Resolved phylogeny of Cleomaceae based on all three genomes

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Abstract Cleomaceae is a small pantropical family that is emerging as a promising system to investigate C_4 photosynthesis, floral evolution, and comparative genomics. However, our understanding of these phenomena is hindered by a lack of a strong phylogenetic hypothesis, despite a number of previous studies. We reconstructed the phylogeny of the family using data from all three genomes, including three cpDNA (*ndhF*, *matK*, *ycf1*), one mtDNA (*rps3*), and one nrDNA (ITS) regions. Analyses strongly supported 15 clades: (1) Clade 1, which includes two Old World species, *Cleome khorassanica* and *C. turkmena*; (2) *Cleome* s.str., which includes the type *C. ornithopodioides* and Old World species; (3) Droserifolia, corresponding to three Old World species, *C. droserifolia*, *C. fimbriata*, *C. quinquenervia*; (4) *Polanisia*, equivalent to this New World genus; (5) Angustifolia, which includes four Old World species; (6) North American cleomoids, which includes four genera, *Cleomella*, *Peritoma*, *Oxystylis*, and *Wislizenia*; (7) Australian, which includes Old world species and worldwide weed *Arivela viscosa*; (8) *Gynandropsis*, equivalent to this monotypic genus; (9) Clade 6, which includes Old World species of *Cleome* and *Dipterygium*; (10) *Dactylaena*, corresponding to this genus and *Physostemon*; (11) African, which includes species distributed in Old World; (12) Andean, which includes *Podandrogyne* and tropical New World species of *Cleome*; (13) *Melidiscus*, which includes New World tropical species; (14) *Cleoserrata*, which includes New World tropical species; and (15) *Tarenaya*, a large New World clade. Major relationships amongst the clades are strongly supported for the first time, including North American cleomoids sister to all remaining Cleomaceae. While five genera are confirmed or newly identified here to be non-monophyletic (*Cleome*, *Cleomella*, *Hemiscola*, *Peritoma*, *Tarenaya*), six are supported (*Cleoserrata*, *Dactylaena*, *Melidiscus*, *Physostemon*, *Podandrogyne*, *Polanisia*). Thus, there are many taxonomic and evolutionary implications to our revised phylogenetic hypothesis.

Keywords Brassicaceae; *Cleome*; Cleomaceae; phylogenetics; *Tarenaya*

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

■ INTRODUCTION

Although Cleomaceae is a small family (18 genera and 150–200 species; Table 1) with few economically important members, it has been the focus of important ecological and evolutionary inquiries. These studies examined floral morphology and development (Nozzolillo & al., 2010; Patchell & al., 2011), the evolution of C_4 photosynthesis (Brown & al., 2005; Marshall & al., 2007; Voznesenskaya & al., 2007; Feodorova & al., 2010; Koteyeva & al., 2011), pollination biology (Cane, 2008), and comparative genomics (Schranz & Mitchell-Olds, 2006; Barker & al., 2009; Cheng & al., 2013) and transcriptomics (Brautigam & al., 2011a, b). Investigations of these intriguing biological phenomena are facilitated by the sister relationship between Cleomaceae and Brassicaceae because the latter family includes the model organism *Arabidopsis thaliana* (L.) Heynh., but have been hindered by the lack of a strong phylogenetic hypothesis of Cleomaceae.

Monophyly and placement of Cleomaceae has been resolved based on molecular data (Hall & al., 2002, 2004; Hall, 2008). Although historically treated as a subfamily of Capparaceae s.l. (Pax & Hoffmann, 1936), Cleomaceae are easily distinguished from closely related Capparaceae s.str. and Brassicaceae by their mostly herbaceous habit, palmately compound leaves, capsular fruits lacking a septum, and seeds with a testa that has a pronounced invagination (Hall & al., 2002; Iltis & al., 2011). Members of the family also have distinctive monosymmetric flowers with a ground plan of four sepals, four petals, generally six stamens, and a bicarpellate gynoecium. Floral monosymmetry arises through upwards curvature of corolla and androecial whorls, which may be complemented by shape, size and color differences between adaxial and abaxial petals as well as variation in nectar gland shape (Kers, 2003; Patchell & al., 2011). The family has a worldwide distribution in both temperate and tropical regions (Kers, 2003; Tucker & Vanderpool, 2010) and a hypothesized origin in central Asia (Feodorova & al., 2010).

Cleomaceae has been the focus of a number of molecular-based phylogenetic studies, all of which reveal that the largest and type genus, *Cleome*, is not monophyletic. In most analyses, taxon sampling has been limited to 13 to 38 species of Cleomaceae (Hall & al., 2002; Sanchez-Acebo, 2005; Hall, 2008; Inda

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& al., 2008; Patchell & al., 2011) with the notable exception of Feodorova & al. (2010) who included 114 representatives of 81 species. Phylogenetic hypotheses were based typically on one marker, mainly ITS (Sanchez-Acebo, 2005; Inda & al., 2008; Feodorova & al., 2010), or at most two chloroplast markers (Hall & al., 2002; Hall, 2008; Patchell & al., 2011). Only two analyses included the type of *Cleome*, *C. ornithopodioides* L., with variable placement (Hall, 2008; Feodorova & al., 2010), thus confounding utility of these studies as a basis for taxonomic changes. Regardless, taxonomic changes are underway in part in consequence of these molecular-based findings. New World species of *Cleome* and some adventive species have recently been placed in *Arivela*, *Cleoserrata*, *Gynandropsis*, *Hemiscola*, *Peritoma*, *Physostemon*, and *Tarenaya* (Iltis & Cochrane, 2007; Tucker & Vanderpool, 2010). However, with one exception (Riser & al., 2013), these taxonomic changes have yet to be tested explicitly using molecular markers.

A second trend of previous phylogenetic studies is lack of support and resolution of early-diverging lineages of the family, although major clades have been identified. Analyses of chloroplast markers with moderate sampling (13–21 species of Cleomaceae), led to the identification of four major clades (Hall & al., 2002; Hall, 2008): western North American cleomoids (hereafter referred to as NA cleomoids), *Cleome droserifolia* (Forssk.) Delile, *Cleome* s.str., and a *Polanisia* clade (further divided into *Tarenaya* and Andean subclades). Greater taxonomic sampling based on ITS (Feodorova & al., 2010) resulted in additional lineages being recognized (Electr. Suppl.: Appendix S1). Feodorova & al. (2010) designated fifteen lineages by number (i.e., 1 through 15) some of which correspond to previously recognized genera or suprageneric classifications and, as such, were given additional informal names: lineage 3 (= Droserifolia), lineage 5 (= "*C. angustifolia*"), lineage 6 (= NA cleomoids), lineage 7 (= Australian), lineage 8 (= *Gynandropsis*), lineage 11 (= *Cleome* s.str.), lineage 13 (= *Melidiscus*), and lineage 14 (= *Cleoserrata*). Because some clades have multiple informal names, we use a single system here based on generic names, when applicable, followed by previously designated names (Hall, 2008; Inda & al., 2008; Feodorova & al., 2010). The NA cleomoids clade plus *C. droserifolia* is sister to the remaining Cleomaceae based on chloroplast data (Hall & al., 2002; Hall, 2008), with moderate support only in maximum likelihood analyses. ITS sequence data suggests either NA cleomoids (maximum parsimony analyses) or lineage 1 (maximum likelihood and Bayesian analyses) as sister to the remaining Cleomaceae with no branch support for either relationship. In sum, a major difficulty in using a phylogenetic framework for interpreting morphological or physiological information is the lack of resolution and support of the backbone of the family.

The major goal of this study was to resolve relationships amongst major clades of Cleomaceae. Towards these ends, we compiled a five gene-region dataset (three chloroplast, one nuclear, and one mitochondrial) for 103 species of Cleomaceae and generated a well-supported phylogenetic hypothesis. This study represents the most thorough taxon and character sampling of the family to date and provides a foundation for future inquiries.

Table 1. Genera, species number (number sampled in this study), and geographic distribution of Cleomaceae.

Taxon	No. species (No. in current study)	Geographic distribution
Arivela Raf.	10 (1)	Asia, Africa, introduced North America
Carsonia Greene	1 (0)	SW United States
Cleome L.	200 (70)	Worldwide
Cleomella DC.	10 (2)	United States, Mexico
Cleoserrata H.H.Iltis	5 (3)	Mexico, Central and South America, West Indes
Dactylaena Schrad. ex Schult.f.	6 (2)	Argentina, Brazil, Haiti
Dipterygium Decne.	1 (1)	Egypt to Pakistan
Gynandropsis DC.	1 (1)	Asia
Haptocarpum Ule	1 (0)	Eastern Brazil
Hemiscola Raf.	6[a] (2)	Mexico, Central and South America
Oxystylis Torr. & Frem.	1 (1)	SW United States
Peritoma DC.	6[a] (4)	North America, Mexico
Physostemon Mart. & Zucc.	17 (5)	Central and South America
Podandrogyne Ducke	36 (6)	Central and South America
Polanisia Raf.	5 (2)	North America, Mexico
Puccionia Chiov.	1 (0)	Somalia
Tarenaya Raf.	33[a] (2)	South America
Wislizenia Engelm.	3 (1)	United States, Mexico

[a] Number of species in genera recently segregated from *Cleome* based on Tucker & Vanderpool (2010).

■ MATERIALS AND METHODS

Character and taxon sampling. — Five loci from all three genomes were sampled: chloroplast (*matK*, *ndhF*, *ycf1*), mitochondrial (*rps3*), and nuclear ribosomal (ITS). A total of 368 sequences were included in the analyses (Appendix 1). We added 108 sequences to published datasets of nuclear ribosomal internal transcribed spacer (ITS; Feodorova & al., 2010), *ndhF* (Hall, 2008; Patchell & al., 2011), and *matK* (Hall, 2008; Patchell & al., 2011). New datasets were generated (107 sequences) for the rapidly evolving chloroplast gene *ycf1* and slower evolving mitochondrial gene *rps3*.

