EVOLUTIONARY RELATIONSHIPS IN POLYGONACEAE WITH EMPHASIS ON *TRIPLARIS*

BY

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ABSTRACT

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EVOLUTIONARY RELATIONSHIPS IN POLYGONACEAE
WITH EMPHASIS ON *TRIPLARIS*

Dissertation under the direction of Kathleen A. Kron, Ph. D.,
Professor

The plant family Polygonaceae Juss. has long been recognized as a distinct group based on the presence of ochrea, a single, basal ovule, a perianth composed of 5 or 6 tepals, and the fruit, an achene. However, the family is morphologically diverse with growth forms varying from small herbs or cushion plants to shrubs, lianas, and trees over 20m tall. Members of Polygonaceae are distributed worldwide (but most species are concentrated on the northern temperate zone), and they colonize virtually all ecosystems. Although few molecular studies have been done in Polygonaceae, the monophyly of the family has been established and a new subfamily classification has recently been proposed. Although the same subfamilies are recognized (Eriogonoideae and Polygonoideae), the circumscription changed: Eriogonoideae was expanded to include the woody Neotropical genera previously placed in Polygonoideae.

In order to test this subfamily circumscription, more molecular regions (three chloroplast and ITS) and an increased taxon sampling (75 species in 40 genera) were included. Based on molecular analyses, there was strong support for both subfamilies, although two genera did not fall definitively into these two clades: *Afrobrunnichia* Hutch. & Dalziel and *Symmeria* Benth. The position of *Afrobrunnichia* is ambiguous: it either is placed sister to Polygonaceae or sister to one of the subfamilies. *Symmeria* consistently

falls as sister to the rest of Polygonaceae, even with increased outgroup sampling in Plumbaginaceae Juss. It was also discovered that there is no support for the traditional delimitation of tribes, with the exception of Eriogoneae and Rumiceae.

A more detailed study on the subfamily Eriogonoideae including 42 accessions from the 12 tropical woody genera, 22 morphological characters and six molecular regions, recovered the relationships within the subfamily with strong support. Tribes Coccolobeae and Triplarideae were not supported as monophyletic, but six clades were strongly supported: *Antigonon-Brunnichia, Coccoloba-Neomillspaughia-Podopterus, Leptogonum, Triplaris-Ruprechtia, Gymnopodium*, and Eriogoneae. Traditional morphological characters used to delimit the tribes are not useful for defining monophyletic groups. The six-tepal condition is derived from the five-tepal condition, and unisexual flowers have arisen multiple times in different sexual systems. Ruminate endosperm has arisen multiple times in the family, suggesting this character is highly plastic.

Two genera in Eriogonoideae, *Ruprechtia* and *Triplaris* (tribe Triplarideae), were explored in more detail. Both genera are very similar morphologically, sharing characters such as strict dioecy and a three winged fruit. However, some of the morphological characters used for distinguishing both genera are still debatable since there were exceptions and therefore their taxonomy had been in constant flux. Different authors had considered that all species of *Ruprechtia* should be merged in *Triplaris*; others considered that more than two genera should be recognized (i.e., *Enneatypus*, *Magonia*), while others kept the two genera as distinct. In order to clarify the relationships between *Triplaris* and *Ruprechtia*, nine species of *Triplaris* were sampled and 19 from

Ruprechtia, and six molecular regions. Our analyses recover *Ruprechtia* as polyphyletic. Two new genera are named in order to recognize monophyletic groups: *Magoniella* and *Salta*. Morphological synapomorphies are given for the four genera in Triplarideae.

Focusing on *Triplaris*, the intraspecific relationships of 12 species were studied using five molecular regions. For seven species, multiple individual accessions were included, and four of the species were not supported as monophyletic (*T. americana, T. cumingiana, T. dugandii*, and *T. poeppigiana*). The ant-plant relationship between *Pseudomyrmex* and *Triplaris* was also studied by comparing the phylogeny of the ants to that of the plants, and by compiling a data set with all the collections of plant hosts and resident ants known mapped on GIS. The pattern of distribution of both organisms reveals that one species of the *Pseudomyrmex triplarinus* subgroup is more specific to its host than others.

CHAPTER I

AN INTRODUCTION TO POLYGONACEAE AND *TRIPLARIS*

The central topic of this dissertation is evolution. I started my dissertation with a broad question (a family-level phylogeny of Polygonaceae) that provided a framework for more in-depth studies such as the ant-plant interactions that occur in one particular genus of Polygonaceae (*Triplaris*). Before this work started, there was virtually no molecular work done in the plant family Polygonaceae. Because I wanted to understand the relationship of *Triplaris*, and its associated ants, to other genera in the family, the evolution of *Triplaris* needed to be investigated in the context of an accurate phylogeny. Therefore, my dissertation begins with a study of the relationships within the plant family Polygonaceae.

Polygonaceae is a morphologically diverse family that contains nearly 1,200 species in 48 genera (Freeman and Reveal 2005). Growth form varies from small herbs or cushion plants to shrubs, lianas, and trees over 20m tall. Leaves are simple and usually alternate, but in some cases they can be opposite (e.g., *Pterostegia*, some species of *Polygonum* and *Eriogonum*). The flowers are small, radially symmetric, and 3-merous, but the number of parts can vary from 4-6 free tepals, 6-9 free stamens, and 2-3 connate carpels. Nectary discs are usually present and the nodes are typically swollen. Despite the variation, and even though some clades within the family apparently have lost several traits, Polygonaceae are monophyletic (Chase et al. 1993; Lledo et al. 1998; Cuénoud et al. 2002; Lamb-Frye and Kron 2003) with morphological synapomorphies such as a sheathing stipule (ocrea), quincuncial aestivation, orthotropous ovules, and achenes. Members of this clade are distributed worldwide, but most of the species are concentrated in the northern temperate zone.

The identity of the family as a natural group was recognized by Jussieu in 1789. However, the taxonomy of Polygonaceae has been constantly changing with respect to the circumscription of subfamilies, tribes, and genera depending on the morphological characters chosen to define the subgroups. In 1893, Dammer subdivided the family into three subfamilies: Rumicoideae, Polygonoideae, and Coccoloboideae, with Eriogonoideae recognized as a tribe within Polygonoideae. Gross (1913) changed the previous circumscription but maintained the three subfamilies. The subfamilies he named were Eriogonoideae, Coccoloboideae, and Polygonoideae (with Gross' Rumicoideae as a tribe within Polygonoideae). In 1925, Jaretzky named two subfamilies: the Eriogonoideae and Polygonoideae, including Coccoloboideae within the latter. Roberty and Vautier (1964) divided the family again into three subfamilies, but this time the groups were Polygonoideae, Eriogonoideae, and Calligonoideae. Reveal (1989), Brandbyge (1993), and Freeman and Reveal (2005) used the two-subfamily division proposed earlier by Jaretzky: Polygonoideae and Eriogonoideae. However, a previous study using the chloroplast gene *rbcL* (Lamb-Frye and Kron 2003) showed that there was no support for the two subfamily circumscription, since a monophyletic Eriogonoideae was nested within Polygonoideae.

A study using more chloroplast genes (*rbcL, matK*, and *ndhF*) and a more inclusive sampling (47 taxa), recovered a phylogeny where the two traditionally recognized subfamilies in Polygonaceae, as most recently circumscribed (Reveal 1989, Brandbyge 1993, and Freeman and Reveal 2005), are not monophyletic (Sanchez and Kron, 2008). The chloroplast data indicated a deep split within Polygonaceae, resulting in two large clades. However the members of the two clades did not correspond to the

traditional subfamilies. Nomenclatural changes to prevent the naming of paraphyletic taxa within Polygonaceae resulted in the re-circumscription of both subfamilies. Subfamily Eriogonoideae was expanded to include currently recognized *Antigonon*, *Coccoloba*, *Triplaris*, and other members of the woody tropical clade plus Eriogonoideae s.s. (or Eriogoneae) (Sanchez and Kron, 2008). Subfamily Polygonoideae was defined by the type genus *Polygonum* and other genera including *Atraphaxis, Emex, Fallopia, Fagopyrum, Koenigia, Muehlenbeckia, Oxyria, Persicaria, Rheum*, and *Rumex*.

