). ***H. glandulosopilosum*** (Tagawa) Ohwi, (TNS 768179), Japan: *rbcL* AB575185 (Ebihara & al., 2010). ***Hypodematium* sp.,** *Boonkerd 2011* (BCU), Thailand: *rbcL* KP271074. ***Hypodematium* sp.,** *Liang Zhang* & *Xin Mao Zhou 002* (CDBI), China (Sichuan): *rbcL* KP271075. ***Hypoderris brauniana*** (H.Karst.) F.G.Wang & Christenh., *Rojas 4134* (NY), Costa Rica: *rbcL* KF667647 (Moran & al., 2014), *trnL-F* spacer KF667618 (Moran & al., 2014). ***H. brauniana*** (H.Karst.) F.G.Wang & Christenh., *Tuomisto 11912* (TUR), Ecuador: *rbcL* KF887171 (Wang & al., 2014), *atpA* KF898007 (Wang & al., 2014), *trnL-F* spacer KF897955 (Wang &al., 2014). ***H. brownii*** J.Sm., *Recart* & *Falcón 95* (UPRRP), Puerto Rico: *rbcL* KF887164 (Wang & al., 2014), *atpA* KF898001 (Wang & al., 2014), *trnL-F* spacer KF897949 (Wang & al., 2014). ***H. brownii*** J.Sm., *Sanchez 19* (NY), Puerto Rico: *rbcL* KF667643 (Moran & al., 2014), *trnL-F* spacer KF667612 (Moran &al., 2014), *rps4-trnS* KF667556 (Moran & al., 2014). ***Lastreopsis subrecedens*** Ching, *Dong 1718* (IBSC), China (Hainan): *rbcL* KJ196864 (Ding & al., 2014). ***Leucostegia pallida*** (Mett.) Copel., *Tsutsumi CT1057* (TI), Micronesia (Ponape Island): *rbcL* AB232389 (Tsutsumi & Kato, 2006); *Schuettpelz 605* (B), cult.: *atpA* EF463709 (Schuettpelz & Pryer, 2007). ***L. immersa*** C.Presl, *Tsutsumi CT1056* (TI), China (Taiwan): *rbcL* AB232388 (Tsutsumi & Kato, 2006); *Kuo 170* (TAIF), China (Taiwan): *atpA* JF304009 (Kuo & al., 2011). ***Lomariopsis marginata*** (Schrad.) Kuhn, *Amorim 1920* (NY), Brazil: *rbcL* AY818677 (Skog & al., 2004), *trnL-F* spacer AY540045 (Skog & al., 2004), *rps4-trnS* AY540049 (Skog & al., 2004). ***L. pollicina*** (Willemet) Mett. ex Kuhn, *Liu 5496* (PE), China: *rbcL* HM748135 (Hennequin & al., 2010), *rps4-trnS* HM748162 (Hennequin & al., 2010). ***L. sorbifolia*** (L.) Fée, *Christenhusz 4070* (TUR), Guadeloupe: *rbcL* EF463236 (Schuettpelz & Pryer, 2007), *atpA* EF463777 (Schuettpelz & Pryer, 2007). ***Lomariopsis* sp.,** (SING F042), cult.: *rbcL* AM946394 (Li & al., 2009), *rps4-trnS* AM947063 (Li & al., 2009). ***L. spectabilis*** (Kunze) Mett., *Tsutsumi & al. IN103* (TI), Indonesia (Cibodas): *rbcL* AB232401 (Tsutsumi & Kato, 2006); *Li 568* (TAIF), China (Taiwan): *atpA* JF304015 (Kuo & al., 2011); *Dong 3597* (IBSC), China (Yunnan): *trnL-F* spacer KJ196685 (Ding & al., 2014). ***Nephrolepis biserrata*** (Sw.) Schott, *Christenhusz 4865* (BM), unknown: *rbcL* HQ157305 (Lehtonen & al., 2012), *atpA* HQ157268 (Lehtonen & al., 2012), *trnL-F* spacer HQ157337 (Lehtonen & al., 2012), *rps4-trnS* HQ157329 (Lehtonen & al., 2012). ***Nephrolepis biserrata*** var. ***furcans*** L.H.Bailey, *Qiu94079* (IND), unknown: 18S DQ629430 (Qiu & al., 2006). ***N. cordifolia*** (L.) C.Presl, *Wolf 309* (UTC): *rbcL* U05933 (Wolf & al., 1994), *atpA* EF452103 (Schuettpelz & al., 2007); Malaysia; HL20000576 (L): *rps4-trnS* HM748173 (Hennequin & al., 2010). ***N. davallioides*** (Sw.) Kunze, *Schuettpelz & al. 716* (DUKE), Malaysia (Pahang): *rbcL* JF832075 (Rothfels & al., 2012a), *atpA* JF832131 (Rothfels & al., 2012a). ***N. hirsutula*** (G.Forst.) C.Presl, *Christenhusz 3580* (TUR), Puerto Rico: *rbcL* EF463237 (Schuettpelz & Pryer, 2007), *atpA* EF463778 (Schuettpelz & Pryer, 2007). ***Oleandra articulata*** (Sw.) C.Presl, *Labiak 3652* (NY), Brazil: *rbcL* KF667644 (Moran & al., 2014), *trnL-F* spacer KF667613 (Moran & al., 2014), *rps4-trnS* KF667557 (Moran& al., 2014). ***O. cumingii*** J.Sm., *Chao 1990* (TAIF), Cambodia (Siem Reap): *rbcL* KJ196816 (Ding & al., 2014), *trnL-F* spacer KJ196690 (Ding & al., 2014). ***O. neriiformis*** Cav., *Chao 1976* (TAIF), Borneo (Sarawak): *rbcL* KJ196815 (Ding & al., 2014), *trnL-F* spacer KJ196689 (Ding & al., 2014). ***O. pilosa*** Hook., *Palacios 2697* (NY), Colombia: *rbcL* KF667646 (Moran & al., 2014), *trnL-F* spacer KF667615 (Moran & al., 2014), *rps4-trnS* KF667559 (Moran & al., 2014). ***O. wallichii*** (Hook.) C.Presl, *Tsutsumi TH10* (TI), Thailand (Chiang Mai): *rbcL* AB212687 (Tsutsumi & Kato, 2005); *Kuo 830* (TAIF), China (Taiwan): *atpA* JF304017 (Kuo & al., 2011). ***Phlebodium decumanum*** (Willd.) J.Sm., *Schuettpelz 216* (DUKE), Ecuador (Napo): *rbcL* EF463256 (Schuettpelz & Pryer, 2007), *atpA* EF463836 (Schuettpelz & Pryer, 2007). ***Polypodium polycarpon*** Cav., *Qiu 94084* (IND), unknown: 18S DQ629431 (Qiu & al., 2006). ***P. virginianum*** L., *Henry s.n.* (OAC 96886), Canada: *rbcL* KF186527 (Henry & Newmaster, unpub.), *atpA* KF186559 (Henry & Newmaster, unpub.). ***P. vulgare*** L., *Lehtonen 714* (TUR), unknown: 18S HQ157247 (Lehtonen & al., 2012). ***Pteridium aquilinum*** (L.) Kuhn, *Wolf 237* (UTC), U.S.A. (Idaho): 18S U18628 (Wolf, P.G., 1995). ***Pteridrys australis*** Ching, *Dong 3560* (IBSC), China (Yunnan): *rbcL* KJ196892 (Ding & al., 2014), *trnL-F* spacer KJ196678 (Ding & al., 2014). ***P. cnemidaria*** (Christ) C.Chr. & Ching, *Wang 5483* (K), China: *rbcL* KF887169 (Wang & al., 