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A cladistic analysis of the genus *Lopezia* (Onagraceae)

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HOCH, P. C., CRISCI, J. V. & TOBE, H., 1993. **A cladistic analysis of the genus *Lopezia* (Onagraceae).** *Lopezia*, a genus of 22 species largely restricted to Mexico, forms a monophyletic group defined by the possession of two stamens in tetramerous flowers. A cladistic analysis of the genus was performed using 16 characters from morphology, anatomy and embryology. Twenty of the 22 species were considered the terminal taxa. Polarity of the characters was based on the outgroup comparison method, using three alternative outgroups: the genus *Fuchsia*, Onagraceae excluding *Ludwigia*, and Epilobieae-Onagreae. Using *Fuchsia* as outgroup, 29 equally parsimonious cladograms were produced, each with 24 steps and a consistency index of 0.75. A successive weighting procedure was applied, resulting in 15 cladograms with consistency index of 0.85. The strict consensus cladogram defines eight monophyletic groups and supports most of the current sectional classification of *Lopezia*, with the exception that no synapomorphy defines section *Jehlia*. The larger sections *Lopezia* and *Pelozia*, although well-defined as clades, are not fully resolved internally. All cladograms support section *Riesenbachia* as monophyletic. The two alternative hypotheses for outgroups produced similar results: 61 most parsimonious trees, reduced after the successive weighting procedure to 15, which are identical to those produced with *Fuchsia* as outgroup. These results are discussed in the context of data on cytology and pollination biology.

ADDITIONAL KEY WORDS:—Cytology – phylogeny – pollination biology.

CONTENTS

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INTRODUCTION

Lopezia Cav. comprises 22 species and seven subspecies in six sections, and is the only genus of tribe Lopezieae (Plitmann, Raven & Breedlove, 1973; Raven, 1988). The genus is largely restricted to Mexico, with three species extending to Guatemala, one to El Salvador, and one subspecies confined to El Salvador, Costa Rica and Panama. *Lopezia* occupies a wide variety of habitats within this range, mainly at middle elevations, but is absent from the northern deserts and the lowland tropics (Plitmann *et al.*, 1973).

Members of the genus *Lopezia* have long fascinated students of floral biology because their flowers are both intricate and reduced in their number of parts. Some species are regularly visited and pollinated by hummingbirds; others by flies, principally Syrphidae, and other insects; and still others are autogamous. The diversity of chromosome numbers, which includes n = 7, 8, 9, 10, 11, 20 and 22 (Plitmann *et al.*, 1975), is remarkable for the small size of the genus. Although the larger genera *Clarkia* and *Epilobium* have similar levels of diversity, no other small genera in the family have so many different numbers (Raven, 1979).

This paper evaluates species and sectional relationships within *Lopezia* using a cladistic analysis based on information available from the morphology (Plitmann *et al.*, 1973, 1975), anatomy (Eyde & Morgan, 1973; Eyde, 1982; Tobe & Raven, unpublished) and embryology (Tobe & Raven, unpublished) of the genus. An independent analysis of relationships in *Lopezia* using molecular techniques involving cpDNA and rDNA (S. O'Kane & B. Schaal, unpublished) will test phylogenetic hypotheses resulting from this study.

MATERIAL AND METHODS

Twenty of the 22 species of *Lopezia* are considered the terminal taxa. Table 1 lists these taxa, their sectional classification and acronyms. Two species known only from their types (*L. sinaloensis* Munz of section *Pelozia*, and *L. conjugens* T. S. Brandegee of section *Lopezia*) were excluded from this analysis. None of the embryological and anatomical characters could be scored for those taxa, and even some of the morphological data were not available. Data from 16 characters were derived from morphology, anatomy and embryology (Table 2). Character polarity was determined by outgroup comparison (Watrous & Wheeler, 1981; Maddison, Donoghue & Maddison, 1984).

Onagraceae are a very well-characterized family within the order Myrtales (Raven, 1988). *Lopezia* is a monophyletic group of species separated from other genera by one major synapomorphy: two stamens in tetramerous flowers. Baehni & Bonner (1948) suggested a polyphyletic origin for *Lopezia*, but this suggestion was rejected in recent studies (Eyde & Morgan, 1973; Plitmann *et al.*, 1973, 1975). Seed tubercles of a particular morphology (Plitmann *et al.*, 1973; Tobe & Raven, unpublished) also appear to be a synapomorphy for the genus, but data are not yet available for all other genera. Tanaka, Oginuma & Toko (1988), in a survey of karyomorphology of Onagraceae, characterized *Lopezia* as having

TABLE 1. Sectional classification, species included and abbreviations

Section//taxa	Abbreviations
Section *Diplandra* (Hooker & Arn.) Plitmann, Raven & Breedlove	
L. lopezioides (Hooker & Arn.) Plitmann, Raven & Breedlove	LOPE
Section *Riesenbachia* (Presl) Plitmann, Raven & Breedlove	
L. semeiandra Plitmann, Raven & Breedlove	SEME
L. riesenbachia Plitmann, Raven & Breedlove	RIES
Section *Jehlia* (Rose) Plitmann, Raven & Breedlove	
L. longiflora Decaisne	LONG
L. grandiflora Zucc	GRAN
L. langmaniae Miranda	LANG
Section *Lopezia*	
L. miniata Lag. ex DC.	MINI
L. suffrutescens Munz	SUFF
L. nuevo-leonis Plitmann, Raven & Breedlove	NUEV
L. cornuta S. Wats.	CORN
L. ciliatula Plitmann, Raven & Breedlove	CILI
L. trichota Schlecht.	TRIC
L. racemosa Cav.	RACE
L. concinna Raven	CONC
L. smithii Rose	SMIT
Section *Nannolopezia* Plitmann, Raven & Breedlove	
L. gracilis S. Wats.	GRAC
Section *Pelozia* (Rose) Plitmann, Raven & Breedlove	
L. laciniata (Rose) M. E. Jones	LAACI
L. ovata (Plitmann, Raven & Breedlove) Plitmann, Raven & Breedlove	OVAT
L. gentryi (Munz) Plitmann, Raven & Breedlove	GENT
L. clavata T. S. Brandegee	CLAV

resting chromosomes of the diffuse-type, unlike any other of the ten genera surveyed, and it appears that the type found in *Lopezia* is autapomorphic within the family.

