Systematic Botany (2004), 29(1): pp. 147–164

Paraphyly in Tribe Onagreae: Insights into Phylogenetic Relationships of Onagraceae Based on Nuclear and Chloroplast Sequence Data

RACHEL A. LEVIN,[1,7] WARREN L. WAGNER,[1] PETER C. HOCH,[2] WILLIAM J. HAHN,[3] AARON RODRIGUEZ,[4] DAVID A. BAUM,[5] LILIANA KATINAS,[6] ELIZABETH A. ZIMMER,[1] and KENNETH J. SYTSMA[5]

[1]Department of Systematic Biology, Botany, MRC 166, Smithsonian Institution, P. O. Box 37012, Washington, District of Columbia 20013-7012;
[2]Missouri Botanical Garden, P. O. Box 299, St. Louis, Missouri 63166-0299;
[3]108 White-Gravenor, Box 571003, Georgetown University, Washington, District of Columbia, 20057-1003;
[4]Departamento de Botaníca y Zoología, Apartado Postal 139, 45101 Zapopan, Jalisco, Mexico;
[5]Department of Botany, University of Wisconsin, 430 Lincoln Drive, Madison, Wisconsin 53706;
[6]Departamento Cientifico de Plantas Vasculares, Museo de Ciencias Naturales, Paseo del Bosque s/n, 1900 La Plata, Provincia de Buenos Aires, Argentina
[7]Author for correspondence (levin@biology.utah.edu)

Communicating Editor: Thomas G. Lammers

ABSTRACT. Onagraceae are a family of 17 genera in seven tribes, with the majority of species in tribes Onagreae and Epilobieae. Despite the species-richness of these two tribes, to date no phylogenetic study has been done with sufficient taxon sampling to examine relationships between and within these tribes. In this study, we used DNA sequence data from one nuclear region (ITS) and two chloroplast regions (*trnL-trnF* and *rps16*) to infer phylogenetic relationships among 93 taxa across the family, with concentrated sampling in the large tribe Onagreae. Results strongly suggest that tribe Gongylocarpeae is sister to tribes Epilobieae + Onagreae, both of which are monophyletic. Within Onagreae, *Camissonia* seems to be broadly paraphyletic, and *Oenothera* is also paraphyletic. In *Oenothera* there appear to be two lineages, one of which has *Gaura* + *Stenosiphon* nested within it. At the base of the Onagraceae phylogeny, we have clarified previous confusion regarding conflicting placements of *Hauya* and *Lopezia* based on nuclear versus chloroplast data. Results of these analyses are supported by morphology and suggest the need for new taxonomic delimitations, which are forthcoming.

The plant family Onagraceae (Evening-primroses) comprises ca. 655 species across 17 genera (Levin et al. 2003), with at least two thirds of the species occurring in tribes Onagreae (8 genera, 262 spp.) and Epilobieae (2 genera, 172 spp.). Onagraceae have a world-wide distribution, with the majority of species concentrated in the New World, especially western North America. Over the past few decades, the family has developed as a model system for studying plant evolution. Comparative studies of cytology, embryology, palynology, anatomy, morphology, reproductive biology, and chemistry have all been completed for various groups within the family (reviewed in Raven 1988). Unfortunately, a limitation of these previous studies has been the absence of a robust phylogenetic framework within which to examine the evolution of these traits.

To date there have been several molecular (Martin and Dowd 1986; Crisci et al. 1990; Sytsma et al. 1991b; Bult and Zimmer 1993; Conti et al. 1993) and morphological (Hoch et al. 1993) phylogenetic studies of the family, although only recently has there been a molecular study that included members of all Onagraceae genera (Levin et al. 2003). There have also been various phylogenetic studies of individual genera within the family, including *Fuchsia* (Sytsma and Smith 1988, 1992; Sytsma et al. 1991a; P. Berry et al., U. Wisconsin-Madison, in mss.), *Lopezia* (O'Kane and Schaal 1998), *Clarkia* (Sytsma and Smith 1988, 1992; Sytsma et al. 1990; Gottlieb and Ford 1996; Ford and Gottlieb 2003; W. J. Hahn et al., in mss.), *Epilobium* and *Chamerion* (Baum et al. 1994), and *Gaura* (Hoggard et al., 2004). However, no such study has focused on relationships among tribes Onagreae and Epilobieae. Furthermore, within Onagreae there have been no molecular phylogenetic studies of the species-rich genera *Camissonia* (62 spp.; western North America, 1 sp. in South America) and *Oenothera* (120 spp.; Americas, the majority of species in western North America).

Using chloroplast *rbcL* and *ndhF* sequence data, Levin et al. (2003) showed that the small genus *Gongylocarpus* (2 spp.), previously included in tribe Onagreae (Raven 1964, 1979; Munz 1965), is strongly supported as sister to the rest of Onagreae + Epilobieae, and should be placed in its own tribe, Gongylocarpeae. That analysis also suggested that neither *Camissonia* nor *Oenothera* is monophyletic, although sampling within these genera was limited. *Camissonia* appears to lack any morphological synapomorphies (Raven 1969; Hoch et al. 1993), and the only character uniting *Oenothera* (stigma with 4 linear elongate non-commissural lobes) also characterizes *Stenosiphon* and *Gaura* (Hoch et al. 1993; Hoggard et al., 2004); however, *Stenosiphon* and *Gaura* differ because of the presence of an indusium at the base of the stigma lobes.

Thus, a major goal of the present study is to understand relationships between and within tribes Onagreae and Epilobieae, with a particular emphasis on evaluating the monophyly of the large and diverse genera *Camissonia* and *Oenothera*. A phylogenetic framework will facilitate comparative analyses of chromosomal evolution and pollination biology of these diverse groups, as well as biogeographical studies of the radiation of these tribes in southwestern North America (Katinas et al. 2004).

While the main focus of this study is on Onagreae and Epilobieae, we have included sampling from members of all Onagraceae genera. This strategy is not only important for examining relationships among tribes Onagreae and Epilobieae, but inclusion of DNA sequence data from both nuclear and chloroplast regions allows examination of previous conflict among evolutionary reconstructions based on these two genomes and on morphology, especially as pertains to the placement of *Hauya* and *Lopezia* (Bult and Zimmer 1993; Conti et al. 1993; Hoch et al. 1993; Levin et al. 2003). The recently described genus *Megacorax* (González Elizondo et al. 2002) may be vital to discerning relationships of *Hauya* and *Lopezia* to the rest of the family, as Levin et al. (2003) found that *Megacorax* is sister to *Lopezia*. Because sampling of *Lopezia* species was limited in that study, it was unclear whether *Megacorax* should be placed within *Lopezia*. Thus, the present study includes additional sampling from various sections of *Lopezia* (Plitmann et al. 1973; O'Kane and Schaal 1998).

In this paper we endeavor to: 1) examine relationships between and within tribes Onagreae and Epilobieae, 2) test the monophyly of *Camissonia*, *Oenothera*, and *Gaura*, 3) compare signal from nuclear vs. chloroplast data, especially as it relates to earlier conflict regarding relationships of *Hauya* and *Lopezia*, and 4) further examine the sister taxon relationship previously reported between *Megacorax* and *Lopezia*. To accomplish these goals, we used DNA sequence data from one nuclear region (ITS) and two chloroplast regions, the *trnL-trnF* region (Taberlet et al. 1991) and the *rps16* intron (Oxelman et al. 1997; Popp and Oxelman 2001). These gene regions evolve more rapidly than the protein-coding *ndhF* and *rbcL* genes used in our earlier study (Levin et al. 2003).

Materials and Methods

Taxon Sampling. This study includes sampling from all eight tribes and 17 genera of Onagraceae, with a concentration on Onagreae and Epilobieae (Table 1). Within these two tribes we included at least one individual per section, subsection, or series, depending on current circumscriptions (Table 1). However, we did not sample from *Chamerion* sect. *Rosmarinifolium*, as *Chamerion* has previously been shown to be strongly monophyletic (Baum et al. 1994). We also did not include all of the subsections of *Clarkia*, as they are the subject of another analysis (W. J. Hahn et al., in mss.), and we discovered late in the analysis that our only sample of *Gaura* sect. *Campogaura* was misidentified. Thus, that section is not in our study, and instead we included both subspecies of *G. hexandra* (sect. *Pterogaura*). In the other six tribes, two taxa were sampled from *Ludwigia* (tribe Jussiaeeae) to serve as a monophyletic outgroup for phylogenetic analyses, given previous studies that unambiguously place this genus sister to the rest of Onagraceae (e.g., Levin et al. 2003). One species each from tribes Hauyeae, Fuchsieae, Circaeeae, and Gongylocarpeae was also included. In order to more precisely determine the relationship of the newly described monotypic genus *Megacorax* to *Lopezia* (tribe Lopezieae), we sampled four *Lopezia* species from various sections plus *Megacorax gracielanus*. The cp *trnL-trnF* and nuclear ITS regions were sequenced from a total of 93 taxa. The cp *rps16* region was sequenced from a subset of 75 species focused mainly in Onagreae, in order to improve resolution within this species-rich tribe. All taxa included in this study are listed in Table 1 with voucher information.

DNA Extraction, Amplification, and Sequencing. Total genomic DNA for the majority of taxa was provided by KJS (see protocols in Conti et al. 1996; Sytsma et al. 2002). However, several taxa were extracted by the senior author from either silica gel-dried or herbarium material using the Qiagen Dneasy™ kit (Qiagen Inc., Valencia, CA). DNAs of *Lopezia lopezioides*, *L. racemosa*, and *L. langmaniae* were provided by S. O'Kane (Univ. Northern Iowa), and DNAs of *Oenothera deltoides* and *O. pallida* were provided by M. Evans (Univ. Arizona).

ITS. Amplification of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA, composed of ITS1, the 5.8S gene, and ITS2 (Baldwin 1992; Baldwin et al. 1995) was mainly conducted by WJH using primers ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′; White et al. 1990) and ITS5HP (5′-GGA AGG AGA AGT CGT AAC AAG G-3′; Hershkovitz and Zimmer 1996); these primers were also used for those amplifications done by the senior author. Standard PCR conditions were used, although Ready-to-go PCR beads (Amersham Pharmacia Biotech Inc.) were employed for a few taxa that were difficult to amplify. PCR products were cleaned using PEG precipitation and ethanol cleaning (Morgan and Soltis 1993). Cycle sequencing used ABI Big Dye chemistry (Applied Biosystems, Foster City, CA), and was done in both directions using the same primers as for amplification. Additional sequencing primers were used by WJH, including ITS2 (5′-CGT AGC TAC TTC TTG CAT CG-3′; White et al. 1990), ITS3B (5′-GCA TCG ATG AAG AAC GTA GC-3′; White et al. 1990), and C5.8S (5′-TGC GTT CAA AGA CTC GAT-3′; Suh et al. 1993). ITS sequences for *Lopezia lopezioides*, *L. racemosa*, and *L. langmaniae* were provided by S. O'Kane (Univ. Northern Iowa), and the sequences for *Chamerion angustifolium*, all *Epilobium* species, and *Clarkia bottae* were previously published by Baum et al. (1994) (see GenBank accession numbers in Table 1).

trnL-trnF. Amplification of the *trnL* intron, *trnL* 3′ exon, and *trnL-trnF* intergenic spacer used primers "c" (5′-CGA AAT CGG TAG ACG CTA CG-3′) and "f" (5′-ATT TGA ACT GGT GAC ACG AG-3′) of Taberlet et al. (1991). PCR products were cleaned as described above. Cycle sequencing used ABI Big Dye chemistry, and was done in both directions using the same primers as for amplification. A few taxa have sequences with long repeats, resulting in incomplete cycle sequence products. For these taxa, cycle sequencing was conducted with additional internal primers (d: 5′-GGG GAT AGA GGG ACT TGA AC-3′, e: 5′-GGT TCA AGT CCC TCT ATC CC-3′; Taberlet et al. 1991).

rps16. Amplification of the *rps16* group II intron used the following primers adapted from Oxelman et al. (1997) and Popp and Oxelman (2001): forward primer P1840 (5′-GTG GTA AAA AGC AAC GCG CGA CTT-3′; similar to rpsF) and reverse primer P1839 (5′-TCG GGA TCG CAC ATC AAT TGC AAC-3′; similar to rpsR2). PCR products were cleaned as previously described. Sequencing used ABI Big Dye chemistry, and was done in both directions using the same primers as for amplification. Due to the same cycle sequencing problem with long repeats mentioned above, two additional internal primers were used in sequencing some taxa: forward primer P1895 (5′-GTG TAT CGT GCG GGA A-3′) and reverse primer P1896 (5′-GTA TTC TCA TAA CTC A-3′).