The study by Sanchez and Kron (2008) was the first subfamilial re-circumscription of the family based on molecular data. It served as a basis for following studies exploring the relationships of genera within Polygonaceae using more genes and even more taxa (e.g., Burke et al. 2010; Sanchez and Kron, 2009). It also served as a basis for current work on the tribal circumscription of the subfamilies Eriogonoideae (Burke and Sanchez, in rev.) and Polygonoideae (Sanchez et al., in press).

The subfamily Eriogonoideae (sensu Sanchez and Kron, 2008) comprises ca. 28 genera and more than 500 species. Seventeen of the described genera are part of the radiation of Eriogoneae (Eriogonoideae sensu Reveal) in North America. *Eriogonum* Michx. is the most species-rich genus with just over 250 species, mainly in western North America. The remaining genera are distributed in North, Central and South America, and the Antilles. In Eriogonoideae several genera remain cryptic and/or poorly known. Some of these genera have restricted geographic distributions and/or numbers of species, making them less likely to be studied. There are others that are less cryptic (e.g., *Antigonon*, *Coccoloba*, *Triplaris*) but number of species and intraspecific relationships among these genera are also not well understood.

Eriogonoideae is highly diverse morphologically; it comprises annual, biennial or perennial herbs, shrubs, trees, or lianas. The ocrea is present, except in Eriogoneae, but it can be persistent, distally deciduous or early caducous, leaving a circular scar. The ocrea can also be hyaline, scarious, membranaceous or foliaceous. Inflorescences can be axillary or terminal, and spicate, racemose, paniculate, umbellate or capitate. Except for Eriogoneae, the subfamily lack involucral structures (or a group of bracts enclosing the flowers) and flowers can be unisexual or bisexual. The perianth is usually accrescent (rarely persistent and non-accrescent), keeled in *Podopterus* Bonpl., *Neomillspaughia* S.F. Blake, awned in *Chorizanthe* Benth. and occasionally glandular. Flowers usually have five or six tepals, eight to nine stamens (three, six or nine in Eriogoneae), and a three carpellate gynoecium with one ovary and one orthotropous ovule. Staminodes are present in *Coccoloba*, *Ruprechtia* C.A. Mey, *Triplaris* Loefl. The fruits are achenes, usually trigonous or globose, rarely winged, but enclosed by perianth. The endosperm is ruminate in some species and embryos can be straight or curved.

Within this subfamily two genera, *Ruprechtia* and *Triplaris*, are important ecological components of the lowland flora of Central and South America. They comprise approximately 55 species, distributed from northern Mexico to Argentina (all Latin American countries except Chile), and the Antilles. Both genera are trees and shrubs (in few instances, lianas) and share characters such as a terminal, conical ocrea enclosing the developing shoot and leaf, three-winged fruits, flowers with six tepals and six stamens, and trigonous achenes. The delimitation of *Ruprechtia* and *Triplaris* has been in constant flux: while some authors merged *Ruprechtia* under *Triplaris* (e.g., Endlicher 1847; Kuntze 1898), others segregated some species of *Ruprechtia* into new

genera such as *Enneatypus* (Herzog 1922; Roberty and Vautier 1964) or *Magonia* (Kuntze 1891). The consensus is to maintain *Ruprechtia* as distinct from *Triplaris* (i.e., Meisner 1856; Bentham 1880; Dammer 1893; Brandbyge and Øllgard 1984; Brandbyge 1986; Pendry 2004); however, the relationships of these genera have not been explored phylogenetically.

Triplaris is a notable component of the rainforests and other lowland ecosystems, due to their interactions with stinging ants (from the genus *Pseudomyrmex*). They also serve an important ecological role since all the species are considered pioneer plants that colonize disturbed areas such as forest clearings, and river and road margins. However, the taxonomy of *Triplaris* is not well studied (see Brandbyge, 1986) and the relationships between species remain poorly known. Understanding the intraspecific relationships in *Triplaris* would permit further exploration of the ant-plant interactions and improve the understanding of the ecological role these plants play.

Since our understanding of the evolutionary relationships in Polygonaceae, and in *Triplaris* in particular, is poor, this dissertation aims to increase our knowledge by studying the relationships at different scales of resolution. The first objective (Chapter II), continuing the work by Sanchez and Kron (2008), is to address the phylogenetic relationships of most of the genera in Polygonaceae. This phylogeny is based on chloroplast and nuclear ITS sequence data and will allow to test whether or not most of the genera sampled are monophyletic and if their status could be maintained as such.

The robust large-scale phylogeny of Polygonaceae (obtained in Chapter II) provides a framework for a more focused study of the relationships among the members of the subfamily Eriogonoideae (where *Triplaris* is placed). The second objective

(Chapter III) explores the relationships within the subfamily by increasing the amount of data by using both nuclear and chloroplast genes, and 22 morphological characters. Relationships among the genera in Eriogonoideae are investigated to understand the utility of traditional morphological characters used to delimit natural groups. This second objective provides a better understanding of the placement of *Triplaris* within Polygonaceae, and its relationships to other genera in Eriogonoideae.

With this context, the relationships of *Triplaris* and its sister genus, *Ruprechtia* can be explore more in-depth (Chapter IV). Since both genera are very similar morphologically and there have been some problems on their delimitation, their relationships and the monophyly of each genus will be explored by sampling several species of *Triplaris* and *Ruprechtia* and two faster evolving chloroplast genes, as well as two nuclear regions.

With the monophyly of *Triplaris* established, details of the relationships between its species can be addressed (Chapter V). In order to have a complete idea of the intraspecific relationships of *Triplaris* and to put them in context with the association to ants, the phylogeny for both organisms (ants and plants) is fundamental to compare patterns of evolution and give possible explanations for the recovered patterns.

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CHAPTER II

A LARGE-SCALE PHYLOGENY OF POLYGONACEAE BASED ON MOLECULAR DATA

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Abstract

Few studies have addressed the evolutionary relationships within Polygonaceae from a global perspective. The convoluted taxonomic history of Polygonaceae is a major barrier to understanding evolution in this group, and only portions of it have been included in systematic treatments. Phylogenetic studies have been limited in both taxon sampling and amount of data. Our objective is to identify clades within Polygonaceae and to provide a global estimate of phylogenetic relationships in this morphologically diverse and geographically widespread group. We include a total of 75 species representing approximately 40 of the 55 named genera in the family. We use three chloroplast regions (*rbcL*, *matK*, and *ndhF*) and the ribosomal internal transcribed spacer (ITS) to understand the phylogenetic relationships in Polygonaceae. Maximum Parsimony and Maximum Likelihood are used to analyze the data. *Symmeria* is the sister group to remaining Polygonaceae sampled, and there is strong support for this placement. *Afrobrunnichia* branches next but has only moderate support. Two large clades comprise Polygonaceae, generally corresponding to those found in previous molecular analyses. Circumscription of most of the currently recognized subgroups within Polygonaceae did not agree with clades identified in the total data analyses, with the exception of Rumiceae Dum.