Taxon sampling of Cleomaceae included 103 species from 15 genera (Table 1). When possible, multiple species were sampled from newly segregated genera of *Cleome* (e.g., *Tarenaya*). We included species from all described clades (Hall, 2008; Inda & al., 2008; Feodorova & al., 2010) as well as 17 taxa not previously included in a molecular phylogenetic study to date (Electr. Suppl.: Appendix S1). Eleven taxa from Brassicaceae were used as outgroups based on previous studies (Hall & al., 2002, 2004). Because sequence data are not available for all taxa across all five genes (Appendix 1), partial sequences were included when available. Uncertainty of analyses due to inclusion of partial sequence data is not expected to obscure relationships between taxa (Wiens, 2006; Galtier & Daubin, 2008; Burleigh & al., 2009; Sanderson & al., 2010).

DNA extraction, amplification, and sequencing. — Total DNA was extracted from fresh or herbarium specimens using Qiagen DNeasy Minikit (Qiagen, Germantown, Maryland, U.S.A.) or a modified CTAB method (Doyle & Doyle, 1987; Smith & al., 1991). PCR reactions were implemented in an Eppendorf Mastercycler Pro gradient thermal cycler (Eppendorf Canada, Mississauga, Ontario, Canada) with a total volume of 20 µl: 2.5 µl of 10× Extaq Buffer (Takara, Tokyo, Japan), sterilized distilled water, 2.5 mM of each dNTP, 0.2–1.0 µM of each primer, 0.625 U Extaq polymerase and less than 250 ng of genomic DNA. Primers used in this study were based on previous studies (White & al., 1990; Olmstead & al., 1993; Koch & al., 2001; Hall & al., 2002; Beilstein & al., 2006; Davis & al., 2007; Wurdack & Davis, 2009; Drew & Sytsma, 2011); see Electr. Suppl.: Appendix S2 for complete primer list). Amplification conditions varied among regions: (1) *matK*, initial denaturation for 10 min at 94°C, followed by 36 cycles of denaturation at 94°C for 0.5 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min, followed by a final extension of 72°C for 10 min; (2) *ndhF*, initial denaturation for 10 min at 94°C, followed by 36 cycles of denaturation at 94°C for 0.5 min, annealing at 48°C for 1 min, and extension at 72°C for 2 min, followed by a final extension of 72°C for 10 min; (3) *ycf1*, initial denaturation for 10 min at 94°C, followed by 36 cycles of denaturation at 94°C for 0.5 min, annealing at 54°C for 1 min, and extension at 72°C for 2 min, followed by a final extension of 72°C for 10 min (4) *rps3*, initial denaturation for 10 min at 94°C, followed by 36 cycles of denaturation at 94°C for 0.5 min, annealing at 55°C for 1 min, and extension at 72°C for 45 s, followed by a final extension of 72°C for 10 min; (5) ITS, initial denaturation for 5 min at 94°C, followed by 36 cycles of denaturation at 94°C for 0.5 min, annealing at 58°C for 1 min, and extension at 72°C for 45 s, followed by a final extension of 72°C for 10 min; and. Problematic extracts required separate amplification of shorter contiguous fragments using different primer pairs (Electr. Suppl.: Appendix S2). PCR products were visualized using 1% gel electrophoreses and then cleaned with QIAquick PCR purification columns (Qiagen). Both strands were cycle-sequenced using BigDye and different primers (Electr. Suppl.: Appendix S2). Reactions were cleaned with Performa DTR V3 96-well Short Plate Kit (Edge BioSystems, Gaithersburg, Maryland, U.S.A.), and sequenced using an ABI-3730 DNA Analyzer (Applied Biosystems, Foster City, California, U.S.A.).

Chromatograms were edited and initially aligned using Sequencher v.4.10.1 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.). Sequences were initially Clustal-aligned in MacVector v.12.0.2 using default settings. Coding regions were then codon-aligned using *Arabidopsis thaliana* sequences in Mesquite v.2.75 (Maddison & Maddison, 2009). Resultant ITS and non-coding *ycf1* alignments were checked by eye and minor adjustments were made. Previous analyses (Feodorova & al., 2010) demonstrated that alternative alignment parameters of ITS do not substantially alter resulting topologies.

Phylogenetic analyses. — Separate maximum parsimony analyses were conducted on each gene region, including separate analysis of coding and noncoding regions of *ycf1*, to assess congruence between datasets using the following search parameters in PAUP* v.4.0b10 (Swofford, 2000): 1000 replicates, tree bisection-reconnection (TBR) branch swapping, simple taxon addition, and saving no more than 1000 trees per replicate. The individual topologies were then considered similar based on visual comparison of clades with greater than 75% maximum parsimony bootstrap values or greater than 95% posterior probabilities (Mason-Gamer & Kellogg, 1996; Seelanan & al., 1997), an approach that has been useful in determining areas of "hard incongruence" between phylogenies (e.g., Daru & al., 2013; Merckx & al., 2013; Penneys & Judd, 2013; Scheunert & Heubl, 2014).

Phylogenetic relationships were determined using Bayesian inference implemented in MrBayes v.3.2.1 (Huelsenbeck & Ronquist, 2001) for four datasets: chloroplast, mitochondrial, nuclear ribosomal, and total evidence (chloroplast, mitochondrial, and nuclear ribosomal). There were no conflicting branches of "hard incongruence" along the backbone between cpDNA and ITS topologies. There were five branches with areas of conflict within clades (see "Phylogenetic relationships" in Results), but since the goal was to determine relationships amongst clades, data were combined. The following regions were subsequently treated as separate partitions in the total evidence analysis: ITS, *matK*, *ndhF*, *rps3*, *ycf1* coding, and *ycf1* non-coding. The most suitable model of evolution was determined independently for each partition using the Akaike information criterion (AIC) implemented in MrModelTest v.2.3 (Nylander, 2004). Bayesian analyses were run with default priors for five million generations. Model parameters for each partition were estimated separately. The number of chains was increased to eight (four is default) and temperature lowered to

0.1 (default 0.2) after initial runs indicated these data were slow to converge. Runs were stopped when the average standard deviation of split frequencies was less than 0.01. Convergence was also confirmed by potential scale reduction factor (PSRF) values approaching 1.0. Stationarity was achieved when a large effective sample size (ESS values >1000) was reached as determined in Tracer v.1.4.1 (Rambaut & Drummond, 2007). The first 25% of trees recovered were discarded as burn-in (trees produced prior to convergence). Branch support was also determined using maximum parsimony bootstrapping (BS) (Felsenstein, 1985) with 1000 replicates of heuristic searching using the following parameters implemented in PAUP* v.4.0b10 (Swofford, 2000): TBR branch swapping, simple taxon addition, and saving no more than 1000 trees per replicate.

■ RESULTS

Sequence data. — The aligned length of the data matrix including *matK* (1589 bp), *ndhF* (1109 bp), *ycf1* (2050 bp, 1315 of which are coding), *rps3* (1558 bp), and ITS (1230 bp) was combined for a total length of 7536 bp for the 114 taxa included in this dataset (Table 2). The most appropriate model of evolution was assessed for each gene separately and applied in partitioned Bayesian analyses: GTR+I+Γ (*ndhF*, *ycf1* coding, *rps3*, ITS) and GTR+Γ (*matK*, *ycf1* non-coding).

Phylogenetic relationships. — Overall analyses of cpDNA (Fig. 1), ITS (Fig. 2), mtDNA (not shown, see below), and total evidence (ITS, cpDNA, mtDNA; Fig. 3; Electr. Suppl.: Fig. S1, Appendix S1) resulted in topologies that support monophyly of 14 of the 15 clades previously identified by Feodorova & al. (2010): (1) Clade 1, including *C. khorassanica* Bunge & Bien. ex. Boiss. and *C. turkmena* Bobrov; (2) *Cleome* s.str., including the type *C. ornithopodioides* and additional Old World species; (3) Droserifolia, corresponding to three Old World species, *C. droserifolia*, *C. fimbriata* Vicary, *C. quinquenervia* DC.; (4) *Polanisia*, equivalent to this New World genus; (5) Angustifolia, which includes four Old World species; (6) North American cleomoids, which includes four genera, *Cleomella*, *Peritoma*, *Oxystylis*, and *Wislizenia*; (7) Australian, which includes Old World species and worldwide weed *Arivela viscosa* (L.) Raf.; (9) Clade 6, which includes Old World species of *Cleome* and *Dipterygium*; (10) *Dactylaena*, corresponding to two New World genera *Dactylaena* and *Physostemon*; (11) African, which includes species distributed in Old World; (12) Andean, which includes *Podandrogyne* and tropical New World species of *Cleome*; (13) *Melidiscus*, which includes New World tropical species; (14) *Cleoserrata*, which includes New World tropical species; and (15) *Tarenaya*, a large New World clade (Figs. 1–2; see Electr. Suppl.: Appendix S1 for full list of species in each clade). There are two notable exceptions to clade monophyly based on cpDNA data: (1) a single representative of *Cleoserrata* is nested within a paraphyletic *Tarenaya* (Fig. 1) and (2) the *Dactylaena* clade is not monophyletic. Four accessions of *Gynandropsis gynandra* (L.) Briq. (Clade 8) were shown to be monophyletic previously (Feodorova & al., 2010). The topology of the mtDNA tree was relatively unresolved (data not shown) due to smaller taxon sampling and lower divergence in this region among Cleomaceae samples.

