Coreopsis sect. *Pseudoagarista* (Asteraceae: Coreopsideae): Molecular phylogeny, chromosome numbers, and comments on taxonomy and distribution

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Abstract *Coreopsis* (Asteraceae) sect. *Pseudoagarista* is the largest section in the genus. It is disjunct between the high mountains of Mexico and high elevations in the Andes. Most species in the section are similar in floral and fruit characters, and are distinguished primarily by leaf characters. Despite the morphological similarity among all species, the monophyly of the section has been called into question by molecular data. Prior studies of tribe Coreopsideae have been equivocal with, cpDNA markers indicating monophyly but nrDNA ITS sequences have not resolved the section as monophyletic. Expanded cpDNA and nrDNA ITS datasets, and statistical (i.e., AU) tests of the ITS and cpDNA topologies provide support that sect. *Pseudoagarista* is not monophyletic. Both data partitions strongly support the Mexican and South American subclades as monophyletic. The low cpDNA sequence variation within each subclade provides no resolution, and thus patterns of evolution within each were examined using a phylogenetic framework estimated from ITS data. Sequences from ITS fail to provide high resolution of relationships among South American species, a likely result of a recent, rapid radiation, as is known in other Andean lineages. Divergence among species is generally higher in the Mexican species, resulting in better resolution of phylogenetic relationships compared to South American species. Three ploidy levels (diploid, tetraploid, hexaploid) are known in species from South America, and diploids and tetraploids have been documented in Mexico. Multiple origins of polyploidy are indicated for both geographic areas.

Keywords Asteraceae; *Coreopsis*; Mexico; Section *Pseudoagarista*; South America

Supplementary Material Electronic Supplement (Tables 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

The two large genera *Bidens* L. and *Coreopsis* L. (Asteraceae: Coreopsideae) as traditionally circumscribed (Sherff, 1936, 1937, 1955) have been considered closely related, and it has been difficult, if not impossible, to identify characters or combinations of characters to delimit each of them (Mesfin Tadesse, 1984, 1986, 1993; Mesfin Tadesse & al., 2001). Several molecular phylogenetic studies using nuclear and plastid sequences have shown that neither genus, as recognized by Sherff (1936, 1937, 1955), is monophyletic (Kim & al., 1999; Kimball & Crawford, 2004; Mort & al., 2008; Crawford & al., 2009). Rather, there are several strongly supported clades of *Bidens* and *Coreopsis* that are interspersed among themselves or with other genera of Coreopsideae (Mort & al., 2008). These results no doubt explain in large measure why diagnostic characters have been difficult to identify for each of the genera as classically recognized. While neither genus is resolved as monophyletic, most of the component clades are comprised of taxa from recognized sections or groups of sections, and are well circumscribed geographically (Crawford & al., 2009).

Far and away, the largest recognized section in *Coreopsis* is *Pseudoagarista* A.Gray, with some 40 species (Mesfin Tadesse & al., 1995). The species are small shrubs or subshrubs (Fig. 1) that inhabit mountainous areas, typically at higher elevations. The characters that define the section are the long trichomes on the fruits and the outer surfaces of the paleae, the paleae detaching with the fruits, and a pappus of two antrorsely barbed awns (Fig. 2; Mesfin Tadesse & al., 1995). *Pseudoagarista* is the only section of *Coreopsis* with a

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continental disjunction, with eight (Mesfin Tadesse, unpub.) to ten (Turner, 2010) species recognized in Mexico and ~29 species in South America (Mesfin Tadesse, unpub.). The species on both continents occur in mountainous areas, almost always above 2000 m. A number of the Andean species are local endemics known from only a few small populations, with a substantial number of them described during the last several decades (e.g., Sagástegui, 1982; Sagástegui & Sánchez Vega, 1971, 1981, 1989; Sánchez Vega & al., 1994). As in South America, new species have been described from Mexico during the past two to three decades (e.g., Turner, 1986, 1992a, b; Panero & al., 1993; Mesfin Tadesse, 1999).

Prior molecular phylogenetic studies based on limited taxon sampling have resolved both the Mexican and South American taxa of sect. *Pseudoagarista* as strongly supported clades (Kim & al., 1999; Kimball & Crawford, 2004), but whether elements from the two areas are resolved as sister groups depends on whether nuclear or plastid sequences are employed (Mort & al., 2008). Morphological delimitation of species in both Mexico and South America is challenging, in large measure because the uniformity of floral and fruit characters often makes it necessary to use foliar features such as leaf dissection, size and pubescence for defining species (Mesfin Tadesse & al., 1995). The few taxa sampled from each section in all previous molecular phylogenetic studies precluded

Fig. 2. Representative fruits from *Coreopsis* sect. *Pseudoagarista*. **Left,** the inner (adaxial) surface; **right,** the outer (abaxial) surface. Note the long trichomes on the fruits (seen best on left), the pappus with two antrorsely barbed awns, and the palea (on right) has long trichomes on its outer surface, is attached at the base of the fruit, and detaches with the fruit from the maternal plant.

