The transfer of two rare monotypic genera, *Neoeplingia* and *Chaunostoma*, to *Lepechinia* (Lamiaceae), and notes on their conservation

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DOI http://dx.doi.org/10.12705/634.6

Abstract We present an updated circumscription of *Lepechinia* (Lamiaceae) based on molecular phylogenetic analyses of chloroplast, nuclear ribosomal, and low-copy nuclear genes. In particular, the relationships between *Lepechinia mexicana*, *Neoeplingia leucophylloides*, and *Chaunostoma mecistandrum*, which range from central Mexico down to northern Central America, are explored in detail. We provide strong evidence for recent hybridization/introgression, and not incomplete lineage sorting, between adjacent populations of *Lepechinia mexicana* and *Neoeplingia leucophylloides*. The molecular data demonstrate that *Neoeplingia leucophylloides* and *Chaunostoma mecistandrum*, two species from monotypic genera with extremely narrow distributions, are embedded within *Lepechinia*. We formally rename the first as *Lepechinia leucophylloides*; the latter was previously renamed as *Lepechinia mecistandrum*. Both of these new *Lepechinia* species are exceedingly rare and worthy of protection.

Keywords *Chaunostoma*; hybridization; *Lepechinia*; Mesoamerica; narrow endemic; *Neoeplingia*; rare plant

Supplementary Material Electronic Supplement (Figs. S1–S6) 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 genus *Lepechinia* Willd. is a morphologically heterogeneous clade of ca. 44 species with a discontinuous distribution ranging from the foothills of northern California in the western U.S.A. to the mountains of central Argentina (Epling, 1948; Hart, 1983). Several species of *Lepechinia* are widely used by indigenous people, and some are being actively investigated for medicinal (Jonathan & al., 1989; Parejo & al., 2004; Perez-Hernandez & al., 2008; Calderón Cevallos & Guerrero Ricaurte, 2013) and/or agricultural (Palacios & al., 2007; Caballero-Gallardo & al., 2011) applications. Based on detailed morphological observations, Bentham (1834, 1848, 1876) and Briquet (1897) treated the species now recognized within *Lepechinia* as two separate genera, *Lepechinia* and *Sphacele* Benth. There are no clear morphological synapomorphies that span *Lepechinia*, and early taxonomists associated *Lepechinia* with a suite of genera including *Hyptis* Jacq., *Stachys* L., *Horminum* L., *Dracocephalum* L., *Rosmarinus* L., *Sideritis* L., *Gardoquia* Ruiz & Pav., and *Buddleja* L. (see comments in Epling, 1948; Hart, 1983).

Carl Epling, who envisioned the current generic breadth of *Lepechinia* (Epling, 1926, 1937, 1948; Epling & Mathias, 1957; Epling & Jativa, 1968), also maintained *Lepechinia* and *Sphacele* as distinct genera initially (Epling, 1926), but later (Epling, 1937, 1948) merged them together as *Lepechinia* with the remark that "the only consistent alternative to recognizing one genus […] is to recognize eight" (Epling, 1948). Epling (1948) also noted the close relationship between *Lepechinia* and the monotypic genus *Chaunostoma* Donn.Sm., a finding echoed by later morphology-based research (Ryding, 1995, 2010; Moon & al., 2008, 2009). Subsequent to Epling, Hart (1983) thoroughly revised the sectional delimitations of the genus as part of his cladistic morphological analysis of the South American section *Parviflorae* Epling. The recent molecular work of Drew & Sytsma (2011, 2012, 2013) has further clarified the relationships within *Lepechinia*, corroborated the close relationship between *Lepechinia* and *Chaunostoma*, and revealed a close relationship between *Lepechinia* and the monotypic genus *Neoeplingia* Ramamoorthy & al.

Chaunostoma mecistandrum Donn.Sm. (Figs. 1, 2G–I) is a straggly shrub (<3 m) found in cloud forest openings and edges, with disjunct occurrences in southern Mexico (Oaxaca and Chiapas), Guatemala, and northeastern El Salvador. *Chaunostoma* was considered closely allied to *Lepechinia* by Epling, but was maintained as a separate genus primarily based on its cauliflorous inflorescences and arched stamens (Epling, 1948), two distinct features not found in *Lepechinia*. The calyx and corolla architecture of *Chaunostoma* are also clearly distinct from those of *Lepechinia*. In *Chaunostoma*, the calyx

Received: 8 Jul 2013 | returned for first revision: 2 Sep 2013 | last revision received: 4 Apr 2014 | accepted: 6 Apr 2014 | not published online ahead of inclusion in print and online issues

is initially large and broadly campanulate before expanding ± two-fold while fruiting, while most *Lepechinia* species have narrowly campanulate calyces during anthesis that become expanded and often pouch-like in fruit. In addition, unlike most *Lepechinia* whose calyces turn purplish after anthesis, the calyx of *Chaunostoma* remains the same color while in fruit. Moreover, the flowers of *Chaunostoma* have very delicate corollas that are easily dislodged from the oversized calyx, which differ from the relatively sturdy corollas generally found in *Lepechinia*; all *Chaunostoma* inflorescences we observed in the field had a very high calyx/corolla ratio (i.e., few corollas were seen in comparison to the relatively abundant calyces). Finally, *Chaunostoma* occurs in cloud forests, while most *Lepechinia* species typically occur in less mesic environments. There are two distinct characters that *Chaunostoma* does share with most other species in *Lepechinia*: the distinct and unique odor that their leaves give off when they are crushed, and bullate leaves. In addition, three micro-morphological traits are known to occur in *Chaunostoma* and at least some *Lepechinia*, and might prove to be synapomorphic with additional sampling: a sclerenchymatous exocarp (Ryding, 1995, 2010), a thick endocarp (Ryding, 2010), and a perforate pollen exine (Moon & al., 2008).

Neoeplingia leucophylloides Ramamoorthy & al. (Figs. 1, 2D–F) is a rounded shrub (1–1.5 m) that grows on sparsely vegetated calcareous soil in the state of Hidalgo in central Mexico. Though initially considered a close relative of *Hedeoma* Pers., *Poliomintha* A.Gray, and *Hesperozygis* Epling (Ramamoorthy & al., 1982) based on calyx, corolla, and staminal features, Drew & Sytsma (2011, 2012) showed that *Neoeplingia leucophylloides* is in fact morphologically (and genetically) more similar to *Lepechinia*. *Neoeplingia leucophylloides* superficially resembles *Lepechinia mexicana* (S.Schauer) Epling (Fig. 2A–C), with which it occurs sympatrically (Fig. 1). The similarities between these two species are striking in terms of flower size and color, leaf size (but not shape), leaf margins and indumentum, and general habitat. Their status as distinct species is clear, however, based on plant size (*L. mexicana* is short and straggly while *Neoeplingia* is a taller, rounded bush), leaf shape (hastate in *L. mexicana* but rounded in *Neoeplingia*), and inflorescences (sparse flowers in upper leaf axils in *L. mexicana*, dense clusters of flowers in upper leaf axils in *Neoeplingia*; Fig. 2A–F). Perhaps the most distinctive difference between the two species is that the leaves and young stems of *Neoeplingia* are so densely tomentose that the whitish color can easily be discerned from a distance of 50 meters or more, while the leaves of *Lepechinia mexicana* appear green until observed more closely. The fact that *L. mexicana* and *Neoeplingia* were collected on the same day at the same locality by Ramamoorthy, Hiriart, and Medrano, but not considered closely related, illustrates nicely that these two species, though very similar in some ways, are indeed quite different. *Lepechinia mexicana* was recently shown to be dioecious (Henrickson & al., 2011), but the breeding system of *Neoeplingia* is unknown. Future investigations of niche overlap, including resource partitioning in the absence of character displacement, would be interesting.

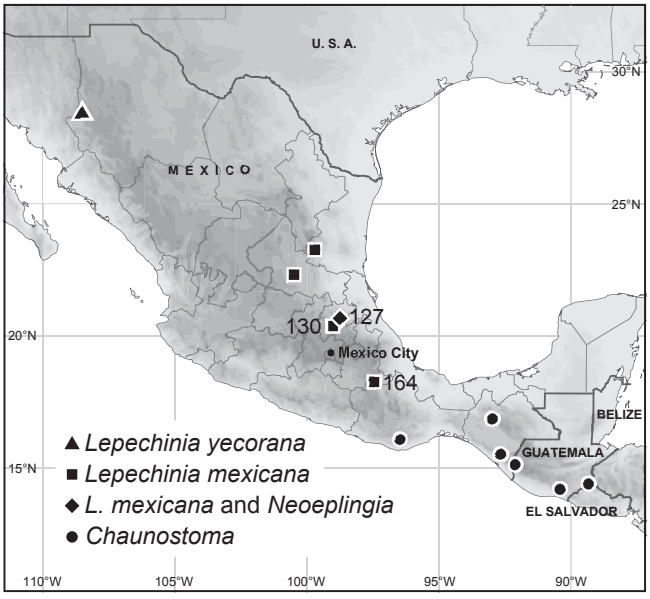

Fig. 1. Distribution of *Lepechinia mexicana*, *L. yecorana*, *Neoeplingia leucophylloides*, and *Chaunostoma mecistandrum*. Note: *Lepechinia mexicana* occurs widely in and around the indicated points, while the latter three taxa are known from only one or a few localities per given occurrence point.