Eleven clades were strongly supported (PP > 95 and/or MP BS > 90 in at least two analyses), whereas four clades have minimal or variable support (Fig. 3). Monophyly of *Cleoserrata* and *Polanisia* was only tested in total evidence and ITS analyses, but both topologies strongly support these genera (Fig. 3). As mentioned above, monophyly of *Tarenaya* is moderately supported by ITS and total evidence, but not by cpDNA. The *Dactylaena* clade, including *Dactylaena* and *Physostemon*, was only supported in Bayesian analyses of total evidence and ITS. *Cleome oxalidea* F.Muell. was only weakly supported as a member of the Australian clade with strong support across all analyses for the rest of the clade (Figs. 1–2; Electr. Suppl.: Fig. S1). Strong branch support for the Australian clade was only provided by Bayesian analyses of cpDNA and total evidence and MP analyses of cpDNA.

Within clades, there were five instances where cpDNA and ITS topologies have high support for alternative relationships. Within Droserifolia, *Cleome quinquenervia* is sister to *C. fimbriata* (cpDNA, Fig. 1) or to *C. droserifolia* (ITS, Fig. 2). There are three areas of incongruence within the African clade: (1) *C. rutidosperma* DC. is sister to C. *iberidella* Welw. ex Oliv. plus *C. schimperi* Pax (cpDNA, Fig. 1) or to *C. briquetii* Polhill plus *C. usambarica* Pax (ITS, Fig. 2); (2) *C. usambarica* is

Table 2. Summary of phylogenetic datasets.

Marker or alignment	Taxa / Cleomaceae	Characters	Parsimony-informative characters	Model of molecular evolution
ndhF	84 / 77	1109	274 (24.7%)	GTR+I+Γ
matK	86 / 77	1589	460 (28.9%)	GTR+Γ
ycf1 coding	59 / 53	1315	436 (33.2%)	GTR+I+Γ
ycf1 non-coding	59 / 53	735	232 (39.4%)	GTR+Γ
rps3	48 / 44	1558	198 (12.7%)	GTR+I+Γ
ITS	91 / 86	1230	490 (39.8%)	GTR+I+Γ
cpDNA (*ndhF*+*matk*+*ycf1*)	90 / 81	4748	1404 (29.6%)	partitioned
Total (*ndhF*+*matk*+*ycf1*+*rps3*+ITS)	114 / 103	7536	2092 (27.8%)	partitioned

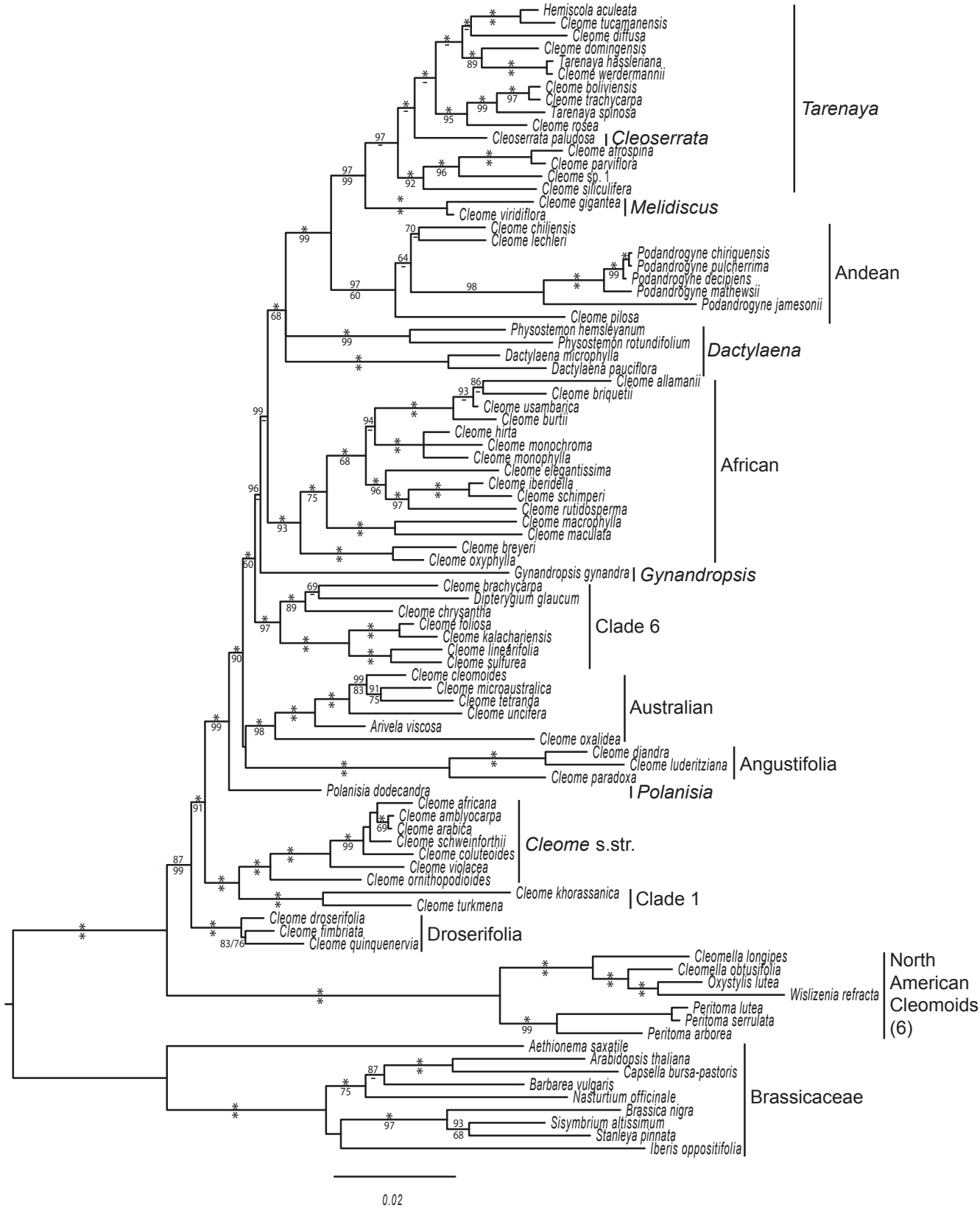

Fig. 1. Bayesian 50% majority-rule consensus tree inferred from chloroplast (*matK*, *ndhF*, *ycf1*) sequence data. Posterior probabilities (PP) greater than 60% are indicated above branches; maximum parsimony bootstrap (MP BS) values greater than 60% are indicated below branches. Branches with 100% PP or MP BS are indicated with an asterisk (*). Informal clade names are indicated at right.

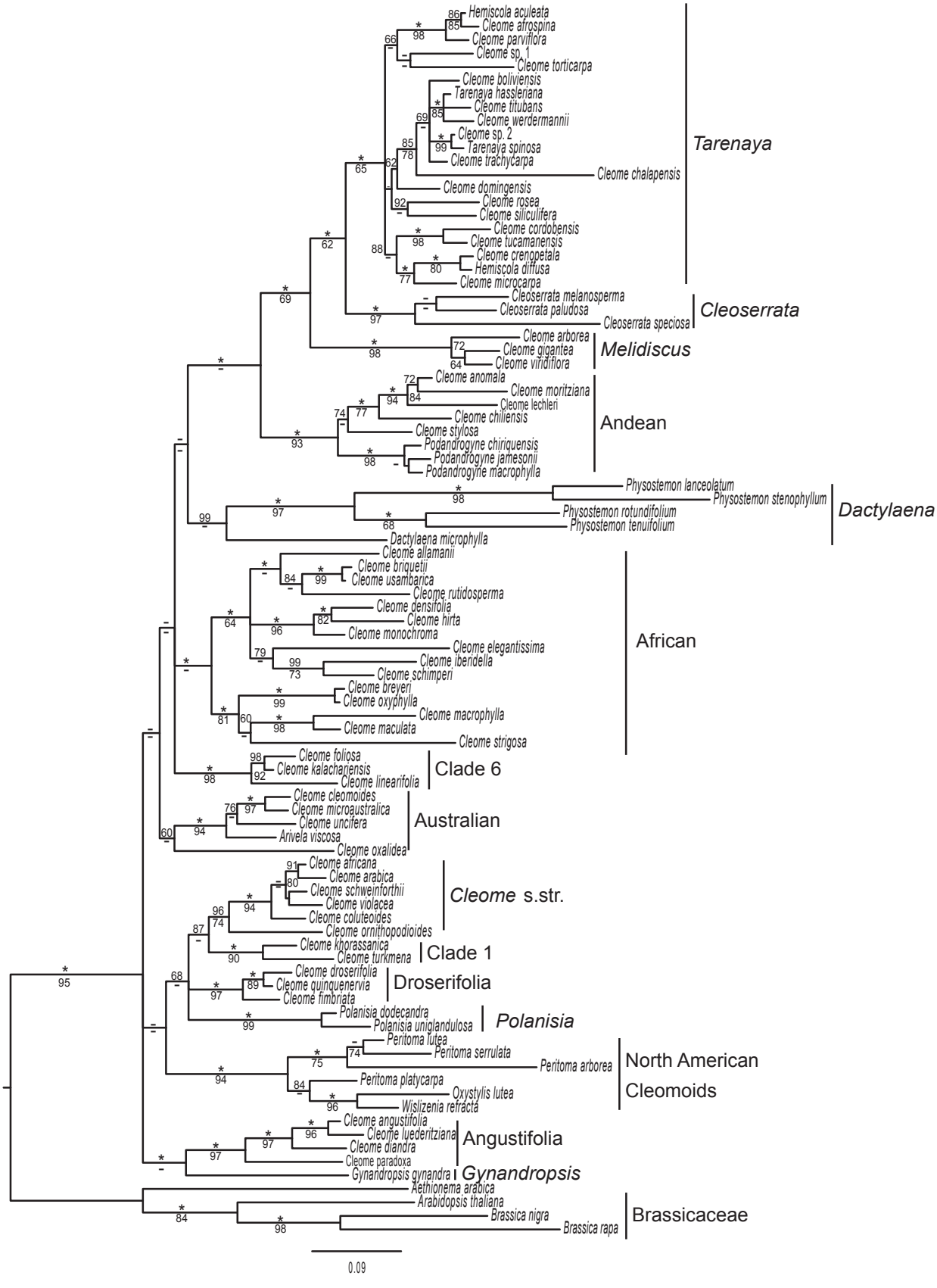

Fig. 2. Bayesian 50% majority-rule consensus tree inferred from nuclear ribosomal ITS sequence data for Cleomaceae. Posterior probabilities (PP) greater than 60% are indicated above branches; maximum parsimony bootstrap (MP BS) values greater than 60% are indicated below branches. Branches with 100% PP or MP BS are indicated with an asterisk (*). Informal clade names are indicated at right.

sister to *C. allamanii* Chiov. plus *C. briquetii* (cpDNA, Fig. 1) or *C. briquetii* (ITS, Fig. 2); and (3) *C. oxyphylla* Bursh plus *C. breyeri* B.Davy varies in placement within the clade (Figs. 1–2). Within the *Tarenaya* clade, *Hemiscola aculeata* (L.) Raf. is sister to *C. tucamanensis* H.H.Iltis (cpDNA, Fig. 1) or to *C. afrospina* H.H.Iltis (ITS, Fig. 2).