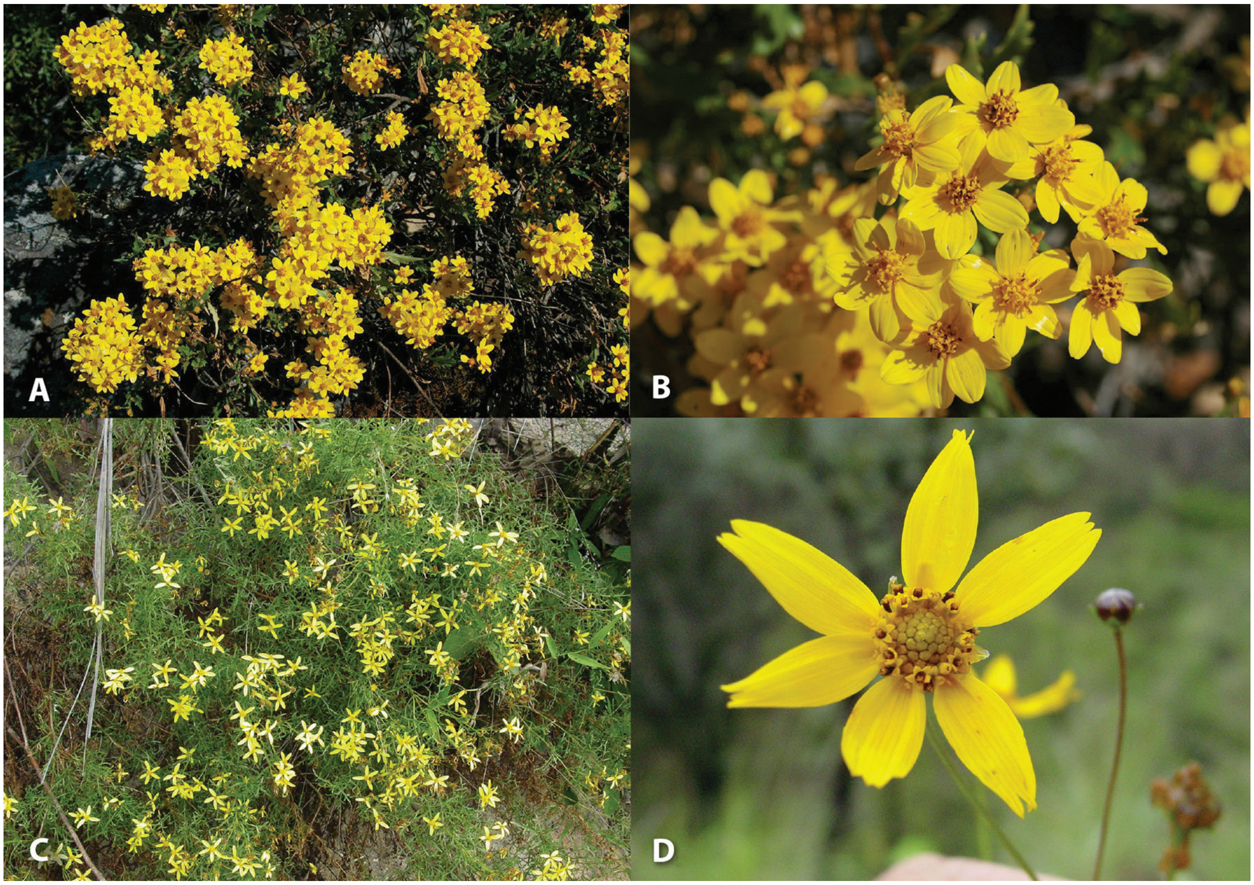

Fig. 1. Photographs of Mexican *Coreopsis* sect. *Pseudoagarista*. **A,** *Coreopsis petrophiloides*; **B,** close-up of capitula of *Coreopsis petrophiloides*; **C,** *Coreopsis petrophila*; **D,** capitulum of *Coreopsis petrophila*. — Photo credits: A–B by Pablo Carrillo-Reyes; C–D by Aaron Rodriguez.

the resolution of species relationships within each of the geographic areas, and prevented testing several of the intuitive hypotheses of relationships proposed by Turner (1986, 1992a, b, 2010), Mesfin Tadesse (1999) and Mesfin Tadesse & al. (1995).

There were several purposes for the present study. One was to test further the monophyly of sect. *Pseudoagarista* using expanded taxonomic sampling for the cpDNA and nuclear datasets, and more sophisticated analyses than employed in previous studies. Second, resolving phylogenetic relationships within the Mexican and South American lineages of *Coreopsis* sect. *Pseudoagarista* will allow evaluation of available hypotheses of relationships inferred from comparative morphology and field studies. Preliminary data (Kimball & Crawford, 2004; Crawford & al., 2012) indicated higher molecular divergence among the Mexican than South American taxa, so another purpose was to see whether more extensive taxon sampling confirmed the differences. The propensity of data had indicated that the base chromosome number for sect. *Pseudoagarista* is $x = 13$, with gametic numbers of $n = 13$, 26, and 39 known in the literature (Crawford, 1971, 1976, 1982; Smith, 1975), as well as several seemingly anomalous determinations (see below). Additional published and unpublished counts since those prior discussions of chromosome number evolution in the section suggest an updated evaluation is warranted. The first counts for several South American species reported here, together with prior determinations and a phylogenetic hypothesis, make it possible to examine polyploidy within a phylogenetic context so that the number of origins of polyploidy may be inferred.

■ MATERIALS AND METHODS

Taxon sampling, DNA extraction, and sequencing. — Plant materials serving as sources for DNA were obtained from several sources (Appendix 1). Most collections from South America were made by T.F. Stuessy, D.J. Crawford and A. Sagástegui Alva (designated as Stuessy & al. in Appendix 1). Additional material from South America was kindly made available by M. Dillon from the Field Museum (F) and T. Wendt from the University of Texas Herbarium (TEX). Sources of DNA for Mexican species were from field collections made by D.J. Crawford, T.F. Stuessy and J. Bruner (designated as Crawford & al. in Appendix 1), and T. Yahara, or herbarium material obtained with permission by P. Carrillo-Reyes (Appendix 1). Two data partitions were constructed by sampling broadly from other clades of Coreopsideae identified in prior molecular phylogenetic studies of the tribe (Kimball & Crawford, 2004; Crawford & al., 2009). The first dataset comprised 39 Coreopsideae taxa sequenced for two cpDNA regions (*matK*, *trnL-trnF*); the representation of sect. *Pseudoagarista* in the cpDNA dataset was expanded from previous studies to include eight species from both geographic subclades. The second dataset comprising 71 taxa was compiled from previously published nrDNA ITS sequences as well as a greatly increased sampling of taxa from sect. *Pseudoagarista* (Appendix 1). A total of 27 species from sect. *Pseudoagarista* were sampled for the nrDNA ITS dataset, including 8 of the 10 species recognized by Turner (2010) for Mexico and 19 of the 29 species recognized for South America by Mesfin Tadesse (unpub.).

Total genomic DNA was extracted using a small amount of silica-dried material or from herbarium collections using a modified CTAB method (Doyle & Doyle, 1987; Mort & al., 2001). For samples with low DNA quality and/or older material, the DNeasy Plant DNA Minikit (Qiagen, Valencia, California, U.S.A.) was used. Two chloroplast loci (*matK*, *trnL-F* spacer) and nuclear ribosomal ITS (including 5.8S) were PCR amplified using primers 1F and 1R (*matK*; Sang & al., 1997), primers C and F (*trnL-trnF*; Taberlet & al., 1991), and primers NNC-18S10 and C26A (ITS; Wen & Zimmer, 1996). PCR reactions included 1× Biomix (Midwest Scientific, St. Louis, Missouri, U.S.A.) and 0.64 μM forward and reverse primer, and in ITS amplifications 0.5% dimethylsulfoxide, to reduce secondary structure. PCR products were visualized via agarose gel electrophoresis and purified prior to sequencing using ExoSap-IT (Affymetrix, Cleveland, Ohio, U.S.A.). DNA sequencing was performed by Macrogen (Rockville, Maryland, U.S.A.). The resulting contigs were assembled and edited using Sequencher v.5.0 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.).

Phylogenetic analyses. — In addition to the sequences newly generated for the present study, previously sequenced members of Coreopsideae were obtained from GenBank (Appendix 1). DNA sequences were aligned using MUSCLE v.3.8.31 (Edgar, 2004) and corrected visually to minimize apparent character state changes with Jalview v.2.7 (Waterhouse & al., 2009). Maximum parsimony (MP) analyses were conducted using PAUP* v.4.0 (Swofford, 2003) with characters equally weighted and using a heuristic search with TBR (tree bisection-reconnection). Levels of support for branches were estimated with 1000 bootstrap replicates. Maximum likelihood (ML) analyses were conducted with GARLI v.2.0 (Zwickl, 2006); bootstrap support values for ML were calculated using 500 replications in GARLI. Bayesian analyses were conducted in MrBayes v.3.2.1 (Ronquist & al., 2012). Two runs of metropolis-coupled Markov chain Monte Carlo simulations each used four linked chains (three heated, one cold) and default priors for all model parameters. To permit a relative comparison of levels of species divergence in Mexico and South America, uncorrected *p*-distances were calculated for all pair-wise comparisons of populations in these geographical regions using PAUP* (Swofford, 2003). To assess whether the alternative relationships (i.e., monophyletic or not monphyletic) between the two subclades of sect. *Pseudoagarista* could be statistically rejected, we performed approximately unbiased (AU) tests (Shimodaira, 2002) for the best ML topologies inferred from the cpDNA (i.e., monophyletic sect. *Pseudoagarista)* and ITS (i.e., non-monophyletic sect. *Pseudoagarista*) and the best constrained ML topologies inferred using CONSEL v.0.1i (Shimodaira & Hasegawa, 2001).