Cycle sequence products for all regions were precipitated and

TABLE 1. Taxa, vouchers, localities, and Genbank accession numbers for all sequences included in this study (**Megacorax* is currently not placed in any tribe; best affinity with Lopezieae). All tribes except Epilobieae and Onagreae contain a single genus; for these tribes, listed are the total number of species in that tribe and the number of sections (if relevant) currently circumscribed for that tribe's genus. For tribes Epilobieae and Onagreae, total number of species per genus and sections per genus are indicated, as are total number of species per section [except *Chamerion* sect. *Rosmarinifolium* (4 spp.) and *Gaura* sect. *Campogaura* (1 sp.)]. Sectional information and total species numbers are based on Raven (1969); Raven and Gregory (1972); Tobe et al. (1987); Baum et al. (1994); O'Kane and Schaal (1998); Levin et al. (2003); and Wagner et al. (in mss.).

OUTGROUP

Tribe Jussiaeeae (81 spp., 23 sects.)
Ludwigia peploides (Kunth) P. H. Raven—Alameda Co., CA, *Sytsma 5010* (WIS); nrITS AY271517, *trnL-trnF* AY264494, *rps16* AY267386. *Ludwigia ravenii* C. Peng—Berkeley Co., SC, *Peng 4402* (MO); nrITS AY271518, *trnL-trnF* AY264495.

INGROUP

Tribe Hauyeae (2 spp.)
Hauya elegans DC.—Esteli, Nicaragua, *Moreno 11352* (MO); nrITS AY271519, *trnL-trnF* AY264496.
Tribe Fuchsieae (105 spp., 10 sects.)
Fuchsia cyrtandroides J. W. Moore—Tahiti, Society Islands (Fr.), *Berry et al. 4618* (MO); nrITS AY271520, *trnL-trnF* AY264497.
Tribe Circaeeae (7 spp.)
Circaea alpina L.—Lincoln Co., WI, *Smith 1052* (WIS); nrITS AY271521, *trnL-trnF* AY264498.
Tribe Lopezieae (22 spp., 6 sects.)
Lopezia
Sect. *Pelozia*
Lopezia laciniata (Rose) Plitm., Raven & Breedl.—Durango, Mexico, *O'Kane 3341* (MO); nrITS AY271522, *trnL-trnF* AY264499.
Sect. *Jehlia*
Lopezia langmaniae Miranda—Chiapas, Mexico, *Breedlove 32300* (CAS); nrITS AY271523, *trnL-trnF* AY264500.
Sect. *Lopezia*
Lopezia racemosa Cav.—Queretaro, Mexico, *O'Kane 3374* (MO); nrITS AY271525, *trnL-trnF* AY264502.
Sect. *Diplandra*
Lopezia lopezioides (Hook. & Arn.) Plitm., Raven & Breedl.—Nayarit, Mexico, *O'Kane 3389* (MO); nrITS AY271524, *trnL-trnF* AY264501.
**Megacorax gracielanus* González & Wagner—Durango, Mexico, *Acevedo et al. 1352* (US); nrITS AY271526, *trnL-trnF* AY264503, *rps16* AY267387.
Tribe Gongylocarpeae (2 spp.)
Gongylocarpus fruticulosus (Benth.) Brandegee—Michoacán, Mexico, *Rzedowski 44253* (IEB); nrITS AY271527, *trnL-trnF* AY264504, *rps16* AY267388.
Tribe Epilobieae
Chamerion (Raf.) Raf. (8 spp., 2 sects.)
Sect. *Chamerion* (4 spp.)
Chamerion angustifolium (L.) Holub—Barron Co., WI, *Sytsma 5500* (WIS); nrITS L28011. King Co., WA, *Wagner 6917* (US); *trnL-trnF* AY264505, *rps16* AY267389.
Epilobium L. (164 spp., 7 sects.)
Sect. *Epilobium* (ca. 150 spp.)
E. ciliatum Raf.—Del Norte Co., CA, *Hoch 3487* (MO); nrITS L28015, *trnL-trnF* AY264508. *E. obcordatum* A. Gray—Harney Co., OR, *Seavey 1151* (MO); nrITS L28027. Lake Co., OR, *Ertter 15067* (JEPS); *trnL-trnF* AY264507. *E. rigidum* Hausskn.—Del Norte Co., CA, *Wiens 6797* (MO); nrITS L28030, *trnL-trnF* AY264506, *rps16* AY267390.
Sect. *Xerolobium* (1 sp.)
E. brachycarpum Presl—Yolo Co., CA, *Sytsma s.n.* (WIS); nrITS L28012, *trnL-trnF* AY264509.
Sect. *Crossostigma* (2 spp.)
E. minutum Lindl. ex Lehm.—Curry Co., OR, *Chambers 4847* (MO); nrITS L28025, *trnL-trnF* AY264510.
Sect. *Cordylophorum* (3 spp.)
E. nevadense Munz—Clark Co., NV, *Hoch 3440* (MO); nrITS L28026, *trnL-trnF* AY264511.
Sect. *Currania* (2 spp.)
E. pygmaeum (Speg.) Hoch & P. H. Raven—Butte Co., CA, *Broyles 1090* (MO); nrITS L28029, *trnL-trnF* AY264512.
Sect. *Boisduvalia* (4 spp.)
E. densiflorum (Lindl.) Hoch & P. H. Raven—Butte Co., CA, *Oswald 794* (CHSC); nrITS L28019, *trnL-trnF* AY264513.
Sect. *Zauschneria* (2 spp.)
E. canum (Greene) P. H. Raven—Los Angeles Co., CA, Cult. UC Bot. Gard. 59.1378; seed from *Beard & Beard,* coll. 1959 (UC); nrITS L28013, *trnL-trnF* AY264514, *rps16* AY267391.
Tribe Onagreae
Xylonagra Donn. Smith & Rose (1 sp.)
Xylonagra arborea (Kellogg) Donn. Sm. & Rose—Baja California, Mexico, *Warshall s.n.* (MO); nrITS AY271528, *trnL-trnF* AY264515, *rps16* AY267392.
Clarkia Pursh (42 spp., 11 sects.)
Sect. *Myxocarpa* (7 spp.)
C. mildrediae (A. Heller) F. H. Lewis & M. R. Lewis—Butte Co., CA, *Weeden 50* (DAV); nrITS AY271529, *trnL-trnF* AY264516, *rps16* AY267393.

TABLE 1. Continued.

Sect. *Rhodanthos* (6 spp.)
C. franciscana F. H. Lewis & P. H. Raven—San Francisco Co., CA, *Gottlieb F28-2-2* (DAV); nrITS AY271530, *trnL-trnF* AY264517, *rps16* AY267394.
Sect. *Clarkia* (1 sp.)
C. pulchella Pursh—Grant Co., OR, *Ford 8357* (DAV); nrITS AY271531, *trnL-trnF* AY264518, *rps16* AY267395.
Sect. *Eucharidium* (2 spp.)
C. concinna (Fischer & Meyer) Greene—Marin Co., CA, *Weeden 146-16-3* (DAV); nrITS AY271532, *trnL-trnF* AY264519.
Sect. *Godetia* (7 spp.)
C. imbricata F. H. Lewis & M. R. Lewis—Sonoma Co., CA, *Gottlieb PG-1* (DAV); nrITS AY271533, *trnL-trnF* AY264520, *rps16* AY267396.
Sect. *Fibula* (2 spp.)
C. bottæ (Spach) F. H. Lewis & M. R. Lewis—Los Angeles Co., CA, *Weeden 35-4* (DAV); nrITS L28016, *trnL-trnF* AY264521.
Sect. *Phaeostoma* (5 spp.)
C. xantiana A. Gray—Tulare Co., CA, *Gottlieb 7436* (DAV); nrITS AY271534, *trnL-trnF* AY264522, *rps16* AY267397.
Sect. *Sympherica* (9 spp.)
C. rostrata W. Davis—Mariposa Co., CA, *Weeden 97a* (DAV); nrITS AY271535, *trnL-trnF* AY264523, *rps16* AY267398.
Sect. *Biortis* (1 sp.)
C. affinis F. H. Lewis & M. R. Lewis—Solano Co., CA, *Weeden 79b* (DAV); nrITS AY271536, *trnL-trnF* AY264524, *rps16* AY267399.
Sect. *Connubium* (1 sp.)
C. delicata (Abrams) Nelson & Macbride—San Diego Co., CA, *Lewis 1461* (LA); nrITS AY271537, *trnL-trnF* AY264525, *rps16* AY267400.
Sect. *Heterogaura* (1 sp.)
C. heterandra (Torrey) F. H. Lewis & P. H. Raven—Tuolumne Co., CA, *Weeden 6* (DAV); nrITS AY271538, *trnL-trnF* AY264526, *rps16* AY267401.
Gayophytum A. Juss. (9 spp.)
G. heterozygum F. H. Lewis & Szweyk.—Shasta Co., CA, *Baldwin 923* (MO); nrITS AY271539, *trnL-trnF* AY264527, *rps16* AY267402.
Camissonia Link (62 spp., 9 sects).
Sect. *Eulobus* (4 spp.)
C. californica (Nutt. ex Torr. & A. Gray) P. H. Raven—Pima Co., AZ, *Schmidt & Merello 2581* (MO); nrITS AY271597, *trnL-trnF* AY264585, *rps16* AY267459. *C. crassifolia* (Greene) P. H. Raven—Baja California, Mexico, RSA seed coll. 16695; nrITS AY271540, *trnL-trnF* AY264528, *rps16* AY267403.
Sect. *Chylismia* (14 spp.)
C. claviformis (Torr. & Frém.) P. H. Raven—Kern Co., CA, RSA seed coll. 16710; nrITS AY271541, *trnL-trnF* AY264529, *rps16* AY267404.
Sect. *Lignothera* (2 spp.)
C. arenaria (A. Nelson) P. H. Raven—Yuma Co., AZ, *Raguso 98-22* (ARIZ); nrITS AY271543, *trnL-trnF* AY264531, *rps16* AY267406.
Sect. *Tetrapteron* (6 spp.)
C. ovata (Nutt. ex Torr. & A. Gray) P. H. Raven—Alameda Co., CA, *Ertter 13924* (JEPS); nrITS AY271544, *trnL-trnF* AY264532, *rps16* AY267407. *C. subacaulis* (Pursh) P. H. Raven—Adams Co., ID, *Smith 2808* (MO); nrITS AY271545, *trnL-trnF* AY264533, *rps16* AY267408. *C. tanacetifolia* (Torr. & A. Gray) P. H. Raven—Washoe Co., NV, *Tiehm 4528* (MO); nrITS AY271546, *trnL-trnF* AY264534, *rps16* AY267409. *C. graciliflora* (Hook. & Arn.) P. H. Raven—Riverside Co., CA, *Boyd 6162* (RSA); nrITS AY271547. Los Angeles Co., CA, *Boyd et al. 10095* (US); *trnL-trnF* AY264535, *rps16* AY267410.
Sect. *Holostigma* (14 spp.)
C. cheiranthifolia (Hornem. ex Spreng.) Raimann—Baja California, Mexico, *Raguso RAR98-16* (ARIZ); nrITS AY271548, *trnL-trnF* AY264536, *rps16* AY267411.
Sect. *Camissonia* (12 spp.)
C. kernensis (Munz) P. H. Raven—Kern Co., CA, *Howell & True 47888* (MO); nrITS AY271549, *trnL-trnF* AY264537, *rps16* AY267412. *C. campestris* (E. Greene) P. H. Raven—Kern Co., CA, RSA seed coll. 16706; nrITS AY271550, *trnL-trnF* AY264538, *rps16* AY267413.
Sect. *Eremothera* (7 spp.)
C. refracta (S. Watson) P. H. Raven—Riverside Co., CA, RSA seed coll. 17552; nrITS AY271551, *trnL-trnF* AY264539, *rps16* AY267414. *C. boothii* (Dougl.) P. H. Raven—Ventura Co., CA, RSA seed coll. 17783; nrITS AY271542, *trnL-trnF* AY264530, *rps16* AY267405. *C. nevadensis* (Kell) P. H. Raven—Washoe Co., NV, *Tiehm 11971* (MO); nrITS AY271552, *trnL-trnF* AY264540, *rps16* AY267415. *C. minor* (A. Nels.) P. H. Raven—Modoc Co., CA, *Bartholomew 6623* (MO); nrITS AY271553, *trnL-trnF* AY264541, *rps16* AY267416.
Sect. *Chylismiella* (1 sp.)
C. pterosperma (S. Watson) P. H. Raven—Inyo Co., CA, *Morefield & McCarty 3364* (MO); nrITS AY271554. Tooele Co., UT, *Windham 93-32* (MO); *trnL-trnF* AY264542, *rps16* AY267417.
Sect. *Nematocaulis* (2 spp.)
C. andina (Nutt.) P. H. Raven—Washoe Co., NV, *Tiehm 8089* (MO); nrITS AY271555, *trnL-trnF* AY264543, *rps16* AY267418.

TABLE 1. Continued.