There are two areas in the Cleomaceae topology that have strong support for relationships amongst the clades. First, NA cleomoids are sister to all remaining Cleomaceae (Figs. 1, 3), albeit with much higher support with cpDNA data than with total evidence (Fig. 3). Interestingly, only MP analyses provide strong support for this relationship, whereas Bayesian topology very weakly supports this relationship in total evidence. The next diverging clades are Droserifolia and Clade 1 plus *Cleome* s.str., again with highest branch support in the cpDNA topology. The deeper nodes of the ITS phylogeny are unresolved with little to minimal support for relationships amongst clades (Fig. 2). We interpret the polytomy at the base of the Cleomaceae as lack of resolution, not as direct conflict with the cpDNA topology (Fig. 2). Both combined and cpDNA analyses strongly support monophyly of *Cleome* s.str. and Clade 1 (Figs. 1, 3), which is moderately supported in the ITS topology (87% MP BS; Fig. 2). In addition, all analyses support a close relationship amongst the Andean, *Cleoserrata*, *Melidiscus*, and

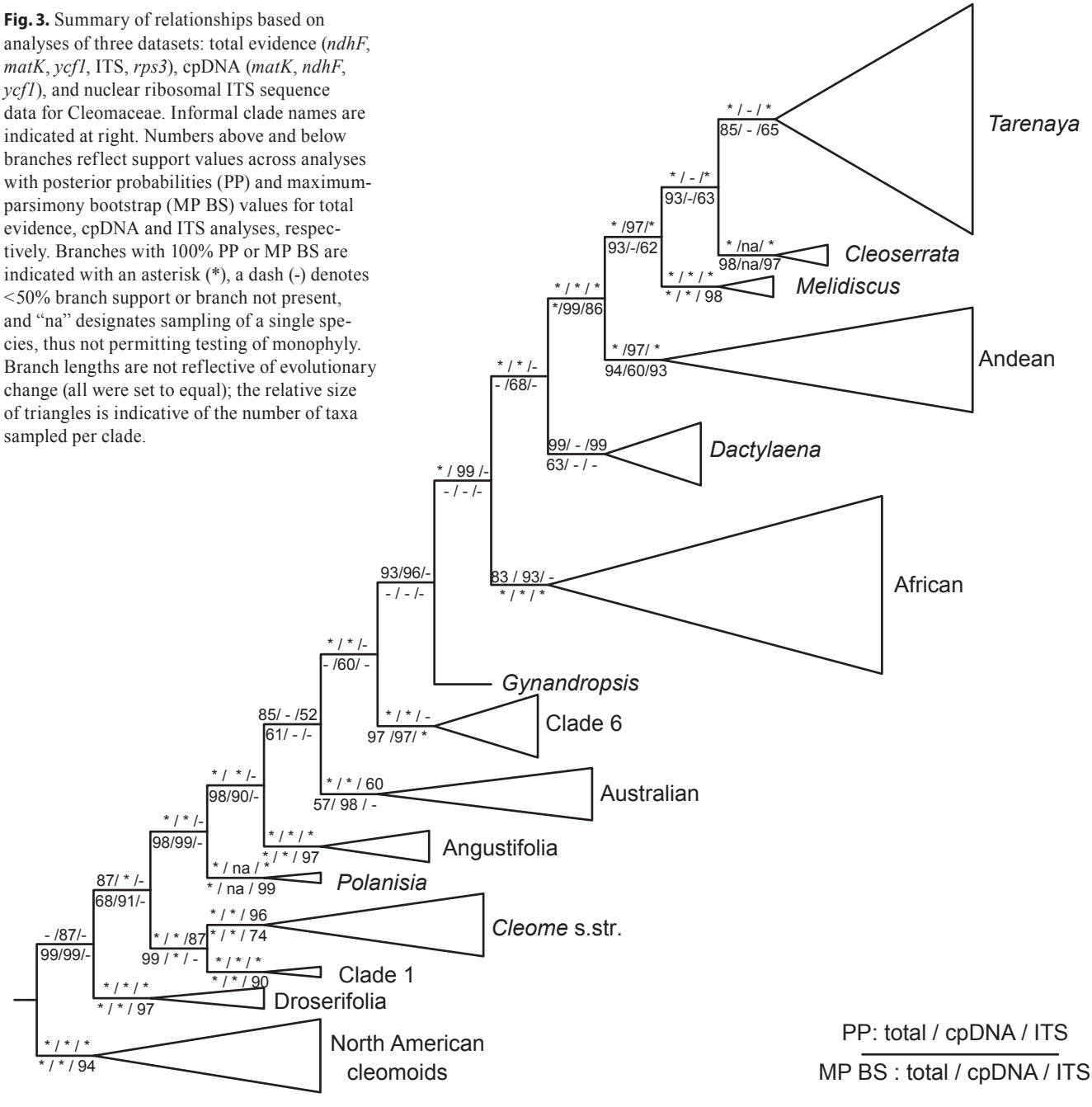

Fig. 3. Summary of relationships based on analyses of three datasets: total evidence (*ndhF*, *matK*, *ycf1*, ITS, *rps3*), cpDNA (*matK*, *ndhF*, *ycf1*), and nuclear ribosomal ITS sequence data for Cleomaceae. Informal clade names are indicated at right. Numbers above and below branches reflect support values across analyses with posterior probabilities (PP) and maximum-parsimony bootstrap (MP BS) values for total evidence, cpDNA and ITS analyses, respectively. Branches with 100% PP or MP BS are indicated with an asterisk (*), a dash (-) denotes <50% branch support or branch not present, and "na" designates sampling of a single species, thus not permitting testing of monophyly. Branch lengths are not reflective of evolutionary change (all were set to equal); the relative size of triangles is indicative of the number of taxa sampled per clade.

Tarenaya clades (Figs. 1–3). Bayesian analyses support Andean as sister to *Cleoserrata*, *Melidiscus*, and *Tarenaya* with *Cleoserrata* and *Tarenaya* as sister.

Several genera were confirmed not to be monophyletic by these analyses. *Cleome* is paraphyletic to all other described genera in the family except *Cleomella*, *Oxystylis*, *Peritoma*, and *Wislizenia*. *Cleomella* is paraphyletic to *Oxystylis* and *Wislizenia*, whereas *Peritoma*, also a member of the NA cleomoids, is paraphyletic to *Cleomella*, *Oxystylis*, and *Wislizenia* (Figs. 1–2; Electr. Suppl.: Fig. S1). These data indicate that the newly described genera *Hemiscola* and *Tarenaya* are not monophyletic, at least based on current taxonomic changes. In contrast, *Cleoserrata*, *Dactylaena*, *Physostemon*, *Podandrogyne*, and *Polanisia* are monophyletic based on our data (Figs. 1–2; Electr. Suppl.: Fig. S1).

■ DISCUSSION

Here we have expanded upon three previous studies to generate a phylogenetic hypothesis of Cleomaceae based on all three genomes. Increased taxon and character sampling provides additional support for the 15 clades that were previously identified based only on ITS data (Feodorova & al., 2010). Although this study represents the largest taxonomic and character sampling of Cleomaceae to date, less than half of the species in the family were included (Table 1). In addition, two monotypic genera remain unsampled in any molecular analysis. These are *Haptocarpum bahiensis* Ule endemic to Brazil and *Puccionia macrodenia* Chiov. distributed in Somalia. Importantly, we present for the first time a topology that resolves basal relationships within the family with moderate to strong support. Thus, these new findings allow for novel and more refined interpretations of the evolutionary history of Cleomaceae.

Phylogenetic relationships. — The NA cleomoids are sister to all remaining Cleomaceae, which is consistent with relationships proposed previously with low statistical support (Hall, 2008) or with no branch support only in MP analyses (Feodorova & al., 2010). Although this relationship is strongly supported in cpDNA analyses, this branch only receives support in parsimony analyses of total evidence. Feodorova & al. (2010) showed that Clade 1 (represented by *C. khorassanica* and *C. turkmena*) was sister to all remaining Cleomaceae in ML and Bayesian analyses of ITS data, albeit with no support. This relationship was not recovered in the ITS analyses presented here (Fig. 2). In fact, all analyses here indicate Clade 1 as sister to *Cleome* s.str. and is further derived within the family. The Droserifolia clade is sister to the remaining taxa in Cleomaceae, excluding the NA cleomoids, a relationship that was identified in previous analyses (Hall, 2008; Patchell & al., 2011).