To evaluate the proper taxonomic placement of *Chaunostoma* and *Neoeplingia* in the context of *Lepechinia*, we analyzed data from three different sources: (1) chloroplast DNA (cpDNA) data consisting of *ycf1*, the *ycf1-rps15* spacer, and the *trnL-trnF* spacer region; (2) nuclear ribosomal DNA (nrDNA) data consisting of the internal and external transcribed spacers (ITS, ETS); and, (3) low-copy nuclear gene (LCN) data consisting of *PPR-AT3G09060* and *GBSSI* (or *waxy*). We discuss the taxonomy and relationships of the three aforementioned genera, propose a formal recircumscription of *Lepechinia* that is consistent with our current and robust knowledge of this group, and end by commenting on the conservation status of *Chaunostoma* and *Neoeplingia*.

■ MATERIALS AND METHODS

Sampling. — We added three accessions of *Lepechinia* to our data matrices from Drew & Sytsma (2013) for a total of 34 *Lepechinia* accessions (including *Chaunostoma* and *Neoeplingia*; Appendix 1). Two additional accessions of *Lepechinia mexicana* were added to better assess the relationship of that species with *Neoeplingia*. One newly sampled *Lepechinia mexicana* accession (*B. Drew 127*) was growing immediately adjacent to the *Neoeplingia* accession included in this study. The second new *Lepechinia mexicana* accession (*B. Drew 130*) was sampled about 10 km east of the former locality, whereas the previously sampled *L. mexicana* accession (*B. Drew 164*) was located ca. 350 km to the south (Fig. 1). The third new accession in this study, *Lepechinia ganderi* Epling, native to southern California (southern San Diego County) and adjacent Baja California, was chosen due to the potentially close

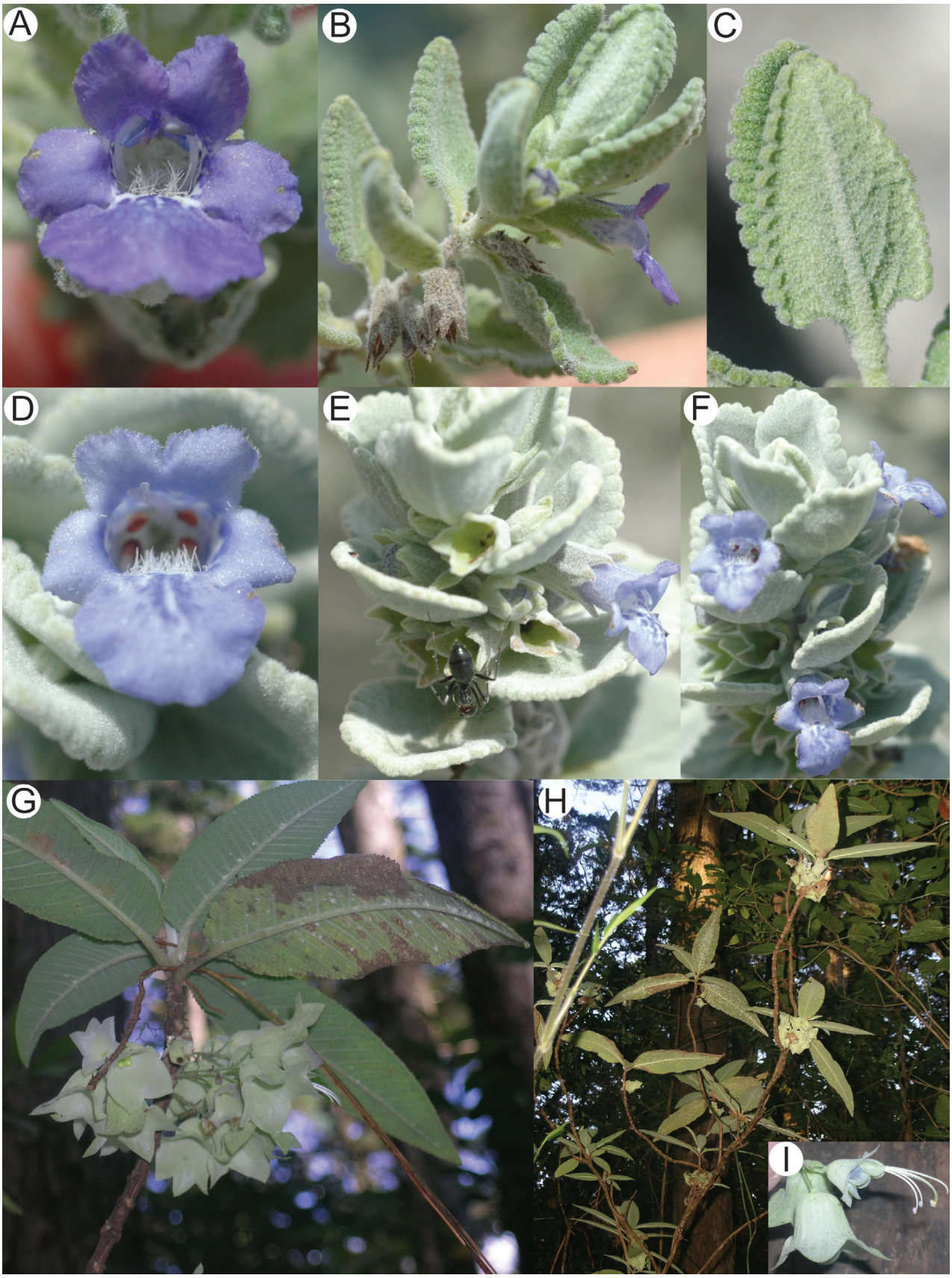

Fig. 2. *Lepechinia*, *Neoeplingia*, and *Chaunostoma*. **A–C,** *Lepechinia mexicana*; **D–F,** *Neoeplingia leucophylloides*; **G–I,** *Chaunostoma mecistandrum*. — Images A–F by N.I. Cacho; images G–I by B.T. Drew.

relationship of the species from the California Floristic Province and *Chaunostoma*, *Neoeplingia*, and *Lepechinia mexicana* (Drew & Sytsma, 2011, 2013). *Melissa officinalis* L. was used as an outgroup based on Walker & Sytsma (2007) and Drew & Sytsma (2011, 2012, 2013).

DNA extraction and sequencing. — DNA was extracted from silica-dried leaves and herbarium specimens (Appendix 1) using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, U.S.A.). PCR thermal cycler settings for the plastid and nrDNA regions were as described in Sytsma & al. (2002). PCR thermal cycling conditions for the nuclear genes *PPR-AT3G09060* (*PPR9060*) and *GBSSI* were as described in Drew & Sytsma (2013). PCR products, obtained using TaKaRa Ex Taq (Otsu, Shiga, Japan), were diluted in water (30×) prior to cycle sequencing and then cleaned using Agencourt magnetic beads (Agencourt, Beverly, Massachusetts, U.S.A.). Cycle sequencing reactions were performed using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, U.S.A.). Samples were electrophoresed on an Applied Biosystems 3730xl automated DNA sequencing instrument, using 50-cm capillary arrays and POP-7 polymer. Data were analyzed using PE-Biosystems v.3.7 of Sequencing Analysis at the University of Wisconsin–Madison Biotechnology Center.

Due to observed polymorphisms and/or suboptimal sequence quality for some sequences, we cloned several *Lepechinia* accessions to investigate the effect that putative allelic variability might have on phylogeny estimation. For cloning, the initial PCR product was obtained as previously cited. The PCR product was then separated on a 2% agarose gel, and the single fluorescent bands were excised and gel purified using the QIAquick Gel Extraction Kit (Qiagen). The purified products were then ligated into a pGEM T-Vector (Promega, Madison, Wisconsin, U.S.A.), cloned using *Escherichia coli* DHB-5a competent cells (Invitrogen, Carlsbad, California, U.S.A.), reamplified and sequenced. Six to ten clones were amplified from the following regions and taxa: ITS—*Lepechinia betonicifolia* (Lam.) Epling, *L. chamaedryoides* (Balb.) Epling, *L. mexicana* 127 and *L. dioica* Hart; *PPR-AT3G09060—L. mexicana* 127, *L. bella* Epling, and *L. calycina* (Benth.) Epling ex Munz; *GBSSI—L. mexicana* 127, *L. yecorana* Henrickson & al., and *Neoeplingia leucophylloides*. We initially analyzed our data with all cloned sequences included, but for subsequent analyses we only retained clones that were distinct (i.e., represented different alleles or distinct paralogues [ITS]).

Phylogenetic analyses. — Sequences were edited in Sequencher v.4.7 (Gene Codes, Ann Arbor, Michigan, U.S.A.), exported, and aligned in MacClade v.4.08 (Maddison & Maddison, 2005). The cpDNA, nrDNA, and LCN regions were each analyzed as separate datasets. In addition, we analyzed the ITS, *PPR-AT3G09060*, and *GBSSI* datasets individually to assess the copy number of the cloned sequences. Maximum likelihood (ML) phylogenetic analysis for each dataset was performed using GARLI v.2.0 (Zwickl, 2006). In GARLI, our analyses employed either the GTR+Γ+I (cpDNA, nrITS, nrDNA), HKY+Γ (*PPR-AT3G09060*), or TrN+I (*GBSSI*, LCN) model of evolution as suggested by the Akaike information criterion (AIC) in ModelTest v.3.7 (Posada & Crandall, 1998), while the other settings in the program were kept at default values. Clade support values were assessed by running 100 bootstrap repetitions using the same GARLI settings as our initial ML analyses.

