Phylogenetic relationships among the North American cleomoids (Cleomaceae): A test of Iltis's reduction series[1]

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- *Premise of Study:* A monophyletic group composed of five genera of the Cleomaceae represents an intriguing lineage with outstanding taxonomic and evolutionary questions. Generic boundaries are poorly defined, and historical hypotheses regarding the evolution of fruit type and phylogenetic relationships provide testable questions. This is the first detailed phylogenetic investigation of all 22 species in this group. We use this phylogenetic framework to assess generic monophyly and test Iltis's evolutionary "reduction series" hypothesis regarding phylogeny and fruit type/seed number.
- *Methods:* Maximum likelihood and Bayesian analyses of four plastid intergenic spacer region sequences (*rpl32-trnL*, *trnQ-rps16*, *ycf1-rps15*, and *psbA-trnH*) and one nuclear (ITS) region were used to reconstruct phylogenetic relationships among the NA cleomoid species. Stochastic mapping and ancestral-state reconstruction were used to study the evolution of fruit type.
- *Key Results:* Both analyses recovered nearly identical phylogenies. Three of the currently recognized genera (*Wislizenia*, *Carsonia*, and *Oxystylis*) are monophyletic while two (*Cleomella* and *Peritoma*) are para- or polyphyletic. There was a single origin of the two-seeded schizocarp in the ancestor of the *Oxystylis–Wislizenia* clade and a secondary derivation of elongated capsule-type fruits in *Peritoma* from a truncated capsule state in *Cleomella*.
- *Conclusions:* Our well-resolved phylogeny supports most of the current species circumscriptions but not current generic circumscriptions. Additionally, our results are inconsistent with Iltis's hypothesis of species with elongated many-seed fruits giving rise to species with truncated few-seeded fruits. Instead, we find support for the reversion to elongated multiseeded fruits from a truncate few-seeded ancestor in *Peritoma*.

  Key words: *Carsonia*; Cleomaceae; *Cleomella*; fruit evolution; *Oxystylis*; *Peritoma*; reduction series; *Wislizenia*.

Within the Cleomaceae, a subset of the genera occurring in North America have been shown to form a monophyletic group based on both morphology (Iltis, 1957; illustrated by Bremer and Wanntorp, 1978) and DNA sequence data (Hall et al., 2002; Hall, 2008; Feodorova et al., 2010). While these more recent molecular studies recovered this North American clade (hereafter referred to as "the NA cleomoids"), the relationships of the genera vary across analyses as a result of differences in sampling. Using cpDNA sequences, Hall et al. (2002) found *Peritoma* (*Isomeris*) sister to *Cleomella* + *Oxystylis* + *Wislizenia*. With more taxon sampling, Hall (2008) recovered a paraphyletic *Cleomella* in relation to the *Oxystylis* + *Wislizenia* clade. Feodorova et al. (2010) used nrDNA sequences and a larger sampling of both *Peritoma* and *Cleomella* and found a monophyletic *Peritoma* and a paraphyletic

Cleomella, with *Cleomella hillmanii* sister to *Peritoma*, and *C. brevipes* and *C. plocosperma* sister to the *Oxystylis* + *Wislizenia* clade. Both Hall's (2008) and Feodorova et al.'s (2010) studies, which sampled two and three *Cleomella* species, respectively, found the genus to be paraphyletic to either *Oxystylis* + *Wislizenia* or *Peritoma*. While it seems clear that *Cleomella* may not be monophyletic, these two studies had no sampled species of *Cleomella* in common, making direct comparisons difficult.

The NA cleomoids are found throughout western North America in a number of arid to seasonally wet habitats such as desert scrub, sandy washes, dry desert flats, coastal and inland sand dunes, saline or alkaline flats and meadows (often near hot springs), and roadsides. Currently, 22 species in five genera are recognized (Villegas-Flores and Ramírez-Delgadillo, 1998; Tucker and Vanderpool, 2010): *Carsonia* Greene (monotypic), *Cleomella* DC. (11 species), *Oxystylis* Torrey & Frémont (monotypic), *Peritoma* DC (six species), and *Wislizenia* Engelmann (three species). *Peritoma serrulata* is the only species in the group that is found throughout North America (Tucker and Vanderpool, 2010). Most of the NA cleomoids are herbaceous annuals. However, *Cleomella perennis*, *C. mexicana*, *Peritoma arborea*, and *Wislizenia palmeri* are perennials with more-or-less woody stems. Leaves in this clade are compound with 3–5 leaflets. In the NA cleomoids, petals are typically yellow, with the notable exception of the purple/pink petals in *P. serrulata* and *P. multicaulis*, and flowers are arranged in elongated (*Cleomella*) to highly compressed (*Oxystylis*) racemes. Fruits are

capsules or schizocarps attached to a more-or-less elongated gynophore.

Historical evolutionary hypotheses regarding both specific and generic relationships among the NA cleomoids were based on morphological characters combined with the geographic distributions of the species. Initially, Iltis (1956) posited that *Cleomella* was derived from a *Peritoma lutea*–like ancestor, and that *C. angustifolia* and *C. longipes* "appear to be the most primitive of all the 10 species of *Cleomella*" (Iltis, 1956, p. 187). Expanding on this, Iltis argued that the Mexican *Cleomella* species form a monophyletic group in which a *C. longipes* ancestor gives rise to *C. perennis*, which, in turn, gives rise to *C. mexicana*. Within this evolutionary context, Iltis (1956) interpreted perenniality as a derived condition in *Cleomella*, associated with a hypothesized north-to-south migration/radiation of the *Cleomella* species into Mexico. This idea of an evolutionary trend within the Cleomaceae of adaptation to progressively more xeric habitats formed the basis for Iltis's evolutionary hypotheses regarding the NA cleomoids. Iltis (1957) expanded on his evolutionary scenario for the NA cleomoids in which a *Peritoma* ancestor (potentially *P. lutea*) gave rise to *Cleomella*. In turn, Iltis proposed that a *Cleomella* species, possibly *C. longipes*, then gave rise to *Wislizenia*. *Wislizenia* subsequently diversified and one subtaxon of *W. refracta* ("*W. r. melilotoides*?") gave rise to *Oxystylis* in the Death Valley (California/Nevada) area (Iltis, 1957, p. 100).

Iltis's idea of a "reduction series" is best seen in his hypothesis of fruit evolution in the NA cleomoids (Iltis, 1957, p. 115). The proposed ancestral fruit condition was an elongated capsule with many seeds (as seen in *Peritoma* and *Cleome*). Derived from this were truncated, fewer-seeded capsules, typically as wide or wider than long, such as those found in *Cleomella* and *Wislizenia*. *Oxystylis lutea* was seen as the end of this fruit reduction with its unusual, indehiscent two-seeded schizocarp fruits.