Chromosome numbers. — Bud material for meiotic chromosome counts was fixed in the field in 4 parts chloroform : 3 parts absolute ethyl alcohol : 1 part glacial acetic acid (Crawford, 1970). Chromosome counts were made by E.B. Smith using anther squashes in 1% propiocarmine (Smith, 1974). Voucher specimens for all of the counts made by E.B. Smith are deposited in OS.

■ RESULTS

Monophyly of *Coreopsis* sect. *Pseudoagarista*. — Both MP and ML analyses of the expanded cpDNA and ITS datasets show results similar to previous, broad phylogenetic studies of Coreopsideae (Kimball & Crawford, 2004; Mort & al., 2008; Crawford & al., 2009). Plastid sequences resolve the Mexican and South American clades as sister with moderate (71%) bootstrap support (data not shown). By contrast, the two geographic subclades are strongly supported as being not monophyletic based on analyses of ITS data (Fig. 3). Comparing the alternative topologies using the AU test (Shimodaira, 2002) indicated that a non-monophyletic sect. *Pseudoagarista* is not a statistically worse cpDNA topology ($P = 0.17$) and that forcing sect. *Pseudoagarista* to be monophyletic for the ITS data partition was rejected ($P = 0.04$). Thus, we tentatively accept the ITS-based hypothesis as the best estimate of phylogeny. Both the cpDNA and ITS data are congruent in resolving the Mexican and South American subclades, with strong bootstrap support for each. The levels of sequence divergence (Electr.

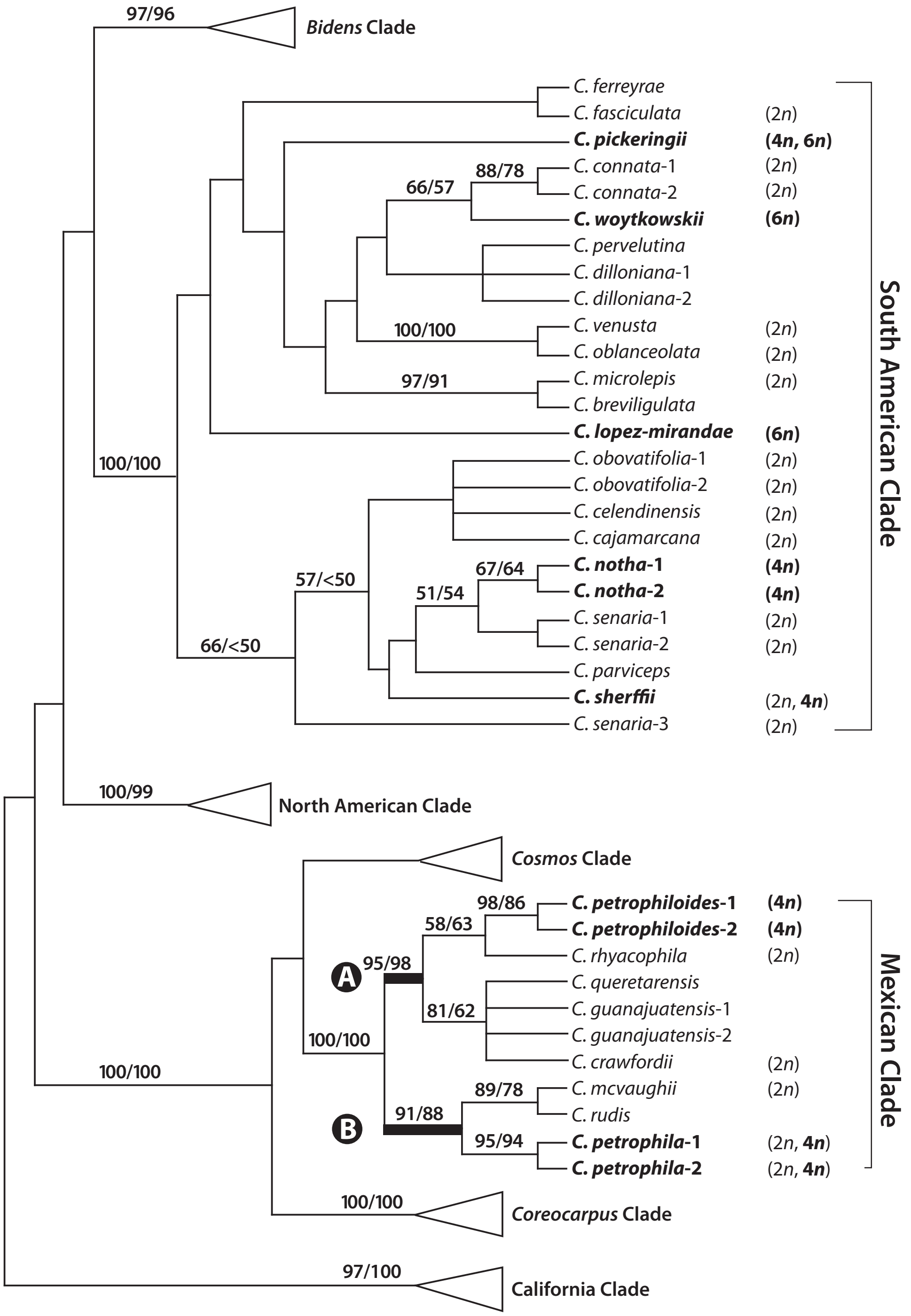

Fig. 3. Maximum likelihood (ML) topology for *Coreopsis* sect. *Pseudoagarista* inferred from nrDNA ITS sequences, with ML/MP bootstrap support greater than 50% indicated. Chromosome numbers that have been reported in the literature or in the present paper for the first time are indicated following the taxon name. Polyploid species are indicated by bold font. The two main subclades within the Mexican Clade are labeled A and B (see text for discussion). Taxa comprising the clades that have been collapsed are provided in Appendix 1.

Suppl.: Tables S1–S2) for the ITS data are an order of magnitude higher than the cpDNA. The low cpDNA sequence divergence within each subclade provides no resolution, and thus subsequent efforts employed only nrDNA ITS data to estimate the phylogeny required to examine patterns of evolution and taxonomic limits of members of the two sect. *Pseudoagarista* subclades.

Phylogeny of *Coreopsis* sect. *Pseudoagarista*. — A total of 71 members of Coreopsideae were sampled for the present study, including 27 species of *Coreopsis* sect. *Pseudoagarista*. The aligned nrDNA ITS dataset comprised 737 characters, of which 224 were potentially parsimony informative. The topologies resulting from MP, ML, and Bayesian analyses resulted in identical topologies, differing only in support for certain clades (Fig. 3; Electr. Suppl.: Fig. S1). Both MP and ML provide strong support for clades comprising the Mexican (100% ML bootstrap) and South American (100% ML bootstrap) *Coreopsis* sect. *Pseudoagarista* species; however, as with previous analyses of nrDNA ITS (e.g., Mort & al., 2008; Crawford & al., 2012), sect. *Pseudoagarista* does not form a monophyletic group; instead, the Mexican clade is much more closely related to *Coreocarpus* Benth. and *Cosmos* Cav. than to other members of sect. *Pseudoagarista* (Fig. 3).

