A PHYLOGENETIC EVALUATION OF *CALLISIA* LOEFL. (COMMELINACEAE) BASED ON MOLECULAR DATA

by

STEPHANIE BERGAMO

(Under the Direction of David E. Giannasi)

ABSTRACT

The genus, *Callisia* (Commelinaceae), of the subtribe Tradescantiinae, currently consists of approximately 20 species confined to the New World tropics and warm temperate zones. Molecular phylogenies using chloroplast gene regions of *ndhF* and *trnL-F* substantiate that the genus is polyphyletic. *Callisia* sensu lato is reinterpreted via the molecular phylogenetic analyses correlated with morphological, anatomical, geographical, and chromosomal data. Molecular topologies and strongly supported tree statistics provide a sound argument for elevating two sections, *Cuthbertia* and *Brachyphylla*, to generic status, and for treating one species of section *Leptocallisia* under *Phyodina*. *Callisia* sensu stricto is redefined to include sections *Callisia* and *Hadrodemas*. The genus *sensu lato* retains the remaining members of section *Leptocallisia* and the one member of section *Lauia* pending further taxon sampling.

INDEX WORDS: *Callisia*, *Cuthbertia*, *Phyodina*, *Brachyphylla*, Commelinaceae, *ndhF*, *trnL-F*, *Tradescantia*, *Tripogandra*, *Gibasis*, Tradescantiinae

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DEDICATION

This dissertation is dedicated to my family:

to my mother, Virginia “Ginny” Bergamo

to my sister, Gillian “Gill” Bergamo

to my father, the late Professor Ralph Bergamo

and

to all additional family members who have brought humor and delight throughout this journey:

to Rio the cat, Kava the horse, Zulu the goat, and others.

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CHAPTER 1

INTRODUCTION

Overview

Commelinaceae R. Br. (1810), nom. cons., a monocotyledon family (ca. 41 genera, 650 species), is currently separated into two subfamilies, Cartonematoideae (Pichon) Faden ex G. Tucker (1989) and Commelinoideae Faden & D. R. Hunt (1991) (Faden 1998). Within subfamily Commelinoideae, tribe Tradescantieae Meisn. (1842), the subtribe Tradescantiinae Rohw. (1956) has been defined to include the New World genera *Tradescantia* L., *Gibasis* Raf., *Tripogandra* Raf., and *Callisia* Loefling., the latter genus with notably problematical taxa (H. Moore 1958, 1961; Hunt 1986b; Faden and Hunt 1991; Faden 1998, 2000a).

Subtribe Tradescantiinae (Table 1) includes perennials or rarely annuals (Faden and Hunt 1991) of erect, climbing, or trailing habit (Mabberley 1997). The inflorescence is organized into paired sessile cincinni (scorpioid cymes) as defined by Weberling (1989). In this type of scorpioid cyme, each successive flower branches alternately from left to right, as opposed to a helicoid cyme (a bostryx), in which each successive flower branches always to the left or always to the right (Weberling 1989). In Tradescantiinae, these scorpioid cymes are partially or completely fused back to back (with the exception of *Gibasis's* unfused stipitate cincinni arranged with 2 or more in a pseudo-umbel) (Faden and Hunt 1991). The typical actinomorphic flowers are usually hermaphroditic (Faden and Hunt 1991). Stamens vary from zero to six, and range from all fertile to

various numbers of antepetalous staminodes (Faden and Hunt 1991). As currently treated, *Callisia* may be distinguished from the other three genera of subtribe Tradescantiinae by the inflorescences composed of paired, sessile cincinni; the absence of spathaceous bracts below the cincinnus-pairs; and the actinomorphic flowers with monomorphic stamens (Faden 1998).

While members of the subtribe do not have significant economic value, some members, including taxa currently included in *Callisia*, are popular ornamental garden or potted plants (H. Moore 1958, 1961; Graf 1976; Hunt 1984; Tucker 1989; Griffiths 1999); others are curiosities in botanical collections (H. Moore 1958, 1961, 1962; Faden 1998). Some members of *Callisia* have escaped from cultivation (Tucker 1989; Faden 1998, 2000), and *C. fragrans* (Lindl.) Woodson is currently included in a comparative study of invasive and non-invasive Commelinaceae in Florida (J. Burns, pers. comm.). Several taxa in the subtribe have been used to monitor drinking water purity and environmental contaminants such as radiation (Tucker 1989); other species have been reported as having culinary or medicinal value to indigenous peoples (Tucker 1989).

Taxonomic History of *Callisia*

Callisia's taxonomic history is complex. The first description of *Callisia* was in dispute. Although *Callisia* purportedly was first described by Loefling in 1758 without a type species designation (H. Moore 1958), various early workers also ascribed the genus to Linnaeus (e.g. Rafinesque 1836, Pichon 1946, Standley and Steyermark 1952, Rohweder 1956, Aristeguieta 1965). The interpretation here, based on scrutiny of Loefling's untranslated *Iter Hispanicum* (1758) and on J. R. Forster's 1771 abstract of Loefling's travels, follows the convention of both Linnaeus and later workers in ascribing

the genus to Loefling. The neotype, *C. repens* (Jacq.) L., was first described as *Hapalanthus repens* by Jacquin in 1760 and transferred to *Callisia* by Linnaeus (1762). The genus remained monotypic for many years, but progressively, additional species were described or transferred to it, some of which were later excluded (H. Moore 1958), such as *C. ciliata* Kunth, *C. cordifolia* (Sw.) Anderson & Woodson, *C. monandra* (Sw.) Schultes f., and *C. multiflora* Standl. Various genera, some currently obsolete (e.g., *Cuthbertia* Small, *Aploleia* Raf., *Phyodina* Raf., *Tradescantella* Small, *Spironema* Lindl., *Rectanthera* Degener, *Hapalanthus* Jacq., *Leptocallisia* Pichon, *Leptorhoeo* C. B. Clarke, *Leiandra* Raf., *Setcreasea* Schumann and Sydow, and monotypic *Hadrodemas* Moore) and some contemporary (e.g. *Tradescantia* L. *Tripogandra* Raf., *Aneilema* R. Br., *Commelina* L., *Gibasis* Raf., and *Dichorisandra* Mikan) have included representatives currently treated under *Callisia.*

Rafinesque (1836) described a profusion of genera, three of which (*Phyodina, Leiandra,* and *Aploleia*) have subsequently been included within or segregated from *Callisia*, and two of which (*Gibasis* and *Tripogandra)* remain in current treatments as two of the four members of subtribe Tradescantiinae (Hunt 1986b, Faden and Hunt 1991, Faden 1998). Regarding *Phyodina, Leiandra, Aploleia*, *Callisia*, *Gibasis* and *Tripogandra*, plus *Tradescantia*, *Tonningia* Necker, *Siphostina* Raf., and *Ftheosanthes* Raf., Rafinesque supported his segregations based largely on staminal characteristics, but with *Phyodina* distinct from *Callisia* by fruit locule number (Rafinesque 1836). Rafinesque noted that if species were treated as in the past, largely under *Tradescantia*, no single character in common would define the genus (Rafinesque 1836). A similar observation has been made in later treatments of *Callisia* (Hunt 1986b).

Lindley (1840) described the monotypic genus *Spironema* (*S. fragrans*), named for the species' garden-worthy qualities: "spiral filaments", "petaloid anthers", and fragrance (Lindley 1840, p. 26), hence the common name, sweet-scented spiralthread. *Spironema* remained as treated by Lindley (1840) until being designated a nomenclatural synonym of Degener's *Rectanthera*; that generic name alludes to the rectangular anther connectives of Degener's combination, *R. fragrans* (Degener 1932).

Hemsley (1880), within Gamopetalae (unfused petals), ascribed to C. B. Clarke both the new species, *C. insignis*, and the new genus, *Leptorhoeo*, with the single entity, *L filiformis* (Hemsley 1880). That species, currently treated under *Callisia filiformis* (Martens & Galeotti) D. R. Hunt, has many synonyms, treated under *Aneilema, Tripogandra,* and *Tradescantia*, with more than one epithet (Hunt 1986b, 1994).

Under *Callisia* (with stamens numbering three, two, or one), Clarke (1881) included *C. repens, C. insignis*, and two other species, *C. umbellulata* (Lamark) C. B. Clarke [currently treated as *C. monandra* (Sw.) Schultes f.] and *C. Martensiana* (Kunth) C. B. Clarke [currently treated as *C. multiflora* (Martens & Galeotti) Standl.]. The latter two species also have many synonymies under *Aploleia, Commelina, Leptocallisia,* and *Tradescantia* (H. Moore 1958, 1961). Under *Tradescantia* section *Eutradescantia* (stamens six, all of the same length or three shorter; anthers essentially monomorphic), Clarke included five species currently assigned to *Callisia* [*C. navicularis* (Ortgies) D. R. Hunt, *C. gracilis* (Kunth) D. R. Hunt, *C. rosea* (Vent.) D. R. Hunt, *C. cordifolia* (Sw.) Anderson & Woodson (1935), and *C. warszewicziana* (Kunth & C. D. Bouché) D. R. Hunt] (Clarke 1881, Hunt 1986b). Clarke (1881) retained the monotypic *Spironema* of Lindley (six stamens).

Bentham and J. D. Hooker (1883) essentially mirrored Clarke, maintaining *Callisia*, *Spironema*, and *Leptorhoeo*, except that *Callisia* was treated with the four species *sensu* Clarke in two sections: section *Hapalanthus* (*C. repens*, *C. insignis*), and section *Leptocallisia* [*C. umbellulata* sensu Clarke (1881) = *C. monandra*, *C. Martensiana* sensu Clarke (1881) = *C. multiflora*].

Grouped under the series Ephemerines (generally consistent with tribe Tradescantieae), treatment of *Callisia*, *Spironema*, and *Leptorhoeo* by Baillon (1895) closely paralleled Bentham and J. D. Hooker (1883) but without sections *Hapalanthus* and *Leptocallisia*. Baillon was ambivalent as to the number of species (three or four) to include in *Callisia* (Baillon 1895).

The *Flora of the Southeastern United States* by Small (1903) includes the original description of the genus *Cuthbertia* (endemic to the southeastern United States) into which Small transferred Ventenat's (1800) *Tradescantia rosea* and added the newly described *C. graminea* Small. At the same time, Small (1903) described *Tradescantella,* transferring to it Watson's (1882) *Tradescantia floridana*, the type of *Tradescantella* (Small 1903). *Tradescantella floridana,* originally described as *Tradescantia cordifolia* by Swartz (1783), was transferred to *Leiandra* by Rafinesque (1836) and was later renamed *Callisia meiandra* by Sauvalle (1873). Reduced, non-spathaceous bracts subtending the cymes distinguished *Cuthbertia* and *Tradescantella* from *Tradescantia* (Small 1903). Small described *Cuthbertia* with simple cymes and *Tradescantella* with "dichotomous cymes" (Small 1903, p. 237). Both genera have two cymes fused dorsally, referable to Small's description of the *Tradescantella* inflorescence. Small used the misunderstood inflorescence structure along with filament pubescence to separate

Cuthbertia from *Tradescantella* (Small 1903). Small added the new Florida endemic, *C. ornata,* in 1933. After the move of the three *Cuthbertia* species back into *Tradescantia* as varieties under *T. rosea* by Anderson and Woodson (1935), separation of the three taxa from *Tradescantia* and the validity of *Cuthbertia* as a viable genus was later given support by chromosome studies of Giles (1942), pigment profiles of Matthews (1966), anatomical surveys of Tomlinson (1966, 1969) and fieldwork of Lakela (1972). Lakela (1972) resurrected *Cuthbertia* with the same three original members as Small (1903, 1933).

In his treatment of Commelinaceae, Pichon (1946) transferred one genus (*Cartonema* R. Br.) to a new family, Cartonemaceae, and split the remaining genera into ten tribes [instead of the traditional two or four (Faden & Hunt 1991)], including Tradescantieae and Callisieae (Pichon 1946, Faden and Hunt 1991). Androecial characters largely separated the two tribes, particularly with the inclusion of *Tripogandra* (dimorphic stamens) in Callisieae. Tribe Tradescantieae included *Phyodina* and *Leptorhoeo* (Pichon 1946). Callisieae totaled four genera: *Callisia, Tripogandra, Palisota* Reichb., and *Dilasia* Raf., characterized by staminal, stigmatic, and stomatal characters (Pichon 1946). Pichon's treatment included the "démembrement du genus" (Pichon 1946, p. 225), *Callisia*, with the transfer of two species, *L. umbellulata* = *C. monandra* and *L. multiflora* = *C. multiflora*, to *Leptocallisia* (Bentham and J. D. Hooker) Pichon under the new tribe Anthericopsideae. The primary distinctions between the two tribes Callisieae and Anthericopsideae were inflorescence structure (which Pichon also misinterpreted as being with single cymes in Callisieae), stamen position (epipetalous in *Callisia*, episepalous in *Leptocallisia*), and cyme characteristics (Pichon 1946).

Anthericopsideae was distinguished from other tribes by androecial and fruit characteristics (Pichon 1946).

Matuda (1954, 1955a) described *C. soconuscensis* (1954) and *C. macdougallii* (1955a; authority ascribed to Miranda) of Mexico, and *C. gentlei* (1955a; as *C. Gentlea*) of Belize. In Matuda's treatment of Mexican Commelinaceae (Matuda 1955b), *Callisia* contained eight species, *C. fragrans, C. macdougallii, C. repens, C. insignis, C. soconuscensis, C. cordifolia,* and included *C. monandra* and *C. multiflora*, the two species of Pichon's *Leptocallisia*, but without reference to the earlier combinations. Additional works by Matuda added the newly described Mexican endemics *C. tehuantepecana* (Matuda 1956) and *C. nizandensis* of Oaxaca (Matuda 1956, 1975), and *C. guerrerensis* of Guerrero (Matuda 1966).

Under the new subtribe, Tradescantiinae, Rohweder (1956) transferred *C. cordifolia*, *Tripogandra rosea*, *T. warszewicziana*, and *Tradescantia navicularis* to *Phyodina*. Rohweder included in the same subtribe of 12 genera *Leptorhoeo* [*L. floribunda* (Hook. and Arnold) Baillon = *Callisia filiformis*], *Leptocallisia* [*L monandra* (Sw.) Ludwig and Rohw. = *C. monandra*, *L. multiflora* = *C. multiflora*]; *Callisia* (*C. repens, C. fragrans, C. insignis*) and *Tripogandra*, and treated *Gibasis* as the only member of another new subtribe, Gibasinae (Rohweder 1956).

Callisia as defined by Moore (1958) included eight species (*C. elegans*, *C. fragrans, C. macdougallii, C. repens, C. insignis, C. soconuscensis, C. gentlei,* and *C. tehuantepecana*), which Moore delineated based on inflorescence, perianth, androecial, and gynoecial characteristics. This resulted in *C. cordifolia* under *Leiandra*, and both *C. monandra* and *C. multiflora* under *Leptocallisia* (H. Moore 1958). The latter two species

were later transferred to *Aploleia* by Moore (1961), based on androecial modifications, particularly the reduced number of episepalous (versus epipetalous) stamens. *Callisia* sensu Moore (1958) mirrors the subsequent *Callisia* section *Callisia* of Hunt (1986b). Moore transferred another problematic taxon to a new and monotypic genus, *Hadrodemas,* as *H. warszewicziana* (H. Moore 1962), which had previously been treated under *Tradescantia* by Kunth and Bouché (1847, 1848) and C. B. Clarke (1890), *Dichorisandra* by Planchon (1854), *Spironema* by Brückner (1927), *Tripogandra* by Woodson (1942), and *Phyodina* by Rohweder (1956) (Standley and Steyermark 1952, H. Moore 1962). Habit, inflorescence, calyx, androecial, and ovary characteristics defined *Hadrodemas* (H. Moore 1962).

Hunt (1978) described *Phyodina laui* and also transferred *Tradescantia micrantha* Torr. Hunt (1978) noted that *Phyodina* sensu Rohweder (1956) included such varied components [as observed by Handlos (1975)], that treatment as *Phyodina* was provisional.

Hunt (1986b) broadened *Callisia* and divided it into six sections (Table 2). This expansion of the genus was Hunt's alternative to recognizing a number of genera with just one or two species (Hunt 1986b). Hunt (1986b) delimited one section, *Callisia*, based upon a set of "relatively highly derived" (Hunt 1986b, p. 408) characters including " a spike-like or paniculate inflorescence and sessile flowers with paleaceous sepals, more or less reduced petals, exserted stamens and penicilliform stigmas, implying a trend towards anemophily" (Hunt 1986b, p. 408). To section *Leptocallisia*, Hunt assigned species exhibiting meiomery, i.e. reduction in stamen, locule, and/or ovary number (Hunt 1986b). The other sections Hunt (1986b) considered less derived in floral structure. Two

of those sections, *Cuthbertia* and *Hadrodemas*, included one species of *Cuthbertia* and the one taxon of *Hadrodemas* sensu Moore (1962), respectively (Hunt 1986b). Two other sections, *Brachyphylla* and *Lauia*, included elements of *Phyodina* sensu Rohweder (1956) and/or Hunt (1978, 1983). Unifying characters of the latter four sections consisted of actinomorphic *Tradescantia*-type pink flowers with six stamens (Hunt 1986b). Hunt (1986b) noted the general succulence of *Callisia* plants, a characteristic less developed in sections *Cuthbertia* and *Lauia*, but then augmented by "geophytism" (Hunt 1986b, p. 408) [i.e. bearing perennial buds underground (Allaby 1992)].

For his treatment of Commelinaceae in the *Generic Flora of the Southeast*, Tucker (1989) transferred the remaining two of the three *Cuthbertia* species *sensu* Small (1933), *C. graminea* and *C. ornata*, to *Callisia* section *Cuthbertia*. Lakela's 1972 treatment of *Cuthbertia* had been overlooked or not recognized by Hunt (1986b), and these two taxa transferred by Tucker (1989) had been left at the varietal rank under *Tradescantia* sensu Anderson and Woodson (1935) by Hunt (1986b).

Callisia as treated *sensu* Hunt (1986b)

Callisia (ca. 20-23 species) *sensu* Hunt (1986b) is tropical to subtropical New World in distribution, comprising perennial and annual herbaceous species (Hunt 1986b, Tucker 1989, Faden 1998). The genus ranges from the southeastern United States, Texas, Mexico, to Central America and the West Indies and Argentina (Hunt 1986b, 1994; Tucker 1989; Faden 1998, 2000). Three species (*C. graminea*, *C. ornata*, *C rosea*) currently treated under *Callisia* are endemic to the southeastern United States (Tucker 1989); one species (*C. warszewicziana*) is endemic to Guatemala (Hunt 1986b, 1994); others are endemic to restricted areas in Mexican (Hunt 1986b).

The concentration of *Callisia* species in tropical and subtropical habitats with seasonal moisture regimes is reflected by their generally succulent habit (Hunt 1986b). Tucker (1989) suggests that *Callisia* commonly thrives in drier habitats than *Tradescantia* or *Tripogandra*. *Callisia fragrans* has been documented to exhibit Crassulacean Acid Metabolism (CAM)-cycling, hypothesized to be a precursor of CAM-idling, a means by which CAM acid fluctuations can be maintained under conditions of stomatal closure, maintaining metabolic activity during periods of severe drought (Martin et al. 1994).

Roots are typically fibrous although a few species have tubers (Hunt 1986b) or rhizomes. Leaves are alternate, sessile, and spiral or two-ranked (Faden 2000a, Hunt 1986b). Those species with spiral leaf arrangement exhibit a "bromeliiform" (Hunt 1986b, p 408) appearance. Three species with linear leaves appear caespitose (Lakela 1972). The remaining species are decumbent, procumbent, prostrate, or ascending, with both elongated and shortened internodes and two-ranked leaves that can deviate from that pattern in prelude to the growth of linear branching (Barcellos de Souza et al. 1986, Hunt 1994). Leaves vary from linear and lanceolate to ovate, lanceolate, and elliptic (Clarke 1881; Matuda 1955a, 1956, 1966, 1975; H. Moore 1958; Hunt 1986b, 1994; Faden 2000). When individual leaves and their sheaths are flattened (in species except the bromeliiform and caespitose), the leaves have an oblique base often not evident when the leaf is attached to the stem. Leaves lack petioles and appear cordate to subcordate and/or amplexicaulis at the base. Leaf surfaces range from glabrous to velutinous.

Cincinni are variously aggregated into a paniculate, spiciform, or umbellate inflorescence with subtending bracts. Bracteoles subtend the flowers at the base of the

pedicel. The three (occasionally two) petals are either readily visible and pigmented white, pink, rose, lavender, or blue, or are much reduced, membranous, and then often dominated by exserted stamens (Hunt 1986b). Stamen number generally is six except for three species that have three stamens (*C. multiflora*, *C. insignis*, and *C. ciliata*); one species that has three or six stamens (*C. repens*), some of which can be staminodial; and one species that has one to three stamens (*C. monandra*) (H. Moore 1958, 1961; Hunt 1986b, 1994). Filaments are glabrous or moniliformly bearded (Hunt 1986b), and anther connective dilation ranges from very little to broad (Hunt 1986b). The superior ovary is usually three-loculed (sometimes two-loculed) with one to two ovules per locule (Hunt 1986b). The style is most often long with penicilliform or papillose-capitate stigma (Hunt 1986b). Fruits are loculicidal capsules; seeds have a punctiform hilum and a dorsally positioned outgrowth of the seed coat called an embryotega (Hunt 1986b), which is a synapomorphy for the family (Judd et al. 1999).

Pollination biology has been little studied in *Callisia*. Highly derived floral parts, such as exserted stamens and much reduced petals, have been proposed to express a trend towards anemophily (Hunt 1986b, Faden 1998). Greenhouse specimens of *C. repens* in full bloom have been observed to release visible clouds of pollen from mild perturbations of the inflorescence (pers. obs.). Staminal characteristics such as dilated anther connectives and filament hairs [uncommon in the family but present in some members of *Callisia* sensu Hunt (1986b)] and other androecial features in the family, coupled with a short flowering time, have been interpreted to be a means of deception to attract pollinators to typically nectar-less flowers that offer only pollen as a reward (Faden 1992, Faden and Evans 1999, Evans et al. 2000b, Faden 2000b). *Callisia multiflora* (in section

Leptocallisia) and *C. fragrans* (in section *Callisia*) are scented; however, the latter exhibits characteristics suggestive of anemophily. The Florida endemic, *C. ornata* (in section *Cuthbertia*), with a relatively large [10-15 mm Lakela (1972)] pink perianth and bearded stamens is host to the bombyliid fly, *Poecilognathus punctipennis* Walker (Deyrup 1988). This pollinator was deemed to prefer other commelinoids, primarily the lightly scented purple-flowered *Tradescantia roseolens* Small, and the blue-flowered *Commelina erecta* L. with fertile stamens and sterile staminodes (Deyrup 1988).

Self-incompatibility has been surveyed in the family (Owens 1980). Of the ten *Callisia* or segregate genera of *Callisia (Aploleia, Phyodina)* species sampled, all were self-incompatible except for the type species, *C. repens,* and its "aggregate" (Owens 1980) (likely *C. insignis).* The self-compatibility exhibited for the type and its aggregate was suggested to complement the reduction in floral morphology previously noted by Owens and others for the type (Owens 1980). Gametophytic self-incompatibility was hypothesized to be the standard cross-pollination mechanism for the self-incompatible members of the family based on the binucleic pollen grains and on other inferential evidence regarding monocotyledon self-incompatibility systems (Owens 1980). Several members of *Callisia* exhibited pollen tube arrest in the style, but most did so at the stigma (Owens 1980). This pattern of generality has been noted for other monocotyledon families, e.g. Poaceae and Liliaceae (Owens 1980). Subsequent reports indicate that most *Callisia* species are self-incompatible, with the exceptions indicated above and *C. cordifolia* (Hunt 1994) [although in an earlier work Moore (1958) reports *C. monandra* and *C. multiflora* to be self-compatible].

The haploid chromosome number in *Callisia* varies from *n* = six, seven, eight (Giles 1942, Heitz 1968b in R. Moore 1970, Jones and Jopling 1972, Hunt 1986b). Polyploidy has also been reported (Anderson and Sax 1936, Giles 1942, Guervin et al. 1975, Le Coq and Guervin 1975, Le Coq et al. 1975). Karyological data indicate that chromosomes are generally asymmetrical, ranging in size from approximately 4 to 12 μm (Jones and Jopling 1972, Hunt 1986b).

Research Goals

Cladistic analyses of the family utilizing morphological, anatomical, and/or molecular characters show incongruence between morphological and molecular data (Evans 1995, Evans et al. 2000a, 2000b). In a study of the family utilizing 47 morphological characters under parsimony analysis for 40 ingroup genera, (one representative species per genus), the very anomalous *Cartonema,* (hypothesized by various workers as a primitive member of the family), was sister to *Callisia,* and *Callisia* was sister to all remaining taxa sampled in the family (Evans et al. 2000a). These results are contrary to the hypothesized recent origin of the New World subtribe under which *Callisia* is treated (Evans et al. 2000a). In another recent analysis, anatomy has been suggested to be less homoplasious, and thus more potentially informative phylogenetically than morphological characters in the family (Evans et al. 2000a, 2000b), and these data need further investigation.

The paraphyly [groups that include the most recent common ancestor and some but not all descendents (Zomlefer 1994)] or polyphyly [groups that include descendents of more than one common ancestor (Judd et al. 1999, Zomlefer 1994)] of *Callisia* based on traditional data sets has been corroborated by molecular phylogenetic analyses (Evans

et al. 2000a, 2000b, 2003), but the sampling of *Callisia* species in those studies was limited.

In this study, the generic boundaries of *Callisia* sensu Hunt (1986b) and Tucker (1989) are investigated utilizing new molecular data along with morphological, anatomical, phytochemical, geographical, and chromosomal information from the literature. Other members of the subtribe will be considered, relative to *Callisia,* as the relationships among the genera of the subtribe have been ambiguous (Evans1995, Faden 2000, Evans et al. 2003).

Table 1. Genera under subtribe Tradescantiinae. Data compiled from Faden 1998.

Callisia	***Gibasis***	***Tradescantia***	***Tripogandra***
- Cymes fused	- Cymes not fused	- Cymes fused	- Cymes fused
- Cymes sessile	- Cymes stipitate	- Cymes sessile	- Cymes sessile
- Inflorescence bracts reduced	- Inflorescence bracts reduced	- Inflorescence bracts developed	- Inflorescence bracts reduced
- Flower sessile or pedicellate	- Flower pedicellate	- Flower pedicellate	- Flower pedicellate
- Androecium of two similar whorls or one whorl lacking	- Androecium of two similar whorls	- Androecium of two similar whorls	- Androecium of two different whorls
- Chromosomes asymmetrical	- Chromosomes asymmetrical	- Chromosomes symmetrical	- Chromosomes asymmetrical

Table 2. Species under *Callisia* sensu Hunt (1986b) and Tucker (1989).

Section	**"Group"**	**Species**
Hadrodemas		***C. warszewicziana*** (Kunth & Bouché) D. R. Hunt
Cuthbertia		***C. graminea*** (Small) G. Tucker
		C. ornata (Small) G. Tucker
		C. rosea (Vent.) D. R. Hunt
Lauia		***C. laui*** (D. R. Hunt) D. R. Hunt
Brachyphylla		***C. navicularis*** (Ortgies) D. R. Hunt
		C. micrantha (Torr.) D. R. Hunt
Leptocallisia		***C. ciliata*** Kunth
		C. cordifolia (Sw.) Anderson & Woodson
		C. filiformis (Martens & Galeotti) D. R. Hunt
		C. gracilis (Kunth) D. R. Hunt
		C. monandra (Sw.) Schultes f.
		C. multiflora (Martens & Galeotti) Standl.
Callisia	I. "Gentlei"	***C. elegans*** Alexander ex H. E. Moore
		C. gentlei Matuda
		C. macdougallii Miranda
		C. nizandensis Matuda
		C. tehuantepecana Matuda
	II. "Fragrans"	***C. fragrans*** (Lindl.) Woodson
		C. guerrerensis Matuda
		C. soconuscensis Matuda
	III. "Repens"	***C. repens*** (Jacq.) L.
		C. insignis C. B. Clarke

CHAPTER 2

MOLECULAR ANALYSIS – *ndhF* and *trnL-F*

Phylogenetic relationships within the Commelinaceae have been studied utilizing molecular data, most commonly the chloroplast *rbcL* (ribulose 1,5-bisphosphate carboxylase large subunit) gene which encodes the large subunit of the ribulose-1,5-bisphoshpate carboxylase/oxygenase photosynthetic enzyme (Evans 1995; Evans et al. 2000a, 2003; Judd et al. 1999). Often this gene is found to evolve too slowly to elucidate lower level phylogenetic relationships (Soltis and Soltis 1998), and the relationships among *Callisia* species, as well as *Callisia* relative to other genera in the family, have not been resolved in these previous molecular studies (Evans et al. 2003). However, these cladistic analyses of the family support the paraphyly or polyphyly of *Callisia* (Evans et al. 2000b, 2003).

In this phylogenetic examination of relationships within *Callisia*, and among *Callisia* and the other genera of the subtribe, molecular sequence data from two chloroplast gene regions were utilized: a portion of the 3′ region of *ndhF* (NADH dehydrogenase subunit F), and the *trnL-F* (transfer RNA leucine, transfer RNA phenylalanine) intergenic spacer region. Sequences from these regions have been shown to change at a rate suitable for analyses of relationships within and below the family level (Soltis and Soltis 1998).

The *ndhF* protein-coding gene is located in the small single-copy chloroplast region near the boundary between that area and the chloroplast inverted repeat. The gene

is thought to encode a subunit protein of NADH dehydrogenase. *NdhF* has been applicable for generic-level resolution of phylogenetic relationships within numerous angiosperm families (Soltis and Soltis 1998). In particular, the 3′ end of the gene has been shown to be the more variable region of the gene and often undergoes higher levels of non-synonymous base substitutions than that of *rbcL* (Olmstead and Palmer 1994, Olmstead and Sweere 1994, Soltis and Soltis 1998).

Located within the large single-copy section of the chloroplast, non-coding regions within *trnL-F* have resolved inter-generic relationships (Soltis and Soltis 1998). These regions include an intron between the 5′ end of the *trn*L (leucine) coding region and its 3′ end, and a spacer between that 3′ end and the *trn*F (phenylalanine) coding region (Taberlet et al. 1991) (Fig. 1). Both the *trnL-F* intron and spacer regions have been shown to diverge at rates equal to or three to five times faster than *rbc*L for some study groups (Soltis and Soltis 1998)

The genus *Commelina* L. (tribe Commelineae) was used as an outgroup of the Tradescantieae tribe, and the genus *Tinantia* Scheidw. (tribe Tradescantieae, subtribe Thyrsantheminae Faden & D. R. Hunt) was included as a representative subtribe related to the Tradescantiinae (Evans 1995). Morphology-based and *rbcL* gene sequence analyses of the order Commelinales place Pontederiaceae Kunth either near Commelinaceae or within a separate but related clade (Evans 1995, Faden 1998, Judd et al. 1999). In a preliminary analysis, the genus *Eichhornia* Kunth in the Pontederiaceae was used as an outgroup to substantiate branch support for the Commelinaceae sampled here (data not shown).

Materials and Methods

A total of twenty *Callisia*, four *Tradescantia*, two *Tripogandra*, and one *Gibasis* species of subtribe Tradescantiinae; one *Tinantia* species of subtribe Thyrsantheminae, and one *Commelina* species of tribe Commelineae were included in this study (Table 3). Fourteen of the species were provided by Dr. R. B. Faden of the Smithsonian Institution's Botany Department and have been cultivated at the University of Georgia Plant Biology Department's greenhouses. One species was sampled from a Smithsonian Institution [US] herbarium specimen. The remaining species were either field collected or were previously growing in the departmental greenhouses. Vouchers are deposited in the University of Georgia Herbarium.

Extraction

Total genomic DNA was extracted from fresh leaf material or, for one taxon, from a herbarium specimen, using the CTAB method of Doyle and Doyle (1987) as modified by Smith et al. (1991). Live specimens housed at the University of Georgia Plant Biology Department greenhouses were brought to the lab and 0.10 to 0.13 g fresh young leaf material was removed from each plant immediately before pulverization in liquid nitrogen. While cumbersome, this method reduced the rapid deterioration of the leaf found to occur when the fresh material was transported from the greenhouse. For the one herbarium specimen sequenced, only 0.01 g was available. Organic compounds were removed with approximately 500 µl of 24:1 chloroform:isoamyl alcohol. DNA was precipitated at -20° C in 600 µl cold isopropanol for two hours for live material and for three weeks for the one herbarium specimen (Fay et al. 1998), then centrifuged and dried. The pellets were re-suspended in 50 to 100 µl distilled deionized water in a 65°C water bath for one to six hours,

cleaned with High Pure filters (High Pure PCR Product Purification, Boehringer Mannheim Corporation or Roche Molecular Biochemicals), and stored in 100 µl distilled deionized water at -20°C until amplification.

Amplification

Gene regions were amplified with an Elmer Perkin Thermal Cycler in 50 µl reactions in 0.5 ml reaction tubes with 10% *Taq* polymerase buffer with magnesium chloride, 2% 10 mM nucleotides, 4% 10 µM primers, 0.4 % *Taq* polymerase (Boehringer Mannheim Corporation), 3% total DNA, and the reminder distilled deionized water. Reactions utilizing bovine serum albumin (BSA) included 4% of a 0.4% aqueous solution adjusted with water accordingly. BSA has been known to be effective in improving the amplification of degraded DNA sequences, particularly those of herbarium specimens (Savoleinen et al. 1995, Fay et al. 1998). While only one of several herbarium specimens was successfully amplified for *ndhF*, the use of BSA generally improved amplification of DNA from fresh leaf material as well. Polymerase chain reactions (PCR) were run for 40 cycles with an annealing temperature of 58°C. The amplified products were visualized by running them on a 1% agarose gel and photographing the gel either with a Polaroid camera placed over a trans-illuminator or with a Stratagene Eagle Eye™ II. PCR products were cleaned with High Pure filters and stored at -20°C in 50 µl distilled deionized water until sequencing.