Iltis (1957) hypothesized that *Oxystylis* was derived from *Wislizenia* in his reduction series. The sister relationship of *Oxystylis* and *Wislizenia* was demonstrated genetically by Vanderpool et al. (1991). They showed that the two genera were united by two shared duplicated gene loci not found in *Peritoma* or *Cleomella*. Although both Iltis (1957) and Vanderpool et al. (1991) agreed that *Oxystylis* and *Wislizenia* were closely related, Vanderpool et al. argued that the separation occurred gradually over time, in agreement with what Bremer and Wanntorp (1978) proposed. By contrast, Iltis (1957) proposed that *Oxystylis* originated within the past 12 000 yr, when Death Valley appeared. More recently, Hall et al. (2002), Hall (2008), and Feodorova et al. (2010) also recovered this close relationship with DNA sequence data, but none of these recent studies examined the potential timing of diversification in this clade.

Keller's (1979) detailed morphological study of *Wislizenia* clarified the confusion regarding the many putative taxa that had been described within this genus (e.g., Greene, 1906). Keller recognized three subspecies within *Wislizenia refracta*: subsp. *californica* from the Central Valley of California, subsp. *palmeri* from the Baja peninsula and coastal Sonora, Mexico, and subsp. *refracta* for individuals found to the east of California. These are currently recognized as separate species (Tucker and Vanderpool, 2010). Keller (1979) also hypothesized that polyploidy played a role in speciation within at least some of the NA cleomoids, an idea not without merit. However, the current cytological information available for the NA cleomoids is incomplete and somewhat ambiguous.

In sum, despite being a small clade in a small plant family, there are a number of testable hypotheses regarding the timing, morphological evolution, and taxonomic boundaries of the NA cleomoids. Our goal is to use comprehensive sampling and multiple molecular markers to test two of these hypotheses. First, are the five currently recognized genera monophyletic, and what are their relationships to one another? Second, using this phylogenetic framework, we test Iltis's reduction-series hypothesis regarding the morphological evolution of the NA cleomoids and the reduction in their fruit size and seed number.

MATERIALS AND METHODS

***Taxon sampling*—**We sampled all 22 currently recognized species (including both varieties of *C. hillmannii*) within the monophyletic NA cleomoids, plus two outgroup species (*Cleome angustifolia* Forssk. and *Polansia dodecandra* [L.] DC.). Seventy-seven specimens of either herbarium or fresh material were sampled, representing a broad geographic coverage of each species' range (Appendix 1).

***DNA extraction, PCR, and sequencing*—**Total genomic DNA was extracted from each sample using either a modified 2× CTAB extraction procedure (Doyle and Doyle, 1987) or a DNeasy Plant Mini Kit protocol (Qiagen, Missisauga, Ontario, Canada). We sequenced one nuclear (ITS) and four plastid (*rpl32-trnL*, *trnQ-rps16*, *ycf1-rps15*, and *psbA-trnH*) intergenic spacer regions. The polymerase chain reaction (PCR) was used to amplify the nrDNA ITS-1, 5.8S, and ITS-2 regions using the ITS-4 (White et al., 1990) and ITS-5 (Suh et al., 1993) primers. The chloroplast DNA spacer regions *rpl32-trnL*, *trnQ-rps16*, and *psbA-trnH* were amplified using the protocols and primer sequences described in Shaw et al. (2005, 2007). The *ycf1-rps15* spacer was amplified and sequenced with primers *ycf1*-5710F and *rps15*-R (Neubig et al., 2009). PCR products were cleaned with either the ExoSAP-IT procedure (USB Corporation, Cleveland, Ohio, USA) or a QIAquick PCR Purification Kit (Qiagen). Cycle sequencing reactions using BigDye Terminator 3 (Applied Biosystems, Foster City, California, USA), and the amplification primers were run using a protocol modified for 1/10th reactions. Cycle sequencing reaction products were purified using Performa DTR V3 96-well short plates (Edge Biosystems, Gaithersburg, Maryland, USA) and were analyzed on an Applied Biosystems 3730 DNA Analyzer at either the Washington State University Center for Integrated Biotechnology or Molecular Biology Service Unit at the University of Alberta.

***Phylogenetic analyses*—**Sequences were edited using Sequencher version 4.10.1 (Gene Codes, Ann Arbor, Michigan, USA), aligned using MUSCLE (EMBL-EBI web version; Edgar, 2004) using default parameters, and manually adjusted in the program SeAl version 2.0a11 (Rambaut, 2002) using the similarity criterion following Simmons (2004). Sequences were submitted to GenBank (Appendix 1), and the aligned data matrix and trees are deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S14446). Maximum likelihood (ML; Felsenstein, 1973) and Bayesian MCMC (Yang and Rannala, 1997) approaches were used to reconstruct phylogenetic relationships in the NA cleomoid clade. Analyses of individual genes were performed using ML using the GTRGAMMA model in RAxML version 7.2.8 (Stamatakis et al., 2008) to assess topological congruence among the different data sets. No major incongruences were found in the separate gene region analyses (no moderately or strongly supported branches differed among the individual analyses; see Supplemental Data with the online version of this article; Appendices S1 and S2), and further analyses were therefore run on a concatenated data set. The combined unpartitioned ML analyses were conducted in RAxML using the GTR+G model with 100 replicates. Analyses were run through the RAxML BlackBox portal (http://phylobench.vital-it.ch/raxml-bb/), which allows only one partition; however, the congruence among all the different analysis types suggests that using a single partition did not negatively influence the results.

Phylogenetic analyses were also performed using the Bayesian uncorrelated lognormal method, which allows for rates of molecular evolution to differ across lineages, each gene region with a separate GTR partition, implemented in BEAST version 1.6.2 (Drummond and Rambaut, 2007). We employed a single secondary calibration point to the crown node of all NA cleomoids, excluding *Carsonia*, based on age estimates produced from an independent ordinal

phylogeny (data not shown). The phylogeny was estimated from 113 taxa representing 15 families in the Brassicales that were sampled for five gene regions: cpDNA *matK*, *ndhF*, *rbcL*, and mtDNA *matR*, *rps3*; for a total aligned length of 7197 bp. Partitioned GTR+I+Γ estimated models were executed in BEAST in two independent runs for 10 million generations using the Yule speciation rate. Those runs were calibrated with 2 lognormal distribution priors based on Cleomaceous seed fossils from the London Clay (~48.6 Ma, Chandler, 1962, 1964) and an *Akania* sp. leaf fossil from Patagonia (61.7 ± 2 Ma, Iglesias et al., 2007). The present node was constrained as a normal distribution age prior with a mean of 22.6187 Ma and standard deviation of 4.2357, reflecting the 95% highest posterior density range of 29.59–15.65 Ma recovered from the broader dating analysis, which is consistent with dates from recent molecular studies (Beilstein et al., 2010, Hernández-Hernández et al., 2013). Two independent BEAST runs were performed for 100 million generations each, using a birth-death prior for branching rates. We assessed convergence of runs and combined the trees from the last 50 million generations of each run based on analyses in Tracer version 1.5 (Rambaut and Drummond, 2007) to create a maximum clade credibility tree.