■ RESULTS

Since the purpose of this paper is to elucidate relationships between *Lepechinia*, *Neoeplingia*, and *Chaunostoma*, the results and discussion focus on the relationships of these three genera, while relationships elsewhere in *Lepechinia* are not discussed.

cpDNA analyses. — The combined cpDNA dataset contained 5959 aligned sites, of which 529 were variable and 191 were potentially parsimony informative (PPI; 3.2%). The large *ycf1* gene had an aligned length of 4611 characters, the *ycf1-rps15* spacer region accounted for 513 aligned positions, and the *trnL-trnF* spacer region alignment numbered 835 aligned characters.

The Mexican/Central American species *Chaunostoma mecistandrum*, *Neoeplingia leucophylloides*, *Lepechinia yecorana* and the three accessions of *L. mexicana* formed a strongly supported clade (100% bootstrap support [BS]) that was sister to the rest of the genus (95% BS; Fig. 3A; Electr. Suppl.: Fig. S1). *Chaunostoma* was in turn sister to the remaining three aforementioned species, and *Neoeplingia* was sister to a clade containing *Lepechinia yecorana* and *L. mexicana*. The two accessions of *L. mexicana* that were collected approximately 10 km apart from one another (Fig. 1) formed a well-supported clade (100% BS), and the cpDNA sequences from the two accessions were identical. The newly sequenced *L. ganderi* formed a clade with *L. calycina*, and the two species together were part of a larger clade (90% BS) consisting of mostly Mexican/Central American taxa.

nrDNA analyses. — Three of the four ITS accessions that were cloned (corresponding to *Lepechinia betonicifolia*, 8 clones; *L. chamaedryoides*, 7; and *L. dioica*, 6) exhibited evidence of pseudogenization (reviewed in Álvarez & Wendel, 2003) as inferred by an elevated substitution rate and/or gaps in the 5.8S region. These clones (*L. betonicifolia*, 3 clones; *L. chamaedryoides*, 1; and *L. dioica*, 2) were considered nonfunctional pseudogenes and were excluded from subsequent analyses. The remaining ITS clone sequences from these three species clustered with their respective directly sequenced analogues in the ML analysis (results not shown). The ten ITS clones from *L. mexicana* 127 showed evidence of paralogy, but exhibited no irregularities (elevated substitution rate and/or frameshifts) in the 5.8S region. Upon visual inspection, one clone (clone 7) showed clear evidence of PCR recombination, and was excluded from subsequent analyses. Among the remaining nine clones, we identified three distinct paralogous copies of ITS (Fig. 3B; Electr. Suppl.: Fig. S2; see also alignment). The majority of the cloned sequences clustered with *L. mexicana* (<50% BS), while one paralogue (clones 2, 5) clustered with *Neoeplingia* (100% BS). Amongst the seven

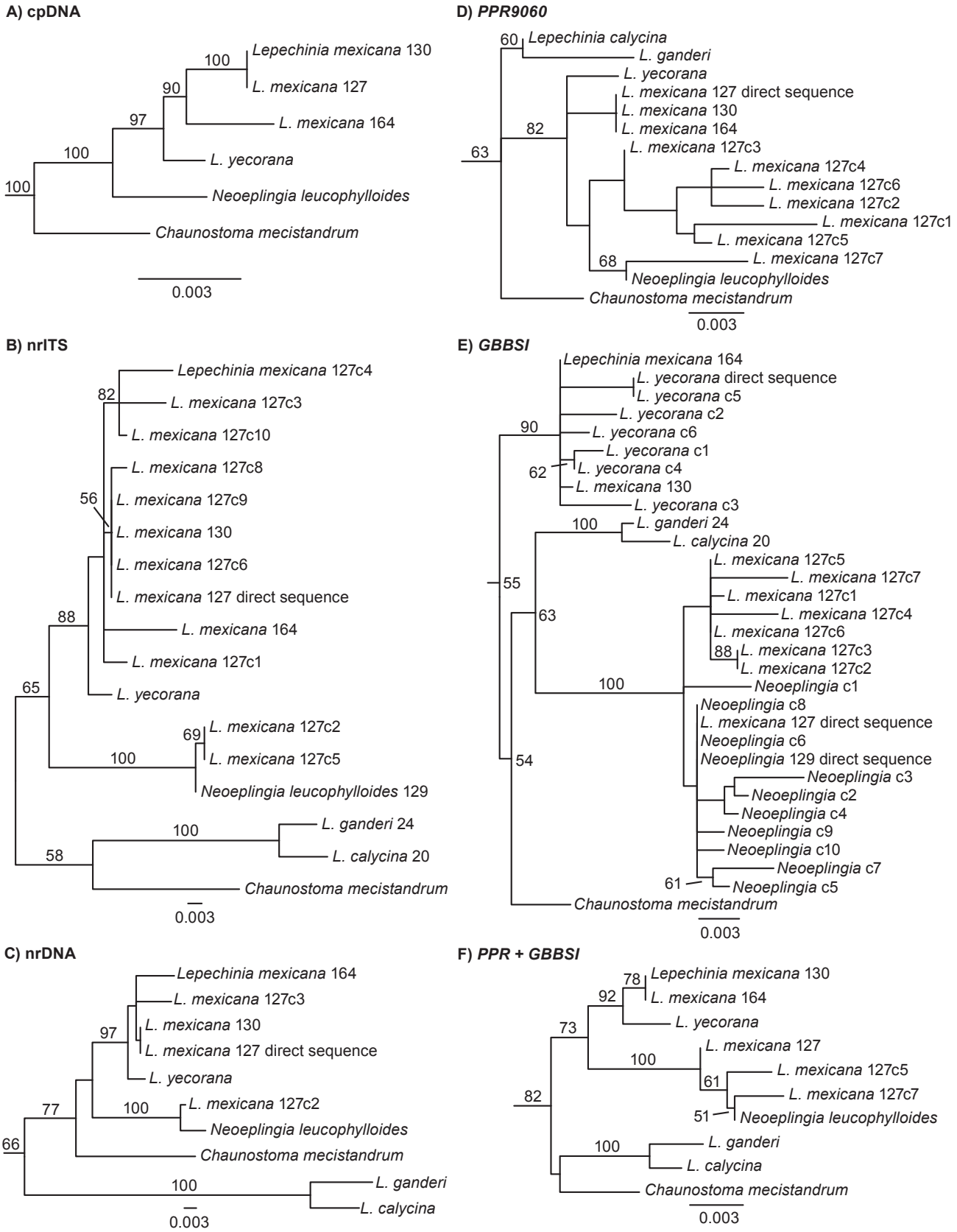

Fig. 3. Excerpts of ML phylograms showing the relationships among *Neoeplingia*, *Chaunostoma*, and *Lepechinia*. ML bootstrap values >50% shown near corresponding nodes. **A,** cpDNA based on *ycf1*, the *ycf1-rpl15* spacer, and *trnL-F*; **B,** nrITS; **C,** nrDNA regions ITS and ETS; **D,** *PPR-AT3G0906*; **E,** *GBSSI*; **F,** combined *PPR-AT3G09060* and *GBSSI*.

clones that clustered with the directly sequenced *L. mexicana*, three formed a clade that we identify as a paralogue (clones 3, 4, 10; 82% BS), and three were practically identical to the directly sequenced accession of *L. mexicana* 127 (clones 6, 8, 9). The last clone (clone 1) could represent an additional paralogue within this group, but an exhaustive analysis of ITS paralogues is beyond the scope of this paper (for a more detailed examination of the ITS sequences of *L. mexicana* 127 and the 10 clones used here please see ITS alignment available online). The two clones that formed a clade with *Neoeplingia* exhibited a ten-nucleotide insertion in the ITS 2 region that was absent in all other *L. mexicana* accessions, but was present in *L. salviae* (Lindl.) Epling, *L. speciosa* (A.St.-Hil. ex Benth.) Epling, *L. chamaedryoides*, *Chaunostoma*, *Neoeplingia*, and *Melissa* L. All seven cloned sequences of *L. chamaedryoides* also had the insertion. *Chaunostoma* formed a clade (58% BS; Fig. 3B; Electr. Suppl.: Fig. S2) with *L. calycina and L. ganderi* that was sister to a clade (65% BS) containing all *L. mexicana* sequences and *Neoeplingia*. For the combined nrDNA analysis we included three ITS sequences of *L. mexicana* 127: (1) one obtained from direct sequencing, (2) one clone (clone 3) that clustered with the other *L. mexicana* accessions, and (3) and a cloned sequence (clone 2) that clustered with *Neoeplingia*. We concatenated each of the above three ITS sequences with the ETS sequence for *L. mexicana* 127 for our combined nrDNA analysis. We did not clone the ETS region, but all chromatogram double peaks were scored as polymorphic characters (as they were in all other gene regions). The combined nrDNA dataset had 37 accessions as opposed to 35.

The combined nrDNA dataset consisted of 1092 aligned characters, of which 324 characters were variable and 181 characters were PPI (16.6%). The ITS dataset (including all *L. mexicana* 127 clones but not clones from the other three species) was 687 aligned characters in length, of which 189 were variable and 111 were PPI (16.1%). The ETS partition contained 405 aligned sites, of which 134 were variable and 75 were PPI (18.5%).