The 3′ portion of *ndhF* was amplified using forward *ndhF*1318 and reverse *ndhF*2110R primers of Olmstead and Sweere (1994) based on *Nicotiana tabacum* L. sequences.

The *trnL-F* region was amplified with the forward c (tab c) and reverse f (tab f) primers of Taberlet et al. (1991) or with the reverse tab f as modified by Sang et al. (1997) (sang f)

with an extra leading 'G' (Table 4). A test was made in order to verify that a preponderance of insertions/deletions (indels) were not manifestations of subsequent sequencing errors. To test this possibility, DNA was amplified for a sampling of taxa using: 1. the forward e (tab e) primer of Taberlet et al. (1991) and the reverse sang f primer (Sang et al. 1997); 2. the Sang et al. (1997) forward e (sang e) and reverse sang f primers (Table 4). Those PCR products were then sequenced in two different labs.

Sequencing

NdhF was sequenced with the forward *ndhF*1318 primer of *N. tabacum*. *TrnL-F* was sequenced with the reverse tab f primer. Where base calls were unclear, sequences were obtained from the reverse *ndhF*2110R primer for *ndhF* or from the forward tab c primer for *trnL-F*

Most sequencing was performed on an ABI version 3703 or 3777 automatic sequencer at the University of Georgia Molecular Genetics Instrumentation Facility (MGIF) following the manufacturer's protocol. Product not sequenced at MGIF I processed in the University of Georgia Department of Plant Biology Genome Analysis Facility (GAF, Table 3) as follows.

The PCR product was quantified with either a Hoefer DyNA Quant 200 fluoromoter or with a Beckman DU® 640B spectrophotometer. For the cycle sequencing protocol used, a range of 10-40 ng/µl DNA per half reaction is recommended (ABI PRISM Big Dye Terminator v3.0 Ready Reaction Cycle Sequencing Kit, Applied Biosystems). For those taxa that sequenced successfully, DNA quantified from 17.9 to greater than 50 ng/µl. Higher concentrations of PCR product did not seem adversely to affect the cycle sequence reaction. Each half-reaction comprised 40% Big Dye, 10% primer, 10% template, and 40%

distilled deionized water in a 0.2 ml reaction tube. Reactions were run in the GAF on a GeneAmp PCR system 9700 for 99 cycles with an annealing temperature of 50°C. Cycle sequence products were cleaned with the Big Dye ethanol precipitation method or with a modified version of the Big Dye ethanol/sodium acetate precipitation method. The modified version was designed by W. M. Whitten (Whitten, pers. comm.). For the taxa sequenced in the GAF, his version yielded cleaner sequences. Whitten's protocol utilizes a higher ratio of ethanol to sodium acetate and requires less stringency during the cleaning process than does the Big Dye version. With both methods, non-denatured ethanol was used as recommended by Applied Biosystems. After cleaning/precipitation, pellets were dried in a Speed Vac SC110 vacuum centrifuge and stored at -20°C until being sequenced.

In preparation for the GAF ABI Prism 310 Genetic Analyzer capillary sequencer (Applied Biosystems) with POP-6 polymer, 15 µl of template suppression reagent was added to each pellet and the samples were briefly vortexed and spun. The DNA was denatured at 95°C for one to two minutes, vortexed and spun again, loaded into the 0.5 ml 310 sequencing tubes, and run in the sequencer for three to four hours.

Alignment

Sequences of the 3′ *ndhF* region were aligned visually using SeqEd version 1.00A (Applied Biosystems) or SequenceNavigator™ (Applied Biosystems). Initial sequences of the *trnL-F* non-coding regions were aligned with Clustal X (Thompson et al. 1997). This computer alignment program generated unparsimonious gaps, so the final alignments were adjusted by hand. Subsequent *trnL-F* sequences were aligned manually.

Phylogenetic analysis

Parsimony analysis of each data set and the combined data sets were conducted with PAUP* Version 4.0b10 (Swofford 2003). All characters were equally weighted. Data sets of *ndhF* and *trnL-F* with indels treated as a fifth character and with indels treated as missing were analyzed both separately and combined using heuristic search algorithms [10 or 100 random sequence addition replicates, tree-bisection-reconnection (TBR) branch swapping, and MulTrees in effect]. Data sets of *ndhF* and *trnL-F* with indels removed were analyzed both separately and combined, with MulTrees in effect and either the heuristic search algorithm [100 random sequence addition replicates, tree-bisection-reconnection (TBR) branch swapping], or with branch and bound. To assess internal branch support for each analysis, 1000 heuristic bootstrap replicates were run with no more than ten trees of length greater than or equal to five saved in each replicate. Trees and corresponding statistics for each run were saved in log files. Bootstrap support values will be designated unsupported (< 50%), weak (50 to 74%), moderate (75 to 84%) and strong (85 to 100%) following Zomlefer et al. (2003). To assess congruence between the *ndhF* and *trnL-F* data sets a partition homogeneity test [incongruence length difference (ILD) test of Farris et al. (1994)], as implemented by PAUP*, was conducted employing the heuristic search with ten random sequence addition replicates, with the steepest descent option in effect, and with indels removed.

Results

Overview of the matrices

The length of the *ndhF* data set was 778 nucleotides with indels included and 714 nucleotides with indels and ambiguous leading and trailing regions excluded. Whether

an indel is considered an insertion or a deletion is determined by the sequence of the outgroup taxon. Two indels are autapomorphic: a six-base insertion present in the type species for *Callisia* (*C. repens*) and a two-base insertion present in *C. navicularis*. A twenty-four-base insertion is synapomorphic for the ingroup taxa.

For *trnL-F*, the intron was unalignable; the variable-length spacer was utilized in these analyses, with a total length of 450 nucleotides including indels, and 193 nucleotides excluding indels.

The test to validate the abundance of *trnL-F* spacer indels, by amplifying the spacer for a sample of species with the forward tab e and sang e primers (both primers being in closer proximity to the spacer than is tab c), and by sequencing the resulting PCR products in two different labs (MGIF and GAF), confirmed that the indels were not the result of technical sequencing errors.

Indels and tandem repeats are complex in the *trnL-F* spacer (Tables 5, 6). In early parsimony analyses conducted in this study, when the taxon sampling was not as complete (data not shown), indels from one to 46 bases could readily be scored as present or absent in a separate binary matrix and analyzed with the remaining sequence data. However, as additional taxa were added to the data set, that scoring scheme became untenable.

Indels have been analyzed in various ways by various workers. In any given DNA sequence, the gain or loss of one or more base pairs is not readily observable (Simmons and Ochoterena 2000). However, if a DNA sequence is aligned with that of at least one other taxon with one or more insertions or deletions, then a gap (or gaps) becomes apparent (Simmons and Ochoterena 2000). To analyze such gaps as missing

data may omit useful information (Simmons and Ochoterena 2000). To analyze each base insertion as a fifth character treats each gap as an alternative base, which it is not. To analyze separately each base of an insertion that contains more than one contiguous base treats each base as a separate insertion event while evidence suggests that such insertions likely evolved as a unit or as units (Simmons and Ochoterena (2000). In this study, both the simple and the complex scoring schemes of Simmons and Ochoterena (2000) were applied to the *trnL-F* spacer indels, but with limited success. Given both the abundant overlap of indels with each other and with tandem repeats, and the uncertain homology of these areas, a separate binary matrix was not utilized in these analyses; instead, a comparison of analyses with and without indels is presented.

For summary results and indices for all parsimony analyses of this study refer to Table 7. The terms MPR (most-parsimonious reconstruction), tree, and cladrogram are used interchangeably.

NdhF *with and without indels*

In one randomly chosen tree of 288 MPRs with indels treated as a fifth character (Fig. 2), *Tinantia pringlei* is sister to all members of subtribe Tradescantiinae. *Tradescantia* plus *Gibasis* form the basal-most clade of that subtribe. Of the remaining taxa, a bifurcating clade, with the members of section *Cuthbertia* sensu Hunt (1986b) and Tucker (1989) (*C. graminea, C. ornata, C. rosea)* as one group, and the members of section *Brachyphylla* (Hunt 1986b) (*C. micrantha, C. navicularis)* as another group, are basal to the remaining sections of *Callisia* sensu lato (s.l.) and *Tripogandra*. Three of the four species of Hunt's (1986b) section *Leptocallisia* (*C. cordifolia, C. monandra, C. multiflora*) sampled here form a clade sister to sections *Callisia* and *Hadrodemas* and to

Tripogandra. The fourth species sampled of section *Leptocallisia* (Hunt 1986b), *C. gracilis*, is within the *Tripogandra* clade. The one species of section *Hadrodemas* sensu Hunt (1986b), *C. warszewicziana*, is basal to all members of Hunt's (1986b) section *Callisia.* Bootstrap support for the tribe Tradescantiinae is high (99%), although the analysis collapses the internal nodes of all clades to which *Tradescantia* is sister (Fig. 3). The *Cuthbertia*/*Brachyphylla* clade and the *Tripogandra*/*Callisia gracilis* clade have high support (100 and 99% respectively), but that support is somewhat reduced for the bifurcation of *T. diuretica* and *C. gracilis*. Section *Leptocallisia* sensu Hunt (1986b) shows high support (99%) for a clade that includes the two species of Moore's (1961) genus, *Aploleia* (*Callisia monandra,* C. *multiflora*). Support is high (94%) for the section *Hadrodemas* (Hunt 1986b) plus section *Callisia* (Hunt 1986b) clade; with an 85% bootstrap value supporting the one member of Hunt's (1986b) section *Hadrodemas* as sister to Hunt's (1986b) section *Callisia.* Within that section, two of the three species sampled from Hunt's (1986b) "Gentlei" group (*C. elegans, C. macdougallii*) have high (99%) bootstrap support as do the three members of Hunt's (1986b) "Fragrans" group *(C. fragrans, C. fragrans* cv 'Melnickoff', *C. guerrerensis*). The two members of the "Repens" group (Hunt 1986b) (*C. repens* and *C. insignis*) are unresolved.

Analysis of the same data set but with indels treated as missing characters yielded similar results regarding the major clades, except that *Gibasis* is excluded from the *Tradescantia* clade, and the clade with sections *Cuthbertia* and *Brachyphylla* of Hunt (1986b), instead of being sister to the remaining taxa, is one of the terminal clades (Fig. 4). Bootstrap support and polytomies (Fig. 5) are similar to the previous analysis. Both

analyses resulted in the same number of MPRs and similar numbers of parsimony-informative characters (Table 7).

With indels removed from the *ndhF* data set, results (Figs. 6, 7) again parallel the two previous analyses.

The percent parsimony informative characters for each of the three *ndhF* data sets are comparable, ca. 16 %.

TrnL-F *with and without indels*

One tree randomly selected from the 24 MPRs of the *trnL-F* data set with indels treated as a fifth character (Fig. 8) makes a few departures from the *ndhF* data set results. A clade containing *Tripogandra serrulata* and *C. guerrerensis* [of section *Callisia* sensu Hunt (1986b)] replaces the *Tradescantia* clade of *ndhF* as sister to the remaining taxa of *Callisia*, *Tripogandra*, *Gibasis*, *Tradescantia* and *Tinantia* (not of the same subtribe as the first four genera). *Callisia gracilis*, the species of section *Leptocallisia* (Hunt 1986b) that consistently grouped with the two *Tripogandra* in the *ndhF* analyses, is embedded in the sections *Callisia*/*Hadrodemas* clade, that clade still with the one entity of section *Hadrodemas* sister to the remaining species, but with the type species, *C. repens* of Hunt's (1986b) "Repens" group sister to the "Gentlei" group instead of to the "Fragrans" group as with *ndhF*. Bootstrap analysis (Fig. 9) collapses internal nodes of the above clades, but support is moderate to high with respect to similar well-supported clades of *ndhF*.

With indels treated as missing data for the *trnL-F* data set, one random representative tree of 105 MPRs (Fig. 10) is similar to the representative tree for the same data set with indels treated as a fifth character except that here *Gibasis* is sister to all

ingroup taxa, *Tradescantia* is basal to the remaining Tradescantiinae and *Tinantia*, and both *Tripogandra* species form a polytomy with *C. guerrerensis* with the one Thrysantheminae taxon, *Tinantia*, basal to them. The bootstrap analysis (Fig. 11) adds further polytomies to the internal branches of the ingroup relative to the previous *trnL-F* analysis, with comparable to reduced support values.

For one randomly selected tree of 84 MPRs of the *trnL-F* analysis with indels removed (Fig. 12), results coincide with that of *trnL-F* with indels treated as missing but with a trend towards lower bootstrap support values (Fig. 13). These two data sets contain a comparable percentage of parsimony-informative characters, 18% and 12% respectively, while the *trnL-F* data set with indels treated as a fifth character has 48% parsimony-informative characters.

NdhF *and* trnL-F *data sets combined with and without indels*

For one randomly chosen tree of six MPRs of the combined data sets analyzed with indels treated as a fifth character (Fig. 14), major clades are similar to those of *trnL-F* except that the sister groups to the subtribe Tradescantiinae and to *Callisia* s.l. plus *Tripogandra* are *Tinantia pringlei* and a *Tradescantia* clade respectively, as in the analyses for *ndhF* alone. Internal nodes are not collapsed completely in the bootstrap analysis (Fig. 15) with support generally moderate to strong.

In the combined *ndhF* and *trnL-F* analyses with indels treated as missing characters and with indels removed, each randomly chosen tree from seven (Fig 16) and six (Fig. 18) MPRs respectively, shows essentially the same topology, which parallels the topology of *ndhF* analyzed alone. Bootstrap support values for these combined analyses likewise are comparable (Figs. 17, 19).

The combined data set with indels treated as a fifth character has 28 % parsimony-informative characters; the other two combined data sets have 15 to 17 % parsimony-informative characters. These figures likely reflect the *trnL-F* data set. When analyzed separately with indels treated as a fifth character, as missing, or with indels removed, this data set has a similar contrast of percentage parsimony-informative characters.

The partition homogeneity test for congruence between the two gene regions without indels resulted in $p = 0.01$. The null hypothesis, that the data partitions (the two gene regions) are homogeneous, must be rejected. Rejection of the null hypothesis usually suggests that the two data sets should not be analyzed together (Gaskin and Schaal 2003). However, arguments have been made for combining such data even with a failed partition homogeneity test (Sullivan 1996, Johnson et al. 2000, Fishbein et al. 2001, Goertzen et al. 2002, Aagesen and Sanso 2003, Loockerman et al. 2003), often depending upon whether or not the incongruence is "hard" or "soft" (Fishbein et al. 2001). If the incongruence is not "hard", that is, if clades that are strongly supported in one data set do not disagree with clades strongly supported in the other data set (Goertzen et al. 2002), a failed congruence test need not negate analysis of the data together (Sullivan 1996, Fishbein et al. 2001, Aagesen and Sanso 2003). The most conflicting clades between the two data sets are the clade with *C. guerrerensis* and one or both *Tripogandra* found with the three analyses of *trnL-F* alone and with the combined analysis of *ndhF* and *trnL-F* with indels treated as a fifth character, versus the clade with *C. gracilis* and both *Tripogandra* found with the three analyses of *ndhF* alone and with the remaining two analyses with the data partitions combined. When *C. gracilis* is not in

a clade with one or both *Tripogandra*, it is in a clade with *C. fragrans* or is sister to sections *Callisia* and *Hadrodemas*, and *C. repens* moves to a clade with *C. elegans* and *C. macdougallii.* These latter groupings have weak to strong bootstrap support, indicating homoplasy in the data set.

The *trnL-F* spacer is a non-coding region and thus is potentially under fewer selective constraints than the protein-coding *ndhF* and is, thus, more likely to mutate. Phylogenetic signals have been found to be additive when data sets from two genes or gene regions with different evolutionary processes are analyzed together under parsimony with equal weighting. Results have been found to provide robust phylogenetic hypotheses even with significant incongruence between the two data sets (Sullivan 1996).

The two data sets analyzed here have the potential to expand understanding of *Callisia* from two perspectives: the perspective that supports pre-existing suppositions of relationships based on traditional data, and the perspective that uncovers anomalies the further scrutiny of which can lead to a clearer understanding of relationships and a more tenable classification scheme.

Discussion

The polyphyly of *Callisia* sensu Hunt (1986b) is readily apparent (H. Moore 1958, 1961; Hunt 1986b; Evans et al. 2000a, 2000b, 2003). In the following discussion refer to Figs. 18 and 19 unless otherwise noted.

The infrageneric scheme of Hunt (1986b) in part parallels molecular results here, as does Moore's work (1958, 1961). The three southeastern United States endemics of section *Cuthbertia* are always in the same clade, as are the two members of section *Brachyphylla.* With the exception of the rearrangements found when *C. gracilis* groups

within section *Callisia* and *C. guerrerensis* joins one or both *Tripogandra* taxa, one clade consistently contains members of Hunt's (1986b) section *Callisia* and those clades branch in accordance with Hunt's (1986b) informal groups within the section. Two members of one small undeviating clade are *C. monandra* and *C. multiflora*, the only two species in Moore's (1961) treatment of the genus *Aploleia*. Consistent inclusion of *C. cordifolia* in that clade reflects Hunt's (1986b) section *Leptocallisia*. Hunt's (1986b) sections are below addressed relative to previous studies, current perspectives, and to the molecular data presented here.

Section Cuthbertia *sensu Hunt (1986b)*

Morphologically, the three members of section *Cuthbertia* (*Callisia graminea, C. ornata, C. rosea*) *sensu* Hunt (1986) and Tucker (1989) include characteristics of *Tradescantia* and Hunt's (1986b) *Callisia*. All three species of section *Cuthbertia* have the two-ranked leaves common in *Callisia* but with long linear blades and a caespitose (tufted) habit more similar to that of some *Tradescantia*. The inflorescence is umbellate as in *Tradescantia* and some *Callisia*, lacks the paired spathaceous or foliaceous inflorescence bracts of *Tradescantia*, and instead has the more typical reduced bracts of *Callisia* (Hunt 1986b, Faden 2000). Flowers have the showy *Tradescantia* size and color unlike a number of *Callisia*, particularly those of section *Callisia*. The bearded moniliform stamens, a characteristic of *Tradescantia,* are found in section *Cuthbertia* and some other members of *Callisia* sensu Hunt (1986b).

Originally described under *Tradescantia* by Ventenat (1800), *C. rosea* was transferred to *Cuthbertia* by Small (1903), with *C. graminea*. These two species and a

third member subsequently added to *Cuthbertia* (Small 1933), *Callisia ornata*, have variously been placed under *Tripogandra, Phyodina*, and *Tradescantia*.

Anderson and Sax (1936) determined that barriers to hybridization occurred among three "units" of North American *Tradescantia*: *T. virginiana* (and its relatives), *T. rosea* (= *C. rosea*), and *T. micrantha* (= *C. micrantha*). Subsequent cytological work by Giles (1942), coupled with morphology and geography, supported *Cuthbertia* as a segregate genus. The base number for the section has been reported as x = six (Tucker 1989); polyploidy is not uncommon (Giles 1942).

Matthews (1966) studied extracts of roots, leaves, and flowers of ten species of *Tradesantia*, including *T. rosea*. The results supported *C. rosea* in *Cuthbertia*.

The homoplasious nature of morphological characters, particularly androecial ones, is considered a basis for the difficulty in understanding relationships within the Commelinaceae (Evans et al. 2000a, 2000b, 2003). Utilization of anatomical features as diagnostic characters has been addressed in several studies (Evans et al. 2000a, 2000b, 2003). Anatomical characters, particularly vegetative ones, are considered to be under fewer selective constraints, and thus more likely to evolve similarly across a lineage with less homoplasy reflected in other lineages (Evans et al. 2000a, 2000b, 2003). Anatomical characters have also been more congruent with molecular data sets than morphological characters (Evans et al. 2000a, 2000b, 2003).

Tomlinson (1966, 1969) found anatomical characters that distinguished the three *Cuthbertia* from *Tradescantia* and *Callisia* (some variously treated under other genera). Most members of the family have four or six stomata subsidiary cells. The three members of section *Cuthbertia* and the African genus *Triceratella* Brenan usually have

two subsidiary cells (Tomlinson 1966, 1969). Tomlinson (1966, 1969) illustrated that *Cuthbertia* could have an additional two subsidiary cells. Recent paraffin-embedded and SEM (scanning electron microscopy)-mounted leaf material of the three species of the section and of other members of the genus require more stringent study, but an initial survey suggests that the number of subsidiary cells needs to be addressed. Longitudinally rectangular adaxial epidermal cells were noted in *Cuthbertia* (Tomlinson 1966, 1969) and were found in just one other distantly related genus of the family, *Cyanotis* D. Don. *Cuthbertia* leaves have a ridged epidermal wall that is homologous with epidermal ridges found in the six members of *Callisia* sensu stricto (s.s.) in Tomlinson's study, but then the ridges are found only on the elongated leaf sheath and stem (Tomlinson 1966).

The lack of resolution of the relationship among this section and other sections of *Callisia* and other genera in the subtribe might advocate against removing the three species of section *Cuthbertia* from *Callisia*. However, bootstrap support for the clade that strictly contains these three species is undeviatingly high (97 to 100%) in all analyses. These three species are the only ones in the genus that occur exclusively in the southeastern United States (Table 8). They are highly recognizable under field conditions. To continue to treat them under *Callisia* is undesirable (see Chapter 3, Taxonomic Considerations). From supplementary molecular, anatomical, and/or other data, the additional question of whether the three species are distinct or whether they are varieties within one species can be further addressed (Anderson and Woodson 1935, Faden 2000).

Section Brachyphylla *sensu Hunt (1986b)*

The two species of Hunt's (1986b) section *Brachyphylla*, *C. navicularis* and *C. micrantha*, are easily recognized from gross morphology but include characters states also typical of *Tradescantia* and *Callisia* s.s. The inflorescence is umbellate, and flowers are showy with bearded stamens, much like *Tradescantia*. The habit of both species is procumbent to decumbent as in some members of *Callisia* s.l. *Callisia navicularis* has small stiff subulate leaves. While leaf shape of *Callisia* s.s. and others of the genus have been described with various qualifications of lanceolate (e.g. oblong-lanceolate, ovate-lanceolate, elliptic-lanceolate), the small leaves of *C. micrantha* are lanceolate. Both *C. micrantha* and *C. navicularis* were originally described under *Tradescantia* by different authorities, and both were transferred to *Phyodina* but at different times and by different workers (Torrey 1859, Ortgies 1877, Rohweder 1956, Hunt 1978). *Phyodina* has been referred to as a grade taxon distinguishable from other genera by an absence rather than a presence of characters (Hunt 1986b).

The work of Anderson and Sax (1936) included *C. micrantha* as the third *Tradescantia* "unit", and *C. micrantha* has hybridization barriers, at least among those genera studied which included one member of Hunt's (1986b) section *Cuthbertia*. Reported chromosome numbers are $n = 13$ (Anderson and Sax 1936), $2n = 24$ (Jones and Jopling 1972), and 26 (Anderson and Sax 1936) for *C. micrantha*; $n = 16$ (H. Moore 1968) with a base of x = eight or 16 (Jones and Jopling 1972), $2n = 32$ (Anderson and Sax 1936, Jones and Jopling 1972) and 48 (Jones and Jopling 1972) for *C. navicularis*.

With *C. navicularis* then treated under *Tradescantia*, Tomlinson (1969) found the leaves to have a ridged epidermis [see *Section* Cuthbertia *sensu Hunt* (*1986*) above].

Tomlinson (1969) found epidermal lens-shaped thickenings in *Callisia* (*C. soconuscensis*, *C. repens*), the one species of section *Hadrodemas* (= *C. warszewicziana*), one species of section *Leptocallisia* [then under *Aploleia* (= *C. monandra*)], and *C. navicularis*.

The question of the association of *C. navicularis* with *Tradescantia* has recently been raised (Faden 2000) based on the presence of a foliaceous inflorescence bract. From my observations, however, the inflorescence can be pedunculate or sessile. When sessile, the subtending leaf can appear to be a bract, but close examination reveals the actual small bracts of the inflorescence distinct from the leaf.

The two sections, *Cuthbertia* and *Brachyphylla*, are distinct from each other vegetatively and anatomically. At least one taxon from each section has been confirmed not to hybridize under experimental conditions (Anderson and Woodson 1935, Anderson and Sax 1936). Geographic ranges do not overlap (Table 8). Yet molecular analyses routinely group the two sections together as sister clades. At this juncture, treatment of one of the two sections as a genus separate from *Callisia* without addressing the rank of the other section would be incomplete (see Chapter 3, Taxonomic Considerations).

Section Leptocallisia *sensu Hunt (1986b)*

This section includes six species, four of which were available for this study, *Callisia cordifolia, C. gracilis*, *C. monandra*, and *C. multiflora*. These species are readily recognizable in the field by a creeping or ascending habit; ovate to ovate-lanceolate to elliptic-lanceolate leaves; terminal or terminal and axillary umbellate, or terminal paniculate, inflorescence; pedicellate flowers; herbaceous sepals; small (ca. four by three mm) white or inconspicuous petals; six or one to three glabrous stamens (one species has

bearded stamens); capitellate or subpenicilliform stigma; glabrous ovary with three, occasionally two locules (H. Moore 1961, Hunt 1994).

Moore (1961) transferred *C. monandra* and *C. multiflora* to *Aploleia* with a conviction that they were more closely allied to *Tripogandra* than to *Callisia* based primarily on androecial characters. Both species have a paniculate inflorescence, small to inconspicuous petals, one to three stamens for *C. monandra* and three for *C. multiflora*, glabrous filaments, and barely dilated (Hunt 1994) or narrow anther connectives (H. Moore 1961). Moore (1961) noted that in *Aploleia*, the one to three stamens are always opposite the sepals (antesepalous), whereas when stamen number is fewer than six in *Callisia*, the stamens are always opposite the petals (antepetalous).

The base chromosome number for Moore's *Aploleia* was x = seven (H. Moore 1961), which conflicts with another report of n = six for *C. multiflora* (Heitz 1968b in R. Moore 1970) and diploid chromosome counts of $2n$ = 12 (Heitz 1968b in R. Moore 1970), 24 (Guervin et al. 1975, Le Coq and Guervin 1975, Le Coq et al. 1975), 28, and 42 (Hunt 1994). Guervin et al (1975), Le Coq and Guervin (1975), and Le Coq et al. (1975) scrutinized chromosome characteristics relative to phylogeny in *C. multiflora* and three species of Hunt's (1986b) section *Callisia*, *C. elegans*, *C. insignis*, and *C. repens.* In their studies, *C. multiflora* with $2n$ = 24 was a tetraploid, *C. insignis* with $2n$ = 28 was an octaploid, and both *C. elegans* and *C. repens* were diploid with $2n$ = 12. Based on chromosome length, volume in metaphase, and DNA quantity and density relative to ploidy level, these workers concluded that *C. multiflora* was part of a polyploid series from the diploid *C. repens* to the tetraploid *C. multiflora* to the octaploid *C. insignis*, with *C. elegans* evolving from an ancestor in common with the other three species but along a

different evolutionary trajectory (Guervin et al. 1975, Le Coq and Guervin 1975, Le Coq et al. 1975). If this were the case, then one would not expect *C. monandra* and *C. multiflora* to consistently group together as they do with the molecular data here (Figs. 2 to 19) with strong bootstrap support (90 to 100%) and never to be in an ambiguous position with *C. repens*. The bi-parental inheritance of the aforementioned studies versus the uni-parental inheritance of the current chloroplast DNA study may influence the discrepancy.

Moore (1961) considered *C. monandra* and *C. multiflora* to be self-compatible, but subsequent reports are to the contrary (Hunt 1993, 1994).

Moore's (1961) connection between *Tripogandra* and *Aploleia* relative to androecial characters is not corroborated by the molecular data here. [Also, Tomlinson (1966) found *C. monandra* to lack the silica cells found in *Tripogandra*.] Nevertheless, Moore's (1961) treatment of *Aploleia* is upheld by the molecular data here (bootstrap 90 to 100%, Figs. 3, 5, 7, 9, 11, 13, 15, 17, 19) for *C. monandra* and *C. multiflora*. From the molecular data, their relationship with other members of *Callisia* requires further investigation since not all taxa attributed to the section have been sampled.

Moore (1958) did not support Anderson and Woodson's (1935) transfer of *C. cordifolia* to *Callisia* based on the inflorescence type (pedunculate cymes not accompanied by sessile cymes), pedicellate flowers, glabrous ovary, and shortly tri-lobed stigma. Moore (1958) suggested retention of *C. cordifolia* under *Leiandra*.

Handlos (1975) excluded *Tripogandra cordifolia* (= *C. cordifolia*) from his comprehensive treatment of *Tripogandra*. *Callisia cordifolia* (and all species of *Callisia* s.l.) lack the dimorphic stamens and verrucate pollen that unite species of *Tripogandra*

(Hunt 1986b). *Callisia cordifolia* is readily distinguished from the two previous members just discussed in this section by its umbellate inflorescence and six glabrous stamens [with anthers nearly contiguous with the connective, as is the case with *C. monandra* (Hunt 1994)].

The base chromosome number of x = seven (Jones and Jopling 1972) is in keeping with Hunt's base number for section *Leptocallisia* (Hunt 1986b). Flowers are self-compatible (Hunt 1994).

Callisia cordifolia is sister to *C. monandra* and *C. multiflora* in these molecular analyses, with an absence of to moderate support with *trnL-F* (Figs. 9, 11, 13) and strong support with *ndhF* (Figs. 3, 5, 7) and the combined data sets (Figs. 15, 17, 19). In this placement, it marks a transition from six stamens to fewer stamens [(one-) three], the inner whorl being lost, and a reduction of petal size. Hunt suggested meiomery (reduction in the number of stamens, ovary locule, and/or ovules) to unite this section (Hunt 1986b). Inclusion of other species in this section not available at the time of this study likely could add clarification to the *C. cordifolia* plus *C. monandra* and *C. multiflora* clade.

The fourth species of section *Leptocallisia* included in this study, *C. gracilis*, most resembles *C. cordifolia* in aspect with small ovate leaves, an umbellate inflorescence, and flowers with small white petals, although its stamens are bearded and it has broad, nearly chevron-shaped connectives. Originally described under *Tradescantia* (Kunth 1815), Rafinesque (1836) transferred the taxon to *Phyodina* and designated it the type, after which it was treated under *Aneilema* by Steyermark (1951). This species was included in a chromosome and classification study of the family by

Jones and Jopling (1972). Their diploid count was 2*n* = 56 (Jones and Jopling 1972). They suggested a base number of seven or 14 from the meiotic pairing, which did not indicate that the specimen they studied was a heptaploid (in which case the base would be eight) (Jones and Jopling 1972). The only other species at the time under *Phyodina* included in their study was *P. navicularis* (= *C. navicularis*). With just these two representatives of the genus, Jones and Jopling (1972) made note of the heterogeneous mix that constituted *Phyodina*.

The molecular grouping of *C. gracilis* with *Tripogandra* when *ndhF* is analyzed alone (Figs. 2 to 7) or is combined with *trnL-F* [with indels treated as missing or with indels removed (Figs. 16 to 19)] is problematical. In the *trnL-F* data set, *C. gracilis* and *T. serrulata* share a lengthy 78-base and a nine-base deletion (Table 5). *Callisia gracilis* lacks the five base deletion found in all other taxa of *Callisia* s.l. (Table 5).

Differences between *Callisia* and *Tripogandra* include radially symmetrical flowers in *Callisia* versus bilaterally symmetrical flowers in *Tripogandra* (Hunt 1993, Faden 1998); zero to six stamens, all equal or subequal, but not alternating long and short in *Callisia* versus three short fertile antesepalous stamens and three long fertile or staminodial antepetalous stamens in *Tripogandra* (Faden 1998).

Chromosomes for both genera are asymmetrical. Jones and Jopling (1972) noted that chromosome differences within the family have been combined with other data satisfactorily to delimit genera; the removal of *C. rosea* from *Tradescantia* as one example. The base chromosome number for *Tripogandra* is likely eight, but 13 has been suggested and polyploidy is not uncommon (Handlos 1975). Two purported

allopolyploids with 21 as their haploid number are suspected of having been derived from bases eight and 13 (Handlos 1975).

Callisia gracilis may be a taxon undergoing more rapid evolution than that of other members of *Callisia* s.l. The chromosome base number, if seven, could represent a single-chromosome loss, through fusion, from an ancestral eight. This is in keeping with the potential base of eight for *Tripogandra*, with *C. gracilis*' propensity to group with *Tripogandra*, and with chromosome loss being a more likely phenomenon within *Callisia* s.l. than a gain (Faden, pers. comm.).

Section Hadrodemas *sensu Hunt (1986b)*

The single species of Hunt's section *Hadrodemas*, *C. warszewicziana*, is morphologically one of the two more robust members of *Callisia* s.l. This species' habit is typically referred to as bromeliiform (Hunt 1994). Long, thick strapping lanceolate leaves, a more spiral than two-ranked leaf arrangement, and short internodes give it the appearance of a pineapple. The inflorescence is much-branched, sepals are persistent and fleshy, and flowers are purple (H. Moore 1962, Hunt 1994). Stamens are six and glabrous or very sparsely bearded (H. Moore 1962). Vivipary has been reported (Hunt 1994). The term "vivipary" has various meanings. From personal observation, the vivipary reported for *C. warszewicziana* manifests as vegetative reproduction of plantlets on the inflorescence peduncle, as opposed to the germination and nourishment of seeds while in the fruit, as exemplified by mangroves (Allaby 1992).