Character reconstructions—Stochastic mapping (SM), as implemented in the program SIMMAP (Bollback, 2006), was used to simulate the evolution of fruit type. SIMMAP uses a stochastic algorithm to map discrete character states onto a distribution of phylogenic trees and then summarizes character history statistics across all individual mappings, thereby incorporating topological and branch-length uncertainty contained in the distribution of trees (Nielsen, 2002; Huelsenbeck et al., 2003). We used the posterior distribution of trees generated in our BEAST analyses to create stochastic mappings of fruit-type change in SIMMAP. Fruit-type state was assumed to vary among the three forms that fall into the general fruit categories of elongated, multiseeded capsules (state 0), truncated, few-seeded capsules (state 1), and two-seeded schizocarps (state 2). Fruit morphology was scored by species on the basis of literature (Iltis, 1956; Villegas-Flores and Ramírez-Delgadillo, 1998; Tucker and Vanderpool, 2010) and observation of herbarium specimens. Although there is considerable variation in the shape and seed number of the truncated capsules of *Cleomella*, they are distinctly shorter and fewer-seeded than the elongated, multiseeded capsules of *Peritoma* and the outgroup species (Table 1).

We generated one character history mapping in SIMMAP for each of the last 1000 trees in the posterior distribution from BEAST. We used a gamma rate prior, with the shape parameters $\alpha = 1.25$ and $\beta = 0.25$. The reconstruction of character histories was robust with respect to this prior; simulations run under other values of α and β generated very similar results. Rates of change among all character states were averaged across all character history reconstructions. Inferred states of internal nodes of the tree are expressed as the proportion of trees in the distribution in which each node was reconstructed to be at each character state. These proportions reflect the uncertainty in the reconstruction of the state of each node, which is dependent, in turn, on the branch lengths and topologies of all the trees in the distribution.

We also used SIMMAP to summarize the number of transitions among character states on each history in the distribution of simulated histories generated by SIMMAP. These per-tree values were then averaged to yield a mean per-tree number of transitions for each possible combination of all character states in the analysis.

Ricklefs (2007) suggested that, because the statistical properties of stochastic mapping and other recent ancestral-state reconstruction (ASR) models are not well understood, results should be compared among them. We chose to compare our SIMMAP results to an inferred ASR history generated by Mesquite version 2.75 (Maddison and Maddison, 2011), under a Markov one-rate model. The Mk1 method was applied to the same distribution of trees as used for the SIMMAP analysis.

RESULTS

Phylogenetic analyses—Bayesian posterior probability (PP) values and ML bootstrap values are generally very high for most nodes in the phylogeny, resulting in a highly resolved and well-supported tree (Fig. 1; also see Supplemental Data with the online version of this article; Appendices S1–S3). Both the Bayesian and ML analyses resulted in nearly identical topologies. The trees differed in the sister relationships of species within only one clade. Both analyses generated clades composed of

C. brevipes, *C. parviflora*, and *C. plocasperma* ("*Brevipes*" clade"). *Cleomella parviflora* was sister to a *brevipes–plocasperma* clade in the Bayesian analysis (Fig. 1), whereas the ML analysis placed *C. plocasperma* as sister to a *C. parviflora–brevipes* clade (see Supplemental Data with the online version of this article; Appendix S4).

Only three of the traditionally circumscribed genera were monophyletic/exclusive lineages: *Wislizenia*, and the monotypic *Carsonia* and *Oxystylis*. *Carsonia* is strongly supported as the sister to all other NA cleomoids. The *Oxystylis–Wislizenia* clade is sister to a *Cleomella–Peritoma muticaulis* clade ("core *Cleomella* clade") that includes *C. longipes*, Iltis's hypothesized ancestor of *Wislizenia*. However, these two clades are nested within a much larger paraphyletic *Cleomella* (*Cleomella* s.l.). This *Cleomella* s.l. clade is sister to a clade composed of the rest of *Peritoma* (*Peritoma* s.s.) and the two varieties of *C. hillmannii* (Fig. 1).

Character reconstructions—Character states were reconstructed for all internal nodes, though posterior support is low (<0.5 PP) for some nodes. Both the SIMMAP implementation of SM and Mesquite ancestral-state reconstructions of fruit types using the Mk1 model showed strong evidence for a single origin of the two-seeded schizocarp state in the ancestor of the *Oxystylis–Wislizenia* clade (Fig. 2 and 3). The elongated, multiseeded capsule-type fruits found in *Peritoma multicaulis* were shown to be a reversion from the truncated, few-seeded capsule state found in most of *Cleomella*. Both ancestral-character reconstruction methods were equivocal in their reconstruction of the *Peritoma–Cleomella hillmannii* node (Fig. 2), with similar relative probabilities for elongated and truncated capsules. However, the node leading to the *Peritoma* s.l. + *Cleomella* s.l. clades has high relative probability for a truncated capsule using both methods. When using the likelihood analysis method, longer branches are often inferred to have more than one state change on them; this is likely the origin of the reconstructions showing two-seeded schizocarp fruits being the ancestral condition found at very low frequencies on the early branches (Fig. 2, right side). This suggests that the elongated, multiseeded capsule-type fruits found in *Peritoma* are derived secondarily from a truncated, few-seeded capsule state and are not the plesiomorphic state found in the outgroup species or the majority of the Cleomaceae.

Inflorescence characters vary considerably in the clade, with a large amount of overlap among species (Table 1). This made interpreting any patterns in this context difficult from a character coding/analysis perspective, and we therefore have presented the data to demonstrate the complexity of these characters.

DISCUSSION

Phylogenetic patterns—Our study represents the first detailed phylogenetic investigation of all species of the NA cleomoids. Importantly, extensive taxon and character sampling enabled us to test two hypotheses regarding this group. Bayesian and ML analyses recovered similar phylogenies for this group, and it is clear from these analyses that neither *Cleomella* nor *Peritoma* is monophyletic as currently circumscribed (Tucker and Vanderpool, 2010). Additionally, the appropriateness of maintaining as distinct the genera *Carsonia*, *Oxystylis*, and *Wislizenia* is called into question.

Table 1. Inflorescence characters and distributions for the NA cleomoids (modified from Iltis, 1956; Villegas-Flores and Ramírez-Delgadillo, 1998; Tucker and Vanderpool, 2010).