In a survey of *N*-terminal sequences of ribulose bisphosphate carboxylase small subunit in Onagraceae, Martin & Dowd (1986) found that *Lopezia* forms the sister group to the rest of the family excluding *Ludwigia*, with *Circaea* and *Fuchsia* forming the closest adjacent branches. In a recent analysis of rDNA sequence, Bult & Zimmer (1993) also found that *Lopezia* forms a sister group to the rest of the family excluding *Ludwigia*. A recent cladistic analysis of all genera of Onagraceae (Hoch *et al.*, 1993) using morphological, anatomical, palynological, embryological and cytological data shows *Lopezia* as sister group of *Fuchsia*, with *Circaea* as sister group to *Lopezia-Fuchsia*, and the three genera forming a distinct clade. *Lopezia* shares with *Fuchsia* a synapomorphy of lipid-containing mature pollen (vs starch-containing; Baker & Baker, 1982). Several molecular analyses have produced cladograms with *Lopezia* as the sister group to the tribes Epilobieae and Onagreae (Sytsma, Smith & Hoch, 1991; Conti, Fischbach & Sytsma, in press).

Thus, there are three hypotheses regarding outgroups for *Lopezia*: (1) *Fuchsia* alone (Hoch *et al.*, 1993), (2) all Onagraceae excluding *Ludwigia* (Martin & Dowd, 1986; Bult & Zimmer, 1993), and (3) Epilobieae and Onagreae (Sytsma *et al.*, 1991). For the characters in this analysis, the outgroup coding for Onagraceae excluding *Ludwigia* (2) and Epilobieae–Onagreae (3) is the same, so we combined these two hypotheses and used the former. Analyses with these alternative outgroups result in similar or identical trees; therefore we present

TABLE 2. Characters and character states used for cladistic analysis of *Lopezia*

Characters	Character states
1 Style–androecium adnation	0 = absent 1 = present
2 Perianth fusion	0 = complete fusion 1 = no fusion 2 = partial fusion
3 Gland on upper sepal	0 = absent 1 = present
4 Glands on upper petals	0 = absent 1 = 1 gland present 2 = 2 glands present
5 Number of fertile stamens	0 = 2 or more 1 = 1
6 Snapping stamens	0 = absent 1 = present
7 Number of cells forming seed tubercle	0 = about 10 cells 1 = 20–30 cells
8 Wax granules on seed surface	0 = absent 1 = present
9 Exotesta	0 = enlarged 1 = collapsed
10 Thickness of mesotesta	0 = 2 or more cells 1 = 1 cell
11 Endotesta	0 = present 1 = absent
12 Exotegmen	0 = present 1 = absent
13 Petal colour	0 = red to red-orange 1 = magenta to white
14 Pollen colour	0 = cream 1 = blue
15 Stylar vascular bundles	0 = 4 well-developed bundles 1 = discrete bundles absent
16 Stamen/staminode bases	0 = free, unfused 1 = fused to form tube around base of style

complete data only for *Fuchsia* as outgroup (Table 3) and compare the results with those using the alternative outgroup.

Character definition and coding

(1) *Style–androecium adnation* (median septum, Eyde & Morgan, 1973): In most species of *Lopezia* and most Onagraceae, including all outgroups, the style and androecium are free from the calyx at or just above the summit of the ovary. In *L. riesenbachia* and *L. semeiandra*, the style and androecium remain fused to each other and to the lower sepal throughout the length of the floral tube, forming a median septum in the floral tube, with two deep lateral nectar-bearing pockets (Eyde & Morgan, 1973).

(2) *Perianth fusion*: Fusion of the lower portions of all eight perianth parts into a floral tube is found in the outgroup *Fuchsia*, in most of Onagraceae except *Ludwigia*, and in *L. riesenbachia* and *L. semeiandra* of section *Riesenbachia*. Two additional states for this character exist in other species of *Lopezia*: (1) complete lack of fusion of any perianth parts beyond the ovary; and (2) fusion of the bases of five perianth lobes (the three upper/lateral sepals and two upper petals)—the

TABLE 3. Data matrix for the cladistic analysis of *Lopezia*, using the genus *Fuchsia* as outgroup