Oenothera L. (120 spp., 14 sects.)
Sect. *Oenothera* (71 spp.)
O. organensis Munz—Doña Ana Co., NM, Cult. DUSS 76-0334 (*Emerson s.n.,* MO); nrITS AY271556, *trnL-trnF* AY264544, *rps16* AY267419. *O. maysillesii* Munz—Durango, Mexico, Cult. DUSS 81-195 (*Breedlove 18812,* MO); nrITS AY271557, *trnL-trnF* AY264545, *rps16* AY267420. *O. macrosceles* A. Gray—Coahuila, Mexico, Cult. DUSS 197 (*Wagner et al. 4096,* MO); nrITS AY271558, *trnL-trnF* AY264546, *rps16* AY267421. *O. stubbei* W. Dietr., W. L. Wagner & P. H. Raven—Nuevo León, Mexico, Cult. DUSS 791 (*Sanders et al. 1203,* MO); nrITS AY271559, *trnL-trnF* AY264547, *rps16* AY267422. *O. heterophylla* Spach—Houston Co., TX, *Wagner 6916* (US); nrITS AY271560, *trnL-trnF* AY264548, *rps16* AY267423. *O. laciniata* Hill—St. Francis Co., AR, *Hecht 21* (MO); nrITS AY271561, *trnL-trnF* AY264549, *rps16* AY267424. *O. pubescens* Willd. ex Spreng.—Michoacan, Mexico, Grown from seeds (*Rzedowski s.n.,* 25 Aug 1986, no voucher); nrITS AY271562, *trnL-trnF* AY264550, *rps16* AY267425. *O. affinis* Cambess—Buenos Aires, Argentina, Cult. DUSS 82-603 (*Hecht 125,* MO); nrITS AY271563, *trnL-trnF* AY264551, *rps16* AY267426. *O. elata* Kunth—San Mateo Co., CA, Cult. DUSS 89-72 (*Cleland s.n.,* MO); nrITS AY271564, *trnL-trnF* AY264552, *rps16* AY267427. *O. biennis* L.—New Brunswick, Canada, Cult. DUSS 91-313 (*Cleland s.n.,* MO); nrITS AY271565, *trnL-trnF* AY264553, *rps16* AY267428.
Sect. *Kleinia* (2 spp.)
O. albicaulis Pursh—Cochise Co., AZ, *Raguso RAR98-52* (ARIZ); nrITS AY271566, *trnL-trnF* AY264554, *rps16* AY267429.
Sect. *Ravenia* (3 spp.)
O. tubifera Ser.—Durango, Mexico, Cult. DUSS 0305, Stubbe s.n. seeds (*Breedlove 14321,* MO); nrITS AY271567, *trnL-trnF* AY264555, *rps16* AY267430.
Sect. *Eremia* (1 sp.)
O. primiveris A. Gray—Maricopa Co., AZ, *Wagner & Mill 4565* (MO); nrITS AY271568, *trnL-trnF* AY264556, *rps16* AY267431.
Sect. *Contortae* (1 sp.)
O. xylocarpa Coville—Mono Co., CA, Not vouchered, from same population as *DeDecker s.n.* (MO); nrITS AY271569, *trnL-trnF* AY264557, *rps16* AY267432.
Sect. *Pachylophus* (5 spp.)
O. caespitosa Nutt.—Ada Co., ID, *Wagner 6267,* no voucher; nrITS AY271570, *trnL-trnF* AY264558, *rps16* AY267433. *O. psammophila* (A. Nels. & J. F. Macbr.) W. L. Wagner, Stockhouse & Klein—Fremont Co., ID, *Raguso RAR01-56* (US); nrITS AY271571, *trnL-trnF* AY264559, *rps16* AY267434.
Sect. *Megapterium* (4 spp.)
O. brachycarpa A. Gray—Grant Co., NM, *Wagner 3811* (MO); nrITS AY271572, *trnL-trnF* AY264560, *rps16* AY267435.
Sect. *Paradoxus* (1 sp.)
O. havardii S. Watson—Brewster Co., TX, *Powell 6175* (MO); nrITS AY271573, *trnL-trnF* AY264561, *rps16* AY267436.
Sect. *Lavauxia* (5 spp.)
O. flava (A. Nelson) Garrett—Apache Co., AZ, *Wagner 3796* (MO); nrITS AY271574, *trnL-trnF* AY264562, *rps16* AY267437. *O. acutissima* W. L. Wagner—Daggett Co., UT, *Raguso RAR01-65* (US); nrITS AY271575, *trnL-trnF* AY264563.
Sect. *Gauropsis* (2 spp.)
O. canescens Torr. & Frém.—Lubbock Co., TX, *Robbins 1820* (MO) (*Sytsma 5030,* WIS); nrITS AY271576, *trnL-trnF* AY264564, *rps16* AY267438.
Sect. *Xylopleurum* (1 sp.)
O. speciosa Nutt.—East Baton Rouge Parish, LA, *Zimmer 48-86* (LSU) (*Sytsma 5024,* WIS); nrITS AY271577, *trnL-trnF* AY264565, *rps16* AY267439.
Sect. *Hartmannia* (10 spp.)
O. rosea L'Hér. ex Ait.—Durango, Mexico, *Wagner & Brown 3960* (MO); nrITS AY271578, *trnL-trnF* AY264566, *rps16* AY267440. *O. tetraptera* Cav.—México, Mexico, Rzedowski s.n. in 1986, no voucher; nrITS AY271579, *trnL-trnF* AY264567, *rps16* AY267441. *O. multicaulis* Ruíz & Pavon—Prov. Loja, Ecuador, *Ellemann 66724* (MO); nrITS AY271580, *trnL-trnF* AY264568, *rps16* AY267442.
Sect. *Kneiffia* (5 spp.)
O. fruticosa L.—Dane Co., WI, Cult. U. WI Bot. Gard., *Sytsma 5025* (WIS); nrITS AY271581, *trnL-trnF* AY264569, *rps16* AY267443. *O. linifolia* Nutt.—St. Francois Co., MO, *Solomon 21279* (MO); nrITS AY271598, *trnL-trnF* AY264586, *rps16* AY267460.
Sect. *Anogra* (9 spp.)
O. neomexicana (Small) Munz—Graham Co., AZ, *Raguso RAR98-167* (ARIZ); nrITS AY271582, *trnL-trnF* AY264570, *rps16* AY267444. *O. pallida* Lindl.—Coconino Co., AZ, *Raguso RAR96-05* (ARIZ); nrITS AY271583, *trnL-trnF* AY264571, *rps16* AY267445. *O. deltoides* Torr. & Frem.—Yuma Co., AZ, *Raguso RAR99-01* (ARIZ); nrITS AY271584, *trnL-trnF* AY264572, *rps16* AY267446.
Calylophus Spach (6 spp., 2 sects)
Sect. *Salpingia* (4 spp.)
C. hartwegii (Benth.) P. H. Raven—Lubbock Co., TX, *Robbins s.n.* (MO); nrITS AY271585, *trnL-trnF* AY264573, *rps16* AY267447.
Sect. *Calylophus* (2 spp.)
C. berlandieri Spach—Lubbock Co., TX, *Robbins s.n.* (MO) (*Sytsma 5021,* WIS); nrITS AY271586, *trnL-trnF* AY264574, *rps16* AY267448.
Stenosiphon Spach (1 sp.)
S. linifolius (Nutt.) Heynh.—Pottawatomie Co., KS, *Barkley s.n.* (KSC); nrITS AY271587, *trnL-trnF* AY264575, *rps16* AY267449.
Gaura L. (21 spp., 8 sects.)
Sect. *Gauridium* (1 sp.)
G. mutabilis Cav.—Hidalgo, Mexico, *Rzedowski s.n.* (MO); nrITS AY271588, *trnL-trnF* AY264576, *rps16* AY267450.

TABLE 1. Continued.

Sect. *Schizocarya* (1 sp.)
G. parviflora Douglas ex Lehm.—Jeff Davis Co., TX, *Clinebell 2017* (MO); nrITS AY271589, *trnL-trnF* AY264577, *rps16* AY267451.
Sect. *Xerogaura* (2 spp.)
G. macrocarpa Rothr.—Brewster Co., TX, *Clinebell 3077* (MO); nrITS AY271590, *trnL-trnF* AY264578, *rps16* AY267452. *G. boquillensis* P. H. Raven & Gregory—Brewster Co., TX, *Clinebell 3074* (MO); nrITS AY271591, *trnL-trnF* AY264579, *rps16* AY267453.
Sect. *Pterogaura* (4 spp.)
G. hexandra Gómez Ortega subsp. *gracilis*—Brewster Co., TX, *Clinebell 2023* (MO); nrITS AY271592, *trnL-trnF* AY264580, *rps16* AY267454. *G. hexandra* Gómez Ortega subsp. *hexandra*—Durango, Mexico, *Clinebell 3031* (MO); nrITS AY271594, *trnL-trnF* AY264582, *rps16* AY267456.
Sect. *Gaura* (6 spp.)
G. demareei P. H. Raven & Gregory—Garland Co., AR, Cult. St. Louis, *Hoch 3574* (MO); seed from Clinebell s.n.; nrITS AY271593, *trnL-trnF* AY264581, *rps16* AY267455.
Sect. *Xenogaura* (1 sp.)
G. drummondii (Spach) Torr. & A. Gray—Archer Co., TX, *Hoggard 409* (OKL); nrITS AY271595, *trnL-trnF* AY264583, *rps16* AY267457.
Sect. *Stipogaura* (5 spp.)
G. villosa Torr.—Union Co., NM, *Clinebell 2052* (MO); nrITS AY271596, *trnL-trnF* AY264584, *rps16* AY267458.

cleaned with isopropanol before sequencing on an ABI 377 automated sequencer. ITS cycle sequence products that were not generated by the senior author were cleaned using Centri-Sep columns (Princeton Separations, Adelphi, NJ).

Sequence Alignment. Sequences were edited in Editview version 1.0.1 (Applied Biosystems, 1996), and the sequences from all primers were aligned and edited using Autoassembler™ DNA Sequence Assembly Software version 1.4.0 (Applied Biosystems, 1989–95) to construct a consensus sequence for each species [Sequencher version 3.0 (Gene Codes Corp., Ann Arbor, Michigan) was used for most ITS sequences]. Species sequences were then aligned manually in SeqApp (Gilbert 1993). These alignments were imported into MacClade 4.0 (Maddison and Maddison 2000) and executed in PAUP*4.0b10 (Swofford 2002). The alignments of all three gene regions are available on TreeBASE (study accession number S941, matrix accession numbers M1559 and M1560).

Phylogenetic Analyses. The three data sets were analyzed separately (Table 2), and in various combinations with other data sets (see below). Parsimony analyses were conducted in PAUP* using heuristic searches with 100 random addition sequence replicates, TBR branch-swapping, and steepest descent. Constant characters were excluded, and gaps were treated as missing data. Following the analysis protocol of Zimmer et al. (2002), each addition replicate was limited to 200 trees that were greater than or equal to the shortest trees for each replicate. This was necessary due to large numbers of equal length trees. The strength of support for individual tree branches was estimated using bootstrap values (BS) (Felsenstein 1985) and decay indices (DI) (Bremer 1988; Donoghue et al. 1992). Bootstrap values were from 500 full heuristic bootstrap replicates, each with 10 random addition sequence replicates. The MulTrees option was not in effect, and constant characters were excluded. Decay values for each branch were determined by first using the PAUP decay index command file in MacClade to prepare a set of trees each with a single branch resolved. To find the shortest trees consistent with each constraint, this file was executed in PAUP* using the heuristic search option with 100 random addition sequence replicates and the Multrees option turned off. The decay index for each branch is the difference in length between the shortest trees consistent with each constraint and the globally shortest trees.

NUCLEAR ITS. Parsimony analysis was conducted as above, with two species of *Ludwigia* defined as a monophyletic outgroup (Levin et al. 2003).

CHLOROPLAST *TRNL-TRNF* AND *RPS*16. Parsimony analyses of each of these two data sets were conducted as described above. The two species of *Ludwigia* were defined as a monophyletic outgroup for the *trnL-trnF* analysis; because only a single species of *Ludwigia*, *L. peploides*, was sampled for *rps16,* this species was defined as the outgroup for the *rps16* analysis. Congruence of the 75 taxon *trnL-trnF* and *rps16* data sets was tested using a partition homogeneity test (ILD; Farris et al. 1994, 1995) as implemented in PAUP*. One hundred heuristic partition homogeneity replicates were completed, each with 10 random addition sequence replicates, TBR branch-swapping, and gaps treated as missing data. Constant characters were excluded, and the MulTrees option was not in effect. Parsimony analysis was also conducted with the *trnL-trnF* and *rps16* data sets combined, with *L. peploides* defined as the outgroup.