Species	Bracts	Raceme length in flower	Raceme length in fruit	Fruit size (L × W)	Seeds per fruit	Distribution
Carsonia sparsifolia	Unifoliate or trifoliate	2–8 cm	3–10 cm	15–45 × 1–3 mm	10–13	US: CA, NV
Cleomella angustifolia	Trifoliate proximally to unifoliate distally	1–6 cm	10–40 cm	4–6 × 6–12 mm	2–4	US: CO, KS, NE, OK, TX
C. brevipes	Unifoliate	Solitary flowers	Solitary flowers	2–3 × 2–3.2 mm	1–4	US: CA, NV
C. hillmannii var. *goodrichii*	Unifoliate	3–10 cm	2–20 cm	3.5–6 × 4–10.5 mm	2–6	US: ID, UT
C. hillmannii var. *hillmannii*	Unifoliate	3–10 cm	2–20 cm	3.5–6 × 4–10.5 mm	2–6	US: CA, ID, NV, OR
C. jaliscensis	Trifoliate proximally to unifoliate distally	(20–) 35–40 (–52) cm	(Unknown)	5–9 × 5–11 mm	10–14	Mexico: Jalisco
C. longipes	Unifoliate	2–12 cm	10–50 cm	4–8 × 6–12 mm	6–16	US: AZ, NM, TX; Mexico: Chihuahua
C. mexicana	Trifoliate	Lax and ill-defined	Not greatly elongated	3–5 × 6–9 mm	6–8	Mexico: Districto Federal, Mexico, Puebla
C. obtusifolia	Unifoliate	0.5–1.2 cm	0.5–1 cm	3.5–4 × 7–10 mm	2–6	US: CA, NV, NM
C. palmeriana	Rudimentary	1–2 cm	2–3.5 cm	2–5 × 3–5 mm	2–4	US: AZ, CO, NM, UT
C. parviflora	Unifoliate or trifoliate	0.5–20 cm	5–30 cm	2.5–5 × 2.5–6 mm	3–12	US: CA, ID, NV
C. perennis	Trifoliate proximally to unifoliate or trifoliate distally	?	Greatly elongated in fruit, up to 25 cm	5–7 × 6–12 mm	5–7	Mexico: Durango, Guanajuato, San Luis Potosi, Zacatecas,
C. plocasperma	Unifoliate	1–15 cm	2–20 cm	2.5–5 × 2.5–6 mm	2–4	US: CA, ID, OR, UT
Oxystylis lutea	Unifoliate	1 cm	2–3 cm	1 × 0.6 mm	2	US: CA, NV
Peritoma arborea	Unifoliate	1–3 cm	6–40 cm	20–30 × 6–12 mm	5–25	US: AZ, CA; Mexico: Baja California, Colima, Sonora
P. jonesii	Unifoliate	1–3 cm	6–40 cm	40–60 × 2–5 mm	15–30	US: AZ, CA; Mexico: Baja California
P. lutea	Unifoliate	1–3 cm	6–40 cm	15–40 × 2–5 mm	10–20	US: CA, CO, ID, MT, NE, NV, NM, OR, UT, WA, WY; Mexico: Baja California
P. multicaulis	Unifoliate	1–3 cm	6–40 cm	15–25 × 1.5 mm	10–20	US: AZ, CO, NM, TX, WY: Mexico: Coahuila, Distrito Federal, Jalisco, Mexico, Sonora
P. platycarpa	Unifoliate	1–3.5 cm	5–40 cm	12–25 × 8–12 mm	10–20	US: CA, ID, NV, OR
P. serrulata	Unifoliate	1–4 cm	4–30 cm	23–76 × 3–6 mm	12–38	Widespread in W. N. Am.
Wislizenia californica	Absent	1–1.5 cm	2–3 cm	1.4–3.4 × 1 mm	2(–4)	US: CA
W. palmeri	Absent	1–1.5 cm	2–3 cm	1–5.6 × 1 mm	2(–4)	US: AZ, CA; Mexico: Baja California, Baja California Sur, Sonora
W. refracta	Absent	1–1.5 cm	2–3 cm	1.2–3.3 × 1 mm	2(–4)	US: AZ, CA, NV, NM, TX, UT; Mexico: Chihuahua, Sonora

Iltis (1957; p. 77) proposed an evolutionary scenario that he termed a "reduction series" describing the phylogeny of the NA cleomoid genera as a series of adaptations to increasing habitat aridity manifested as (1) reductions in fruit size and seed number per capsule, (2) reductions in inflorescence size and inflorescence bract size, and (3) increases in size and complexity of the stipules. We do not address the evolution of stipule characters here because the quantitative nature of the variation in this character makes it rather difficult to analyze in this context. *Peritoma* and *Carsonia* have oblong to elongated capsules containing 5–38 seeds. *Cleomella* capsules are characterized as being approximately as wide as they are long, resulting in rhomboidal, globose, or deltoid shapes. Seed number in *Cleomella* is variable, with capsules typically containing 2–6 seeds, but *C. longipes* can have as many as 16 seeds in a capsule. Fruits in *Wislizenia* are indehiscent schizocarps, generally with two seeds. *Oxystylis* represents the extreme in that its fruits are highly reduced indehiscent schizocarps that might be better termed nutlets with two seeds

per fruit (Tucker and Vanderpool, 2010). Regardless of terminology, the seeds in *Wislizenia* and *Oxystylis* are dispersed quite differently than most Cleomaceae: they are dispersed as protected propagules retained within the pericarp.