The 15 identified clades are correlated with geographic distribution of the species such that clades either comprise New World or Old World species (Feodorova & al., 2010). A few Old World species are now widespread weeds (*A. viscosa*, *C. rutidosperma*, and *Gynandropsis gynandra*; Iltis, 1960), which slightly obscures this pattern. The seven clades comprised of taxa distributed in the New World are Andean, *Cleoserrata*, *Dactylaena*, NA cleomoids, *Melidiscus*, *Polanisia*, and *Tarenaya*. The eight Old World clades include African, Angustifolia, Australian, Clade 1, Clade 6, *Cleome* s.str., Droserifolia, and *Gynandropsis*. New World and Old World clades are dispersed across the phylogeny, regardless of analysis, reflecting the complicated biogeographical history driven by long-distance dispersal (Feodorova & al., 2010).

Andean, *Cleoserrata*, *Melidiscus*, and *Tarenaya* lineages represent a strongly supported clade of New World taxa, and the New World *Dactylaena* clade is also closely related to these with moderate support. *Cleome afrospina* is the only member of these clades that is not distributed in the New World, and this species has been proposed to be a recent introduction to Africa from the American tropics (Iltis, 1967). The close relationship among these clades was recovered in a number of studies (Hall, 2008; Inda & al., 2008; Feodorova & al., 2010). Although there is not a clear morphological synapomorphy for this larger New World clade, many members have bracteate inflorescences and echinate pollen (Sanchez-Acebo, 2005).

In general, the cpDNA partition appears to be determining the topology of the total evidence tree, as expected given the larger number of characters in this portion of the data (Table 2). There is no evidence of conflict at deeper nodes between cpDNA and ITS because both analyses support monophyly of most clades and the ITS topology is not well supported (Figs. 1–2). However, the decreased bootstrap values when the data are combined suggest data incongruence (Gehrke & al., 2010), perhaps beyond the minor areas of conflict within clades that we identified. Additional data from low-copy nuclear genes would distinguish whether this incongruence is the result of nuclear versus cpDNA conflict or whether it is the result of inherent difficulties with aligning ITS across this phylogenetic distance.

Taxonomic implications. — Based on this study and previous publications (Hall & al., 2002; Sanchez-Acebo, 2005; Hall, 2008; Inda & al., 2008; Feodorova & al., 2010; Riser & al., 2013), it is clear that generic boundaries in the family are problematic and that taxonomic revision is needed. The current trend is to split *Cleome* into many smaller segregates, an approach that has been criticized (Stevens, 2001–) and is occurring in a somewhat haphazard manner. For example, formal taxonomic changes were made only for a limited number of New World taxa (Iltis & Cochrane, 2007; Tucker & Vanderpool, 2010). Iltis & Cochrane (2007) proposed ten new taxonomic combinations based on morphology, chromosome numbers, and molecular-based phylogenies. However, these taxonomic changes were incomplete as illustrated by the following quote: "Although recognizing that some two dozen species presently assigned to *Cleome* have yet to be transferred to *Tarenaya* for the first time, only one such transfer is proposed here … [i.e., *T. hassleriana* (Chodat) H.H.Iltis]" (Iltis & Cochrane, 2007: 450). A recent Flora reported 33 species of *Tarenaya* (Tucker & Vanderpool, 2010), including *T. spinosa* (Jacq.) Raf., but the name changes have not been published formally to the best of our knowledge. In other words, as Floras are being published, new taxonomic changes are integrated (e.g., resurrection of *Physostemon*;

Tropicos), but no comprehensive suite of formal changes has been made. In light of this problematic situation, we identify four major questions which we address in turn: (1) What group should retain the name *Cleome*, assuming the continued splitting of the genus? (2) What are taxonomic solutions for the Old World taxa that have not been evaluated to date? (3) Are New World taxonomic changes robust with regards to our current phylogenetic hypothesis? (4) How can we conduct a more comprehensive approach to the taxonomic revision of the family?

The position of the type of *Cleome*, *C. ornithopodioides*, is particularly important. Because the genus is currently being split into many smaller genera, the clade that includes *C. ornithopodioides* will be the only group to retain the name *Cleome* (designated *Cleome* s.str. in Figs. 1–3). Importantly, data presented here and in Hall (2008) specify a different position of the type than the Feodorova & al. (2010) topologies where their specimen was placed in the African clade. Re-examination of the voucher used in Feodorova & al. (2010) puts the identification of that specimen in question. As such, we did not include sequences from this voucher in our analyses. Inclusion of additional accessions of this taxon is necessary to clarify the circumscription of *Cleome* s.str. Although this clade was previously identified (Hall, 2008), its relationship to *C. droserifolia* and NA cleomoids was ambiguous. Because all data indicate a well-supported sister relationship between *Cleome* s.str. and Clade 1, *Cleome* s.str. can be expanded to include members of Clade 1.

Few taxonomic changes have been proposed for the other Old World taxa, which are found in eight clades: African, Angustifolia, Australian, Clade 1, Clade 6, *Cleome* s.str., Droserifolia, and *Gynandropsis*. The most notable exception is that *Gynandropsis* was re-instated as a generic name (Tucker and Vanderpool, 2010), although this taxonomic change has not been embraced in all of the recent literature (e.g., Brautigam & al., 2011a, b). Accounting for *Cleome* s.str. (including Clade 1) and *Gynandropsis*, five Old World clades will require new names: African, Angustifolia, Australian, Clade 6, and Droserifolia. None of these five clades correspond to different sections or subsections of *Cleome* (Pax & Hoffmann, 1936), thus excluding the option of raising previous intrageneric names to genus rank. *Arivela* has been proposed and used for the Old World adventive species *A. viscosa* (Tucker & Vanderpool, 2010), a member of the Australian clade. Historically, this taxon was placed in sect. *Eucleome* subsect. *Foliolosae* ser. *Herbaceae* (Pax & Hoffmann, 1936). However, members of this series are scattered across much of the phylogeny, revealing that traditionally used characters do not reflect phylogeny (e.g., sect. *Eucleome* included species with six fertile stamens). Further, members of sect. *Eucleome* subsect. *Simplicifolia* were characterized by the presence of simple leaves (Pax & Hoffmann, 1936). This subsection is not monophyletic, even after excluding unifoliate New World members now placed in *Physostemon* (= sect. *Physostemon*; Iltis, 1959). Based on current sampling, species traditionally placed in subsect. *Simplicifolia* are found in the African (*C. densifolia* C.H.Wright, *C. monophylla* L.), Droserifolia (*C. droserifolia*, *C. quinquenervia*) and Clade 6 (*C. chrysantha* Decne.) clades (Electr. Suppl.: Appendix S2). Iltis (1959) predicted that Old World unifoliate species are not related to one another, but did not provide information about what characteristics led him to this conclusion.

Alternative taxonomic solutions are also problematic when renaming Old World clades. For example, *Dipterygium* is a member of Clade 6 (Fig. 2; Electr. Suppl.: Fig. S1), but expanding that name to all species in the clade is unsatisfactory, in part due to the intriguing taxonomic history of this monotypic genus. *Dipterygium glaucum* Decne. has been placed in Brassicaceae (Hutchinson, 1967), treated as a subfamily of Capparaceae (Pax & Hoffmann, 1936; Hedge & al., 1980), or even been proposed to possibly warrant family status (Kers, 2003). The flattened and winged nut-like fruits are quite unusual for Cleomaceae, and the flowers are small, inconspicuous and cruciferlike. This taxon is so unusual that it would be challenging, if not impossible, to come up with a morphologically cohesive *Dipterygium* that includes large-flowered taxa like *C. foliosa* Hook.f. Examination of New World *Cleome* indicates that other characters, such as seed and morphology, are potentially useful in delimiting clades (Sanchez-Acebo, 2005). Careful examination of Old World taxa is needed to determine if there are previously overlooked features that can be used to unite these seemingly disparate taxa. In sum, we are left with no clear-cut solution to revising generic names of Old World taxa.

The results presented here suggest that recent New World segregates of *Cleome* are not monophyletic. Because not all taxonomic changes have been made for New World taxa, and those that were made were based on the detailed research of Dr. Iltis, we use his unpublished work (1952; pers. comm.) to evaluate generic boundaries. Neither *Hemiscola* nor *Tarenaya* are monophyletic based on data presented here. When all putative members are considered, *Hemiscola* is monophyletic (*C. cordobensis* Eichler ex Griseb., *C. crenopetala* DC., *C. microcarpa* Ule, *C. tucamensis* H.H.Iltis, *H. aculeata*, *H. diffusa* (Banks ex DC.) H.H.Iltis) based on cpDNA and total evidence only if *C. torticarpa* H.H.Iltis & T.Ruiz Zapata, informally placed in *Tarenaya* (Iltis, pers. comm.), is also included. Because *Hemiscola* is embedded within a paraphyletic *Tarenaya*, the name *Hemiscola* can not be maintained. A close relationship of *Hemiscola* and *Tarenaya*, based on the shared characteristic of stipular spines, has been documented (Iltis & Cochrane, 2007). However, the two genera differ in seed characters and putative chromosome numbers (*Hemiscola* $2n = 18$; *Tarenaya* $2n = 20$; Iltis & Cochrane, 2007). *Cleome siliculifera* Eichler has not been previously associated with *Tarenaya*, in contrast to data presented here (Figs. 1–2; Electr. Suppl.: Fig. S1). This species is an annual from eastern Brazil with unknown affinities and is tentatively placed in its own genus (Iltis, pers. comm.). *Cleome siliculifera* has small, delicate flowers, whereas members of *Tarenaya* generally have larger petals (Tucker & Vanderpool, 2010). With the exception of *C. siliculifera*, all species placed in the *Tarenaya* clade with these data (Figs. 1–2; Electr. Suppl.: Fig. S1) were previously identified as being closely related, thus tentatively supporting a broad concept of this genus (including *Hemiscola*).