Originally described under *Tradescantia* by Kunth and Bouché (1847), *C. warszewicziana* has variously been treated under *Dichorisandra* (Planch. 1854), *Spironema* (Brückner 1927), *Tripogandra* (Woodson 1942), *Phyodina* (Rohweder 1956),

and the monotypic genus *Hadrodemas* (H. Moore 1962). Moore (1962) transferred this species to *Hadrodemas* from *Phyodina* which then also included *C. cordifolia*, *C. rosea*, *C. gracilis*, and would later include *C. micrantha* and another species not available for this study. Moore (1962) distinguished *Hadrodemas* from *Phyodina* based on habit, leaf size and shape, and inflorescence, androecial, and gynoecial characteristics.

Tomlinson (1969) found two kinds of silica cells in *C. warszewicziana*. At that time, Brenan (1966) had proposed that the family be divided into fifteen groups. The presence of silica cells in the *Tripogandra*, *Callisia*, and *Hadrodemas* species that Tomlinson studied, as well as a similar micro-hair morphology supported Brenan's (1966) Group XI, which included *Callisia* and *Tripogandra* and endorsed the placement of the *Hadrodemas* taxon in that group instead of the one that Brenan proposed (Tomlinson 1969).

Contrary to Tomlinson, Jones and Jopling (1972) found the *Hadrodemas* chromosome karyotype and diploid number ($2n = 16$) dissimilar to *Callisia*, and Jones and Jopling (1972) found little reason to follow Tomlinson's (1969) suggestion for placement of the *Hadrodemas* taxon. The group into which *C. warszewicziana* had been placed according to Brenan (1966) was Group XIII, which included *Gibasis* and *C. filiformis* of section *Leptocallisia* sensu Hunt (1986b), a species not available for this study nor included in Jones and Jopling's (1972) study (then treated under *Leptorhoeo*).

The basal position of *C. warszewicziana* to section *Callisia* in the molecular data here is quite unvarying (Figs. 2 to 19) although bootstrap support ranges from weak (55-68%, Figs. 13, 15) to moderate (79%, Fig. 11) to strong (94 to 100%, Figs. 3, 5, 7, 9, 17, 19). Such a position could, again, reflect a chromosome loss from a base of eight to six.

The spiral leaf arrangement of *C. warszewicziana* is found only in *C. fragrans* of section *Callisia*, although that species is stoloniferous while *C. warszewicziana* is not. From a putatively basal *C. warszewicziana* to members of section *Callisia* is a transition from less to more derived inflorescence and perianth and a progression towards anemophily (Hunt 1986b, Faden, pers. comm.).

All members of section *Hadrodemas* and section *Callisia* sampled here share a seven-base *trnL-F* deletion in the position where all other species sampled have a tandem repeat except for *Tripogandra diuretica*, *Tradescantia spathacea*, and *Commelina erecta* (Table 6).

The basal position of *C. warszewicziana* as suggested by this molecular analysis is most likely valid (Faden, pers. comm.). Moore (1962) considered the one member of his *Hadrodemas* to represent an "evolutionary endpoint" in relation to its relatives (H. Moore 1962, p. 134), but from these analyses, *C. warszewicziana* marks an evolutionary step towards section *Callisia* sensu Hunt (1986b).

Section Callisia *sensu Hunt (1986b)*

Section *Callisia* sensu Hunt (1986b) parallels Moore's treatment of the genus (1958). With foliar characteristics similar to those of previous sections (two-ranked or spiral arrangement, variations on ovate and lanceolate shapes), the inflorescence in this section ranges from a much-branched open panicle to a stipitate form with sessile to pedunculate cincinnus-pairs and very short pedicels. In this section, reduction of the corolla is correlated with an even more enlarged anther connective; for some species (e.g. *C. repens*, *C. fragrans*, *C. guerrerensis*, *C. fragrans* cv 'Melnickoff') the exserted stamen/anther connective combination is the "showy" aspect of the flower. For some

species, the corolla remains prominent (e.g. *C. elegans*, *C. macdougallii*). Stamens are (one-) three or six, glabrous, and the antepetalous are longest or the only ones developed (H. Moore 1958). The ovary apex is pubescent to pilose (H. Moore 1958, Hunt 1994). The type species, *C. repens*, exhibits the most reduction in floral parts and is the only species of the genus to be monogynoecious (Hunt 1986b, Faden 1998).

Tomlinson (1966) found that those species of *Callisia* included in his study were among the relatively few members of the family (in addition to *Tripogandra* species and *C. warszewicziana*) to have two types of silica cells. The only other genera in the family to have silica cells, but of only one type, are *Gibasis* and two genera not in the subtribe of interest here (Tomlinson 1966). Tomlinson found two-celled prickle-hairs and/or uniseriate hair distribution on leaf surfaces and margins, leaf surface epidermal cell shape, and hypodermis presence or absence (and number of hypodermal layers if present) to be potential diagnostic characters for *Callisia* and allied genera (Tomlinson 1966). From these characters, Tomlinson (1966) informally suggested several groupings; one included *C. elegans*, *C. macdougallii*, and *C. tehuantepecana*, and another included *C. fragrans*, *C. repens*, and *C. soconuscensis*. These two groups are similarly reflected in Hunt's (1986b) treatment of the genus and in molecular data here.

Hunt (1986b) divided section *Callisia* into three taxonomically informal "groups" (Table 2) based on petal blade expansion, number of stamens, and chromosome karyotype.

All *Callisia* s.l. with known karyotypes have asymmetrical chromosomes (Anderson and Sax 1936; Giles 1942; Jones and Jopling 1972; Hunt 1979, 1986b). In Hunt's (1986b) section *Callisia*, the informal groups are in part distinguished by diploid

karyotype: the "Gentlei" group has two submeta- and ten subtelocentric chromosomes; the "Fragrans" group has six submeta- and six subtelocentric chromosomes; the "Repens" group has four submeta- and eight subtelocentric chromosomes.

The molecular analyses presented here in part support the groups of Hunt (1986b). The two members of the "Gentlei" group available for this study, *C. elegans* and *C. macdougallii*, form a consistent clade within the larger section *Callisia* clade with usually strong bootstrap support (98 to 100% for *ndhF* alone and for the combined data sets; Figs. 3, 5, 7, 15, 17, 19) and with two indels in common (Table 5). An expanded petal blade and six stamens distinguish Hunt's (1986b) "Gentlei" group in addition to karyotype. The members of the "Fragrans" group included in this study, *C. fragrans*, *C. fragrans* cv 'Melnickoff', and *C. guerrerensis* typically form a clade. Diagnostic for this group *sensu* Hunt (1986b), in addition to karyotype, are petals not expanded into a blade and six stamens. Bootstrap support for this clade is strong (86 to 100%; Figs. 3, 5, 7, 9, 11, 13, 15, 17, 19). Hunt's (1986b) "Repens" group includes two species, *C. repens* and *C. insignis*. In addition to karyotype, petals not expanded into a blade and (one-) three or six stamens diagnose the group. For *ndhF*, the two species collapse into a polytomy (Figs. 3. 5. 7), but in randomly chosen representative trees of all the MPRs generated for each *ndhF* analysis (Figs 2, 4, 6), *C. repens* is sister to the "Fragrans" group, which also has an unexpanded petal blade. (Only *ndhF* data was obtainable from the herbarium specimen available for *C. insignis*, so that species was excluded from the combined analyses.) For the combined analysis, however, *C. repens* is sister to the "Gentlei" group with bootstrap support varying from weak (66%, Fig. 19) to moderate (78%, Fig. 17) to strong (100%, Fig. 15). Two indels are shared by the taxa representative here of the

"Gentlei" and "Repens" groups *sensu* Hunt (1986b) (Table 5). The placement of *C. guerrerensis* into clades with one or both *Tripogandra*, and the corresponding placement of *C. gracilis* into the section *Callisia* clade require further study.

Prior to these molecular analyses, examination of the live specimen of the taxon mislabeled *C. fragrans* cv 'Melnickoff' and of its voucher specimen suggested that the plant is neither *C. fragrans* nor a cultivar thereof. Determination of whether the taxon is *C. guerrerensis* or *C. soconuscensis* (also of the section *Callisia* "Fragrans" group) was still unsettled, despite descriptions from the literature and without a valid *C. soconuscensis* specimen with which to compare to known *C. guerrerensis* vouchers. In the molecular analyses, the mislabeled taxon groups with *C. guerrerensis* in the *ndhF* data set with mostly weak branch support (61 to 75%, Figs. 3, 5, 7) and a considerable amount of character state change differences (Figs. 2, 4, 6). In *trnL-F*, the plant in question groups with *C. gracilis* with weak (62%) or no bootstrap support (Figs. 9, 11, 13) and with very little difference in character state changes (Fig. 8, 10, 12). In the combined data set, the taxon groups with *C. fragrans* both with high branch support (bootstrap 91 to 99%, Figs. 15, 17, 19) and with numbers of character state changes (Figs. 14, 16, 18) similar to or exceeding those found in *ndhF* alone (Figs. 2, 4, 6). The inflorescence and the vegetation of this taxon more resemble *C. guerrerensis*, which, in gross morphology more resembles *C. soconuscensis* than *C. fragrans.* Given the molecular results, the taxon likely is not *C. guerrerensis*, nor does it at all resemble the illustration of *C. fragrans* cv 'Melnickoff' depicted in Moore (1958). If it is a cultivar of *C. fragrans*, then it certainly has been selected in that artificial environment to resemble *C. guerrerensis*, and perhaps obtains from *C. fragrans* a greater adaptation to dry

conditions. Indeed, *C. fragrans* and *C. repens* are known to adapt to dry conditions in cultivation in Africa (R. B. Faden, pers. comm.). In chapters subsequent to this discussion, the taxon will be referred to as "*C. soconuscensis*".

The section *Callisia*/section *Hadrodemas* clade appears to be a monophyletic group [which is composed of the most recent common ancestor and all descendents (Judd et al. 1999, Zomlefer 1994)] based on these molecular data and supported by characters states of more traditional data. The relationships of the taxa within that clade are not as ambiguous as are relationships of those of other clades within *Callisia* s.l.

Table 3. Taxa included in *ndhF* and *trnL-F* molecular analyses. [a] Species provided by Dr. R. B. Faden of the Smithsonian Institution Botany Department's greenhouses; [b] species sequenced from herbarium specimen; [c] species for which *trnL-F* sequence was not obtainable. (GenBank accession numbers are pending submission for publication.)

Taxon	**Original Collector/Source**	**Voucher (deposited at GA)**	**GenBank Accession Number**
Subfamily Commelinoideae Faden & D. R. Hunt			
Tribe Tradescantieae Meisn.			
Subtribe Tradescantiinae Rohw.			
Callisia Loefl.			
C. cordifolia (Sw.) Anderson & Woodson [a]	Faden 83/37	Bergamo 99-192	
C. elegans Alexander ex H. E. Moore [a]	excult Tim Chapman	Bergamo 99-196	
C. fragrans (Lindl.) Woodson [a]	Hort. U. of Chicago	Bergamo 99-198	
C. fragrans cv 'Melnickoff' [a] ("*C. soconuscensis*")	Munich Bot. Gart. 84/3362	Bergamo 02-265	
C. gracilis (Kunth) D. R. Hunt[a]	Grant 3984	Bergamo 02-267	
C. graminea (Small) G. Tucker	Giles, 93L-1	Bergamo 99-189	
C. guerrerensis Matuda[a]	Hunt 9733	Bergamo 99-193	
C. insignis C. B. Clarke[b,c]	US 1945801		
C. macdougallii Miranda[a]	D. Gold, s.n.	Bergamo 00-218	
C. micrantha (Torr.) D. R. Hunt	T.F. Patterson s.n.	Bergamo 00-268	
C. monandra (Sw.) Schultes f.[a]	Munich Bot. Gart., J. Bogner	Bergamo 99-194	
C. multiflora (Martens & Galeotti) Standl.[a]	Faden 76/116A	Bergamo 99-195	
C. navicularis (Ortgies) D. R. Hunt[a]	Fryxell s.n	Bergamo 00-217	
C. ornata (Small) G. Tucker	Bergamo 99-187	Bergamo 99-187	
C. repens (Jacq.) L.[a]	Spencer 92-351	Bergamo 99-197	
C. rosea (Vent.) D. R. Hunt	Giles s.n.	Bergamo 99-198	
C. warszewicziana (Kunth & Bouché) D. R. Hunt[a]	D. Hunt BH 60-511	Bergamo 99-191	

Gibasis Raf.			
Gibasis pellucida (Martens & Galeotti) D. R. Hunt	UGA Plant Biology Dept. Greenhouses	Bergamo 99-190	
Tradescantia L.			
T. roseolens Small	Bergamo 99-186	Bergamo 99-186	
T. spathacea Sw.	UGA Plant Biology Dept. Greenhouses	Bergamo 99-201	
T. virginiana L.	UGA Plant Biology Dept. Greenhouses	Bergamo 00-214	
T. zebrina hort. ex Bosse	UGA Plant Biology Dept. Greenhouses	Bergamo 00-215	
Tripogandra Raf.			
T. diuretica (Martius) Handlos[a]	Plowman 10171	Bergamo 99-200	
T. serrulata (Vahl) Handlos[a]	Brenner 10/81	Bergamo 99-199	
Subtribe Thyrsantheminae			
Tinantia Scheidw.			
T. pringlei (S.Watson) Rohw.[a]	Avent AIM-773	Bergamo 00-215	
Tribe Commelineae Meisn.			
Commelina L.			
C. erecta L.	Bergamo 99-185	Bergamo 99-185	

Table 4. Difference between Taberlet et al (1991) and Sang et al. (1997) primer sequences used for *trnL-F* amplification and sequencing.

Primer	**Primer sequence**
tab e	5′- G GTT CAA GTC CCT CTA TCC C-3′
sang e	5′-AAA ATC GTG AGG GTT CAA GTC -3′
tab f	5′- ATT TGA ACT GGT GAC ACG AG-3′
sang f	5′-G ATT TGA ACT GGT GAC ACG AG-3′

Table 5. Insertions/deletions (indels) in the *trnL-F* spacer region, excluding indels of fewer than three bases and ambiguous ones.

	Indel position, # bases	**Present**	**Overlaps with another indel or tandem repeat**	**Absent**
1.	53-58 6-base insertion	Sect. *Cuthbertia (C. graminea, C. ornata, C. rosea*	yes	All other taxa
2.	75-79 5-base deletion	*Gibasis pellucida*	no	All other taxa
3.	171-248 78-base deletion	*Callisia gracilis, Tripogandra serrulata*	yes	All other taxa
4.	241-249 9 base deletion	*C. repens*	yes	All other taxa
5.	254-275 21-base insertion	*C. repens*	no	All other taxa
6.	276-295 20-base insertion	*C. elegans, C. macdougallii, C. repens*	no	All other taxa
7.	296-304 13-base insertion	*C. elegans, C. macdougallii*	no	All other taxa
8.	305-308 4-base insertion	*C. elegans, C. macdougallii, C. repens*	no	All other taxa
9.	309-317 9-base deletion	*C. gracilis, T. serrulata*	no	All other taxa
10.	335-343 10-base deletion	*T. diuretica*	yes	All other taxa
11.	336-339 4-base deletion	Sect. *Brachyphylla (C. micrantha, C. navicularis)*	yes	All other taxa
12.	348-350 3-base insertion	All other taxa	yes	Sect. *Brachyphylla (C. micrantha, C. navicularis)*

13.	351-357 7-base deletion	All other taxa	yes	Sect. *Brachyphylla (C. micrantha, C. navicularis), Commelina erecta*
14.	440-444 5-base deletion	All members of *Callisia* s.l. except *C. gracilis*	yes	All other taxa

Table 6. Tandem repeats in the *trnL-F* spacer region.

	Tandem Repeat position, # bases repeated	**Present**	**Overlaps with indel**	**Absent**
1.	46-65 10 bases	Sect. *Brachyphylla (Callisia micrantha, C. navicularis)*	yes	All other taxa
2.	335-357 14 bases	*Tripogandra diuretica*	yes	All other taxa
3.	364-377 7 bases	All other taxa	no	Sects. *Callisia* and *Hadrodemas* as deletion; (nucleotides present in *Tripogandra diuretica, Tradescantia spathacea, Commelina erecta*, but not as a tandem repeat)
4.	387-419 16 bases	*Tripogandra serrulata*	yes	All other taxa

Table 7. Summary results and indices for the *ndhF*, *trnL-F*, and combined data sets. Analyses are from heuristic search algorithms except for the combined data set without indels which is from branch and bound. CI = consistency index excluding uninformative characters; RI = retention index.

Gene region	**# characters**	**# parsimony-informative characters**	**# trees**	**Tree length**	**CI**	**RI**
ndhF						
with indels						
as 5th	778	127	288	406	.65	.79
as missing	778	125	288	362	.65	.79
without indels	714	117	300	329	.64	.79
trnL-F						
with indels						
as 5th	450	215	24	495	.71	.78
as missing	450	56	105	170	.62	.74
without indels	193	34	84	105	.59	.73
ndhF & trnL-F						
with indels						
as 5th	1228	342	6	951	.64	.72
as missing	1228	181	7	555	.60	.73
without indels	907	151	6	452	.60	.74

Table 8. Geographic distribution of *Callisia* sensu Hunt (1986b), Tucker (1989).

Distribution compiled from H. Moore (1958); Hunt (1986b, 1993, 1994); Faden (2000).

Section	**"Group"**	**Species**	**Distribution**
Hadrodemas		***C. warszewicziana*** (Kunth & Bouché) D. R. Hunt	Guatemala, endemic
Cuthbertia		***C. graminea*** (Small) G. Tucker	Florida, Georgia, North Carolina, South Carolina, Virginia
		C. ornata (Small) G. Tucker	Florida
		C. rosea (Vent.) D. R. Hunt	Florida, Georgia, North Carolina, South Carolina
Lauia		***C. laui*** (D. R. Hunt) D. R. Hunt	Oaxaca, Mexico, endemic
Brachyphylla		***C. navicularis*** (Ortgies) D. R. Hunt	southeast Texas, eastern Mexico
		C. micrantha (Torr.) D. R. Hunt	southeast Texas, eastern Mexico
Leptocallisia		***C. ciliata*** Kunth	Panama, Colombia
		C. cordifolia (Sw.) Anderson & Woodson	Yucatan, Mexico to Venezuela, Peru; West Indies; Florida, Georgia
		C. filiformis (Martens & Galeotti) D. R. Hunt	Mexico to Brazil
		C. gracilis (Kunth) D. R. Hunt	Panama to Peru
		C. monandra (Sw.) Schultes f.	Baja CA; West Indies to Brazil and Peru
		C. multiflora (Martens & Galeotti) Standl.	Jalisco, Mexico to Nicaragua
Callisia	I. "Gentlei"	***C. elegans*** Alexander ex H. E. Moore	Guatemala, Honduras; allegedly from Oaxaca, Mexico
		C. gentlei Matuda	Belize
		C. macdougallii Miranda	Chiapas, Mexico, endemic

		C. nizandensis Matuda	Oaxaca, Mexico, endemic
		C. tehuantepecana Matuda	Oaxaca, Mexico, endemic
	II. “Fragrans”	***C. fragrans*** (Lindl.) Woodson	Tamaulipas to Yucatan, Mexico; Florida introduction
		C. guerrerensis Matuda	Guerrero, Mexico
		C. soconuscensis Matuda	Southwest Mexico to Guatemala
	III. “Repens”	***C. repens*** (Jacq.) L.	Mexico (native); West Indies (type from Martinique); Central America; South America (to Argentina); Florida, Georgia, Louisiana
		C. insignis C. B. Clarke	Highlands of east central Mexico

Figure 1. Schematic of *trnL-F* with intron and spacer region. Primers are designated by b, c, d, e, and f, with the direction of amplification indicated by the arrow alongside the primer designation. Redrawn in part from Taberlet et al. (1991).

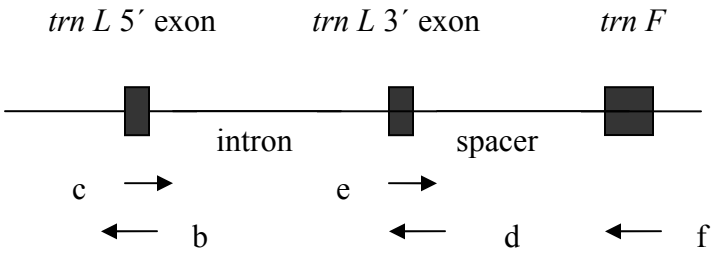

trn L 5′ exon
trn L 3′ exon
trn F
intron
spacer
c
e
b
d
f

Figure 2. One randomly chosen variable length tree of 288 most parsimonious trees from parsimony analysis of the 3′ region of *ndhF* with indels treated as a fifth character. Character state changes are indicated above branch lines. CI without uninformative characters = 0.65; RI = 0.79.

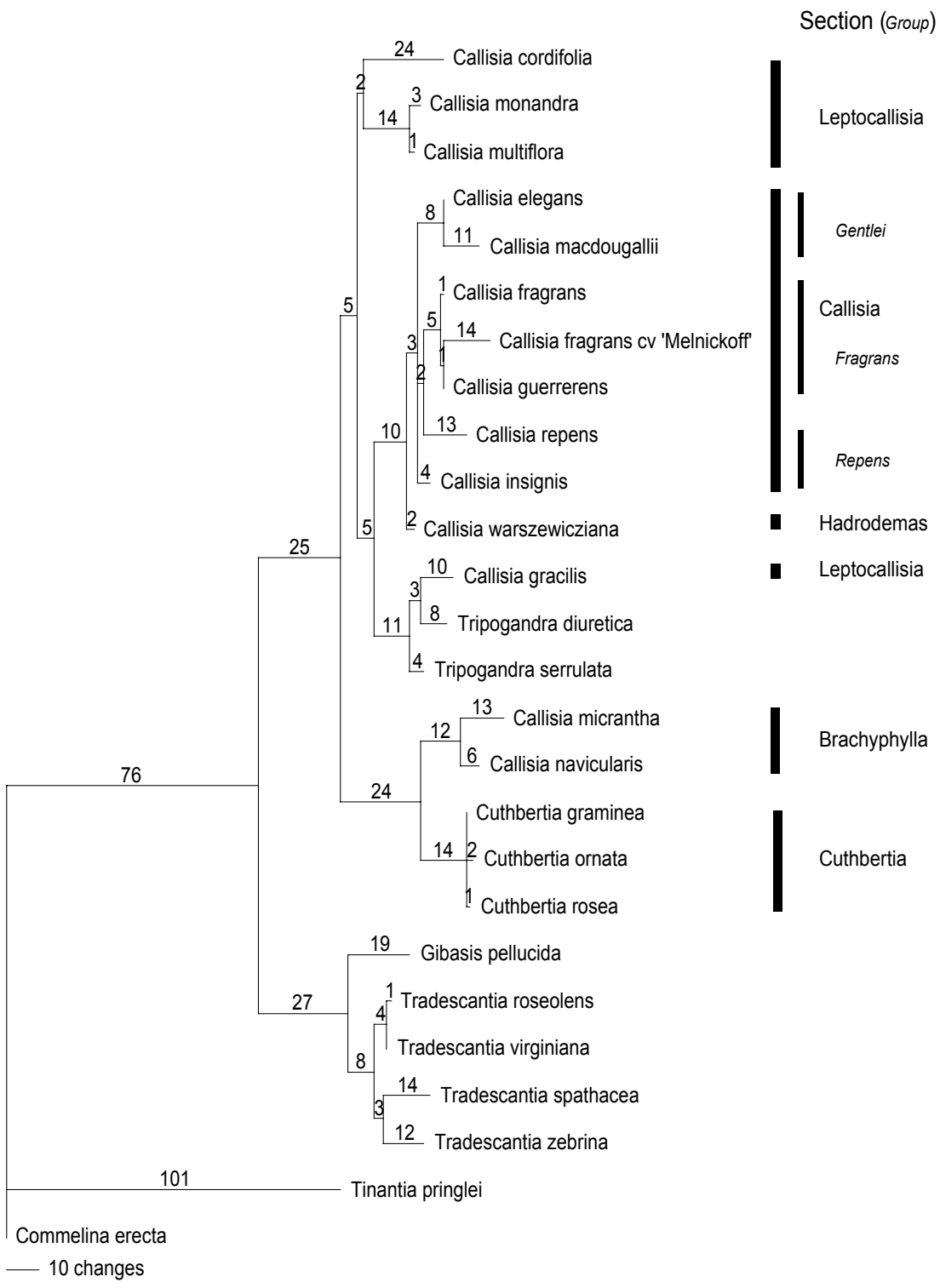

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Leptocallisia
Callisia elegans
Callisia macdougallii
Gentlei
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia guerrerens
Callisia
Fragrans
Callisia repens
Callisia insignis
Repens
Callisia warszewicziana
Hadrodemas
Callisia gracilis
Tripogandra diuretica
Tripogandra serrulata
Leptocallisia
Callisia micrantha
Callisia navicularis
Brachyphylla
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Cuthbertia
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
10 changes

Figure 3. Bootstrap analysis of the 3′ region of *ndhF* with indels treated as a fifth character. Bootstrap percentages greater than 50% are indicated above branch lines.

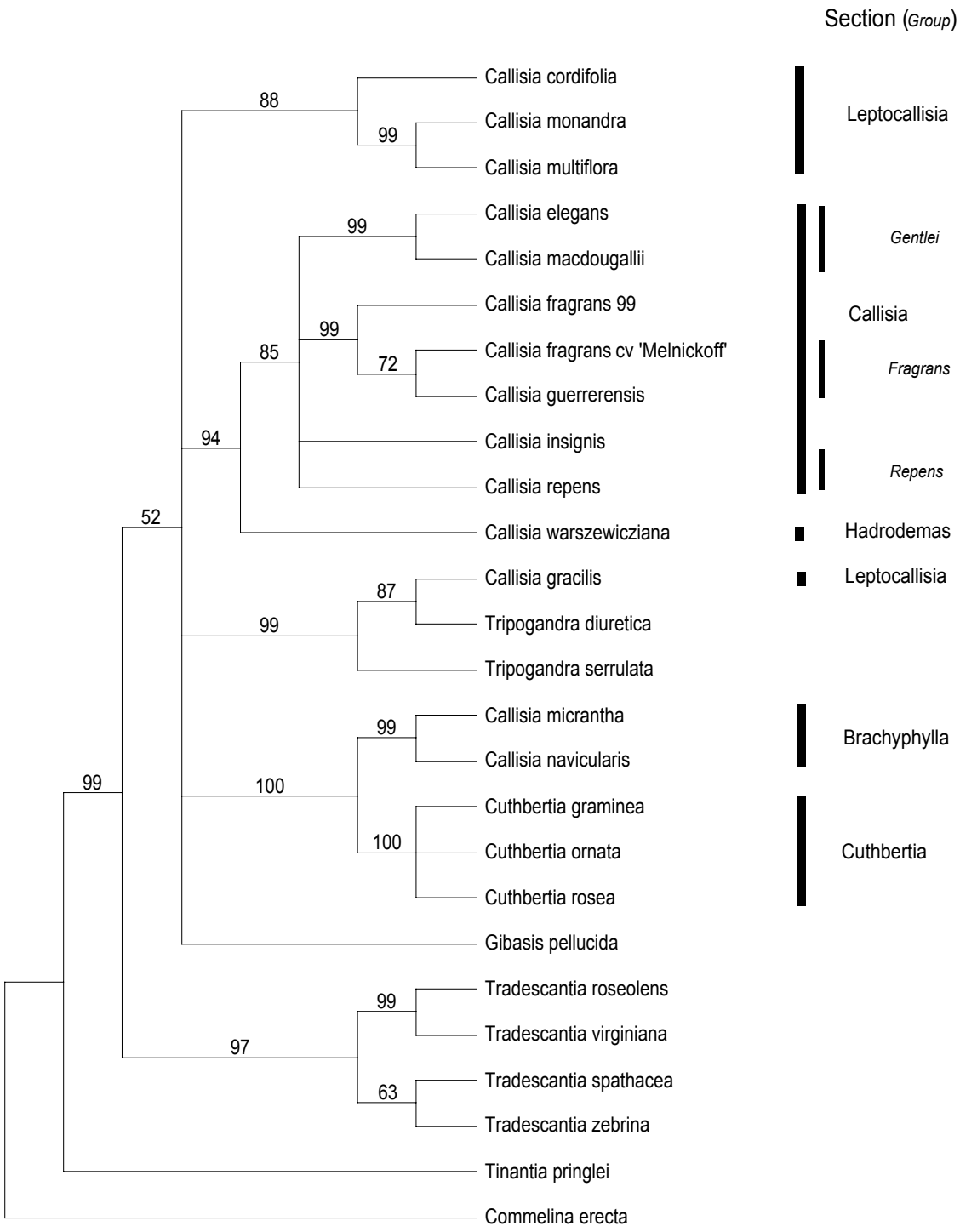

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia fragrans 99
Callisia fragrans cv 'Melnickoff'
Callisia guerrerensis
Callisia insignis
Callisia repens
Callisia warszewicziana
Callisia gracilis
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
88
99
99
99
85
72
94
52
87
99
99
100
100
99
97
63
Leptocallisia
Gentlei
Callisia
Fragrans
Repens
Hadrodemas
Leptocallisia
Brachyphylla
Cuthbertia

Figure 4. One randomly chosen variable length tree of 288 most parsimonious trees from parsimony analysis of the 3′ region of *ndhF* with indels treated as missing. Character state changes are indicated above branch lines. CI without uninformative characters = 0.65; RI = 0.79.

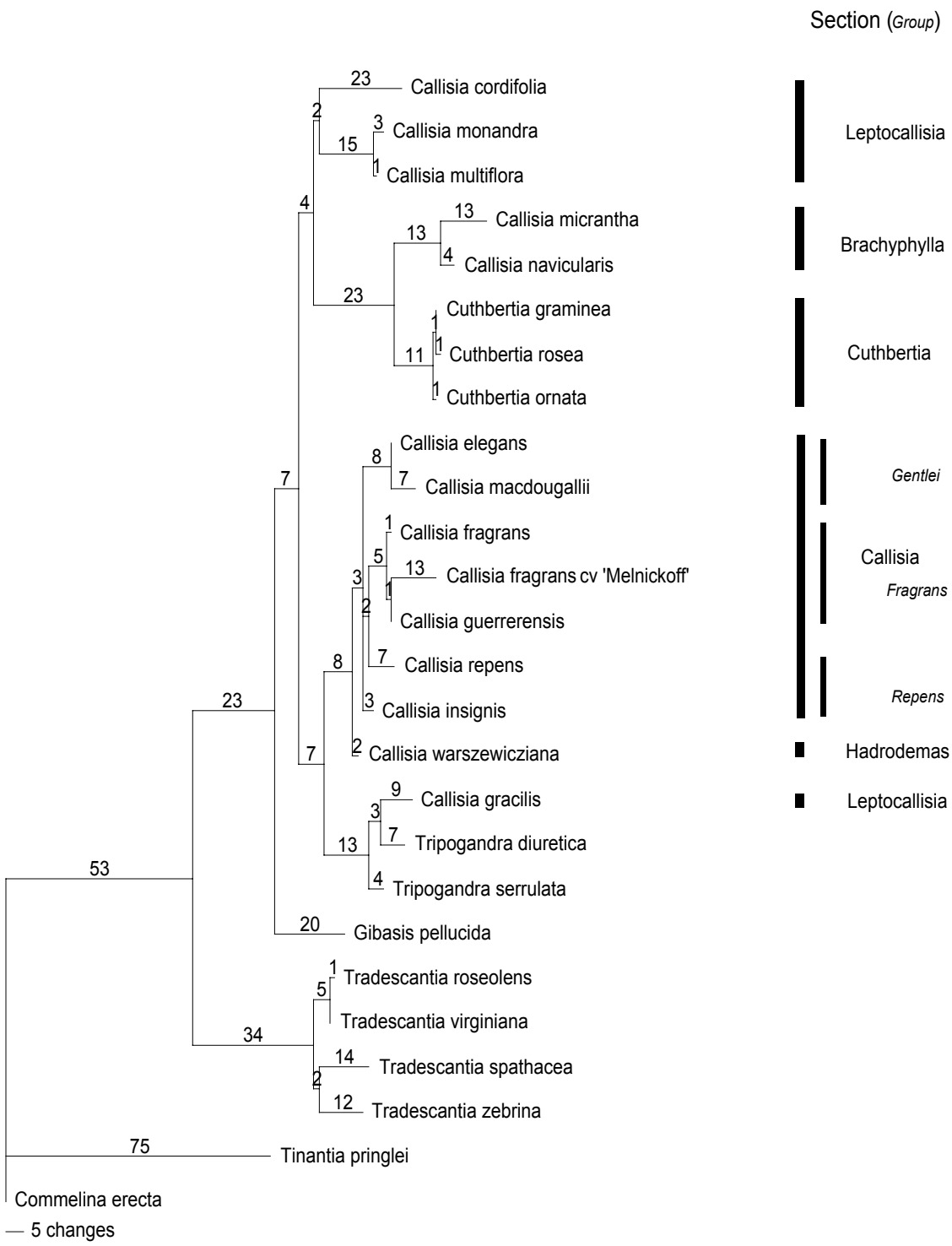

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia rosea
Cuthbertia ornata
Callisia elegans
Callisia macdougallii
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia guerrerensis
Callisia repens
Callisia insignis
Callisia warszewicziana
Callisia gracilis
Tripogandra diuretica
Tripogandra serrulata
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
— 5 changes
Leptocallisia
Brachyphylla
Cuthbertia
Gentlei
Callisia
Fragrans
Repens
Hadrodemas
Leptocallisia

Figure 5. Bootstrap analysis of the 3′ region of *ndhF* with indels treated as missing. Bootstrap percentages greater than 50% are indicated above branch lines.