NUCLEAR AND CHLOROPLAST DATA. To test for congruence among the nrITS data set and the chloroplast data sets, PAUP* was used to conduct pairwise ILD tests of the 93 taxon ITS and *trnL-trnF* data sets and the 75 taxon ITS and *rps16* data sets, and a simultaneous ILD test with the 75 taxon data sets for all three regions (settings for ILD tests are as above). Parsimony analyses were then conducted using a combined data set of ITS and *trnL-trnF* sequence data, as well as a data set including all three genomic regions. Because a subset of taxa were sampled for *rps16,* fewer taxa are included in the analysis of all data sets than in the analysis of the ITS + *trnL-trnF* data sets. Two species of *Ludwigia* were defined as a monophyletic outgroup for the ITS + *trnL-trnF*

TABLE 2. Comparison of the 75 taxa data sets for the nrITS and two cp regions. Parsimony-informative = PI; consistency index = CI (RC = rescaled CI); retention index = RI.

	nrITS	*trnL-trnF*	*rps16*
Range of raw length	572–606 bp	743–957 bp	790–838 bp
Aligned length	663 bp	1174 bp	1009 bp
Variable sites (proportion)	312 (0.47)	347 (0.30)	318 (0.32)
PI sites (proportion)	176 (0.27)	153 (0.13)	163 (0.16)
Pairwise distance ranges	0–0.26	0–0.077	0–0.092
CI (RC); RI	0.56 (0.37); 0.66	0.73 (0.59); 0.80	0.72 (0.58); 0.81

analysis. For the analysis of all three data sets combined, *Ludwigia peploides* was defined as the outgroup.

INDELS. Using the combined data set of all three regions, indels greater than 1 bp were coded as separate binary characters. Only indels that were identical in length and bases were included. Parsimony analysis was conducted on all three data sets combined, with indels included as additional characters.

MAXIMUM LIKELIHOOD. An analysis using a maximum likelihood (ML) model was conducted with all three data sets combined. ML model parameters were determined by using Modeltest v. 3.06 (Posada and Crandall 1998). This program tests the fit of 56 substitution models to the data; based on a hierarchical likelihood ratio test, a model that best fits the data is identified. The best model was used in a ML analysis in PAUP*, using the heuristic search option, starting tree determined by neighbor-joining, TBR branch-swapping, and MulTrees option in effect. As above, *Ludwigia peploides* was defined as the outgroup.

ALTERNATIVE TOPOLOGIES. Constraint trees were constructed in MacClade to test alternative phylogenetic hypotheses, including the monophyly of each of the following three genera: *Camissonia*, *Oenothera*, and *Gaura*. These trees were loaded into PAUP*, and heuristic searches were conducted to find the shortest trees consistent with each constraint. The number of additional steps required for a given constraint is the difference between the shortest trees consistent with a particular constraint and the globally shortest trees. Further, one-tailed non-parametric Shimodaira-Hasegawa tests (S-H test; Shimodaira and Hasegawa 1999; see also Goldman et al. 2000) were conducted in PAUP* to assess the statistical support for these constraints, using the same ML parameters outlined above. In this procedure, the likelihoods of all the shortest trees constrained to contain a particular lineage of interest were compared with the likelihood of a random most-parsimonious (MP) tree from the unconstrained analysis. The time efficient RELL method was used, with 1000 bootstrap replicates.

RESULTS

Nuclear ITS. ITS sequences for 93 taxa ranged in length from 572–617 bp, with an aligned length of 680 characters, including ITS1, the 5.8S rRNA gene, and ITS2. Of these 680 characters, 243 were parsimony-informative across all 93 taxa. Among these species, % missing data ranged from 0–13.1%, with a mean of 0.5% and a median of 0%. Although a number of clades near the tips are well supported, overall there was not strong signal (i.e., low CI/RI) in the ITS data set (CI=0.51, RI=0.73, RC=0.37), resulting in a general lack of resolution and low support for many nodes (tree not shown). However, *Fuchsia* + *Circaea* (BS=100; DI=14) and *Lopezia* + *Megacorax* (BS=81; DI=4) are well supported clades. Further, the genus *Epilobium* is strongly monophyletic (BS=100; DI=13), as is *Clarkia* (BS=100; DI=12). In general, the topology is as in Fig. 1 (ITS + *trnL-trnF*, see below), but with less support for relationships.

***Chloroplast* trnL-trnF.** *trnL-trnF* sequences for 93 taxa ranged in length from 729–957 bp, with an aligned length of 1204 characters. Of these characters, 218 were parsimony-informative. Taxon sequences contained no missing data, except for one with 0.2% and another with 0.6% missing data. Compared to the phylogeny inferred from the nrITS data alone, the phylogeny inferred from *trnL-trnF* data is somewhat more resolved (tree not shown). There is strong support for Gongylocarpeae + Epilobieae + Onagreae (BS=94; DI=4). Additionally, Onagreae + Epilobieae are well supported (BS=97; DI=5), and Epilobieae is strongly supported as monophyletic (BS=98; DI=7) including *Chamerion angustifolium*, which appears sister to a monophyletic *Epilobium* (BS=97; DI=5). Additionally, *trnL-trnF* yielded better resolution within Onagreae than did ITS (see details from combined analysis below). Results of an ILD test for congruence between the ITS and *trnL-trnF* data sets strongly suggest that the data sets are congruent (P=0.33). Thus, the two data sets were combined (Fig. 2).

***Nuclear ITS and cp* trnL-trnF.** In general, analysis of the two data sets combined (Fig. 1) yielded more robust support and greater clade resolution than the analyses of the separate data sets. There is moderate support for *Hauya* as sister to all Onagraceae except *Ludwigia* and for the monophyly of the rest of Onagraceae above *Ludwigia* and *Hauya* (BS=73; DI=5). As with the analyses of the separate data sets, the sister relationship between *Fuchsia* and *Circaea* is well supported (BS=100; DI=19). Although *Megacorax gracielanus* clearly forms a clade with the genus *Lopezia* (BS=99; DI=14), it is apparent that *M. gracielanus* is sister to a monophyletic *Lopezia* (BS=100; DI=16). However, there is only limited support for the monophyly of Onagraceae above the early diverging tribes of Jussiaeeae, Hauyeae, Circaeeae, and Fuchsieae (BS=57; DI=2).

Tribes Gongylocarpeae + Epilobieae + Onagreae are well supported as a monophyletic group (Node A; BS=92; DI=5), with *Gongylocarpus* sister to Epilobieae + Onagreae (BS=98; DI=9). Within this lineage, the monophyly of tribe Epilobieae is strongly supported (BS=97; DI=8), and *Chamerion angustifolium* is sister to a monophyletic *Epilobium* (BS=100; DI=21). In the phylogeny inferred from ITS data alone, the relationship of *Chamerion* to *Epilobium* was not resolved.

Onagreae is also supported as monophyletic (Node B; BS=82; DI=5), with very weak support for *Xylonagra* as sister to the rest of Onagreae (BS<50; DI=1). Within Onagreae relationships are somewhat equivocal, but a number of lineages are well supported. *Clarkia* is clearly monophyletic (BS=100; DI=21), as is *Camissonia* sect. *Tetrapteron* excluding *C. graciliflora* (*C. ovata* + *C. subacaulis* + *C. tanacetifolia*; BS=100; DI=14). Relationships among these three *Camissonia* species and *Clarkia*, *Gayophytum heterozygum*, and *Camissonia pterosperma* remain unclear (BS<50, DI=1). Among the rest of the *Camissonia* species sampled, the monophyly of sections *Eremothera* (*C. boothii* + *C. minor* + *C. nevadensis* + *C. refracta*; BS=100; DI=13) and *Camissonia* (*C. kernensis* + *C. campestris*; BS=100; DI=11) is strongly supported. Further, *Camissonia graciliflora* (sect. *Tetrapteron*) and *C. cheiranthifolia* (sect. *Holostigma*) form a weakly supported clade (BS=64; DI=1). Although

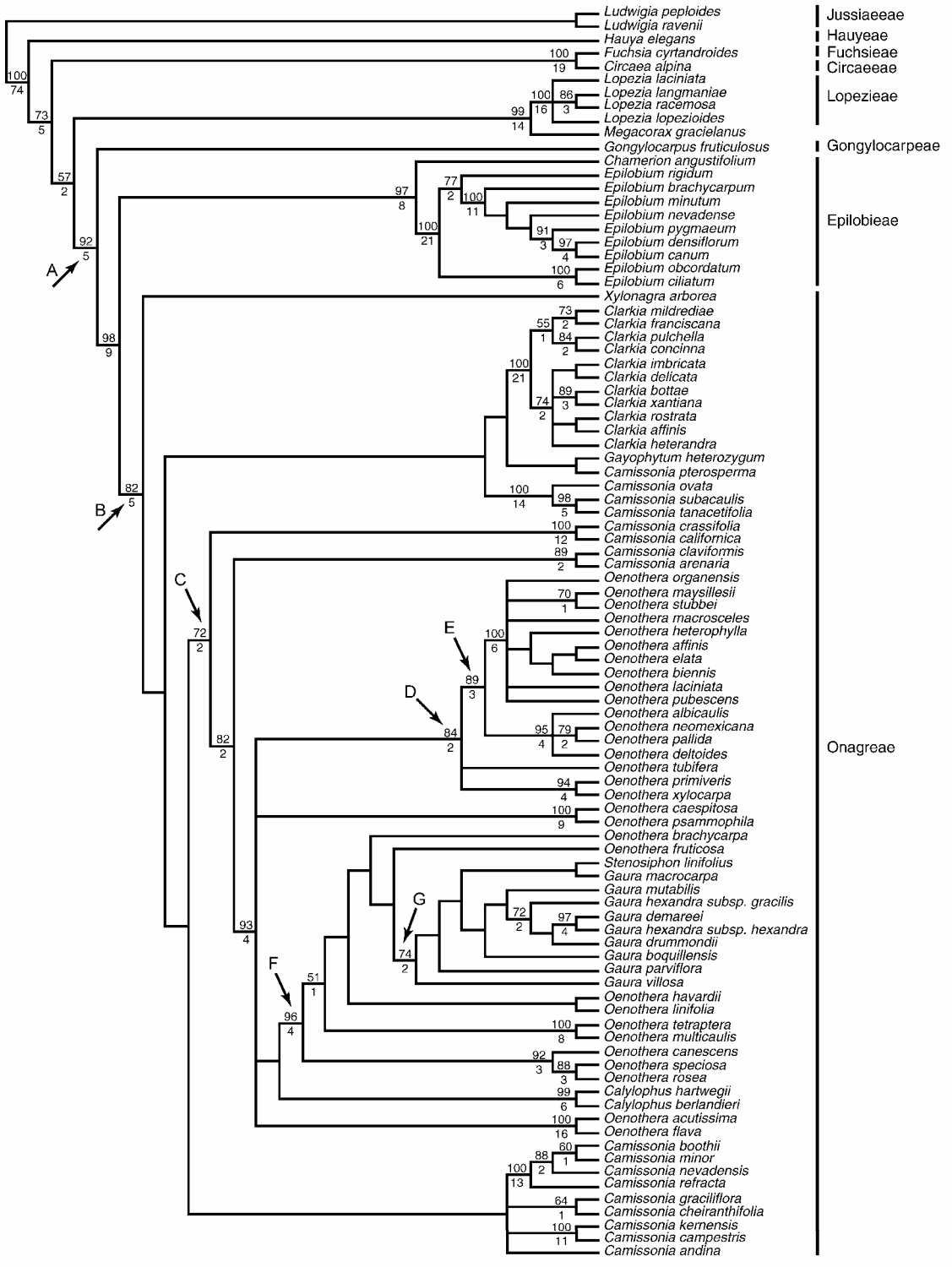

FIG. 1. Strict consensus of 13275 MP trees (TL=1912, rescaled CI=0.44, RI=0.76) from the combined analysis of ITS + *trnL-trnF* data (93 taxa). Nodes with bootstrap values (BS) >50% and decay indices (DI) >0 are indicated, with BS listed above the node and DI below. Tribes to which the taxa belong are listed to the right, and specific nodes of interest are labeled with letters; see text for discussion.