As suggested previously (Hall, 2008; Feodorova & al., 2010), relationships within NA cleomoids reveal that generic

boundaries in this early-diverging clade need to be reexamined. Specifically, neither *Cleomella* nor *Peritoma* are supported as monophyletic, contrary to the assertion that "*Peritoma* is an exceptionally robust" genus (Tucker & Vanderpool, 2010: 205) based on leaf and nectary gland features. A recent analysis sampled all species of this clade (Riser & al., 2013), thus providing a complete picture of relationships and putative taxonomic changes in this clade. First, this work has shown that *Carsonia sparsifolia* S.Watson (unsampled in our analyses) belongs to this clade. More importantly, they conclude that this clade might be best served if it is treated as a single genus, albeit one that encompasses substantial morphological heterogeneity.

Five New World genera remain supported as monophyletic with the additional data presented here: *Dactylaena* (two species sampled), *Physostemon* (five species sampled), *Podandrogyne* (six species sampled), and *Polanisia* (two species sampled). These results are consistent with previous work (Hall, 2008; Feodorova & al., 2010) and are supported by morphological data. Members of *Dactylaena* are unusual in that their flowers have one fertile and four sterile stamens as opposed to the typical six fertile stamens found in most Cleomaceae. *Physostemon* is characterized as New World taxa with simple (one-foliate) leaves and open flower aestivation (Foster, 1945; Iltis, 1959). Given these morphological differences, they can be maintained as separate genera belonging to the *Dactylaena* clade or recognized as two clades given the weak statistical support for their sister relationship. *Polanisia* is distinguished from other Cleomaceae by flowers with notched petals, large adaxial glands, and more than six stamens (Iltis, 1958; Tucker & Vanderpool, 2010). In contrast, members of *Podandrogyne* do not have a single clear-cut feature that separates them from *Cleome*, but are instead recognized by the combination of unisexual flowers, elongate androphores, and seeds with an open cleft and white aril (Woodson, 1948; Cochrane, 1977, 2011). *Podandrogyne* was shown previously to be closely related to *Cleome* in the Andean clade, which in turn may be placed in yet another genus which has not yet been named (Iltis and Cochrane, pers. comm.). Members of the Andean clade are united by seed characteristics and chromosome number ($2n$ = 58; Cochrane, 1978; Iltis & Cochrane, 1989).

The taxonomic situation in Cleomaceae is, quite simply, a mess. Ongoing taxonomic changes of New World taxa are being made and accepted. For the most part, these newly raised or resurrected genera are supported by molecular data and, fortunately, also by morphological data. In contrast, Old World taxa have remained relatively unstudied and molecular-based clades reveal relationships amongst taxa that have not been previously suggested. Careful and detailed morphological analyses are needed to support any taxonomic changes. The situation is compounded by these clades containing highly divergent morphologies. However, we have identified the clade that should retain the name *Cleome*. What is needed is a comprehensive taxonomic revision of the entire family. Before this can be made, the on-going New World taxonomic changes need to be finalized and scrutinized with molecular data and Old World clades need to be examined in the light of revised phylogenetic hypotheses.

Evolutionary implications. — The phylogenetic framework presented here impacts interpretation of evolutionary patterns in Cleomaceae, of which we highlight a few examples here. Members of Cleomaceae are the closest C_4 relatives to the model plant *Arabidopsis*. This important photosynthetic pathway increases photosynthetic efficiency under certain conditions and impacts angiosperm diversification, biogeography, and community assembly (Brown & al., 2005, 2011; Roalson, 2007, 2008, 2011, 2012; Christin & al., 2008; Besnard & al., 2009; Roalson & al., 2010). *Gynandropsis gynandra* is the best-studied C_4 species in Cleomaceae, although additional C_4 and C_3-C_4 intermediate species exist (Marshall & al., 2007; Voznesenskaya & al., 2007; Koteyeva & al., 2011). The phylogeny presented here provides strong evidence for multiple origins of this pathway with C_4 and C_3-C_4 intermediate species found in the African (*C. allamanii*), Angustifolia (*C. angustifolia* Forssk., *C. luederitziana* Schinz, *C. paradoxa* R.Br. ex DC.), Australian (*C. oxalidea*), *Gynandropsis* (*G. gynandropsis*), and *Tarenaya* (*Cleome siliculifera*) clades. Other studies demonstrate the position of *Carsonia* within the NA cleomoids (Riser & al., 2013), a putative sixth clade with C_4-like characteristics. Furthermore, topologies presented here indicate that *Gynandropsis* is an isolated C_4 clade (Feodorova & al., 2010), but not basal as previously suggested (Inda & al., 2008).

Greater resolution of Cleomaceae relationships also alters interpretation of biogeographical patterns. NA cleomoids as sister to remaining Cleomaceae implicates a New World origin of the family, although the next three clades (Droserifolia, Clade 1, *Cleome* s.str.) are Old World. Feodorova & al. (2010) preferred a topology with Clade 1 (represented by *C. khorassanica* and *C. turkmena*) as sister to all remaining Cleomaceae in their biographical analyses, which indicated an Asian origin for the family. Because there was limited supported for their tree based on ITS data, they reconstructed a biogeographical history of the family with an alternative topology of the NA cleomoids as sister to all remaining Cleomaceae (suppl. fig. 4 in Feodorova & al., 2010). Because the biogeographical history of the family was previously evaluated, we do not re-analyze the data here. However, they did not use age estimated for the family to interpret their ancestral areas. This decision was based on the fact that estimated ages within Brassicales are contentious as evidenced by the widely variable estimates for Brassicaceae (Beilstein & al., 2010; Couvreur & al., 2010; Franzke & al., 2011). It is estimated that Brassicaceae and Cleomaceae diverged around 38 million years ago (Schranz & Mitchell-Olds, 2006; Couvreur & al., 2010), which is consistent with dispersal explaining Old/New World transitions in Cleomaceae (Feodorova & al., 2010).

Phylogenetic relationships within Cleomaceae establish a stronger framework in which to interpret floral evolution. The earliest diverging clade, the NA cleomoids, has monosymmetric flowers, although some genera are interpreted as polysymmetric (e.g., *Cleomella*; Tucker & Vanderpool 2010). In these flowers, monosymmetry is due to upward curvature of petal bases, but no abaxial/adaxial differentiation within the petal or sepal whorls. In more derived clades (e.g., *Cleome* s.str.), monosymmetry is additionally due to differentiation of

adaxial/abaxial organs within perianth whorls (Patchell, 2012), which is then subsequently lost in most taxa of the *Dactylaena*, Andean, and *Tarenaya* groups. However, some species in these clades exhibit monosymmetry different from that in the early-diverging Cleomaceae (e.g., the single sterile stamen in *Dactylaena*). There is evidence that derived clades also share a different developmental trajectory towards monosymmetry (Patchell & al., 2011), an intriguing pattern that can be explored with additional comparative developmental data of flowers.

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Appendix 1. Taxon; collector with collection number and herbarium acronym; GenBank no. *ndhF*; *matK*; *ycf1*; *rps3*; ITS. Missing sequence data is indicated by an n-dash (–). Sequences generated in this study are indicated by an asterisk (*).

