Origin and diversification of the Milla Clade (Brodiaeoideae, Asparagaceae): A Neotropical group of six geophytic genera

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1. Introduction

The Milla clade currently comprises six genera of geophytic plants distributed from Arizona to Guatemala. Three genera (Behria, Jaimehintonia and Petronymphe) are monotypic while the remaining genera (Bessera, Dandya and Milla) contain from two to ten (Milla) species. Parsimony, Maximum Likelihood and Bayesian Inference analyses were conducted with plastid and nuclear DNA sequences from a total of 181 plants belonging to 15 species in all six genera. Molecular dating was performed under a relaxed clock model. We examined the phylogenetic relationships of the genera and species, estimated origin-divergence times for the clade and genera and determined the ancestral distribution area of the clade by optimizing ancestral areas given current biogeographic distributions. The phylogenetic results suggest that final decisions on limits of the six genera in the Milla clade will have to be established until further taxonomic work is completed for Milla, in particular for the group of populations included under the name M. biflora. The later genus is rendered polyphyletic by other genera of the family. The origin of the Milla clade is estimated at 15.8 Ma. Ancestral area of the clade most likely was located in the California Floristic Province and dispersed most likely occurred into the Chihuahuan-Coahuilla Plateaus and the Trans-Mexican Volcanic Belt and from there to Baja California and the Sierra Madre del Sur. Two hypotheses that need further testing are proposed to explain complex relationships of genera and polyphyly of Milla, one in relation to fragmentation of populations and pollinator shifts and another suggesting that populations remained in refugia in the Trans-Mexican Volcanic Belt.

all six genera) as sister to the Brodiaea complex (Fay and Chase, 1996; Pires et al., 2001; Pires and Sytsma, 2002). The clade is supported by two synapomorphic character states: an ovary stipe adnate to the perianth tube and a membranous corm (Pires and Sytsma, 2002). Sampling in earlier phylogenetic studies of the Milla clade has, however, been incomplete. The selection of taxa in the study of Brodiaea by Pires and Sytsma (2002) was extensive but did not include eight species of Milla, three of Dandya and Behria tenuiflora. Moreover, analyses to establish the generic limits of Behria and Bessera included only a few taxa from the other genera in the Milla Clade (Gándara et al., 2009). The status of Jaimehintonia needs further clarification as the most recent studies indicate its position as nested within a clade of Milla species (Gándara et al., 2009). The status of Jaimehintonia needs further clarification as the most recent studies indicate its position as nested within a clade of Milla species (Gándara et al., 2009). In contrast, diagnostic floral anatomical characters (Gutiérrez et al., 2010) indicate that Dandya is likely to retain its taxonomic position. Milla has been retrieved as polyphyletic group (Pires et al., 2001; Pires and Sytsma, 2002; Gándara et al., 2009). Thus, the Milla clade is in need of further phylogenetic analysis to clarify the limits of the genera, and to understand their biogeographic and floral evolution.

The diversification of a number of plant lineages from arid lands in North America has been correlated with events from different geological periods. For example, the rapid aridification that started during the Cenozoic allowed for the diversification of Tiquilia (Boraginaceae) at the Eocene/Oligocene boundary in the Chihuahuan Desert (Moore and Jansen, 2007), and habitat expansion from the Pliocene to the Pleistocene promoted speciation events in the North American species of Ephedra (Loera et al., 2012) and Baja California species of Encelia (Asteraceae) (Fehlberg and Ranker, 2009). Hence, lineages of the Milla clade that correspond to different arid regions of North America offer an ideal group to further investigate timing of origin and diversification and to determine if speciation and dispersal events are associated with geological and/or climatic events.

Our study focuses on the evolutionary history of the Milla clade to (1) determine the phylogenetic relationships of the genera and species in order to identify monophyletic groups, (2) estimate the origin-divergence times of the clade and its lineages, and (3) determine the ancestral distribution area of the clade.