Introduction

Polygonaceae Juss. is a monophyletic group with the morphological synapomorphies of an ocrea, orthotropous ovules, trigonal (usually) achenes, and quincuncial aestivation (Judd et al. 2007). The family is distributed worldwide and present in almost all ecosystems, from tundra and alpine (e.g., *Polygonum*, *Rheum*, *Rumex*) to sand dunes and deserts (e.g., *Calligonum*, *Coccoloba*, *Eriogonum*); some plants are found in periodically inundated rainforests (e.g., *Ruprechtia*, *Triplaris*) and others are aquatic (e.g., some species of *Persicaria*, *Polygonum*). The family is morphologically diverse and shows extensive plasticity in growth form (e.g., lianas, trees, mat forming shrubs).

Polygonaceae have been described to include anywhere from 43 (Brandbyge 1993) to 55 (Qaiser 2001) genera. The disparity in the number of genera recognized depends on the fact that many treatments are restricted regionally (e.g., Flora North America), politically (e.g., Flora of Pakistan) or within a group of Polygonaceae (e.g., Ronse Decraene and Akeroyd 1988). Additionally, characters that are considered important by one author are not by another, and this results in many synonyms used in different treatments (Qaiser 2001; Li et al. 2003). Genera that are newly described add to the confusion. At the subfamily level, varying classifications have also contributed to confusion regarding the number of subfamilies recognized, subfamily circumscription, and generic placement. In one of the earliest treatments of the family, Meisner (1856) recognized four subfamilies: Eriogonoideae, Polygonoideae, Brunnichioideae, and Symmerioideae. Later workers (Bentham and Hooker 1880; Dammer 1893; Perdrigeat 1900; Gross 1913) recognized three subfamilies: Eriogonoideae, Polygonoideae, and

Coccoloboideae. More recently Jaretzky (1925), Haraldson (1978) and Brandbyge (1993) recognized two subfamilies: Eriogonoideae and Polygonoideae. However, Haraldson's (1978) treatment dealt only with Polygonoideae. Most workers have used characters of the ocrea and calyx in their subfamily delimitations, and some have also considered habit as an important feature in classification (e.g., Dammer 1893; Gross 1913). Recent treatments (e.g., Brandyge 1993) recognize two subfamilies: Eriogonoideae (on the basis of the lack of an ocrea and the flowers enclosed in an involucre) and Polygonoideae (on the basis of the presence of a well-developed ocrea and usually five tepals). Haraldson's (1978) treatment changed the circumscription of several tribes and genera. For example, she recognized *Millspaughia* as distinct from *Gymnopodium*, contrary to Brandbyge (1993; table 1). Brandbyge (1993) also changed the delimitation of several taxa, but many of these were not in agreement with previous authors (Dammer 1893; Gross 1913; Jaretzky 1925; Ronse Decraene and Akeroyd 1988; Hong et al. 1998; Ronse Decraene et al. 2000) or Haraldson's (1978) classification. Since Brandbyge's treatment of Polygonaceae (1993), some taxa have been segregated from larger groups (e.g., *Knorringia*) and several genera have been described in the traditionally named subfamily Eriogonoideae (Reveal 2004*a*, 2004*b*, 2005). Obviously, the nomenclature of this group is complex, and we endeavor to streamline the names used and focus on those taxa that have been recognized by Brandbyge (1993) and Haraldson (1978; table 1). As discussed by Kim and Donoghue (2008*b*), we will refer to groups by names that are in accordance with general usage but will limit the use formal ranks in our discussion.

Molecular data have only recently begun to address the phylogenetic relationships within the group (Lamb-Frye and Kron 2003; Kim and Donoghue 2008*a*, Kim and

Donoghue 2008*b*; Kim et al. 2008; Sanchez and Kron 2008, 2009). Both of the large-scale studies done by Lamb-Frye and Kron (2003) and Sanchez and Kron (2008) indicate a deep split in Polygonaceae that forms two large clades. However, the Lamb-Frye and Kron (2003) analyses used only *rbcL* and had a restricted sample size. The study did not support the monophyly of Polygonoideae as defined by recent workers such as Haraldson (1978) and Brandbyge (1993). In Lamb-Frye and Kron (2003) *Eriogonum* was placed in a clade containing representatives of *Antigonon*, *Coccoloba*, and *Triplaris*, with *Brunnichia* as sister to this group. The remaining taxa sampled formed a clade in which the first branching node was *Fagopyrum*. A molecular study by Sanchez and Kron (2008) focused on the relationships of selected woody genera in Polygonaceae used three chloroplast genes (*rbcL*, *matK*, *ndhF*) and more taxa. That study also indicated that Polygonoideae as previously recognized (Meisner 1856; Dammer 1893; Gross 1913; Jaretzky 1925; Hutchinson and Dalziel 1927; Haraldson 1978; Brandbyge 1993; Li et al. 2003) was not monophyletic. In both the Lamb-Frye and Kron (2003) and Sanchez and Kron (2008) studies, each of the two major groups had moderate to low bootstrap support levels. More recently Sanchez and Kron (2009) investigated the relationship of *Brunnichia* to the new members of an expanded Eriogonoideae (Sanchez and Kron 2008). In that study, they sampled 39 taxa, representing ~22 genera, and used sequence data from the chloroplast genes *rbcL*, *matK* and *ndhF* and the nuclear internal 18s-26s spacer region (ITS) region. The results showed that *Brunnichia ovata* is not closely related to *Brunnichia africana* (= *Afrobrunnichia erecta*). *Brunnichia ovata* is placed sister to *Antigonon*, but is not in the same clade as *Afrobrunnichia*. The Sanchez and Kron (2009) study also included more representatives of the tropical lowland forest trees *Triplaris* and

Ruprechtia, found in northern South America, than in previous analyses. This study samples nearly twice as many taxa than in Sanchez and Kron (2009), representing 16 additional genera.

The nomenclatural confusion in Polygonaceae has been a major barrier to our understanding of evolution in this geographically widespread and morphologically diverse group. In view of this problem, our aim in this study is to identify clades within the group and to provide a first estimate of phylogenetic relationships based on a global taxon sampling.

Materials and Methods

Taxon sampling

Taxon names within Polygonaceae are problematic for sampling purposes. Usually in studies of groups with poorly known phylogenetic relationships, current classifications are the initial basis for sampling, in addition to morphological diversity and geographical distribution. However, classifications of Polygonaceae have varied so significantly in recognition of rank and scope that we have chosen to approach sampling with an emphasis on segregate genera named recently (Li 1981; Hong et al. 1989) as well as those from more comprehensive classifications (Haraldson 1978; Brandbyge 1993). We include representatives of 75 species of Polygonaceae. These represent 40 of the approximately 55 recognized genera (Brandbyge 1993; Qaiser 2001; Li et al. 2003). The 15 genera not sampled include *Oxygonum* (because of lack of suitable material) and 14 from '*Eriogonum* and allies' (table 1). Many of the latter genera are monospecific or comprise few species and are probably within a large clade that includes *Eriogonum s. str.* (Sanchez and Kron 2008). In this study we sample nine representatives from *Eriogonum* and allies; four species of *Eriogonum s. str*., one each of *Chorizanthe*, *Dedeckera*, *Gilmania*, *Johanneshowellia* and *Pterostegia* (the latter was placed in a separate group by Brandbyge [1993] and Reveal [2005]; see table 1). The *Eriogonum* and allies clade is strongly supported as monophyletic (Sanchez and Kron 2008; E. Kempton, personal communication) and is in need of a more complete revision; this is beyond the scope of our study (for a complete list of the genera recognized, see Reveal 2005; table 1). In our analyses, most genera are represented by at least two species (appendix in the online edition of the *International Journal of Plant Sciences*). Three chloroplast regions (*rbcL*,

matK [excluding the flanking spacer regions] and *ndhF*) and one nuclear region (the ribosomal ITS) were used. The number of taxa sampled for each region was 74 for *rbcL*, 66 for *matK*, 71 for *ndhF* and 76 for ITS, for a total of 287 sequences (appendix; 5.6% missing data). For *Parapteropyrum tibeticum* we only had access to the ITS sequence (GenBank accession no. EU718499), and therefore it was included in the ITS and total combined analysis in order to understand its general placement within Polygonaceae.

