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# FAMILY-LEVEL RELATIONSHIPS OF ONAGRACEAE BASED ON CHLOROPLAST *RBCL* AND *NDHF* DATA[1]

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Despite intensive morphological and molecular studies of Onagraceae, relationships within the family are not fully understood. One drawback of previous analyses is limited sampling within the large tribe Onagreae. In addition, the monophyly of two species-rich genera in Onagreae, *Camissonia* and *Oenothera*, has never been adequately tested. To understand relationships within Onagraceae, test the monophyly of these two genera, and ascertain the affinities of the newly discovered genus *Megacorax*, we conducted parsimony and maximum likelihood analyses with *rbcL* and *ndhF* sequence data for 24 taxa representing all 17 Onagraceae genera and two outgroup Lythraceae. Results strongly support a monophyletic Onagraceae, with *Ludwigia* as the basal lineage and a sister-taxon relationship between *Megacorax* and *Lopezia*. *Gongylocarpus* is supported as sister to Epilobieae plus the rest of Onagreae, although relationships within the latter clade have limited resolution. Thus, we advocate placement of *Gongylocarpus* in a monogeneric tribe, Gongylocarpeae. Most relationships within Onagreae are weakly resolved, suggesting a rapid diversification of this group in western North America. Neither *Camissonia* nor *Oenothera* appears to be monophyletic; however, increased taxon sampling is needed to clarify those relationships. Morphological characters generally agree with the molecular data, providing further support for relationships.

**Key words:** *Camissonia*; *Megacorax*; *ndhF*; *Oenothera*; Onagraceae; Onagreae; phylogeny; *rbcL*.

The family Onagraceae in the order Myrtales are composed of about 650 species in 17 genera with a worldwide distribution, although the family is most species-rich in the New World (Table 1; Raven, 1988; Mabberley, 1997). Raven (1979, 1988) divided the Onagraceae into seven tribes (Table 1); only tribe Onagreae, with nine genera, contains more than two genera. Although much information is available regarding the systematics and evolution of Onagraceae, including detailed systematic revisions and surveys of embryology, palynology, pollination biology, and other features (summarized in Raven, 1988), relationships among genera within the family are not yet fully understood. Previous efforts to address phylogenetic relationships within Onagraceae include a morphological study (Hoch et al., 1993) and molecular analyses of chloroplast (Sytsma, Smith, and Hoch, 1991) and nuclear restriction site data (Crisci et al., 1990), 18S and 26S nuclear ribosomal RNA sequence data (Bult and Zimmer, 1993), *rbcS* amino acid sequence data (Martin and Dowd, 1986), and *rbcL* sequence data (Conti, Fischbach, and Sytsma, 1993). The morphological and molecular analyses strongly support the monophyly of Onagraceae, the basal position of *Ludwigia* within the family, a near-basal clade of *Fuchsia* + *Circaea*, and a clade of Epilobieae + Onagreae.

One drawback of these previous phylogenetic analyses is that only the morphological study (Hoch et al., 1993) included all recognized genera (at that time, *Boisduvalia* was recognized separately from *Epilobium*, and *Chamerion* was not, a situation now reversed). Specifically, sampling within the complex tribe Onagreae was limited in the molecular analyses to 1–3 genera out of nine. The morphological analysis suggested that tribe Onagreae may be polyphyletic, indicating a need to broaden molecular sampling of Onagreae to test its monophyly and clarify intergeneric relationships. Subsequent to all of these analyses, a new genus of Onagraceae (*Megacorax*; González Elizondo, López Enriquez, and Wagner, in press) has been discovered in Durango, Mexico, and its phylogenetic relationship to the rest of the family should be established.

Molecular analyses of several genera of Onagraceae including *Fuchsia* (Sytsma and Smith, 1988, 1992; P. Berry et al., University of Wisconsin-Madison, unpublished data), *Lopezia* (O'Kane and Schaal, 1998), *Clarkia* (Sytsma and Smith, 1988, 1992; Sytsma, Smith, and Gottlieb, 1990; W. Hahn et al., Columbia University, unpublished data), *Epilobium* and *Chamerion* (Baum, Sytsma, and Hoch, 1994), and *Gaura* (G. Hoggard, University of Oklahoma, unpublished data) have shown them to be monophyletic. Although no broad phylogenetic study of *Ludwigia* has been conducted, analyses including divergent species strongly imply the monophyly of the genus (Conti, Fischbach, and Sytsma, 1993). The two other large genera of Onagraceae that have not yet received intensive molecular study are *Camissonia* and *Oenothera*. Raven (1964) noted that *Camissonia* (62 spp.) is ''probably the most diverse genus'' in tribe Onagreae, and Hoch et al. (1993) did not find any morphological synapomorphies for the genus. *Oenothera* (120 spp.) also includes species exhibiting great morphological diversity (Tobe, Wagner, and Chin, 1987); a four-lobed noncomissural stigma (Raven, 1964; Heslop-Harrison, 1990) ap-

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TABLE 1. Tribes and genera of Onagraceae with number of sections, species, and geographical distribution.

| Taxon | No. sections/spp. | Distribution |
|---|---|---|
| *Megacorax* González & W. L. Wagner (incertae sedis) | –/1 | Central Durango, Mexico |
| Tribe Jussiaeeae | | |
| *Ludwigia* L. | 23/81 | Pantropical, extending to temperate North America and Asia |
| Tribe Hauyeae | | |
| *Hauya* DC. | –/2 | Southern Mexico to Costa Rica |
| Tribe Fuchsieae | | |
| *Fuchsia* L. | 10/105 | Andean South America, extending to Mexico and Hispaniola; New Zealand and Tahiti |
| Tribe Circaeeae | | |
| *Circaea* L. | –/7 | North temperate, especially Asia |
| Tribe Lopezieae | | |
| *Lopezia* Cav. | 6/22 | Mexico, extending to Panama |
| Tribe Epilobieae | | |
| *Chamerion* (Raf.) Raf. | 2/8 | North temperate, especially Asia |
| *Epilobium* L. | 7/164 | Cosmopolitan at high altitudes and latitudes; all seven sections occur in western North America |
| Tribe Onagreae | | |
| *Calylophys* Spach | 2/6 | Rocky Mountains to central U.S. and central Mexico |
| *Camissonia* Link | 9/62 | Western North America, mostly California; 1 sp. in temperate South America |
| *Clarkia* Pursh | 11/42 | Western North America, esp. California; 1 sp. in temperate South America, and another common to both continents |
| *Gaura* L. | 8/21 | Southwestern to central U.S. with center in Texas, extending to Atlantic coast and south to Guatemala |
| *Gayophytum* A. Juss. | –/9 | Mainly western North America; 1 sp. in temperate South America, and another common to both continents |
| *Gongylocarpus* Schlecht. & Cham | –/2 | One endemic on islands off coast of western Baja California, Mexico, and the other in central Mexico to Guatemala |
| *Oenothera* L. | 15/121 | All sections in North America, esp. western U.S., with center of diversity in Arizona and Texas to northern Mexico; four sections and >50 spp. in Central to South America; 2 spp. of European hybrid origin from North American introduced taxa |
| *Stenosiphon Spach* | –/1 | Great Plains of central U.S. |
| *Xylonagra* Donn. Smith & Rose | –/1 | Central Baja California, Mexico |