	1	2*	3	4*	5	6	7	8	9	10†	11	12	13†	14†	15	16
Fuchsia	0	0	0	0	0	0	?	0	0	0	0	0	0	?	0	0
L. lopezioides	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
L. semeiandra	1	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0
L. riesenbachia	1	0	0	0	1	0	0	1	0	1	0	0	1	0	0	0
L. longiflora	0	1	0	0	1	0	0	1	0	1	0	0	0	1	1	0
L. grandiflora	0	1	0	0	1	0	0	1	0	1	0	0	0	1	1	1
L. langmaniae	0	1	0	1	1	0	0	1	0	1	0	0	0	1	1	1
L. miniata	0	1	0	2	1	1	0	1	0	1	0	0	1	1	1	0
L. suffrutescens	0	1	0	2	1	1	0	1	0	1	0	0	1	1	1	0
L. nuevo-leonis	0	1	0	2	1	1	0	1	0	1	0	0	1	1	1	0
L. cornuta	0	1	0	2	1	1	0	1	0	1	0	0	1	1	?	?
L. ciliatula	0	1	0	2	1	1	0	1	0	1	0	0	1	1	?	?
L. trichota	0	1	0	1	1	1	0	1	0	1	0	0	1	1	1	0
L. racemosa	0	1	0	1	1	1	0	1	0	1	0	0	1	1	1	0
L. smithii	0	1	0	1	1	1	0	1	0	1	0	0	1	1	?	?
L. concinna	0	1	0	0	1	1	0	1	0	1	0	0	1	1	?	?
L. gracilis	0	1	0	1	1	0	1	1	0	1	0	0	1	1	1	0
L. laciniata	0	2	1	1	1	0	0	0	1	1	1	1	1	1	?	?
L. ovata	0	2	1	1	1	0	0	0	1	1	1	1	1	1	1	0
L. gentryi	0	2	1	1	1	0	0	0	1	1	1	1	1	1	1	0
L. clavata	0	2	1	1	1	0	0	0	1	1	0	1	1	0	1	0

*Characters 2 and 4 are treated as non-additive (unordered).

†The alternative outgroups are scored differently than *Fuchsia* in the following characters: 10(?), 12(?) and 14 (0).

remaining three perianth parts are free—in the highly zygomorphic flowers of section *Pelozia* (Plitmann *et al.*, 1973). There are no data that suggest that this partially fused zygomorphic perianth is ontogenetically or otherwise intermediate between presence and absence of floral tube, so these apomorphic states are treated as non-additive (i.e. an unordered multistate character).

(3) *Gland on upper sepal*: In all other genera of Onagraceae, including all outgroups, and in most species of *Lopezia*, all sepals are without glands. In the taxa of section *Pelozia*, however, the upper median sepal has a prominent gland at the level of divergence from the adjacent floral parts (Eyde & Morgan, 1973; Plitmann *et al.*, 1973).

(4) *Glands on upper petals*: All other Onagraceae including the outgroups and some species of *Lopezia* lack glands on the petals, whereas in many species of *Lopezia* the upper petals have one or two glands located near the level of divergence of the petals or just above a distinct stalk at the base of the petals (Plitmann *et al.*, 1973). The petals of *L. langmaniae* are less than half the size of the sepals (subequal in all other species), and are considered vestigial; they have distinct swellings that Eyde & Morgan (1973) and Plitmann *et al.* (1973) considered to be glands, even though they are scarcely visible within the tube-shaped flower during normal flowering phenology. Presence of one or two glands is treated as two separate, non-additive states.

(5) *Number of fertile stamens*: In all genera of Onagraceae, there are as many, or twice as many stamens as sepals; in *Fuchsia*, all species have eight stamens. In *Circaea* (dimerous flowers; Boufford, 1982; Boufford *et al.*, 1990) and *Lopezia*, stamen number is reduced to two. In *Lopezia* only *L. lopezioides* has two fertile stamens; all other species have one fertile stamen and one staminode. This reduction to a single fertile stamen is considered the apomorphic character state.

(6) *Snapping stamens*: In the species of section *Lopezia*, the sterile staminode enfolds the fertile stamen in bud and holds it under tension below the central axis of the flower as it opens. The stimulation of a flower-visiting insect causes the stamen to snap upwards, depositing pollen onto the lower abdomen of the visitor. This phenomenon is not known elsewhere in Onagraceae, even in other species of *Lopezia* with a staminode.

(7) *Number of cells forming seed tubercle*: In most species of *Lopezia*, seeds are more or less finely tuberculate, with each tubercle formed by about ten exotestal (epidermal) cells and fewer underlying mesotestal cells (Tobe & Raven, unpublished). *Lopezia gracilis* differs from this pattern in having coarsely tuberculate seeds, with each tubercle formed by 20–30 exotestal and a few mesotestal cells. All potential outgroups in Onagraceae lack comparable tubercles on the seed surface and are scored as undefined.

(8) *Wax granules on seed surface*: Abundant granules occur on the seed surface in most species except in *L. lopezioides* and the four species of section *Pelozia* (Tobe & Raven, unpublished). Wax granules do not occur on the seed surface of *Fuchsia* (Tobe, unpublished observations), and they have not been reported elsewhere in Onagraceae, so their presence is considered a synapomorphy.

(9) *Exotesta*: In most species of *Lopezia*, exotestal cells are more or less enlarged, at least at the position of the tubercles, but in the four species of section *Pelozia*, they are collapsed even at the tubercle (Tobe & Raven, unpublished). Exotestal cells remain uncollapsed in *Fuchsia* and other genera of Onagraceae (Tobe, unpublished observations).

(10) *Thickness of mesotesta*: In all species of *Lopezia* except *L. lopezioides*, the mesotesta is consistently one cell-layer thick; *L. lopezioides* has a mesotesta of two cell-layers. In *Fuchsia* and many other genera of Onagraceae, the mesotesta is always more than two cells thick, often much more so (Tobe & Raven, unpublished). In *Hauya*, Epilobieae, and most genera of Onagreae, the mesotesta is absent (Tobe, unpublished observations), so outgroups other than *Fuchsia* are scored as undefined.

(11) *Presence of an endotesta*: A crystalliferous endotesta is common to most species of *Lopezia* and all other species of Onagraceae. The endotesta is lacking, however, in *L. laciniata*, *L. ovata* and *L. gentryi* (Tobe & Raven, unpublished).

(12) *Presence of an exotegmen*: A fibrous exotegmen is found in most species of *Lopezia* except for the four species of section *Pelozia* (Tobe & Raven, unpublished). The exotegmen is also found in all species examined of other genera of the family (Tobe, unpublished).