Phylogenetic relationships within the Mexican clade of *Coreopsis* sect. *Pseudoagarista* are well resolved, with a few exceptions noted below. There are two well-supported subclades, with subclade A (Fig. 3) comprised of *C. crawfordii* Mesfin, *C. queretarensis* B.L.Turner, *C. rhyacophila* Greenm., and two accessions each of *C. petrophiloides* B.L.Rob. & Greenm. and *C. guanajuatensis* B.L.Turner. Subclade B consists of *C. mcvaughii* D.J.Crawford, *C. rudis* (Benth.) Benth. & Hook.f. ex Hemsl., and *C. petrophila* A.Gray (Fig. 3). In contrast to the Mexican clade, phylogenetic relationships within the South American lineage are less resolved and not as robustly supported. Two main subclades are also recovered within this clade; however, the support for these subclades is weak (see Fig. 3). The ranges and mean (in parentheses) *p*-distances for ITS sequences between all pairwise comparisons of species in each of the geographic areas are 0.00–0.048 (0.021) for Mexico and 0.00–0.024 (0.009) for the South American clade (Electr. Suppl.: Tables S1–S2).

Chromosome numbers. — Plotting chromosome numbers onto the phylogeny indicates that $x = 13$ is the ancestral base chromosome number for both clades of *Coreopsis* sect. *Pseudoagarista* (Fig. 3). There are three ploidy levels within the section; diploids and tetraploids occur in both Mexico and South America, and hexaploids are present in the latter area. First reports are presented for six species, and a new number is reported for one species (Table 1). Chromosome counts made in the present study show two ploidy levels within *C. sherffii* S.F.Blake. Two determinations for *C. woytkowskii* Sherff are the first hexaploid reports for the species, and they differ from a previous rather anomalous report of $n =$ ca. 10 for the species (Table 1). Prior reports for *C. boliviana* S.F.Blake (= *C. pickeringii* A.Gray) have included two ploidy levels (Table 1). Both diploid and tetraploid determinations have been made for plants identified as *C. petrophila* (Table 1).

■ DISCUSSION

No prior phylogenetic studies have been reported for *Coreopsis* sect. *Pseudoagarista*, thus the results of the present investigation must, by necessity, be compared to comments on relationships based primarily on comparative morphology for the Mexican (Turner, 2010; Mesfin Tadesse, unpub.) and South American taxa (Mesfin Tadesse & al., 1995).

Monophyly of *Coreopsis* sect. *Pseudoagarista*. — The cpDNA and nrITS sequences give conflicting results for the monophyly of the section as a whole, with the former data partition indicating monophyly, and the latter partition not resolving taxa from the two geographical areas as monophyletic. However, a statistical test on an expanded dataset indicated that a non-monophyletic sect. *Pseudoagarista* is not a statistically worse cpDNA topology than a monophyletic section, but that forcing sect. *Pseudoagarista* to be monohyletic for the ITS data partition is strongly rejected. Available data thus suggest that sect. *Pseudoagarista* as a whole is not monophyletic, but that taxa from each of the geographic areas form strongly supported clades.

The results of the present molecular phylogenetic study suggesting that sect. *Pseudoagarista* is not monophyletic may seem highly problematic, given the suite of floral and fruit characters that have served to diagnose the section; however, reproductive characters are labile in tribe Coreopsideae (Kimball & Crawford, 2004). More specifically, species with paleae attached to the fruits and other characters typical of sect. *Pseudoagarista* are also known in the California clade, which has been recognized as the separate genus *Leptosyne* D.C. (Keil, 2014; Mesfin Tadesse & Crawford, 2014). Variation among taxa and even within the same capitula of individual species exists for several of the characters present in sect. *Pseudoagarista* (Sharsmith, 1938; Smith, 1984). Mort & al. (2004) showed that these features have a complex evolutionary history within this clade with several equally likely reconstructions involving reversals and/or parallelisms. Given the observations of evolutionary lability in floral/fruit characters in other clades in Coreopsideae, it is perhaps not that surprising that sect. *Pseudoagarista* may not be monophyletic despite the seemingly distinctive features that have been used to define it. It appears likely that these characters that have been used to define the section are plesiomorphic within Coreopsideae rather than shared derived features. Elucidation of morphological features distinguishing the Mexican and South American lineages awaits further study, but several quantitative, technical features have been identified from examination of extensive herbarium collections (Mesfin Tadesse, unpub.). The Mexican taxa tend to have larger leaves (up to 15 cm long) than South American species (0.5–7 cm long). There are also subtle differences in the outer phyllaries, with those of the Mexican species often 3/4 the length of the inner phyllaries and rarely exceeding them in length whereas in the South American plants the outer whorl of involucral bracts is shorter relative to the inner whorl, usually only 1/4 to 3/4 the length. In addition, the texture of the phyllaries of the Mexican taxa tend to be herbaceous or membranous, while they are somewhat thicker and leathery

Version of Record (identical to print version).

in the Andean species (Mesfin Tadesse, unpub.). Additional characters for distinguishing the two lineages are to be desired.

Mexican clade: resolution and species relationships. — This clade is highly, though not completely, resolved, with generally high internal support for subclades. Mexican *Coreopsis* sect. *Pseudoagarista* is resolved into two major subclades, each with high support (Fig. 3). In general, members of subclade A (95% ML bootstrap) are distributed in central and southern Mexico while members of subclade B (91% ML bootstrap) occur to the north and west of subclade A members.

The ITS sequences of *C. crawfordii*, *C. guanajuatensis*, and *C. queretarensis* are identical and these taxa form a strongly supported (81% ML bootstrap) group within subclade A. These results support Turner's (2010) hypothesis that *C. crawfordii* and *C. guanajuatensis* are closely related. However, they are not in agreement with his placement of *C. rhyacophila* close to the former two species because it is sister to *C. petrophiloides* in another part of subclade A (Fig. 3), albeit with relatively low support (58% ML bootstrap). By contrast, Mesfin Tadesse (unpub.) considered *C. crawfordii* as most closely related to *C. pringlei* B.L.Rob (not included in this study) and *C. rudis*. The ITS topology does not support this hypothesis, as *C. rudis* is strongly supported (91% ML bootstrap) within subclade B, whereas *C. crawfordii* is nested well within subclade A (Fig. 3).

When Turner (1986) described *C. queretarensis*, he considered the species to be closely related to *C. mcvaughii*. Later, Turner (1992b) indicated that the newly described *C. guanajuatensis* was allied with *C. mcvaughii* and *C. queretarensis*. As mentioned above, the present study provides support for *C. guanajuatensis* and *C. queretarensis* being closely related, but neither is close to *C. mcvaughii*, which, like *C. rudis*, is placed within subclade B with strong support (Fig. 3). The identical ITS sequences for the three species *C. crawfordii*, *C. guanajuatensis*, and *C. queretarensis* are in sharp contrast

Table 1. Chromosome numbers in *Coreopsis* sect. *Pseudoagarista*.