In the combined nrDNA tree there was no supported resolution along the backbone of *Lepechinia* (Electr. Suppl.: Fig. S3). Accessions of *Lepechinia mexicana*, *L. yecorana*, *Chaunostoma*, and *Neoeplingia* formed a moderately supported clade (77% BS; Fig. 3C; Electr. Suppl.: Fig. S3). This clade was nested within a larger group (66% BS) that included *Lepechinia calycina* and *L. ganderi* as a well-supported (100% BS) sister clade. *Chaunostoma* was sister to a clade consisting of *Lepechinia mexicana*, *L. yecorana*, and *Neoeplingia.* Within the latter clade two well-supported clades were recovered, one containing *Neoeplingia* and a *Lepechinia mexicana* 127 sequence (*L. mexicana* directly sequenced ETS with ITS clone 2; 100% BS), and the other containing the other *L. mexicana* accessions and *L. yecorana* (BS = 97%).

Low-copy nuclear gene analyses. — An initial ML analyses found that all cloned sequences of *Lepechinia bella* and *L. calycina* clustered with their respective directly sequenced analogues (results not shown), and clones for these taxa were excluded from subsequent analyses. The final *PPR9060* alignment contained 1121 characters and no insertions or deletions. Of the 1121 characters, 160 were variable and 55 (4.9%) were PPI. This dataset consisted of 42 accessions and included seven cloned sequences of *L. mexicana* 127. A clade (63% BS; Fig. 3D; Electr. Suppl.: Fig. S4) containing six taxa (*L. mexicana*, *L. yecorana*, *L. calycina*, *L. ganderi*, *Chaunostoma*, *Neoeplingia*) was sister to a clade (93% BS) representing the remainder of the genus (Electr. Suppl.: Fig. S4). Within the former group, a clade (82% BS) containing *L. mexicana*, *L. yecorana*, and *Neoeplingia* was recovered (Fig. 3D). There was only poorly supported resolution within the latter clade, but the cloned accessions of *L. mexicana* 127 grouped with *Neoeplingia* while the directly sequenced *L. mexicana* accessions formed a separate group (both clades <50% BS). The seven *L. mexicana* clones formed at least two groups (Fig. 3D). One sequence (clone 7), the most divergent from the rest, was sister *to Neoeplingia* (68% BS), while the remaining six clones clustered in a clade (<50% BS). In total, the directly sequenced *L. mexicana* 127 displayed 12 variable sites with double peaks in the chromatograms, and clones 1 and 3 exhibited signs of PCR recombination (although this recombination was not as clear as with the ITS recombinant and we retained the samples in this case). *Neoeplingia* had 13 nucleotide sites showing double peaks (8 of these were common with the directly sequenced *L. mexicana* 127). For comparison, *L. mexicana* 164 had five variable sites, *L. mexicana* 130 had three, and *L. yecorana* had two. With the available data it is difficult to assess if this variation represents more than one copy of this gene. However, the data seem to indicate that two copies are present in *L. mexicana* 127, one represented by clone 7, and the other by the rest of the sequences. Of this latter copy, two alleles could exist, one that is functional (potentially clone 3), and one that has subfunctionalized and shows increased variation (clones 1, 2, 4, 5, 6).

The *GBSSI* dataset contained 1372 aligned characters and included numerous insertions/deletions. Of the 1372 aligned characters, 206 characters were excluded (mostly due to long single-taxa insertions); the 1166 included characters contained 181 variable and 54 PPI (4.6%) characters. *Lepechinia mexicana*, *L. yecorana*, *L. calycina*, *L. ganderi*, *Chaunostoma*, and *Neoeplingia* again formed a clade in this analysis (55% BS; Fig. 3E; Electr. Suppl.: Fig. S5). Within this clade, *L. yecorana* (direct sequence and clones), *L. mexicana* 130, and *L. mexicana* 164 formed a clade (90% BS) sister to the other taxa (54% BS). Amongst the remaining taxa, *Chaunostoma* was sister to a clade containing two subclades (63% BS), one consisting of *L. ganderi*+*L. calycina* (100% BS), and the other of *L. mexicana* 127 (direct sequence and clones)+*Neoeplingia* (direct sequence and clones; 100% BS). Within the latter clade, all but one of the clones (*Neoeplingia* clone 1) segregated into two clades that reflected species designations (<50% BS). The only variation exhibited within the clones of *L. mexicana* 127 and *Neoeplingia* was the result of PCR mis-incorporation; that is, variation in the clones did not correspond to any double peaks in the direct sequences, and was not consistent among clones. In *L. mexicana* 127 only two positions were scored as polymorphic in the direct sequence, which appear to be the result of locally poor-quality sequences rather than allelic variation. In *Neoeplingia*, no nucleotides in the direct sequence were initially scored as polymorphic; it was cloned due to

suboptimal sequence quality in the first ~300 reads at the 5′ end. The reduced sequence quality may have been due to primer infidelity. In *L. yecorana*, we were only able to obtain ~415 nucleotides of good quality direct sequence due to apparent allelic variation. Upon cloning, two alleles were inferred: one, represented by clone 2, exhibited an eight-base pair insertion; the other, represented by the other five clones, lacked the insertion. There were only 19 additional variable sites amongst the six clones, which appeared to be PCR error (i.e., they were not consistently shared among clones). No polymorphisms (chromatogram double peaks) were detected within the ~415 nucleotides of directly sequenced *L. yecorana.*

For the combined low-copy nuclear (LCN) gene analysis we included three *PPR9060* sequences of *L. mexicana* 127: (1) one obtained from direct sequencing, (2) one clone (c5) that clustered with the other *L. mexicana* cloned sequences, and (3) and a clone sequence (c7) that was sister to *Neoeplingia* in the *PPR9060* phylogeny. We concatenated the *GBBSI* sequence for *L. mexicana* 127 to each of the above three *L. mexicana* 127 *PPR9060* sequences for our combined LCN analysis. Thus, as in the nrDNA analysis, the combined LCN dataset had 37 accessions as opposed to 35. In the combined LCN analysis, *L. mexicana*, *L. yecorana*, *L. calycina*, *L. ganderi*, *Chaunostoma*, and *Neoeplingia* composed a clade (82% BS; Fig. 3F; Electr. Suppl.: Fig. S6) that was sister to the remainder of *Lepechinia* (99% BS). Within the clade, two main subclades were identified; one contained *L. mexicana*, *L. yecorana*, and *Neoeplingia* (73% BS), while the other consisted of *Chaunostoma* as sister (<50% BS) to a clade of *L. calycina* and *L. ganderi* (100% BS). Within the first subclade, *Neoeplingia* and all the *L. mexicana* 127 sequences (direct sequences and clones) formed a clade with 100% BS that was sister to a clade (92% BS) consisting of *L. yecorana* and the other two *Lepechinia mexicana* accessions (130, 164) that clustered together with 78% BS.

■ DISCUSSION

***Chaunostoma* and *Neoeplingia* are nested in *Lepechinia*.** — Molecular phylogenetic analyses using cpDNA, nrDNA, and LCN genes (Fig. 3; Electr. Suppl.: Figs. S1–S6; Drew, 2011; Drew & Sytsma, 2011, 2012, 2013) conclusively demonstrate that *Chaunostoma* and *Neoeplingia* are embedded within *Lepechinia*. *Neoeplingia* is most closely related to *L. mexicana* and the recently described *L. yecorana* Henrickson & al. (Henrickson & al., 2011; Drew & Sytsma, 2013). However, results from cpDNA, nrDNA, and LCN DNA vary somewhat as to the relationships among the aforementioned four species and to the remainder of *Lepechinia*. In the cpDNA analysis, *Chaunostoma*, *L. mexicana*, *L. yecorana*, and *Neoeplingia* form a clade (100% BS) that is sister to the rest of the genus, whereas in the nrDNA tree the backbone of *Lepechinia* is poorly supported but the aforementioned clade of four species is sister to a clade of two species (*L. calycina*, *L. ganderi*) from California/northern Baja California (Mexico). In the combined LCN DNA analyses, *L. calycina*, *L. ganderi*, and *Chaunostoma* form a poorly supported clade (<50% BS) that is sister to a clade of *L. mexicana*, *L. yecorana*, and *Neoeplingia* (73% BS). Together, these two clades form a clade (BS = 82%) that is sister to the remainder of the genus.

Gene discordance in *Lepechinia* and *Neoeplingia*: hybridization/introgression or incomplete lineage sorting? — The relationships among accessions of *Neoeplingia*, *Lepechinia mexicana* and *L. yecorana* are particularly intriguing and complex. In the cpDNA phylogeny all three *L. mexicana* accessions (127, 130, 164) group together with *L. yecorana* as sister, and these two species in turn are sister to *Neoeplingia leucophylloides*. In sharp contrast, nuclear genes (either direct sequence and/or cloned products) of *L. mexicana* accession 127 (sympatric with *Neoeplingia leucophylloides*, see Fig. 1) cluster with *Neoeplingia* either in part or as a whole (Fig. 3). *Lepechinia mexicana* accessions 130 and 164 strongly group with *L. yecorana* (and with some *L. mexicana* 127 cloned or direct sequences) with nuclear genes. In the nrDNA analyses (Fig. 3B–C), only two *L. mexicana* 127 cloned sequences form strongly supported clades with *Neoeplingia*; the direct sequence and all other cloned sequences are placed with *L. yecorana* and the other *L. mexicana* accessions. The LCN *PPR9060* gene placed one *L. mexicana* 127 clone/allele with *Neoeplingia*, although support values overall were low (Fig. 3D). However, the LCN *GBBSI* gene strongly placed all clones/alleles of *L. mexicana* 127 with *Neoeplingia* and not with other accessions of *L. mexicana* (Fig. 3E).