Plumbago capensis was used as outgroup because Plumbaginaceae has repeatedly received strong support as sister to a monophyletic Polygonaceae (Chase et al. 1993; Lledo et al. 1998; Cuénoud et al. 2002). Although it is possible that the use of a single taxon as an outgroup could mistakenly place taxa such as *Symmeria* within Polygonaceae, the morphological characteristics of *Symmeria*, such as presence of an ocrea make its placement outside of Polygonaceae unlikely. The GenBank numbers for all sequences used in this study are found in the appendix.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from silica gel–dried leaves or herbarium material, using the modified CTAB method (Doyle and Doyle 1987) or the Qiagen (Valencia, California) DNeasy Plant Mini Kit. In some instances, we also obtained extracted DNA through the generosity of the Royal Botanical Gardens at Kew, the Donoghue Lab (Yale University), Janelle Burke (Cornell University), and Toby Pennington (Royal Botanical Gardens at Edinburgh). Protocols for standard polymerase chain reaction (PCR) generally follow those of Sanchez and Kron (2009). The primers used were *rbcL* (Lledo et al. 1998); *matK* (Johnson and Soltis 1994; Steele and Vilgalys 1994; Plunkett et al. 1996); *ndhF* (Olmstead and Sweere 1994), and ITS (White et al.

1990; Sun et al. 1994). The PCR products were cleaned using Qiagen QIAquick PCR purification columns, followed by direct sequencing. All sequences were run on an ABI (**Ramsey, MN)** 377 Automated DNA Sequencer at Wake Forest University's Automated DNA Sequencing Facility. Sequences were edited with Sequencher v.3.1.1 (Gene Codes, Ann Arbor, MI).

Alignment and Phylogenetic Analysis

Sequences were aligned using MAFFT (Katoh et al. 2005) and adjusted manually in MacClade, version 4.0 (Maddison and Maddison 2002), as needed. Maximum parsimony (MP) analyses of the combined chloroplast, ITS-only and total combined (chloroplast and ITS) data sets were conducted using TNT (Goloboff et al. 2008), under the traditional search option (or heuristic search), with one random seed, tree-bisection-reconnection branch swapping, 10 random sequence additions and saving 10 trees per replication. Bootstrap analysis was used for evaluating node support (Felsenstein 1985), and was estimated by 500 replicates with heuristic search settings identical to those of the original search.

Modeltest, version 3.6 (Posada and Crandall 1998, 2001), was run in PAUP* (Swofford 2002) to estimate the best-fitting model of sequence evolution. Maximum likelihood (ML) analyses were run using Garli (Zwickl 2006) with 100 bootstrap replicates. The RAxML analysis (Stamatakis et al. 2005; Stamatakis 2006) was performed under the GTRMIX model with 500 fast bootstrap replicates. Fast bootstrap in RAxML is not comparable to the same option in PAUP* (for information on this analysis refer to the online manual - http://icwww.epfl.ch/~stamatak/index-Dateien/software/RAxML-Manual.7.0.4.pdf). Three data sets were analyzed in this study:

(1) the combined chloroplast data set of *rbcL*, *matK* (coding region) and *ndhF*; (2) the ITS data set; and (3) the total combined chloroplast and ITS data set.

Results

Aligned DNA Sequences

The aligned matrix for the combined chloroplast analysis of *rbcL* (1299 base pairs [bp]), *matK* (835 bp) and *ndhF* (1209 bp) consisted of 3343 bp. For the MP analysis 1891 characters were constant, 670 parsimony uninformative, and 782 were potentially parsimony informative (PI; 23.4%). The alignment of these three chloroplast regions was straightforward with few indels for *matK* and *ndhF*. We did not include indel information in the phylogenetic analyses (they were treated as missing data) because their sizes were variable and did not always overlap. The ITS alignment by MAFFT consisted of 1066 bp and was straightforward across representatives of Polygonaceae in the conserved regions (e.g., 5.8S), but the introns showed high levels of variability. A total of 93 ambiguous base pairs were excluded from the second intron because of questions of alignment. The final alignment of ITS consisted of 973 sites, with 361 characters constant, 178 parsimony-uninformative and 434 PI sites (44.6%). The combined matrix of chloroplast and ITS data consisted of 4316 characters with 2252 constant sites, 848 parsimony uninformative and 1216 PI sites (28.2%).

Phylogenetic analyses

Parsimony and ML analyses were highly congruent, although support for several clades differed, depending on the analysis. In general, parsimony bootstrap scores were lower than those calculated in the Garli and RAxML analyses. Support values were usually higher for the RAxML analyses than they were for the Garli analyses. In this study we regard strong support values as 86%-100%; moderate support as 71%-85%; low

support 61%-70%. Values from 50%-60% (<50% not shown) are considered not reliable because of their wide range of error.

Chloroplast data. Polygonoideae form a clade that is strongly supported by ML analyses but only weakly supported in the MP results (fig. 1). Within this clade four subclades are moderately to strongly supported (clades A-C, and E; fig. 1). Clade A contains *Aconogonon, Koenigia*, *Bistorta* and *Rubrivena*, as well as other members of Eupersicarieae (as defined by Kim and Donoghue [2008*b*]). Clade B includes two smaller clades. *Atraphaxis, Polygonella, Polygonum aviculare* and *Polygonum erectum* form a monophyletic group that is sister to a clade that comprises species of *Fallopia*, *Reynoutria, Muehlenbeckia* and the monospecific *Homalocladium*. Sister to these two small clades is *Knorringia sibirica*. Clade C corresponds to the genus *Fagopyrum* and is strongly supported in both parsimony and ML results. Clade E, although not supported in parsimony results, is strongly supported in the ML analyses and corresponds to the currently recognized Rumiceae. This clade includes representatives of *Oxyria*, *Rheum*, *Rumex* and the noxious weed *Emex spinosa*. Poorly supported in the ML results is clade D. This group includes representatives from *Calligonum*, *Pteropyrum,* and *Pteroxygonum*. Bootstrap scores from parsimony analyses are below 50% and the next node below clade D has no support from either ML or parsimony results. Thus, the relationships among clades B, C, D and E are not strongly supported by chloroplast data in this study (fig. 1).

The results from chloroplast data analyses give support to a "core" Eriogonoideae that include the three strongly supported clades designated F-H (fig.1). Clade F contains *Antigonon* and *Brunnichia*. This clade is strongly supported (by ML) as sister to remaining core Eriogonoideae. Clades G and H form a trichotomy with *Leptogonum*.

Clade G contains the *Eriogonum* and allies group, which includes *Chorizanthe*, *Dedeckera*, *Johanneshowellia*, *Pterostegia*, and *Gilmania*; in addition to *Eriogonum s. str.*, *Gymnopodium* successively branch at the base of the *Eriogonum* and allies node. The placement of *Gymnopodium* as sister to the remaining Clade G is strongly supported in the ML analyses but only moderately supported by MP. Clade H is very strongly supported in all analyses and contains representatives of *Ruprechtia* and *Triplaris*. Clade I is composed of four species of *Coccoloba*. Although *Podopterus* is placed as sister to clade I, this relationship is poorly supported. The placement of *Neomillspaughia* is unresolved with respect to clade I and the trichotomy of clades G, H and *Leptogonum domingense*. *Afrobrunnichia* is placed as sister to remaining Eriogonoideae but this is essentially not supported by the chloroplast data. *Symmeria* is placed as sister to all remaining Polygonaceae sampled (fig. 1). This position is moderately to strongly supported in ML results but weakly supported (50%) by parsimony.