(13) *Petal colour*: Variation in the colour of the petals in *Lopezia* is striking, but can be divided broadly into two types: red to red-orange; and lavender, rose-purple, magenta, pink to white (Plitmann *et al.*, 1973). Some taxa in this second category are quite variable infraspecifically, but no taxa have both colour types. These categories correlate closely with flower size and pollination syndrome: the red flowers are generally larger and more tubular, and are hummingbird-pollinated; the others smaller, more open and zygomorphic, and are fly-pollinated or autogamous. Most species of *Fuchsia* have red flowers (Berry, 1982), so we designate red petal colour as the plesiomorphic state (and *Fuchsia* as 0), and the magenta-purplish-white category as the apomorphy. Variation in flower colour for other Onagraceae is enormous, both within and between genera, so the alternative outgroups are undefined.

(14) *Pollen colour*: Most Onagraceae have cream-coloured pollen, but some species of *Fuchsia* (Crisci & Berry, 1990), *Epilobium* (Raven, 1976), and *Lopezia* have blue pollen, which is rare in the angiosperms. In fact, only three species of *Lopezia* (*L. semeiandra*, *L. riesenbachia* and *L. clavata* (SEME, RIES, CLAV)) have cream pollen (Plitmann *et al.*, 1973). In view of the distribution of blue pollen in the family, we treat it as the apomorphic state. Only the species of *Fuchsia* section *Skinnera* have blue pollen; however, that section appears to be the sister group to the rest of the genus, therefore we treat outgroup *Fuchsia* as mixed. Because cream pollen characterizes *Circaea*, *Hauya*, and all genera of Onagreae, we have scored the alternative outgroups as 0 (cream pollen).

(15) *Stylar bundles*: Eyde & Morgan (1973) reported four well-developed stylar bundles in three species of *Lopezia* (*L. lopezioides*, *L. semeiandra* and *L. riesenbachia* (LOPE, SEME, RIES)); they also reported this 'typical angiosperm style' in other Onagraceae. Stylar vasculature in other species of *Lopezia* was poorly defined or a network of anastomosing strands. Species that were not examined by Eyde & Morgan are treated as unknown. Even though data are lacking for at least some taxa in the outgroups, there are no reports of any state for this character other than four well-developed stylar bundles, so we have treated them all as 0.

(16) *Stamen/staminode bases*: In most species of *Lopezia* and all others of Onagraceae, the stamens, or the stamen and staminode, are free to their bases, but in *L. grandiflora* and *L. langmaniae*, the stamen and staminode are basally united around the free-standing base of the style (Eyde & Morgan, 1973). Some species of *Lopezia* were not examined for this character, which can only be studied in living or fresh-fixed material.

Characters 2 and 4 are the only multistate characters, and both are treated as non-additive (unordered in the analyses). As noted under characters 10, 13 and 14, the two alternative outgroups (*Fuchsia* and Onagraceae excluding *Ludwigia*) are scored differently for those three characters; in all other characters, the outgroups are scored identically. Table 3 contains the data matrix used in this analysis. The data were analysed using a Wagner parsimony algorithm (Kluge & Farris, 1969; Farris, 1970) from Farris's phylogenetic package, HENNIG86 (version 1.5; Farris, 1988; Platnick, 1989) run on a CompuAdd 320sc computer applying the implicit enumeration option for calculating trees. When the analyses yielded more than one tree, we calculated the strict consensus tree (Page, 1989), defined as the tree containing only those groups occurring in all trees, using the 'nelsen' option in HENNIG86. We also used the successive weighting procedure in HENNIG86, which calculates weights from the best fits to the most parsimonious trees, using rescaled consistencies (*rc*), which are the products of the character consistency (*c*) and the character retention index (*r*). The products are scaled to lie in the range 0–10. The weighting procedure is repeated on successively produced trees until the trees no longer change (Farris, 1989). The program CLADOS (version 1.1; Nixon, 1992) was used for examination of character distributions and production of the published figures.

RESULTS

With *Fuchsia* as outgroup, 29 equally parsimonious cladograms were generated by our data matrix, each with 24 steps and a consistency index of 0.75. The strict

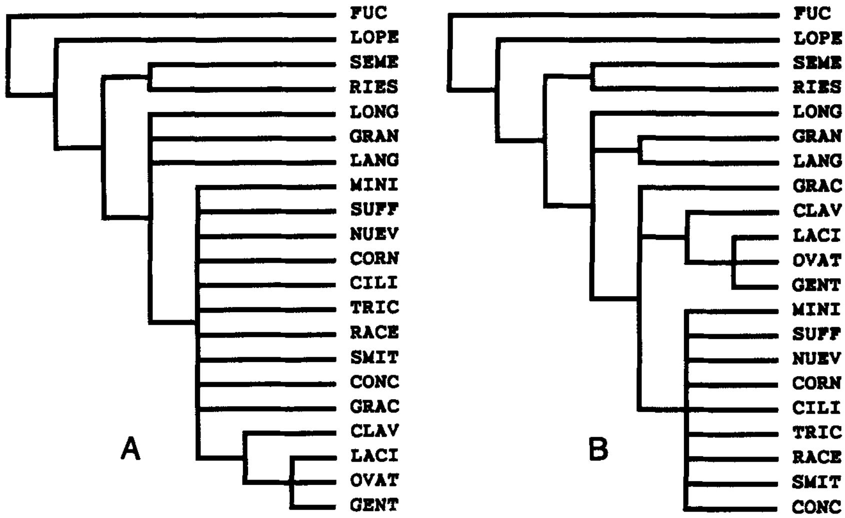

Figure 1. Strict consensus trees of species of *Lopezia*, using *Fuchsia* as outgroup. A. Consensus of 29 most-parsimonious trees (length = 24; consistency index = 0.75). B. Consensus of 15 minimum-length trees resulting after successive weighting procedure (length = 161; consistency index = 0.85) See Table 1 for abbreviations.