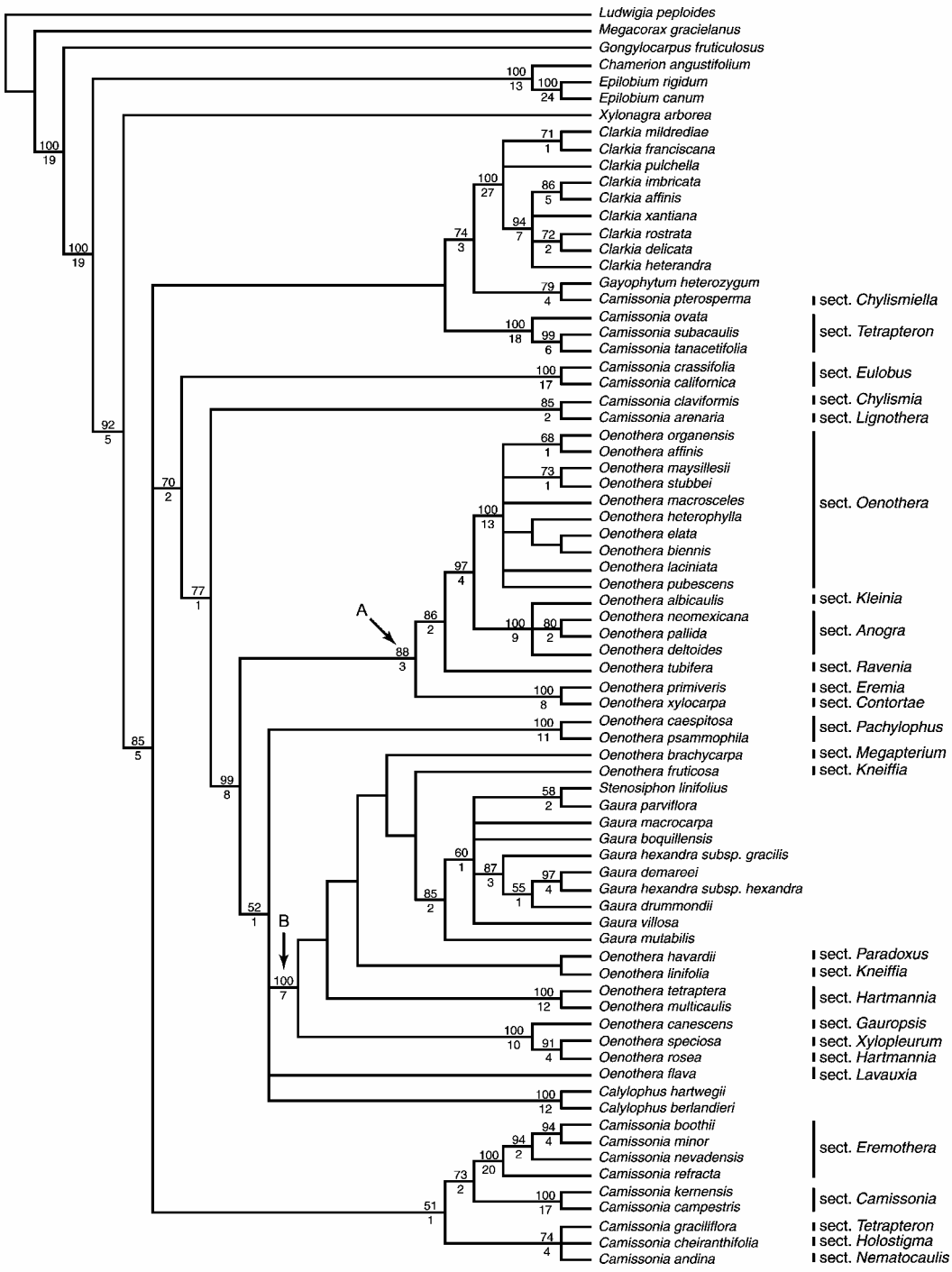

FIG. 2. Strict consensus of 18200 MP trees (TL=2018, rescaled CI=0.47, RI=0.73) from the combined analysis of nrITS and cp *trnL-trnF* and *rps16* data (75 taxa). Nodes with bootstrap values (BS) >50% and decay indices (DI) >0 are indicated, with BS listed above the node and DI below. Current sectional affiliations are listed to the right for all *Camissonia* and *Oenothera* species. The two main lineages of *Oenothera* species are indicated as "A" and "B"; see text for discussion.

many of the sections within *Camissonia* are strongly supported, there is little resolution for relationships among these lineages.

However, there is moderate support for a clade comprising the remainder of Onagreae, including four species of *Camissonia* + *Oenothera* + *Calylophus* + *Gaura* + *Stenosiphon* (Node C; BS=72; DI=2). Within this lineage the monophyletic *Camissonia* sect. *Eulobus* (*C. crassifolia* + *C. californica*; BS=100; DI=12) appears sister to the rest of the lineage (BS=82; DI=2). The next branch within this clade consists of *Camissonia claviformis* (sect. *Chylismia*) + *C. arenaria* (sect. *Lignothera*) (BS=89; DI=2), which is strongly supported as sister to *Oenothera* + *Calylophus* + *Gaura* + *Stenosiphon* (BS=93; DI=4). In this well supported clade, *Oenothera* is paraphyletic relative to the other three genera. A lineage composed of *Oenothera* sects. *Oenothera*, *Kleinia*, *Anogra*, *Ravenia*, *Eremia*, and *Contortae* has moderate support (Node D; BS=84; DI=2), as does a nested clade of sects. *Oenothera* + *Kleinia* + *Anogra* (Node E; BS=89; DI=3). Further, sects. *Kleinia* + *Anogra* (*O. albicaulis* + *O. neomexicana* + *O. pallida* + *O. deltoides*; BS=95; DI=4) are clearly sister to a monophyletic sect. *Oenothera* (*O. organensis* through *O. pubescens*; BS=100; DI=6).

The monophyly of *Oenothera* sects. *Pachylophus* (*O. caespitosa* + *O. psammophila*; BS=100; DI=9) and *Lavauxia* (*O. acutissima* + *O. flava*; BS=100; DI=16) is strongly supported, but their relationships to the rest of the genus are obscure (Fig. 1). Similarly, the small genus *Calylophus* appears strongly monophyletic (BS=99; DI=6), but relationships of this genus to specific sections of *Oenothera* are uncertain. However, there is strong support for a lineage composed of the rest of *Oenothera* + *Gaura* + *Stenosiphon* (Node F; BS=96; DI=4). Among the *Oenothera* species in this group there is limited resolution, although there is strong support for *O. canescens* + *O. speciosa* + *O. rosea* (BS=92; DI=3) and for a sister taxon relationship between *O. speciosa* and *O. rosea* (BS=88; DI=3). *Oenothera tetraptera* + *O. multicaulis* are also well supported as sister taxa (BS=100; DI=8). Further, there is moderate support for the monophyly of *Gaura* + *Stenosiphon linifolius* (Node G; BS=74; DI=2).

***Chloroplast* rps16.** Sequences of *rps16* for 75 taxa ranged in length from 790–838 bp, with an aligned length of 1009 bp (Table 2). Of these characters, 163 were parsimony-informative. Within this data set, percent missing data per species ranged from 0–2.6%, with a mean of 0.1% and a median of 0%. The strict consensus of most-parsimonious trees differs from Fig. 2 (all data sets combined, see below) only in the level of support and resolution of a few nodes; thus, the topology is not shown. Results of an ILD test conducted with *trnL-trnF* and *rps16* data suggest that the two cp data sets are highly congruent (P=0.63).

***Chloroplast* trnL-trnF *and* rps16.** There is strong agreement between the topologies inferred from the nrITS and *trnL-trnF* data sets combined (Fig. 1) and the *trnL-trnF* and *rps16* data sets combined (tree not shown), but note that the former includes 93 taxa and the latter 75 taxa. Differences in the two topologies are mainly due to differing levels of support for nodes, with clades inferred from the *trnL-trnF* + *rps16* data set often having higher bootstrap support than those inferred from the ITS + *trnL-trnF* analysis. The majority of conflicts, especially those relating to *Camissonia*, are associated with low resolution in at least one of the two combined analyses. However, within *Clarkia* the nuclear and cp data support different topologies. For example, the ITS data strongly support *Clarkia rostrata* as sister to *C. affinis* (BS=97; DI=3), whereas the cp analysis suggests strong support for the sister relationships of *C. rostrata* + *C. delicata* (BS=91; DI=3) and *C. imbricata* + *C. affinis* (BS=99; DI=8).

All Data Sets Combined. Results of an ILD test comparing all three data sets simultaneously suggest significant incongruence (P=0.01). However, there is evidence that a value between 0.01 and 0.001 (rather than the traditional P=0.05) is the more appropriate critical value for incongruence (Cunningham 1997). Otherwise a significant ILD test may simply reflect differing amounts of signal among data sets (Davis et al. 1998; Yoder et al. 2001; but see Hipp et al. in press), a difference that is also found in our data sets (Table 2). When the pairwise comparisons are done across all the data sets, it is clear that the incongruent data sets are ITS and *rps16* (P=0.01). The above-mentioned conflict between the placement of *Clarkia* species in the ITS-only and cp-only topologies suggests that these taxa are the source of the data set incongruence. Thus, additional ILD tests were conducted using the ITS and *rps16* data sets excluding both *Clarkia rostrata* and *C. affinis* and excluding each of these taxa separately. All three of these additional analyses yielded insignificant P-values (both taxa excluded, P=0.53; *C. rostrata* excluded, P=0.07; *C. affinis* excluded, P=0.39); however, it appears that the allopolyploid *C. affinis* is the greatest source of incongruence. Therefore, the data sets are not completely incongruent, only the nrITS and *rps16* sequence data for a few taxa. Consequently, we believe that it is useful to analyze all data sets together. The combined data set included 2893 characters for 75 taxa, of which 492 were parsimony-informative.

Overall, the phylogeny inferred from the combined analysis of all data sets (Fig. 2) is very similar to that shown in Fig. 1, although generally with greater (occasionally less) support for many branches. There is strong support for a clade of Onagreae + Epilobieae (BS=100; DI=19), with *Gongylocarpus* sister to this clade. Tribe Epilobieae is also monophyletic (BS=100; DI=13), and Onagreae is supported as monophyletic

(BS=92; DI=5), with moderate support for *Xylonagra* as sister to the rest of Onagreae (BS=85; DI=5). Within this clade relationships are less well resolved. However, *Clarkia* is clearly monophyletic (BS=100; DI=27), and there is moderate support for *Gayophytum* + *Camissonia pterosperma* (BS=79; DI=4) as sister to *Clarkia* (BS=74; DI=3).

In accord with the other analyses of the data sets individually and in combination, the majority of *Camissonia* species do not appear to comprise a monophyletic group; rather species groups form monophyletic lineages (often corresponding to recognized sections) that are basal to the remainder of the taxa in the tribe (Fig. 2). Thus, there is moderate support for a clade composed of *Camissonia claviformis* + *C. arenaria* + *Oenothera* + *Calylophus* + *Gaura* + *Stenosiphon* (BS=77; DI=1). Within this clade there is strong support for *Oenothera* + *Calylophus* + *Gaura* + *Stenosiphon* (BS=99; DI=8), but *Oenothera* is paraphyletic. A clade composed of *Oenothera* sects. *Oenothera*, *Ravenia*, *Kleinia*, *Eremia*, *Anogra*, and *Contortae* is well supported (A in Fig. 2; BS=88; DI=3). In this lineage, *O. primiveris* + *O. xylocarpa* (BS=100; DI=8) are sister to the rest of the clade (BS=86; DI=2), and *O. tubifera* is sister to sects. *Oenothera* + *Kleinia* + *Anogra* (BS=97; DI=4). Sections *Kleinia* and *Anogra* form a monophyletic lineage (BS=100; DI=9) that is sister to the strongly supported sect. *Oenothera* (BS=100; DI=13).

Sister to lineage A is a weakly supported group composed of the other *Oenothera* sections + *Calylophus* + *Gaura* + *Stenosiphon* (BS=52; DI=1). Although *Oenothera* sect. *Pachylophus* is strongly supported as monophyletic (BS=100; DI=11), as is *Calylophus* (BS=100; DI=12), relationships of these clades to the other taxa in this group remain equivocal. Likewise, the relationship of *Oenothera flava* (sect. *Lavauxia*) to other taxa in this group is uncertain.

A lineage including the rest of *Oenothera* + *Gaura* + *Stenosiphon* is well supported (B in Fig. 2; BS=100; DI=7), although there is generally limited resolution among the *Oenothera* species in this clade. Strongly supported groups include *O. canescens* (sect. *Gauropsis*) + *O. speciosa* (sect. *Xylopleurum*) + *O. rosea* (purple-flowered sect. *Hartmannia*) (BS=100; DI=10), *O. speciosa* + *O. rosea* (BS=91; DI=4), and *O. tetraptera* (white-flowered sect. *Hartmannia*) + *O. multicaulis* (yellow-flowered sect. *Hartmannia*) (BS=100; DI=12). The lineage comprised of *Gaura* + *Stenosiphon* has moderate support (BS=85; DI=2).

Indels. A total of 24 indels was identified across all three data sets; no indels were coded in the nrITS data, 13 indels were identified in the *trnL-trnF* data, and 11 indels were coded in the *rps16* data. Parsimony analysis of the combined data set plus indels yielded a topology (not shown) that is nearly identical to that shown in Fig. 2. The only differences are due to increased resolution, an expected result of the addition of indels as separate characters. For example, two indels support the monophyly of tribe Epilobieae, five indels support the monophyly of *Clarkia*, and three indels support the monophyly of *Camissonia* sect. *Camissonia*.