Iltis (1957) hypothesized that the elongated, multiseeded capsules found in *Peritoma* were ancestral with the truncated, few-seeded capsules (*Cleomella*), and schizocarps (*Wislizenia* and *Oxystylis*) the derived condition. He viewed this reduction in fruit size and seed number as a direct response to increasing habitat aridity. In other lineages, other factors are also involved, including seed size and dormancy (Baker, 1972; Brown and Venable, 1986; Delph, 1986; Venable and Brown, 1988; Ehrman and Cocks, 1996; Lu et al., 2010; Volis and Bohrer, 2013). Iltis (1957) also proposed a reduction in inflorescence size and a loss of inflorescence bracts. Again, the putative primitive genus is *Peritoma* with its elongate bracteate racemes, and *Oxystylis* is seen as the derived species with its highly compressed ebractate(?) racemes.
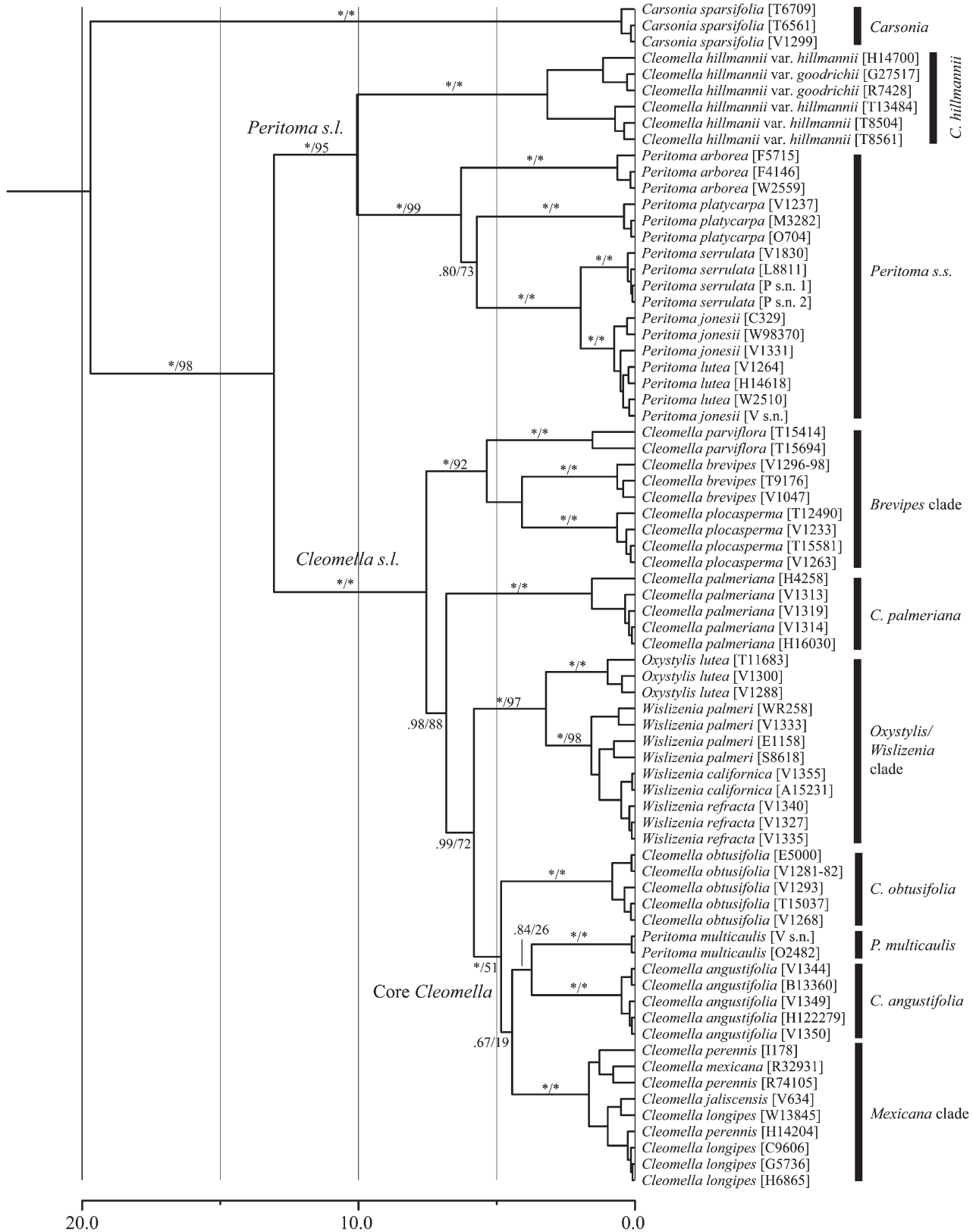
Fig. 1. Maximum clade credibility tree from the BEAST 100-million-generations analysis with posterior probability values (number preceding slash) and maximum likelihood bootstrap values (number following slash) for each node. Asterisks denote a posterior of 1.0 or bootstrap value of 100. Support values for short branches near the tips of the tree and within species were excluded for clarity and can be found in Supplemental Data with the online version of this article (Appendix S3).
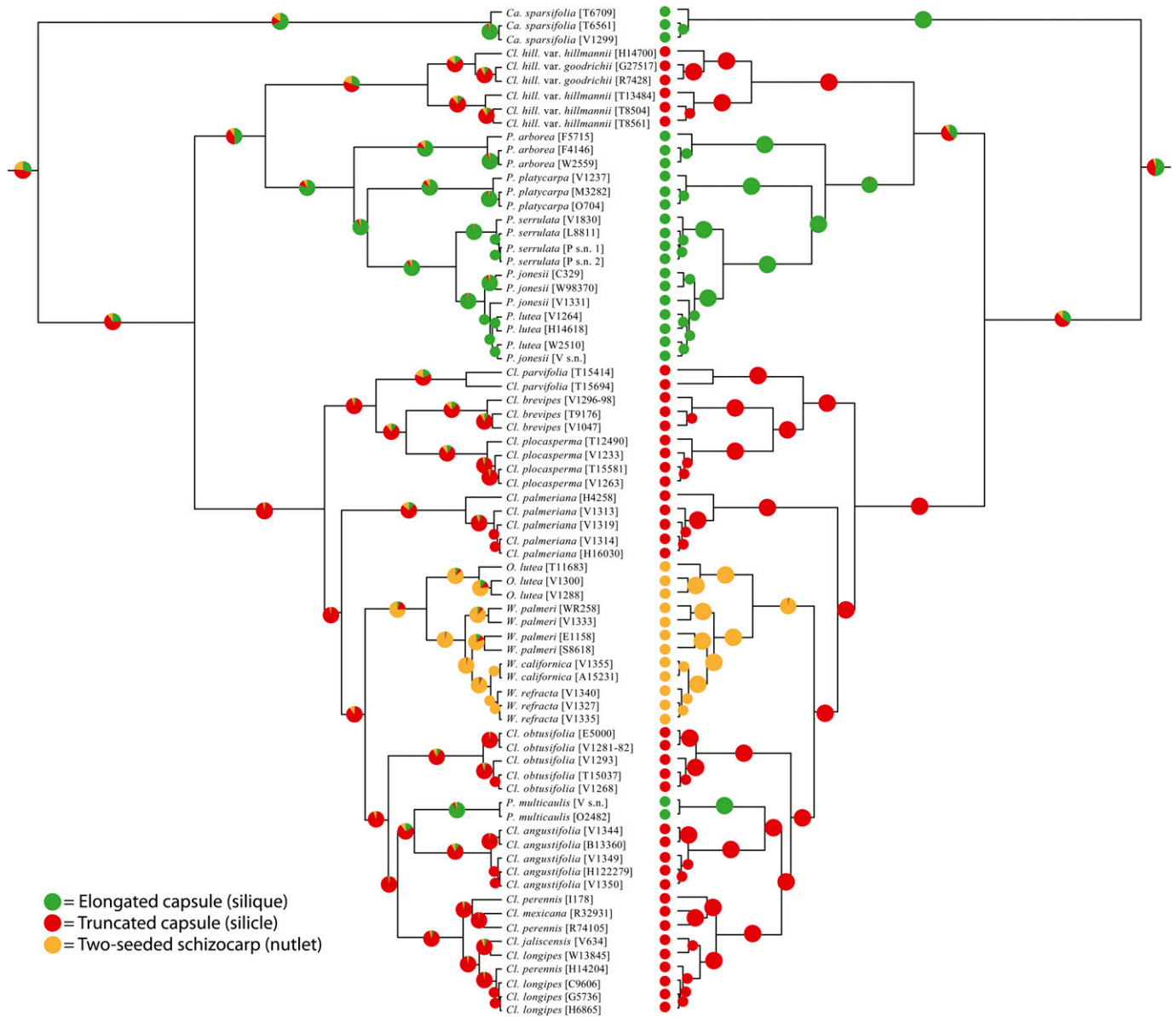
Fig. 2.　Ancestral-state reconstructions of fruit types in the NA cleomoids, performed using both stochastic mapping, as implemented in the program SIMMAP (Bollback, 2006) (left tree), and Mk1 likelihood optimization generated by the program Mesquite version 2.75 (Maddison and Maddison, 2011) (right tree). Both methods were applied to the last 1000 trees from the BEAST analysis to incorporate topological uncertainty.

The clade including all three species of *Wislizenia* forms a clade sister to *Oxystylis*; however, relationships among the species of *Wislizenia* are less clear. *Wislizenia californica* and *W. refracta* both form exclusive lineages; however, *W. palmeri* does not. Instead, *W. palmeri* forms a paraphyletic grade leading to the other two *Wislizenia* species (Fig. 1). These relationships are not strongly supported, and when other individuals of *Wislizinia* species are sampled with sequence data, *W. refracta* is paraphyletic and *W. palmeri* is exclusive (data not shown). Given the inferred recency of divergence of the *Wislizenia* species (~1.5 Ma), it is possible that lineage-sorting effects are influencing our inference of species monophyly. Our results are in agreement with Vanderpool et al. (1991), who demonstrated that *Oxystylis* is sister to all *Wislizenia* taxa, indicating a divergence prior to the diversification of *Wislizenia*.