consensus tree of these 29 is shown as Fig. 1A. Six monophyletic groups in the consensus tree, listed with their synapomorphies, are as follows: (1) all species except *L. lopezioides* (LOPE) (one fertile stamen; presence of wax granules on seed surface, with reversion in section *Pelozia*; and mesotesta only one cell layer thick); (2) *L. semeiandra* and *L. riesenbachia* (SEME–RIES) (presence of style–androecium adnation; pollen colour blue, parallel with *L. clavata* (CLAV); and reversion to complete fusion of the perianth); (3) all species except *L. lopezioides*, *L. semeiandra* and *L. riesenbachia* (LOPE, SEME and RIES) (discrete stylar bundles absent); (4) all species except *L. lopezioides* (LOPE), *L. semeiandra* (SEME), *L. riesenbachia* (RIES), *L. longiflora* (LONG), *L. grandiflora* (GRAN) and *L. langmaniae* (LANG) (one or two glands present on upper petals, parallel with *L. langmaniae* (LANG); petal colour magenta to white, parallel with *L. riesenbachia* (RIES)); (5) *L. clavata* (*L. laciniata*, *L. ovata* and *L. gentryi*) (CLAV(LACI–OVAT–GENT)) (partial perianth fusion; gland on upper sepal; exotesta collapsed; exotegmen absent; and reversion to wax granules absent on seeds); and (6) *L. laciniata*, *L. ovata* and *L. gentryi* (LACI–OVAT–GENT) (endotesta absent).

When the successive weighting procedure was applied, 15 minimum-length trees resulted after the third round of weighting, with length 161 and the consistency improved to 0.85. (Note that the high value for the length is a function of the weight being scaled up to a value of ten.) The strict consensus tree for these cladograms is shown in Fig. 1B. The values for the range and number of steps, consistency index (*c*), retention index (*r*), and weight (*rc* × 10) for each character in the weighted trees are listed in Table 4. After the procedure, the maximum weight of ten was assigned to characters 1, 3, 5–7, 9–12, 15–16; a weight of five to character 2; a weight of four to characters 4, 8 and 13; and a weight of two to character 14.

TABLE 4. Character consistencies (c) and retention indices (r) as the best fits on the 29 most parsimonious trees from HENNIG86 using *Fuchsia* as outgroup, and used to calculate weights. Final weights were obtained after the third round of the successive weighting procedure in HENNIG86. Weights were truncated to integers

Character	Range of steps	Number of steps	Consistency index (c)	Retention index (r)	Weight ($rc \times 10$)	Final weight
1	1	1	1	1	10	10
2	2	3	0.66	0.80	5	5
3	1	1	1	1	10	10
4	2	3	0.66	0.90	5	4
5	1	1	1	1	10	10
6	1	1	1	1	10	10
7	1	1	1	1	10	10
8	1	2	0.5	0.80	4	4
9	1	1	1	1	10	10
10	1	1	1	1	10	10
11	1	1	1	1	10	10
12	1	1	1	1	10	10
13	1	2	0.50	0.80	4	4
14	1	2	0.50	0.50	2	2
15	1	1	1	1	10	10
16	1	1	1	1	10	10

Eight monophyletic groups appear in the consensus tree, six of which also appear in the 29 original cladograms as noted above; the two additional groups, listed with their synapomorphies, are as follows: (1) *L. grandiflora* and *L. langmaniae* (GRAN–LANG) (stamen–staminode bases fused into tube); and (2) *L. miniata*, *L. suffrutescens*, *L. cornuta*, *L. ciliatula*, *L. smithii*, *L. trichota*, *L. nuevo-leonis*, *L. racemosa* and *L. concinna* (MINI–SUFF–CORN–CILI–SMIT–TRIC–NUEV–RACE–CONC) (snapping stamens).

The 15 trees resulting after the successive weighting procedure can be further simplified to just three topologies without loss of parsimony. That is, one of the 15 trees (Fig. 2A) is the strict consensus tree of 11 others, a second (Fig. 2B) is the consensus of itself and one additional tree, and a third topology (Fig. 2C) is unique. The reason for this is that the implicit enumeration option of HENNIG86 regards trees as distinct if there is any possible character interpretation that will distinguish them. If a strict consensus tree is not longer than several trees, this shows that the extra branches in the latter trees, although possibly supported, are not necessary to account for the characters (Carr, Crisci & Hoch, 1990). Using one of these three trees (Fig. 2A), we illustrate character evolution, and have included chromosome numbers and sectional names (Fig. 3).

When the analysis is repeated using all Onagraceae except *Ludwigia* (same using Epilobieae–Onagreae) as outgroup, this results in 61 most parsimonious trees, with a strict consensus very similar to that found with *Fuchsia* as outgroup, differing only in that it does not resolve as monophyletic the group of all species except *L. lopezioides*. All 61 trees have 25 steps and a consistency index of 0.72. When the successive weighting procedure was applied, 15 minimum-length trees resulted after the third round of weighting, with length 158 and consistency index 0.86. These trees and the strict consensus tree from them are identical in topology to the 15 weighted trees derived using *Fuchsia* as outgroup.