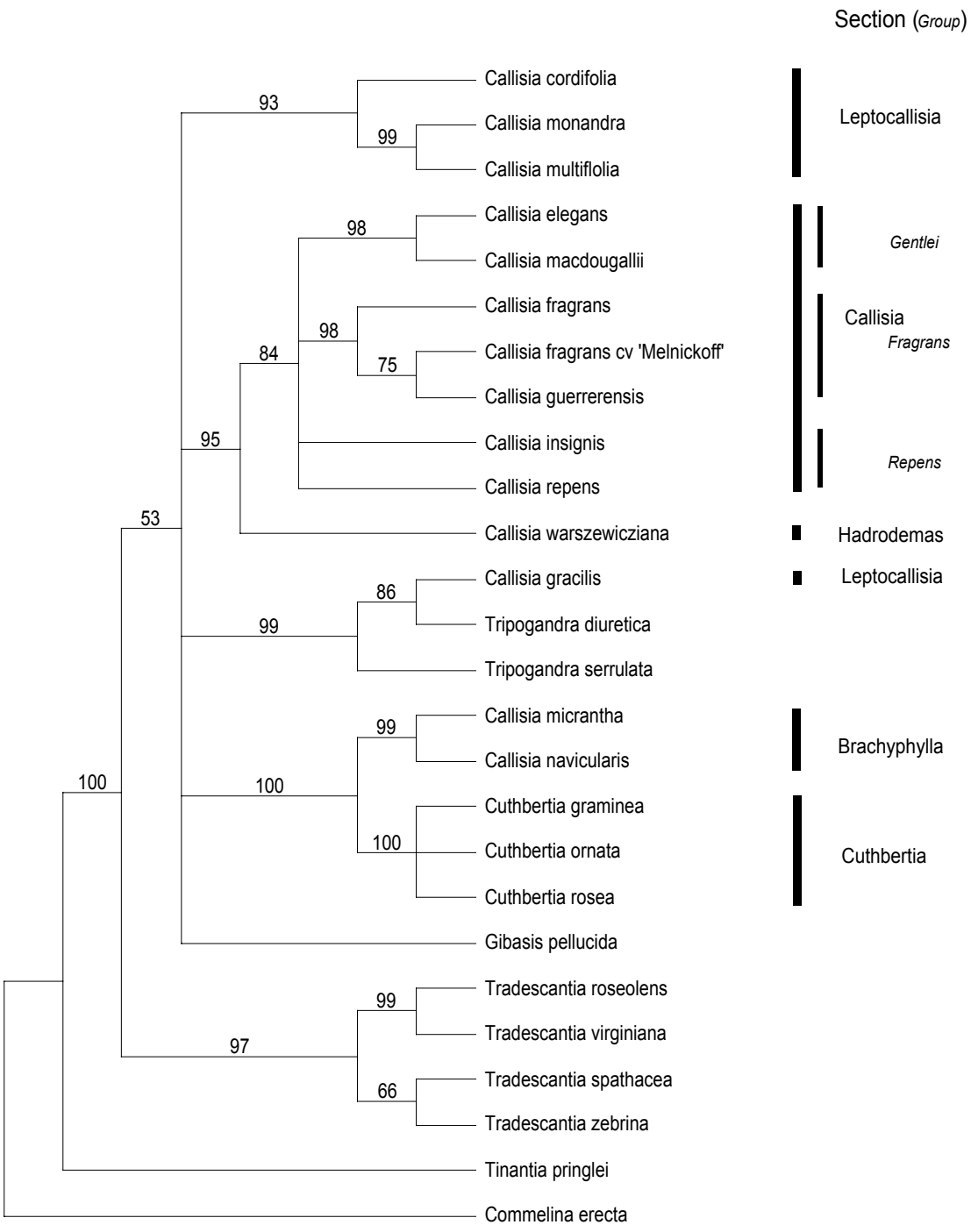

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflolia
Callisia elegans
Callisia macdougallii
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia guerrerensis
Callisia insignis
Callisia repens
Callisia warszewicziana
Callisia gracilis
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
93
99
98
98
84
75
95
53
86
99
99
100
100
100
97
99
66
Leptocallisia
Gentlei
Callisia
Fragrans
Repens
Hadrodemas
Leptocallisia
Brachyphylla
Cuthbertia

Figure 6. One randomly chosen variable length tree of 300 most parsimonious trees from parsimony analysis of the 3′ region of *ndhF* with indels removed. Character state changes are indicated above branch lines. CI without uninformative characters = 0.64; RI = 0.79.

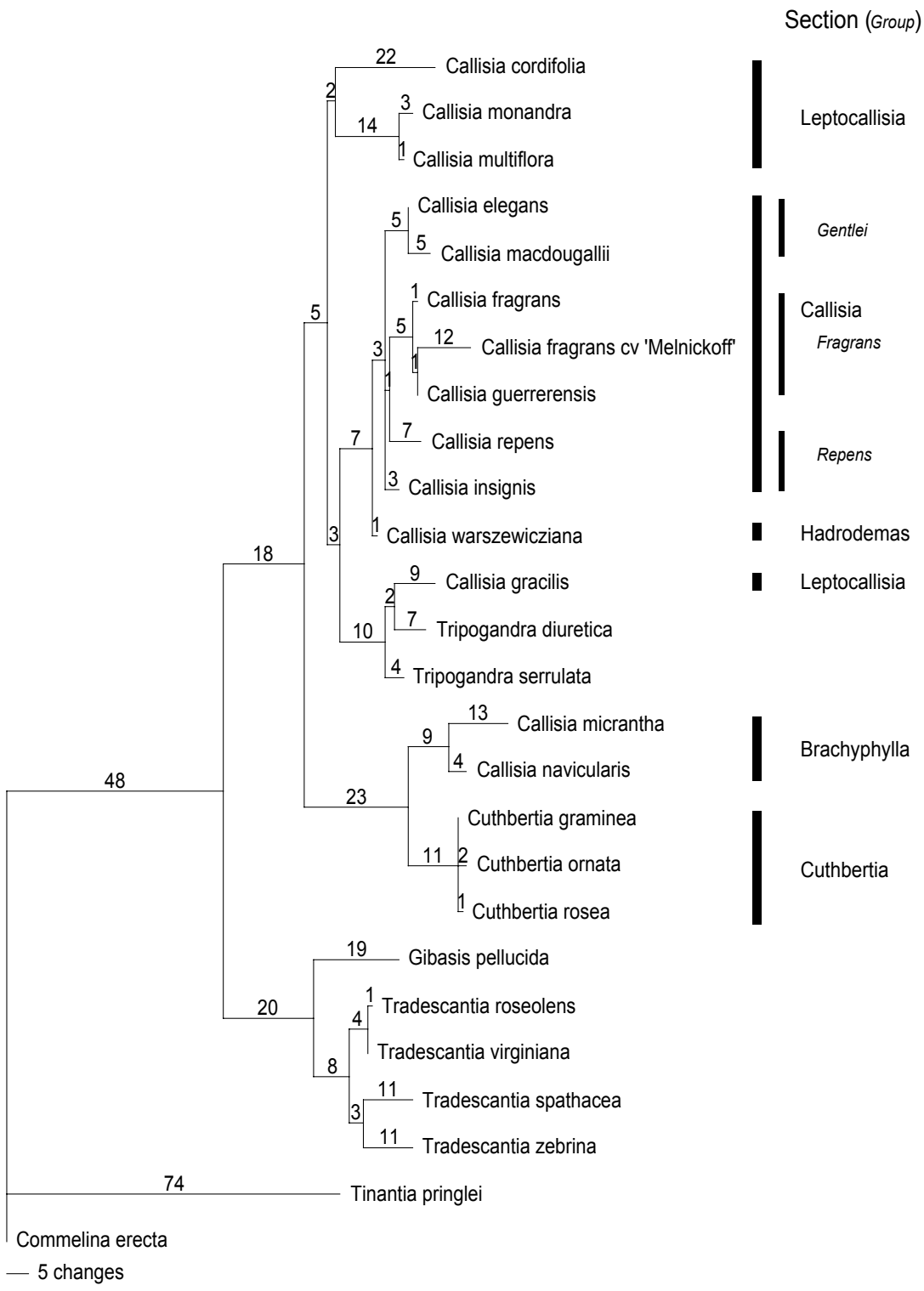

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Leptocallisia
Callisia elegans
Callisia macdougallii
Gentlei
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia guerrerensis
Callisia
Fragrans
Callisia repens
Repens
Callisia insignis
Callisia warszewicziana
Hadrodemas
Callisia gracilis
Leptocallisia
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Brachyphylla
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Cuthbertia
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
5 changes

Figure 7. Bootstrap analysis of the 3′ region of *ndhF* with indels removed. Bootstrap percentages greater than 50% are indicated above branch lines.

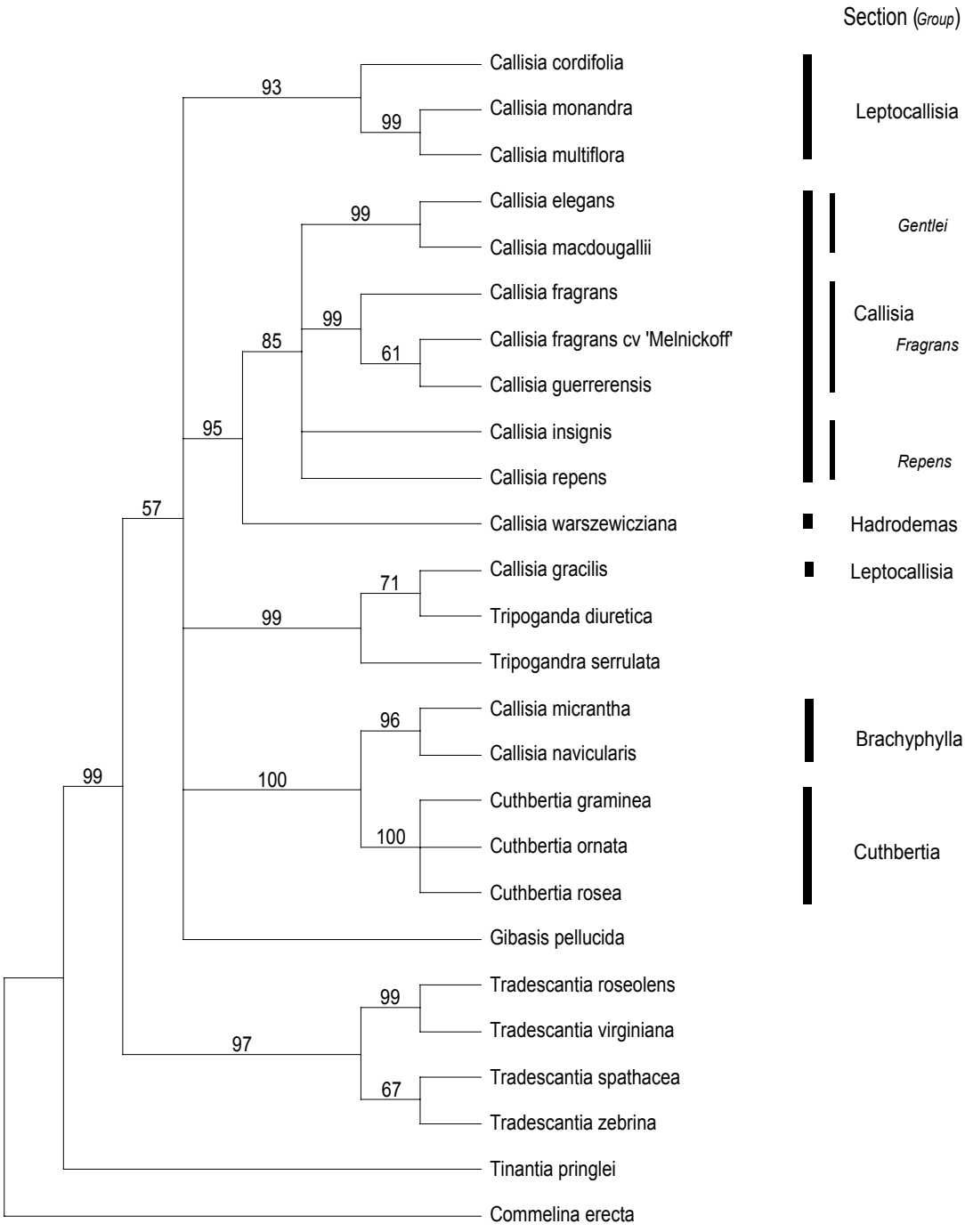

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia guerrerensis
Callisia insignis
Callisia repens
Callisia warszewicziana
Callisia gracilis
Tripoganda diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
93
99
99
85
99
61
95
57
71
99
96
99
100
100
99
97
67
Leptocallisia
Gentlei
Callisia
Fragrans
Repens
Hadrodemas
Leptocallisia
Brachyphylla
Cuthbertia

Figure 8. One randomly chosen variable length tree of 24 most parsimonious trees from parsimony analysis of the *trnL-F* spacer region with indels treated as a fifth character. Character state changes are indicated above branch lines. CI without uninformative characters = 0.64; RI = 0.78.

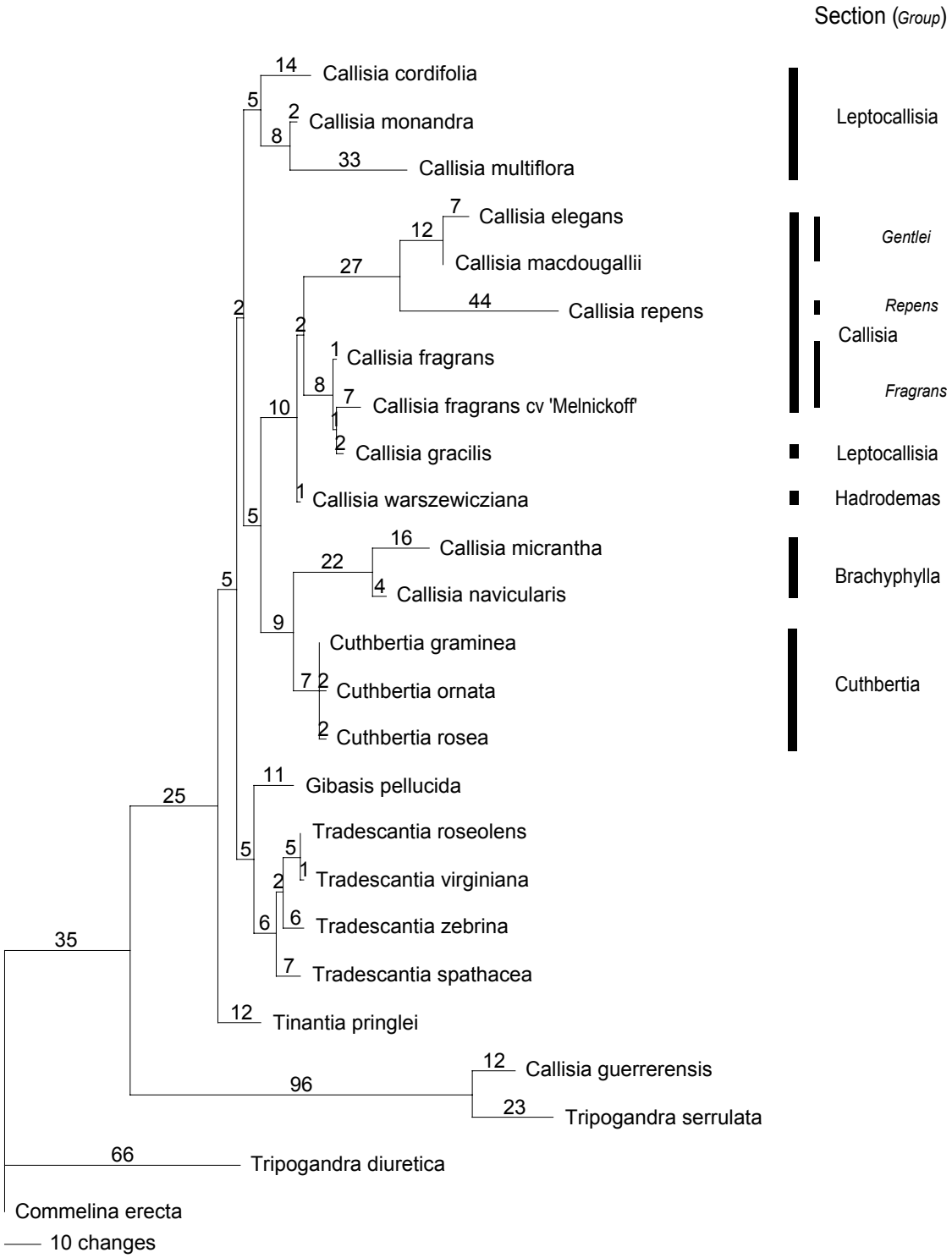

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia gracilis
Callisia warszewicziana
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia zebrina
Tradescantia spathacea
Tinantia pringlei
Callisia guerrerensis
Tripogandra serrulata
Tripogandra diuretica
Commelina erecta
10 changes
Leptocallisia
Gentlei
Repens
Callisia
Fragrans
Leptocallisia
Hadrodemas
Brachyphylla
Cuthbertia

Figure 9. Bootstrap analysis of the *trnL-F* spacer region with indels treated as a fifth character. Bootstrap percentages greater than 50% are indicated above branch lines.

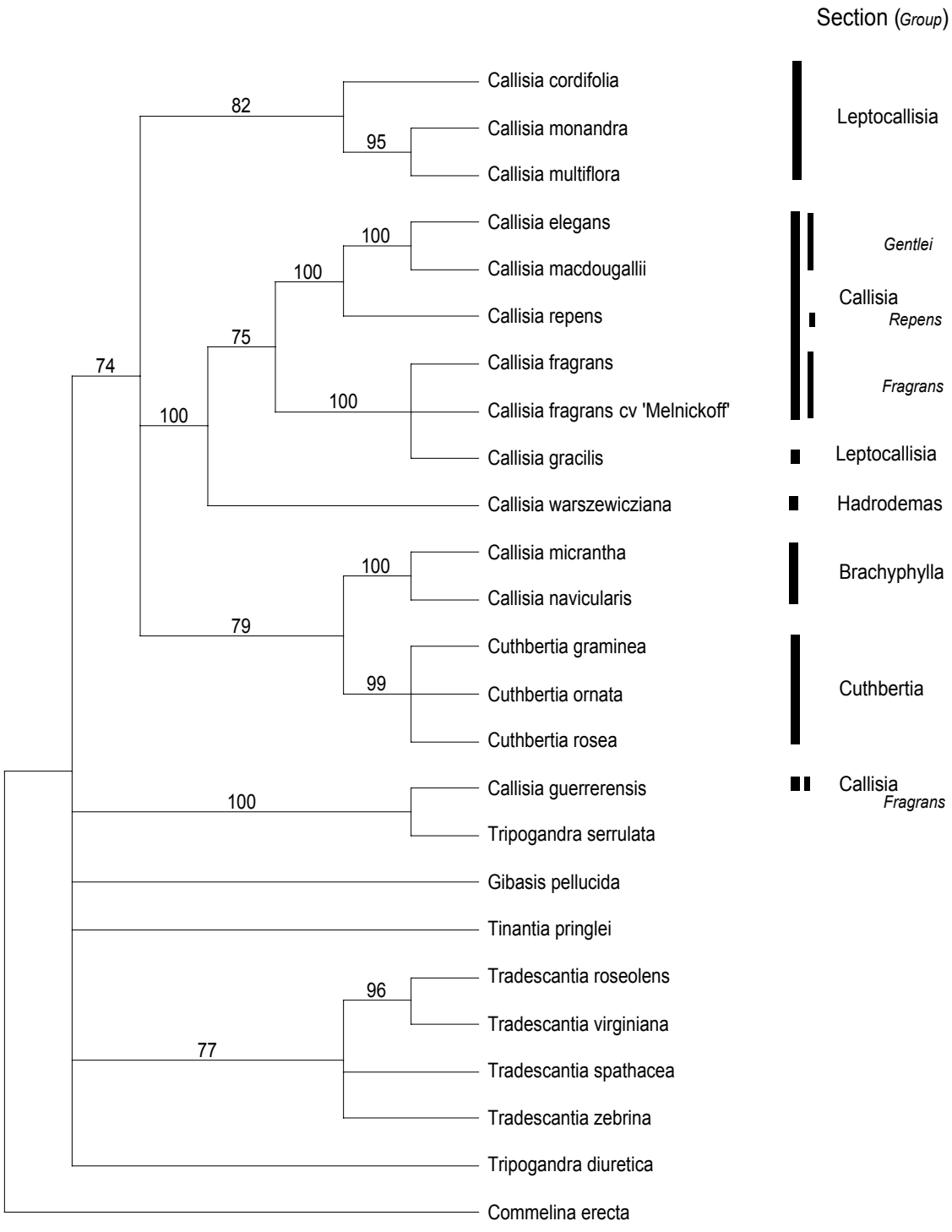

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia gracilis
Callisia warszewicziana
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Callisia guerrerensis
Tripogandra serrulata
Gibasis pellucida
Tinantia pringlei
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tripogandra diuretica
Commelina erecta
82
95
100
100
75
74
100
100
79
99
100
96
77
Leptocallisia
Gentlei
Callisia
Repens
Fragrans
Leptocallisia
Hadrodemas
Brachyphylla
Cuthbertia
Callisia
Fragrans

Figure 10. One randomly chosen variable length tree of 105 most parsimonious trees from parsimony analysis of the *trnL-F* spacer region with indels treated as missing. Character state changes are indicated above branch lines. CI without uninformative characters = 0.62; RI = 0.74.

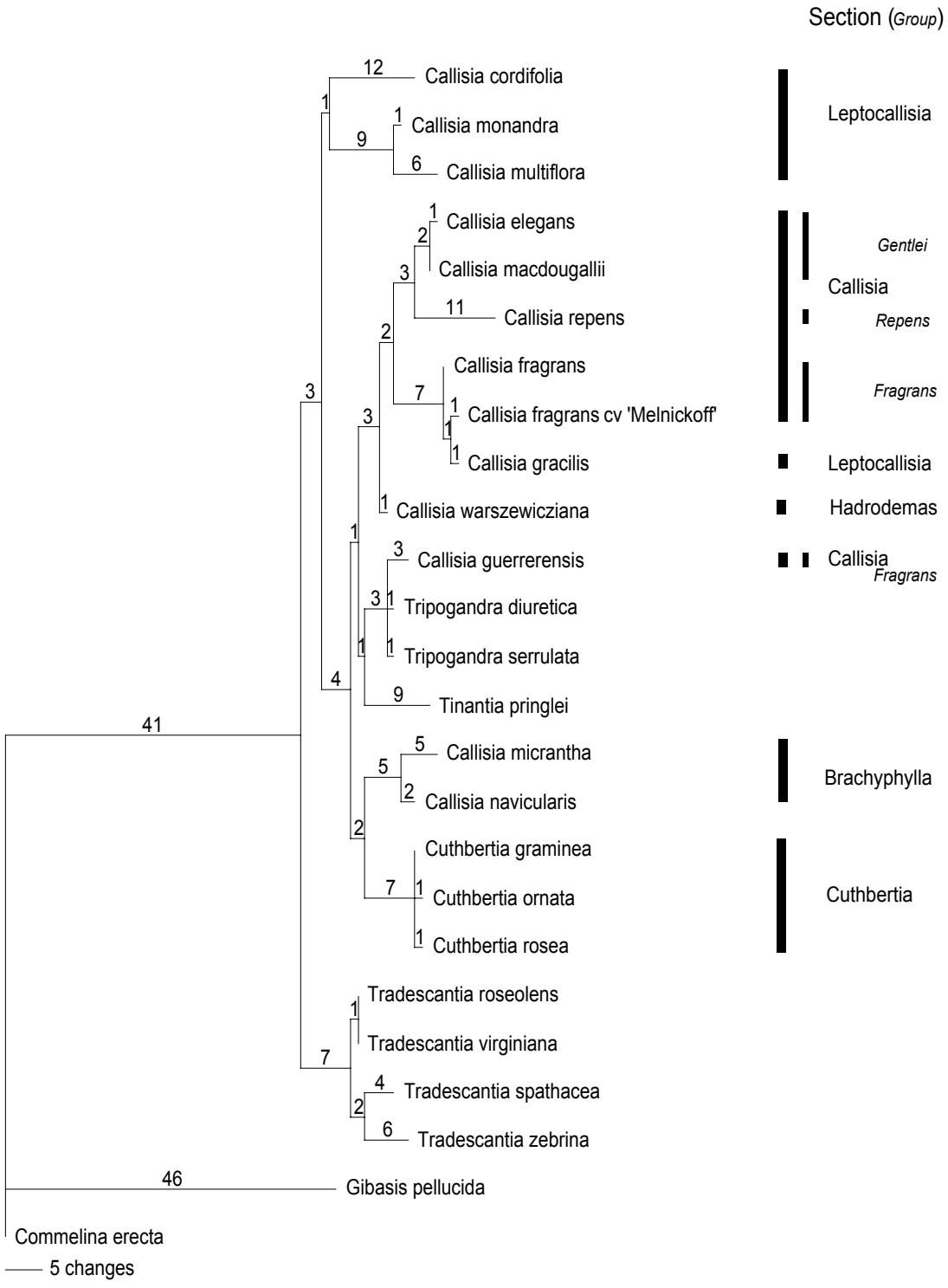

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia gracilis
Callisia warszewicziana
Callisia guerrerensis
Tripogandra diuretica
Tripogandra serrulata
Tinantia pringlei
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Gibasis pellucida
Commelina erecta
5 changes
Leptocallisia
Gentlei
Callisia
Repens
Fragrans
Leptocallisia
Hadrodemas
Callisia
Fragrans
Brachyphylla
Cuthbertia

Figure 11. Bootstrap analysis of the *trnL-F* spacer region with indels treated as missing. Bootstrap percentages greater than 50% are indicated above branch lines.

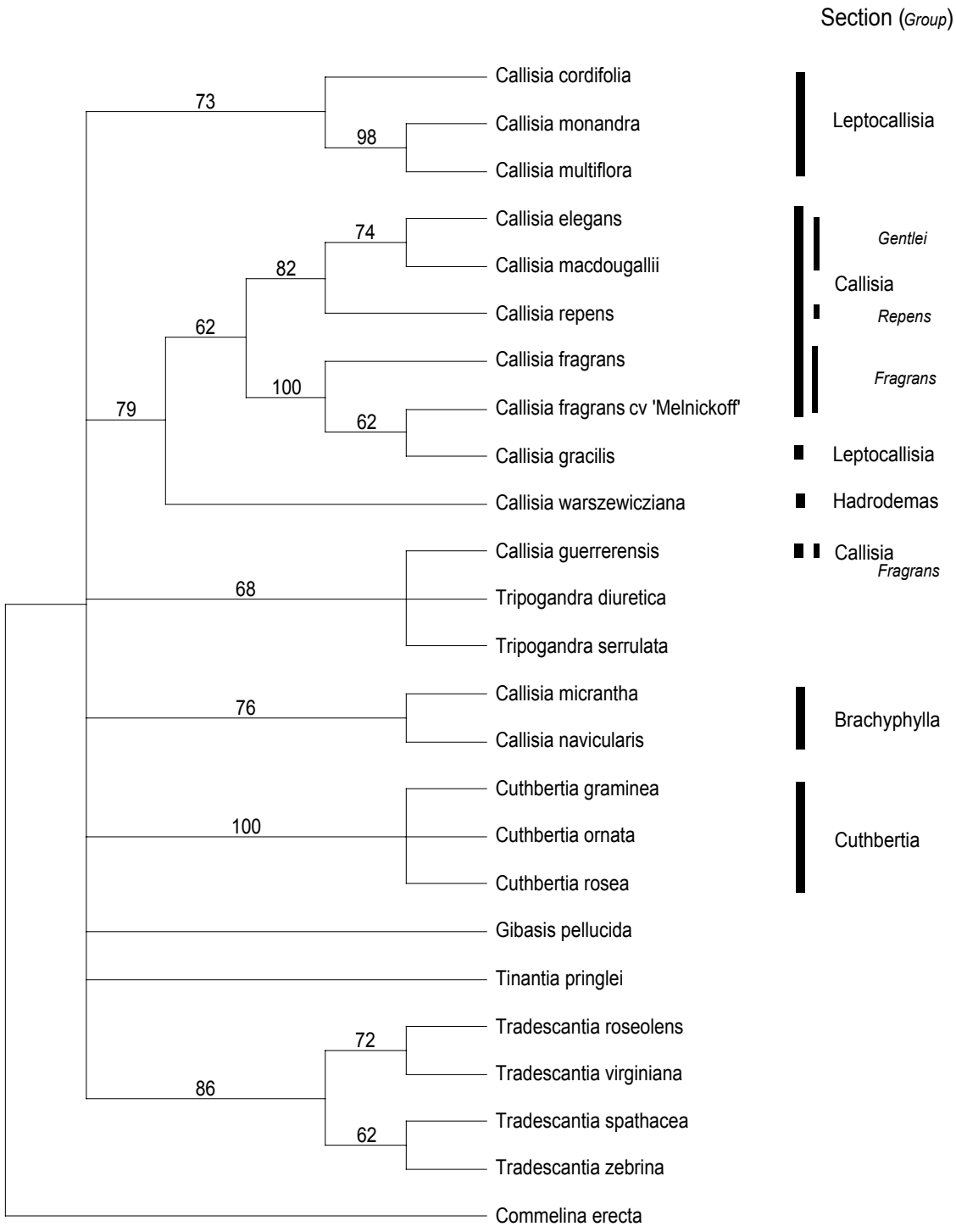

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia gracilis
Callisia warszewicziana
Callisia guerrerensis
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Gibasis pellucida
Tinantia pringlei
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Commelina erecta
73
98
74
82
62
100
62
79
68
76
100
72
86
62
Leptocallisia
Gentlei
Callisia
Repens
Fragrans
Leptocallisia
Hadrodemas
Callisia
Fragrans
Brachyphylla
Cuthbertia

Figure 12. One randomly chosen variable length tree of 84 most parsimonious trees from parsimony analysis of the *trnL-F* spacer region with indels removed. Character state changes are indicated above branch lines. CI without uninformative characters = 0.59; RI = 0.73.

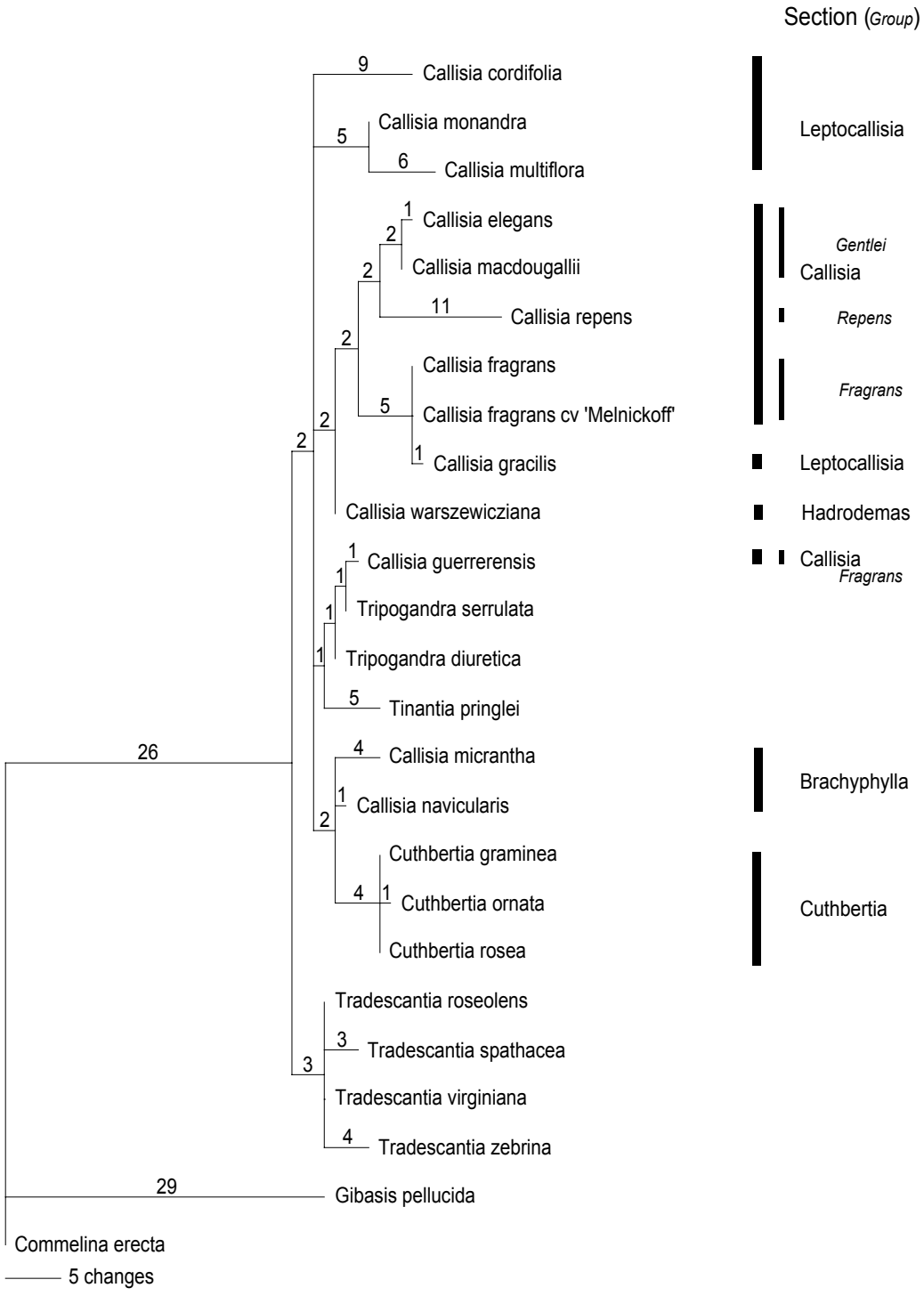

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia gracilis
Callisia warszewicziana
Callisia guerrerensis
Tripogandra serrulata
Tripogandra diuretica
Tinantia pringlei
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Tradescantia roseolens
Tradescantia spathacea
Tradescantia virginiana
Tradescantia zebrina
Gibasis pellucida
Commelina erecta
5 changes
Leptocallisia
Gentlei
Callisia
Repens
Fragrans
Leptocallisia
Hadrodemas
Callisia
Fragrans
Brachyphylla
Cuthbertia

Figure 13. Bootstrap analysis of the *trnL-F* spacer region with indels removed. Bootstrap percentages greater than 50% are indicated above branch lines.