pears to be the single uniting morphological character for the genus.

Thus, the present study endeavors to (1) elucidate relationships among all genera of Onagraceae, including the recently described *Megacorax*, and compare these findings with phylogenetic hypotheses inferred in previous studies using molecular and morphological characters, (2) provide a preliminary test of the monophyly of the large genera *Camissonia* and *Oenothera*, (3) examine these molecular results in the context of morphological characteristics, and (4) test the monophyly of the tribes as currently circumscribed. In order to address these questions, we have added taxa to the earlier published analysis of *rbcL* sequence data by Conti, Fischbach, and Sytsma (1993), sequencing species from all genera of tribe Onagreae that were not included in that study, as well as the newly described *Megacorax*. To strengthen phylogenetic signal, we have combined these *rbcL* data with new molecular sequence data from the 3′ end of the *ndhF* chloroplast gene.

## MATERIALS AND METHODS

***Taxon sampling***—One species each from all Onagraceae genera was included in this study; for a few genera (*Lopezia*, *Ludwigia*) two species were included. Two species of *Lopezia* were included in an attempt to resolve previous confusion over the phylogenetic placement of *Hauya* and *Lopezia*; two divergent species of *Ludwigia* were included to demonstrate that *Ludwigia*, although large, is clearly a monophyletic sister group to the rest of the family. To explore the monophyly of *Camissonia* and *Oenothera*, one additional species was added for the former and two additional species for the latter. Conti, Litt, and Sytsma (1996), Conti et al. (1997), and Sytsma et al. (University Wisconsin-Madison, unpublished data) showed that within Myrtales Lythraceae are sister to Onagraceae; therefore, from Lythraceae we included *Lythrum salicaria* and *Cuphea llavea* as outgroups. The 22 ingroup and 2 outgroup taxa are listed at http://ajbsupp.botany.org/v90 with voucher information.

***DNA extraction, amplification, and sequencing***—Total genomic DNA for all taxa was provided by K. J. Sytsma (see protocols in Conti, Litt, and Sytsma, 1996; Sytsma et al., 2002) except for *Chamerion angustifolium* (3′ *ndhF* sequence; see taxa list at http://ajbsupp.botany.org/v90) and *Megacorax gracielana*, which were extracted by the senior author using the Qiagen Dneasy kit (Qiagen, Valencia, California, USA). Some of the *rbcL* sequences used in this study were previously published by Conti, Fischbach, and Sytsma (1993) (Genbank: L10216–L10217, L10219–L10222, L10225, L10227; see details at http://ajbsupp.botany.org/v90). Those amplified for this study by the senior author include *Calylophus hartwegii*, *Camissonia arenaria*, *C. boothii*, *Epilobium rigidum*, *Gaura mutabilis*, *Gayophytum heterozygum*, *Gongylocarpus fruticulosus*, *Megacorax gracielana*, *Oenothera brachycarpa*, *O. fruticosa*, *Stenosiphon linifolius*, and *Xylonagra arborea*; sequences for the 3′ *ndhF* region only were amplified for *Chamerion angustifolium* and *Circaea alpina*.

Sequences for *Lopezia langmaniae*, *Cuphea llavea*, and *Lythrum salicaria* (*rbcL* and 3′ *ndhF*) and 3′ *ndhF* sequences of *Clarkia xantiana*, *Fuchsia cyrtandroides*, *Hauya elegans*, *Ludwigia peploides*, and *L. peruviana* were amplified and sequenced in the Sytsma laboratory at the University of Wisconsin. Conditions for amplification and sequencing details for those sequences generated by the Sytsma laboratory are presented elsewhere (Conti, Fischbach, and Sytsma, 1993; Conti, Litt, and Sytsma, 1996; Sytsma et al., 2002; and references therein). For *rbcL*, up to ten primers were used (Conti, Fischbach, and Sytsma, 1993; Conti, Litt, and Sytsma, 1996), and for 3′ *ndhF* up to 10 primers from Olmstead and Sweere (1994) were used.

The *rbcL* and 3′ *ndhF* sequences generated by the senior author were polymerase chain reaction (PCR) amplified using a combination of specially designed and universal plant primers. The sequences were amplified with the primer pair P1630 (adapted from primer Z-1 of Zurawski [DNAX Research Institute, Palo Alto, California, USA]; 5′-ATG TCA CCA CAA ACA GAG ACT AAA GC-3′) at the 5′ end and P1782 (5′-ATA CTT CAC AAG CAG CAG CTA GTT CC-3′) at the 3′ end. The 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, California, USA) and was done in both directions using the same primers as for amplification, as well as two additional internal primers: P1628 (corresponds to primer Z-895R of Zurawski [DNAX Research Institute, Palo Alto, California, USA]; 5′-ACC ATG ATT CTT CTG CCT ATC AAT AAC TGC-3′) and P1626 (corresponds to primer Z-674 of Zurawski [DNAX Research Institute]; 5′-TTT ATA AAT CAC AAG CCG AAA CTG GTG AAA TC-3′).