Species	Chromosome number (*n*)	Published count or voucher sampled
Mexican Clade		
C. lucida (= *C. petrophiloides*)	26	Turner & King, 1964; Crawford, 1971
C. mcvaughii	13	Crawford, 1969
C. petrophila	13	Hartman & Crawford, 1971; Crawford, 1982
C. petrophila	26	T. E. Melchert, pers. comm.
C. petrophiloides	26	Turner & King, 1964; Crawford, 1971
C. pringlei (= *C. crawfordii*)	13	Crawford, 1982
C. rhyacophila	13	Hartman & Crawford, 1971
C. teotepecensis (= *C. petrophiloides*)	26	Crawford, 1971
South American Clade		
C. capillacea	13	Stuessy & Jansen, unpub.
C. celendinensis	13*	*Stuessy & al. 12522*
C. connata	13*	*Stuessy & al. 12517; 12519; 12529*
C. fasciculata	26	Dillon & Turner, 1982
C. lopez-mirandae	39*	*Stuessy & al. 12573; 12575*
C. microlepis	13	Turner & al., 1967; Carr & al., 1999
C. notha	26*	*Stuessy & al. 12653; 12660; 12676*
C. oblanceolata	13	Dillon & Turner, 1982
C. obovatifolia	13*	*Stuessy & al. 12480; 12579*
C. boliviana (= *C. pickeringii*)	ca. 23	Casas & Piqueras, 1981
C. boliviana (= *C. pickeringii*)	39	Carr & al., 1999
C. senaria	13	Dillon & Turner 1982; Carr & al., 1999; *Stuessy & al. 12469; 12471; 12584; 12600*
C. sherffii	13*	*Stuessy & al. 12487; 12489*
C. sherffii	26*	*Stuessy & al. 12614*
C. triloba (= *C. capillacea*)	13	Strother & Panero, 1994
C. venusta	ca. 13	Strother & Panero, 1994
C. woytkowskii	ca. 10	Dillon & Turner, 1982
C. woytkowskii	39*	*Stuessy & al. 12514; 12553*

* Counts reported here for the first time.

to the divergence seen between other Mexican species. These three species, together with *C. pringlei*, are centered in a relatively small geographic area in the states of Guanajuato and Querétaro. The sequence data suggest that the three species (and possibly *C. pringlei*) have diverged recently, and perhaps additional field, morphological and molecular studies will cast doubt on their taxonomic recognition.

Coreopsis petrophiloides is a widespread, variable species occurring primarily in central Mexico, but collections identified as this species have been made from more northern areas in Durango as well as south into Oaxaca (Turner, 2010; Mesfin Tadesse, unpub.). Two additional species were described to accommodate variation found within *C. petrophiloides*. In the state of Mexico a variant of this species with highly dissected leaves was described as *C. lucida* by Paray (1957), and a year later Paray (1958) described *C. teotepecensis* from Guerrero based on features of the capitula. Citing data from biosystematics and secondary chemistry, Crawford (1971) placed both of the species erected by Paray in synonymy under *C. petrophiloides*. Turner (2010), however, opined that forms referable to *C. teotepecensis* are worthy of taxonomic recognition at some level. *Coreopsis petrophiloides* appears to be a uniformly tetraploid species, with counts made from plants referable to both of the segregate species as well as from different populations within the range of the species (Table 1; Crawford, 1971). The ITS sequences group the two accessions of *C. petrophiloides* (98% ML bootstrap), one from the state of Mexico and one from Michoacan, and they, in turn, are sister to *C. rhyacophila* (58% ML bootstrap) in subclade A (Fig. 3). Additional molecular sampling, together with field, morphological, chromosomal, and biosystematic studies are needed to ascertain whether additional taxa should be recognized within this variable, widespread complex presently recognized as *C. petrophiloides.* The diploid *C. rhyacophila* (Table 1; Hartman & Crawford, 1971) has been collected most frequently in the lava fields between Mexico City and Cuernavaca, but Turner (2010) and Mesfin Tadesse (unpub.) view several collections from other locations in central Mexico as belonging to this species. The one accession of *C. rhyacophila* included in this study is from the lava fields.

Subclade B (Fig. 3) includes two strongly supported lineages, one consisting of *C. mcvaughii* and C. *rudis* (89% ML bootstrap), and the other comprising two accessions of *C. petrophila* (95% ML bootstrap). With regard to *C. mcvaughii* and *C. rudis*, Turner (2010: 80) suggested that additional collections could show that *C. rudis* might “encompass the closely related *C. mcvaughii*”. These two species are distributed largely in the states of Jalisco and Aguascalientes; Crawford (1969, 1976, 1982) indicated characters distinguishing the two taxa and the data presented here show divergent ITS sequences. However, additional studies are needed to understand better variation within and among populations of these two species.

As discussed by Crawford (1976, 1982), Turner (2010) and Mesfin Tadesse (unpub.), *Coreopsis petrophila* is a variable species, particularly in leaf dissection, and is most often distinguished from the morphologically similar species *C. rhyacophila* and *C. petrophiloides* (Crawford, 1976, 1982; Turner, 2010; Mesfin Tadesse, unpub.) by its smaller capitula with mostly five ray florets and fewer disk florets (cf. Fig. 1B, D). The two ITS sequences obtained in the present study, while from the adjacent states of Jalisco and Nayarit, still represent rather distant locations within the range of the species. Sequences from the two populations are an order of magnitude more divergent than are the two populations of *C. petrophiloides*. Turner (2010) suggested, based on the extensive morphological variation in *C. petrophila,* that it might be advisable to recognize infraspecific categories in this species. The level of divergence in ITS sequences (Electr. Suppl.: Table S1) between the two populations of *C. petrophila* lends support to this view, and suggests that more widespread geographic sampling for molecular and chromosomal variation could be informative.

South American clade: resolution and species relationships. — There is lower resolution among the Andean taxa compared to the Mexican taxa of *Coreopsis* sect. *Pseudoagarista* (Fig. 3). More than one population was sequenced for four taxa (Appendix 1), and in all but one, *C. senaria* S.F.Blake & Sherff, the sequences of each species were identical. In several instances, identical ITS sequences were found among species. For example, *C. cajamarcana* Sagást. & Sánchez Vega, *C. celendinensis* Sagást. & Sánchez Vega, and *C. obovatifolia* Sagást. have identical ITS sequences; all three are somewhat localized in the Peruvian provinces of Cajamarca and San Marcos. While *C. cajamarcana* and *C. obovatifolia* are known to be sympatric along the Cajamarca River basin, the former species occurs mostly at lower elevations than the latter species. *Coreopsis celendinensis* is isolated from the other two species by a high-elevation mountain range. To our knowledge, no one has questioned the distinctiveness of these three *Coreopsis* species (Mesfin Tadesse & al., unpub.), but their identical sequences suggest recent divergence into different habitats.