ITS. Analyses of the ITS data resulted in trees with many fewer well-supported nodes than found in the chloroplast analyses, especially toward the base of the tree (fig. 2). Clade A is moderately to strongly supported (inML) and includes the same taxa recovered in the chloroplast analyses (fig. 2). However, relationships among taxa at the tips of this clade are not the same in the group that contains *Bistorta* and *Rubrivena*. Note that with ITS, support for these differences is generally low to <50% (fig. 2). Resolution is lacking among most clades within clade B, but *Reynoutria* and *Fallopia* are each strongly to moderately supported as monophyletic. *Knorringia sibirica* is placed as sister to the remaining taxa of clade B, as is found in the chloroplast analyses, although without support in the parsimony analyses. Clade C is composed of *Fagopyrum* and

Parapteropyrum tibeticum, and this group is moderately to strongly supported (chloroplast data for *Parapteropyrum* were not available for this study). Clade D is not supported, but a clade containing all sampled *Calligonum* is monophyletic, with strong support in all analyses. This is also the case for *Pteropyrum*, which is monophyletic on the basis of the two species sampled here. Clade E, which corresponds to Rumiceae (fig. 1), is not recovered in the ITS analyses. However, the currently recognized genera *Oxyria*, *Rheum*, and *Rumex* are each strongly (ML) supported as monophyletic (fig. 2).

Many of the phylogenetic relationships in the Eriogonoideae identified in the chloroplast analyses are not resolved or are poorly supported in the ITS results. Clade G is strongly supported by ML but by <50% in the MP results. The same taxa are found in clade G as in the chloroplast analyses, but the differences in relationships among exemplars are weakly or very weakly supported. The *Coccoloba* clade (I) is supported as monophyletic (supported only in the ML results), and the sampled taxa of *Ruprechtia* and *Triplaris* also form a monophyletic group (H; fig. 2), as in the chloroplast results (fig. 1). Support for clade F is low in the MP results and moderate to strong in the ML results (Garli 75%, RAxML 90%). The deepest nodes of the Polygonaceae outside the Polygonoideae have little or no (<50%) support in any of the ITS analyses. Thus, relationships are uncertain among *Afrobrunnichia*, *Symmeria*, clade F, clade G, and the remaining sampled taxa within Eriogonoideae (fig. 2).

Combined Analysis. Differences between the results of the chloroplast and ITS analyses were determined by inspection, and there were no strongly supported conflicts between the trees obtained in the individual analyses. Therefore, the two data sets were

combined and analyzed, using parsimony and ML. The results show better resolution and often more strongly supported nodes throughout the tree than individual data set analyses.

In the total evidence analyses, Polygonoideae are strongly supported by both parsimony and likelihood (fig. 3). Within this monophyletic group, two major clades are supported as sister groups. Clade A is strongly supported by parsimony and likelihood results, and this group is sister to the remaining Polygonoideae (clades B–D, E). As in the chloroplast data set, clades A–C and E are strongly supported. Clade A is composed of two main clades. One clade contains *Aconogonon*, *Koenigia*, and *Polygonum paniculatum* (= *Aconogonon molle* var. *paniculatum*), a group that is sister to the clade composing Bistorta and Rubrivena. Sister to this pair of clades is a strongly supported clade composed of species of *Persicaria* and *Antenoron filiforme* (= *Persicaria filiforme*). In clade B the sister group relationship of *K. sibirica* to the remaining clade is very strongly supported. Phylogenetic structure within clade B is well supported on the basis of the sampled taxa. *Atraphaxis*, *Polygonella*, *Fallopia*, and *Reynoutria* are each strongly supported as monophyletic. Currently recognized *Polygonum* s.str. is also supported as monophyletic (*P. aviculare* and *P. erectum*) on the basis of the sampling here. *Muehlenbeckia* is not supported as a clade unless the monospecific *Homalocladium* platycladum is included within it. In agreement with the currently recognized Rumiceae, clade E is strongly supported and contains *Emex*, *Oxyria*, *Rheum*, and *Rumex*. The strongly supported clade C includes *P. tibeticum* as sister to *Fagopyrum urophyllum* and this subclade as sister to *Fagopyrum cymosum* and *Fagopyrum esculentum*. In this combined analysis (fig. 3) there is weak support for clade D, as was evident in the individual chloroplast (fig. 1) and ITS (fig. 2) results. As in the ITS analyses, *Calligonum*

and *Pteropyrum* are each supported as monophyletic. There is strong support in the ML analyses for clade C as a sister group to clades B, D, and E, but the relationships among the latter three clades are still uncertain because their placement is not supported by the combined data (<50%).

Within the core Eriogonoideae, clade G is strongly supported, with *Gymnopodium floribundum* branching from the node just below this group. *Gymnopodium*'s placement is weakly supported (ML). Another weakly supported relationship is the placement of *L. domingense* as sister to clades G + H. The *Coccoloba* clade (I) is strongly supported as sister to *Neomillspaughia*, with *Podopterus* branching at the next node below. As in the chloroplast analyses, clade F is strongly supported as sister to the remaining core Eriogonoideae. *Afrobrunnichia* is placed as sister to core Eriogonoideae plus Polygonoideae but with only moderate to low support. *Symmeria paniculata* is placed as sister to remaining Polygonaceae with strong support (fig. 3).

Discussion

This study discovered two large clades within Polgonaceae; Polygonoideae and Eriogonoideae. As in studies by Sanchez and Kron (2008, 2009) and Lamb-Frye and Kron (2003), these clades do not correspond to the traditional circumscriptions of the subfamilies. Members of the Eriogonoideae clade are noticeably different than in most previous classifications, where the subfamily consisted essentially of *Eriogonum*, its segregates, and *Chorizanthe*. Within the Polygonoideae clade, five subclades (A-E) were identified that had strong support, and four subclades (F-I) were discovered within the Eriogonoideae clade.

In the total evidence analyses, Polygonoideae are strongly supported by both parsimony and likelihood (fig. 3). Within this monophyletic group, two major clades are supported as sister groups. Clade A is strongly supported by parsimony and likelihood results, and this group is sister to the remaining Polygonoideae (clades B–D, E). As in the chloroplast data set, clades A–C and E are strongly supported. Clade A is composed of two main clades. One clade contains *Aconogonon*, *Koenigia*, and *Polygonum paniculatum* (= *Aconogonon molle* var. *paniculatum*), a group that is sister to the clade composing *Bistorta* and *Rubrivena*. Sister to this pair of clades is a strongly supported clade composed of species of *Persicaria* and *Antenoron filiforme* (= *Persicaria filiforme*). In clade B the sister group relationship of *K. sibirica* to the remaining clade is very strongly supported. Phylogenetic structure within clade B is well supported on the basis of the sampled taxa. *Atraphaxis*, *Polygonella*, *Fallopia*, and *Reynoutria* are each strongly supported as monophyletic. Currently recognized *Polygonum* s.str. is also supported as monophyletic (*P. aviculare* and *P. erectum*) on the basis of the sampling here.

Muehlenbeckia is not supported as a clade unless the monospecific *Homalocladium* platycladum is included within it. In agreement with the currently recognized Rumiceae, clade E is strongly supported and contains *Emex*, *Oxyria*, *Rheum*, and *Rumex*. The strongly supported clade C includes *P. tibeticum* as sister to *Fagopyrum urophyllum* and this subclade as sister to *Fagopyrum cymosum* and *Fagopyrum esculentum*. In this combined analysis (fig. 3) there is weak support for clade D, as was evident in the individual chloroplast (fig. 1) and ITS (fig. 2) results. As in the ITS analyses, *Calligonum* and *Pteropyrum* are each supported as monophyletic. There is strong support in the ML analyses for clade C as a sister group to clades B, D, and E, but the relationships among the latter three clades are still uncertain because their placement is not supported by the combined data (<50%). Within the core Eriogonoideae, clade G is strongly supported, with *Gymnopodium floribundum* branching from the node just below this group. *Gymnopodium*'s placement is weakly supported (ML). Another weakly supported relationship is the placement of *L. domingense* as sister to clades G + H. The *Coccoloba* clade (I) is strongly supported as sister to *Neomillspaughia*, with *Podopterus* branching at the next node below. As in the chloroplast analyses, clade F is strongly supported as sister to the remaining core Eriogonoideae. *Afrobrunnichia* is placed as sister to core Eriogonoideae plus Polygonoideae but with only moderate to low support. *Symmeria paniculata* is placed as sister to remaining Polygonaceae with strong support (fig. 3).