Maximum Likelihood. Maximum likelihood (ML) analysis of the three region combined data set was conducted using parameters estimated from the data set with Modeltest v. 3.06 (Posada and Crandall 1998). This procedure indicated that the GTR + G + I model best fit the data. The ML model parameters included a nucleotide frequency of A=0.3215, C=0.1824, G=0.2023, and T=0.2938; substitution rate matrix of A to C: 1.3557, A to G: 1.07, A to T: 0.3806, C to G: 0.6649, C to T: 1.7271, and G to T: 1.000; proportion of invariant sites=0.289; and a gamma rate distribution at variable sites with shape (alpha)=0.795. Using this model, the analysis conducted 20,097 rearrangements and was stopped before completion, after retaining a tree with the same –ln value for 72 hours. This analysis yielded one tree with –ln=16321.085 (Fig. 3).

Generally, the ML tree has a similar topology to that inferred using parsimony. Any differences are due to the increased resolution of the ML tree (Fig. 3) compared to Fig. 2; this is not surprising as Fig. 2 is a strict consensus of many MP trees, whereas the ML algorithm generally yields a single tree. For example, the ML analysis yields a topology with various clades of *Camissonia* species forming a grade at the base of a lineage of *Oenothera* + *Calylophus* + *Gaura* + *Stenosiphon*, and there is a sister taxon relationship between *Calylophus* and *Oenothera* sect. *Pachylophus* (i.e., *O. caespitosa* and *O. psammophila*). In general, the internal branches are much shorter in the Onagreae above *Clarkia* + *Gayophytum* + *Camissonia pterosperma*, likely contributing to the low resolution among the various *Camissonia* clades in Figs. 1, 2. Further, within this *Camissonia* + *Oenothera* + *Calylophus* + *Gaura* + *Stenosiphon* clade, branch lengths are especially short at the tips of many *Oenothera* and *Gaura* species.

Discussion

Relationships within Epilobieae. In agreement with the nrITS analysis of Baum et al. (1994), this tribe is well supported as a monophyletic lineage, with *Chamerion* sister to a monophyletic *Epilobium* (Figs. 1–3). Relationships within *Epilobium* (Fig. 1) are generally consistent with the earlier analysis of Baum et al. (1994), with strong support for a clade of all sections excluding sect. *Epilobium* (i.e., *E. ciliatum*, *E. obcordatum*, and *E. rigidum*) (BS=100; DI=11) and for a lineage composed of sects. *Currania* (*E. pygmaeum*) + *Boisduvalia* (*E. densiflorum*) + *Zauschneria* (*E. canum*) (BS=91; DI=3). In addition, the present study concurs with Baum et al. (1994) in supporting a sister relationship

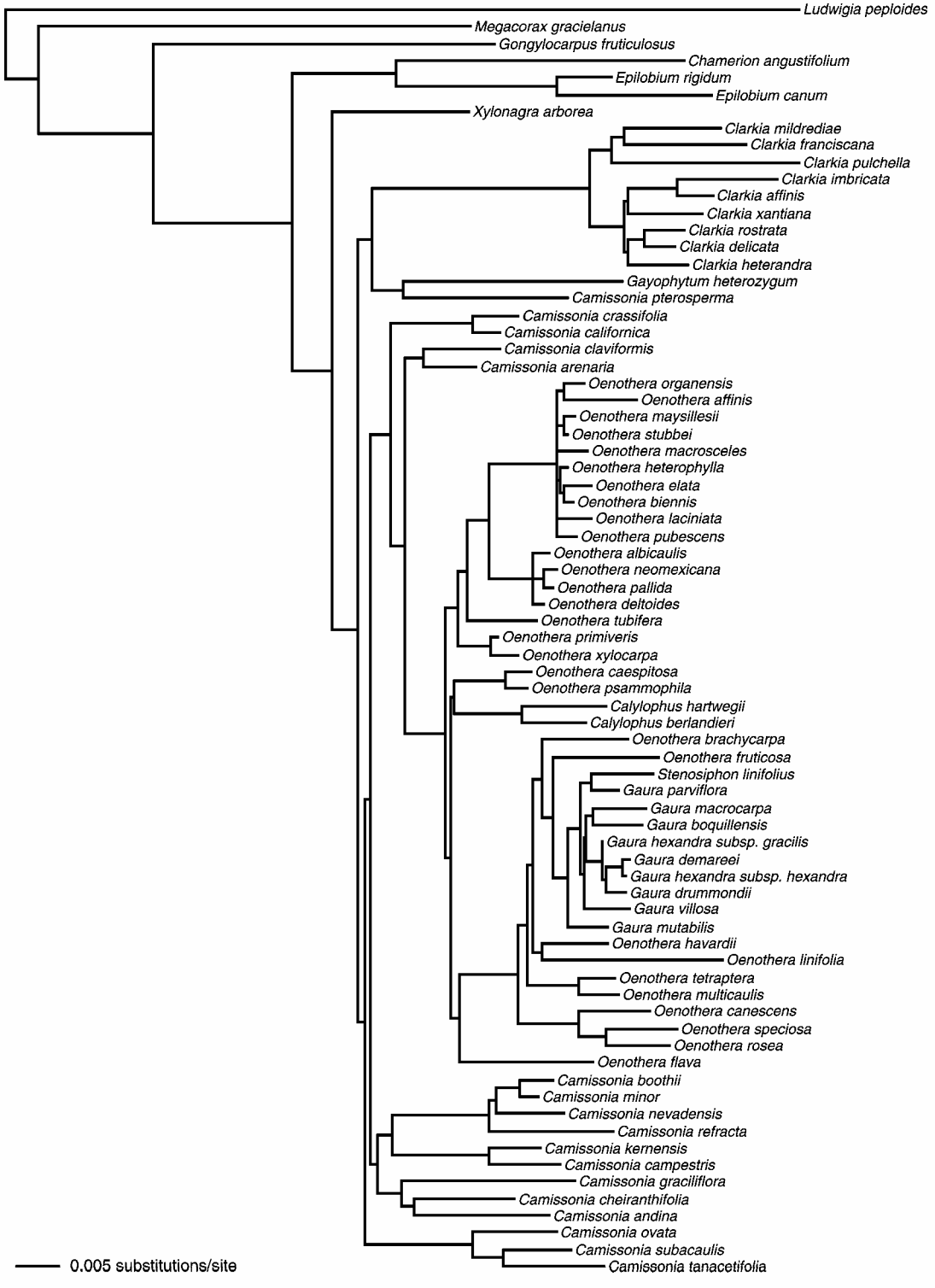

FIG. 3. Phylogram from the ML analysis of the combined nrITS and cp *trnL-trnF* and *rps16* data (75 taxa; −ln=16321.085).

between sects. *Boisduvalia* and *Zauschneria* (BS=97; DI=4). Interestingly, although analysis of the ITS data alone suggests that sect. *Epilobium* may be monophyletic (this study and Baum et al. 1994), support for the inclusion of the enigmatic species *E. rigidum* (Raven 1976) is weak. Addition of cp *trnL-trnF* data provides moderate support for the placement of this taxon outside of sect. *Epilobium* and sister to all sections except sect. *Epilobium* (BS=77; DI=2; Fig. 1), a position supported by seed morphology (Seavey et al. 1977).

Relationships within Onagreae. The genus *Xylonagra* has not often been included in phylogenetic analyses of Onagraceae. The morphological analysis of the family (Hoch et al. 1993) placed *Xylonagra* in a basal polytomy in tribe Onagreae. Recently, a family-wide study of Onagraceae based on a combined analysis of cp *rbcL* and *ndhF* sequence data (Levin et al. 2003) provided weak support for *Xylonagra* as sister to *Gayophytum*, with *Clarkia* sister to *Xylonagra* + *Gayophytum*. In the present study, there is moderate support for *Xylonagra* as sister to the rest of Onagreae (BS=85; DI=5; Fig. 2). *Xylonagra* is clearly morphologically distinct, with tubular red flowers, woody capsules, and asymmetrically winged seeds. These characters are homoplastic within the family, but are undoubtedly independently derived and autapomorphic in *Xylonagra*.

Within the rest of Onagreae, *Clarkia* is strongly monophyletic. Previous studies of this genus used a variety of outgroups, including single species of *Epilobium* (Sytsma et al. 1990), *Oenothera* (Gottlieb and Ford 1996) and *Gayophytum* (W. J. Hahn et al., in mss.). The monophyly of *Clarkia* is supported by a *PgiC* gene duplication (Gottlieb and Ford 1996; Ford and Gottlieb 2003) and the shared presence of unicellular papillae on the stigmatic surface (Heslop-Harrison 1990; Hoch et al. 1993). Relationships among the sections of *Clarkia* generally agree with other more detailed analyses (Sytsma et al. 1990; Gottlieb and Ford 1996; Ford and Gottlieb 2003; W. J. Hahn et al., in mss.); the few differences between our results and previous studies were not strongly supported in one or both analyses being compared. However, our results show incongruence between the nrITS and *rps16* data for *C. affinis* and *C. rostrata*. This appears to be the result of an allopolyploid origin of *C. affinis* ($n = 26$), which according to Lewis and Lewis (1955) arose via hybridization between a common ancestor of *C. davyi* and *C. tenella* (sect. *Godetia*; $n = 17$) and a species of sect. *Sympherica* ($n = 9$), and the resulting divergence between nuclear DNA and the maternally inherited plastid DNA. The other two allopolyploids in our study, *C. delicata* (sect. *Connubium*) and *C. pulchella* (sect. *Clarkia*), associate with the sectional representative of one of their presumed parents determined from more detailed studies (Lewis and Lewis 1955; Gottlieb and Ford 1996; Ford and Gottlieb 2003). Thus, *Clarkia delicata* groups with *C. heterandra* (sect. *Heterogaura*; ''*Sympherica*'' clade of Ford and Gottlieb 2003) in the ITS analysis and with *C. rostrata* (sect. *Sympherica*) in the cp analyses. Of the species included in the present study, *C. heterandra* and *C. rostrata* are the closest to the parental species (Ford and Gottlieb 2003), as *C. delicata* is presumed (Lewis and Lewis 1955) to have arisen as an allopolyploid ($n = 18$) between *C. unguiculata* ($n = 9$; sect. *Phaeostoma*; ''*Sympherica*'' clade of Ford and Gottlieb 2003) and *C. epilobioides* ($n = 9$; sect. *Sympherica*).

Our results show moderate support for *Gayophytum* + *Camissonia pterosperma* as sister to *Clarkia*. The apparently close relationship between *Gayophytum* and *Camissonia pterosperma* (sect. *Chylismiella*) supports speculation by Raven (1962, 1969) and Lewis and Szweykowski (1964), but neither suggested a close relationship of these species with *Clarkia*. This is the first phylogenetic study to include all of these taxa. Although an earlier morphological phylogeny (Hoch et al. 1993) showed *Gayophytum* as sister to Epilobieae, Levin et al. (2003), using similar taxon sampling to the Hoch et al. (1993) study and the more slowly-evolving *ndhF* and *rbcL* genes, showed a close relationship among *Xylonagra, Gayophytum* and *Clarkia*. In the present study, the nrITS data do not suggest a close relationship between *Gayophytum* and *C. pterosperma*, although these relationships were poorly resolved. By contrast, both chloroplast data sets strongly support a sister relationship between *Gayophytum* and *C. pterosperma*. Raven (1962, 1969) suggested the possibility of a close relationship between these taxa based on similarities in habit and unique white petals with a yellow base. However, he also noted that these taxa were quite distinct, with *C. pterosperma* marked by strongly autapomorphic seeds with thick papillate wings, and *Gayophytum* by 2-loculed ovaries.

Relationships among the rest of the *Camissonia* species included in this study are less clear, but, as previously suggested by Levin et al. (2003), there seems little doubt that the genus is not monophyletic. Constraining *Camissonia* to be monophyletic requires eight more steps (0.4% longer trees), and topologies with *Camissonia* constrained to be monophyletic have lower likelihood values, although most comparisons were not significant after Bonferroni correction (one-tailed S-H test; values varied from $P=0.064$ to $P=0.015$ across all constrained topologies, Bonferroni-corrected $P=0.017$). Instead, a number of well supported clades of *Camissonia* species appear to form a broadly paraphyletic grade within which is nested a monophyletic lineage composed of *Oenothera* + *Calylophus* + *Gaura* + *Stenosiphon*. All but one of these monophyletic groups of *Camissonia* species correspond to sections or groups of sections as delimited by Raven (1969); only *C. graciliflora*, placed in sect. *Tetrapteron* by Raven (1969), appears outside that section. Sections *Eremothera* and

Camissonia are strongly monophyletic in all of the analyses (Figs. 1–3), and together they form a clade with limited support (Fig. 2). These two sections are distinctive (Raven 1969); all of the species of sect. *Eremothera* have white petals, while those of sect. *Camissonia* have yellow ones. The two sections do share several characters (enumerated below), but these are also shared with some of the other *Camissonia* species.