2014), *atpA* KF898005 (Wang & al., 2014), *trnL-F* spacer KF897953 (Wang & al., 2014). ***P. lofouensis*** (Christ) C.Chr. & Ching, *Liu BJ01* (PE), China: *rbcL* EF460687 (Liu & al., 2007a). ***P. microthecia*** (Fée) C.Chr. & Ching, *Cicuzza 947* (BM), Indonesia (Sulawesi): *rbcL* KF709489 (Liu & al., 2014), *atpA* KF709460 (Liu & al., 2014), *rps4-trnS* KF709503 (Liu & al., 2014); *Cicuzza 676* (BM), Indonesia (Sulawesi): *trnL* intron & *trnL-F* spacer KF709518 (Liu & al., 2014). ***P. syrmatica*** (Willd.) C.Chr. & Ching, *Dong 3439* (IBSC), Indonesia (Sumatra): *rbcL* KJ196875 (Ding & al., 2014), *trnL-F* spacer KJ196654 (Ding & al., 2014); *Kessler 13702* (BM), Indonesia (Sulawesi): *rps4-trnS* KF709507 (Liu & al., 2014). ***Rumohra adiantiformis*** (G.Forst.) Ching, *Lehtonen 566* (TUR), unknown: 18S HQ157243 (Lehtonen & al., 2012). ***Tectaria decurrens*** (C.Presl) Copel., *Li Bing Zhang, Liang Zhang* & *Ngan Thi Lu 6497* (CDBI, MO, VNMN), Vietnam (Hanoi): *rbcL* KP271076, *atpA* KP271065, *trnL* intron & *trnL-F* spacer KP271093, *rps4-trnS* KP271084. ***T. devexa*** (Kunze) Copel., *Li Bing Zhang, Liang Zhang* & *Ngan Thi Lu 6591* (CDBI, MO, VNMN), Vietnam (Lang Son): *rbcL* KP271077, *atpA* KP271066, *trnL* intron & *trnL-F* spacer KP271094, *rps4-trnS* KP271085. ***T. fuscipes*** (Wall. ex Bedd.) C.Chr., *Li Bing Zhang, Liang Zhang* & *Ngan Thi Lu 6639* (CDBI, MO, VNMN), Vietnam (Lang Son): *rbcL* KP271078, *atpA* KP271067, *trnL* intron & *trnL-F* spacer KP271095, *rps4-trnS* KP271086. ***T. incisa*** Cav., *Christenhusz 4983* (BM), unknown: 18S HQ157245 (Lehtonen & al., 2012). ***T. kusukusensis*** (Hayata) Lellinger, *Li Bing Zhang, Liang Zhang* & *Ngan Thi Lu 6466* (CDBI, MO, VNMN), Vietnam (Vinh Phuc): *rbcL* KP271079, *atpA* KP271068, *trnL* intron & *trnL-F* spacer KP271096, *rps4-trnS* KP271087. ***T. moorei*** (T.Moore) C.Chr., *Alanko 81-1889* (H), New Caledonia: *rbcL* KF887162 (Wang & al., 2014), *trnL-F* spacer KF955995 (Wang & al., 2014). ***T. phaeocaulis*** (Rosenst.) C.Chr., *Li Bing Zhang, Liang Zhang* & *Ngan Thi Lu 6492* (CDBI, MO, VNMN), Vietnam (Hanoi): *rbcL* KP271080, *atpA* KP271069, *trnL* intron & *trnL-F* spacer KP271097, *rps4-trnS* KP271088. ***T. quinquefida*** (Baker) Ching, *Li Bing Zhang, Liang Zhang* & *Ngan Thi Lu 6317* (CDBI, MO, VNMN), Vietnam (Hoa Binh): *rbcL*KP271081, *atpA* KP271070, *trnL* intron & *trnL-F* spacer KP271098, *rps4-trnS* KP271089. ***T. subtriphylla*** (Hook. & Arn.) Copel., *Li Bing Zhang, Liang Zhang* &*Ngan Thi Lu 