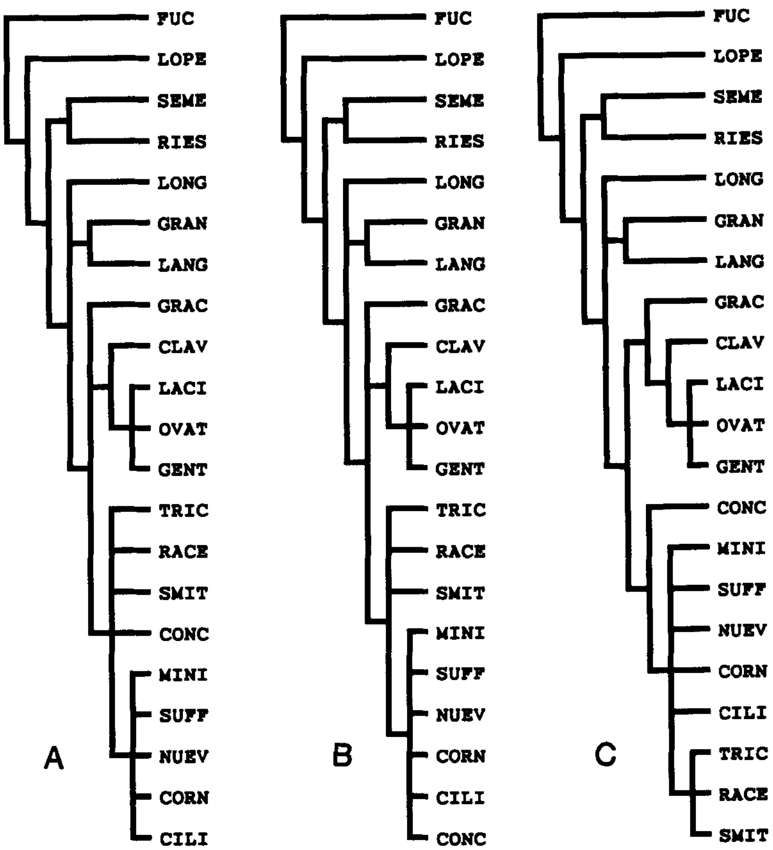

Figure 2. Three of the 15 minimum-length trees resulting after successive weighting; these three are the only fully supported topologies, as explained in text. A. Strict consensus of 12 trees. B. Strict consensus of two trees. C. Unique topology. See Table 1 for abbreviations.

DISCUSSION

Our analysis supports the current infrageneric classification of *Lopezia*, with the exception that there is no synapomorphy that defines section *Jehlia*. In particular, section *Pelozia* is extremely distinct, marked by four synapomorphies and a shared reversion to a plesiomorphic state, although the species of the section are only partially resolved. Section *Lopezia* is marked by one synapomorphy, but without any clear infrasectional delimitation.

All trees support the monophyly of section *Riesenbachia*, which is defined by three characters (stamen–androecium adnation; reversion to presence of a floral tube; and pollen cream-coloured, in parallel with *L. clavata* in section *Pelozia*). Prior to 1973, no authors postulated any direct connection between *L. riesenbachia* and *L. semeiandra*, and in fact these species were treated as the monotypic genera *Riesenbachia* and *Semeiandra* (Munz, 1965). Nevertheless, the similarity in basic floral structure, noted above by two of the synapomorphies for the section, caused Eyde & Morgan (1973) and Plitmann *et al.* (1973) to hypothesize the monophyletic nature of this group. Plitmann *et al.* (1973) also used similarities in pollen colour and chromosome number between these two species to argue their close relationship, but ambiguity in the character states for

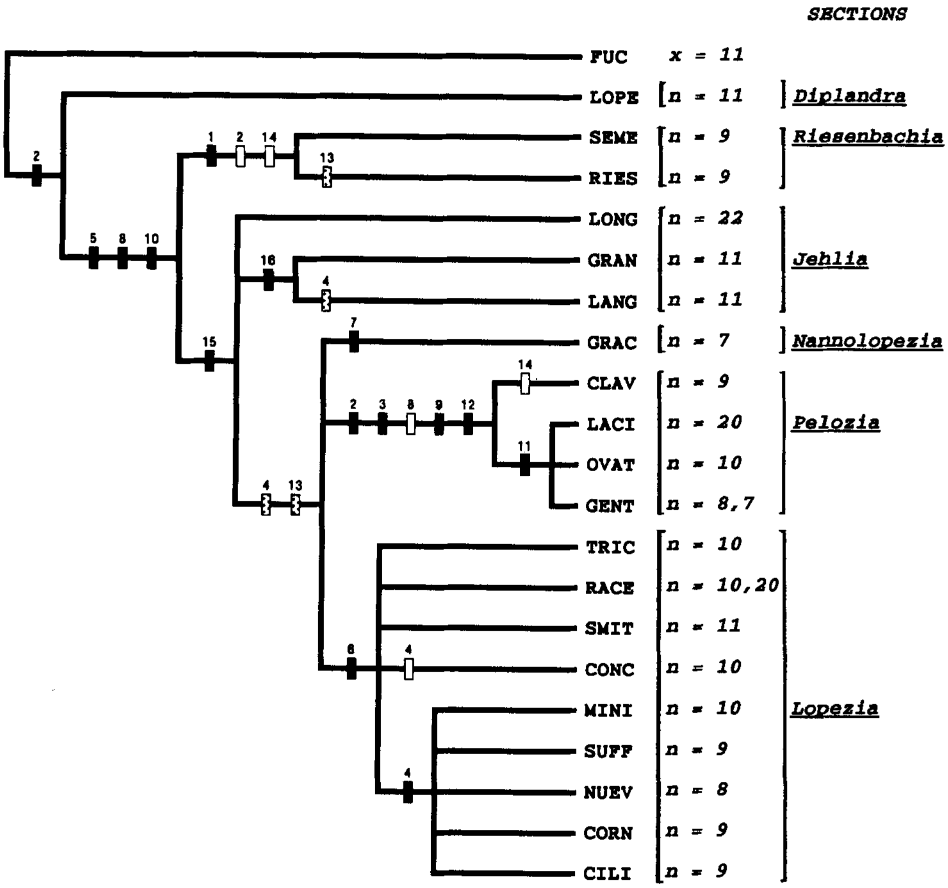

Figure 3. One of the 15 minimum-length trees of *Lopezia* species, using *Fuchsia* as outgroup, resulting after successive weighting procedure; also illustrated in Fig. 2A. Character state changes are superimposed; solid black = synapomorphies; shaded = homoplasies (parallel or convergent evolution); open = reversals. See Table 1 for abbreviations. Chromosome numbers and sectional names also superimposed; see text for discussion.