2. Materials and methods

2.1. Taxon sampling

For each species, three to ten plants were collected in the field or from herbarium specimens (vouchers and GenBank accessions are provided in Appendix S1). The ingroup contains 15 species belonging to the six genera of the Milla clade. Dandya purpusii and three species of Milla (M. filifolia, M. mortoniana and M. rosea) were not included because we were not able to found them in their type localities. D. purpusii is known from a single specimen in Sierra La Paila, Coahuila without a precise locality. The type of M. filifolia was collected in the east of Cuernavaca near Yautepec and Cuautla, which is currently an urban area and we were not able to find plants. M. mortoniana was described from Tierras Blancas, Guerrero, without an accurate location. M. rosea type comes from Cerro del Obispado in Nuevo León and at present it is a recreational park with the Bishopric Palace. Four species of Dichelostemma (Brodiaea clade) were used as outgroup and D. volubile was selected as the functional outgroup (Pires and Sytsma, 2002).
2.2. DNA, Extraction, Amplification, and Sequencing

DNA was isolated using either a modified 2XCTAB method (Cota-Sánchez et al., 2006) or the DNeasy Plant MiniKit (Qiagen, Valencia, California) according to the manufacturer's instructions. The following plastid and nuclear ribosomal markers were amplified and sequenced: rpl16 intron [primers “F71” and “R1661” as per Pires and Sytsma (2002)], psbK-psbI intergenic spacer [primer psbK and psbI as per Lahaye et al. (2008)], ITS [primers ITS4 and IT5S as per White et al. (1990) and as per Li et al. (2010)] and ETS [primers 18S-IGS as per Baldwin and Markos (1998)]. PCR products were purified with QIAquick columns (Qiagen, Valencia, USA) or ExoSAP-IT (Affymetrix, Santa Clara, USA) sequenced with the TaqBigDye Terminator Cycle Sequencing kit (Perkin Elmer Applied Biosystems, Foster City, USA) and visualized on a 310AB DNA sequencer (Perkin Elmer Applied Biosystems, Foster City, USA). The sequences were edited in Se-Rev v. 2.0a11 (Rambaut, 2002).

2.3. Phylogenetic analyses

Two sets of analyses were performed; in the first set Parsimony (P), Maximum Likelihood (ML) analyses and Bayesian Inference (BI) were used to infer the phylogeny of a plastid DNA (rpl16, psbK-psbI) matrix of 181 terminals to test for genetic differences among individuals of the same species from different localities. Based on these results, the number of terminals was reduced to 37 for a second set of analyses including all four genes (rpl16, psbK-psbI, ITS, ETS) as the following set of combined matrices: cpDNA alone (rpl16 + psbK-psbI), nrDNA alone (ITS + ETS), and a total evidence analysis (combined cp + nr DNA). Parsimony analyses were run in TNT (Goloboff et al., 2008) under equal weights with 500 iterations of parsimony ratchet (Nixon, 1999). Gaps were coded in SeqState ver. 1.4.1 (Müller, 2005), following the simple method of Simmons and Ochoterena (2000). Clade support was estimated by jackknife, with 1000 replicates with 30% deletion on a traditional search in TNT (Goloboff et al., 2008). For ML analyses the software RAXML version 7.0.3 (Stamatakis, 2006) was utilized with the same partitioning scheme used for the Bayesian Inference. The GTR + GAMMA model was used with 1000 nonparametric bootstrap replicates to assess nodal support. The model of molecular evolution for each partition was determined with jModelTest v.0.1.1 (Posada, 2008) using the Akaike information criterion (AICc) and default search values for each plastid and nuclear marker. The models obtained were, HKY (psbK-psbI), TIM3 + I (rpl16), TIM1 + G (ITS) and TIM3 + G (ETS). The Bayesian Markov chain Monte Carlo (MCMC) was performed in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) and carried out with two separate chains for each run, with three hot and one cold chains, running 100000000 generations, sampling a tree every 1000 generations. Stationarity was determined based on convergence of likelihood scores, and sample points generated prior to stationarity were eliminated as burn-in (20%). Gaps were coded in the same way as in Parsimony analyses. The posterior probabilities (PP) of the clades were determined by a 50% majority consensus of the trees retained.