Coreopsis dilloniana Sánchez Vega & al. and *C. pervelutina* Sagást. from northern Peru (several provinces in the Cajamarca region) have identical ITS sequences, but appear to be separable by several morphological characters. In fact, when Sagástegui (1982) described *C. pervelutina*, he compared it to *Coreopsis holodasya* S.F.Blake (not included in the present study), a species restricted to the high mountains in the Abancay province in southern Peru. Later, when Sánchez & al. (1994) described *C. dilloniana* they suggested that it was most similar to *C. lopez-mirandae* Sagást. Mesfin Tadesse (unpub.) suggested that *C. dilloniana* is closest to *C. woytkowskii* or *C. dentifolia* Sánchez Vega & al. The latter species is known only from the type collection and was not included in the present study. One collection of *C. dilloniana* and the accession of *C. pervelutina* sequenced in this study were collected from the same location (i.e., the type locality of *C. dilloniana*) on the same day but the vouchers are distinct morphologically. The second accession of *C. dilloniana* sampled in the present study was from a different province (Appendix 1). These observations indicate that *C. dilloniana* and *C. pervelutina* are morphologically distinct, but closely related species.

Rather surprisingly, the ITS sequences of *C. fasciculata* Wedd. and *C. ferreyrae* Sagást. & Sánchez Vega are identical. The former species is widely distributed in Chile, Ecuador, and Peru while the latter is rare and known only from near its type locality in northern Peru. Both species occur in dry, rocky habitats, and Sagástegui & Sánchez (1989) commented on the xeromorphic aspects of the leaves when describing *C. ferreyrae*. *Coreopsis ferreyrae* occurs in an isolated area that converges to the Marañón River in northern Peru; the molecular data, narrow geographic distribution, and xeromorphic features of the species suggest that it could represent a recent divergence into its present isolated habitat.

The strong grouping (100% ML bootstrap) of the rare *C. oblanceolata* S.F.Blake, which is known from very few populations in northern Peru, and the widespread and morphologically variable *C. venusta* Kunth was unexpected because, to our knowledge, no one has suggested a close relationship between them. The latter is known from southern Ecuador into east central Peru, and there are collections of the two species from several nearby localities around the city of Huancabamba in northern Peru. The sequences for the two species in this study were obtained from geographically distant populations.

The very rare *C. breviligulata* Sagást. & Sánchez Vega, known from a localized area in the province of Cajamarca, groups with *C. microlepis* S.F.Blake & Sherff (97% ML bootstrap) from the Amazonas region to the north and east. As the specific epithet indicates, *C. breviligulata* is distinguished by its small ray florets. Mesfin Tadesse (unpub.) notes both the similarity of *C. microlepis* and *C. parviceps* S.F.Blake & Sherff, and in turn the similarity of the latter species to *C. breviligulata*. In fact, he places the latter species in synonymy under the former. The results of the present study, while supporting the close relationship of *C. breviligulata* and *C. microlepis*, do not place *C. parviceps* close to the other two species (Fig. 3).

The modest support for the grouping of the two populations of *C. connata* Cabrera with *C. woytkowskii* (66% ML bootstrap) is somewhat surprising, as no one has apparently suggested a close relationship between these morphologically distinct species with different ploidy levels (diploid and hexaploid, see below). Rather, Sagástegui (1969) stated that *C. woytkowskii* is close to *C. lopez-mirandae*, and presented a list of contrasting features. Mesfin Tadesse (unpub.) likewise noted the morphological similarity of *C. woytkowskii* and *C. lopez-mirandae*. As discussed below, the two species are hexaploids. *Coreopsis woytkowskii* is distributed in eastern Celendín province in the Marañón River watershed while *C. lopez-mirandae* is distributed east of the Marañón River basin and has been collected in the Amazonas region. The present study provides support for distinguishing the morphologically similar *C. lopez-mirandae* and *C. woytkowskii* (Fig. 3).

Polyploidy. — Polyploidy has unquestionably played a role in the diversification of both the Mexican and South American clades of *Coreopsis* sect. *Pseudoagarista*, although the nature and extent of its role remain unknown because of the limited taxon sampling for phylogenetic analyses of plants with documented chromosome numbers. In Mexico, published documentation of polyploidy (tetraploids) is available only for the so-called *Coreopsis petrophiloides* complex (but see below). Chromosome counts have been obtained from several widespread localities in different mountain ranges within the distribution of this species, and these counts encompass the morphological variation that has been described as two segregate species (Table 1; Crawford, 1971). While more counts are to be desired, available data indicate that tetraploidy is widespread within this variable complex, and probably originated once.

Next to the *Coreopsis petrophiloides* complex, *C. petrophila* is the most morphologically variable species in Mexico (Crawford, 1976, 1982; Turner, 2010; Mesfin Tadesse, unpub.). As noted above, all published counts for *C. petrophila* are diploid and come from geographically widespread localities in the states of Durango, Jalisco, and Nayarit (Crawford, 1982), and these counts are from populations that encompass considerable morphological variation. However, there is one unpublished tetraploid count of $n = 26$ by T.E. Melchert (pers. comm.) based on a collection (*Melchert, Ballard & Hart 71-290*, TEX) made west of the city of Durango. The voucher generally has six ray florets, which would be more typical of *C. petrophila* (Fig. 1D), rather than the larger, eight-rayed capitula characteristic of *C. petrophiloides* (Fig. 1B) (Sherff, 1936; Mesfin Tadesse, unpub.). This tetraploid count is from a population occurring a short distance from diploid plants of more “typical” *C. petrophila* (Crawford, 1982). Turner (2010) noted the morphological variation in plants from around the city of Durango, especially in the number and size of capitula. Molecular phylogenetic studies indicate that the two species are distantly related, and only additional phylogenetic analyses of plants with known chromosome numbers will determine whether tetraploidy has originated once or multiple times in Mexico.

Polyploidy appears to be more common in South American *Coreopsis* sect. *Pseudoagarista*; in this clade, six taxa are reported as polyploids. The present study provides the first chromosome count for *C. sherffii*, and documents that both diploid and tetraploid races occur in this species (Table 1). Two ploidy levels also occur in *C. pickeringii*, with both tetraploids and hexaploids reported (Casas & Piqueras, 1981; Carr & al., 1999) (Table 1); this species is morphologically variable, and widespread over various altitudes (Mesfin Tadesse, unpub.). *Coreopsis sherffii* is more restricted geographically, occurring in several provinces in Peru, but like *C. pickeringii*, it is variable morphologically and occurs over a range of altitudes. However, the voucher specimens for the diploid and tetraploid cytotypes of *C. sherffii* are not morphologically distinguishable. The occurrence of polyploidy within these two species suggests two independent origins of polyploidy in the Andean clade. However, additional data are needed to determine the frequency, geographic distribution, and morphological-ecological correlates of polyploidy in *C. pickeringii* and *C. sherffii*.

The first reports for *C. notha* S.F.Blake & Sherff show that all three populations are tetraploid, which indicates yet another independent origin of polyploidy in South America. Based on determinations made in the present study, the two morphologically similar species *C. lopez-mirandae* and *C. woytkowskii* appear to be exclusively hexaploid, the only published exception is the count of $n =$ ca. 10 for *C. woytkowskii* (Dillon & Turner, 1982), which seems rather anomalous in light of other determinations in the South American clade. The positions of the two species in the phylogeny (Fig. 3) provide some support for the independent origin of hexaploidy in the two species.