Some previously mentioned characters are supported as useful in identifying monophyletic groups, such as the occurrence of extrafloral nectaries (pit nectaries; Brandbyge 1993) on the abaxial surface of the petiole as a potential synapomorphy for the clade containing *Fallopia*, *Reynoutria*, and *Muehlenbeckia* + *Homalocladium* (for

Homalocladium; T. Schuster, personal observation). Brandbyge (1993) placed *Homalocladium* within *Muehlenbeckia*, and this is supported in this study. A thorough study of morphological character evolution will likely reveal more characters that are useful in determining phylogenetic relationships.

The tribe Rumiceae, as circumscribed by Haraldson (1978) and Brandbyge (1993), is strongly supported as monophyletic (clade E), as is the *Fagopyrum* clade, if *Parapteropyrum* is included (clade C). The *Eriogonum* and allies group is a strongly supported clade that has molecular support from previous studies and was also recognized by Brandbyge (1993). Other than these groups, this study indicates that phylogenetic relationships in Polygonaceae are poorly reflected in current classifications of the family (table 1). For example, tribe Polygoneae as recognized by Haraldson (1978) is polyphyletic because members thought to belong to this group are placed in different parts of the tree (clades A–D). This arrangement does not correspond to either Haraldson's (1978) or Brandbyge's (1993) classifications. Representatives of Brandbyge's (1993) tribe Coccolobeae are found in clade B (*Muehlenbeckia*), clade F (*Antigonon* and *Brunnichia*), and also in clade I (*Coccoloba*, *Podopterus*).

Ronse Decraene and Akeroyd (1988) and Ronse Decraene et al. (2000) recognized a tribe Persicarieae on the basis of shared morphological characters; however, our results do not support this because *Fagopyrum* (including *Parapteropyrum*) is not included in clade A (fig. 3). This is in agreement with Haraldson's (1978) circumscription of Persicarieae, restricted to *Aconogonon*, *Bistorta*, *Koenigia*, and *Persicaria* (table 1), and is currently recognized as Eupersicaria (Kim and Donoghue 2008b). Traditional Persicarieae are also not monophyletic because of the position of

Knorringia, which in this analysis is sister to the remaining clade B (fig. 3). *Knorringia* has been considered part of *Aconogonon* (Soják 1974) or *Persicaria* (Brandbyge 1993), but Hong (1989) recognized *Knorringia* as distinct from *Persicaria* on the basis of features of tepal venation, palynology, and seed anatomy. He suggested a close relationship of *Knorringia* to *Fallopia*, *Muehlenbeckia*, and *Reynoutria*, which is supported by the results presented here.

There are some relatively long branches seen in both the individual chloroplast (fig. 1) and the ITS (fig. 2) analyses. In the chloroplast analysis there are long branches leading to *Coccoloba uvifera*, *Emex spinosa*, *Pteroxygonum giraldii*, and *Triplaris poeppigiana*. In the total evidence tree (fig. 3), only *C. uvifera* retains a long branch. *Parapteropyrum tibeticum* has a long branch in the ITS and combined analysis and is placed within clade C. The relationship of this species to *Fagopyrum* has not been suggested before and is questionable because of its extremely long branch. *Parapteropyrum tibeticum* was not available for chloroplast analysis; therefore, its evaluation as a possible long-branch attraction problem cannot be thoroughly analyzed. This species is endemic to the Xizang Plateau (Tibet) in China and was described by Li (1981) as a segregate of *Pteropyrum* on the basis of the presence of an acute apex in the achenes, a calyx with entire wings in fruit (vs. beaked achenes and wings divided in two), glabrous filaments, and inflorescences in racemes. Hong (1995) and Hong et al. (1998) suggested the placement of *Parapteropyrum* within *Pteropyrum* on the basis of their similar tepal surface and pollen morphology. Our results place *Pteropyrum* in a weakly supported clade with *Calligonum* and *Pteroxygonum* (clade D). Dammer and Diels (1905) recognized *Pteroxygonum* as distinct from *Fagopyrum*. However, Haraldson (1978)

included *Pteroxygonum* within *Fagopyrum*. The results of our study place *Pteroxygonum* in clade D, while *Fagopyrum* is in clade C and includes *Parapteropyrum*. *Calligonum*, *Pteropyrum*, and *Pteroxygonum* are plants of arid environments, with most of their diversity distributed in Central Asia, extending southwest into the Middle East and northeast Africa. As one of the larger groups within Polygonaceae, *Calligonum* and its relatives (>80 species; Qaiser 2001) may represent a diversification in response to the extreme conditions that occur in desert habitats. A parallel example within Polygonaceae is *Eriogonum*, which has more than 200 described species and is most diverse in the arid southwest of North America (Reveal 2005).

Many of the clades recovered in this analysis are well supported in the ML results, but the relationships among several of the larger clades are less so, and their placement is uncertain. We have endeavored to increase taxon sampling significantly in this study in order to increase the reliability of the results. However, taxon sampling effects are somewhat problematic, and additional taxon sampling may be targeted in order to break up long branches (Graybeal 1998). Increasing taxon sampling in phylogenetic analyses is usually considered the best approach when evaluating phylogenetic relationships (Poe 1998; Soltis et al. 1998; Pollock et al. 2002). This is often a problem in poorly known groups such as Polygonaceae. Previous large-scale studies in Polygonaceae have gradually increased the number of taxa, but these analyses sampled more intensively in some clades than in others, making comparison of the results difficult. Hovencamp (2006) tested the reliability of using branch support for a predictor of clade stability when making decisions about taxon sampling in phylogenetic analyses. Although his results indicated that higher bootstrap values generally are a good predictor of reliability, when

taxon sampling is of concern, this was often not the case. Soltis et al. (1998) suggested that when analyzing large-scale phylogenetic relationships, taxon sampling in addition to more nucleotide data is desirable. Our study does not represent all genera currently recognized in the *Eriogonum* and allies group and does not include species of *Oxygonum*. *Oxygonum* is restricted to Africa, with the exception of two species (one is endemic to Madagascar), and is usually found in dry or weedy habitats (Graham 1958). It shares the possession of an ocrea with other members of the Polygonoideae and is considered a member of that group (Haraldson 1978; Brandbyge 1993). The lack of representatives of *Oxygonum* in our analysis may influence the placement of some clades within the group, but it is unlikely that this renders most of the relationships indicated in this study unreliable. From a geographical viewpoint, it will be interesting to see whether the addition of *Oxygonum* in future studies may influence the placement of the other African clades: *Symmeria* (South America/Africa) and *Afrobrunnichia* (Africa only) within Polygonaceae. A South American sample of *Symmeria paniculata* was included here, but the West African representative was not available. In most cases, we have included only two species per recognized group (usually a named genus or tribe), and additional representatives may change the current results. The effect of outgroup choice is also important, and it is well known that this can affect the inferred phylogenetic relationships within the ingroup (Hillis 1996; Rydin and Källersjö 2002). Until recently, Polygonaceae was considered an isolated group within angiosperms because of its unique morphological, chemical, and embryological characteristics (Cronquist 1988; Thorne 2000). However, recent molecular systematic work has shown that Polygonaceae are strongly supported as sister to Plumbaginaceae (Chase et al. 1993; Lledo et al. 1998;

Cuénoud et al. 2002), and the Plumbaginaceae + Polygonaceae clade is now included in an expanded Caryophyllales (Cuénoud et al. 2002; Stevens 2008). The closest relative to the Plumbaginaceae + Polygonaceae clade is the Tamaricaceae + Frankeniaceae clade (Cuénoud et al. 2002; Stevens 2008). So far, molecular analyses addressing relationships within Polygonaceae or parts thereof have used representatives of Plumbaginaceae or Polygonoideae (as circumscribed in Sanchez and Kron 2008) as outgroups. The use of additional outgroups such as *Tamarix* (Tamaricaceae) and *Frankenia* (Frankeniaceae) may also result in changes, especially at the deepest nodes of the Polygonaceae tree.