2.4. Molecular dating

Divergence times were estimated in BEAST v. 1.6.2 (Drummond and Rambaut, 2007) using evolutionary models as determined by jModelTest 0.1.1 (Posada, 2008). To estimate the divergence of the Milia clade from remaining Asparagaceae, sequences from three cpDNA markers (rbcL, ndhF, trnL-F) of 36 representative taxa of Asparagales were selected. Sampling included nine species of subfamily Brodiaeoidae as well as a representative species from each of the remaining six subfamilies of Asparagaceae. Thirteen representative taxa from the remaining Asparagales families were chosen as outgroup for this analysis (Vouchers and GenBank accessions in Appendix S2). Two node-based calibration ages were set based on (1) a fossil taxon within Asteliaceae and (2) a secondary calibration point constraining Asparagales with a mean of 98.5 Ma (SD = 9.3, 33–114 Ma) based on estimates by Bell et al. (2010). Seven species of Asteliaceae were utilized to calibrate the node of the position of Astelia antiqua, the only fossil known from this group (19–23.2 Ma, with an exponential distribution, with a mean of 3.333, and offset = 19) (Macunias et al., 2011). Liliurn (Liliales) was selected as the functional outgroup based on previous phylogenetic analyses (Appendix S2) (Jansen and Bremer, 2004; Bell et al., 2010). The evolutionary model GTR + I + G was used (AICc selected model in jModelTest 0.1.1 (Posada, 2008)) for the combined chloroplast matrices (rbcL; ndhF; trnL-F) and analyses were run under an uncorrelated lognormal relaxed clock model. To model the tree, the Yule speciation process was used as a prior.

A second estimate of divergence times was conducted to investigate the temporal diversification within the Milia clade. The model GTR + I + G was used (AICc selected model in jModelTest 0.1.1 (Posada, 2008)) for the combined Milia matrix (psbK-I; rpl16, ITS, ETS) minus the coded gap characters. Analyses were run under an uncorrelated lognormal relaxed clock model. Yule speciation process was used as a prior for tree modeling. Two calibration points were set, constraining minimum ages with a normal distribution: Brodiaeoidae was constrained with a mean of 27.2 Ma (16.63–37.46 Ma), and the Milia clade was constrained with a mean of 16.67 Ma (7.99–21.29 Ma). For both estimates four independent 100 000 000 generation runs were performed with random starting trees, sampling every 1000 generations. TRACER v. 1.5 ([http://tree.bio.ed.ac.uk/software/tracer]) was used to assess convergence and effective sample sizes (ESS) for all parameters and also to combine tree files from the four BEAST runs. If convergence between the independent chains was evident, we combined the samples from each run using LogCombiner v1.6.2 (part of the BEAST distribution). After discarding the first 10000 trees as burn-in, the remaining trees were summarized in a maximum clade credibility tree with TreeAnnotator v1.6.1. (Drummond and Rambaut, 2007), with the PP limit set to 0 and including mean node heights. The single tree was visualized with FIGTREE v. 1.5.4 ([http://tree.bio.ed.ac.uk/software/figtree])

2.5. Ancestral area optimization

The distribution range of the Milia clade (i.e. the six genera) was divided into four areas based on eleven morpho-tectonic provinces (Ferreras-Villafranca, 1993): (A) Baja California Province; (B) Trans-Mexican Volcanic Belt; (C) Sierra Madre del Sur; (D) Chihuahua-Coahuila Plateau, and (E) the California Floristic province that corresponds to the distribution of Dichelostemma, the taxon designated as the outgroup. To infer ancestral areas, the Bayesian Binary MCMC (BBM) analysis approach was followed (implemented in Reconstruct Ancestral States in Phylogenies RASP 2.0; Yan et al., 2011). The input file for RASP consisted in the fifty percent majority rule consensus tree from the 4000 trees retrieved by the Bayesian Inference run in BEAST based on the a data matrix that included only a single individual for the 20 terminal taxa. The number of maximum areas was kept at 5. The possible ancestral ranges at each node on a selected tree were estimated. The MCMC chains were run simultaneously for 50000000 generations and the reconstructed state was sampled every 1000 generations. The fixed model JC + G (Jukes– Cantor + Gamma) was used for BBM analysis with a null root distribution.
3. Results