BRASSICACEAE: ***Aethionema arabicum*** (L.) Rothm.; [no country listed]; [no voucher listed]; –; –; –; –; AY254539. ***Aethionema saxatile*** (L.) W.T.Aiton.; [no country listed]; *Moore s.n.* (WIS); AY483250; EU371817; KF923022*; KF923188*; –. ***Arabidopsis thaliana*** (L.) Heynh.; [no country listed]; *Hall s.n.* (WIS); –; –; –; –; AJ232900. ***Arabidopsis thaliana*** (L.) Heynh.; [no country listed]; [no voucher listed]; AY122394; AF144348; KF923023*; KF923189*. ***Barbarea vulgaris*** W.T.Aiton.; [no country listed]; *Moore 9* (WIS); AY122395; EU371818; –; –; –. ***Brassica nigra*** (L.) W.D.J.Koch; [no country listed]; [no voucher listed]; –; JN584951; –; –; AF128103. ***Brassica rapa*** L.; [no country listed]; [no voucher listed]; –; –; –; –; AF128098. ***Capsella bursa-pastoris*** (L.) Medik.; [no voucher listed]; *Moore 4* (WIS); KF923080*; KF923122*; KF923024*; –; –. ***Iberis oppositifolia*** Pers.; [no country listed]; *Cochrane 6*; AY122398; EU371819; –; KF923230*; –. ***Nasturtium officinale*** R.Br.; [no country listed]; *Stahmann 233* (WIS); AY122399; AY483225; KF923071*; –; –. ***Sisymbrium altissimum*** L.; [no country listed]; *Leach & al. 1939* (WIS); –; JN585004; KF923077*; –; –. ***Stanleya pinnata*** (Pursh) Britton; [no country listed]; *Hall 1* (AZ); AY122401; AY483226; KF923078*; KF923234; –. ***Stanleya pinnata*** (Pursh) Britton; [no country listed]; *R. Price s.n.* (GA); –; –; –; –; AF531620. **CLEOMACEAE:** ***Arivela viscosa*** (L.) Raf. [=*Cleome viscosa* L.]; [no country listed]; *J.D. Sauer 3492* (WIS); EU373714; EU371806; KF923067*; –; KF923186*. ***Cleome africana*** Botsch.; Egypt; *E. Voznesenskaya 1* (WS); –; –; –; –; HM044222. ***Cleome africana*** Botsch.; [no country listed]; *Hall & Taggart s.n.* (ALTA); HQ452951; HQ452946; –; –; –. ***Cleome afrospina*** Iltis; Gabon; *F.J. Breteler 696* (MO); KF923081*; KF923123*; –; KF923191*; HMO44290. ***Cleome allamani*** Chiov.; Kenya; *Agnew & al. 10879* (MO); KF923082*; KF923124*; KF923026*; –; HMO44270. ***Cleome ambylocarpa*** Baratte & Murb.; [no country listed]; *Mankowski* (ALTA); HQ452952; HQ452947; –; –; –. ***Cleome angustifolia*** Forssk.; South Africa: National Park Kruger; *Feodorova, O. Maurin s.n.* (WS); –; –; –; –; HM044250. ***Cleome anomala*** Kunth; Venezuela: Tachira; *T. Ruiz & L. Hernandez 4980* (MY); –; –; –; –; DQ455782. ***Cleome arabica*** L.; [no country listed]; *J.C. Hall s.n.* (ALTA); EU373701; EU371791; KF923027*; –; KF923164*. ***Cleome arborea*** Kunth.; Venezuela: Las Chorreras de las Gonzalez, Merida; *T. Ruiz & L. Hernandez 4981* (MY); –; –; –; –; DQ455783. ***Cleome boliviensis*** Iltis; Bolivia; *S. Aizama & C. Saravia 1122* (MO); KF923083*; KF923125*; –; –; DQ455785. ***Cleome brachycarpa*** Vahl ex DC.; [no country listed]; *Hall & Taggart s.n.* (ALTA); HQ452953; HQ452948; KF923028*; –; –. ***Cleome breyeri*** Burt Davy; South Africa: Norscot; *A.O.D. Mogg & al. 19159* (MO); KF923084*; KF923126*; KF923029*; –; HM044258. ***Cleome briquetii*** Polhill; Kenya; *R.B. & A.J. Faden 74* (MO); KF923085*; KF923127*; KF923030*; KF923192*; KF923165*. ***Cleome burttii*** R.A.Graham; Tanzania; *S. Bidgood, L. Mwasumbi & K. Vollesen s.n.* (MO); KF923086*; KF923128*; –; KF923193*; –. ***Cleome chapalensis*** Iltis; Mexico: Michoacan; *H.H. Iltis & al. 832* (USZ); –; –; –; –; DQ455800. ***Cleome chilensis*** DC.; Chile; *F. Billiet & B. Jadin s.n.* (MO); KF923087*; KF923129*; –; KF923194*; –. ***Cleome chrysantha*** Decne.; Libya; *J. Leonard 4879* (MO); KF923088*; KF923130*; –; –; –. ***Cleome cleomoides*** (F.Muell.) Iltis; [no country listed]; "Accession # 55989901" (MO); KF923089*; KF923131*; –; –; KF923166*. ***Cleome coluteoides*** Boiss. [= *Buhsea coluteoides* (Boiss.) Bunge]; Turkmenistan; *V. Btachanzev 136* (LE); KF923090*; KF923132*; KF923031*; KF923195*; HM044224. ***Cleome cordobensis*** Eichl. ex Griseb.; Argentina; *S. Victoria 1733* (MO); –; –; –; –; KF923167*. ***Cleome crenopetala*** DC; Brazil: Parana; *P. Dusen 7365* (MO); –; –; –; –; DQ455788. ***Cleome densifolia*** C.H.Wright; [no country listed]; "Accession # 3245723" (MO); –; –; –; –; KF923168*. ***Cleome diandra*** Burch.; Ethiopia; *J.J.F.E. De Wilde 5456* (MO); KF923091*; –; –; –; KF923169*. ***Cleome domingensis*** Iltis; [no country listed]; "2/17/89 [85-01-4]" (MO); AY122383; EU371793; KF923033*; KF923197*; KF923171*. ***Cleome droserifolia*** (Forssk.) Delile; [no country listed]; *A.G. Miller 6387* (WIS); EU373703; EU371794; KF923034*; KF923198*; –. ***Cleome droserifolia*** (Forssk.) Delile; Egypt; *E. Voznesenskaya 41* (WS); –; –; –; –; HM044229. ***Cleome elegantissima*** Briq.; Angola; *L.E. Kers 3651* (MO); KF923092*; KF923133*; –; –; HM044272. ***Cleome* sp. 1;** Peru; *C. Grandez, G. Baquero & G. Criollo 17060* (MO); KF923093*; –; –; –; KF923172*. ***Cleome fimbriata*** Vicary; Uzbekistan; *V. Botchanzv 159a* (LE); KF923094*; KF923134*; KF923035*; KF923199*; HM044227*. ***Cleome foliosa*** Hook.f.; [no country listed]; *L.E. Kers 1750* (WIS); EU373704; EU371795; KF923036*; KF923200*; KF923173*. ***Cleome gigantea*** Blanco.; Prague Bot. Garden; *M. Smith s.n.* (WS); KF923095*; KF923135*; KF923037*; KF923201*; HM044283. ***Cleome hirta*** (Klotzch) Oliv.; Bayliss 10731; HQ452949; HQ452954; –; –; –. ***Cleome hirta*** Oliv.; Tanzania; *N.A. Mwangulango 791* (MO); –; –; –; –; HM044264. ***Cleome iberidella*** Welw.; Tanzania; *Bidgood & al.* (MO); KF923097*; KF923138*; –; –; KF923174*. ***Cleome kalachariensis*** Gilg. & Gilg-Ben.; Namibia; *P.M. Burgoyne & N. Snow 4984* (MO); KF923098*; KF923139*; KF923040*; KF923203*; HM044277. ***Cleome khorassanica*** Bunge & Bien. ex Boiss.; Afganistan; *D. Bukinich s.n.* (LE); KF923099*; KF923140*; KF923041*; KF923204*; HM044230. ***Cleome lechleri*** Eichler; [no country listed]; *J.C. Solomon & M. Morales 17236* (WIS); KF923100*; KF923141*; –; –; KF923176*. ***Cleome linearifolia*** (Stephens) Dinter; Namibia; *W. Giess & al. 5785* (MO); KF923101*; KF923142*; KF923042*; KF923205*; HM044278. ***Cleome luederitziana*** Schinz; Namibia; *M. Bourele & al. 2827* (MO); KF923102*; KF923143*; KF923044*; –; HM044256. ***Cleome macrophylla*** Briq. var. ***macrophylla*;** Zambia; *H.H. Schmidt & al. 2346* (MO); KF923103*; –; KF923045*; KF923207*; HM044262. ***Cleome maculata*** Szyszyl.; South Africa; *Balkwill & al. 5421* (MO); KF923104*; KF923144*; KF923046*; KF923208*; HM044263. ***Cleome microaustralica*** Iltis; Australia; *A.V. Slee s.n.* (CANB); KF923105*; KF923145*; KF923047*; KF923209*; HM044246. ***Cleome microcarpa*** Ule; Brazil; *R.M Harley 27228* (MO); –; –; –; –; DQ455793. ***Cleome monochroma*** J.F.Macbr.; Tanzania; *P. Kuchar 23051* (MO); KF923106*; KF923146*; KF923048*; KF923210*; HM044267. ***Cleome monophylla*** L.; [no country listed]; *R.E. Gereau & C.J. Kayombo 3951* (MO); AY122384; EU371798; KF923049*; KF923211*; –. ***Cleome moritziana*** Klotzsch ex Eichler; Venezuela; *T. Ruiz & L. Hernandez 4984* (MY); –; –; –; –; DQ455794. ***Cleome ornithopodioides*** L.; [no country listed]; Botany Department Garden (WIS); EU373707; EU371799; KF923050*; KF923212*; KF923178*. ***Cleome oxalidea*** F.Muell; Australia: Western Australia; *P.A. Fryxell 3958* (NY); –; KF923147*;

Appendix 1. Continued.