For *ndhF* only the 3′ region was sequenced, and all primers were Onagraceae-specific except for P1740. Sequences were amplified with primer P1785 (adapted from the forward primer at *Nicotiana* position 972 in Olmstead and Sweere [1994]; 5′-GTC TCA ACT GGG TTA TAT GAT G-3′) at the 5′ end and P1786 (adapted from the reverse primer at *Nicotiana* position 2110 in Olmstead and Sweere [1994]; 5′-CCC CGA AAT ATT TGA GAC TTT CT-3′) at the 3′ end. The PCR products were cleaned as described above. Cycle sequencing used ABI Big dye chemistry (Applied Biosystems and both amplification primers as well as two internal primers: P1740 (same as forward primer at *Nicotiana* position 1318 in Olmstead and Sweere [1994]; 5′-GGA TTA ACY GCA TTT TAT ATG TTT CG-3′), and P1783 (5′-TTA AAA GGA ATT CCT ATG GCT GC-3′), a reverse primer at ca. 1667 in *Nicotiana*. Cycle sequence products for *rbcL* and 3′ *ndhF* were precipitated and cleaned with isopropanol before sequencing on an ABI 377 automated sequencer.

***Sequence alignment and analysis***—Sequences were edited in Editview version 1.0.1 (Applied Biosystems, Foster City, California, USA), and the sequences from all four primers were aligned and edited using Autoassembler DNA Sequence Assembly Software version 1.4.0 (Applied Biosystems) to construct a consensus sequence for each species (in the Sytsma laboratory, editing was done using Sequence Navigator [Applied Biosystems] and Sequencher version 3.0 [Gene Codes Corporation, Ann Arbor, Michigan, USA]). Species sequences were then aligned manually in Se-Al (Rambaut, 1996) and SeqApp (Gilbert, 1993). These alignments were imported into MacClade 4.0 (Maddison and Maddison, 2000) and executed in PAUP* version 4.0b8 (Swofford, 2002). To test for congruence of the *rbcL* and 3′ *ndhF* data sets, a partition homogeneity test (Farris et al., 1994, 1995) was conducted in PAUP*. One hundred heuristic partition homogeneity replicates were completed, each with ten random-addition-sequence replicates, tree bisection-reconnection (TBR) branch swapping, and gaps treated as missing data. Using the combined *rbcL* and 3′ *ndhF* data, and with *Lythrum salicaria* and *Cuphea llavea* defined as a monophyletic outgroup, we conducted a parsimony analysis using the branch and bound search option with gaps treated as missing data.

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 ten random addition sequence replicates. 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. This file was then executed in PAUP* using the heuristic search option to find the shortest trees consistent with each constraint. The decay index for each branch is the difference in length between the shortest trees consistent with each constraint and the globally shortest trees.

Constraint trees were constructed in MacClade to test alternative phylogenetic hypotheses, including the monophyly of each of the following three groups: Onagreae, *Camissonia*, and *Oenothera*. These trees were loaded into PAUP*, and branch and bound 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, Templeton's tests (Templeton, 1983) were conducted in PAUP* to assess the statistical support for these constraints. In this procedure, Wilcoxon signed-ranks tests are used to compare a most-parsimonious (MP) tree from the unconstrained analysis to the shortest trees constrained to contain a particular lineage of interest.

A maximum likelihood (ML) analysis was also conducted in PAUP* using the combined *rbcL* and 3′ *ndhF* data set. Nucleotide frequencies were empirically determined, and estimates were made of the transition : transversion ratio, proportion of invariable sites, and gamma distribution shape parameter. Due to constraints on time and memory, this analysis was stopped before completion. These estimated values were then specified in an analysis that was completed using the heuristic search option and 100 random-addition sequence replicates.

***Morphological characters***—Much information regarding structural and other biological characters is available for Onagraceae. Thus, we discuss morphological characters as they provide support for the phylogeny inferred from the *rbcL* + *ndhF* sequence data. We have included 11 of the 17 characters used by Hoch et al. (1993), two that were suggested by Tobe, Wagner, and Chin (1987) in a study of capsule and seed anatomy in *Oenothera*, and several others not previously used. The characters from Hoch et al. (1993) that were not included here are either highly homoplastic, present major problems of interpretation and/or missing data, or are used in a modified form (e.g., the dry stigmatic surface type of the wet/dry character of Hoch et al. [1993] appears here as two apomorphic types of dry stigma papillae).

## RESULTS

The aligned length for the *rbcL* gene was 1289 base pairs (bp), and the 3′ end of *ndhF* was 1081 bp. Unaligned sequences for *rbcL* varied from 1287 bp (*Lythrum salicaria*) to a length of 1289 bp for all others except *Clarkia xantiana* (1288 bp). For *ndhF* sequence length ranged from 1036 bp (*Megacorax*) to 1078 bp (*Lopezia langmaniae*), with the other 22 sequences having a length of 1072 bp. For the *rbcL* and *ndhF* data sets combined, pairwise distances across all taxa ranged from 0.00212 (between *Gaura mutabilis* and *Stenosiphon linifolius*) to 0.10058 (between *Cuphea llavea* and *Chamerion angustifolium*). Results of the partition homogeneity test suggested that the two data sets were somewhat incongruent ($P = 0.04$); however, the lengths of the random partitions exceeded the summed length of the original partition by maximally six steps (0.7% longer). Thus, we conducted a combined analysis of *rbcL* and *ndhF* sequence data.

***Parsimony analysis***—The combined analysis of 285 parsimony-informative characters across 24 taxa resulted in a single most-parsimonious tree (Fig. 1). There is strong support for a monophyletic Onagraceae (BS = 100; DI = 32) and for placement of *Ludwigia* as sister to the rest of the family (BS = 100; DI = 23). The placement of *Hauya* as sister to all Onagraceae except *Ludwigia* has limited support (BS = 66; DI = 2). *Fuchsia* and *Circaea* are strongly supported as sister