The concatenated matrix of the \textit{Milla} Clade consisted of 3205 characters, 1581 bp of plastid DNA, 1260 bp of nuclear DNA and 149 indels (Table 1). A large insertion of approximately 220 bp in the plastid intergenic spacer \textit{psbK-I} was found in \textit{Dichelostemma congestum}, \textit{Milla bryanii} and \textit{Milla} sp. nov. Bayesian Inference (BI) with the \textit{cp} + \textit{nr} DNA retrieved the \textit{Milla} clade as monophyletic (Posterior probability (PP) = 1), Jackknife percentage for parsimony (jcK) = 98 and bootstrap percentage for ML (bst) = 94 (Fig. 2). The relationships among \textit{Milla} clade genera were poorly resolved. \textit{Milla} was identified as polyphyletic and \textit{Dandya} as paraphyletic. Only two clades received good and moderate support; the first is the \textit{Behria-Bessera} clade (bst = 73), within this clade a well-supported group (jck = 69, bst = 95, PP = 1) included the two individuals of \textit{Behria tenuiflora} (Fig. 2), and another well-supported group (bst = 73, PP = 0.95) comprised individuals of the two species of \textit{Bessera} (\textit{B. elegans} and \textit{B. tuitensis}). The second clade with a moderate support (PP = 0.79, bst = 68) included \textit{M. delicata} from the State of Mexico, \textit{M. biflora} from Puebla and \textit{Milla} sp. from Querétaro. \textit{Petronymphe decora} resulted the sister taxon to a large unsupported clade in which a small group formed by the individuals of \textit{Dandya bahlsiensis} and \textit{D. thadhowardii} received support (bst = 97, PP = 0.94), as well as another small group including two individuals of \textit{Milla bryanii} (jck = 99, bst = 96, PP = 1).

3.1. Phylogenetic analyses

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3.2. Divergence time

The \textit{Milla} clade is estimated to have diverged from the \textit{Brodiaea} clade in the Mid-Miocene (mean = 15.28 Ma; 95% HDP = 10.31–20.48). The \textit{Behria-Bessera} clade is estimated to have diverged some time in the Late Miocene to the Early Pliocene (mean = 8.6 Ma; 95% HDP = 3.89–13.82); while the clade formed by \textit{Jaimehintonia} and \textit{Milla bryanii} is estimated to have diverged in the Late Miocene to Early Pliocene (mean = 7.88 Ma; 95% HDP = 2.46–13.50) (Fig. 3, see also Fig. S2).

3.3. Ancestral area optimizations

Results of the Bayesian Binary Analysis MCMC (BBM) suggest a complex biogeographic history of the \textit{Milla} clade in which 12 dispersal and six vicariance events have shaped the current distribution areas of the taxa. Pie charts at nodes indicate the relative frequencies of ancestral area optimization across the entire ancestral area reconstruction chronogram shown in Fig. 3. BBM analysis found the ancestral area of the \textit{Milla} clade as ambiguous (abbreviations of the areas are indicated in Fig. 2; $E = 0.48$, $D = 18\%$, $B = 0.13\%$, $A = 0.07\%$) being the California Province (E) the most likely ancestral area. Three dispersal events were identified: from the California Province (E), to the Chihuahua Coahuila Plateau + Trans-Mexican Volcanic Belt (BD) followed by a vicariant event. These results suggested a northern to southern migration route. The most likely ancestral range of the \textit{Behria-Bessera-Milla biflora} clade was the Trans-Mexican Volcanic Belt (0.48%). Two dispersal events were identified from this area to the Baja California Peninsula, and a vicariance event. The BBM model identified the Chihuahua-Coahuila Plateaus (0.65%) as the ancestral area of the \textit{Dandya} clade.

4. Discussion

4.1. Phylogenetic relationships

As in previous analyses, the \textit{Milla} clade was recovered as a well supported lineage; however groups within received poor support. \textit{Dichelostemma congestum}, \textit{Milla bryanii} and \textit{Milla} sp. nov. from Querétaro are on long branches due to a large insertion of approximately 200 bp in the plastid intergenic spacer \textit{psbK-I}. This spacer has been reported having gaps as large as 40 bp (Yoo and Jang, 2010) and large insertions are common in other plastid spacers like in \textit{trnH-psbA} in which 150 bp have been reported (Sosa et al., 2009). However, when this insertion is eliminated or when complete taxonomic sampling is used to reduce the long branch, the topology remains the same (see Fig. S3). Thus we can discount long-branch attraction as a mechanism for driving the lack of resolution for relationships of taxa in the \textit{Milla} clade. When compared with the plastid DNA matrix, the topology of the trees retrieved by the nuclear DNA markers is not congruent (see Fig. S4). The nrDNA tree is similar to the total evidence topology. In the cpDNA tree, the most remarkable differences are that accessions of \textit{M. bryanii} were the sister clade to the rest of taxa in the \textit{Milla} clade, while in the nrDNA and total evidence trees \textit{Petronymphe} was the sister taxon to the rest of the groups of this clade. Incongruence with ITS and plastid markers is common in monocots (e.g. Ronsted et al. 2013; Yang et al., 2013), and processes such as hybridization and incomplete plastid lineage sorting have been cited as the cause of incongruent phylogenetic positions (Ronsted et al., 2013; Yang et al. 2013).