chromosome number (see below) prevent us from using this evidence in our analysis.

Two species, *L. conjugens* and *L. sinaloensis*, were excluded from this analysis due to insufficient data. Both are known only from type material collected more than 70 years ago, and for neither was it possible to examine embryology, seed anatomy or floral morphology in detail. Plitmann *et al.* (1973) placed *L. conjugens* in section *Lopezia* near *L. smithii* based on the few floral characters available, and *L. sinaloensis* in section *Pelozia* based on the partial fusion of perianth parts so characteristic of that group; our results suggest no better placement for either species.

Data from chromosome numbers were not used in our analysis due to the complex variation of this character (Plitmann *et al.*, 1975); nevertheless several observations may be appropriate. The basic chromosome number for Onagraceae appears to be $x = 11$ (Raven, 1979, 1988), a number that characterizes all species of *Circaea* and *Fuchsia*, but not most of the rest of

Onagraceae; it also characterizes *L. lopezioides* and the three species of section *Jehlia*. One species of section *Lopezia* (*L. suffrutescens*) also has $n = 11$, but other species of the section have $x = 8$, 9 and 10. *Lopezia riesenbachia* and *L. semeiandra* share a chromosome number of $n = 9$, but this number is also found in five other species of sections *Pelozia* and *Lopezia*. The chromosome numbers have been added to Fig. 3 as a way to contrast our results with this information. This analysis supports the hypothesis that $x = 11$ is the basic number for *Lopezia*, and does not suggest that $n = 11$ ever re-evolved from another number ($n = 11$ in *L. smithii* in section *Lopezia* could be basic to the section). The number $n = 9$ appears to be a synapomorphy for section *Riesenbachia*, but the number arose independently at least two other times. Aneuploid reductions appear to have occurred independently in sections *Riesenbachia*, *Lopezia*, *Pelozia* and *Nannolopezia*, although the placement of *L. gracilis* (section *Nannolopezia*) is not fully resolved.

For such a relatively small genus, *Lopezia* has a remarkably diverse array of pollination syndromes, from bird and insect pollination to modal autogamy (Plitmann *et al.*, 1973). This diversity is reflected in our characters, since at least eight of them (1–4, 6, 13–14 and 16) involve features that relate directly to pollination mechanisms. Five species (*L. lopezioides*, the three species of section *Jehlia*, and *L. semeiandra*) are pollinated by hummingbirds; all have red to red-orange flowers (character 13) and except for *L. semeiandra* have relatively unspecialized flowers. *Lopezia semeiandra* shares with *L. riesenbachia* the presence of a floral tube (character 2) and the apomorphic 'median septum' (character 1, but is otherwise similar to the other hummingbird-pollinated species and quite unlike the small-flowered, modally autogamous *L. riesenbachia*. Raven (1979, 1988) cites these two species as the only known example of a close sister-species relationship between a bird-pollinated and an autogamous species.

The remaining species, in sections *Lopezia*, *Pelozia* and *Nannolopezia*, are pollinated by flies, bees, small moths or other insects, or are autogamous (Plitmann *et al.*, 1973; Raven, unpublished observations). These changes come about in part through partial floral fusion and zygomorphy (character 2), various glands or 'pseudonectaries' (characters 3 and 4; Plitmann *et al.*, 1973), the snapping stamen feature (character 6), and changes in floral colour (character 13). In many taxa there are additional autapomorphies that influence pollination, such as the striking petal colour banding of *L. concinna* (Raven, 1977), petals fringed with teeth (*L. ciliatula*) or ciliate hairs (*L. trichota*), or petals deeply cleft or laciniate (most of section *Pelozia*). Many species in these sections also have very distinctive auricles near the bases of the upper petals and/or geniculate stalks on the upper petals (Plitmann *et al.*, 1973). However, these features were not always well defined or described even in the fine monograph of Plitmann *et al.* (1973), especially since many can only be seen clearly in living plants. Also, some of these characters vary within taxa. For these reasons, these floral characters could not be included in our cladistic analysis, with the result that relationships of the taxa of sections *Lopezia* and *Pelozia* are largely unresolved.

The three hypotheses for outgroup of *Lopezia* result in nearly identical hypotheses of relationships within the genus. The fact that the position of the genus in this well-studied family is so uncertain is striking, but this uncertainty does not seem to be an obstacle to establishing the relationships among the species of the genus.