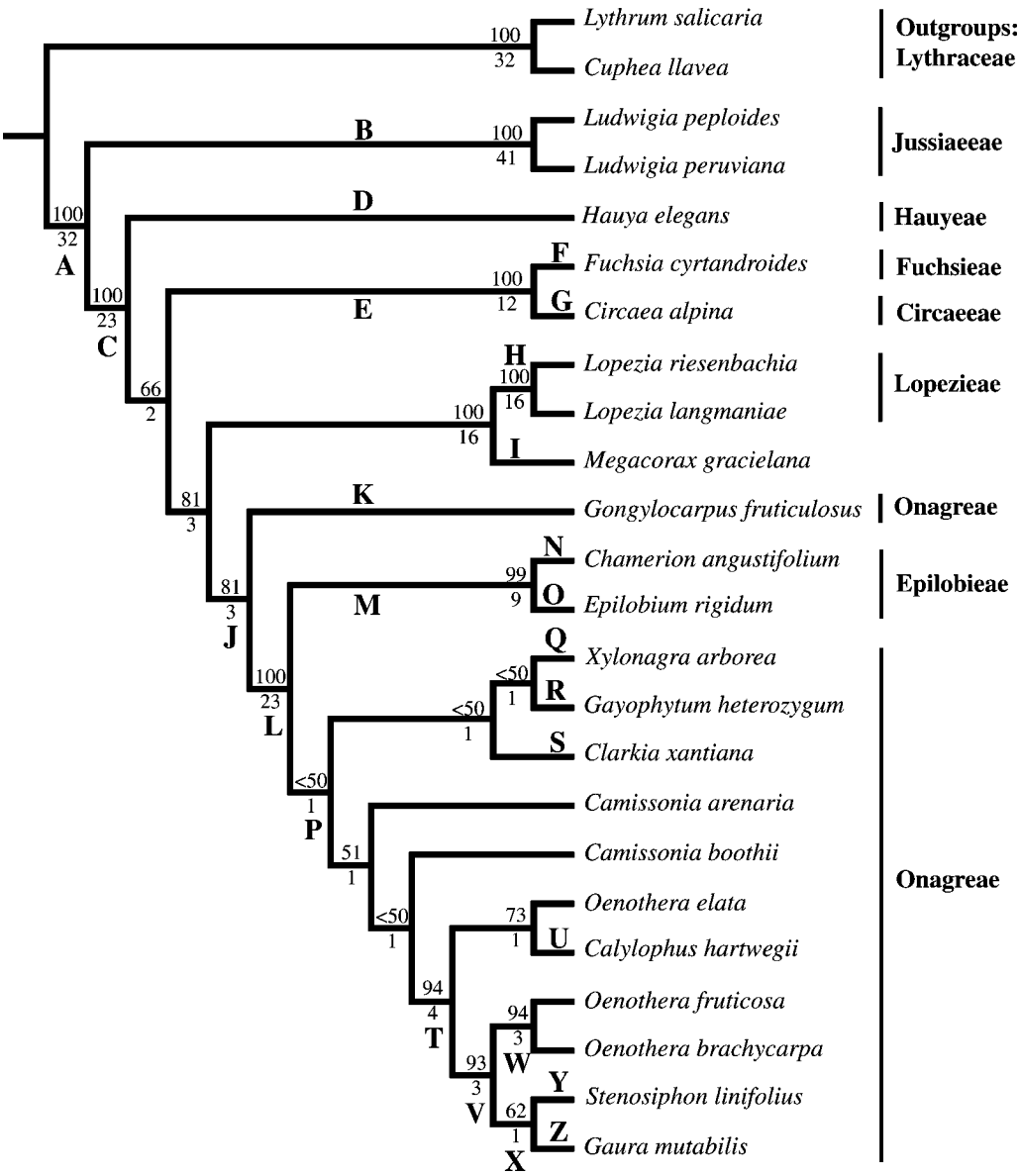


Fig. 1. The single most-parsimonious tree for all genera of Onagraceae inferred from *rbcL* and 3′ *ndhF* sequence data (tree length = 903, consistency index = 0.74, retention index = 0.74, rescaled consistency index = 0.55). Bootstrap values are shown above the branches, decay indices below. Also indicated are those lineages with morphological support; letters above the branches show the presence of characters that define genera, and letters below indicate characters that support relationships among genera. Table 2 gives the morphological characters indicated by each letter.

taxa (BS = 100; DI = 12), and the placement of *Fuchsia* + *Circaea* as sister to the remainder of the family (tribes Lopezieae + Epilobieae + Onagreae) has moderate support (BS = 81; DI = 3). Further, the two species of *Lopezia* clearly comprise a monophyletic group (BS = 100; DI = 16) and form a strongly supported lineage with *Megacorax* (BS = 100; DI = 16). This clade of *Lopezia* + *Megacorax* is moderately supported (BS = 81; DI = 3) as sister to tribes Epilobieae + Onagreae. Additionally, *Gongylocarpus* is well supported (BS = 100; DI = 23) as sister to tribe Epilobieae plus the rest of Onagreae (= Onagreae sensu stricto [s.s.]).

Within the clade of Epilobieae + Onagreae s.s., there is only weak support for Epilobieae as sister to Onagreae s.s. (BS < 50; DI = 1), although the monophyly of Epilobieae is strongly supported (BS = 99; DI = 9) (Fig. 1). Also weakly supported are the relationships of *Xylonagra*, *Gayophytum*, and *Clarkia* to each other and to the rest of Onagreae s.s. (BS < 50; DI = 1). Further, a lineage composed of *Camissonia*, *Calylophus*, *Oenothera*, *Gaura*, and *Stenosiphon* (BS = 51; DI = 1) is not well supported. However, within this lineage, the placement of the two species of *Camissonia* as a paraphyletic grade basal to a monophyletic lineage composed of *Calylophus*, *Oenothera*, *Gaura*, and *Stenosiphon* is strongly supported (BS = 94; DI = 4). *Oenothera elata* has limited support as sister to *Calylophus* (BS = 73; DI = 1), and this clade is well supported as sister to *Oenothera brachycarpa* + *O. fruticosa* + *Gaura* + *Stenosiphon* (BS = 93, DI = 3). *Gaura* + *Stenosiphon* comprise a lineage with limited support (BS = 62; DI = 1), and *Oenothera brachycarpa* + *O. fruticosa* form a strongly supported clade (BS = 94; DI = 3).