Using this phylogenetic framework, we examine Iltis's insights into the evolution of the NA cleomoids via his reduction-series hypothesis. Specifically, is there a phylogenetic relationship coupled to a reduction in fruit size and seed number? Fruit type has been one of the traditional characters used in defining genera in the NA cleomoids. *Carsonia* and *Peritoma* generally possess elongated, multiseeded capsules; *Cleomella* has shorter, few-seeded truncated capsules that are typically as wide (or wider) as they are long. The putatively more derived genera *Wislizenia* and *Oxystylis* are characterized by their two-seeded schizocarps. Iltis (1957) proposed that this reduction in fruit size and seed number followed an evolutionary trajectory from elongated, multiseeded fruits (*Peritoma*) to truncated, few-seeded fruits (*Cleomella*) finally giving rise to the small, two-seeded schizocarps of *Wislizenia* and *Oxystylis*. This reduction
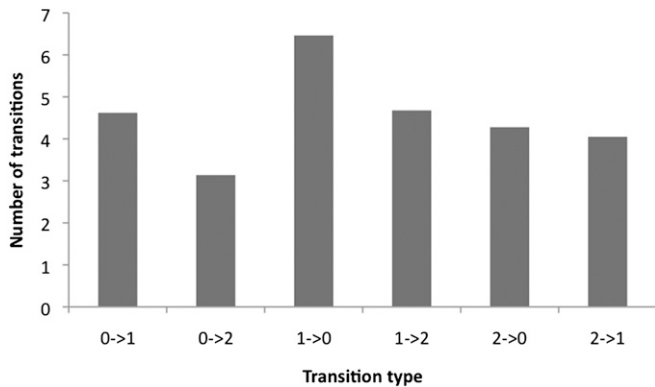
Fig. 3. Probabilities of transitions from each character to all other character states as generated by stochastic mapping in the program SIM-MAP (Bollback, 2006). Character states are as follows: (0) elongated, multiseeded capsule; (1) truncated, few-seeded capsule; (2) two-seeded schizocarps.

in fruit size and seed number was proposed to be in response to the increasing habitat aridity experienced by each successive species. We did not find evidence for this linear transition between fruit types (Fig. 2). Instead we found high relative probabilities that most of the NA cleomoids ancestrally had truncated, few-seeded fruits (*Cleomella*-type) from which elongated, multiseeded fruits were derived in *Peritoma* and from which two-seeded schizocarps were derived in the *Wislizenia–Oxystylis* clade (Fig. 2). In other words, indehiscence and seed reduction is a shared characteristic of the *Wislizenia–Oxystylis* clade. Iltis (1957) proposed that this reduction series was directly related to habitat aridity; however, no one has ever quantified any differences in habitat aridity among the NA cleomoid species. Particularly, overall habitat aridity is less important than the actual conditions under which these predominantly annual species germinate, grow, and flower. It is expected that the seeds dispersed within pericarpy might be more protected from harsh environmental conditions (Gutterman, 2002), but the association of habitat characteristics with adaptation will require further study to quantify, particularly the tradeoffs among seed number, seed size, and seed dormancy (Baker, 1972; Brown and Venable, 1986; Delph, 1986; Venable and Brown, 1988; Ehrman and Cocks, 1996; Lu et al., 2010; Volis and Bohrer, 2013).

One of the other "reduction series" characteristics discussed by Iltis (1957) is the reduction in inflorescence size and bract presence. Raceme length in *Peritoma* is relatively consistent, with considerably more variation present in *Cleomella*. *Cleomella* shows a broad range of inflorescence lengths and varying degrees of inflorescence bract reduction (Table 1). When bract presence is mapped onto the tree (data not shown), this character changes only once, at the ancestor of *Oxystylis* + *Wislizenia*. There are some cases of bract presence varying within species (e.g., *Cleomella palmeriana*), but this seems to be a different condition from that found in the *Oxystylis–Wislizenia* clade. Further, although there appears to be a general reduction in inflorescence size from *Peritoma* to *Cleomella* to *Wislizenia* to *Oxystylis*, it is unclear whether there is an affiliated loss of inflorescence bracts.

Timing of diversification—The NA cleomoid clade began to diversify in the early Miocene with the divergence of the *Carsonia* lineage at ~20 Ma, and continued in the mid-Miocene with

the divergence of the *Peritoma* s.l. and *Cleomella* s.l. clades at ~13 Ma (Fig. 1; also see see Supplemental Data with the online version of this article; Appendices S5 and S6). Our phylogenetic hypothesis suggests that most of the extant species originated no later than ~6 Ma (Fig. 1), and the divergence of *Oxystylis* from *Wislizenia* at ~3 Ma. These estimates are much older than those suggested by Iltis (1957), who proposed that *Oxystylis* originated within the past 12 000 yr, when Death Valley was formed. In fact, our data support more recent work (Stebbins and Major, 1965; Vanderpool et al., 1991) that proposed that *Oxystylis* and *Wislizenia* are older taxa. Given that nearly all of these species are adapted to seasonally dry to extremely arid habits and are predominantly distributed in the southwestern United States and northern Mexico, this corresponds nicely to the aridification of these areas in the late Miocene and Pliocene associated with the uplift of the Sierra Nevada Mountains and Colorado Plateau.

Chromosome evolution—The hypothesis that polyploidy has played a role in speciation within at least some of the NA cleomoids is less certain than previously reported by Keller (1979). Keller (1979) expanded upon Iltis's (1957) reduction series hypothesis by proposing that *Peritoma* (n = 10) was the ancestral genus, with *Cleomella*, *Oxystylis*, and *Wislizenia* being derived and subsequently (and independently of each other) becoming polyploid (n = 20). Unfortunately, the published record of chromosome numbers for the NA cleomoids does not support such a scenario (Table 2), nor does Keller's (1979) own table 3. In fact, the chromosome numbers reported for the NA cleomoids are quite complicated: *Peritoma* is n = 16, 17, 20, and 30; *Cleomella* is n = 17 and 20; *Carsonia* is n = 16; *Oxystylis* is n = 10 and 20; and *Wislizenia* is n = 20. In light of the phylogenetic hypothesis, it is hard to discern any clear pattern based on changes in ploidy levels (Fig. 1). The case for a polyploid

TABLE 2. Previously published chromosome counts for the NA cleomoids.