Low support for lineages within the \textit{Milla} clade and the unresolved position of key genera like \textit{Petronymphe} raise questions about the generic delimitations recognized for this clade. This monotypic genus, which has an extremely local distribution on the Pacific Slopes, was proposed as related to \textit{Milla} (Moore, 1951). Our results, however, did not resolve its position.

The most controversial genus in the clade is \textit{Milla}, as it appears polyphyletic in every analysis. Species of \textit{Milla} are grouped with individuals from \textit{Behria}, \textit{Bessera}, \textit{Dandya} and \textit{Jaimehintonia}. Furthermore, the only clade formed entirely by species of \textit{Milla} (\textit{Milla oaxacana}, \textit{M. mexicana}, \textit{M. potosina}) did not receive strong support. \textit{Milla} is the largest genus, with ten species, three of which are only known from a single collection that we were not able to locate again (\textit{M. filifolia}, \textit{M. mortoniana} and \textit{M. rosa}) (Moore, 1953; Howard, 1999). The most widespread species in the genus is \textit{M. biflora}, the specimens of which fall in four clades indicating that there is a complex of species under the name \textit{M. biflora} that needs further work. Furthermore the accession of \textit{M. biflora} in the phylogenetic analysis of Asparagales was conflicting, it did not even formed part of Asparagaceae but of Orchidaceae (Chen et al., 2013). The flower morphology of \textit{Jaimehintonia} is identical to that of \textit{Milla}, with the
exception of perianth color that is bluish (rather than white) in 
Jaimehintonia (Turner, 1993). Topologies generated by our analyses 
retrieved accessions of *J. gypsophila* in a clade with *M. bryani* (with 
low support). These species occupy areas with gypsum soils. More-
over, the flower color of *J. gypsophila* is similar to that of *M. morton-
iana*, another species inhabiting this type of substrate.

Species of Dandya were retrieved in a large clade with *Milla* spe-
cies such as *M. potosina, M. mexicana, M. oaxacana* and *M. magnifica.* 
Gutiérrez et al. (2010) found several diagnostic anatomical charac-
ters for Dandya. Our results, however, did not retrieve Dandya as 
monophyletic. It is possible that these characters are plesiomor-
phic for this large clade but have been lost or partially lost in the 
species of this clade currently placed in *Milla.*

Behria and Bessera are both recovered as monophyletic, how-
ever these genera are each nested within a broader clade that con-
tains individuals of *Milla biflora*. A *Milla biflora* accession from 
Jalisco is sister to Bessera, and a *Milla biflora* accession from Quer-
etaro is sister to Behria.
Our results suggest further research is necessary to determine the generic limits of *Milla* and especially to resolve the taxonomic uncertainty encompassed by the populations placed under the name of *Milla biflora*. It is possible that species identified as *Milla biflora* maintain plesiomorphic character states that make it impossible to identify them as separate species, however our phylogenetic analyses of molecular data make it clear that *Milla biflora* is comprised of genetically distinct populations with differing evolutionary histories. Generic limits of the rest of *Milla* clade cannot be established until *Milla* is completely revised. Thus, all genera in the *Milla* clade will need re-circumscription pending further taxonomic work focused on the species complexes that are contained within the genus *Milla*.