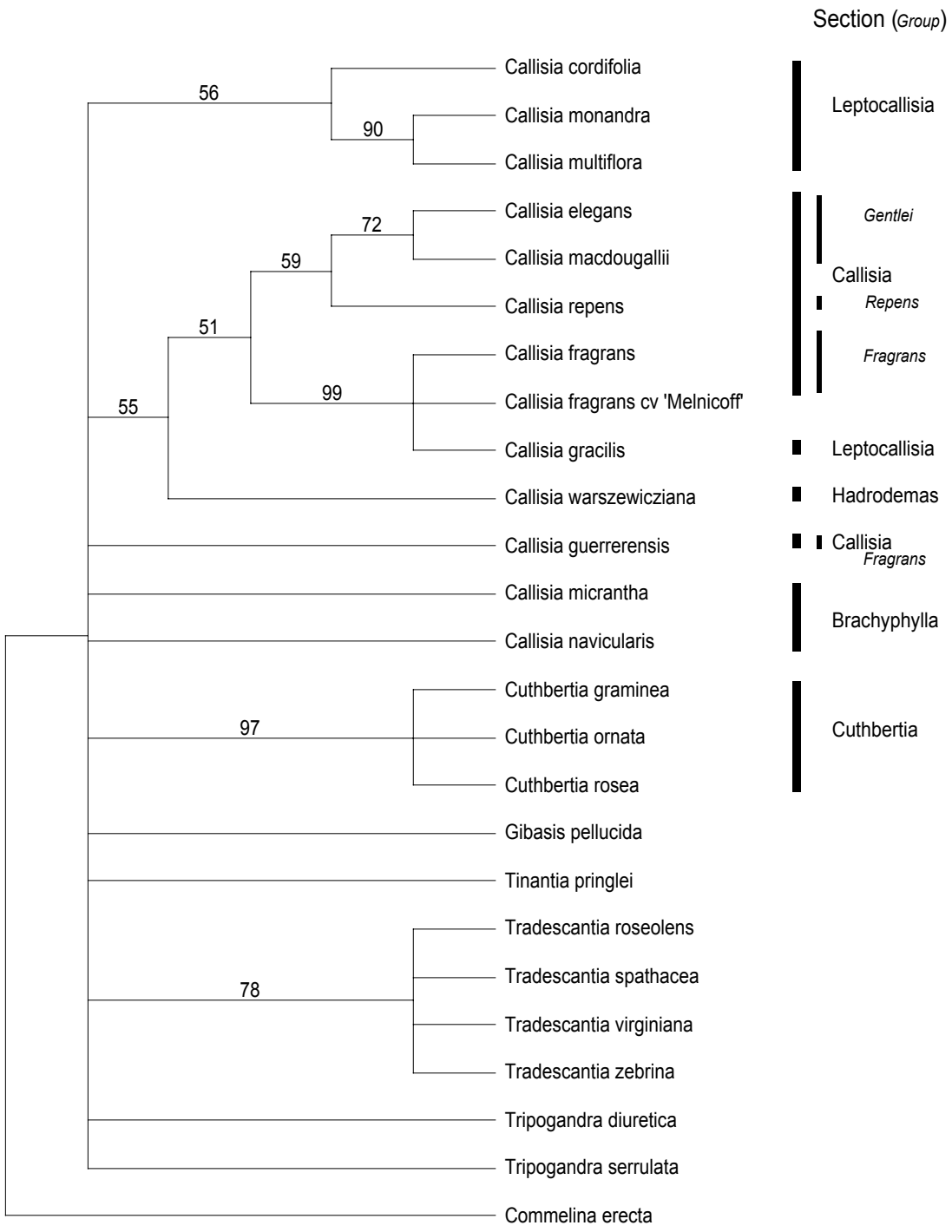

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
Callisia fragrans cv 'Melnicoff'
Callisia gracilis
Callisia warszewicziana
Callisia guerrerensis
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Gibasis pellucida
Tinantia pringlei
Tradescantia roseolens
Tradescantia spathacea
Tradescantia virginiana
Tradescantia zebrina
Tripogandra diuretica
Tripogandra serrulata
Commelina erecta
56
90
72
59
51
99
55
97
78
Leptocallisia
Gentlei
Callisia
Repens
Fragrans
Leptocallisia
Hadrodemas
Callisia
Fragrans
Brachyphylla
Cuthbertia

Figure 14. One randomly chosen variable length tree of 6 most parsimonious trees from parsimony analysis of the 3′ region of *ndhF* and the *trnL-F* spacer with indels treated as a fifth character. Character state changes are indicated above branch lines. CI without uninformative characters = 0.64; RI = 0.72.

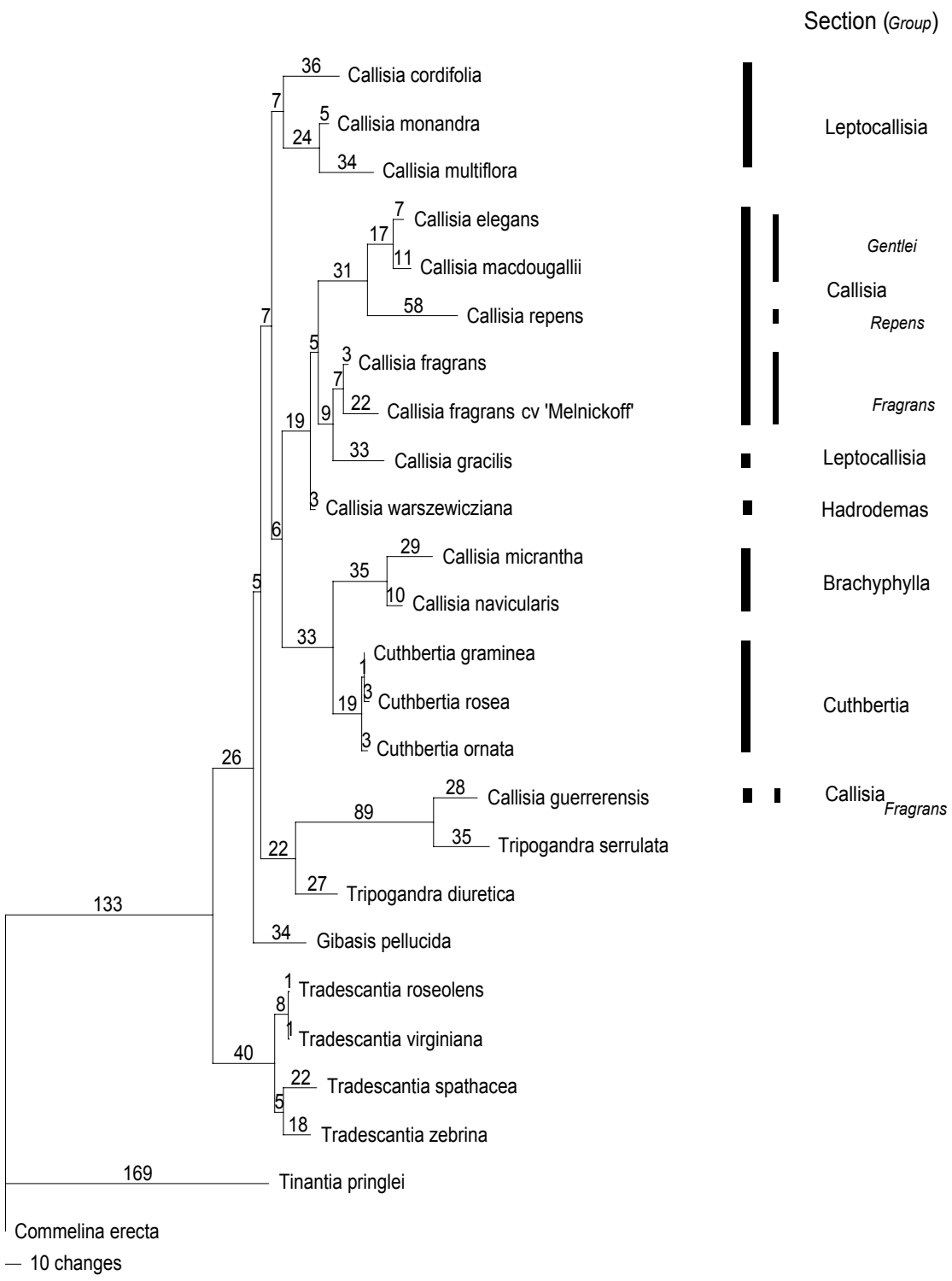

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia gracilis
Callisia warszewicziana
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia rosea
Cuthbertia ornata
Callisia guerrerensis
Tripogandra serrulata
Tripogandra diuretica
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
10 changes
Leptocallisia
Gentlei
Callisia
Repens
Fragrans
Leptocallisia
Hadrodemas
Brachyphylla
Cuthbertia
Callisia Fragrans

Figure 15. Bootstrap analysis of the 3′ region of *ndhF* and the *trnL-F* spacer with indels treated as a fifth character. Bootstrap percentages greater than 50% are indicated above branch lines.

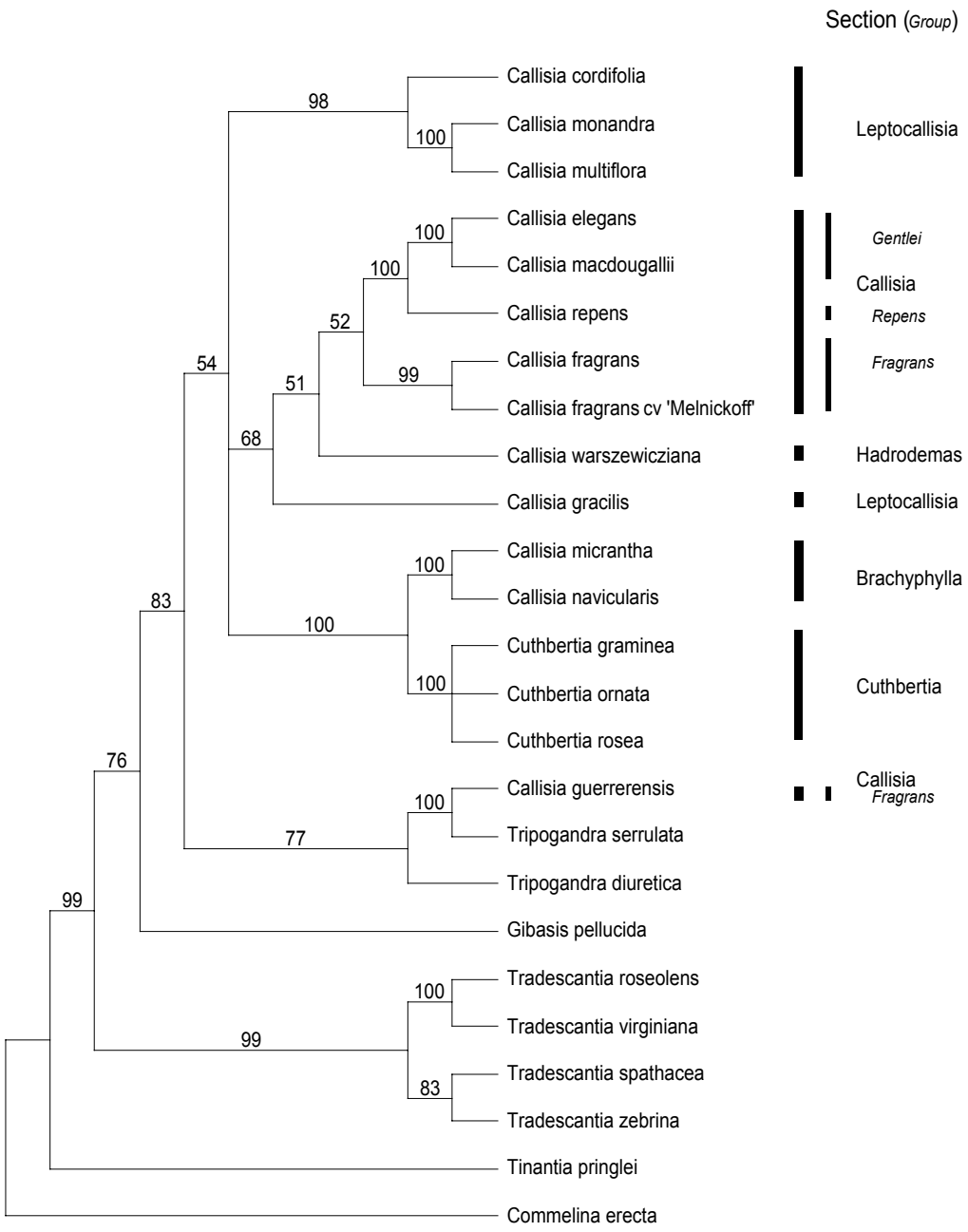

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia warszewicziana
Callisia gracilis
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Callisia guerrerensis
Tripogandra serrulata
Tripogandra diuretica
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
98
100
100
100
52
99
54
51
68
83
100
100
100
76
100
77
99
100
99
83
Leptocallisia
Gentlei
Callisia
Repens
Fragrans
Hadrodemas
Leptocallisia
Brachyphylla
Cuthbertia
Callisia
Fragrans

Figure 16. One randomly chosen variable length tree of 7 most parsimonious trees from parsimony analysis of the 3′ region of *ndhF* and the *trnL-F* spacer with indels treated as missing. Character state changes are indicated above branch lines. CI without uninformative characters = 0.60; RI = 0.73.


Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia guerrerensis
Callisia warszewicziana
Callisia gracilis
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia rosea
Cuthbertia ornata
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
10 changes
Leptocallisia
Gentlei
Callisia
Repens
Fragrans
Hadrodemas
Leptocallisia
Brachyphylla
Cuthbertia

Figure 17. Bootstrap analysis of the 3′ region of *ndhF* and the *trnL-F* spacer with indels treated as missing. Bootstrap percentages greater than 50% are indicated above branch lines.

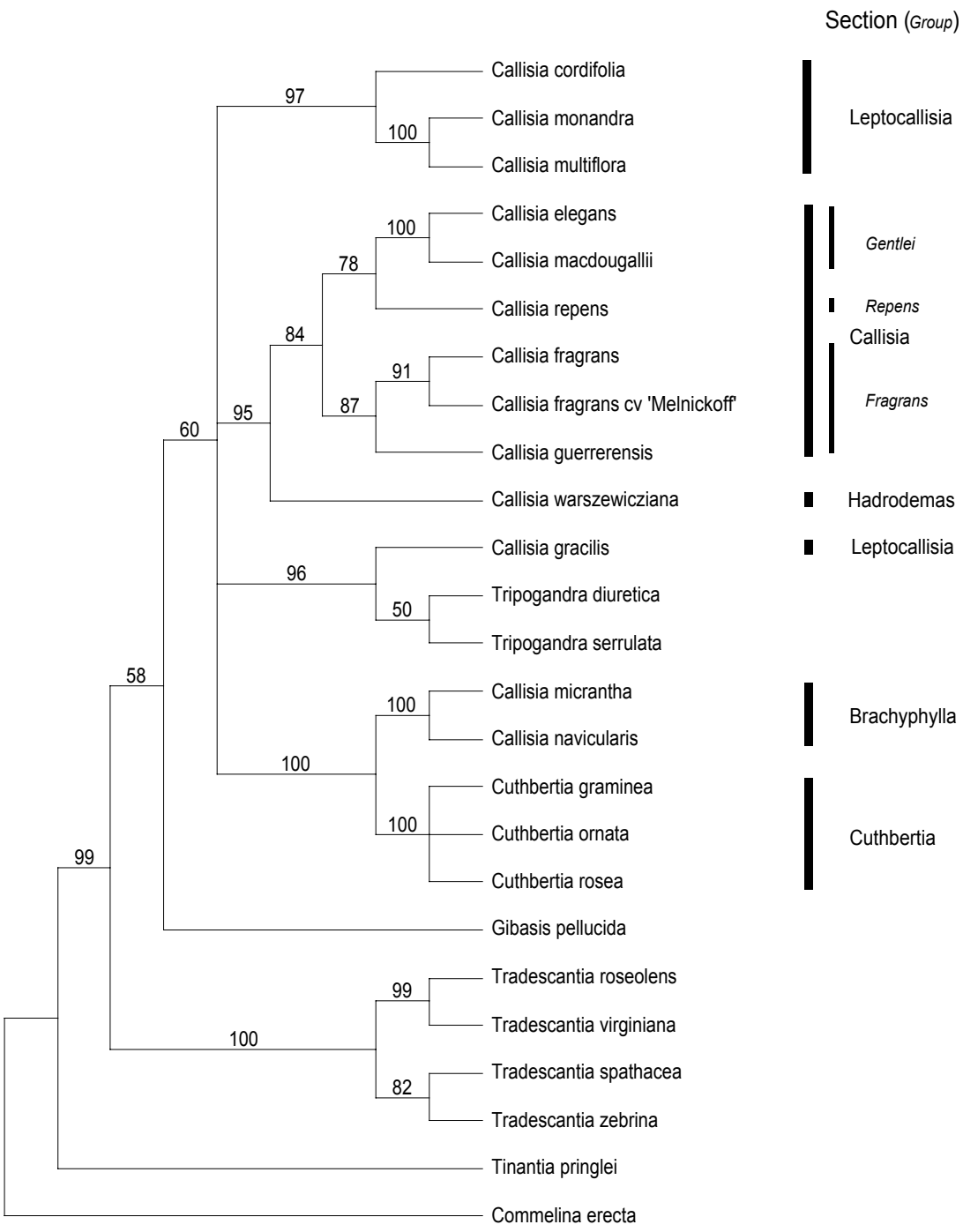

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia guerrerensis
Callisia warszewicziana
Callisia gracilis
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
97
100
100
78
84
91
87
95
60
96
50
58
100
100
100
99
99
100
82
Leptocallisia
Gentlei
Repens
Callisia
Fragrans
Hadrodemas
Leptocallisia
Brachyphylla
Cuthbertia

Figure 18. One randomly chosen variable length tree of 6 most parsimonious trees from parsimony analysis of the 3′ region of *ndhF* and the *trnL-F* spacer with indels removed. Character state changes are indicated above branch lines. CI without uninformative characters = 0.60; RI = 0.74.

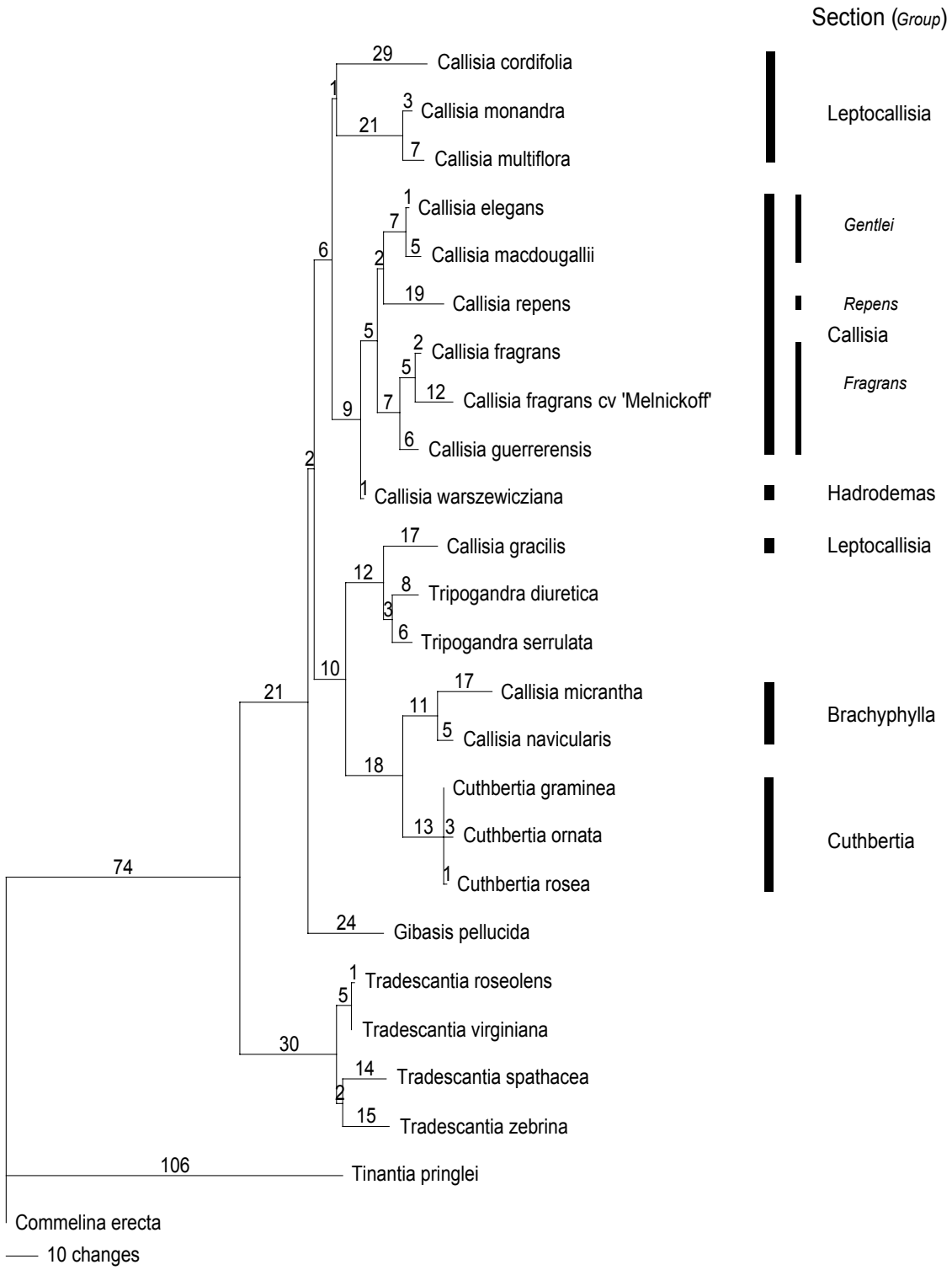
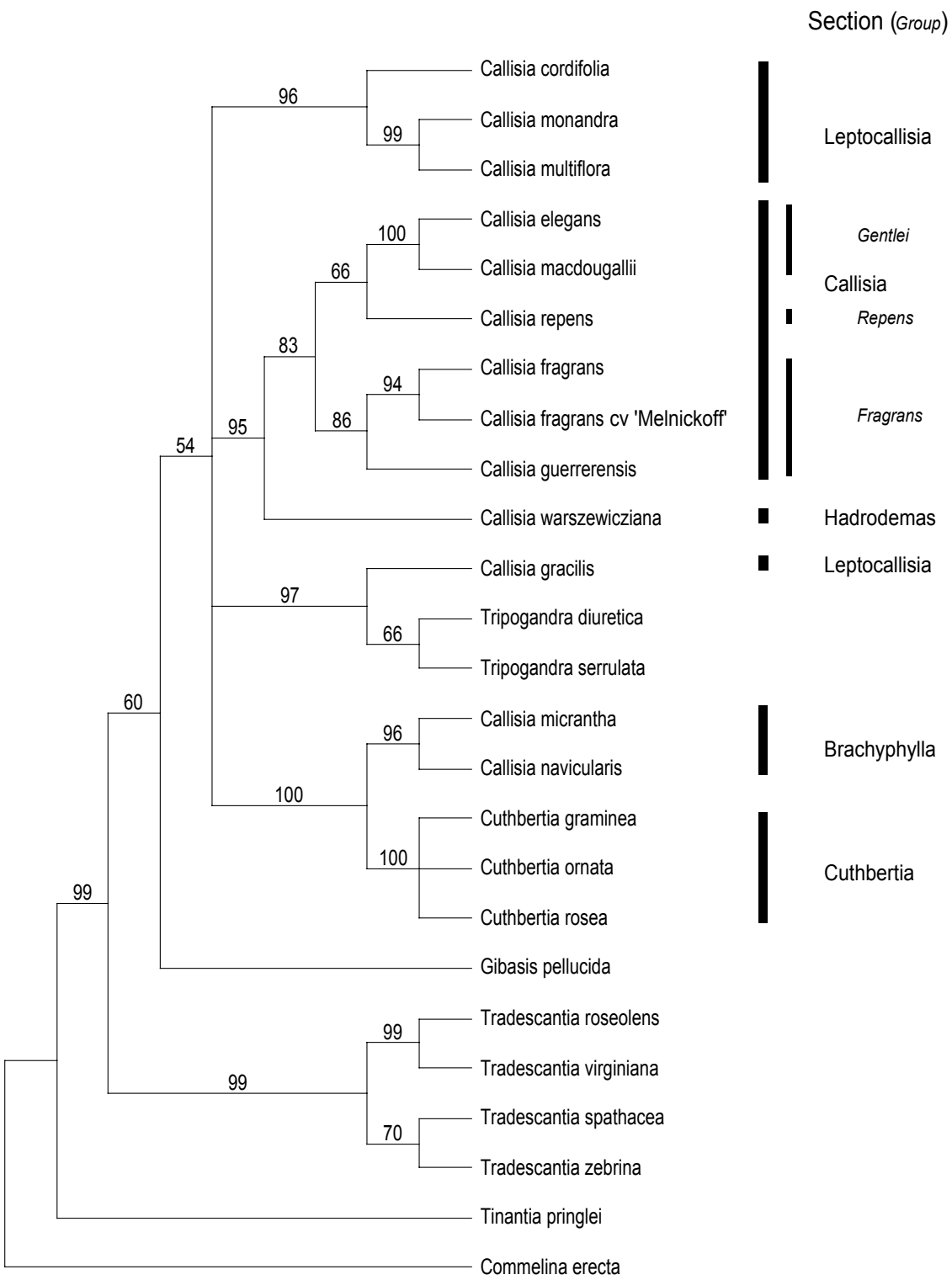

Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Callisia guerrerensis
Callisia warszewicziana
Callisia gracilis
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
10 changes
Leptocallisia
Gentlei
Repens
Callisia
Fragrans
Hadrodemas
Leptocallisia
Brachyphylla
Cuthbertia

Figure 19. Bootstrap analysis of the 3′ region of *ndhF* and the *trnL-F* spacer with indels removed. Bootstrap percentages greater than 50% are indicated above branch lines.


Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Leptocallisia
Callisia elegans
Callisia macdougallii
Gentlei
Callisia
Callisia repens
Repens
Callisia fragrans
Callisia fragrans cv 'Melnickoff'
Fragrans
Callisia guerrerensis
Callisia warszewicziana
Hadrodemas
Callisia gracilis
Leptocallisia
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Brachyphylla
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Cuthbertia
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
96
99
100
66
83
94
86
95
54
97
66
60
96
100
100
99
99
99
70

CHAPTER 3

NON-MOLECULAR DATA with a MOLECULAR HYPOTHESIS

Morphology

Although molecular phylogenies have become the main driver for reconsidering both relatedness and classification at almost all taxonomic ranks for plants and other organisms, a practical consideration in systematics still remains that which is directly observable, morphology. Ideally, molecular data helps to clarify or support traditional morphological data sets.

A trend in previous treatments of *Callisia* (Hunt 1986b, Faden and Hunt 1991), some of its segregate genera [e.g. *Phyodina* (Rohweder 1956)], and in analyses addressing relationships within and among members of the tribes Tradescantieae and Commelineae (Evans 1995; Evans et al. 2000a, 2000b, 2003), is the lack of a unifying morphological character that defines *Callisia* (Evans et al. 2000a, 2003). Early treatments pre-date molecular data, with its purported independence from morphology, and current studies at the familial level must, by nature, limit numbers of taxa sampled within genera to exemplar OTU's (Operational Taxonomic Units) (Evans 1995, 2000a, 2000b, 2003). Without a synapomorphy for *Callisia* s.l., any taxon within the genus utilized for investigations at the familial level has the potential to produce misleading results (Evans 2000a, 2000b).

Morphology of *Callisia* has in part been considered in the previous chapters. Here, morphology and other data will be further scrutinized utilizing parsimony and the

phylogenetic hypothesis (cladogram) of the bootstrap consensus of the combined *ndhF* and *trnL-F* data sets analyzed with indels removed (Fig. 19).

Homoplasies and Sympleisiomorphies

Homoplasy within the family has contributed to the difficulty in treating both the family and its genera (Evans et al. 2000a, 2000b, 2003). Di- or polymorphic stamens, although not characteristic of *Callisia* s.l., are homoplasious in the family, as illustrated by the results of this study (Fig. 20). In taxa included in this study, the character occurs only in species of *Tripogandra* and in the more distantly related outgroup taxa, *Tinantia* and *Commelina*. Also homoplasious in the subtribe Tradescantiinae are one and/or two types of silica which are rare in the family and occur only in section *Callisia* and in *Tripogandra* and *Gibasis* (Fig. 21). The character state of dorsally fused cymes with complete or partial fusion (Fig. 22) may be a synapomorphy for *Callisia* s.l. and *Tripogandra*, but occurs in close outgroup *Tradescantia*. In another example of homoplasy, the zygomorphy of *Tripogandra* flowers (Fig. 23), and in some flowers of another unrelated genus, *Murdannia*, arises from a reorientation of the stamens at anthesis. Other manifestations of androecial zygomorphy in the family include the differentiation of stamens or stamens and staminodes into anterior and posterior arrangements usually accompanied by a zygomorphic corolla, e.g. in *Commelina* and *Aneilema* (Faden 1998). Thus floral zygomorphy may develop in several ways.

Androecial characters, often utilized in older treatments [e.g. Meisner (1842), Clarke (1881), Haaskarl (1870)] are homoplasious (Evans et al. 2000b). Such characters are intricately related to pollination syndromes thought to be largely based upon pollinator deception, that is, the tendency of flowers to appear to have more pollen

reward than they actually yield (Faden 1992, 2000b; Evans et al. 2000b). Members of *Callisia* s.l. are no exception regarding such characters as an expanded, showy anther connective (Fig. 24). Here the character appears in nearly all taxa (including *Gibasis*) except in species of *Callisia* section *Leptocallisia*, in species of *Tripogandra*, and in the outgroup taxa (*Commelina* and *Tinantia*). In *Tripogandra*, the character is variable and is absent in the two members of the genus included in this study, *T. diuretica* and *T. serrulata* (Handlos 1975). Similar random associations are seen in filament bearding (Fig. 25), floral scent (Fig. 26), reduction of the perianth (Fig. 27), inflorescence structure (Fig. 28), presence of above-ground stolons (Fig. 29), vegetative reproduction from the inflorescence rachis (Fig. 30), and antesepalous stamen fertility (Fig. 31).

Within the subtribe, the reduction of inflorescence bracts as seen in *Callisia*, *Tripogandra*, and *Gibasis* but not in *Tradescantia* (Hunt 1986a, Faden 1998) constitutes a sympleisiomorphy at least insofar as with the molecular hypothesis of relationships utilized here. This sympleisiomorphy is in contrast to the paired-cyme unit of the inflorescence found in *Callisia*, *Tripogandra*, and *Tradescantia* but not in *Gibasis* (Faden 1998). However, only one species of *Gibasis* was available for this study and its placement as sister to *Callisia* s.l. may or may not be supported by additional sampling.

Autapomorphies and Synapomorphies

Individual species of *Callisia* exhibit autapomorphies, useful for individual taxon identification but not useful for the establishment of monophyly of a group. Some autapomorphies include the subulate leaf of *C. navicularis* (section *Brachyphylla*); pistillate flowers of *C. repens* (section *Callisia*) which are rare in the family, the fleshy sepals of *C. warszewicziana* (section *Hadrodemas*), the bilocular ovary of *C. repens*

(section *Callisia*) and the bi- or trilocular ovary of *C. monandra* (section *Leptocallisia*). No morphological synapomorphies are known for *Callisia* s.l.

A cladistic analysis of morphological characters states compiled from the literature and from personal observation for those members of the subtribe included in these molecular analyses, with the addition of another species of *Gibasis*, was undertaken. Results of this work clearly exemplify the difficulty in detecting phylogenetically useful morphological characters. Figure 32 shows one randomly chosen tree of a heuristic parsimony analysis of 27 morphological characters commonly used in *Callisia.* Note that one clade is composed of some members of sections *Callisia* and *Leptocallisia* while other clades contain a scattering of *Callisia* species *sensu* Hunt (1986b) among other genera. Submission of these data to bootstrap analysis results in a mass of unresolved polytomies and conglomerate taxa (Fig. 33).

Chromosomal Perspective

Chromosome numbers have been variously reported in the subtribe (Fig. 34) and have been addressed in Chapter 2. As noted, the tendency towards reduction in chromosome number is the likely trend in the family, and such a trend may well be exemplified by the basal position of *C. warszewicziana* to the members of Hunt's (1986b) section *Callisia*. However, a comprehensive body of chromosome data remains incomplete. For some taxa (e.g. *C. repens*, *C. multiflora*, and *C. warszewicziana*), historical counts are ambiguous. Hunt (1986b) proposed a base number of x = six, seven, and eight for the sections *Callisia*, *Leptocallisia*, and *Hadrodemas* respectively.

Moore (1961) designated n = seven as the haploid number for the genus *Aploleia* and its only two members, currently under section *Leptocallisia* (*C. monandra* and *C.*

multiflora). Reported chromosome counts of Guervin et al. (1975), Le Coq and Guervin (1975), and Le Coq et al. (1975) dispute H. Moore's (1961) purported haploid number, unless chromosomal changes in addition to polyploidy have occurred or the species sampled as *C. multiflora* was misidentified.