Species	Gametophytic	Sporophytic	Reference
Carsonia sparsifolia	16		Löve, 1987
Cleomella hillmannii var. *goodrichii*	17		Mulligan, 1967
C. longipes	20		Keller, 1979
		34	Tucker and Vanderpool, 2010
Oxystylis lutea	20		Raven et al., 1965
	10		Reveal and Styer, 1973
Peritoma arborea		40	Snow, 1959
	20		Raven et al., 1965
	20		Löve, 1985
P. lutea	ca. 16		Rollins, 1939
	17		Raven et al., 1965
	17		Löve, 1966
P. platycarpa	20		Löve, 1987
P. serrulata	16		Rollins, 1939
		60	Löve, 1965
	17		Raven et al., 1965
	17		Löve, 1966
	17		Löve, 1976
		34	Löve, 1982
	16		Ward and Spellenberg, 1988
Wislizenia refracta	20		Bell, 1965
	20		Reveal and Moran, 1977
	20		Keller, 1979
	20		Spellenberg, 1979

origin of *Cleomella*, *Oxystylis*, and *Wislizenia* from a *Peritoma* ancestor is difficult to reconcile with the distribution of chromosome counts on the phylogeny. In fact, the recognition of *Carsonia* based, in part, on its "unique cytology (2n = 32)" (Tucker and Vanderpool, 2010; p. 208) is unsupported because its ploidy level is not unique among the NA cleomoids. Currently, only 9 of the 22 NA cleomoid species have had their chromosome numbers characterized, and of these nine species, several have had different counts reported in different studies (Table 2). Whether this reflects variation in chromosome complement within species or errors in the literature is unclear. Further chromosome studies will be necessary to resolve the evolution of chromosome variation in the clade.

Concluding remarks—Within the NA cleomoid group, species are well defined and typically form well-supported clades in our analyses (Fig. 1). However, the currently recognized multispecies genera are not supported. The two largest genera (*Cleomella* and *Peritoma*) are para- or polyphyletic as currently circumscribed (Tucker and Vanderpool, 2010). Although *Carsonia* was strongly supported as sister to all the rest of the NA cleomoids, there is little morphological (or cytological) evidence to maintain it as a distinct genus. It is clear that fruit evolution has been a dynamic process of reduction and expansion and not a linear reduction series as proposed by Iltis (1957). Although the extremes of variation in this clade (e.g., *Oxystylis*) seem distinctive in relation to much of the morphological variation, it is clearly the end point of a continuum of variation and reduction in the clade. This pattern of morphologically distinct species derived within a clade has been found in other groups (e.g., *Zauschneria* derived from *Epilobium* [Raven, 1976] and *Cymophyllus* derived from *Carex* [Roalson et al., 2001]), and in most cases it has been clear that although the species may be distinctive, these specialized monotypic genera are usually submerged into the larger genus to provide monophyletic genera. The phylogenetic relationships recovered here, and the difficulty in morphologically defining several of these genera, lead us to conclude that these genera need to be recircumscribed. This lineage might be best treated as a single genus; however, it is unclear whether there might be other morphological data that support a different generic circumscription. A careful reevaluation of morphological variation in the clade is necessary to evaluate possible synapomorphies for clades in the NA cleomoids.

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APPENDIX 1. Cleomaceae species and voucher specimens used in the present study. Entries are as follows. Taxon: voucher, geographic location (county [if known], state, country) (collection year), herbarium. GenBank accessions: *psbA-trnH*, *trnQ-rps16*, *rpl32-trnL*, *ycf1-rps15*, ITS.

Carsonia sparsifolia (S. Watson) Greene: *A. Tiehm 6561*, Mineral, CA, USA (1981), WS. KF258776, KF241568, KF241602,—, KF217172; *A. Tiehm 6709*, Inyo, CA, USA (1981), WS. KF258777, KF241569,—,—, KF217173; *S. Vanderpool 1299*, Nye, NV, USA (1986), OKL.—,—,—, KF128736, KF217174. *Cleomella angustifolia* Torrey: *L.C. Higgins 122279*, Hemphill, TX, USA (1978), NY. KF258779, KF241571, KF241604,—, KF217179; *R. Brooks 13360*, Harvey, KS, USA (1977), NY. KF258778, KF241570, KF241603,—, KF217178; *S. Vanderpool 1344*, Jefferson, OK, USA (1986), OKL.—,—,—, KF128737, KF217175; *S. Vanderpool 1349*, Archer, TX, USA (1986), OKL.—,—,—, KF128738, KF217176; *S. Vanderpool 1350*, Witchita, TX, USA (1986), OKL.—,—,—, KF128739, KF217177. *Cleomella brevipes* S. Watson: *A. Tiehm 9176*, Churchill, NV, USA (1984), NY. KF258780, KF241572,—,—, KF217182; *S. Vanderpool 1296-98*, Nye, NV, USA (1986), OKL.—,—,—, KF128740, KF217180; *S. Vanderpool 1047*, Inyo, CA, USA (1982), OKL.—,—,—, KF128741, KF217181. *Cleomella hillmannii* A. Nelson var. *hillmannii*: *N. H. Holmgren 14700*, Malheur, OR, USA (2002), NY. KF258783, KF241575, KF241607,—, KF217187; *A. Tiehm 13484*, Mineral, NV, USA (2001), NY. KF258784, KF241576, KF241608,—, KF217188; *A. Tiehm 8561*, Mineral, NV, USA (1986), WIS.—,—,—, KF128742, KF217183; *A. Tiehm 8504*, Churchill, NV, USA (1984), WIS.—,—,—, KF128743, KF217184. *Cleomella hillmannii* A. Nelson var. *goodrichii* (S.L. Welsh) P.K. Holmgren: *J. L. Reveal 7428*, Lemhi, ID, USA (1995), NY. KF258782, KF241574, KF241606,—, KF217186; *S. Goodrich 27517*, Uintah, UT, USA (2009), NY. KF258781, KF241573, KF241605,—, KF217185. *Cleomella jaliscensis* E. Villegas-Flores & R. Ramírez Delgadillo: *Villegas 634*, Jalisco, Mexico (1996), WIS.—,—,—, KF128744, KF217179. *Cleomella longipes* Torrey: *R.D. Worthington 13845*, Hudspeth, TX, USA (1985), NY. KF258785, KF241577, KF241609,—, KF217190; *Gillis 5736*, Brewster, TX, USA (1964), WIS.—,—,—, KF128745, KF217191; *Chiang et al. 9606*, Coahuila, Mexico (1972), WIS.—,—,—, KF128746, KF217192; *Henrickson 6865*, Chihuahua, Mexico (1971), WIS.—,—,—, KF128747, KF217193. *Cleomella mexicana* M. Sessé y Lacasta & J.M. Mociño ex de Candolle in A.P. de Candolle and A.L.P.P. de Candolle: *J. Rzedowski 32931*, Veracruz, Mexico (1975), NY. KF258786, KF241578, KF241610,—, KF217194. *Cleomella obtusifolia* Torrey & Frémont in J.C. Frémont: *B. Ertter 5000*, San Bernardino, CA, USA (1983), NY. KF258787, KF241579, KF241611,—, KF217198; *A. Tiehm 15037*, Nye, NV, USA (2005), NY. KF258788, KF241580, KF241612,—, KF217199; *S. Vanderpool 1293*, Nye, NV, USA (1986), OKL.—,—,—, KF128748, KF217195; *S. Vanderpool 1281-82*, San Bernardino, CA, USA (1986), OKL.—,—,—, KF128749, KF217196; *S. Vanderpool 1268*, Inyo, CA, USA (1986), OKL.—,—,—, KF128750, KF217197. *Cleomella palmeriana* M.E. Jones: *N. H. Holmgren 16030*, Kane, UT, USA (2009), NY. KF258789, KF241581, KF241613,—, KF217203; *R. R. Halse 4258*, Montezuma, CO, USA (1991), NY. KF258790, KF241582, KF241614,—; KF217204; *S. Vanderpool 1313*, Montezuma, CO, USA (1986), OKL.—,—,—, KF128751, KF217200; *S. Vanderpool 1314*, Wayne, UT, USA (1986), OKL.—,—,—, KF128752, KF217201; *S. Vanderpool 1319*, Garfield, UT, USA (1986), OKL.—,—,—, KF128753, KF217202. *Cleomella parviflora* A. Gray: *A. Tiehm 15414*, Humbolt, NV, USA (2007), NY. KF258791, KF241583, KF241615,—, KF217205; *A. Tiehm 15694*, Eureka, NV, USA (2008), NY. KF258792, KF241584, KF241616,—, KF217206. *Cleomella perennis* H.H. Iltis: *Rollins & Roby 74105*, Zacatecas, Mexico (1974), NY. KF258794, KF241586,—,—, KF217209; *J. Henrickson 14204*, Coahuila, Mexico (1974), NY. KF258793, KF241585, KF241617,—, KF217208; *H. Iltis 178*, Durango, Mexico (1978), WIS.—,—,—, KF128754, KF217207. *Cleomella plocasperma* S. Watson: *A. Tiehm 15581*, Humbolt, NV, USA