The documentation of discordance in placement of species (or populations) between cpDNA and nuclear DNA evidence has a long record in plants (e.g., Smith & Sytsma, 1990; Rieseberg & Soltis, 1991; Rieseberg & Brunsfeld, 1992; Mason-Gamer & al., 1995; Soltis & Kuzoff, 1995; Sang & al., 1997; Wendel & Dolye, 1998). Such incongruence is a special case of the more general issue of genes trees in species trees (Maddison, 1997), and can be attributed to any of the processes of rapid diversification, introgressive hybridization (including cpDNA capture), incomplete lineage sorting involving either or both the chloroplast and nuclear genomes, and horizontal gene transfer. Deciding among these processes (or acknowledging the impact of more than one) is difficult, especially when the level of divergence is low among the taxa examined (Mason-Gamer & al., 1995; Wendel & Doyle, 1998; Yu & al., 2011; Xu & al., 2012). However, multiple lines of evidence can assist in teasing apart the importance of these potential processes operating within *Lepechinia*. First, introgressive hybridization (and cpDNA capture) is more likely in groups in which interspecific barriers to crossing are known to fail (e.g., Mason-Gamer & al., 1995). Furthermore, introgressive hybridization is more likely between species that have overlapping geographical distributions (e.g., Smith & Sytsma, 1990; Mason-Gamer & al., 1995; Xu & al., 2012).

We argue that introgressive hybridization is the primary mechanism within *Lepechinia* generating the discordance between and among cpDNA and nuclear genes seen primarily with one population of *L. mexicana* (accession 127) and with *Neoeplingia leucophylloides*. A number of lines of evidence support recent hybridization and subsequent introgression

between these two populations. First, interspecific hybridization including cpDNA capture in areas of species overlap is not uncommon in *Lepechinia*, as evidenced by discordance between morphology, cpDNA, and nuclear DNA in other North American, Mexican, and especially South American clades (Drew, 2011; Drew & Sytsma, 2013; Drew & Sytsma, unpub. data); hybridization within *Lepechinia* has also been noted by previous workers within the group (e.g., Epling, 1948; Hart, 1983; Wood, 1988). Second, the only occurrence of gene discordance (amongst *L. mexicana*, *L. yecorana*, and *Neoeplingia*) in this study involves the one population of *L. mexicana* (127) growing sympatrically with *Neoeplingia* (Fig. 1). As the cpDNA of *L. mexicana* 127 is almost identical to the other accessions of *L. mexicana*, and these in turn are sister to *L. yecorana* and not *Neoeplingia*, these data support the hypothesis that this accession is the result of a past hybridization event in which *Neoeplingia* functioned as the pollen source and *Lepechinia mexicana* as the maternal parent. Third, the pattern of gene discordance within the group of three species (*L. mexicana*, *L. yecorana*, *Neoeplingia leucophylloides*) supports a hypothesis of nuclear introgression following recent hybridization, rather than just ongoing lineage sorting within the group. Finally, the fossil-calibrated chronogram of *Lepechinia* (Drew & Sytsma, 2013) indicates that speciation for *L. mexicana* (164) and *L. yecorana* occurred ca. 2.0 Ma and that the cladogenesis event separating *Neoeplingia* from these two species occurred ca. 5.5 Ma. These ages are more consistent with the hypothesis of hybridization in areas of contact among already differentiated species rather than of lineage sorting within a recent and actively differentiating species complex (although effective population size also affects this calculus). It has been estimated that the chloroplast genome coalesces at roughly two times the rate of a nuclear gene (Birky & al., 1983).

Incomplete lineage sorting, although possible, would mandate an unlikely scenario in which a complex of populations have recently diverged, some uniquely derived morphological traits have coincidentally been fixed in some populations so that three "species" (*L. mexicana*, *L. yecorana*, *Neoeplingia leucophylloides*) are recognized, but that: (1) *Lepechinia mexicana* is not monophyletic; (2) ancestral cpDNA polymorphism has been fixed fortuitously along these "species" lineages; and (3) gene trees still provide evidence of a complex of populations that have not sorted out yet. The incomplete lineage sorting hypothesis would predict that nuclear genes should show ongoing lineage sorting in populations of all "species" in similar fashion, whereas the data indicate that it is restricted only to *L. mexicana* and, furthermore, specifically to an accession (*L. mexicana* 127) sampled in sympatry with *Neoeplingia leucophylloides*. Direct LCN sequencing of *Neoeplingia*, *L. yecorana*, and other accessions of *L. mexicana* exhibited few polymorphic sites (sites with double peaks in sequences) relative to *L. mexicana* 127 (although in the nrDNA sequences all *L. mexicana* accessions had elevated polymorphism rates relative to other *Lepechinia*; see alignments available online). Cloned nrITS sequences of *L. mexicana* 127 show evidence of incomplete concerted evolution (Buckler & al., 1997) as is evidenced by some clones segregating with the other *L. mexicana* accessions (130, 164) and other clones grouping with *Neoeplingia*. In the LCN gene trees, clones of *L. mexicana* 127 group with *Neoeplingia* in both the *PPR9060* and *GBSSI* analyses. Based upon the *GBSSI* analyses, however, it does appear that enough time has passed since the putative hybridization event to allow at least some nuclear genes to display sequence heterogeneity between the two populations. We are currently analyzing additional *L. mexicana* accessions from the vicinity of the *Neoeplingia* type locality as well as elsewhere in Mexico in an effort to clarify the interrelated history of these two species.

Taxonomic treatment. — Our findings clearly demonstrate, despite some ambiguity as to the exact relationships of *Chaunostoma* and *Neoeplingia* within *Lepechinia*, that there is no question that the two monotypic genera are indeed part of *Lepechinia*. Consequently, the most prudent course of action is to formally recognize *Chaunostoma* and *Neoeplingia* as part of *Lepechinia*. The only other viable options are to transfer *L. mexicana* and *L. yecorana* to a new genus or have *L. mexicana* and *L. yecorana* subsumed into *Neoeplingia*. We consider these latter options unsatisfactory because they imply more taxonomic changes, generate taxonomies that are more likely to be unstable than the one we propose, and ignore the relationship of the aforementioned four taxa with the California clade (*L. calycina*, *L. ganderi*) shown with nrDNA and LCN markers. We therefore proceed to formalize *Neoeplingia* as part of *Lepechinia* (*Chaunostoma* recently was combined by Moon, 2012) and thereafter briefly comment on their conservation status.

Lepechinia Willd., Hort. Berol.: 20, t. 21. 1804 – Type: *L. spicata* Willd.

= *Chaunostoma* Donn.Sm. in Bot. Gaz. 20: 9. 1895, **syn. nov.** – Type: *Ch. mecistandrum* Donn.Sm.

= *Neoeplingia* Ramamoorthy, Hiriart & Medrano in Bol. Soc. Bot. México 43: 61. 1982, **syn. nov.** – Type: *N. leucophylloides* Ramamoorthy, Hiriart & Medrano.

Lepechinia leucophylloides (Ramamoorthy, Hiriart & Medrano) B.T.Drew, Cacho & Sytsma, **comb. nov.** ≡ *Neoeplingia leucophylloides* Ramamoorthy, Hiriart & Medrano in Bol. Soc. Bot. México 43: 62–65. 1982 –Holotype: MEXICO. Hidalgo; municipio de Cardonal, Barranca de Tolantongo (1800 m), 0.5 km north of Molanguito, 5 Aug 1982, *Medrano & Hiriart 12792* (MEXU!; isotype: GH n.v.)

Lepechinia mecistandra (Donn.Sm.) H.K.Moon in Phytotaxa 71: 52. 2012 ("*mecistandrum*") ≡ *Chaunostoma mecistandrum* Donn.Sm. in Bot. Gaz. 20: 9–10, pl. 3. 1895 – Holotype: GUATEMALA. Buena Vista, Depart. Santa Rosa (6000 ft.), Dec 1892, *E.T. Heyde & E. Lux 4368* (US n.v.; isotype: MO!).