Tobe & Raven (unpublished) present data on seed morphology and anatomy in *Lopezia*; we have used six characters (7–12) from that analysis. One additional character that they describe concerns the presence of wings or ridges on the adaxial surface of the seeds. *Lopezia trichota* has well-developed wings; *L. riesenbachia*, *L. concinna*, *L. nuevo-leonis* and *L. racemosa* have small ridges visible only in microscopic cross-section; all other species lack either ridges or wings in that position. As reported earlier by Plitmann *et al.* (1973), the seeds of *L. lopeziodes* also have wings, but they do not appear to be homologous with those described by Tobe & Raven; the seeds of *L. lopeziodes* are more than twice the length of any other seed in the genus (3–4.5 mm long), and are nearly equally broad with large flattened wings. The seeds of *L. trichota*, by contrast, are *c.* 1 mm long, *c.* 0.6 mm wide; with the wings, they are *c.* 1.5 mm wide. We repeated our entire analysis with a character for seed wings/ridges, treating *L. lopezioides* as lacking wings, since they are not homologous to those found in the five species listed above. Using *Fuchsia* as outgroup, this resulted in 164 most-parsimonious trees (length 28, consistency index 0.71); the consensus is nearly identical to that derived without this character, differing only in that section *Lopezia* is resolved as a monophyletic unit. The successive weighting procedure produced 46 trees (length 165, consistency index 0.84), with an identical strict consensus to that in the main analysis. These results suggest that use of this character, although creating many additional possible resolutions within section *Lopezia*, and despite our uncertainty about the extent of this character within the genus, improves the unweighted consensus topology.

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REFERENCES

Baehni C, Bonner CEB. 1948. La vascularization des fluers chez les Lopezieae (Onagracées). *Candollea* **11:** 305–322.

Baker HG, Baker I. 1982. Starchy and starchless pollen in the Onagraceae. *Annals of the Missouri Botanical Garden* **69:** 748–754.

Berry PE. 1982. The systematics and evolution of *Fuchsia* sect. *Fuchsia*. *Annals of the Missouri Botanical Garden* **69:** 1–198.

Boufford DE. 1982. The systematics and evolution of *Circaea* (Onagraceae). *Annals of the Missouri Botanical Garden* **69:** 804–994.

Boufford DE, Crisci JV, Tobe H, Hoch PH. 1990. A cladistic analysis of *Circaea* (Onagraceae). *Cladistics* **6:** 171–182.

Bult CJ, Zimmer EA. 1993. Nuclear ribosomal RNA sequences for inferring tribal relationships within Onagraceae. *Systematic Botany* **18:** 48–63.

Carr BL, Crisci JV, Hoch PH. 1990. A cladistic analysis of the genus *Gaura* (Onagraceae). *Systematic Botany* **15:** 454–461.

Conti E, Fischbach A, Sytsma KJ. in press. Tribal relationships in Onagraceae: implications from *rbc*L sequence data. *Annals of the Missouri Botanical Garden* *80*(3).

Crisci JV, Berry PE. 1990. A phylogenetic reevaluation of the Old World species of *Fuchsia* (Onagraceae). *Annals of the Missouri Botanical Garden* **77:** 517–522.

Eyde RH. 1982. Evolution and systematics of the Onagraceae: floral anatomy. *Annals of the Missouri Botanical Garden* **69:** 735–747.

Eyde RH, Morgan JT. 1973. Floral structure and evolution in Lopezieae (Onagraceae). *American Journal of Botany* **60:** 771–787.

Farris JS. 1970. Methods for computing Wagner Trees. *Systematic Zoology* **19:** 83–92.

Farris JS. 1988. *HENNIG86 Version 1.5.* Documentation.

Farris JS. 1989. The retention index and the rescaled consistency index. *Cladistics* **5:** 417–419.

Hoch PC, Crisci JV, Tobe H, Berry PB. 1993. A cladistic analysis of the plant family Onagraceae. *Systmatic Botany* **18:** 31–47.

Kluge AG, Farris JS. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* **18:** 1–32.

Maddison WP, Donoghue MJ, Maddison DR. 1984. Outgroup analysis and parsimony. *Systematic Zoology* **33:** 83–103.

Martin PG, Dowd JM. 1986. Phylogenetic studies using protein sequences within the order Myrtales. *Annals of the Missouri Botanical Garden* **73:** 441–448.

Munz PA. 1965. Onagraceae. In: *North American flora*, series II, Vol. 5. Bronx, NY: New York Botanical Garden, 1–278.

Nixon KC. 1992. *CLADOS ver. 1.1. IBM PC-compatible character analysis program.* Documentation published by the author.

Page RDM. 1989. Comments on component-compatibility in historical biogeography. *Cladistics* **5:** 167–182.

Platnick NI. 1989. An empirical comparison of microcomputer parsimony programs, II. *Cladistics* **5:** 145–161.

Plitmann U, Raven PH, Breedlove DE. 1973. The systematics of Lopezieae (Onagraceae). *Annals of the Missouri Botanical Garden* **60:** 478–563.

Plitmann U, Raven PH, Tai W, Breedlove DE. 1975. Cytological studies in Lopezieae (Onagraceae). *Botanical Gazette* **136:** 322–332.

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

Raven PH. 1977. A new species of *Lopezia* (Onagraceae) from Sinaloa, Mexico. *Annals of the Missouri Botanical Garden* **64:** 638–641.

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

Raven PH. 1988. Onagraceae as a model of plant evolution. In: Gottlieb LD, Jain SK, eds. *Plant evolutionary biology. A symposium honoring G. Ledyard Stebbins.* London: Chapman & Hall, 85–107.

Sytsma KJ, Smith JF, Hoch PC. 1991. A chloroplast DNA analysis of tribal and generic relationships within Onagraceae. *American Journal of Botany* **78** (6/Suppl.): 222 (abstract).

Tanaka R, Oginuma K, Toko S. 1988. Karyomorphological studies on 26 species of ten genera of the Onagraceae. *La Kromosomo* **II-51-52:** 1675–1696.

Watrous L, Wheeler Q. 1981. The out-group comparison method of character analysis. *Systematic Zoology* **30:** 1–11.