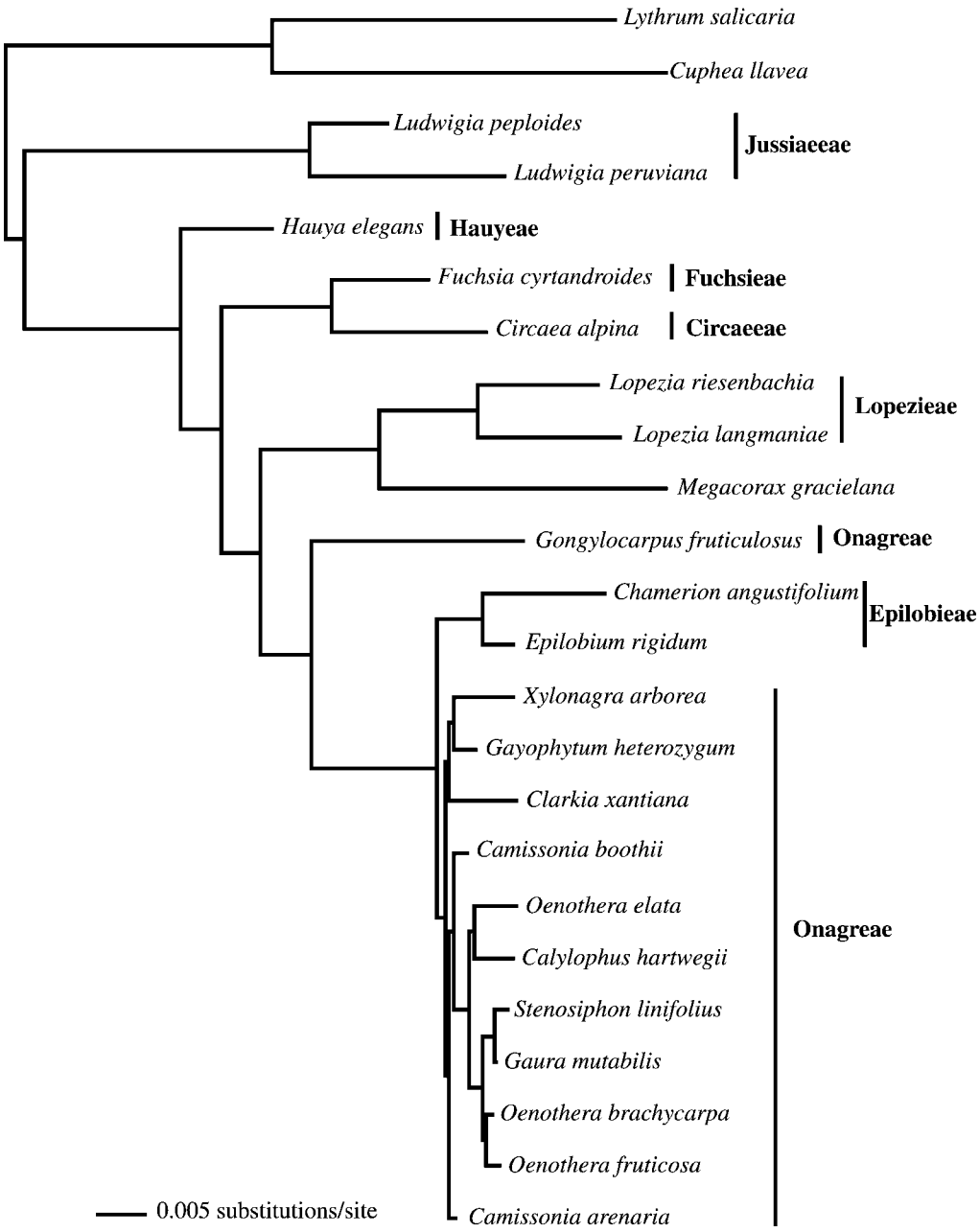


Fig. 2. The phylogram of all genera of Onagraceae with the optimal maximum likelihood score (−ln = 8597.86172) inferred from *rbcL* and 3′ *ndhF* sequence data.

***Maximum likelihood analysis***—Maximum likelihood analysis yielded one tree with an optimal likelihood score (Fig. 2), and this tree is identical to the MP tree (Fig. 1). The phylogram shown in Fig. 2 demonstrates the striking difference in branch lengths of taxa within Onagreae s.s. and Epilobieae relative to those in the other tribes.

## DISCUSSION

This combined analysis of chloroplast *rbcL* and 3′ *ndhF* sequence data was the first to include all 17 genera within Onagraceae and, thus, offers valuable insight into relationships within the family. Further, in contrast to previous analyses with reduced taxon sampling, the relationships among lineages are generally well supported. In addition to molecular support, we have outlined a number of morphological characters that strengthen many of these hypotheses of relationships (Fig. 1, Table 2).

As previous studies have shown, the family Onagraceae is clearly monophyletic. In addition to strong molecular support, all Onagraceae are characterized by a number of morphological synapomorphies, including the presence of pollen with viscin threads (A; Fig. 1, Table 2). Further, in concordance with all previous studies, the genus *Ludwigia* is clearly sister to all other Onagraceae, a position first proposed by Eyde (1977) based on floral anatomy. The well-supported genus *Ludwigia* is united by a number of morphological characters including the presence of pollen in tetrads, loss of the floral

TABLE 2. Morphological characters supporting lineages and genera in Fig. 1. Most characters are adapted from Hoch et al. (1993) and Tobe, Chin, and Wagner (1987) and are discussed in detail there. In a few cases new ones are added here or modified from earlier studies. (p) = parallelism; (r) = reversal. Plesiomorphic states for the family are not given in the table, but are discussed in detail by Hoch et al. (1993).

Morphological characters supporting lineages or genera of Onagraceae

A. Abundant raphides in vegetative cells.
Viscin threads present in the pollen.
Paracrystalline beaded pollen ektexine.
4-nucleate embryo sac.
Septa present dividing sporogenous tissue.

B. Floral tube absent (p).
Pollen in tetrads (p; lost in some sections, occasionally polyads).
Ovule archesporium single-called.
Outer integument dermal (p).

C. Flowers 4-merous (2-merous in *Circaea*).
Sepals deciduous.
Nectaries on the floral tube.
Central ovary vasculature absent.
Minor stylar bundles absent.
$x = 11$.

D. Flowers vespertine (p).
Fruit a woody capsule.
Seed winged, asymmetrical (p).
Outer integument partially dermal (p).
$x = 10$.