In conclusion, this study has found that many groups currently recognized within Polygonaceae are in need of taxonomic reassessment. However, any formal rearrangements are premature until a more intensive taxon sampling throughout the Polygonaceae is achieved. This work suggests that many morphological characteristics, such as tepal number and the presence of an ocrea, previously used to determine groups within Polygonaceae have evolved or have been lost more than once and likely are not reliable indicators of relationship. One notable exception is the extrafloral (pit) nectaries found in *Fallopia*, *Muehlenbeckia*, and *Reynoutria*, which form a clade in the total combined analyses. Other characters such as palynology and seed coat morphology may also prove to be reliable indicators of phylogenetic relationship. A global approach to studying morphological character evolution is likely to reveal new synapomorphies for many of the clades identified here.

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Appendix 2.1.

Voucher information for DNA extractions used in this study. Sequences obtained from Genbank are given with their respective site specific numbers. New sequences generated for this study provide the following information: Taxon, collector(s), collection number (in italics), and Genbank accession numbers. Specimens are deposited at WFU unless another herbarium is given. Herbarium acronyms follow Index Herbariorum, K = Royal Botanic Gardens Kew, E = Royal Botanic Gardens Edinburgh, BH= Cornell University, MO = Missouri Botanical Garden, NCU = University of North Carolina WFU = Wake Forest University. Gene abbreviations are as follows: R = *rbcL*, M = *matK*, N = *ndhF* and I = ITS. NA= not used in this study.

1. Sequences obtained from Genbank. ***Aconogonon molle*** (D. Don) Hara, R- EF653764, I- EF653687; ***Afrobrunnichia erecta*** Hutch. & Dalziel, R- FJ154447, M- FJ154489, N- FJ154501, I- FJ154459; ***Antigonon guatimalense*** Meisn., R- FJ154449, M- FJ154491, N- FJ154503, I- FJ154461; ***Antigonon leptopus*** Hook. & Arn., R- AF297146, M- EF437988, N- EF438027, I- FJ154462; ***Atraphaxis spinosa*** L., R- AF297123, M- EF437989, N- EF438028, I- FJ154463; ***Brunnichia ovata*** (Walter) Shinners, R- FJ154451, M- EF437990, N- EF438029 I- FJ154465; ***Chorizanthe brevicornu*** Torr. var. ***brevicornu***, R- EF437974, M- EF437991, N- EF438030, I- FJ154466; ***Coccoloba peltata*** Schott, R- FJ154452, M- FJ154493, N- FJ154504, I- FJ154467; ***Coccoloba pyrifolia*** Desf., R- Z97647, M- EF437994, N- EF438033, I- FJ154468; ***Coccoloba swartzii*** Meisn., R- AF297150, M- EF437995, N- EF438034, I- FJ154469; ***Coccoloba uvifera*** (L.) L., R- AF206753, M- EF437996, N- EF438035; ***Dedeckera eurekensis*** Reveal & J. T. Howell, R- EF437976, M- EF437997, N-

EF438036, I- FJ154470; ***Emex spinosa*** (L.) Campd., R- AF297142, M- AY042582, N- EF438037, I- FJ154471; ***Eriogonum alatum*** Torr. var. ***alatum***, R- EF437977, M- EF437998, N- EF438038, I- FJ154472; ***Eriogonum clavellatum*** Small, R- EF437979, M- EF438000, N- EF438040; ***Eriogonum esmeraldense*** S. Watson var. ***toiyabense*** J. T. Howell, R- EF437981, M- EF438003, N- EF438043; ***Eriogonum inflatum*** Torr. & Frém., R- EF437984, M- EF438006, N- EF438046; ***Fagopyrum cymosum*** Meisn., R- D86286, M- EF438008, I- AB000329; ***Fagopyrum esculentum*** Moench, R- D86285, M- AB093087, N- EU254477, I- AB000331; ***Fagopyrum urophyllum*** (Bureau & Franch.) H. Gross, R- 86288, M- AB026332, N- NA, I- AB000342; ***Fallopia dumetorum*** (L.) Holub, R- NA, M- AM503813, N- AM503835, I- AF040068; ***Fallopia scandens*** (L.) Holub, R- EF653785, M- NA, N- NA, I- AF040069; ***Gilmania luteola*** (Coville) Coville, M- EF438010, N- EF438049; ***Homalocladium platycladum*** (F.J. Müll.) L.H. Bailey, I- AF189738; ***Johanneshowellia crateriorum*** Reveal, R- EF437986, M- EF438011, N- EF438050; ***Koenigia forrestii*** (Diels) Měsíček and Soják, R- AF297144, M- EF438012, N- EF438051; ***Koenigia islandica*** L., R- EF653789, M- NA, N- NA, I- EF653686; ***Muehlenbeckia complexa*** (A. Cunn.) Meisn., I- AF040076; ***Muehlenbeckia tamnifolia*** Meisn. R- FJ154453, M- FJ154499, N- FJ154511, I- FJ154473; ***Oxyria digyna*** Hill, R- FJ154454, M- FJ154500, N- FJ154512, I- FJ154474; ***Oxyria sinensis*** Hill, R- AF297148, M- EF438013, N- EF438053; ***Parapteropyrum tibeticum*** A.J. Li, I- EU718499;***Persicaria pensylvanica*** (L.) M. Gómez, R- AF297133, M- EF438017, N- EF438056, I- FJ154475; ***Persicaria sagittata*** (L.) H. Gross, R- AF287141, M- EF438018, I- FJ154476; ***Persicaria virginiana*** (L.) Gaertn., R- AF297135, M- EF438019, N- EF438058, I- FJ154477; ***Podopterus cordifolius*** Rose & Standl., R- FJ154455, M-

FJ154494, N- FJ154505, I- FJ154479; ***Polygonella articulata*** (L.) Meisn., R- EF653760, I- EF653683; ***Polygonum aviculare*** L., R- AF297127, M- EF438020, N- EF438059, I- FJ154478; ***Polygonum erectum*** L. R- AF297128; ***Polygonum paniculatum*** Blume, R- AF297129; ***Pteroxygonum giraldii*** Dammer & Diels, I- EU580725; ***Reynoutria japonica*** Houtt., R- AF297131, M- AY042586, N- EF438048, I- AF189734; ***Reynoutria sachalinensis*** F. Schmidt Petrop., R- AF297125, M- EF438009, I- AF189737; ***Rheum nobile*** Hook. & Thomson, R- AF297147, M- EF438021, N- EF438060; ***Rumex acetosella*** L., R- D86290, M- EF438022; ***Rumex induratus*** Boiss. & Reut., R- AF297122, M- AY042647, N- EF438061, I- FJ154480; ***Rumex obtusifolius*** L., R- AF297126, M- EF438023, N- EF438062, I- FJ154481; ***Ruprechtia chiapensis*** Lundell ex Standl. & Steyerm. R- FJ154456, M- FJ154495, N- FJ154506, I- FJ154482; ***Ruprechtia laxiflora*** Meisn., R- EF437987, M- EF438024, N- EF438063, I- FJ154484; ***Ruprechtia tangarana*** Standl., M- EF438025, N- EF438064, I- FJ154485; ***Triplaris americana*** L., R- Y16910, M- AY042668, N- FJ154508; I- FJ154486; ***Triplaris poeppigiana*** Wedd., R- AF297137 M- FJ154497, N- FJ154509, I- FJ154487; ***Triplaris setosa*** Rusby, R- FJ154458, M- FJ154498, N- FJ154510, I- FJ154488; ***Plumbago capensis*** Thunb., R- M77701, M- EF438026, N- EF438065.