As previously mentioned, *C. gracilis* remains an anomaly both in the results of the molecular analysis here, and in its reported chromosome count. Jones and Jopling (1972) discounted the possibility of a base of x = eight based on meiotic pairing, yet here, *C. gracilis* joins the *Tripogandra* clade, that genus with a suggested base of x = eight or 13 (Handlos 1975). The pollen grains of *C. gracilis* need to be tested with lactophenol blue to determine if pollen viability is low and thus suggestive of an allopolyploid hybridization resulting in a sterile or semi-sterile species. This possibility is intimated by its molecular position with *Tripogandra*. Indeed, a 4*n* x 6*n* cross would yield a 5*n* allopolyploid which, depending upon the mode of chromosome set segregation, might occasionally produce viable pollen/ovule recombinations.

Alternatively, *C. gracilis* could be the result of a cross between *6n* and *8n* parents. Such a cross would yield a potentially sterile amphidiploid with $2n = 14$. A doubling of chromosomes occurring twice would produce a fertile plant with $2n = 56$. Such phenomena have been documented to have occurred in wild populations of *Arabidopsis* and have been further substantiated by experimental crosses (Nasrallah et al. 2000). The experimental *Arabidopsis* interspecific hybrids were morphologically intermediate between the parents in some character states (e.g. petal size) but also exhibited growth characteristics more similar to one parent than to the other (e.g. plant stature and vernalization) (Nasrallah et al. 2000). For other traits, the hybrid phenotype exceeded

that of both parents (e.g. stigma size) and hybrid vigor was apparent (Nasrallah et al. 2000). During their study of the experimental hybrids, Nasrallah et al. (2000) discovered the spontaneous generation of viable amphidiploids.

In studies of the ancestry of the Hawaiian silversword alliance, artificial hybrids have been produced from parental species of two different genera, one with *2n* = six and one with *2n* = eight. The F1 generations commonly underwent mitosis when meiosis was expected, yielding viable allotetraploids (Carr and Kyhos 1981, 1986; Carr et al. 1996).

If *C. gracilis* is an allotetraploid from an amphidiploid hybridization, its affinity to *Tripogandra* in these molecular analyses, and its morphological similarity to *Callisia* s.l. (i.e. to *C. cordifolia*) are potentially explained. Further investigation of this hypothesis is warranted.

Jones and Jopling (1975) found that species of *Callisia* s.l. then treated under *Tradescantia* (e.g. *C. micrantha* and *C. cordifolia)* as well as species under the segregate genera *Phyodina* and *Cuthbertia* lacked the characteristic chromosome symmetry for typical *Tradescantia*. The chromosome symmetry and a base of *x* = six, that base being suggested as that of the family, constituted a complex of species for *Tradescantia* in contrast to the asymmetry of chromosomes for some species then under *Tradescantia* or segregate genera.

Generally, all species of *Callisia* sensu Hunt (1986b) have asymmetrical chromosomes. Jones and Jopling (1975) determined that the five species they studied then under *Callisia,* [including five of the ten taxa of section *Callisia* sensu Hunt (1986b) plus seven unidentified *Callisia* species], have a recognizably distinct karyotype morphology and a single base of *x* = six, with diploidy common and also tetraploidy and

hexaploidy. Other members of *Callisia* sensu Hunt (1986b) with karyotype asymmetry and a base of x = six or otherwise were not placed within the *Callisia* alliance *sensu* Jones and Jopling (1975). Again, the molecular analyses here support the distinction between members of section *Callisia* and other members *sensu* Hunt (1986b), despite anomalous counts that need further scrutiny.

One exception to the above is the conclusion of Jones and Jopling (1975) that *C. warszewicziana* karyotype and base number were not similar to other *Callisia*. The molecular data here and Tomlinson's (1966, 1969) anatomical study, however, support *C. warszewicziana* as within the section *Callisia* alliance. Both *C. fragrans* and *C. warszewicziana* are heteromorphic for a tandem satellite (Jones and Jopling 1975).

Biogeographical Perspective

An Old World origin for the 14 genera of the tribe Commelineae has been suggested, with distributions of six genera in both the Old World and the New World and with eight genera found exclusively in Asia, Africa, and Australia (Martínez and Swain 1985, Evans et al. 2003). Tribe Tradescantieae contains four paleotropical subtribes and three subtribes distributed only in the Americas, including Tradescantiinae (Evans et al. 2003). Phylogenetic analyses and biogeographic patterns of the family and other commelinoid monocots suggest an eastern Gondwanaland origin for Commelinaceae and closely related families (Evans et al. 2003). Families closely related to Commelinaceae: Mayacaceae Kunth, Zyridaceae C. Agardh, Rapataceae Dumort., and sometimes Eriocaulaceae Desv., are based on non-molecular data; families closely related to Commelinaceae: Pontederiaceae Kunth, Philydraceae Link, Haemodoraceae R. Br., and Hanguanaceae Airy Shaw, are based on molecular data (Faden 1998, APG II 2003).

Combined analyses of non-molecular and molecular data sets are the same or similar to analyses with molecular data (Faden 1998). Within the Commelinaceae, the current disjunct distribution of tribe Commelineae may be either the result of one ancient vicariance event or the result of multiple causes (Evans et al. 2003). Molecular analyses in which members of two strictly Old World subtribes of the tribe Tradescantieae nested within New World members of the tribe suggest inconclusive and juxtaposed hypotheses of origin: one long-distance dispersal event to the Old World from the New World versus more than one separate introduction to the New World from the Old World (Evans et al. 2003).

The possibility that the Boreotropical Flora hypothesis (representing relict distributions of a once widespread north temperate range) could explain extant distributions of the Tradescantieae tribe was discounted in part because of the relatively derived placement of the Tradescantiinae subtribe (Evans et al. 2003). Additionally, the ease with which Faden and Hunt (1991) were able to define the subtribes of Tradescantieae was thought in part to stem from the relatively old age of most genera, but subtribe Tradescantiinae is thought to have fairly recent origins (Faden and Hunt 1991, Faden 1998, Evans et al. 2003). If that is the case, then the accumulation of synapomorphies that would define monophyletic genera in the subtribe, particularly those of troublesome systematics such as *Callisia* sensu Hunt (1986b), may well not have had sufficient time to evolve as suggested by the distribution of morphological character states overlain on the molecular phylogeny of the combined *ndhF* and *trnL-F* data sets (Figs. 20 to 31).

For subtribe Tradescantiinae and other members of the family, major centers of diversity include northern Central America and Mexico (particularly Chiapas and Oaxaca) (Faden 1998). The approximately 22 species of *Tripogandra* are distributed in tropical America with 13 species in Mexico (Hunt 1993, 1994). The approximately 11 species of *Gibasis* are mostly endemic to Mexico with some exceptions, e.g. one species [*G. geniculata* (Jacq.) Rohw.] is found throughout tropical America; one species [*G. pellucida* (Martens and Galeotti) D. R. Hunt] extends to Guatemala (Hunt 1994). Hunt (1986a) recognized that tuberous *Gibasis* were largely confined to Mexican uplands, while non-tuberous species were generally found at lower Mexican elevations and typically on the Atlantic side (Hunt 1986a). The approximately 70 species of *Tradescantia* are found in American tropics, subtropics, temperate forests, and grasslands (Hunt 1993). For *Callisia* sensu Hunt (1986b), the distribution ranges from South America to the southeastern United States, with a center of diversity in Mexico (Hunt 1993, Faden 2000). Geographic distributions of taxa included in this study are designated on the combined *ndhF* and *trnL-F* molecular phylogeny (Fig. 35).

Faden (1988) suggested that suites of attributes could be characteristic for forest and non-forest species of African Commelinaceae and proposed that these suites evolved along lineages in response to ecology. Forest species have adapted to low-light conditions and tend to have white flowers for greater visibility, an axillary inflorescence, and spirally arranged leaves (Faden 1988, Evans et al. 2003). Non-forest species, on the other hand, have adapted to low water availability during dry seasons with accompanying succulence (Faden 1988, Evans et al. 2003). While *Callisia*, *Tradescantia, Tripogandra*, and *Gibasis* have been considered to be non-forest species (Evans et al. 2003), within

Callisia, at least, the suite of characteristics outlined by Faden (1988) for forest and non-forest species have applicability to the molecular phylogenetic hypothesis.

Members of sections *Leptocallisia* and *Callisia* sensu Hunt (1986b), such as *C. cordifolia*, *C. gracilis*, *C. monandra*, *C. repens*, and other species not represented in this study, tend to inhabit moist thickets and woodlands, shady banks, and damp shade (Watson 1882; Hunt 1993, 1994). These species usually have white flowers or much reduced, inconspicuous petals; in two species (*C. monandra*, *C. repens*), the gynoecium and androecium are reduced. *Callisia warszewicziana* and *C. fragrans*, the two species with pronounced spirally arranged leaves, favor shade, but withstand desiccation (Hunt 1994). The above taxa are predominately either endemic to Mexico or range in Central America, the West Indies, and parts of South America. The hypothesis of relationships proposed here mirrors both habitat and distribution. Morphologically, the most derived [reduced (Hunt 1986b)] floral characteristics of *Callisia* s.l. are found in some of these species, and the molecular analyses suggest that they are of more recent descent than are other members.

The three members of section *Cuthbertia* and the two members of section *Brachyphylla* sensu Hunt (1986b) not only are found in xeric habitats, but also exhibit non-forest characteristics *sensu* Faden (1988). These species have pigmented showy flowers (as opposed to white), an inflorescence that is umbellate and all terminal or axillary and terminal, very succulent leaves and/or rhizomes or other geophytic structures (Lakela 1972, Hunt 1986b, Tucker 1989). From the molecular data, these species may have evolved from a purported most recent common ancestor (MRCA) of *Callisia* sensu

Hunt (1986b) along a northern trajectory accompanied by morphological adaptations to brighter, drier habitats.

A number of species of *Callisia*, of *Tradescantia* and *Tripogandra* included in this study, and some *Gibasis* range in the West Indies (e.g. *C. cordifolia*, *C. monandra*, *C. repens*, *Tradescantia spathacea*, *Tripogandra serrulata*). One suggestion for the presence of *C. cordifolia* on the archipelago is hurricane dispersal (Faden, pers. comm.). A recent symposium focused on the investigation of the biogeography of the Caribbean flora (Fritsch and McDowell 2003). Debates have ensued about the origin of three tectonic plates that border the Caribbean plate and the pre-historic position of the islands; each proposed model shapes the understanding of contemporary fauna and flora (Graham 2003). Nevertheless, a consensus has been reached regarding the origin of the Greater Antilles near the contemporary Isthmian area. This land mass connected North and South America until the early Tertiary when it migrated easterly and then fragmented in the Paleogene into land masses that ultimately formed into the contemporary Greater Antilles. The emersion/submersion pattern of separate land masses is unknown and the clarification would be useful to biologists. At least by the early Pleistocene, upland habitats had developed (Graham 2003).

Caribbean flora with African affinities has been addressed. Hurricanes have been considered one avenue for dispersal based upon models of the paleoclimate and on the proximity at one time of South America/Caribbean land masses to Africa. (Graham 2003). This is not to suggest that taxa ancestral to *C. cordifolia* originated in Africa, but does pose the question as to whether or not hurricane activity would disperse ancient *Callisia* from a Mexican center of diversity to the Caribbean. The sprinkling of *Callisia*

species documented in the West Indies could represent dispersal/vicariance or introductions.

Tripogandra has been thought to have originated from *Callisia* based on *rbcL* (Evans et al. 2003). Hunt (1986b) and Faden (pers. comm.) have viewed members of *Callisia* as having derived (reduced) reproductive characters. Radford et al. (1974) adapt and illustrate Leppik's (1957) "classification based on evolutionary flower-pollinator relationships" (Radford et al. 1974, p. 102), which suggests that pleomorphic flowers arose during the Cretaceous to Tertiary (Fig. 36). Such flowers are defined as actinomorphic with a reduced number of parts, and a *Tripogandra* flower was used as one of the reference examples illustrated. Such a proposition, coupled with molecular data here suggests that *Callisia* evolved from *Tripogandra.* Both occur in Mexico and South America, with *Tripogandra* and *C. gracilis* more prevalent in South America (Faden, pers. comm.). Ancestors of *Callisia* and related taxa might have evolved from *Tripogandra* amidst the drier habitats of Mexico. Mexico is thought to be the center of distribution of *Callisia* (Faden 2000). From an extinct MRCA *Callisia* may have further diverged into the extant eastern Mexican and southern North American relatives, i.e. the members of sections *Cuthbertia* and *Brachyphylla* of Hunt (1986b), whose inflorescence and floral morphology retain a *Tripogandra*-like characteristic. Molecular data here suggest that the species of these two sections *sensu* Hunt (1986b) may well have evolved into their current morphological characteristics and geographical distributions prior to the reduction of floral parts and the development of a paniculate or spiciform inflorescence found within section *Callisia* and some members of section *Leptocallisia*.

Taxonomic Considerations

Despite the paucity of unifying (synapomorphic) characters for *Callisia* s.l., the results of molecular analyses here, coupled with evidence from traditional data, provide a compelling argument for reinterpretation of the genus. Criteria for this reinterpretation and for recognition of taxa include the strength of the evidence supporting the monophyly of a group, e.g. bootstrap support for a clade; a set of characters by which a group is distinguishable from other groups; and the presence of obvious morphological characters (Judd et al. 1999). Morphological synapomorphies as evidence of monophyly remain problematical. Since a character state which at one point in time is synapomorphic will later become ancestral, the synapomorphy(s) of a rapidly evolving or recently evolved group of taxa may quickly recede, may be retained in portions of clades and not in others, and/or may become transitional and thus not recognizable in the extant group (Judd et al. 1999). Such may be the case for the taxa of *Callisia* sensu Hunt (1986b) and Tucker (1989).

Molecular data here support the monophyly of section *Cuthbertia* and warrant the elevation of the section to that of genus *sensu* Lakela (1972) and Small (1933) (Table 9). The three members of the section constitute a robust clade with high bootstrap support (97 to 100%) in all analyses, and in the *trnL-F* data set they share a six-base synapomorphic insertion (Table 5). Morphologically, the three members are readily distinguishable from all other members of the subtribe including those of *Callisia*, although not one character, but a suite of distinctive characters in concert, must be considered (Table 10). Linear leaves with a grass-like, caespitose habit distinguish the three from all other members of *Callisia*, *Tripogandra*, and *Gibasis* and from some

members of *Tradescantia*. Reduced inflorescence bracts distinguish the three *Cuthbertia* taxa from all members of *Tradescantia*. Petals are pink to rose (with one report of a white form), stamens are bearded, and the ovary is glabrous (Lakela 1972). As previously mentioned (see Chapter 2), anatomical characters also set these three taxa apart from *Callisia* (Tomlinson 1966, 1969). The three species are endemic to the southeastern United States where they are either allopatric to remaining members of *Callisia*, naturalized introductions notwithstanding, or have been shown neither readily nor successfully to hybridize with *Tradescantia* (Anderson and Woodson 1935, Anderson and Sax 1936). These three segregates likely represent an evolutionary product of migration from ancestral taxa of Mexico often considered the center of distribution of *Callisia* (Faden 2000a).

The only two members of section *Brachyphylla* (*C. navicularis* and *C. micrantha*) similarly constitute a highly supported clade [bootstrap 96 to 100% in all but two (Figs. 11, 13) analyses]. In the *trnL-F* data set, they have a four-base synapomorphic deletion, a ten-base synapomorphic tandem repeat, and lack a three-base insertion found in all other taxa sampled (Tables 5, 6). They are native to southeast Texas and eastern Mexico and may be the intermediate end product of segregates between Mexico and the southeastern United States, a phenomenon found in other recent genera (Kim et al. 1999; Giannasi, pers. comm.). These two species are morphologically distinct from *Cuthbertia*, from remaining members of *Callisia*, and from all other members of the subtribe. Distinctive characters (Table 10) include a procumbent or decumbent (Tucker 1989, Hunt 1994) habit; small (to 3.5 cm [Hunt 1994, Faden 2000]), succulent, lanceolate or subulate leaves; the inflorescence is terminal (Hunt 1986b), and flowers have a well-developed

perianth (in contrast to sections *Leptocallisia* and *Callisia*; see below) (Hunt 1986b). The proposal here is to treat these two members under a segregate genus. The provisional new genus, *Brachyphylla*, in keeping with Hunt's (1986b) *Callisia* section *Brachyphylla*, is proposed under which to treat these two species (Tables 9, 10).

An insufficient sampling for section *Leptocallisia* interferes with more decisive treatment. The relationship between *C. cordifolia* and the two species that Moore (1961) treated under *Aploleia* (*C. monandra* and *C. multiflora*) has been ambiguous, and the molecular data do not resolve the relationship. However, bootstrap support values are high (98 to 100%) for the clade containing *C. multiflora* and *C. monandra* for all analyses. *Callisia multiflora* has three stamens, *C. monandra* has one to three. These are the only two species of *Callisia* s.l. in this study in which the fertile stamens are antesepalous (H. Moore 1961). Antesepalous fertile stamens occur in one other taxon of the section, *C. ciliata* (Hunt 1994), and in those members of *Tripogandra* that have three fertile stamens and three staminodes, e.g. *T. diuretica* (Handlos 1975) (Fig. 31).

Callisia cordifolia, *C. monandra*, and *C. multiflora* constitute a clade not including *C. gracilis*. Within this clade, *C. cordifolia* has an umbellate inflorescence and six fertile stamens, a condition also found in *C. gracilis*.

Callisia gracilis groups with *Tripogandra* with high bootstrap support (96 to 99%) in all *ndhF* analyses and in two of the three combined analyses; shares with *T. serrulata* a 78-base insertion and a nine-base deletion; and lacks the five-base deletion found in all other members of *Callisia* sensu Hunt (1986b) (Table 5). This taxon, as evidenced by molecular data here, is not a *Callisia*, despite its gross morphological

similarity to *C. cordifolia* (H. Moore 1961). A transfer to *Phyodina* is proposed (Tables 9, 10), the genus under which it was designated the type by Rafinesque (1836).

Among the sampled species of the section *Leptocallisia*, bearded stamens are present or absent in *C. multiflora* (Hunt 1994), and absent in *C. cordifolia* and *C. monandra*. This gradual reduction in bearding and in frequency of bearding extends to the terminal-most clade of this analysis, to which the *Leptocallisia* clade is sister. The terminal-most clade includes section *Hadrodemas* plus section *Callisia* (Fig. 19).

In this terminal clade, bearded stamens are only found occasionally in the one taxon of section *Hadrodemas*, *C. warszewicziana*, and then the bearding is exceedingly sparse (H. Moore 1962). This species and one member of section *Callisia*, *C. fragrans*, are the only two taxa of *Callisia* s.l. with a bromeliiform habit (Hunt 1994). *Callisia warszewicziana* is in a position basal to the section *Callisia* clade. The cyme pairs in this clade, in *C. warszewicziana*, plus in *C. monandra* and *C. multiflora* of the *Leptocallisia* clade, are contained in a compound spiciform or paniculate inflorescence in contrast to the umbellate cyme-pairs of all other sampled taxa of Hunt's (1986b) *Callisia* (Fig. 28).

The section *Callisia* clade is morphologically united by characters defined by Moore (1962): an inflorescence with sessile cyme-pairs sometimes accompanied by a pedunculate cyme-pair in the same axil; largely sessile flowers; three to six glabrous stamens with the longest ones or the only ones developed antepetalous. A pilose ovary apex is evident in all members with the possible exception of *C. tehuantepecana* (H. Moore 1958). Moore (1958) nevertheless included this character as defining *Callisia*. Until further sampling and analyses are undertaken, the section *Callisia* plus *Hadrodemas* clade, as evidenced here, likely constitutes a monophyletic group (Fig. 19) despite the

fact that *C. warszewicziana,* from a gross morphology perspective, lies at one extreme with its relatively large pink corolla and its thick robust strapping leaves, while the type species for the genus, *C. repens*, lies at the other extreme with a much-reduced perianth and gynoecium and smaller ovate leaves. Bootstrap values supporting this clade are high (94 to 100%) for all *ndhF* analyses and for two of the three combined analyses. This clade should be treated as *Callisia* s.s. (Tables 9, 10).

Table 9. Proposed generic and sub-generic treatment of *Callisia* based on molecular and non-molecular data (compare to Table 2). Taxa in bold were sequenced for this analysis. [a] indicates members of *Callisia* s.s.

Genus	**Section**	**"Group"**	**Species**
Brachyphylla			***Brachyphylla navicularis*** (Ortgies) D. R. Hunt
			B. micrantha (Torr.) D. R. Hunt
Cuthbertia			***Cuthbertia graminea*** (Small) G. Tucker
			C. ornata (Small) G. Tucker
			C. rosea (Vent.) D. R. Hunt
Callisia	*Callisia*[a]	I. "Gentlei"	***Callisia elegans*** Alexander ex H. E. Moore
			C. gentlei Matuda
			C. macdougallii Miranda
			C. nizandensis Matuda
			C. tehuantepecana Matuda
		II. "Fragrans"	***C. fragrans*** (Lindl.) Woodson
			C. guerrerensis Matuda
			C. soconuscensis Matuda
		III. "Repens"	***C. repens*** (Jacq.) L.
			C. insignis C. B. Clarke
	Hadrodemas[a]		***C. warszewicziana*** (Kunth & Bouché) D. R. Hunt
	Leptocallisia		*C. ciliata* Kunth
			C. cordifolia (Sw.) Anderson & Woodson
			C. filiformis (Martens & Galeotti) D.R. Hunt
			C. monandra (Sw.) Schultes f.
			C. multiflora (Martens & Galeotti) Standl.
	Lauia		*C. laui* (D. R. Hunt) D. R. Hunt

Phyodina			***P. gracilis*** (Kunth) D. R. Hunt

Table 10. Taxonomic characters delimiting *Brachyphylla*, *Callisia* sensu stricto/*Callisia* section *Leptocallisia*, *Cuthbertia*, and *Phyodina*.

	Brachyphylla	***Callisia* s.s and section *Leptocallisia***	***Cuthbertia***	***Phyodina***
Habit	Procumbent or decumbent	Bromeliiform or decumbent to erect	Caespitose	Decumbent
Leaves	Lanceolate or subulate	Lanceolate or varying ovate to elliptic to lanceolate	Linear	Ovate
Inflorescence	Umbellate	Paniculate or umbellate	Umbellate	Umbellate
Flowers	Pedicellate to nearly sessile	Sessile to nearly sessile to pedicellate	Pedicellate	Pedicellate
Corolla	Well-developed, showy (petals to 8 mm long)	Well-developed (petals to 9.5 mm) to inconspicuous (scarcely exceeding sepals)	Well-developed, showy (petals to 15 mm)	Conspicuous (petals to 4 mm long)
Stamen #	Six	One to three; three or six; six	Six	Six
Filaments	Bearded	Glabrous	Bearded	Bearded
Ovary apex	Glabrous	Pilose, pubescent, glabrous	Glabrous	Glabrous

Figure 20. Evolution of stamen di- and polymorphism plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from Handlos (1975), Hunt (1994), Faden (1998, 2000).

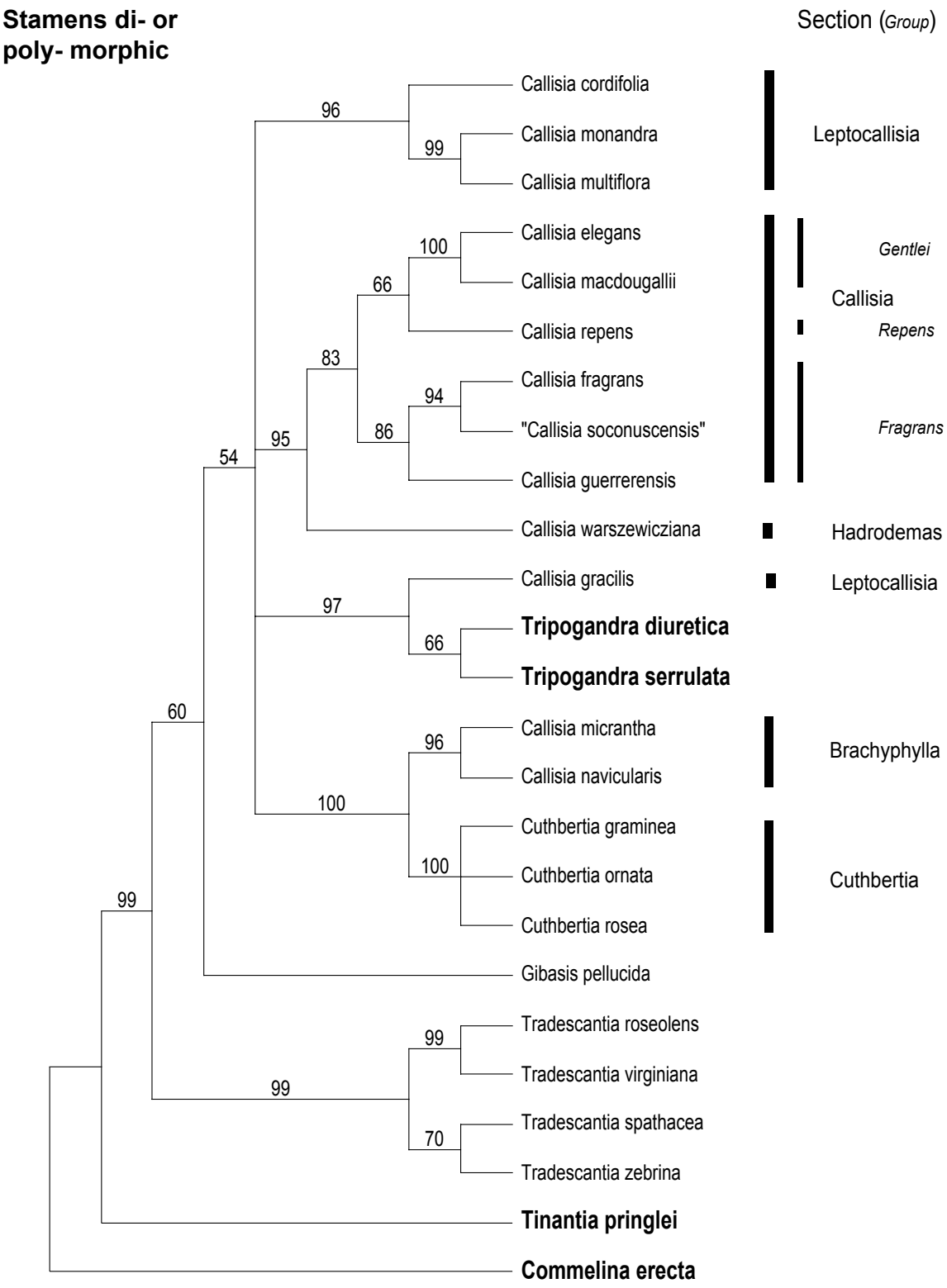
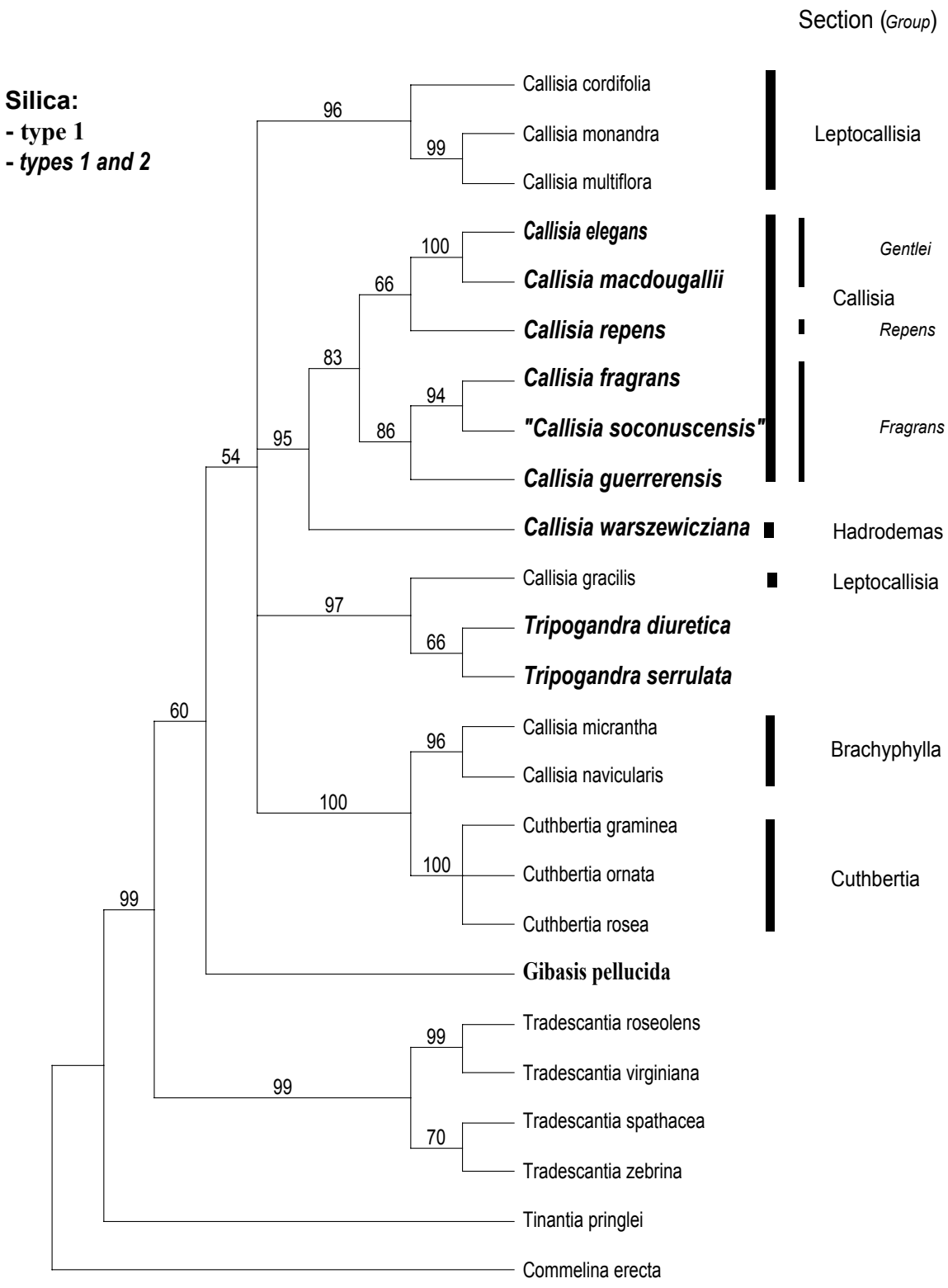

Stamens di- or poly- morphic
Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Leptocallisia
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
"Callisia soconuscensis"
Callisia guerrerensis
Gentlei
Callisia
Repens
Fragrans
Callisia warszewicziana
Hadrodemas
Callisia gracilis
Leptocallisia
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Brachyphylla
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Cuthbertia
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
96
99
100
66
83
94
86
95
54
97
66
60
96
100
100
99
99
99
70

Figure 21. Evolution of silica, type 1 and types 1 and 2, plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from Tomlinson (1969, 1966), personal observation.