E. Fruit indehiscent (p).
Pollen with prominent apertural protrusions (p)

F. Fruit a berry.
Pollen 2-aperturate.

G. Flowers 2-merous.
Petals notched (p).
Fruit with hooked hairs.
Seed I/locule.

H. Flowers structurally zygomorphic.
Stamens 2 or 1 + 1 staminode.

I. Leaves linear.
Corolla presentation zygomorphic.
Capsule wall thin, seeds distending wall.

J. Stipules absent.
Ovule parietal tissue thick (lost in *Gayophytum* and Epilobieae).
Outer integument dermal (p).

K. Mature fruit embedded in stem.

L. Chromosome number change?

M. Commissural stigmas (p).
Stigma papillae multicellular.
Seeds comose.
$x = 18$.

N. Floral tube absent (p).
Stamens equal.
Style reflexed, then stamens reflexed.

O. Basal leaves opposite.
Petals notched (p).
Pollen in tetrads (p; also in *Camissonia* sect. *Lignothera*).

P. Pollen with prominent apertural protrusions (p).
$x = 7$.

Q. Corolla red, tubular.
Seeds asymmetrical, winged (p).

R. Capsule bilocular.
Chromosomal translocations common (p).

S. Commissural stigmas (p).
Stigma papillae unicellular.

T. Flowers vespertine (p).
Outer integument partially subdermal (r).
Stigma noncommissural, divided (lobes 0 or short in *Calylophus*).
Chromosomal translocations common (p; also in *Gayophytum*).

U. Stigma without lobes or lobes short, receptive adaxially.

V. Fruit sharply angled or winged.

W. Seed endotestal cells radially flattened (p; in *Oenothera* sect. *Anogra*).

X. Fruits condensed, indehiscent (p).
Ovule number reduced (1–8).
Septa in fruit incomplete, fragile and absent at maturity or wholly absent.

Y. Ovary with 1 locule.

Z. Ovary with 3(4) locules, but septa not evident at maturity.

tube, and two embryonic features (B in Fig. 1, Table 2). There are also many morphological synapomorphies for all of Onagraceae minus *Ludwigia*. These include features of the ovary vasculature as well as deciduous sepals, the presence of nectaries on the floral tube, and usually four-merous flowers (C in Fig. 1, Table 2). The placement of *Hauya* as sister to all Onagraceae minus *Ludwigia* has limited support and concurs with a phylogenetic hypothesis based on *rbcL* data alone (Conti, Fischbach, and Sytsma, 1993). However, Crisci et al. (1990; nrDNA restriction site), Sytsma, Smith, and Hoch (1991; 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) and Hoch et al. (1993; morphology) placed it into the clade with tribes Onagreae and Epilobieae. Although this latter position seems untenable on the balance of evidence, the position of *Hauya* inferred in the present study is not strongly supported (BS = 66; DI = 2), and currently is being addressed using additional data from both nuclear and chloroplast regions (R. Levin et al., Smithsonian Institution, unpublished data).

***The Fuchsia + Circaea lineage***—In the present study there is a strong sister-taxon relationship between *Fuchsia* and *Circaea*, a result consistent with the findings of previous molecular studies (Sytsma, Smith, and Hoch, 1991; Bult and Zimmer, 1993; Conti, Fischbach, and Sytsma, 1993). Despite the strong molecular support, there are few recognized morphological synapomorphies for this lineage; indehiscent fruits (albeit very different types in the two genera) and pollen with prominent apertural protrusions characterize this lineage, although these features are both homoplastic (E in Fig. 1, Table 2). One of the reasons that this lineage lacks uniting characters may be the highly distinctive morphologies of each genus, including a berry fruit in *Fuchsia* and two-merous flowers in *Circaea* (F, G in Fig. 1, Table 2). These morphological characteristics, in addition to divergent habits and biogeography (Berry, 1982; Boufford, 1982), have supported the traditional placement of each genus in separate tribes. *Fuchsia* + *Circaea* are moderately supported as sister to Lopezieae + *Megacorax* + Epilobieae + Onagreae, a finding with limited agreement among previous studies, mainly due to the shifting placement of *Hauya* and *Lopezia* (see above and below).

***The Lopezia + Megacorax lineage***—There is strong support for the monophyly of *Lopezia* + *Megacorax*. Surprisingly, this lineage has no clear morphological synapomorphy, but

few comparable data are available for the recently discovered *Megacorax*. Its corolla presentation in a zygomorphic manner suggests a trend toward the structural zygomorphy found in *Lopezia*, and the capsules of *Megacorax* are similar in structure to those of some species of *Lopezia*. There is moderate support for the *Lopezia* + *Megacorax* lineage as sister to Onagreae + Epilobieae; this relationship is consistent with previous cpDNA analyses with *Lopezia* alone (Sytsma, Smith, and Hoch, 1991; Conti, Fischbach, and Sytsma, 1993). However, nuclear rRNA sequence (Bult and Zimmer, 1993) and amino acid data (Martin and Dowd, 1986) suggest that *Lopezia* is sister to all tribes except Jussiaeeae. In general, *Hauya* diverges earlier and *Lopezia* is more closely related to Onagreae in phylogenies based on plastid DNA (as here), whereas *Lopezia* is earlier diverging and *Hauya* forms a clade with *Fuchsia* + *Circaea* when using nuclear DNA (Bult and Zimmer, 1993). Further analyses including the closely related *Megacorax* may help clarify the conflict observed in the position of *Lopezia* in previous studies (R. Levin et al., Smithsonian Institution, unpublished data).

***Relationships among and within Onagreae + Epilobieae***—A clade comprising all genera in tribes Onagreae and Epilobieae has moderate support and is consistent with the majority of previous family-level phylogenetic analyses of Onagraceae. Additionally, Epilobieae + Onagreae are well supported by morphological characters such as the absence of stipules (J in Fig. 1, Table 2). However, within this lineage, the strongly supported position of *Gongylocarpus* as sister to all other members of this clade makes tribe Onagreae paraphyletic relative to Epilobieae. This finding disagrees with that of Hoch et al. (1993), the only previous phylogenetic study of Onagraceae to have included *Gongylocarpus*; their results suggest that *Gongylocarpus* is nested within Onagreae. Raven (1964; Carlquist and Raven, 1966) included *Gongylocarpus* in tribe Onagreae because of the absence of stipules, absence of pollen in tetrads, occurrence in dry habitats, and cytological characteristics (pollen mother cells tolerate intense hydrolysis, and reciprocal translocations are a regular occurrence in populations). It is clear now that none of these characteristics except the cytological ones serve to link *Gongylocarpus* more closely to other Onagreae ; rather, they are equally consistent with its placement sister to a clade of Epilobieae + Onagreae s.s. (i.e., excluding *Gongylocarpus*). Further, *Gongylocarpus* is highly distinctive morphologically, with its fruit embedded in the stem (Carlquist and Raven, 1966) (K in Fig. 1, Table 2), and cytologically, with its base chromosome number ($x$ = 11) not otherwise found in Onagreae.