Notwithstanding the limited sampling to date, it appears that polyploidy has originated twice in Mexican *Coreopsis* sect. *Pseudoagarisata*, and more frequently, perhaps six or more times, in South America. There is little doubt that polyploidy has been important in the diversification and radiation of both clades of sect. *Pseudoagarisata*, and this is especially true in South America.

Comparison of Mexican and South American clades. — A notable difference between the Mexican and South American clades of *Coreopsis* sect. *Pseudoagarista* is the lower molecular divergence (Elect. Suppl.) among South American as compared to Mexican species. These differences between the two geographic areas are also reflected in the greater resolution and higher support for clades of the Mexican species (Fig. 3). All species in the two regions have similar life histories: they are more or less fruticose, self-incompatible perennials that generally occur in small populations (Crawford, unpub.). Thus, factors such as generation time and mating systems are ostensibly not responsible for differences in molecular divergence. Various other factors could be responsible for the differences. For example, it is possible that taxonomic concepts in the two regions are different, with more "splitting" in the South American than the Mexican clade. While this hypothesis cannot be tested without additional genetic, phylogenetic, and biosystematic data, it is questionable whether finer taxonomic distinctions have been drawn in South America than in Mexico because similar characters, primarily from the leaves, have been employed in distinguishing species.

Another possibility for lower divergence among the South American taxa is that radiation and speciation has, in general, been more recent in the Andes than in Mexico. The low divergence among members of the Andean clade fits a pattern seen in several other groups in the Andes where relatively recent uplift has provided environments for colonization (Hughes & Eastwood, 2006; Drummond, 2008; Luebert & al., 2011). Even the most divergent species in South America are only half as divergent as the most divergent Mexican taxa. The species in Mexico occur in several different major mountain ranges, with the Trans-Mexican Volcanic Belt being especially rich in diversity. However, species and species complexes, such as *C. petrophila* and *C. petrophiloides*, are distributed across different ranges. While Mexican taxa in different geographic areas tend to occur in different clades, it also it noteworthy that several species occurring in more localized areas have identical sequences. It may be that Mexican species represent both recent local divergence and taxa with older divergence times that are associated with occurrence in different geographic areas.

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Appendix 1. Accessions of Coreopsideae sampled, with voucher information (*Stuessy & al.*: collection by T.F. Stuessy, D.J. Crawford and A. Sagástegui; *Crawford & al.*: collected by D.J. Crawford, T.F. Stuessy and J. Bruner) and GenBank accession number for each taxon used in the present study. Members of *Coreopsis* sect. *Pseudoagarista* are organized by area of geographic origin (i.e., Mexico and South America): Mexican states and municipalities are given; Peruvian regions and provinces are provided; and, the Bolivian department and province is given for the one voucher from that country. The component taxa for the clades condensed in Fig. 3 are listed under their respective clade names. NS indicates taxa not sequenced for a specific region.

Taxon, Location, Accession (herbarium), Genbank - ITS [or ITS1 + ITS2], *matK*, *trnL-F*;

Coreopsis **sect.** ***Pseudoagarista*****—South America:** ***C. breviligulata*** Sagást. & Sánchez Vega, Peru: Cajamarca: Cajamarca, *Sánchez 2394* (OS), KM269124, NS, NS; ***C. cajamarcana*** Sagást. & Sánchez Vega, Peru: Cajamarca: Cajamarca, *Stuessy & al. 12476* (OS), KM269122, NS, NS; ***C. celendinensis*** Sagást. & Sánchez Vega, Peru: Cajamarca: Celendín, *Stuessy & al. 12522* (OS), KM269123, NS, NS; ***C. connata***-1 Cabrera, Peru: Cajamarca: Celendín, *Stuessy & al. 12517* (OS), EU049333, EU049358, EU047865; ***C. connata***-2 Cabrera, Peru: Cajamarca: Celendín, *Stuessy & al. 12529* (OS), KM269114, NS, NS; ***C. dilloniana***-1 Sánchez Vega, Sagást. & D.J.Crawford, Peru: Cajamarca: Hualgayoc, *Dillon & al. 6471* (OS), KM269128, NS, NS; ***C. dilloniana-***2 Sánchez Vega, Sagást. & D.J.Crawford, Peru: Cajamarca: Chota, *Sánchez 6538* (OS), KM269129, NS, NS; ***C. fasciculata*** Wedd., Peru: La Libertad: Otuzco, *Ságastegui 11666* (MO), KM269125, NS, NS; ***C. ferreyrae*** Sagást. & Sánchez Vega, Peru: Cajamarca: Celendín, *Stuessy & al. 12526* (OS), KM269112, NS, NS; ***C. lopez-mirandae*** Sagást., Peru: Cajamarca: Celendín, *Stuessy & al. 12573B* (OS), KM275375 + KM275376, NS, NS; ***C. microlepis*** S.F.Blake & Sherff, Peru: Amazonas: Chachpoyas, *Cowan & al. 4316* (TEX), KM269116, NS, NS; ***C. notha***-1 S.F.Blake & Sherff, Peru: Cajamarca: Contumazá, *Stuessy & al. 12653* (OS), EU049334, EU049357, EU049649; ***C. notha***-2 S.F.Blake & Sherff, Peru: Cajamarca: Contumazá, *Stuessy & al. 12676* (OS), KM269119, NS, NS; ***C. oblanceolata*** S.F.Blake, Peru: Cajamarca: Chota, *Dillon & al. 6415* (F), KM269126, NS, NS; ***C. obovatifolia***-1 Sagást., Peru: Cajamarca: Cajamarca, *Stuessy & al. 12475* (OS), KM269118, NS, NS; ***C. obovatifolia***-2 Sagást., Peru: Cajamarca: Cajamarca, *Stuessy & al. 12480* (OS), KM269117, NS, NS; ***C. parviceps*** S.F.Blake & Sherff, Peru: Cajamarca: Hualgayoc, *Sánchez-Vega & al. 3782* (F), KM269127, NS, NS; ***C. pervelutina*** Sagást., Peru: Cajamarca: Hualgayoc, *Dillon & al. 6470* (F), AY429083, EU049347, EU047866; ***C. pickeringii*** A.Gray (**=** ***C. boliviana*** S.F.Blake), Bolivia: Cochabamba: Carrasco, *RM King 9648* (MO), KM269113, NS, NS; ***C. senaria***-1 S.F.Blake & Sherff, Peru: Cajamarca: Celendín, *Stuessy & al. 12528* (OS), KM269120, NS, NS; ***C. senaria***-2 S.F.Blake & Sherff, Peru: Cajamarca: Cajamarca, *Stuessy & al. 12600* (OS), AY429085, EU049360, EU047869; ***C. senaria***-3 S.F.Blake & Sherff, Peru: Cajamarca: Cajamarca, *Stuessy & al. 12469* (OS), KM269139, NS, NS; ***C. sherffii*** S.F.Blake, Peru: Cajamarca: Cajamarca, *Stuessy & al. 12487* (OS), KM269121, NS, NS; ***C. venusta*** Kunth (**=** ***C. integra*** S.F.Blake), Peru: Cajamarca: Hualgayoc, *Sánchez & al. 6857* (F), KM269115, NS, NS; ***C. woytkowskii*** Sherff, Peru: Cajamarca: Celendín, *Stuessy & al. 12520* (OS), AY429086, NS, NS; — ***Coreopsis*** **sect.** ***Pseudoagarista*****—Mexico:** ***C. crawfordii*** Mesfin, Mexico: Queretaro: Colón, *Crawford & al. 1342* (OS), KM269133, NS, NS; ***C. guanajuatensis***-1 B.L.Turner, Mexico: Queretaro: El Marqués, *Carrillo-Reyes & al. 1301* (IEB), KM269134, NS, KM517567; ***C. guanajuatensis***-2 B.L.Turner, Mexico: Queretaro: Colón, *Rzedowski 54043* (IEB), KM269135, NS, KM517568; ***C. mcvaughii*** D.J.Crawford, Mexico: Aguascalientes: San José de Gracia, *Rzedowski & McVaugh 857* (MICH), KM269130, NS, NS; ***C. petrophila***-1 A.Gray, Mexico: Jalisco: Guadalajara, *Crawford & al. 1389* (OS), AY561292 + AY561293, EU049359, EU047867; ***C. petrophila***-2 A.Gray, Mexico: Nayarit: Ahuacatlán, *Tenario 17013* (MICH), KM269136, NS, KM517566; ***C. petrophiloides***-1 B.L.Rob. & Greenm., Mexico: Mexico: Donato, *Cornejo-Tenorio & al. 1037* (IEB), KM269131, NS, NS; ***C. petrophiloides***-2 B.L.Rob. & Greenm., Mexico: Michoacan: Morelia, *Carrillo-Reyes & Torres 5272* (IEB), KM269132, NS, NS; ***C. queretarensis*** B.L.Turner, Mexico: Queretaro: Pinal de Amoles, *Zamudio 6859* (IEB), KM269138, NS, NS; ***C. rhyacophila*** Greenm., Mexico: Morelos: Cuernavaca, *Yahara & Spejima, 225* (OS), EU049346 + EU047868, EU049346, EU047868; ***C. rudis*** (Benth.) Benth. & Hook.f. ex Hemsl., Mexico: Zacatecas: Tlaltenango, *Rzedowski & McVaugh 907* (MICH), KM269137, NS, NS