–; –; HM044247. ***Cleome oxyphylla*** Burch.; [no country listed]; *L.E. Kers 3003* (WIS); EU373708; EU371800; KF923051*; –; KF923179*. ***Cleome paradoxa*** R.Br. ex DC.; Yemen; *E. Voznesenskaya 43* (WS); KF923108*; KF923149*; KF923052*; KF923214*; HM044257. ***Cleome parviflora*** Kunth; [no country listed]; *R. Seidel 321* (WIS); EU373709; EU371801; KF923053*; KF923215*; KF923180*. ***Cleome* sp. 2;** Brazil: Pernambuco; *C. Silva 1529* (MO); –; –; –; –; DQ455798. ***Cleome pilosa*** Benth.; [no country listed]; *H.H. Iltis 30585* (WIS); AY122385; AY483231; KF923054*; KF923216*; –. ***Cleome quinquenervia*** DC.; Turkmenistan; *E. Leontieva 127* (LE); KF923109*; KF923150*; KF923055*; KF923217*; HM044228. ***Cleome rosea*** Vahl ex DC.; [no country listed]; JH greenhouse (WIS); EU373710; EU371802; KF923056*; KF923218*; KF923181*. ***Cleome rutidosperma*** DC.; [no country listed]; *A.A. Mitchell 6380* (WIS); EU373712; EU371804; KF938993*; –; –. ***Cleome rutidosperma*** DC.; Venezuela: Maracay; *T. Ruiz 4360* (MY); –; –; –; –; DQ455802. ***Cleome schimperi*** Pax; Tanzania; *L. Festo & W. Bayona 1729* (MO); KF923111*; KF923152*; KF923050*; KF923219*; HM044273. ***Cleome schweinfurthii*** Gilg; Ethiopia; *W.J.J.O de Wilde & B.E.E de Wilde-Duyfjes s.n.* (MO); –; KF923153*; –; KF923220*; KF923183*. ***Cleome siliculifera*** Eichler; Brazil: Bahia; *R.M. Harley 26987* (NY); KF923113*; KF923155*; KF923059*; KF923222*; HM044286. ***Cleome strigosa*** Oliv.; U.S.A.: Colorado; *F.R. Fosberg s.n.* (MO); –; –; –; –; KF923184*. ***Cleome stylosa*** Eichler; Venezuela: Tachira; *R. Ruiz & L. Hernandez 4977* (MY); –; –; –; –; DQ455812. ***Cleome sulfurea*** Bremek. & Oberm.; Zimbabwe; *H. Wild 5131* (MO); KF923114*; KF923156*; –; –; –. ***Cleome tenuifolia*** (Mart. & Zucc.) Iltis; Brazil: Bahia; *R.M. Harley 163525* (NY); –; –; –; –; HM044280. ***Cleome tetrandra*** Banks ex DC.; Australia; *Mitchell 3659* (MO); KF923115*; KF923157*; –; –; –. ***Cleome titubans*** Speg.; Argentina: Buenos Aires; *A. Krapovickas 2897* (MO); –; –; –; –; DQ455813. ***Cleome torticarpa*** Iltis & T.Ruiz Zapata; Venezuela: Falcon; *T. Ruiz & R. Villafane 5011* (MO); –; –; –; –; DQ455810. ***Cleome trachycarpa*** Klotsch ex Eichler; Argentina; *A. Drapovickas & C.L. Critobal 46421* (MO); KF923116*; KF923158*; –; KF923224*; HM044297. ***Cleome tucumanensis*** Iltis; Argentina; *R. Fortunato 6639* (MO); KF923117; * KF923159*; KF923061*; KF923225*; HM044291. ***Cleome turkmena*** Bobrov; Turkmenistan; *Feodorova & D. Kurbanov 1055* (MO); KF923118*; KF923160*; KF923062*; KF923226*; HM044231. ***Cleome uncifera*** Kers; Western Australia; *B.J. Pepschi & L.A. Craven 5624* (CANB); KF923119*; KF923161*; KF923063*; –; HM044249. ***Cleome usambarica*** Pax ex Engl.; Tanzania; *M.A. Mwangoka 2967* (MO); KF923120*; KF923162*; KF923064*; –; HM044274. ***Cleome violacea*** L.; [no country listed]; *M. Bolton s.n.* (ALTA); HQ452955; HQ452950; KF923065*; KF923227*; KF923185*. ***Cleome viridiflora*** Schreb.; Venezuela: Barinitas, Barinas; *T. Ruiz & L. Herbandez 4987* (MY); –; –; –; –; DQ455820. ***Cleome viridiflora*** Schreb.; [no country listed]; *Solomon s.n.* (MO); AY122386; AY483232; KF923066*; KF923228; * –. ***Cleome werdermannii*** Alf.Ernst; Bolivia: Santa Cruz; *Sanchez 111a* (MO); KF923121*; KF923163*; –; –; DQ455809 ***Cleomella longipes*** Torr.; [no country listed]; *S. Vanderpool 1334* (OKL); AY122387; EU371807; KF923043*; –; –. ***Cleomella obtusifolia*** Torr. & Frem.; [no country listed]; *S. Vanderpool 1293* (OKL); EU373715; EU371808; –; –; –. ***Cleoserrata melanosperma*** (S.Watson) Iltis [= *Cleome melanosperma* S.Watson]; Mexico: Sonora; *R.L. Reina G. 98-853* (NY); –; –; –; –; HM044284. ***Cleoserrata paludosa*** (Willd. Ex Eichler) Iltis [= *Cleome paludosa* Willd. ex Eichler]; Argentina; *R.H. Fortunato 2874* (MO); KF923107*; KF923148*; –; KF923213*; HM044285. ***Cleoserrata speciosa*** (Raf.) Iltis [= *Cleome speciosa* Raf.]; Venezuela: Tachira; *R. Ruiz & L. Hernandez 4978* (MY); –; –; –; –; DQ455806. ***Dactylaena microphylla*** Eichler; [no country listed]; *R.M. Harley 26503 B. Stannard & D.J.N. Hind* (MO); EU373716; EU371809; KF923068*; KF923229*; –. ***Dactylaena microphylla*** Eichler; Brazil: Bahia; *Callejas & A.M de Carvalho 1729* (NY); –; –; –; –; HM044279. ***Dactylaena pauciflora*** Griseb.; [no country listed]; *J.C. Solomon & M. Nee 18108* (MO); EU373717; EU371810; KF923069*; –; –. ***Dipterygium glaucum*** Decne.; [no country listed]; *M.I. Bajwa 972-75* (MO); EU373718; EU371811; –; –; –. ***Gynandropsis gynandra*** (L.) Briq. [= *Cleome gynandra* L.]; [no country listed]; *Hall 238* (WIS); AY122388; EU371812; KF923038*; KF923202*; –. ***Gynandropsis gynandra*** (L.) Briq. [= *Cleome gynandra* L.]; Australia: Queensland; *I.D. Cowie s.n.* (CANB); –; –; –; –; HM044253. ***Hemiscola aculeata*** (L.) Raf. [= *Cleome aculeata* L.]; [no country listed]; *Iltis 30563a* (WIS); AY122382; EU371790; KF923025*; KF923190*; –. ***Hemiscola aculeata*** (L.) Raf. [= *Cleome aculeata* L.]; French Guiana; *F. Billeit & B. Jadin 7445* (MO); –; –; –; –; HM044288. ***Hemiscola diffusa*** (Banks ex DC.) Iltis [= *Cleome diffusa* Banks ex DC.]; [no country listed]; *Follii 3782* (WIS); EU373702; EU371792; KF923032*; KF923196*; KF923170*. ***Oxystylis lutea*** Torr & Frem.; U.S.A.; *S. Vanderpool 1228* (OKL); AY122390; EU371814; KF923072*; KF923231*; KF217215*. ***Peritoma arborea*** (Nutt.) Iltis [= *Isomeris arborea* Nutt ex. Torr. & Gray]; U.S.A.; *M. Fishbein 4146* (WS); AY122389; EU371813; KF923070*; –; –. ***Peritoma arborea*** (Nutt. ex Torr. & A.Gray) Iltis [= *Isomeris arborea* Nutt ex. Torr. & Gray]; [no country listed]; *E. Voznesenskaya 6* (WS); –; –; –; –; HM044239. ***Peritoma lutea*** (Hook.) Raf. [= *Cleome lutea* Hook. subsp. *jonesii* (Macbr.) Iltis]; [no country listed]; *S. Vanderpool 1007* (OKL); EU373706; EU371797; –; KF923206*; KF923177*. ***Peritoma playtcarpa*** (Torr.) Iltis [= *Cleome platycarpa* Torr.]; U.S.A.: Nevada; *A. Tiehm 8030* (WS); –; –; –; –; HM044234. ***Peritoma serrulata*** (Pursh) DC. [= *Cleome serrulata* Pursh]; Canada: Alberta; *M. Patchell* (ALTA); KF923112*; KF923154*; KF923058*; KF923221*; KF217234*. ***Physostemon hemsleyanus*** (Bullock) R.C.Foster [= *Cleome hemsleyana* (Bullock) Iltis]; Mexico; *R.L. Wilbur 36639* (MO); –; KF923137*; –; –; –. ***Physostemon lanceolatum*** Mart. & Zucc. [= *Cleome lanceolata* (Mart. & Zucc.) Iltis]; Brazil; *R.W. Harley s.n.* (MO); –; –; –; –; KF923175*. ***Physostemon rotundifolius*** Mart. & Zucc. [= *Cleome rotundifolia* Mart. & Zucc.]; Brazil; *R.M. Harley 27032* (MO); KF923110*; KF923151*; –; –; KF923182*. ***Physostemon stenophyllum*** (Klotzsch ex Urban). Iltis [= *Cleome stenophylla* Klotzsch ex Urban]; Venezuela: Guarico; *T. Ruiz & R. Villafane 4987* (MY); –; –; –; –; DQ455814. ***Podandrogyne chiriquensis*** (Standl.) Woodson; Costa Rica; *J. & K. Utley 4533* (MO); AY122393; AY483233; –; –; HM044281. ***Podandrogyne decipiens*** (Triana & Planch.) Woodson; [no country listed]; *G. Mora 380*; EU373719; EU371815; –; –; –. ***Podandrogyne jamesonii*** (Briq.) Cochrane; Ecuador; *G.P. Lewis & al. 3438* (MO); –; –; –; –; HM044282. ***Podandrogyne jamesonii*** (Briq.) Cochrane; [no country listed]; *Hall 276*; –; –; KF923073; –; –. ***Podandrogyne macrophylla*** (Turcz.) Woodson; Venezuela: Merida; *T. Ruiz & L. Hernandez 4982*; –; –; –; –; DQ455815. ***Podandrogyne mathewsii*** (Briq.) Cochrane; [no country listed]; *J.R.I. Wood 11536* (K); EU373720; EU371816; KF923074*; –; –. ***Podandrogyne pulcherrima*** (Standley) Woodson; [no country listed]; *M.N. 45* (WIS); AY122393; AY483233; –; KF923232*; –. ***Podandrogyne pulcherrima*** (Standley) Woodson; [no country listed]; *M.N. s.n.* (WIS); –; –; KF923075*; –; –. ***Polanisia dodecandra*** DC.; [no country listed]; *D.F. Grether 8603* (WIS); AY483251; AY483234; KF923076*; KF923233*; KF923187*. ***Polanisia uniglandulosa*** DC.; Mexico; *Stanford & al. 2098* (WS); –; –; –; –; HM044225. ***Tarenaya hassleriana*** (Chodat) Iltis [= *Cleome hassleriana* Chodat]; Harris Seeds #2285, Rochester, New York; *E. Voznesenskaya 6* (WS); KF923096*; KF923136*; KF923039*; –; HM044293. ***Tarenaya spinosa*** (Jacq.) Raf. [= *Cleome spinosa* Jacq.]; [no country listed]; *G. Ayala 91-11* (WIS); EU373713; EU371805; KF923060*; KF923223*; –. ***Tarenaya spinosa*** (Jacq.) Raf. [= *Cleome spinosa* Jacq.]; Puerto Rico; *A. Grable 11178* (WS); –; –; –; –; HM0455296. ***Wislizenia refracta*** Engelm. subsp. ***refracta*;** U.S.A.; *S. Vanderpool 1340* (OKL); AY122391; AY483235; KF923079*; KF923235*; KF217247*.