A base chromosome number of $x = 11$ has been suggested as plesiomorphic for Onagraceae (Raven, 1964, 1979, 1988), as it also characterizes the early diverging lineages of *Circaea*, *Fuchsia*, and *Lopezia* (with subsequent aneuploid reduction to $x = 10$ in *Hauya*). However, Graham and Cavalcanti (2001) recently proposed that $x = 8$ is the base chromosome number for the family Lythraceae, which is sister to Onagraceae. As *Ludwigia*, sister to all other Onagraceae, also has $x = 8$ (Raven and Tai, 1979), it now appears that $x = 8$ is plesiomorphic for Onagraceae, with an early shift to $x = 11$ along the branch leading to the rest of the family (C in Fig. 1, Table 2). This chromosome number of $x = 11$ has been retained in *Circaea*, *Fuchsia*, *Lopezia*, and *Gongylocarpus*. We infer that a change in chromosome number is most likely to have occurred along the branch leading to Epilobieae + Onagreae s.s. (L in Fig. 1, Table 2); interestingly, this change in chromosome number is the only nonmolecular character that appears to unite Epilobieae + Onagreae s.s., with subsequent shifts to $x = 18$ in Epilobieae and to $x = 7$ in Onagreae s.s. (M, P in Fig. 1, Table 2; Raven, 1979). The nature of these changes and, indeed, the basis for the significant chromosomal differences throughout the family (Kurabayashi, Lewis, and Raven, 1962) are not well understood. Clearly, a dramatic cytological/genomic revolution took place with the origin of this lineage in western North America. This resulted in an explosive radiation of the distinctive ''generic'' lineages we recognize in these tribes today, yet left virtually no discernible morphological traces.

Within Onagreae s.s. + Epilobieae, relationships are generally poorly resolved (Fig. 1). Tribe Epilobieae is clearly monophyletic (see also Baum, Sytsma, and Hoch, 1994), with strong molecular and morphological support, including commisural stigmas and comose seeds (M in Fig. 1, Table 2). Although, the position of Epilobieae as sister to Onagreae s.s. has only weak support, this finding concurs with all other studies; however, the morphological analysis of Hoch et al. (1993) was the only other study to include sufficient sampling within Onagreae.

The clade of Onagreae s.s., which has <50% bootstrap support, has at least two apparent morphological synapomorphies: base chromosome number $x = 7$ and pollen with prominent apertural protrusions (also found in *Fuchsia* + *Circaea*; P in Fig. 1, Table 2). Both parsimony and ML analyses weakly support a clade of *Clarkia*, *Gayophytum*, and *Xylonagra* that is sister to the rest of Onagreae s.s (i.e., *Camissonia* + *Calylophus* + *Oenothera* + *Gaura* + *Stenosiphon*), but more data and increased sampling are needed to properly evaluate these relationships. Further, although each of these three genera (i.e., *Clarkia*, *Gayophytum*, and *Xylonagra*) has distinctive morphological characteristics (Q, R, and S in Fig. 1, Table 2), we are not aware of any features that unite these three related taxa.

***The monophyly of Camissonia and Oenothera***—Neither *Camissonia* nor *Oenothera* appears to be monophyletic, although the present study did not include sufficient sampling to strongly support these findings. The two sampled species of *Camissonia* form a paraphyletic grade basal to a well-supported clade of *Calylophus*, *Oenothera*, *Gaura*, and *Stenosiphon*; however, a monophyletic *Camissonia* requires only one more step (Templeton's test was invalid [Sokal and Rolf, 1981] for such a small sample size [$N = 1$], with sample size = number of characters optimized differently onto the alternative topologies). Not surprisingly, Hoch et al. (1993) report no morphological characters uniting *Camissonia*, and characters used by Raven (1969) to delimit the genus are currently thought symplesiomorphic. The absence of support for the monophyly of these two species of *Camissonia*, and for the genus as a whole, indicates the need for a more comprehensive analysis of *Camissonia*.

Within the *Calylophus* + *Oenothera* + *Gaura* + *Stenosiphon* lineage, the most surprising result is that *Oenothera* appears to be paraphyletic; however, a monophyletic *Oenothera* costs only four steps (0.4% longer). As above, low sample size ($N = 4$) rendered a Templeton's test invalid. Interestingly, the synapomorphy previously suggested for *Oenothera*, linear noncommissural stigma lobes, now seems to be a synapomorphy for the lineage of *Oenothera* + *Calylophus* + *Stenosiphon* + *Gaura* (T in Fig. 1, Table 2). There appear to be two distinct

lineages of *Oenothera*, which correspond to the two lineages described by Tobe, Wagner, and Chin (1987) based on fruit and seed characters. One of these lineages has limited support as sister to *Calylophus*, and the other is strongly supported as sister to *Gaura* + *Stenosiphon*. The presence of fruits that are sharply angled or winged characterizes this lineage of *O. fruticosa* (sect. *Kneiffia*) + *O. brachycarpa* (sect. *Megapterium*) + *Gaura* + *Stenosiphon* (V in Fig. 1, Table 2). Although weakly supported by molecular data, a number of characters unite *Gaura* + *Stenosiphon*; these include condensed (clavate with a sterile basal region), indehiscent fruits, a reduction in ovule number, and incomplete or absent fruit septa (X; Fig. 1, Table 2). Some species of *Oenothera*, including *O. fruticosa*, also have condensed, capsular fruits, although these are dehiscent, rather than indehiscent, as in *Gaura*.