(2008), NY. KF258795, KF241587, KF241618,—, KF217212; *A. Tiehm 12490*, Mineral, NV, USA (1998), NY. KF258796,—,—,—, KF217213; *S. Vanderpool 1233*, Churchill, NV, USA (1986), OKL.—,—,—, KF128755, KF217210; *S. Vanderpool 1263*, Inyo, CA, USA (1986), OKL.—,—,—, KF128756, KF217211. *Oxystylis lutea* Torrey & Frémont in J.C. Frémont: *A. Tiehm 11683*, Esmeralda, NV, USA (1988), WS. KF258797, KF241588, KF241619,—, KF217214; *S. Vanderpool 1288*, Inyo, CA, USA (1986), OKL.—,—,—, KF128757, KF217215; *S. Vanderpool 1300*, Nye, NV, USA (1986), OKL.—,—,—, KF128758, KF217216. *Peritoma arborea* (Nuttall) H.H. Iltis: *M.D. Windham 2559*, San Bernardino, CA, USA (2002), NY. KF258799, KF241590, KF241620,—, KF217218; *M.A.(Ben) Franklin 5715*, Baja California, Mexico (1987), NY. KF258798, KF241589,—,—, KF217217; *M. Fishbein 4146*, Baja California, Mexico (2000), WS.—,—,—, KF128759, KF217219. *Peritoma jonesii* (J.F. Macbride) H.H. Iltis: *M. D. Windham 98-370*, Yavapai, AZ, USA (1998), NY. KF258801, KF241591, KF241622,—, KF217221; *C. M. Christy 329*, Yavapai, AZ, USA (1990), RSA. KF258800,—, KF241621,—, KF217220; *S. Vanderpool 1331*, Yavapai, AZ, USA (1986), OKL.—,—,—, KF128760, KF217222; *S. Vanderpool s.n.*, Yavapai, AZ, USA (1995), OKL.—,—,—, KF128761, KF217223. *Peritoma lutea* (Hooker) Rafinesque: *N. H. Holmgren 14618*, Mohave, AZ, USA (2002), NY. KF258802, KF241592, KF241623,—, KF217224; *M. D. Windham 2510*, Mono, CA, USA (2001), NY. KF258803, KF241593, KF241624,—, KF217225; *S. Vanderpool 1264*, Inyo, CA, USA (1986), OKL.—,—,—, KF128761, KF217226. *Peritoma multicaulis* (de Candolle) H.H. Iltis: *S. O'Kane 2482*, Alamosa, CO, USA (1986), NY. KF258804, KF241594, KF241625,—, KF217227; *S. Vanderpool s.n.*, Alamosa, CO, USA (1986), OKL.—,—,—, KF128763, KF217228. *Peritoma platycarpa* (Torrey) H.H. Iltis: *M. Mancuso 3282*, Payette, ID, USA (2008), NY. KF258805, KF241595, KF241626,—, KF217229; *S. Vanderpool 1237*, Lassen, CA, USA (1986), OKL.—,—,—, KF128764, KF217231; *R. Olmstead 704*, Crook, OR, USA (1985), MO.—,—,—, KF128764, KF217230. *Peritoma serrulata* (Pursh) de Candolle in A.P. de Candolle and A.L.P.P. de Candolle: *P. Lesica 8811*, Beaverhead, MT, USA (2004), NY. KF258806, KF241596,—,—, KF217232; *S. Vanderpool 1830*, Union, NM, USA (2003), OKL.—,—,—, KF128766, KF217233; *M. J. Patchell s.n. #1*, AB, Canada (2010), ALTA.—,—,—, KF128767, KF217234; *M. J. Patchell s.n. #2*, AB, Canada (2010), ALTA.—,—,—, KF128768, KF217235. *Wislizenia californica* Greene: *L. Ahart 15231*, Merced, CA, USA (2008), RSA. KF258807, KF241598, KF241628,—, KF217239; *S. Vanderpool 1355*, Kern, CA, USA (1987), OKL.—,—,—, KF128771, KF217240. *Wislizenia palmeri* A. Gray: *Wiggins & Rollins 258*, Sonora, Mexico (1941), WS. KF258810, KF241601, KF241629,—, KF217243; *J. F. Emmel 1158*, Riverside, CA, USA (1991), RSA. KF258808, KF241599,—,—, KF217241; *J. M. Stewart 86-18*, Riverside, CA, USA (1986), RSA. KF258809, KF241600,—,—, KF217242; *S. Vanderpool 1333*, Pima, AZ, USA (1986), OKL.—,—,—, KF128772, KF217244. *Wislizenia refracta* Engelmann in F.A. Wislizenus: *S. Vanderpool 1340*, Dona Ana, NM, USA (1986), OKL.—,—,—, KF128773, KF217245; *S. Vanderpool 1335*, Cochise, AZ, USA (1986), OKL.—,—,—, KF128774, KF21746; *S. Vanderpool 1327*, Coconino, AZ, USA (1986), OKL.—,—,—, KF128775, KF21747. *Cleome angustifolia* Forssk.: *I. Friis et al. 8609*, Ethiopia, K. KF258812,—,—,—, HM044251. *Polanisia dodecandra* (Linnaeus) de Candolle in A.P. de Candolle and A.L.P.P. de Candolle: *D.F. Grether 8603*, Sterns, MN, USA (1957), WIS.—,—,—, KF128769, KF217237; *J. Hall s.n.*, Cultivated (ALTA) (2008), ALTA.—,—,—, KF128770, KF217238; *E. Voznesenskaya 17*, Kiev Bot. Garden (WSUG), WS. KF258811, KF241597, KF241627,—, HM044226.