Section *Tetrapteron* excluding *C. graciliflora* also appears monophyletic. It should not be too surprising that *C. graciliflora* does not, in fact, belong within sect. *Tetrapteron* as circumscribed by Raven (1969); this species differs from all other members of its section except for *C. palmeri* (not included in this study) by having an annual habit and winged capsules (all others are perennials and lack wings; Raven 1969). However, the section as delimited by Raven is characterized by a unique sterile projection of the ovary, elevating the flowers above the leaves and connecting to a very short floral tube with a fleshy disk at the tube entrance. Close re-examination (by WLW) of this sterile ovary projection has shown that in the perennial members of this section the projection appears continuous with both the short floral tube and the fertile part of the ovary, whereas in the two annual species there is a clear abscission line at both of these junctures. Therefore, this character appears homoplastic as originally described. Raven (1969) suggested an evolutionary link between these two annuals and sect. *Holostigma* based on the similarity of the entire leaves in both groups. Our results support this proposed relationship, as *Camissonia graciliflora* (sect. *Tetrapteron*) + *C. cheiranthifolia* (sect. *Holostigma*) + *C. andina* (sect. *Nematocaulis*) form a moderately supported clade (Fig. 2). This clade then forms a weakly supported lineage with sects. *Camissonia* and *Eremothera*. There is additional morphological support for this relationship: all of these taxa have sessile capsules that are often coiled or contorted, seeds in a single row per locule, simple subentire leaves, no fleshy disk at the floral tube entrance, and the absence of non-ultraviolet-reflective areas on the petals. Additionally, all but one species in this group has an annual habit, although this last characteristic seems to have evolved several times independently in adjacent clades. This entire group (i.e., sects. *Eremothera*, *Camissonia*, *Holostigma*, *Nematocaulis*, and *C. graciliflora*) may best be recognized as a more narrowly delimited genus *Camissonia*.

Two of the analyses presented here weakly support the position of *Camissonia* sect. *Tetrapteron* s.s. (i.e., excluding *C. graciliflora*) as sister to *Clarkia* + *Gayophytum* + *Camissonia pterosperma* (Figs. 1, 2), but clearly more data are needed. Unlike most taxa of Onagreae, all species of *Camissonia* sect. *Tetrapteron*, including *C. graciliflora* and *C. palmeri* (Raven 1969), as well as all species of *Clarkia* (Lewis and Lewis 1955), have basifixed anthers. However, measurements taken by WLW from one species each of *Clarkia*, *Gayophytum*, and every section of *Camissonia* indicate that the situation is not quite so simple, with anthers exhibiting a range of attachment points. Nevertheless, that analysis verifies that all taxa examined of *Clarkia* and *Camissonia* sect. *Tetrapteron* (including *C. graciliflora*), as well as *Gayophytum* and *C. pterosperma*, have anther attachments closer to the base than do other taxa of *Camissonia*. Thus, this anther character generally supports the relationships inferred from the DNA sequence data, except that it suggests a closer relationship of *C. graciliflora* to the rest of sect. *Tetrapteron* than do the DNA data.

Our results suggest a strongly monophyletic *Camissonia* sect. *Eulobus* (*C. californica* + *C. crassifolia*) that is moderately supported as sister to *C. claviformis* + *C. arenaria* + *Oenothera* + *Calylophus* + *Gaura* + *Stenosiphon*. Species of *Camissonia* sect. *Eulobus* are characterized by deeply pinnatifid leaves that are mostly restricted to the base of the plant, a floral tube closed by a fleshy disk, petals finely flecked with red near the base, seeds brown with maroon dots, virgate inflorescences, and usually pubescent anthers. The last three characters may be synapomorphies, but the others appear to be homoplastic. Additionally, our results support a sister taxon relationship between *C. claviformis* (sect. *Chylismia*) and *C. arenaria* (sect. *Lignothera*). Raven (1969) suggested that sect. *Chylismia* is closely related to sect. *Lignothera*, based on the shared presence of pedicellate, noncontorted capsules, seeds in two rows per locule, and relatively broad, primarily basal leaves.

Phylogenetic relationships within the monophyletic group of *Oenothera* + *Calylophus* + *Gaura* + *Stenosiphon* show that *Oenothera* is not monophyletic as currently circumscribed. When *Oenothera* is constrained to be monophyletic, there is a cost of 16 steps (trees 0.8% longer), and such topologies have significantly lower likelihood values (one-tailed S-H test; significance values from $P<0.001$ to $P=0.003$ across all constrained trees, Bonferroni-corrected $P=0.017$). Nevertheless, as in *Camissonia*, there is strong support for many monophyletic groups within *Oenothera*. These groups largely correspond to recognized sections, which together form a grade within which *Calylophus*, *Gaura*, and *Stenosiphon* are nested (Figs. 1–3). Levin et al. (2003) first suggested these relationships, although taxon sampling was limited. This broad ''*Oenothera* clade'' appears to comprise two primary lineages that generally correspond to the two groups defined by Tobe et al. (1987), based on capsule and seed coat anatomy. The group containing *O. fruticosa* and *O. brachycarpa* is more closely related to *Gaura* + *Stenosiphon* (lineage B; Fig. 2) than to other *Oenothera* species that comprise lineage A (Fig. 2). However, unlike results from Levin et al. (2003), *Calylophus* appears more closely related to lineage B than to lineage A; in the earlier study *Calylophus*

hartwegii is closer to *Oenothera elata*, which is in lineage A in the present study. Neither of these studies provides strong support for the exact placement of *Calylophus*, although it is clearly within the ''*Oenothera* clade''. Together with *Oenothera* sects. *Pachylophus* and *Lavauxia*, *Calylophus* forms a grade at the base of the strongly monophyletic lineage B (Fig. 2).

Analyses of the combined DNA sequence data sets (Figs. 1–3) strongly support the monophyly of *Oenothera* lineage A and relationships within this group. There is also morphological support for this clade, but it is complicated by the position of *Oenothera* sect. *Pachylophus* and by some apparently homoplastic characters. *Oenothera* lineage A + sect. *Pachylophus* share a unique thick seed endotesta (Tobe et al. 1987). These two groups also share a reticulate to papillate seed surface (also in sect. *Megapterium*), exotesta cells irregularly swollen or collapsed (also in sect. *Megapterium*), seed mesotesta with 1–3 cell layers (also in *O. havardii*), and seed mesotesta cells crushed (also in *O. havardii* and sect. *Megapterium*) (Tobe et al. 1987). In the analyses presented here, there is limited resolution of whether sect. *Pachylophus* is more closely related to lineages A or B; ML analysis places this section as sister to *Calylophus*, and together they are sister to lineage B, although the branch lengths involved are very short (Fig. 3). In addition, it costs only one additional step for section *Pachylophus* to be constrained to *Oenothera* lineage A. In view of the equivocal nature of these results, additional data are needed to clarify the relationships of *Calylophus* and *Oenothera* sects. *Pachylophus* and *Lavauxia* to one another and to the rest of *Oenothera*.

The well supported lineage B is composed of *Oenothera* sects. *Megapterium*, *Paradoxus*, *Gauropsis*, *Xylopleurum*, *Hartmannia*, and *Kneiffia*, as well as the monophyletic *Gaura* + *Stenosiphon* clade, with these taxa all sharing condensed, winged or angled capsules (Tobe et al. 1987). Among these *Oenothera* taxa, sect. *Hartmannia* does not appear to be monophyletic as currently circumscribed, with flower color apparently useful in dividing the section. *Oenothera rosea* (sect. *Hartmannia* with rose-purple petals) is sister to *O. speciosa* (sect. *Xylopleurum* with white to pink flowers), and together they are sister to *O. canescens* (sect. *Gauropsis* with white petals with pink flecks) in a strongly supported clade (Figs. 1–3). The other two species sampled from sect. *Hartmannia*, *O. tetraptera* (white petals) and *O. multicaulis* (yellow petals or yellow with red center), comprise a separate monophyletic lineage. However, relationships of these two clades to each other and to the rest of lineage B are equivocal, suggesting the need for more data.

Nested among these *Oenothera* species is the moderately supported clade of *Gaura* + *Stenosiphon*. Constraining *Gaura* to be monophyletic (i.e., excluding *Stenosiphon*) costs only an additional 4 steps (0.2% longer), and, not surprisingly, the likelihood of the constrained topologies is not statistically lower (S-H test; *P*-values from 0.264 to 0.735). However, in addition to the molecular support, morphology supports a close relationship between *Gaura* and *Stenosiphon*. *Stenosiphon* has a habit and inflorescence structure very similar to *Gaura parviflora*, and was only separated from *Gaura* based on the autapomorphy of fruits having one locule and four ovules, with only one maturing (Spach 1835; Johansen 1931; Raven 1964). Four morphological characters support the *Stenosiphon* + *Gaura* lineage: 1) fruits condensed, indehiscent; 2) ovule number reduced (1–8) from the much greater numbers in *Oenothera*; 3) septa fragile, incomplete and absent at maturity or wholly absent; and 4) presence of an indusium at the base of the stigma lobes. In addition, *Stenosiphon* + all species of *Gaura* except *G. mutabilis* share clawed, white to pink petals. Considering the present support for *Stenosiphon* nested within *Gaura* and similar results from Hoggard et al. (2004), it may be best to include *Stenosiphon* in a taxonomic group with *Gaura*, and both of these genera in an expanded *Oenothera*.

Within *Gaura*, the monophyly of *G. hexandra* is not supported, a finding consistent with results of G. Hoggard, U. Oklahoma (unpubl. data). The two subspecies of *Gaura hexandra* have been treated as distinct species in the past (e.g., Munz 1965), in part because they differ consistently in having 3 vs. 4-merous flowers. By contrast, they were grouped together by Raven and Gregory (1972) because of their shared predominant autogamy and general similarity. However, both subspecies share a unique 21 bp insertion in *rps16*; there is also a 5 bp deletion in *trnL-trnF* shared by them and *G. demareei*, a species that appears closely related in our analyses and in Hoggard et al. (2004).

***Relationships Among* Hauya, Circaea, Fuchsia, Lopezia, *and* Megacorax.** The placement of these taxa within the family has varied considerably among previous phylogenetic studies of Onagraceae (see review in Levin et al. 2003). Strong consensus has emerged for a sister relationship between *Fuchsia* and *Circaea* (Sytsma et al. 1991b; Bult and Zimmer 1993; Conti et al. 1993; Levin et al. 2003; also observed in the present study). Further, based on our results and those of Levin et al. (2003), *Megacorax* is sister to a monophyletic *Lopezia*. However, conflicts remain regarding the placement of *Hauya* and the *Lopezia* lineage. Crisci et al. (1990; nrDNA restriction sites), Sytsma at al. (1991b; cpDNA restriction sites), and Bult and Zimmer (1993; nrRNA sequence data) placed *Hauya* as sister to *Circaea* + *Fuchsia*, whereas Martin and Dowd (1986; amino acid sequence data) and Hoch et al. (1993; morphology) placed *Hauya* in a clade with tribes Onagreae and Epilobieae. In contrast, Conti et al. (1993; *rbcL* sequence data) and Levin et al. (2003; *rbcL* and *ndhF* sequence

data) placed *Hauya* as sister to all Onagraceae excluding *Ludwigia*. Our combined ITS + *trnL-trnF* analysis (Fig. 1) provides moderate support for this latter placement of *Hauya*; the ITS data alone also yield this relationship, but with limited support.

Similarly, some previous studies (nrRNA sequence data: Bult and Zimmer, 1993; and amino acid data: Martin and Dowd 1986) placed *Lopezia* as sister to all Onagraceae except *Ludwigia*, whereas others (cpDNA: Sytsma et al. 1991b; Conti et al. 1993) placed *Lopezia* as sister only to tribes Epilobieae and Onagreae. Analyses including both *Lopezia* and *Megacorax* (cpDNA sequence data: Levin et al. 2003) concurred with this latter placement. Our results, both from ITS alone and the combined analysis of nrITS and cp *trnL-trnF* (Fig. 1), confirm these relationships. These differences in topology prompted us to explore whether the addition of newly discovered *Megacorax gracielanus* is the key to resolving these relationships. Parsimony analyses were conducted using the ITS-only data set with: 1) *Megacorax* excluded, and 2) *Megacorax* and all *Lopezia* species except *L. langmaniae* excluded (most previous studies have only included one *Lopezia* species; more taxa were included in the present study to test the relationship of *Megacorax* to *Lopezia*). When only *Megacorax* was excluded, relationships did not change; however, when the additional *Lopezia* species were also excluded, the placement of *Hauya* and *Lopezia* switched. Thus, it appears that the previously reported incongruence between nuclear and chloroplast data is likely due to a lack of sampling within the *Lopezia* + *Megacorax* lineage.

From the present analyses it is now clear that Hauyeae are sister to all Onagraceae minus Jussiaeeae. The Fuchsieae + Circaeeae lineage was next to diverge, and Lopezieae + *Megacorax* are sister to Gongylocarpeae + Epilobieae + Onagreae. In accord with Levin et al. (2003), there is strong support for the placement of *Gongylocarpus* in its own tribe Gongylocarpeae (Smith and Rose 1913; Levin et al. 2003) and sister to a redefined Onagreae + Epilobieae.