Previous molecular studies of generic relationships did not include sufficient taxon sampling to examine relationships within Onagreae, and the morphological study of Hoch et al. (1993) did not find either *Oenothera* or *Stenosiphon* as sister to *Gaura*. Martin and Dowd (1986) found a close relationship between species of *Oenothera* and *Gaura*, but their sampling was insufficient to generalize this result. Preliminary analyses with greater taxon sampling and more variable molecular regions appear to support the relationships presented here among *Oenothera*, *Calylophus*, *Gaura*, and *Stenosiphon* (R. Levin et al., Smithsonian Institution, unpublished data).

It should not be surprising that relationships within and among *Camissonia*, *Oenothera*, and *Calylophus* are especially complex. Until the early 1960s, all three genera were combined in a broadly defined *Oenothera* (Munz, 1965), which included plants with mostly yellow, actinomorphic flowers that opened in the evening and were mainly distributed in western North America (note that it did not include *Gaura* or *Stenosiphon*). Even earlier, as many as 19 segregate genera were recognized in the 19th century, but Munz brought much-needed order to the Onagraceae in a series of valuable revisions during the mid-20th century. Raven (1964, 1969) further refined our understanding and proposed the current classification for tribe Onagreae, including segregation of three genera (*Calylophus*, *Camissonia*, and *Oenothera*) from Munz's broader concept of *Oenothera*. The results of the present study suggest that some changes in generic delimitation within Onagreae are warranted, in turn necessitating nomenclatural changes in these groups. However, such changes await results from analyses in progress with increased taxon sampling.

***Rates of diversification***—Variation in branch lengths among tribes is quite striking (Fig. 2). Branches leading to Jussiaeeae, Hauyeae, Circaeeae, Fuchsieae, Lopezieae, *Megacorax*, and *Gongylocarpus* are comparatively much longer than those leading to or within the Epilobieae + Onagreae s.s. clade. Specifically, the very short branch lengths within Onagreae s.s. suggest that this group experienced a period of rapid diversification and speciation, likely coincidental with its expansion into the Madrean Floristic Region of northern Mexico and the southwestern United States (Raven, 1964, 1976; L. Katinas et al., Museo de La Plata, unpublished data).

***Tribal delimitations***—Results of this study suggest possible changes in the current tribal circumscriptions (Table 1). Jussiaeeae (*Ludwigia*) is well marked and unambiguous, and although the exact phylogenetic placement of Hauyeae (*Hauya*) remains equivocal, the tribe possesses a unique base chromosome number ($x = 10$) and morphological synapomorphies, though many of these are homoplastic (D in Fig. 1, Table 2). The strong relationship between Fuchsieae (*Fuchsia*) and Circaeeae (*Circaea*) suggests that they might be merged into a single tribe. However, this lineage has few morphological synapomorphies compared to the numerous autapomorphies for each genus (E, F, and G in Fig. 1, Table 2), and they occur entirely allopatrically (see Table 1), emphasizing what is clearly a long period of separation. Placement of tribe Lopezieae (*Lopezia*) is somewhat ambiguous, but the tribe is very clearly distinguished. However, our results showing that the new genus *Megacorax* belongs in a monophyletic lineage with Lopezieae suggest that *Megacorax* may best be considered within an expanded Lopezieae. But the long branch lengths (Fig. 2) separating these two genera indicate the need for caution, at least until additional information is available about the new genus. *Lopezia* is strongly supported morphologically by having only two stamens (or one, plus a staminode) in a fourmerous flower, a character that *Megacorax* lacks (H, I in Fig. 1, Table 2).

Our results challenge the current circumscription of the two remaining tribes, Epilobieae (*Chamerion* and *Epilobium*) and Onagreae (nine genera). The position of *Gongylocarpus* as sister to Epilobieae + Onagreae s.s. and phylogenetically distant from the rest of Onagreae has strong support. A tree that forces a monophyletic Onagreae including *Gongylocarpus* requires an additional 23 steps (2.6% longer), which is a significant length difference ($P < 0.0001$; one-tailed Wilcoxon signed-ranks test). The strength of that position suggests that *Gongylocarpus* be placed in its own tribe (Gongylocarpeae; Smith and Rose, 1913). The alternative would be an expanded, monophyletic Onagreae that includes Epilobieae, which would result in a tribe with 11 of the 17 genera in the family. We prefer the option to segregate *Gongylocarpus* in its own tribe; such a circumscription acknowledges the very distinctive nature of that genus (including a high rate of divergence; Fig. 2), while still allowing for recognition of both Epilobieae and a reconfigured Onagreae, which we consider to be useful phylogenetic units.

The monophyly of the lineage comprising *Epilobium* (i.e., the traditional Epilobieae [Raven, 1976; Baum, Sytsma, and Hoch, 1994]) + *Chamerion* appears certain, based on strong bootstrap support and morphological synapomorphies (M in Fig. 1, Table 2). Although the relationships of *Gayophytum*, *Xylonagra*, *Clarkia*, and *Camissonia* relative to each other and to Epilobieae are poorly resolved in the present analysis, Onagreae s.s. still appears to comprise a recognizable evolutionary lineage (marked, for example, by a base chromosome number of $x = 7$) that, with the exception of its closeness to Epilobieae, is distinct from other tribal lineages in the family. For the present, we prefer to maintain the two tribes, Epilobieae and Onagreae s.s., and to pursue additional studies of these groups, using more rapidly evolving molecular sequences on a more extensive sampling of taxa in Onagreae (R. Levin et al., Smithsonian Institution, unpublished data).

***Conclusions***—This is the first phylogenetic study of Onagraceae to include complete sampling of all 17 genera, and the results greatly improve our understanding of relationships among lineages within the family. As predicted by morphology, the newly discovered genus *Megacorax* is clearly sister to *Lopezia*. Further, *Gongylocarpus* does not belong within a paraphyletic Onagreae, but rather is sister to Epilobieae + On-

agreae s.s. and should be recognized as constituting its own monogeneric tribe Gongylocarpeae. Within Onagreae s.s., *Camissonia* and *Oenothera* appear paraphyletic, and *Gaura* + *Stenosiphon* are nested within a paraphyletic *Oenothera* + *Calylophus*. Clearly, increased taxon sampling and more data should yield greater insight into relationships within Onagreae.

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