Section (Group)
Silica:
- type 1
- types 1 and 2
Callisia cordifolia
Callisia monandra
Callisia multiflora
Leptocallisia
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
"Callisia soconuscensis"
Callisia guerrerensis
Gentlei
Callisia
Repens
Fragrans
Callisia warszewicziana
Hadrodemas
Callisia gracilis
Leptocallisia
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Brachyphylla
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Cuthbertia
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
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86
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97
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96
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100
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99
99
70

Figure 22. Evolution of dorsally (back-to-back) fused cymes with complete or partial fusion, plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from Faden (1998, 2000)

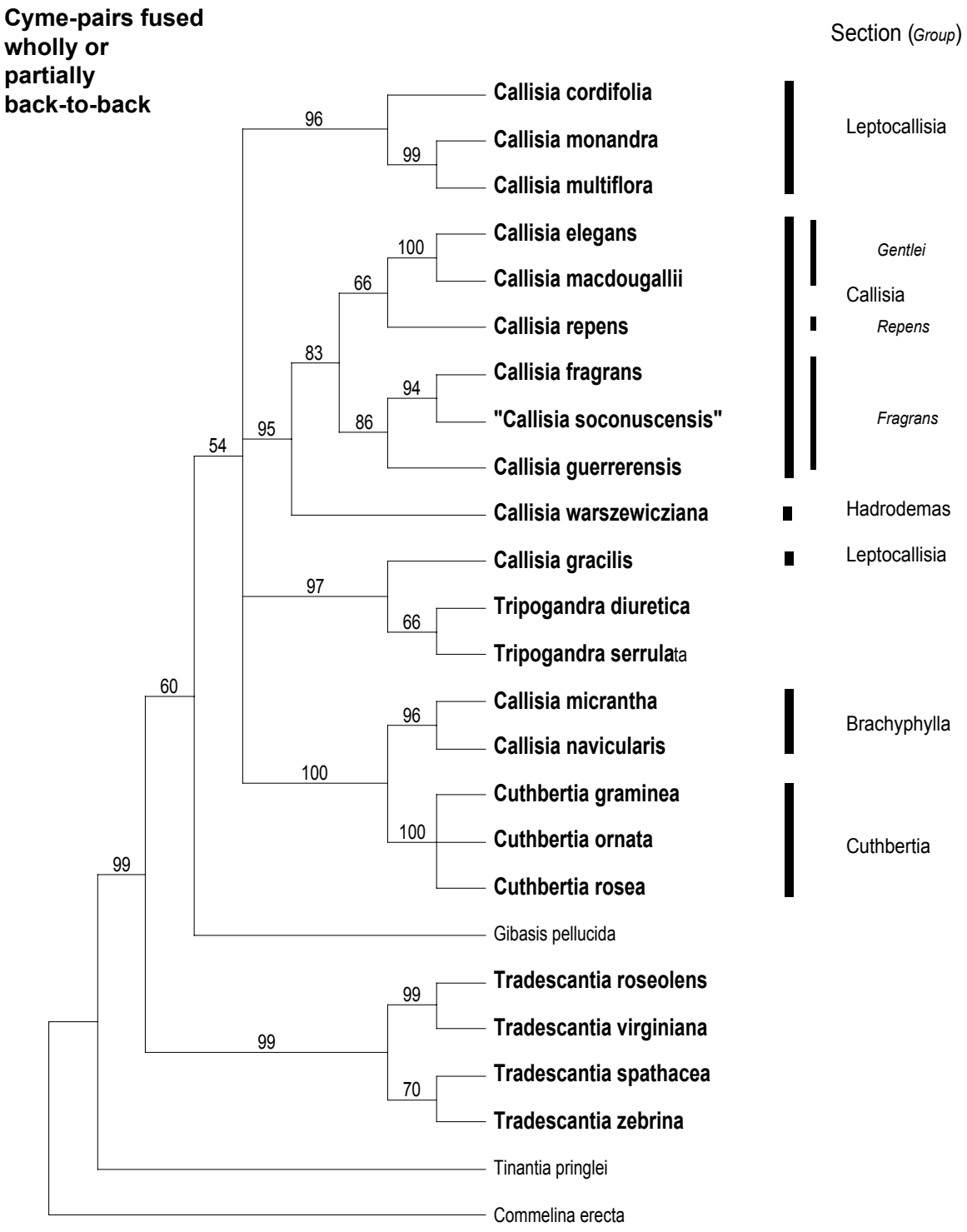
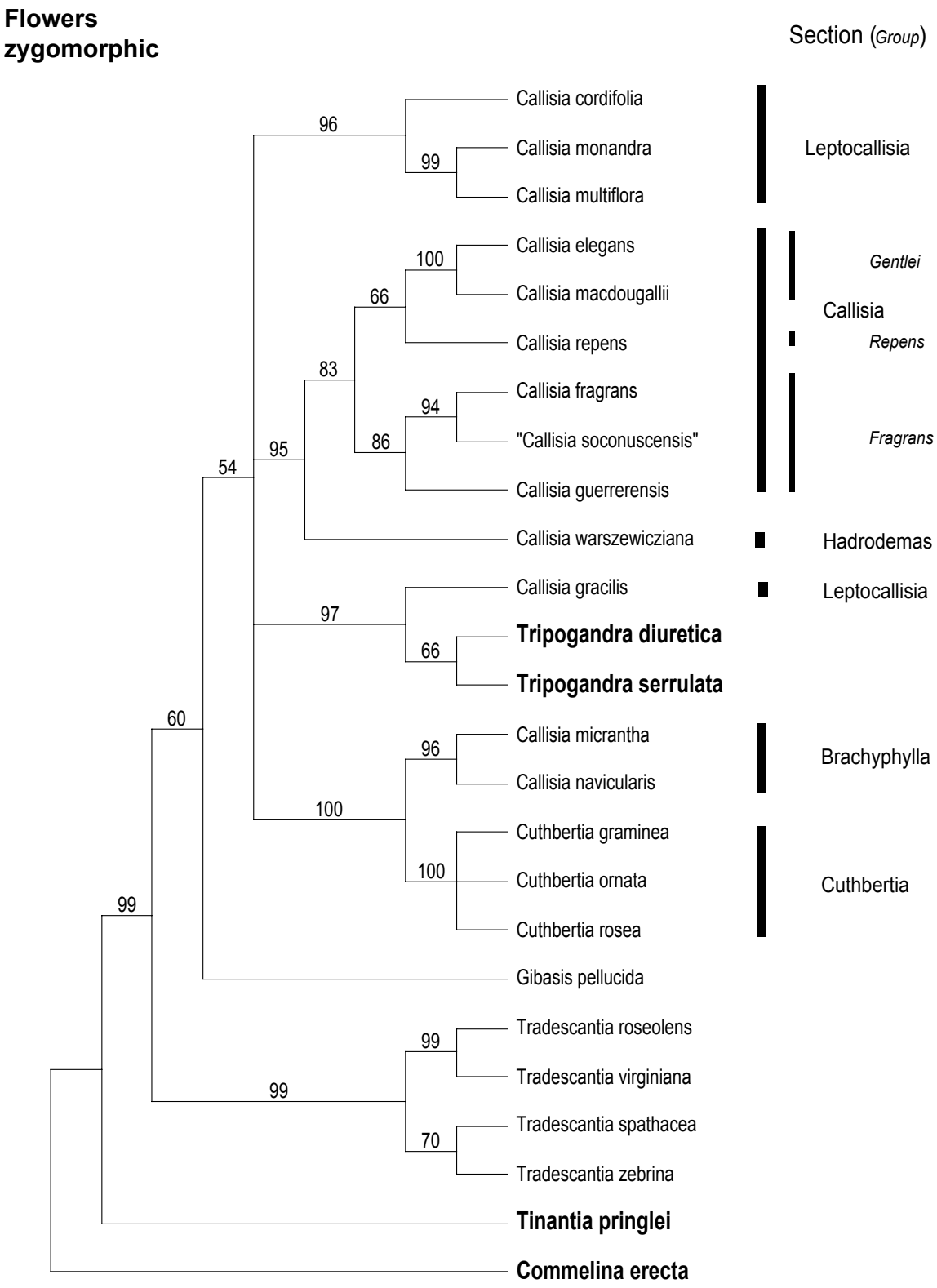

Cyme-pairs fused wholly or partially back-to-back
Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
"Callisia soconuscensis"
Callisia guerrerensis
Callisia warszewicziana
Callisia gracilis
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
96
99
100
66
83
94
86
95
54
97
66
60
96
100
100
99
99
99
70
Leptocallisia
Gentlei
Callisia
Repens
Fragrans
Hadrodemas
Leptocallisia
Brachyphylla
Cuthbertia

Figure 23. Evolution of zygomorphic flowers plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from Handlos (1975), Hunt (1986b), Faden (2000, 1998).


Flowers zygomorphic
Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Leptocallisia
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
"Callisia soconuscensis"
Callisia guerrerensis
Gentlei
Callisia
Repens
Fragrans
Callisia warszewicziana
Hadrodemas
Callisia gracilis
Leptocallisia
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Brachyphylla
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Cuthbertia
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
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96
100
100
99
99
99
70

Figure 24. Evolution of expanded (showy) anther connective plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from Lakela (1972), H. Moore (1958, 1961, 1962), Handlos (1975), Hunt (1986a, 1986b, 1994) and personal observation. * Outer whorl narrow, inner whorl various (Hunt 1993); both whorls with narrow connectives for the *Tripogandra* species in these analyses.

Expanded anther connective

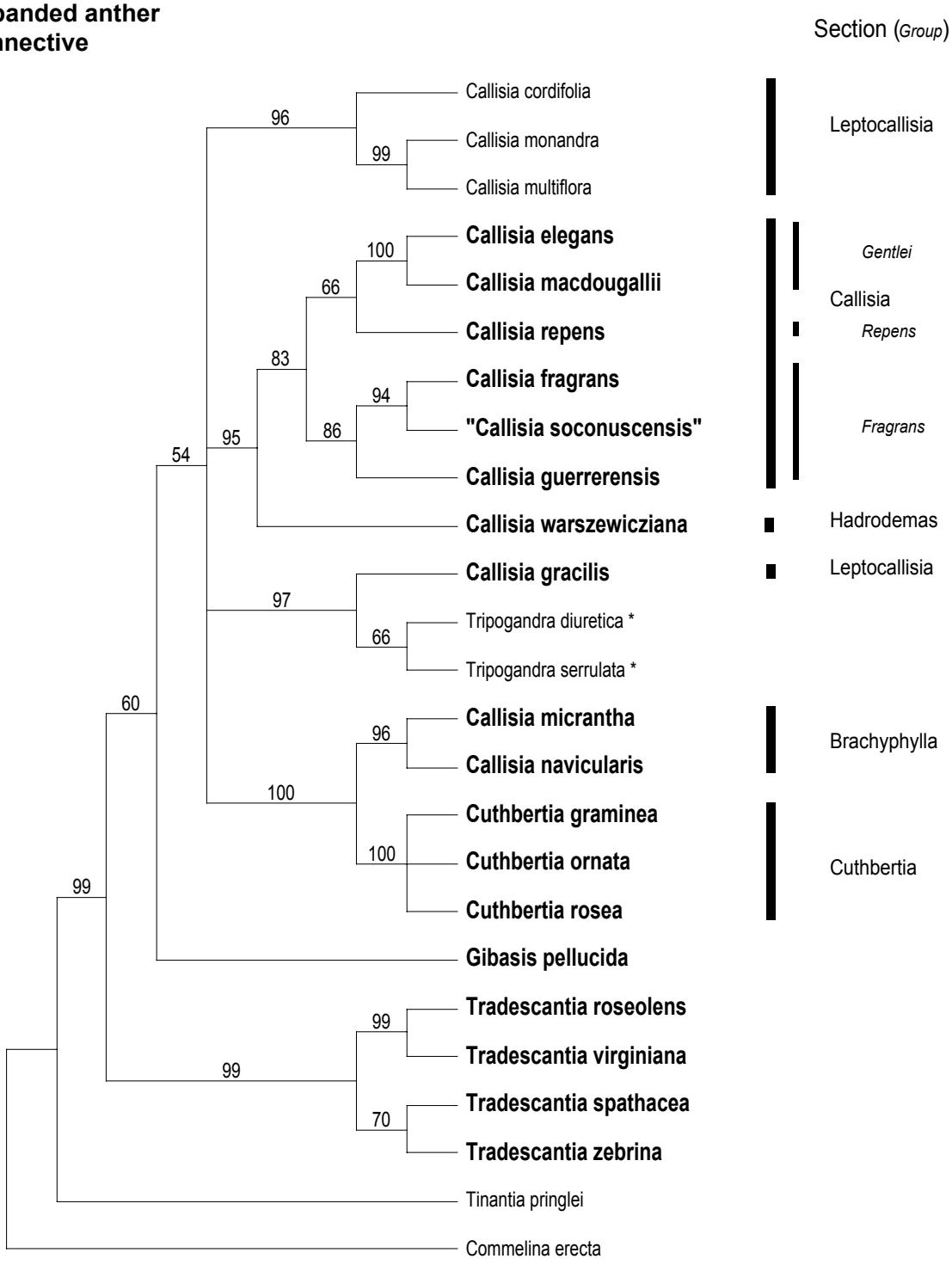


Figure 25. Evolution of filament bearding plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from H. Moore (1958, 1961, 1962), Lakela (1972), Handlos (1975), , Hunt (1986a, 1986b, 1994), and personal observation. * *Callisia multiflora*: Hunt (1994): glabrous or bearded; Moore (1958): glabrous. ** *Callisia warszewicziana*: Hunt 1994: usually glabrous; Moore (1962): glabrous or with a few moniliform hairs.

Filaments bearded

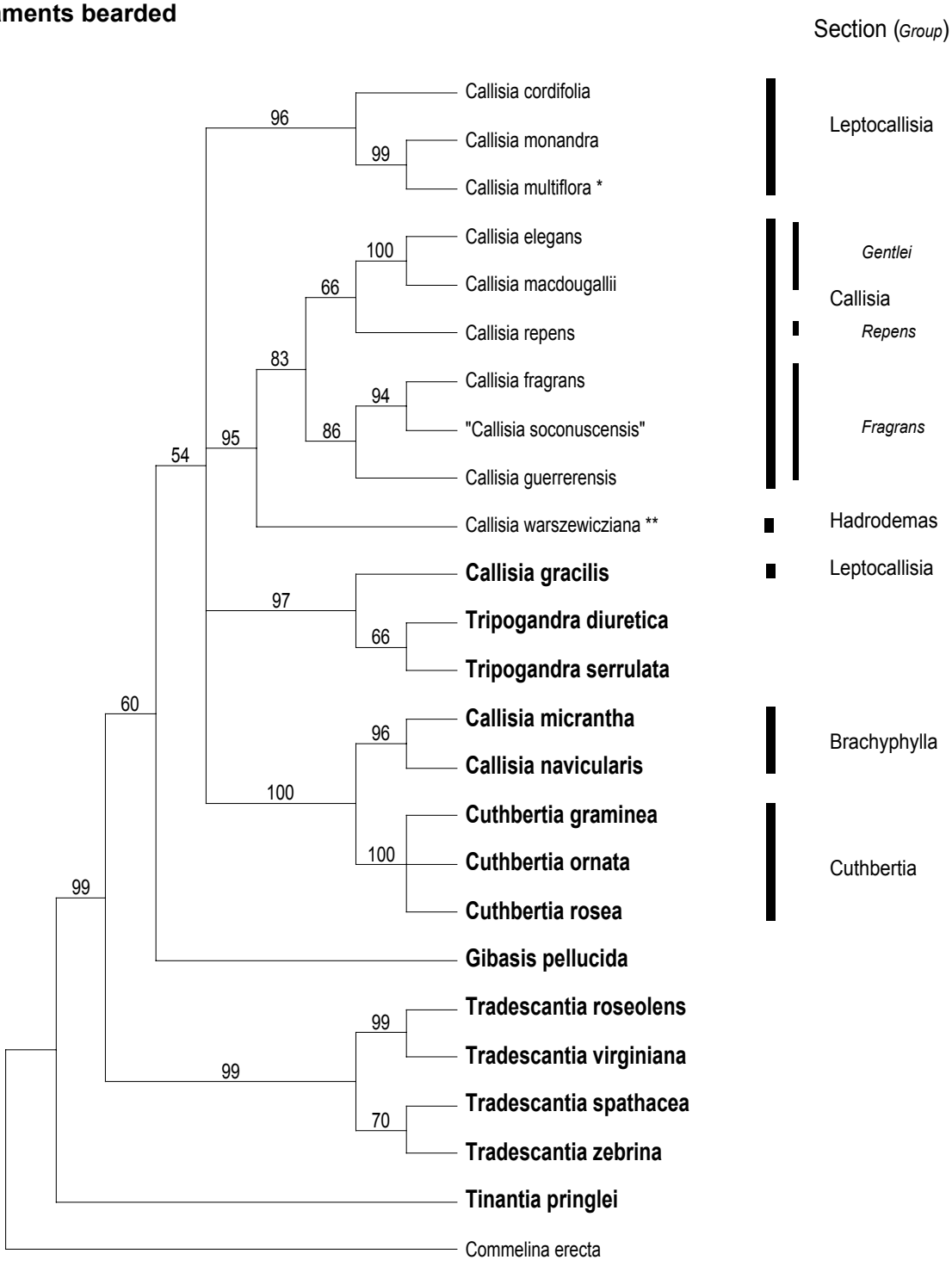
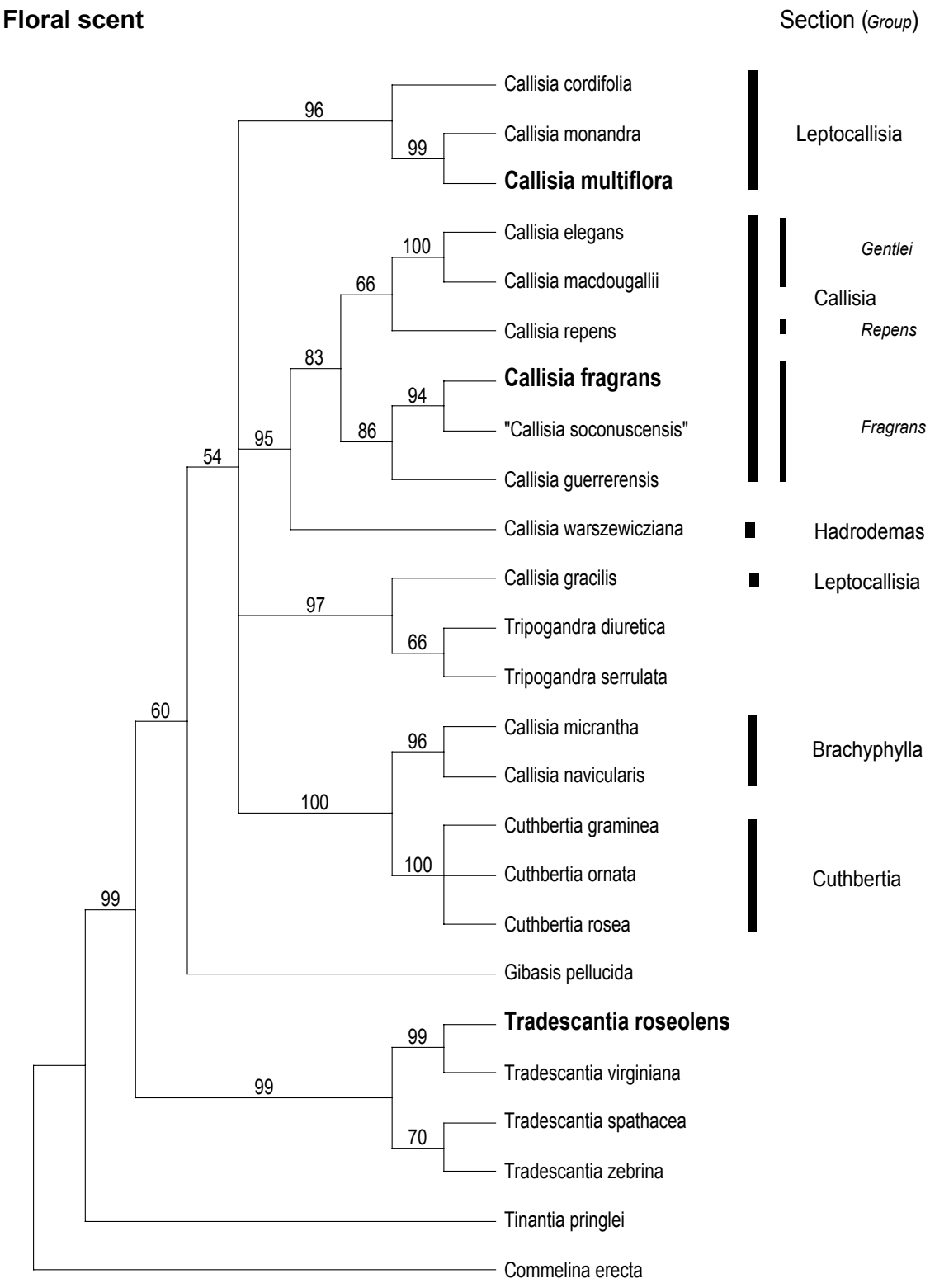


Figure 26. Evolution of floral scent plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines.


Floral scent
Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
"Callisia soconuscensis"
Callisia guerrerensis
Callisia warszewicziana
Callisia gracilis
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
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100
100
99
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99
70
Leptocallisia
Callisia
Gentlei
Repens
Fragrans
Hadrodemas
Leptocallisia
Brachyphylla
Cuthbertia

Figure 27. Evolution of a perianth reduction plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from H. Moore (1958, 1961), Hunt (1986b, 1994).

Perianth reduced

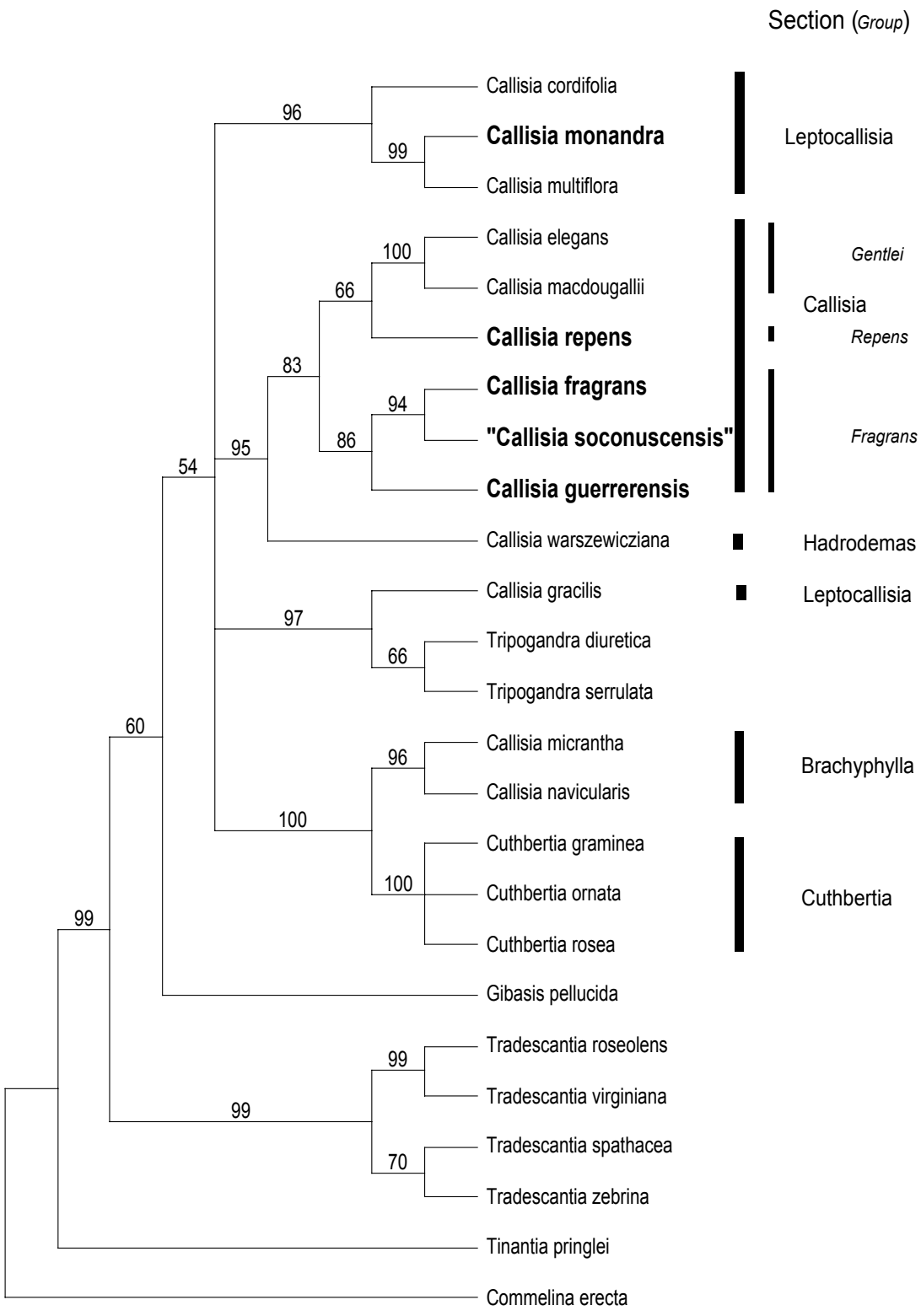
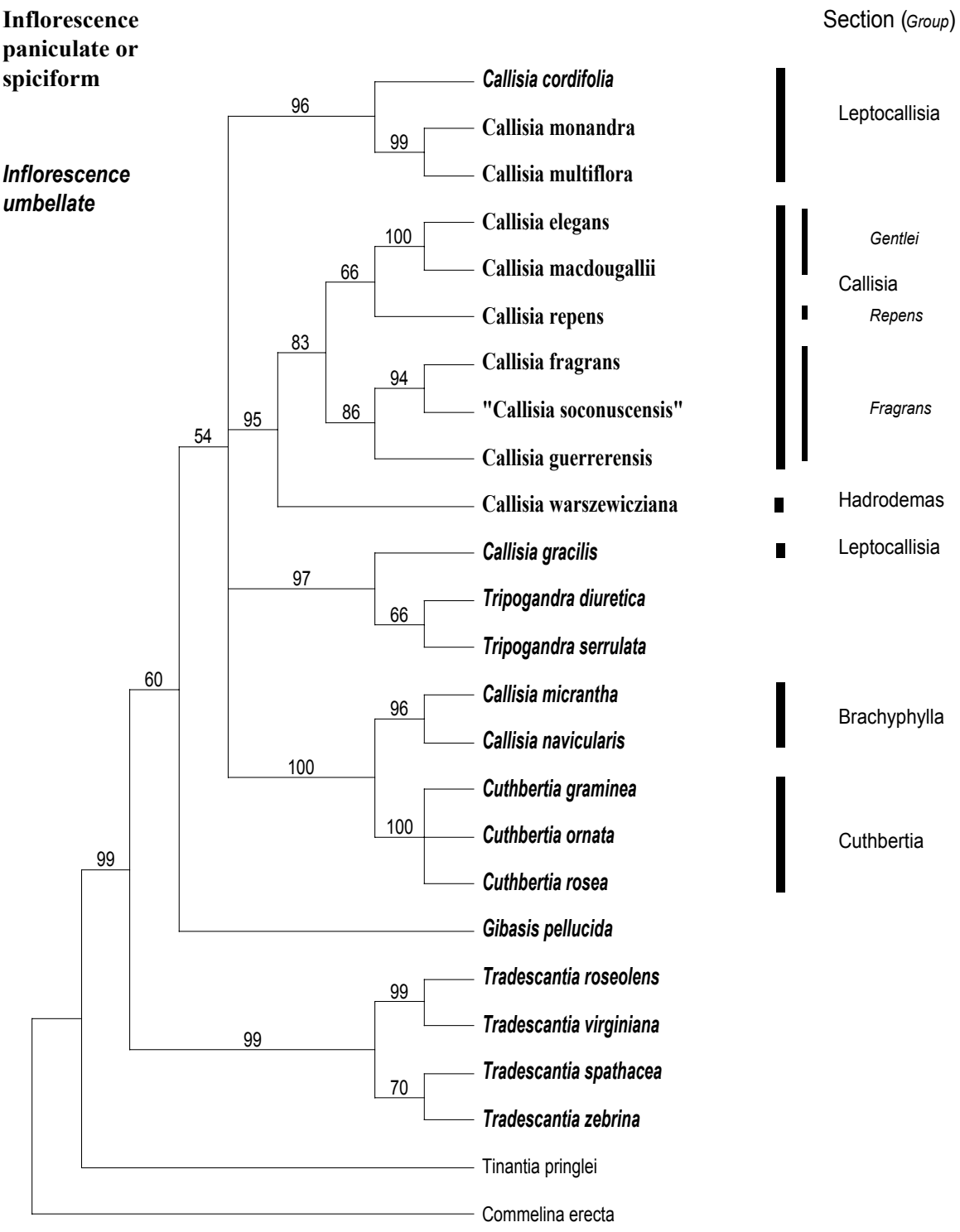


Figure 28. Evolution of inflorescence structure plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from H. Moore (1958, 1961, 1962), Lakela (1972), Handlos (1975), Hunt (1986a, 1986b, 1994), and personal observation.


Inflorescence paniculate or spiciform
Inflorescence umbellate
Section (Group)
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
"Callisia soconuscensis"
Callisia guerrerensis
Callisia warszewicziana
Callisia gracilis
Tripogandra diuretica
Tripogandra serrulata
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta
96
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54
97
66
60
96
100
100
99
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99
70
Leptocallisia
Gentlei
Callisia
Repens
Fragrans
Hadrodemas
Leptocallisia
Brachyphylla
Cuthbertia

Figure 29. Evolution of above-ground stolons plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from Barcellos de Souza (1986), Hunt (1984), and personal observation.

Above-ground stolons present

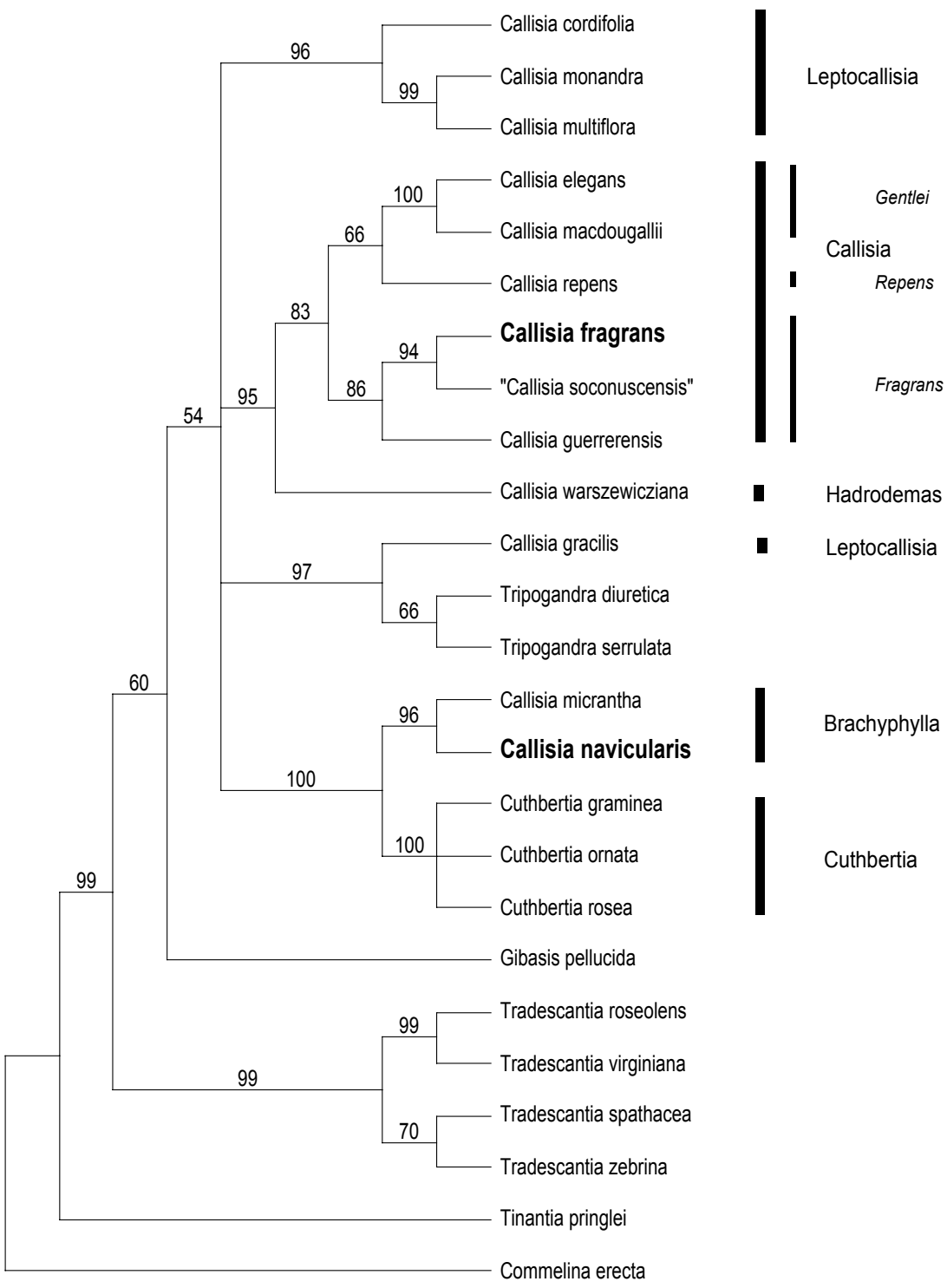


Figure 30. Evolution of vegetative reproduction from the inflorescence rachis plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from H. Moore (1962), Lakela (1972), and personal observation. * Reported by Lakela (1972) for *Cuthbertia graminea* forma *leucantha*.

Vegetative reproduction from inflorescence rachis

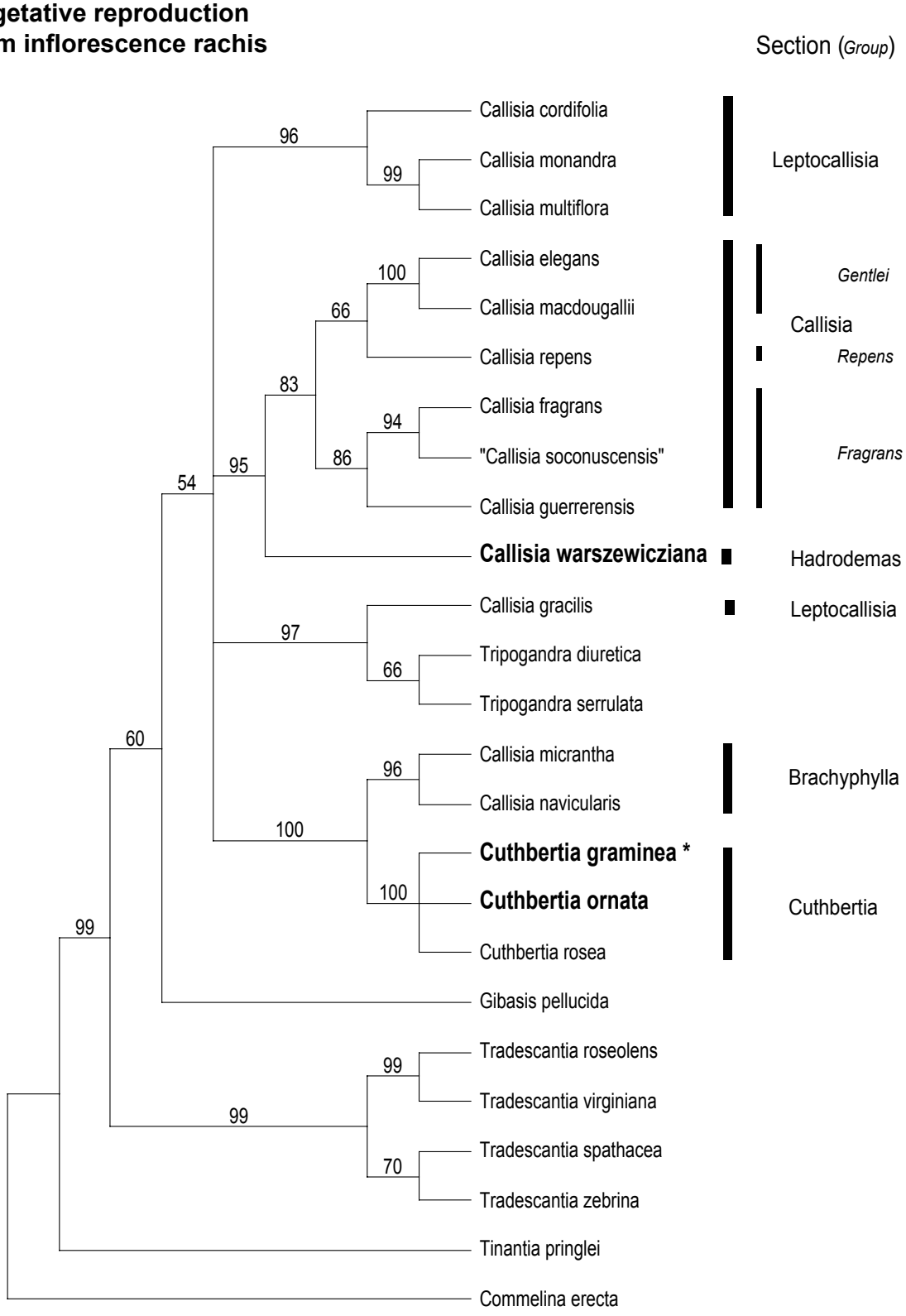


Figure 31. Evolution of antesepalous fertile stamens plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from H. Moore (1958, 1961), Handlos (1975), Hunt (1994), Faden (2000). * The outer and inner whorl of the dimorphic stamens of this species are both fertile. ** The position of the three sterile and three fertile stamens is posterior and anterior respectively, for this genus.