During the preparation of this manuscript, *Chaunostoma mecistandrum* was renamed *Lepechinia mecistandrum* (Donn. Sm.) H.K.Moon by Moon (2012) based largely on the results of Drew & Sytsma (2011); as noted above, the appropriate specific epithet in this case should be "*mecistandra*" as *Lepechinia* is feminine. Drew & Sytsma (2011) had specifically laid out three possible scenarios for nomenclatural changes

(including those we advocate here) involving *Chaunostoma mecistandrum*, *Neoeplingia leucophylloides*, and *Lepechinia mexicana* (*L. yecorana* was not yet described), but advocated caution for any formal change until additional accessions were sampled for phylogenetic study (as done in this study). Several important points should be noted regarding the paper by Moon (2012): (1) the distribution of *Chaunostoma* is purported to be limited to southern Mexico (Chiapas) and Guatemala, but *Chaunostoma* also occurs Oaxaca State in Mexico and in El Salvador (e.g., *Carrillo-Reyes & Lomelí-Sención 3762* in IBUG; *Monterrosa & Carballo 842* in MEXU, MO, LAGU, B); (2) Epling (1948) and Walker & Sytsma (2007) are cited in claiming *Lepechinia* have arched stamens and this claim is used to support the inclusion of *Chaunostoma* within *Lepechinia*. In fact, Epling (1948) mentions arched stamens as a key difference between *Lepechinia* and *Chaunostoma*, while Walker & Sytsma (2007) make no mention of arched stamens whatsoever in their study; (3) Moon & al. (2009) is cited in postulating that an areolate nutlet abscission scar represents a synapomorphy for *Lepechinia* and *Chaunostoma*, but Moon & al. (2009) only included two *Lepechinia* species in their study. Furthermore, since *Dorystaechas* Boiss. & Heldr. ex Benth., a member of the *Salvia* clade sensu Walker & Sytsma (2007), and ten other *Salvia* species were posited by Moon & al. (2009: fig. 7) to also possess an areolate abscission scar, the condition may be plesiomorphic within Salviinae, and certainly (at least at this time) should not be considered a synapomorphy of just *Lepechinia* and *Chaunostoma* (and thus justification for the transfer of the latter genus by Moon & al., 2009).

Conservation status of *Lepechinia leucophylloides* and *L. mecistandrum*. — *Lepechinia leucophylloides* (*Neoeplingia leucophylloides*) is only known to occur in open sites on calcareous soils near the Barranca de Tolantongo in central Mexico. The Barranca de Tolantongo has floristic affinities with the now far-removed Chihuahuan desert, and is a hotspot of species-richness and endemism (Sosa & DeNova, 2012). *Lepechinia leucophylloides* has only been documented from the type locality and should be considered globally extremely threatened or probably endangered. Edaphically similar nearby localities were surveyed for *L. leucophylloides* (by the first and second authors), and although similar communities and particular floral associates (e.g., *Lepechinia mexicana*, *Salvia coulteri* Fernald, *Opuntia* sp.) of *L. leucophylloides* were found, *L. leucophylloides* was not observed. In all, only a handful of mature specimens, and no seedlings, of *L. leucophylloides* were observed at the Barranca de Tolantongo, which remains the only locality from which it has been collected. Moreover, being adjacent to a busy crossroads and near a tourist area, this population is vulnerable to extirpation due to any development, even as minor as a bus stop. The breeding system of *L. leucophylloides* is unknown, but the closely related *L. yecorana* and *L. mexicana* have recently been shown to be dioecious (Henrickson & al., 2011). If *L. leucophylloides* is also dioecious, it makes the species survival even more precarious.

We document here that *L. leucophylloides* has most likely hybridized with *L. mexicana*, and our data so far suggest that *L. leucophylloides* has been the pollen donor rather than receptor. Future work on characterizing the barriers to reproduction between these two species is needed and should be informative. For example, it would be of interest to quantify if there is extant gene flow between individuals of these two species, so as to examine if levels of gene exchange are high enough that they could lead to a breakdown of the current barriers separating these two species as distinct evolutionary lineages.

Lepechinia mecistandrum (*Chaunostoma mecistandrum*) is found in clearings and edges of cloud forests from southern Oaxaca in Mexico to northeastern El Salvador. It has only been collected at six localities, all between 1300 and 2300 meters in elevation. It is so poorly known that until recently its corollas, which are shed promptly, were thought to be red, not blue. Although apparently more common and widely distributed than *L. leucophylloides*, *L. mecistandrum* is also very rare and should be considered threatened (or possibly endangered). Only three populations of *L. mecistandrum* have been documented within the past 40 years, two from Mexico and one from El Salvador. The two recent collections of *L. mecistandrum* from Mexico were from central Chiapas (2013) and southern Oaxaca (2002), and represent the only *L. mecistandrum* collections from those localities. The population from El Salvador occurs in the understory of a Mexican cypress (*Hesperocyparis lusitanica* (Mill.) Bartel) plantation, and could be easily extirpated by a small fire. This population had few if any seedlings or juvenile plants, suggesting low levels of recruitment. Judging by the size of the plants, the apparently low recruitment, the fairly dense shade present under the cypress plantation, and the fact that other collections of *L. mecistandrum* have been from open habitats, this population seems unlikely to withstand environmental or other perturbations (although, admittedly, the ecological preferences of *L. mecistandrum* are largely unknown). Open areas immediately adjacent to the (fenced in) cypress plantation were grazed by sheep or goats and did not contain any *L. mecistandrum* individuals. The population of *L. mecistandrum* in El Salvador is distinct from collections in Mexico and Guatemala in terms of calyx color (tannish-green as opposed to blue) and stem vestiture (much less hairy than northern localities), and may even warrant varietal status after further examination (although observed morphological differences could be caused by environmental or edaphic conditions). Efforts by the first and second authors to locate a previously documented population (from 1969) of *L. mecistandrum* near Chiquihuites in southern Chiapas were not successful. *Lepechinia mecistandrum* was also collected on Mt. Ovando (Matuda, 1950) multiple times, and owing to Mt. Ovando's remote and relatively inaccessible location, *L. mecistandrum* probably still occurs there. However, *L. mecistandrum* was only collected at an elevation of 2300 m on Mt. Ovando. Because the peak of Mt. Ovando is 2346 m, global climate change could easily drive environmental conditions beyond the physiological tolerance conditions of *L. mecistandrum* (Thomas & al., 2004). La Laguna Botanical Garden in San Salvador has living specimens of *L. mecistandrum*, but we recommend its cultivation elsewhere to ensure survival.

In conclusion, these two species have very narrow niches and discrete distributions, with one (*L. leucophylloides*) or

six (*L. mecistandrum*) known populations. We raise serious concerns as to their viability due to: (1) their narrow distributions and rarity, which have been shown to increase risk of extinction (Brown, 1984; Ohlemüller & al., 2008); (2) their low population and low recruitment numbers—especially with *L. leucophylloides*; (3) their shrubby habit, which might pose a challenge to recruitment, particularly in the presence of grazing (Crisp, 1978); and (4) their potentially dioecious breeding system (*L. leucophylloides*). Low recruitment, scattered distribution, and specific habitat requirements of the two species indicate they may be eco-displaced (Wiens & al., 2012) and slowly marching towards extinction even in the absence of human pressure. Species of *Lepechinia* are rich in bioactive compounds, and several species are being actively investigated to assess their medicinal properties and/or agricultural utility. It stands to reason that *L. leucophylloides* and *L. mecistandrum* also harbor interesting secondary compounds that may prove useful to humans. Thus, we make a call for measures to ensure their protection, including their cultivation in local botanical gardens in Mexico, Guatemala, and El Salvador. These efforts should also include increasing local awareness of the biological value of these populations since local communities and governments currently play a pivotal role in their conservation.

■ ACKNOWLEDGEMENTS

Thanks to the many collectors who made this study possible, especially Carlos Hinostrosa and Gerardo Salazar from the UNAM herbarium (MEXU) in Mexico City. We gratefully acknowledge James Henrickson for providing plant material, and Marybel Morales and Asunción Cano for collecting assistance in Peru. We acknowledge Pablo Carrillo-Reyes and Jesús González-Gallegos for very helpful discussion regarding *L. mecistandrum* distribution in Mexico. Thanks also to Sarah Friedrich for assistance with constructing figures and to Beverley Becker for editing. Funding from the UW-Madison Botany Department Davis Fund, the Wisconsin State Herbarium Fund, UW-Madison LACIS, NSF (DDIG DEB-0910336 to BTD and KJS), and a Conacyt Fellowship (to NIC) provided funding for this study. Richard Olmstead and an anonymous reviewer provided insightful comments and suggestions on an earlier version of this manuscript.

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Appendix 1. Voucher information and GenBank accession numbers for taxa used in this study. Information is as follows: taxon name and authority, collecting locality, collector(s) name and collection number (herbarium), GenBank numbers for previously submitted loci (where applicable): *ycf1* & *ycf1-rpl15* spacer region, *trnL-F*, ITS, ETS, *PPR-AT3G09060*, and *GBBSI*, respectively. Abbreviation: RSABG, Rancho Santa Ana Botanical Garden.