Bidens **Clade (tropical-subtropical America and Africa):** ***B. cronquistii*** (Sherff) Melchert, *Smith 3944* (OS), AF330102, EU049353, EU047838; ***B. hintonii*** (Sherff) Melchert, *Villasenor & Soto s.n.* (OS), AF330101EU049354, EU047839; ***B. prestinaria*** (Sch.Bip.) Cufod., *Mesfin s.n.* (OS), KM275379 + KM275380, EU049338, EU047841; ***B. schimperi*** Sch.Bip. ex Walp, *Mesfin s.n.* (OS), KM275377 + KM275378, EU049343, EU047840; ***B. segetum*** Mart. ex Colla, *Ganders s.n.* (UBC), AY553661 + AY553662, NS, EU047836

North American Clade (*Bidens*, *Coreopsis*, *Thelesperma*) — ***C. auriculata*** L., *Smith 3514* (OS), AY553677, AY553678, EU047848; ***C. basalis*** (A.Dietr.) S.F.Blake, *Roberts 5785* (OS), AY553705 + AY553706, AY551492, EU047849; ***C. delphiniifolia*** Lam., *Crawford & Lewis 1461* (OS), AY553689 + AY553690, NS, NS; ***C. floridana*** E.B.Sm., *Crawford & Giannasi 1806* (GA), AY553663 + AY553664, AY551483, EU047855; ***C. gladiata*** Walter, *Crawford & Giannasi 1802* (GA), AY553685 + AY553686, AY551484, EU047856; ***C. grandiflora*** Hogg ex Sweet var. ***grandiflora***, *Smith 3738* (OS), AY553707 + AY553708, AY551493, EU047850; ***C. integrifolia*** Poir., *Crawford & Giannasi 1801* (GA), AY553665 + AY553666, AY551485, EU047857; ***C. intermedia*** Sherff, *Hornberger 1021* (OS), AY553675 + AY553676, AY551494, EU047851; ***C. latifolia*** Michx., *Crawford & Lewis 1466* (OS), AY553671 + AY553672, NS, NS; ***C. linifolia*** Nutt., *Crawford & Giannasi 1809* (GA), AY553667 + AY553668, AY551486, EU047858; ***C. major*** Walter, *Crawford 1444* (OS), AY553669, AY551479, EU047860; ***C. nuecensis*** A.Heller, *Crawford 1163* (OS), AY553703 + AY553704, AY551497, EU047852; ***C. nuecensoides*** E.B.Sm., *Crawford 1193* (OS), AY553679 + AY553680, AY551496, EU047853; ***C. palmata*** Nutt., *Crawford s.n.* (OS), AY553673, AY551480, EU047861; ***C. paludosa*** M.E.Jones, *Crawford & al. 1430* (OS), AY553697 + AY553698, NS, NS; ***C. pubescens*** Elliott var. ***pubescens***, *Smith 3739* (OS), AY553701 + AY553702, NS, NS; ***C. pulchra*** F.E.Boynton, *Crawford 1456* (OS), AY553687 + AY553688, AY551481, EU047862; ***C. rosea*** Nutt., *Roberts s.n.* (OS), AY553693 + AY553694, AY551487, EU047859; ***C. tripteris*** L., *Crawford s.n.* (OS), AY553691 + AY553692, NS, NS; ***C. verticillata*** L., *Crawford 1933* (OS), EU053653, EU049856, EU047863; ***Bidens comosa*** (A.Gray) Wiegand, *Roberts 6050* (OS), AY553655 + AY553656, NS, NS; ***Thelesperma marginatum*** Rydb., *Evert 19279* (RM), AY429100, EU049365, EU047882; ***Thelesperma megapotamicum*** (Spreng.) Kuntze, *Nelson 27184* (RM), AY429101, AY551501, EU047883

Cosmos **Clade:** ***Cosmos bipinnatus*** Cav., *Ganders & al. 2000* (UBC), U67114, EU049361, EU047873; ***Coreocarpus congregatus*** (S.F.Blake) E.B.Sm., *Smith 3959* (OS), AF330089, EU049352, EU047843

Coreocarpus **Clade:** ***C. arizonicus*** (A.Gray) S.F.Blake, *Smith 3977* (OS), AF330092, EU053651, EU047842; ***C. parthenioides*** Benth., *Smith 3915* (OS), AF330093, EU053652, EU047844; ***C. sonoranus*** Sherff, *Smith 3928* (OS), AF330097, EU049355, EU047845

California Clade (*Leptosyne*): ***Leptosyne californica*** Nutt., *Crawford & al. 1278* (OS), KM275385 + KM275386, EU049336, EU047864; ***Leptosyne calliopsidea*** (DC.) A.Gray, *Crawford 1476* (OS), KM275381 + KM275382, NS, NS; ***Leptosyne gigantea*** Kellogg, *Elisens s.n.* (OS), KM275383 + KM275384. NS, NS