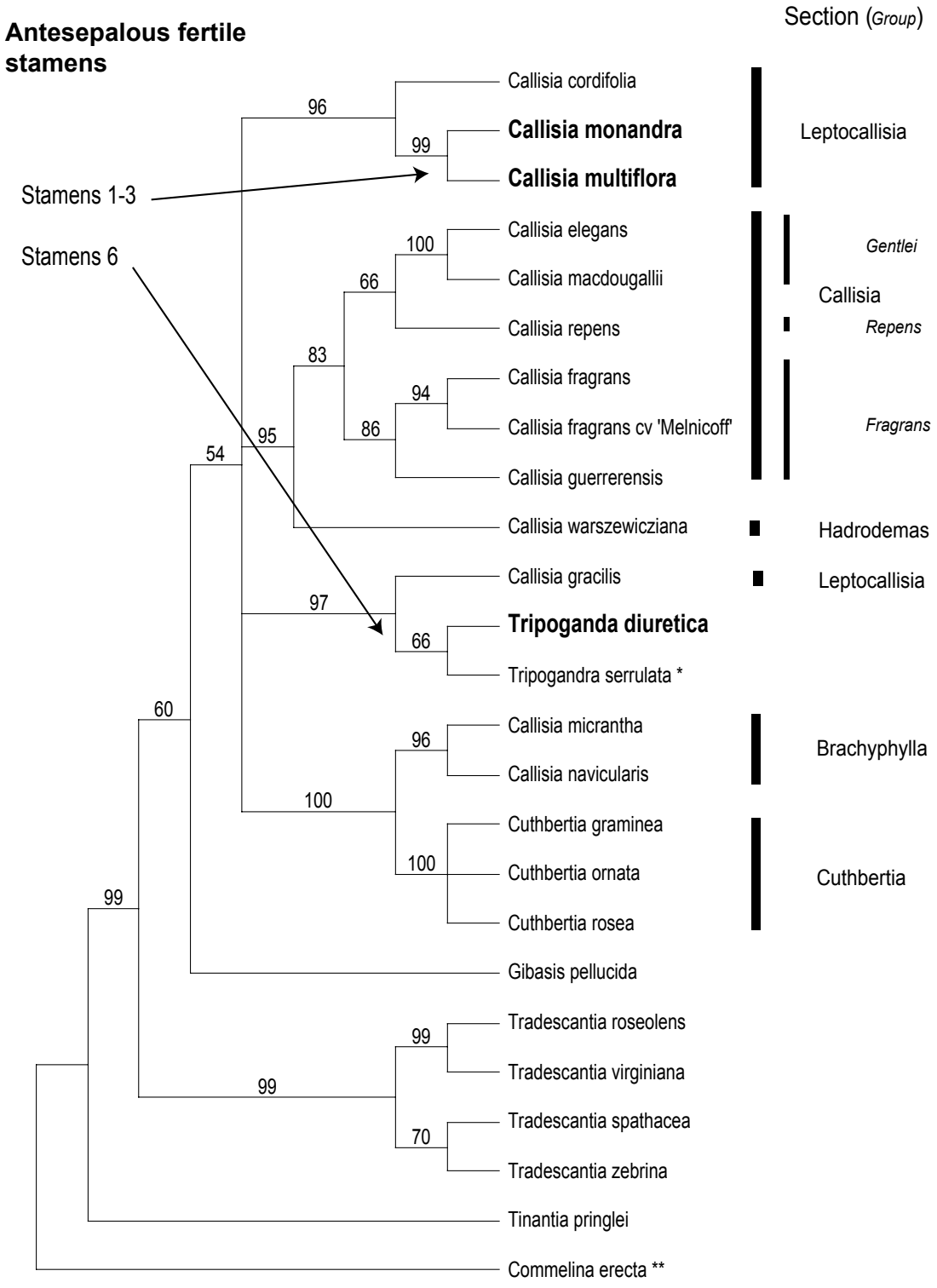

Antesepalous fertile stamens
Section (Group)
Stamens 1-3
Stamens 6
Callisia cordifolia
Callisia monandra
Callisia multiflora
Callisia elegans
Callisia macdougallii
Callisia repens
Callisia fragrans
Callisia fragrans cv 'Melnicoff'
Callisia guerrerensis
Callisia warszewicziana
Callisia gracilis
Tripoganda diuretica
Tripogandra serrulata *
Callisia micrantha
Callisia navicularis
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Gibasis pellucida
Tradescantia roseolens
Tradescantia virginiana
Tradescantia spathacea
Tradescantia zebrina
Tinantia pringlei
Commelina erecta **
96
99
100
66
83
94
86
95
54
97
66
60
96
100
100
99
99
99
70
Leptocallisia
Callisia
Gentlei
Repens
Fragrans
Hadrodemas
Leptocallisia
Brachyphylla
Cuthbertia

Figure 32. One randomly chosen variable length tree of 2175 most parsimonious trees from parsimony analysis of 27 morphological characters. Character state changes are indicated above branch lines. CI without uninformative characters = 0.52; RI = 0.66.

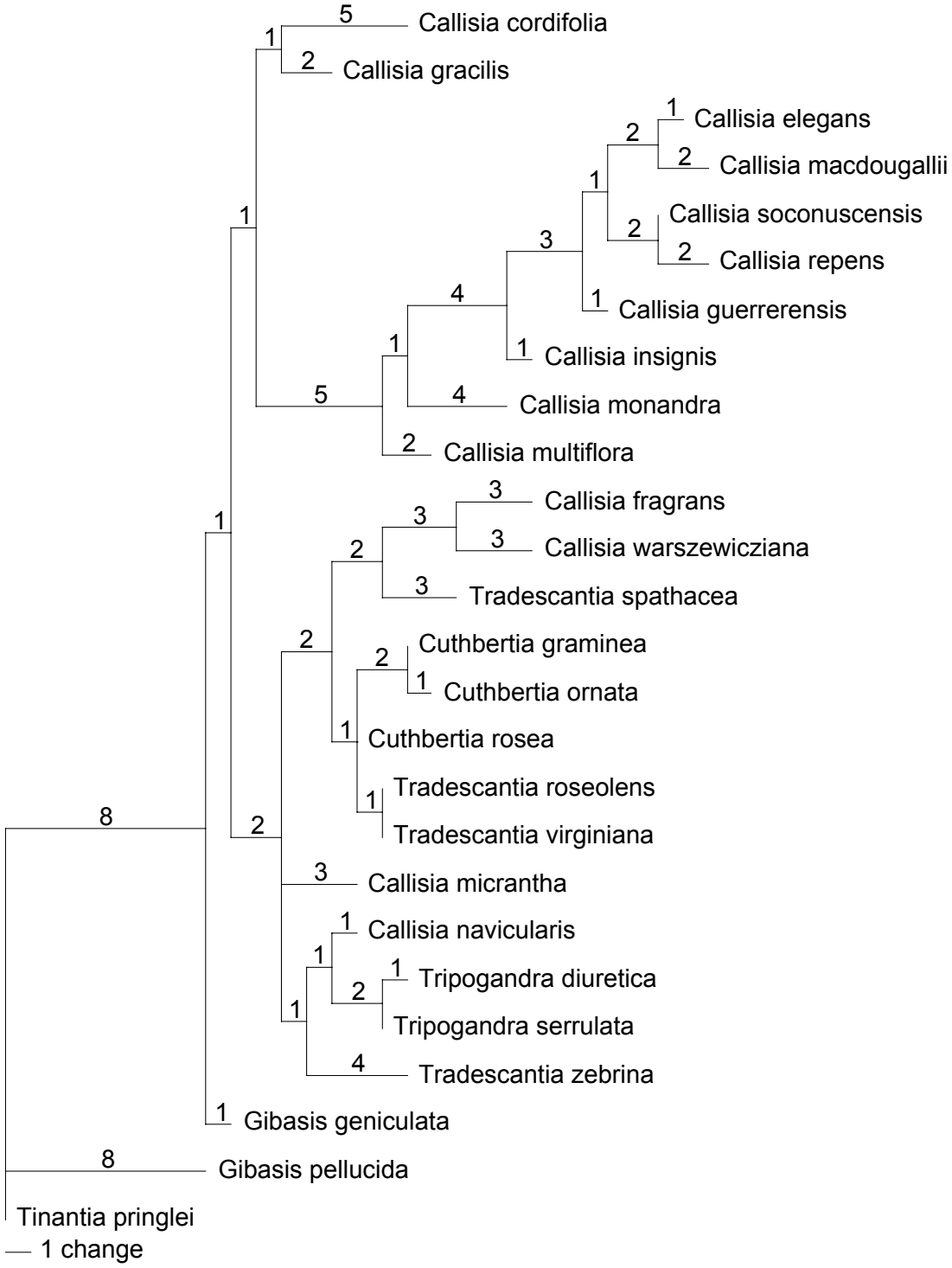

Callisia cordifolia
Callisia gracilis
Callisia elegans
Callisia macdougallii
Callisia soconuscensis
Callisia repens
Callisia guerrerensis
Callisia insignis
Callisia monandra
Callisia multiflora
Callisia fragrans
Callisia warszewicziana
Tradescantia spathacea
Cuthbertia graminea
Cuthbertia ornata
Cuthbertia rosea
Tradescantia roseolens
Tradescantia virginiana
Callisia micrantha
Callisia navicularis
Tripogandra diuretica
Tripogandra serrulata
Tradescantia zebrina
Gibasis geniculata
Gibasis pellucida
Tinantia pringlei
1 change

Figure 33. Bootstrap analysis of 27 morphological characters. Bootstrap percentages greater than 50% are indicated above branch lines.

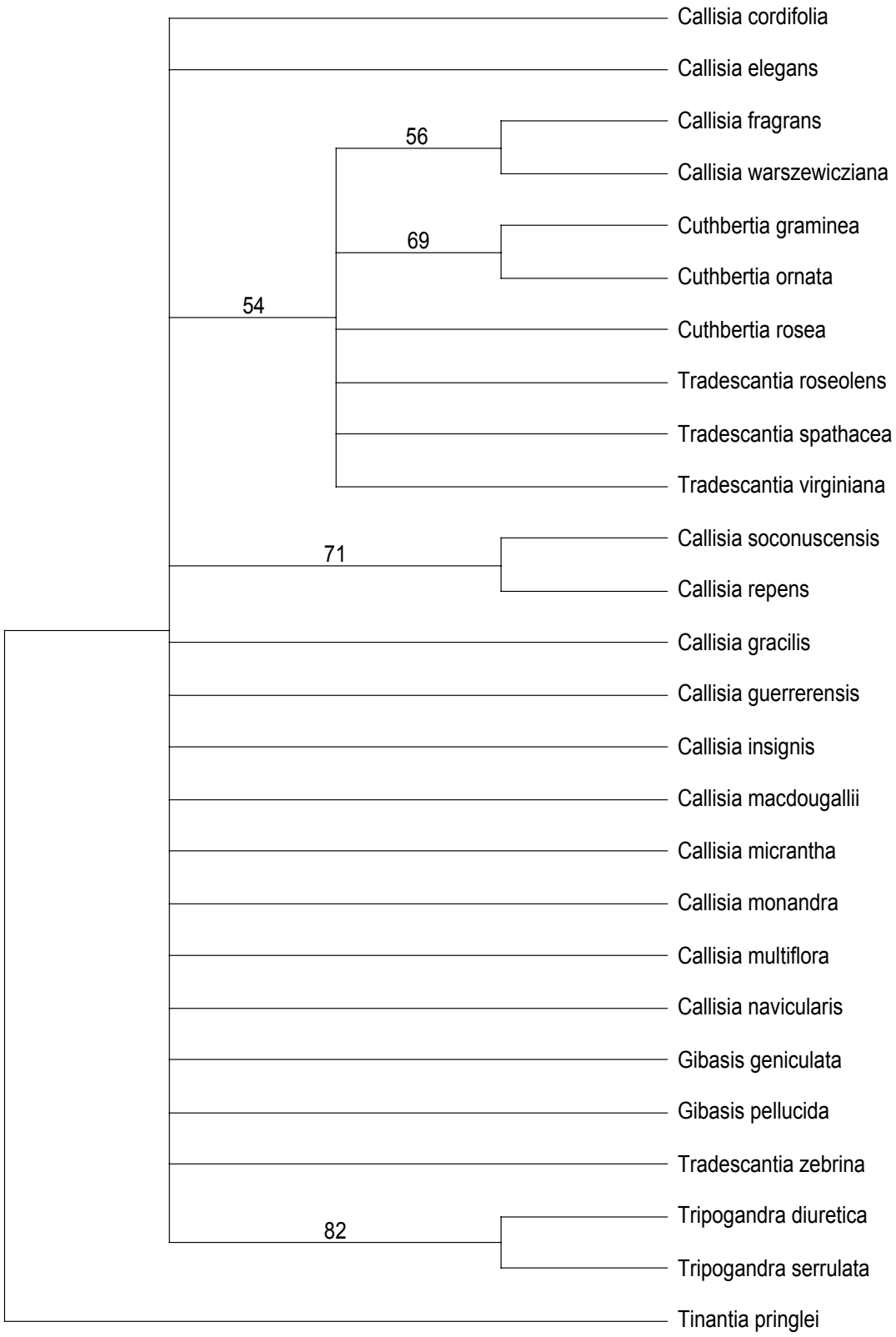

Callisia cordifolia
Callisia elegans
Callisia fragrans
56
Callisia warszewicziana
Cuthbertia graminea
69
Cuthbertia ornata
54
Cuthbertia rosea
Tradescantia roseolens
Tradescantia spathacea
Tradescantia virginiana
Callisia soconuscensis
71
Callisia repens
Callisia gracilis
Callisia guerrerensis
Callisia insignis
Callisia macdougallii
Callisia micrantha
Callisia monandra
Callisia multiflora
Callisia navicularis
Gibasis geniculata
Gibasis pellucida
Tradescantia zebrina
Tripogandra diuretica
82
Tripogandra serrulata
Tinantia pringlei

Figure 34. Chromosome numbers plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from Anderson and Sax (1936); Giles (1942); H. Moore (1961); Jones and Jopling (1972); Bailey and Luquire (1967) in R. Moore (1973); Lewis et al. (1967) in R. Moore (1973); Le Coq and Guervin (1968) in R. Moore (1973); Celarier (1955) in Federov (1974); Lewis et al. (1962) in Federov (1974); Morton (1965) in Federov (1974); Guervin et al. (1975); Le Coq and Guervin (1975); Le Coq et al. (1975); Handlos (1975); Bhattacharya (1975) in Goldbladt (1981); Lin & Paddock (1978) in Goldbladt (1984); Rao (1978) in Goldbladt (1984); Begum and Zamum (1980) in Goldbladt (1984) Uhrikova & Ferakova (1980) in Goldbladt (1984); Hunt (1986a, 1986b, 1994); Tucker (1989); Faden (1998, 2000).

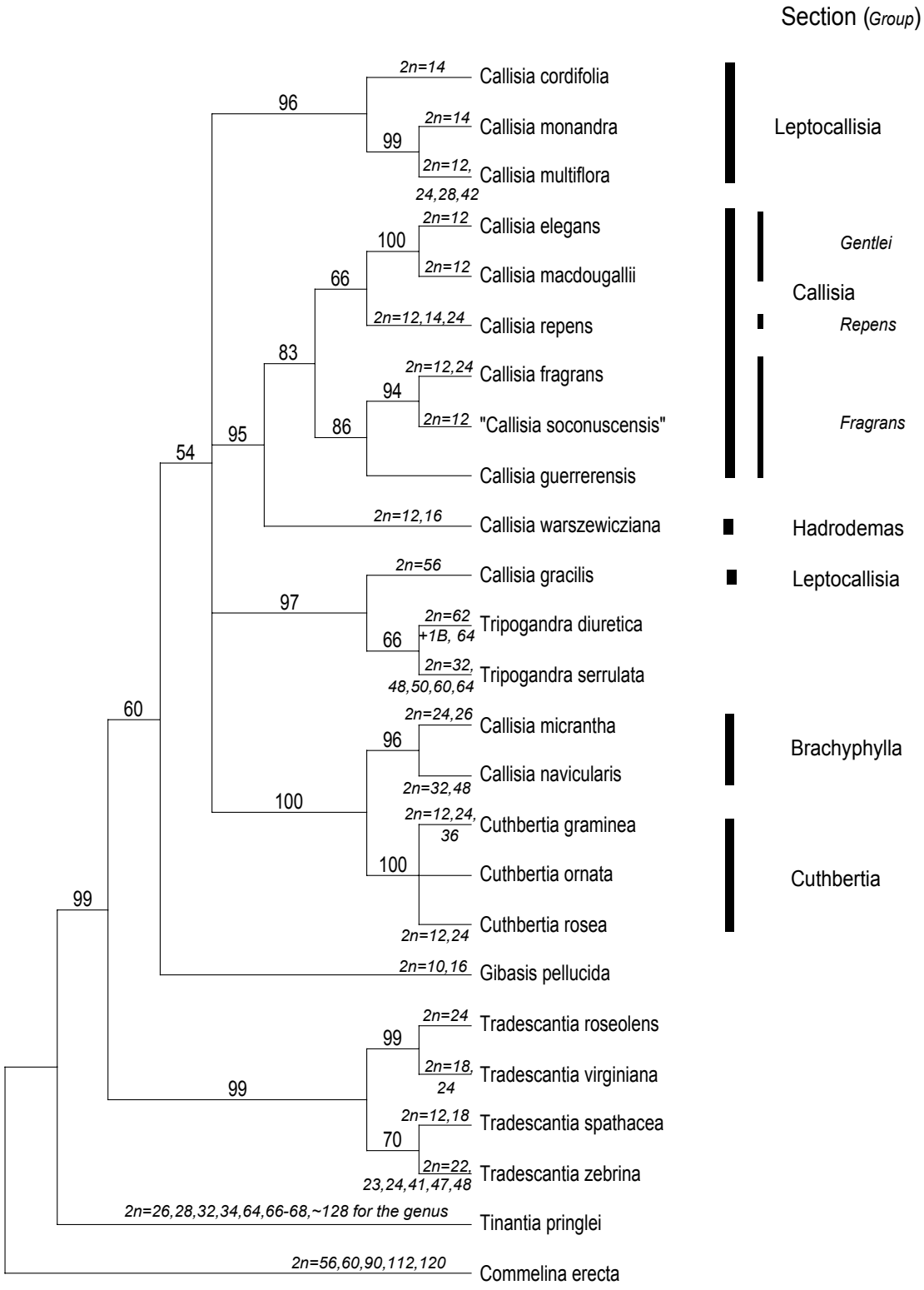

Section (Group)
2n=14
Callisia cordifolia
96
2n=14
Callisia monandra
99
2n=12, 24,28,42
Callisia multiflora
Leptocallisia
2n=12
Callisia elegans
100
2n=12
Callisia macdougallii
66
2n=12,14,24
Callisia repens
Gentlei
Callisia
Repens
83
2n=12,24
Callisia fragrans
94
2n=12
"Callisia soconuscensis"
86
95
54
Callisia guerrerensis
Fragrans
2n=12,16
Callisia warszewicziana
Hadrodemas
2n=56
Callisia gracilis
Leptocallisia
97
2n=62 +1B, 64
Tripogandra diuretica
66
2n=32, 48,50,60,64
Tripogandra serrulata
60
2n=24,26
Callisia micrantha
96
Callisia navicularis
2n=32,48
Brachyphylla
100
2n=12,24, 36
Cuthbertia graminea
100
Cuthbertia ornata
99
Cuthbertia rosea
2n=12,24
Cuthbertia
2n=10,16
Gibasis pellucida
2n=24
Tradescantia roseolens
99
2n=18, 24
Tradescantia virginiana
99
2n=12,18
Tradescantia spathacea
70
2n=22, 23,24,41,47,48
Tradescantia zebrina
2n=26,28,32,34,64,66-68,~128 for the genus
Tinantia pringlei
2n=56,60,90,112,120
Commelina erecta

Figure 35. Geographic distributions plotted on bootstrap consensus of the combined *ndhF* and *trnL-F* data set with indels removed. Bootstrap values greater than 50% are indicated above branch lines. Data compiled from Tharp (1927), Lakela (1972), Handlos (1975), Hunt (1986a, 1986b, 1994), Tucker (1989), Faden (2000).

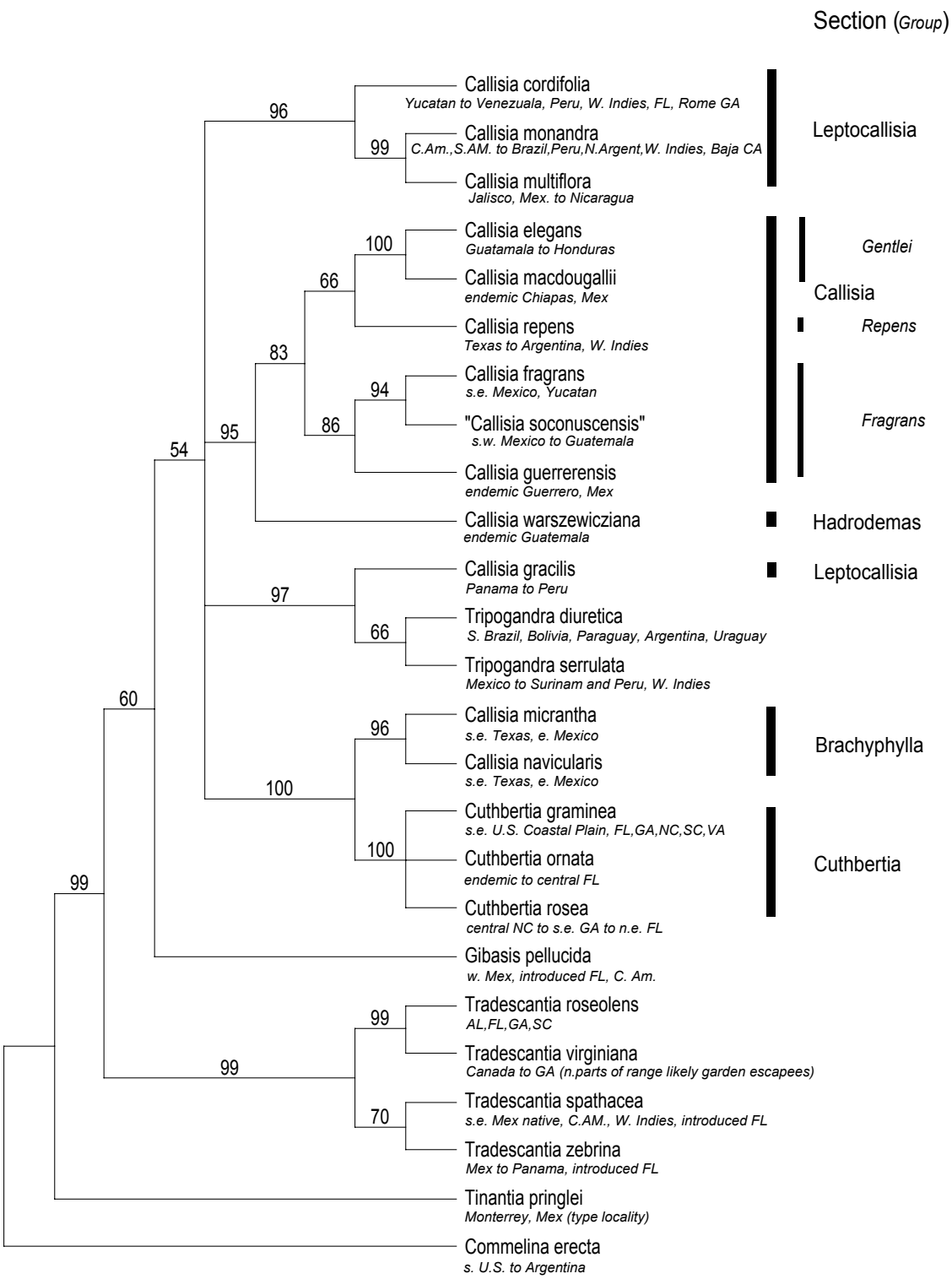
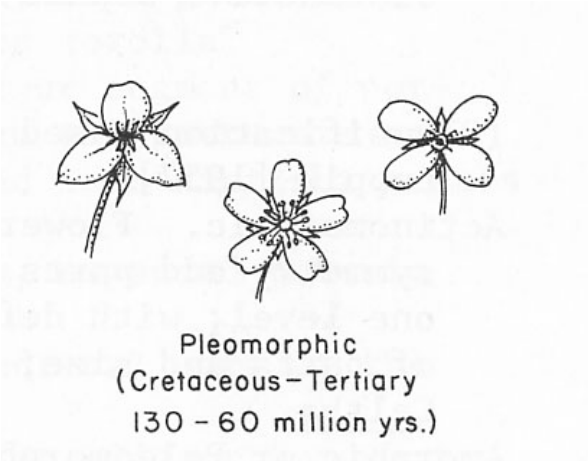

Section (Group)
Callisia cordifolia
Yucatan to Venezuala, Peru, W. Indies, FL, Rome GA
Callisia monandra
C.Am.,S.AM. to Brazil,Peru,N.Argent,W. Indies, Baja CA
Callisia multiflora
Jalisco, Mex. to Nicaragua
Leptocallisia
Callisia elegans
Guatamala to Honduras
Callisia macdougallii
endemic Chiapas, Mex
Callisia repens
Texas to Argentina, W. Indies
Gentlei
Callisia
Repens
Callisia fragrans
s.e. Mexico, Yucatan
"Callisia soconuscensis"
s.w. Mexico to Guatemala
Callisia guerrerensis
endemic Guerrero, Mex
Fragrans
Callisia warszewicziana
endemic Guatemala
Hadrodemas
Callisia gracilis
Panama to Peru
Leptocallisia
Tripogandra diuretica
S. Brazil, Bolivia, Paraguay, Argentina, Uraguay
Tripogandra serrulata
Mexico to Surinam and Peru, W. Indies
Callisia micrantha
s.e. Texas, e. Mexico
Callisia navicularis
s.e. Texas, e. Mexico
Brachyphylla
Cuthbertia graminea
s.e. U.S. Coastal Plain, FL,GA,NC,SC,VA
Cuthbertia ornata
endemic to central FL
Cuthbertia rosea
central NC to s.e. GA to n.e. FL
Cuthbertia
Gibasis pellucida
w. Mex, introduced FL, C. Am.
Tradescantia roseolens
AL,FL,GA,SC
Tradescantia virginiana
Canada to GA (n.parts of range likely garden escapees)
Tradescantia spathacea
s.e. Mex native, C.AM., W. Indies, introduced FL
Tradescantia zebrina
Mex to Panama, introduced FL
Tinantia pringlei
Monterrey, Mex (type locality)
Commelina erecta
s. U.S. to Argentina
96
99
100
66
83
94
86
95
54
97
66
60
96
100
100
99
99
99
70

Figure 36. Leppik's (1957) pleomorphic flowers (from Radford et al. 1974, p 102) suggesting that these floral types arose during the Cretaceous to Tertiary (130-60 million years ago). The exemplar flower in the upper left is from *Tripogandra*; such flowers are also typical of *Tradescantia*, *Callisia* sections *Cuthbertia* and *Brachyphylla*, and some flowers of sections *Callisia, Hadrodemas,* and *Leptocallisia* sensu Hunt (1986b).



Pleomorphic
(Cretaceous–Tertiary
130 – 60 million yrs.)

CHAPTER 4

SUMMARY and CONCLUSIONS

Historically, *Callisia* has confounded systematists. Treatments have spanned the extremes of "lumping" (e.g. Hunt 1986b) and "splitting" (e.g. H. Moore 1958, 1961, 1962). Molecular data has substantiated certain close relationships previously ascertained [e.g. members within section *Cuthbertia* and members within section *Brachyphylla* sensu Hunt (1986b)], but has not resolved other relationships (e.g. *C. cordifolia*; the relationships among the genera of the subtribe).

Taxa difficult to resolve with morphological data often tend to be difficult to resolve with molecular data. One hypothesis for this pattern proposes that such groups have speciated very rapidly and morphological and/or molecular changes have not had time to accumulate (Hillis and Wiens 2000). Subtribe Tradescantiinae presents a good example of such difficulties spanning different data sets.

Where morphological characters may be decoupled from the molecular and those same characters have been interpreted from a general perspective, incongruence between the molecular and the morphological is not a surprise (Giannasi, pers. comm..). Workers in the Commelinaceae have described the nature of zygomorphic flowers in a broad sense, e.g. a different manifestation of zygomorphy found in *Tripogandra* versus that of *Commelina* (see Chapter 3, Fig. 23). Nevertheless, zygomorphic flowers are homoplasious. In a recent talk at the 2003 Monocot Conference, Dennis Stevenson, who has worked with the Commelinaceae, showed that molecular data can only point out that

something may be homoplasious, but that does not indicate why (Giannasi, pers. comm.). The "why" might only be discovered by specific analysis of differences in developmental pathways within a character that has only been described in its broadest sense.

In another context but along the same vein, Handlos' (1975) quote of Woodson (1942) is relevant:

"the Commelinaceae always have been difficult subjects for herbarium study because of their deliquescent flowers. It is not easy to understand, therefore, why previous systematists of the family have focused almost all their whole attention upon floral structure in the delimitation of subfamilies, tribes and genera." (Handlos 1975, p. 217).

The genetic basis for the development of the Commelinaceae inflorescence has not been determined, but evidence has accumulated to suggest that variation in the type of inflorescence is under the control of one or two regulatory genes. With such structures under simple genetic controls, and with those same structures also under strong selective pressures relative to pollination syndromes, homoplasious evolution of the inflorescence might be the most parsimonious outcome (Evans et al 2000b).

Evans et al. (2000a, 2000b, 2003) and Faden (pers. comm.) have provided evidence that anatomical features in the Commelinaceae have potential to be less homoplasious than morphological characters. Subtribe Tradescantiinae would be a good candidate for further study of anatomical data. Such features as the longitudinal laminar epidermal cells of *Cuthbertia* versus the polygonal cells of *Callisia* and the presence of two types of silica in *Callisia* s.s. (Tomlinson 1966, 1969) provide an initial framework. Other characters need further scrutiny for phylogenetic utility, such as the determination

of whether *Cuthbertia* stomatal subsidiary cells are two or four, comparison of glandular microhair distal to middle to basal cell size (Tomlinson 1969. 1966), and the validity of lobed palisades of *Callisia* s.s. These potential avenues for future analysis could in part add evidence to or dispute the taxonomic considerations proposed here and in part further test the conviction that anatomical characters are less homoplasious than are morphological characters.

The molecular analyses undertaken here contribute to the understanding of relationships within the subtribe Tradescantiinae and thus contribute to the understanding of lineages within the family. Hunt's (1986b) "experiment with an amplification" of *Callisia* (Hunt 1986b, p. 407) was valid in the context of wrestling with the difficulty of delimiting *Callisia* s.s. and segregates. But Hunt's (1986b) treatment has here been shown to include members within *Callisia* that should not be treated under that genus. *Cuthbertia*, and the provisional genus, *Brachyphylla*, are related to *Callisia,* but should be segregated from it. Resurrection of *Phyodina* to include the one taxon, "*gracilis*", better reflects that taxon's evolutionary history and contemporary relationship with *Tripogandra* than does retaining that species under *Callisia*. The species' potential amphidiploid origin further explains its anomalous systematic position exemplified by the molecular analyses presented here.

The derivation of *Callisia* from *Tripogandra* has been proposed, in contrast to the reverse hypothesis of other workers (i.e. *Tripogandra* as having been derived from *Callisia*). Members of *Callisia* s.s. are the terminal-most clade in the molecular analyses here. In that clade are species with reduced floral characteristics, a reduction in base chromosome number, a largely Mexican center of distribution, and a prevalence of

endemism. Further studies of biogeography and comparisons of developmental morphological character-state pathways among *Callisia*, its segregates, and *Tripogandra* sensu Handlos (1975) would be useful further to address this hypothesis.

Additional molecular sampling of section *Leptocallisia* under *Callisia* s.l. can potentially resolve the position of the two species, *C. monandra* and *C. multiflora*, previously treated as the sole members under Moore's (1961) *Aploleia*. Data here suggest that the two species are closely related segregates of *Callisia* s.s. The implications of a segregation of *C. monandra* and *C. multiflora*, upon the treatment of *C. cordifolia*, would be clarified by additional taxon sampling.

The suggested polyphyly of *Callisia* as previously treated (Hunt 1986b) has been demonstrated in this phylogenetic evaluation of the problematic genus based on molecular data. The de-amplification of the genus *sensu* Hunt (1986b) and Tucker (1989) herein recommended initiates the resolution of that polyphyly. By extension, the result of these molecular analyses and the accompanying taxonomic proposals contribute to the monophyletic treatment of taxa of the southeastern United States and southeastern Texas/eastern Mexican floras, and provide both foundation and fuel for future studies of *Callisia* s.s. and other members of the subtribe Tradescantiinae.

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