6915* (CDBI, MO, VNMN), Vietnam (Ha Giang): *rbcL* KP271082, *atpA* KP271071, *trnL* intron & *trnL-F* spacer KP271099, *rps4-trnS* KP271090. ***T. zeilanica*** (Houtt.) Sledge, *Li Bing Zhang, Liang Zhang* & *Ngan Thi Lu 6387 (*CDBI, MO, VNMN), Vietnam (Hoa Binh): *rbcL* KP271083, *atpA* KP271072, *trnL* intron & *trnL-F* spacer KP271100, *rps4-trnS* KP271091. ***Thelypteris palustris*** Schott, *Wolf 284* (UTC), unknown: 18S AY612742 (Pryer & al., 2004); *Christenhusz 5863* (H), unknown: 18S HQ157253 (Lehtonen & al., 2012). ***Triplophyllum crassifolium*** Holttum, *Tuomisto 15691* (TUR), Brazil: *rbcL* KF887203 (Wang & al., 2014), *atpA* KF898037 (Wang & al., 2014), *trnL-F* spacer KF897985 (Wang & al., 2014). ***T. dicksonioides*** (Fée) Holttum., *Moran 6291* (NY), Ecuador: *rbcL* KF667656 (Moran & al., 2014), *trnL-F* spacer KF667629 (Moran & al., 2014), *rps4-trnS* KF667571 (Moran & al., 2014). ***T. funestum*** (Kunze) Holttum, *Christenhusz 4107* (TUR), Guadeloupe: *rbcL* EF463276 (Schuettpelz & Pryer, 2007), *atpA* EF463872 (Schuettpelz & Pryer, 2007), *trnL-F* spacer KJ196682 (Ding & al., 2014). ***T. funestum*** (Kunze) Holttum, *Hormia 2092* (H), Peru: *rbcL* KF887205 (Wang & al., 2014), *atpA* KF898039 (Wang & al., 2014), *trnL-F* spacer KF897987 (Wang & al., 2014). ***T. glabrum*** J.Prado & R.C.Moran, *Tuomisto 15444* (TUR), Brazil: *rbcL* KF887207 (Wang & al., 2014), *atpA* KF898041 (Wang & al., 2014), *trnL-F* spacer KF897989 (Wang & al., 2014). ***T. heudelotii*** Pic. Serm., *Fraser-Jenkins 11416* (H), Cameroon: *rbcL* KF887208 (Wang & al., 2014), *atpA* KF898042 (Wang & al., 2014), *trnL-F* spacer KF897990 (Wang & al., 2014). ***T. hirsutum*** (Holttum) J.Prado & R.C.Moran, *Pereira-Silva5029* (NY), Brazil; *Tuomisto 15438* (TUR), Brazil: *rbcL* KF667659 (Moran & al., 2014), *atpA* KF898043 (Wang & al., 2014), *trnL-F* spacer KF667632 (Moran & al., 2014), *rps4-trnS* KF667659 (Moran & al., 2014). ***T. lanigerum*** Holttum, *Fay 1158* (NY), Sierra Leone: *rbcL* KF667661 (Moran & al., 2014), *trnL-F* spacer KF667634 (Moran & al., 2014), *rps4-trnS* KF667576 (Moran & al., 2014). ***T. jenseniae*** (C.Chr.) Holttum, *Fay 1165* (NY), Sierra Leone: *rbcL* KF667660 (Moran & al., 2014), *trnL-F* spacer KF667633 (Moran & al., 2014), *rps4-trnS* KF667575 (Moran & al., 2014). ***T. pentagonum*** (Pic. Serm.) Holttum, *Daly 13102* (NY) Madagascar: *rbcL* KF667662 (Moran & al., 2014), *trnL-F* spacer KF667635 (Moran & al., 2014), *rps4-trnS* KF667577 (Moran & al., 2014).