Lepechinia bella Epling, Bolivia, *R. Jabaily s.n.* (WIS); KF307566, KF307411, KF307436, KF307471, KF307358, KF307498; ***Lepechinia betonicifolia*** (Lam.) Epling, Ecuador, *B. Drew 224* (WIS); KF307567, KF307412, KF307437, KF307472, KF307359, KF307499; ***Lepechinia bullata*** (Kunth) Epling, Ecuador, *B. Drew 223* (WIS); KF307568, KF307413, KF307438, KF307473, KF307360, KF307500; ***Lepechinia calycina*** (Benth.) Epling ex Munz, U.S.A., *B. Drew 20* (WIS); KF307569, KF307414, KF307439, KF307474, KF307361, KF307501; ***Lepechinia caulescens*** (Ortega) Epling, Mexico, *B. Drew 149* (WIS); KF307570, KF307415, KF307440, KF307475, KF307362, KF307502; ***Lepechinia chamaedryoides*** (Balb.) Epling, Chile, cult. RSABG, *J. Walker 2537* (WIS); JF289031, AY570459, DQ667231, JF301317, KF307363, KF307503; ***Lepechinia codon*** Epling, Peru, *B. Drew 177* (WIS); KF307571, KF307416, KF307441, KF307476, KF307364, KF307504; ***Lepechinia dioica*** J.A.Hart, Ecuador, *B. Drew 232* (WIS); KF307572, KF307417, KF307442, KF307477, KF307365, KF307505; ***Lepechinia flammea*** Martínez-Gordillo & Lozada-Pérez (previously treated as *L. glomerata* Epling in Drew & Sytsma, 2011, 2012, 2013), Mexico, *B. Drew 155* (WIS); JF289032, JF301377, JF301346, JF301318, KF307368, KF307508; ***Lepechinia floribunda*** (Benth.) Epling, Peru, *B. Drew 172* (WIS); KF307573, KF307418, KF307443, KF307478, KF307367, KF307507; ***Lepechinia ganderi*** Epling, U.S.A., *B. Drew 24* (WIS); KF307574, KF30741, KF307444, KF307479, KF307366, KF307506; ***Lepechinia graveolens*** (Regel) Epling, Bolivia, *Fuentes & al. 10351* (M); KF307575, KF307420, KF307445, KF307480, KF307369, KF307509; ***Lepechinia hastata*** (A.Gray) Epling, Mexico, *B. Drew 44* (WIS); JF289033, JF301378, JF301347, JF301319, KF307370, KF307510; ***Lepechinia***

Appendix 1. Continued.

heteromorpha (Briq.) Epling, Peru, *B. Drew 192* (WIS); KF307576, KF307421, KF307446, KF307481, KF307371, KF307511; ***Lepechinia lamiifolia*** (Benth.) Epling, Peru, *B. Drew 178* (WIS); JF289034, JF301379, JF301348, JF301320, KF307372, KF307512; ***Lepechinia*** (*Neoeplingia*) ***leucophylloides*** Ramamoorthy, Hiriart & Medrano, Mexico, *B. Drew 129* (WIS); JF289047, JF301390, JF301354, JF301327, KF307391, KF307531, *GBBSI* clones 1–10: KF307545, KF307546, KF307547, KF307548, KF307549, KF307550, KF307551, KF307552, KF307553, KF307554; ***Lepechinia*** (*Chaunostoma*) ***mecistandrum*** Donn.Sm., El Salvador, *J.A. Monterrosa & R.A. Carballo 213* (MO); JF289005, JF301361, JF301342, JF301311, KF307357, KF307497; ***Lepechinia mexicana*** (S.Schauer) Epling, Mexico, *B. Drew 127* (WIS); JF289036, JF301381, JF301350, JF301322, KF307373, KF307513, ITS clones 1–10: KF307461, KF307462, KF307463, KF307464, KF307465, KF307466, KF307467, KF307468, KF307469, KF307470, *PPR-AT3G09060* clones 1–7: KF307392 KF307393, KF307394, KF307395, KF307396, KF307397, KF307398, *GBBSI* clones 1–7: KF307532, KF307533, KF307534, KF307535, KF307536, KF307537, KF307538; ***Lepechinia mexicana*** (S.Schauer) Epling, Mexico, *B. Drew 130* (WIS); KF307577, KF307422, KF307447, KF307482, KF307374, KF307514; ***Lepechinia mexicana*** (S.Schauer) Epling, Mexico, *B. Drew 164* (WIS); JF289035, JF301380, JF301349, JF301321, KF307375, KF307515; ***Lepechinia meyenii*** (Walp.) Epling, Peru, *B. Drew 173* (WIS); KF307578, KF307423, KF307448, KF307483, KF307376, KF307516; ***Lepechinia mollis*** Epling, Peru, *B. Drew 182* (WIS); KF307579, KF307424, KF307449 KF307484, KF307377, KF307517; ***Lepechinia mutica*** (Benth.) Epling, Ecuador, *B. Drew 229* (WIS); KF307580, KF307425, KF307450, KF307485, KF307378, KF307518; ***Lepechinia paniculata*** (Kunth) Epling, Ecuador, *B. Drew 241* (WIS); KF307581, KF307426, KF307451, KF307486, KF307379, KF307519; ***Lepechinia radula*** (Benth.) Epling, Ecuador, *B. Drew 237* (WIS); KF307582, KF307427, KF307452, KF307487, KF307380, KF307520; ***Lepechinia rufocampii*** Epling & Mathias, Ecuador, *B. Drew 245* (WIS); KF307583, KF307428, KF307453, KF307488, KF307381, KF307521; ***Lepechinia salviae*** (Lindl.) Epling, Chile, *R. Jabaily s.n.* (WIS); KF307584, KF307429, KF307454, KF307489, KF307382, KF307522; ***Lepechinia salviifolia*** (Kunth) Epling, Colombia, *R. Jabaily s.n.* (WIS); JF289038, JF301383, JF301352, JF301324, KF307383, KF307523; ***Lepechinia schiedeana*** (Schltdl.) Vatke, Mexico, *B. Drew 157* (WIS); KF307585, KF307430, KF307455, KF307490, KF307384, KF307524; ***Lepechinia scobina*** Epling, Peru, *B. Drew 184* (WIS); KF307586, KF307431, KF307456, KF307491, KF307385, KF307525; ***Lepechinia speciosa*** (A.St.-Hil. ex Benth.) Epling, Brazil, *Cordeno 3060* (WIS); KF307587, KF307432, KF307457, KF307492, KF307386, KF307526; ***Lepechinia urbanii*** Epling, Dominican Republic, *B. Drew 135* (WIS); KF307588, KF307433, KF307458, KF307493, KF307387, KF307527; ***Lepechinia vesiculosa*** (Benth.) Epling, Peru, *B. Drew 175* (WIS); KF307589, KF307434, KF307459, KF307494, KF307388, KF307528; ***Lepechinia yecorana*** Henrickson, Fishbein & T. van Devender, Mexico, *Henrickson 24,691* (WIS); KF307590, KF307435, KF307460, KF307495, KF307389, KF307529, *GBBSI* clones 1–6: KF307539, KF307540, KF307541, KF307542, KF307543, KF307544; ***Melissa officinalis*** L., cult. UW-Madison, *B. Drew 70* (WIS); JF289042, JF301386, JF301353, JF301325, KF307390, KF307530;

Vol. 63 (4) • August 2014

TAXON

International Journal of Taxonomy, Phylogeny and Evolution

Electronic Supplement to

The transfer of two rare monotypic genera, *Neoeplingia* and *Chaunostoma*, to *Lepechinia* (Lamiaceae), and notes on their conservation

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Taxon 63: 831–842

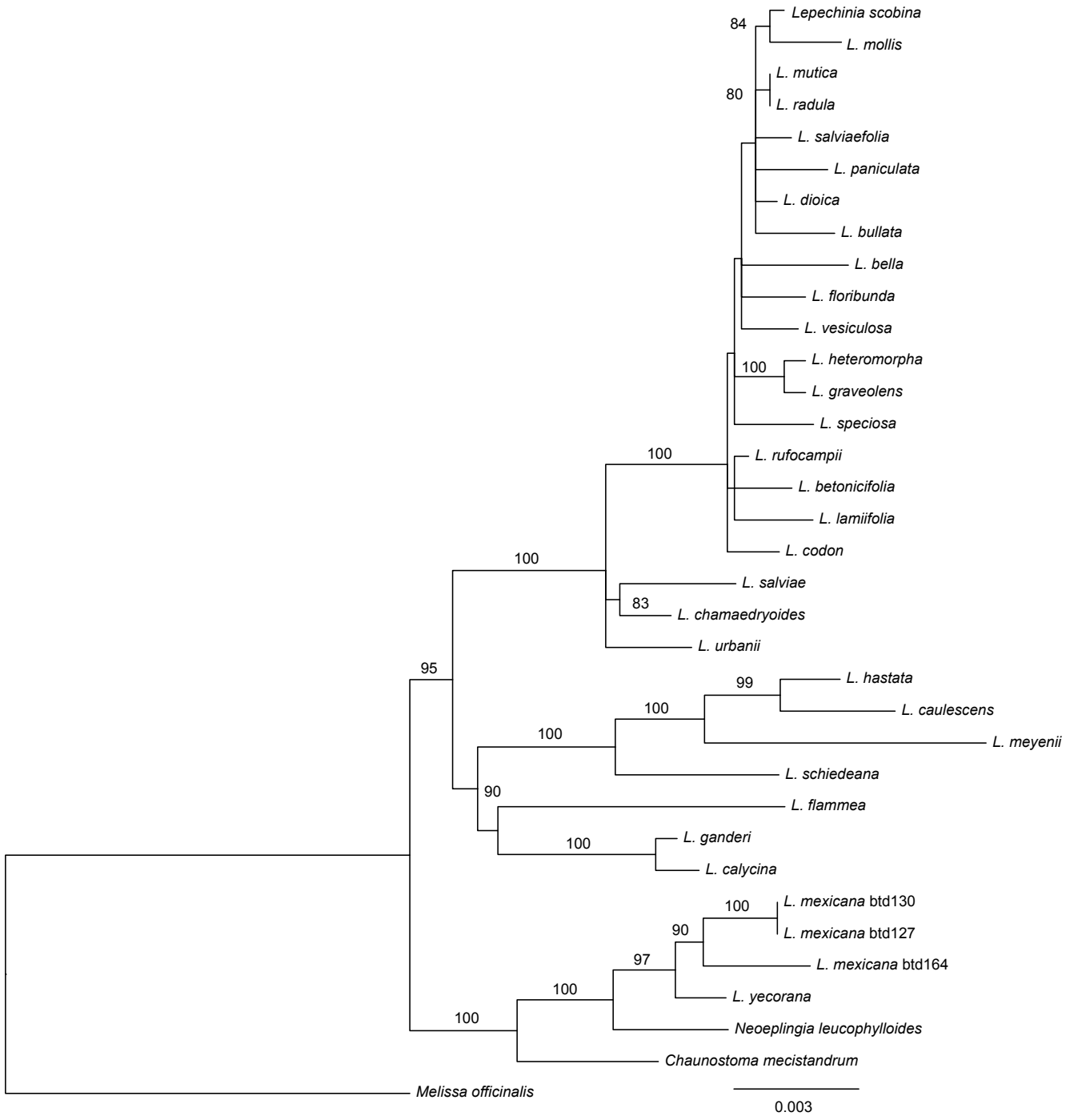

Fig. S1. ML phylogram based upon cpDNA regions *ycf1*, the *ycf1-rpl15* spacer, and *trnL-F*; ML bootstrap values >70% shown near corresponding nodes.