Molecular Evolution. Comparison of the nrITS region and the two cp regions (Table 2) shows that the ITS data have the highest proportion of parsimony-informative (PI) characters, with the *trnL-trnF* data having the lowest, but fairly similar to *rps16*. Although ITS has the highest pairwise distance between sequences, yielding more PI characters, there is greater conflict among these characters than in the cpDNA, as exemplified by the low CI and RI values for ITS compared to *trnL-trnF* and *rps16* (Table 2). It is interesting to consider why the ITS data have such a low CI; possibly in this fast evolving region there has been too much divergence, resulting in multiple changes per site. Alternatively, it may be due to incomplete concerted evolution of the multiple ITS copies in the nuclear genome.

Several conclusions can be drawn from this study. In general, nuclear and chloroplast DNA sequence data sets were congruent, particularly regarding placement of *Hauya* and *Lopezia*. Further, *Megacorax* definitely does not belong within *Lopezia*, but it is strongly supported as sister to it. Tribes Epilobieae and Onagreae are monophyletic and are supported as sister taxa. Within Onagreae current generic delimitations are in question, though *Clarkia* is well supported as a monophyletic lineage. Neither *Camissonia* nor *Oenothera* are monophyletic as currently circumscribed, with the former broadly paraphyletic and comprising a grade of monophyletic lineages that correspond to traditional sections or groups of sections. Further data are needed to clarify relationships of *Camissonia* lineages relative to each other and to *Clarkia*, *Gayophytum*, and *Oenothera*. *Oenothera* is also paraphyletic, but becomes monophyletic if *Gaura*, *Stenosiphon*, and *Calylophus* are included. This broadly defined *Oenothera* would then contain all of the species that have a unique stigma with four noncommissural lobes (further modified in the peltate stigma of *Calylophus*). Within this group, *Gaura* is monophyletic only if *Stenosiphon linifolius* is included, a result consistent with Hoggard et al. (2004). Pending a more thorough review of morphological characters, the taxonomy of these genera clearly need to be reevaluated, and revised circumscriptions are forthcoming (Wagner et al., in prep.).

ACKNOWLEDGEMENTS. The authors thank Steve O'Kane for generously providing *Lopezia* genomic DNAs and ITS sequences, Carrie McCracken for sequencing help, Margaret Evans for sharing genomic DNAs, two anonymous reviewers whose comments greatly improved the manuscript, and gratefully acknowledge the Smithsonian Institution Andrew W. Mellon Fellowships in Structure and Evolution of Terrestrial Ecosystems and the National Science Foundation DEB 9407270 to KJS.

LITERATURE CITED

BALDWIN, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3–16.

———, M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, S. S. CAMPBELL, and M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.

BAUM, D. A., K. J. SYTSMA, and P. C. HOCH. 1994. A phylogenetic analysis of *Epilobium* (Onagraceae) based on nuclear ribosomal DNA sequences. *Systematic Botany* 19: 363–388.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.

BULT, C. J. and E. A. ZIMMER. 1993. Nuclear ribosomal RNA sequences for inferring tribal relationships within Onagraceae. *Systematic Botany* 18: 48–63.

CONTI, E., A. FISCHBACH, and K. J. SYTSMA. 1993. Tribal relationships in Onagraceae: implications from *rbcL* sequence data. *Annals of the Missouri Botanical Garden* 80: 672–685.

———, A. LITT, and K. J. SYTSMA. 1996. Circumscription of Myr-

tales and their relationships to other rosids: evidence from *rbcL* sequence data. *American Journal of Botany* 83: 221–233.

Crisci, J. V., E. A. Zimmer, P. C. Hoch, G. B. Johnson, C. Mudd, and N. Pan. 1990. Phylogenetic implications of ribosomal DNA restriction site variation in the plant family Onagraceae. *Annals of the Missouri Botanical Garden* 77: 523–538.

Cunningham, C. W. 1997. Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14: 733–740.

Davis, J. I., M. P. Simmons, D. W. Stevenson, and J. F. Wendel. 1998. Data decisiveness, data quality, and incongruence in phylogenetic analysis: an example from the monocotyledons using mitochondrial *atp* A sequences. *Systematic Biology* 47: 282–310.

Donoghue, M. J., R. G. Olmstead, J. F. Smith, and J. D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on *rbcL* sequences. *Annals of the Missouri Botanical Garden* 79: 333–345.

Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.

———, ———, ———, and ———. 1995. Constructing a significance test for incongruence. *Systematic Biology* 44: 570–572.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

Ford, V. S. and L. D. Gottlieb. 2003. Reassessment of phylogenetic relationships in *Clarkia* sect. *Sympherica*. *American Journal of Botany* 90: 284–292.

Gilbert, D. G. 1993. SeqApp: A biosequence editor and analysis application. ftp://iubio.bio.indiana.edu/molbio/seqapp/.

Goldman, N., J. P. Anderson, and A. G. Rodrigo. 2000. Likelihood-based tests of topologies in phylogenetics. *Systematic Biology* 49: 652–670.

González Elizondo, M. S., I. L. López Enriquez, and W. L. Wagner. 2002. *Megacorax gracielanus* (Onagraceae), a new genus and species from Durango, Mexico. *Novon* 12: 360–365.

Gottlieb, L. D. and V. S. Ford. 1996. Phylogenetic relationships among the sections of *Clarkia* (Onagraceae) inferred from the nucleotide sequences of *PgiC*. *Systematic Botany* 20: 45–62.

Hershkovitz, M. A. and E. A. Zimmer. 1996. Conservation patterns in angiosperm rDNA ITS2 sequences. *Nucleic Acids Research* 24: 2857–2867.

Heslop-Harrison, Y. 1990. Stigma form and surface in relation to self-incompatibility in the Onagraceae. *Nordic Journal of Botany* 10: 1–19.

Hipp, A. L., J. C. Hall, and K. J. Sytsma. In press. Phylogenetic accuracy and the ILD. *Systematic Biology*.

Hoch, P. C., J. V. Crisci, H. Tobe, and P. E. Berry. 1993. A cladistic analysis of the plant family Onagraceae. *Systematic Botany* 18: 31–47.

Hoggard, G. D., P. J. Kores, M. Molvray, and R. K. Hoggard. 2004. The phylogeny of *Gaura* (Onagraceae) based on ITS, ETS, and *trnL-F* sequence data. *American Journal of Botany* 91: 139–148.

Johansen, D. A. 1931. Studies on the morphology of Onagraceae IV. *Stenosiphon linifolium*. *Bulletin of the Torrey Botanical Club* 57: 315–326.

Katinas, L., J. Crisci, W. L. Wagner, and P. C. Hoch. 2004. Geographical Diversification of Tribes Epilobieae, Gongylocarpeae, and Onagreae (Onagraceae) in North America, based on Parsimony Analysis of Endemicity and Track Compatibility Analysis. *Annals of the Missouri Botanical Garden* 91: 159–185.

Levin, R. A., W. L. Wagner, P. C. Hoch, M. Nepokroeff, J. C. Pires, E. A. Zimmer, and K. J. Sytsma. 2003. Family-level relationships of Onagraceae based on chloroplast *rbcL* and *ndhF* data. *American Journal of Botany* 90: 107–115.

Lewis, H. and M. E. Lewis. 1955. The genus *Clarkia*. *University of California Publications in Botany* 20: 241–392.

——— and J. Szweykowski. 1964. The genus *Gayophytum* (Onagraceae). *Brittonia* 16: 343–391.

Maddison, W. P. and D. R. Maddison. 2000. MacClade 4: analysis of phylogeny and character evolution. Sunderland, Massachusetts: Sinauer Associates.

Martin, P. G. and J. M. Dowd. 1986. Phylogenetic studies using protein sequences within the order Myrtales. *Annals of the Missouri Botanical Garden* 73: 442–448.

Morgan, D. R. and D. E. Soltis. 1993. Phylogenetic relationships among members of Saxifragaceae sensu lato based on *rbcL* sequence data. *Annals of the Missouri Botanical Garden* 80: 631–660.

Munz, P. A. 1965. Onagraceae. *North American Flora* II 5: 1–278.

O'Kane, S. L., Jr. and B. A. Schaal. 1998. Phylogenetics of *Lopezia* (Onagraceae): evidence from chloroplast DNA restriction sites. *Systematic Botany* 23: 5–20.

Oxelman, B., M. Lidén, and D. Berglund. 1997. Chloroplast *rps*16 intron phylogeny of the tribe *Sileneae* (*Caryophyllaceae*). *Plant Systematics and Evolution* 206: 393–410.

Plitmann, U., P. H. Raven, and D. E. Breedlove. 1973. The systematics of Lopezieae (Onagraceae). *Annals of the Missouri Botanical Garden* 60: 478–563.

Popp, M. and B. Oxelman. 2001. Inferring the history of the polyploid *Silene aegaea* (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. *Molecular Phylogenetics and Evolution* 20: 474–481.

Posada, D. and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Raven, P. H. 1962. The systematics of *Oenothera* subgenus *Chylismia*. *University of California Publications in Botany* 34: 1–122.

———. 1964. The generic subdivision of Onagraceae, tribe Onagreae. *Brittonia* 16: 276–288.

———. 1969. A revision of the genus *Camissonia* (Onagraceae). *Contributions from the United States National Herbarium* 37: 161–396.

———. 1976. Generic and sectional delimitation in Onagraceae, tribe Epilobieae. *Annals of the Missouri Botanical Garden* 63: 326–340.

———. 1979. A survey of reproductive biology in Onagraceae. *New Zealand Journal of Botany* 17: 575–593.

———. 1988. Onagraceae as a model of plant evolution. Pp. 85–107 in *Plant evolutionary biology: a symposium honoring G. Ledyard Stebbins*, eds. L. D. Gottlieb and S. K. Jain. London: Chapman and Hall.

——— and D. P. Gregory. 1972. A revision of the genus *Gaura* (Onagraceae). *Memoirs of the Torrey Botanical Club* 23: 1–96.

Seavey, S. R., R. E. Magill, and P. H. Raven. 1977. Evolution of seed size, shape, and surface architecture in the tribe Epilobieae (Onagraceae). *Annals of the Missouri Botanical Garden* 64: 18–47.

Shimodaira, H. and M. Hasegawa. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* 16: 1114–1116.

Smith, J. D. and J. N. Rose. 1913. A monograph of the Hauyeae and Gongylocarpeae, tribes of the Onagraceae. *Contributions from the United States National Herbarium* 16: 287–298.

Spach, E. 1835. Monographia Onagrearum. *Nouvelles Annales du Muséum d'Histoire Naturelle, Paris* 4: 321–407.

Suh, Y., L. B. Thien, H. E. Reeves, and E. A. Zimmer. 1993. Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. *American Journal of Botany* 80: 1042–1055.

Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.

Sytsma, K. J. and J. F. Smith. 1988. DNA and morphology: comparisons in the Onagraceae. *Annals of the Missouri Botanical Garden* 75: 1217–1237.

——— and ———. 1992. Molecular systematics of Onagraceae: examples from *Clarkia* and *Fuchsia*. Pp. 295–323 in *Molecular systematics of plants*, eds. P. S. Soltis, D. E. Soltis, and J. J. Doyle. New York: Chapman and Hall.

———, J. Morawetz, J. C. Pires, M. Nepokroeff, E. Conti, M. Zjhra, J. C. Hall, and M. W. Chase. 2002. Urticalean rosids: circumscription, rosid ancestry, and phylogenetics based on *rbcL*, *trnL-F*, and *ndhF* sequences. *American Journal of Botany* 89: 1531–1546.

———, J. F. Smith, and P. E. Berry. 1991a. The use of chloroplast DNA to assess biogeography and evolution of morphology, breeding systems, and flavonoids in *Fuchsia* sect. *Skinnera* (Onagraceae). *Systematic Botany* 16: 257–269.

———, ———, and L. D. Gottlieb. 1990. Phylogenetics in *Clarkia* (Onagraceae): restriction site mapping of chloroplast DNA. *Systematic Botany* 15: 280–295.

———, ———, and P. C. Hoch. 1991b. A chloroplast DNA analysis of tribal and generic relationships within Onagraceae. *American Journal of Botany* 78: 222 (Abstract).

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

Tobe, H., W. L. Wagner, and H.-C. Chin. 1987. A systematic and evolutionary study of *Oenothera* (Onagraceae): seed coat anatomy. *Botanical Gazette* 148: 235–257.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in *PCR protocols: a guide to methods and applications*, eds. M. Innis, D. Gelfand, J. Sninsky, and T. White. San Diego: Academic Press.

Yoder, A. D., J. A. Irwin, and B. A. Payseur. 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. *Systematic Biology* 50: 408–424.

Zimmer, E. A., E. H. Roalson, L. E. Skog, J. K. Boggan, and A. Idnurm. 2002. Phylogenetic relationships in the Gesnerioideae (Gesneriaceae) based on nrDNA and cpDNA *trnL-trnF* and *trnE-T* spacer region sequences. *American Journal of Botany* 89: 296–311.