### 4.2. Divergence time and ancestral area

Time of origin of the *Milla* clade coincides to a certain extent with the origins of other Asparagaceae geophyte groups such as section *Apodanthi* of *Narcissus* (13.2 Ma), the *Hyancinthoideae* (16.3 Ma) and *Massoniaeae* (17.92 Ma) (Santos-Gally et al., 2011; Ali et al., 2012). Our data also indicate that the *Milla* clade arose along with other xerophyte taxa in North America including the...
Papaveraceae genera *Humennamia* and *Escholtzia* (16.03 Ma) (Ruiz-Sanchez et al., 2012). In addition, the time of divergence for the *Dandyia* clade, distributed in the Balsas River Basin, overlaps with the origin of the canyon tree frog species in the *Hyla eximia* group (2.4–12.1 Ma) (Bryson et al., 2010) which shares a narrow distribution with *Dandyia* and likely indicates the rise of suitable habitat in this area. It was recently shown that habitat expansion during aridification might play a major role in the origin and divergence of species that are adapted to arid conditions (Loera et al., 2012). Hence it is possible that habitat expansion with an increase in the extent of the North American deserts, as occurred with Ephe-dra (Loera et al. 2012) may have influenced speciation and divergence in the *Milla* clade. Moreover, divergence time estimates for clades within *Bursera*—one of the most important genera of the seasonal deciduous tropical forest in America—coincides with our estimate of 15.8 Ma for the *Milla* clade (De-Nova et al., 2012). *Milla magnifica*, *M. biflora*, *Bessera elegans*, *B. tuitensis* and *B. tenuiflora* have all been reported in this type of forest, from Baja California to the Pacific slopes. The divergence time of *Behria tenuiflora* occurred during the Late Pliocene to Early Miocene (2.46–13.50 Ma), after the separation of La Paz–Los Cabos from Puerto Vallarta (14–12 Ma) (Ferrari, 1995).

Our results detect a high probability that the ancestor of the *Milla* clade inhabited the California Floristic Province, and that there was a southward dispersal from California to the Trans-Mexican Volcanic Belt and Chihuahua-Coahuila Plateaus and from these areas there was a secondary dispersal from the Trans-Mexican Volcanic Belt to the Baja California Peninsula and from the Chihuahua-Coahuila Plateaus to the Sierra Madre del Sur (Fig. 3). This pattern of dispersal has been found in other dry land taxa, such as *Tiquilia* (Moore and Jansen, 2006).

The identification of the California Floristic Province as the ancestral area for the *Milla* clade coincides with previous hypotheses on the origin of the flora that colonized the North American Deserts. Axelrod (1979, 1983) indicated the taxa of desert floras were confined to dry areas during the Tertiary drying trend, and that the arid-adapted species from boreal shrub-steppes, among them the California chaparral, gradually extended their distribution ranges from the Late Pliocene to the Early Oligocene.

5. Conclusions

Our results suggest that *Milla* is the most controversial genus in the clade, and that it needs further research to determine its limits as well as the species involved under the name of *M. biflora*. Until this work is completed, generic limits of the genera of the rest of *Milla* clade can be established. The time of origin of the *Milla* clade is estimated to be around 15.8 Ma, and our results indicate that the ancestral taxon of the clade had a distribution range within the California Floristic Province. In two groups, dispersal occurred to the Chihuahua-Coahuila Plateaus and to the Trans-Mexican Volcanic Belt and from there to the Baja California Province and to the Pacific slopes, to Sierra Madre del Sur.

We propose two hypotheses that need further testing but could explain the polyphyletic origin of *Milla*: (1) Given the position of individuals of *Milla biflora* in every clade is as the sister taxon to the rest of species in the group (see Fig. 1), we suggest that *M. biflora* was once a widespread panmictic population extending from the California Floristic Province through Mexico and Guatemala. Over time gene flow became restricted, populations were fragmented, and in each area populations remained isolated and pollinator selection influenced independent divergence and diversification. Filipowicz and Renner (2012) suggested that switches between hawk-moth pollination and hummingbird pollination do not require drastic morphological changes, and such changes in pollinator may have driven genetic isolation and speciation of the various clades in different geographic regions. 2) The polyphyletic origin of *Milla* could be related with populations remaining in refugia during the Pliocene–Pleistocene and later dispersing from refugia and diverging locally across the landscape. These hypotheses are not mutually exclusive scenarios. Our ancestral area reconstruction suggests that one of the clades that received strong statistical support is located in Sierra Madre del Sur, including three species of *Milla*. We suggest, based on a recent phylogeographic study (Velo-antón et al., 2013), that the Trans-Mexican Volcanic Belt acted as a refugia for populations of *Milla biflora*. One of its individuals is the sister taxon to the rest of the clade, located to the north of this mountain complex. Populations later dispersed and diverged in the Sierra Madre del Sur that includes the Balsas Basin. Sampling for further studies must focus on obtaining population level sampling for all *Milla* species.

6. Unicted reference

Axelrod (1983).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.02.014.

References