Fig. S2. ML phylogram based upon nrITS; ML bootstrap values >50% shown near corresponding nodes.

Fig. S3. ML phylogram based upon combined datasets of nrITS and nrETS; ML bootstrap values >65% shown near corresponding nodes.

Fig. S4. ML phylogram based upon *PPR-AT3G09060*; ML bootstrap values ≥60% shown near corresponding nodes.

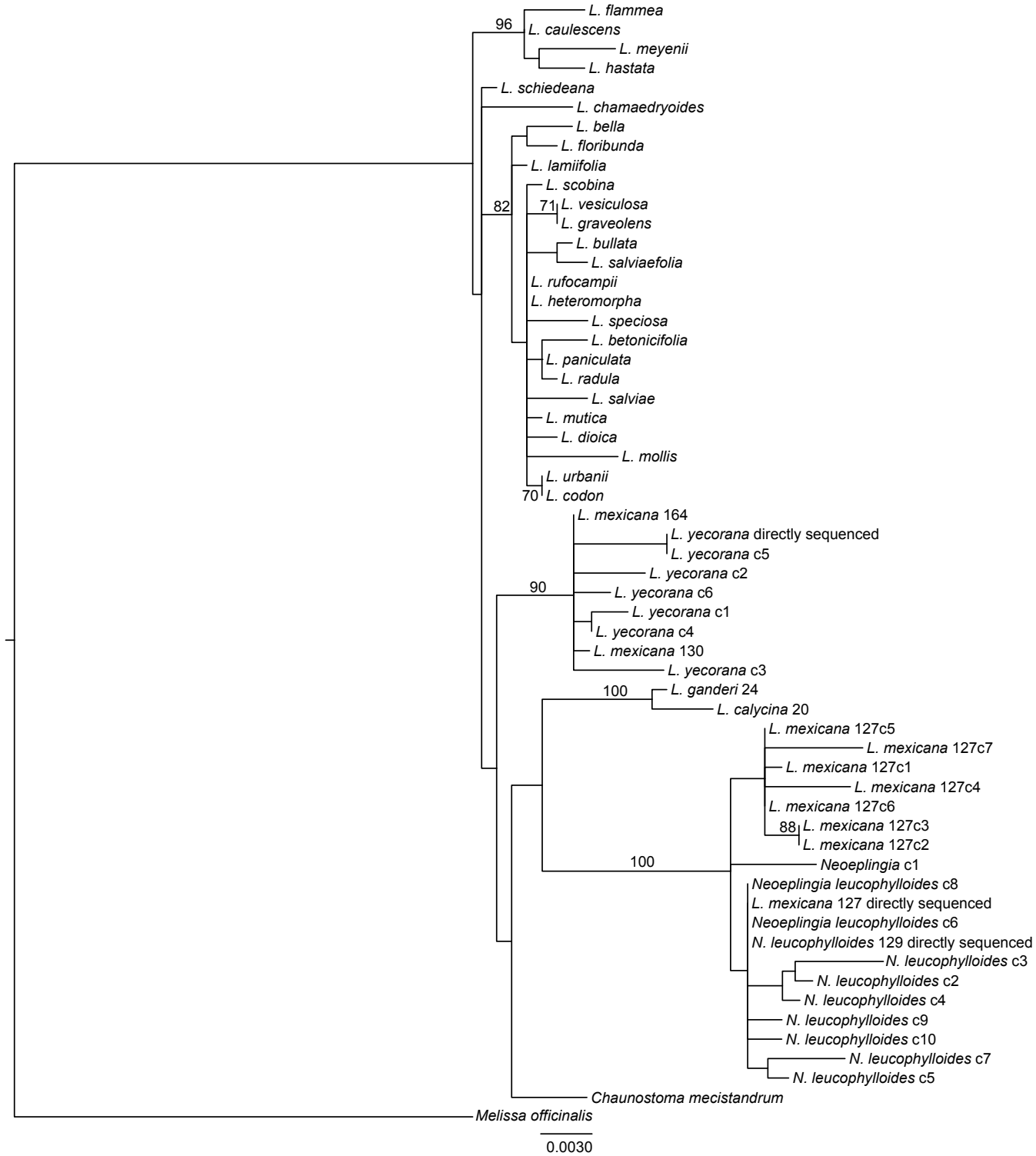

Fig. S5. ML phylogram based upon *GBSSI*; ML bootstrap values ≥70% shown near corresponding nodes.

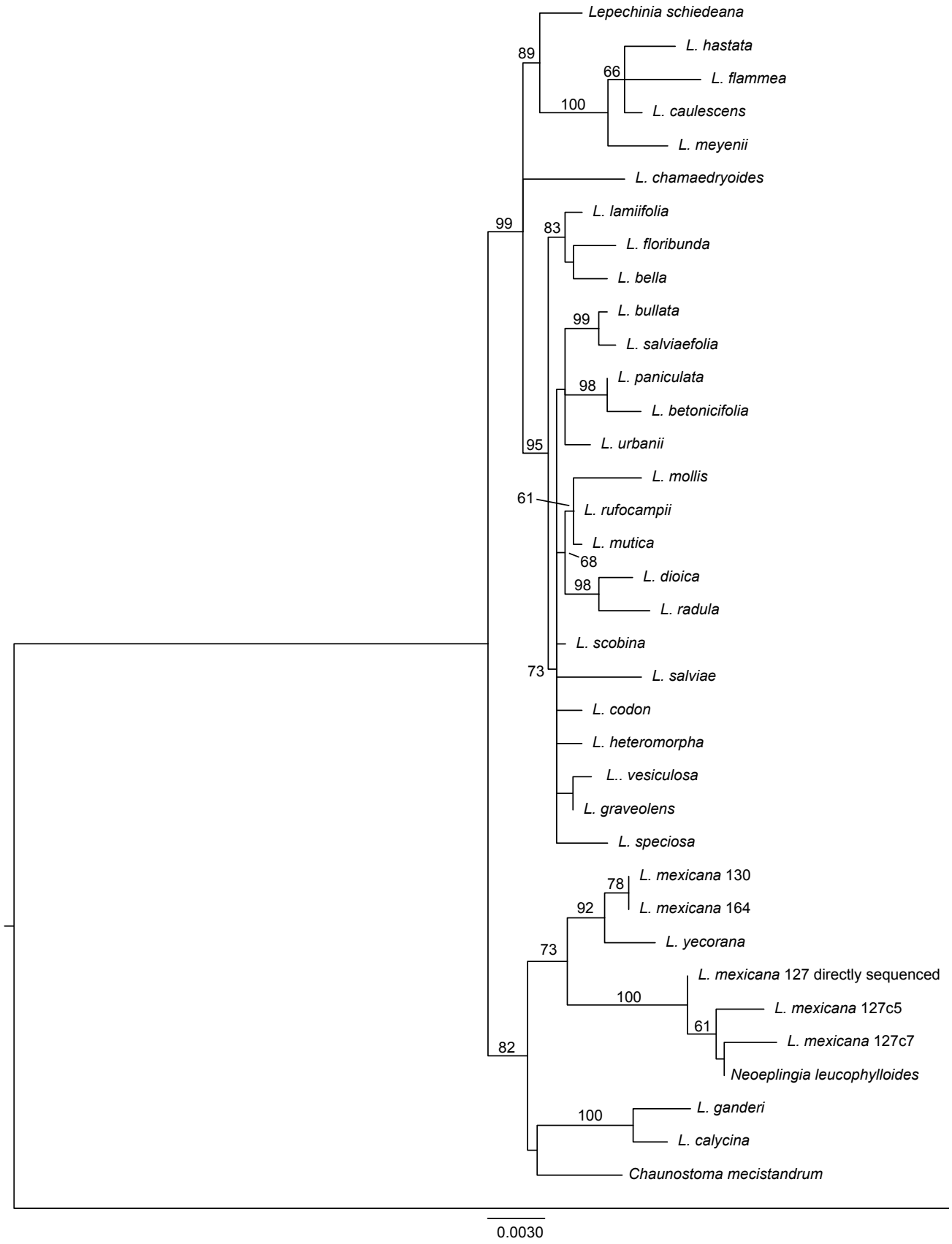

Fig. S6. ML phylogram based upon combined datasets of *PPR-AT3G09060* and *GBSSI*; ML bootstrap values >60% shown near corresponding nodes.