2. Sequences generated in this study. ***Aconogonon molle*** (D. Don) Hara, *Kim & Deng Ch-Ko-54* (YU) M-GQ206190, N-GQ206271; ***Antenoron filiforme*** (Thunb.) Roberty & Vautier (***Polygonum filiforme*** Thunb. ***subsp. neofiliforme*** (Nakai) Kitam.), *Zhonghui H* (MO), R-GQ206211, M-NA, N-GQ206272, I- GQ206237; ***Atraphaxis pyrifolia*** Bunge., *Kron s.n.*, R-GQ206212, M-GQ206191, N-GQ206273, I- GQ206238; ***Bistorta attenuatifolia*** Miyam. & H. Ohba, *Bufford et al. 35161* (MO), R-GQ206213, M-

NA, N-NA, I-GQ206239; ***Bistorta tenuicaulis*** Petrov, *Landrein* (K), R-GQ206214, M-NA, N-GQ206274, I-GQ206240; ***Calligonum aphyllum*** (Pall.) Gürke, *Kron s.n.*, R-GQ206215, M-GQ206192, N-GQ206275, I- GQ206241; ***Calligonum eriopodum*** Bunge, *Kron s.n.*, R-GQ206216, M-GQ206193, N-GQ206276, I- GQ206242; ***Calligonum junaceum*** (Fisch.& Mey.) Litv., *Kron s.n.*, R-GQ206217, M-GQ206194, N-GQ206277, I- GQ206243; ***Calligonum microcarpum*** Borszcz., *Kron s.n.*, R-GQ206218, M-GQ206195, N-GQ206278, I- GQ206244; ***Calligonum molle*** Litv., *Kron s.n.*, R-GQ206219, M-GQ206196, N-GQ206279, I- GQ206245; ***Coccoloba uvifera*** (L.) L., *Kron s.n.*, I- GQ206246; ***Eriogonum clavellatum*** Small, *Reveal & Broome 8478,* I- GQ206247; ***Eriogonum esmeraldense*** S. Watson var. ***toiyabense*** J. T. Howell, *Tiehm 14537* (WFU), I- GQ206248; ***Eriogonum inflatum*** Torr. & Frém., *Reveal 8458,* I- GQ206249; ***Fagopyrum cymosum*** Meisn., *Chase 893* (K), N- GQ206280; ***Gilmania luteola*** (Coville) Coville, *Reveal 8465*, I- GQ206250; ***Gymnopodium floribundum*** Rolfe, *Burke 48* (BH), R-GQ206220, M-GQ206197, N-GQ206282, I- GQ206251; ***Homalocladium platycladum*** (F.J. Müll.) L.H. Bailey, *Schuster s.n.*, R-GQ206221, M-GQ206198, N-GQ206283; ***Johanneshowellia crateriorum*** Reveal, *Reveal 8469,* I- GQ206252; ***Knorringia sibirica*** (Laxm.) S.P. Hong (= ***Polygonum sibiricum*** Laxm.), *Boufford et al*. *31660* (MO), R-GQ206222, M-NA, N-GQ206284, I- GQ206253; ***Koenigia forrestii*** (Diels) Měsíček and Soják, *Chase 888* (K), I- GQ206254; ***Leptogonum buchii*** Urb. **(*Leptogonum domingense*** Benth). *Gustafson 3077* (RSA), R-GQ206223, M-GQ206199, N-GQ206285, I- GQ206256; ***Muehlenbeckia complexa*** (A. Cunn.) Meisn., *MJC 425* (K), R-GQ206224, M- GQ206200, N- GQ206286; ***Neomillspaughia emarginata*** (H. Gross) S.F. Blake, *Burke 66* (BH), R-GQ206225, M-GQ206201, N-GQ206287, I- GQ206257;

Oxyria sinensis Hill, *Chase 895* (K), I- GQ206258; ***Persicaria sagittata*** (L.) H. Gross, *Kron s.n.*, N- GQ206288; ***Polygonella americana*** (Fisch. & C.A. Mey.) Small, *Kron s.n.*, R-GQ206226, M-GQ206202, N-GQ206289, I- GQ206259; ***Polygonella articulata*** (L.) Meisn., *Kron s. n.*, M- NA; N- GQ206290; ***Polygonum erectum*** L., *Kron s.n.*, M-GQ206203, N-NA, I- GQ206260; ***Polygonum paniculatum*** Blume, *Chase 899* (K), M-GQ206204, N-GQ206291, I- GQ206255; ***Pteropyrum aucheri*** Jaub. & Spach, *Alava 11002* (RSA), R-GQ206227, M-GQ206205, N-GQ206292, I- GQ206261; ***Pteropyrum olivieri*** Jaub. & Spach, *Ertter 188848* (MO), R-GQ206228, M-NA, N-GQ206293, I-GQ206262; ***Pterostegia drymarioides*** Fisch. & C.A. Mey., *Reveal 8807* (RSA), R-GQ206229, M-GQ206206, N-GQ206294, I- GQ206263; ***Pteroxygonum giraldii*** Dammer & Diels, *Wang et al. 2835* (RSA), R-GQ206230, M-GQ206207, N-GQ206295; ***Reynoutria sachalinensis*** F. Schmidt Petrop., *Chase 896* (K), N- GQ206281; ***Rheum nobile*** Hook.f. & Thomson, *Prahan s.n.*, (E), I- GQ206264; ***Rheum pichonii*** Pierre ex F.B.Forbes & Hemnsl., *Chase 926* (K), R-GQ206231, M-GQ206208, N-GQ206296, I-GQ206265; ***Rubrivena polystachya*** (Wall. ex Meisn.) M. Král ***(=Polygonum polystachyum*** Wall. ex Meisn.), *Heng 11894* (MO), R-GQ206232, M-NA, N-GQ206297, I- GQ206266; ***Rumex acetosella*** L., *Lamb-Frye s.n.*, N- GQ206298; ***Ruprechtia tangarana*** Standl, *Silman s.n.*, R- GQ206233; ***Ruprechtia triflora*** Griseb., *Pendry* (E), R-GQ206234, M-NA, N-GQ206299, I- GQ206267; ***Symmeria paniculata*** Benth., *Hoffman 1515* (NY), R-GQ206235, M-GQ206209, N-GQ206300, I- GQ206268; ***Triplaris cumingiana*** Fisch. & C.A. Mey. ex C.A. Mey., *Michelangeli s.n.*, R-GQ206236, M-GQ206210, N-GQ206301, I- GQ206269; ***Plumbago capensis*** Thunb., *Kron s.n.*, I-GQ206270.

Table 2.1.

Comparison of Polygonaceae classifications to tribe by Haraldson (1978) and Brandbyge (1993).

Haraldson (1978)	Brandbyge (1993)
N/A	Eriogonoideae Arn. [a]
	Eriogoneae Benth.
	Aristocapsa Reveal & Hardham [b]
	Centrostegia A.Gray ex Benth. [b]
	Chorizanthe R.Br. ex Benth.
	(G) *Dedeckera* Reveal & J. T. Howell
	Dodecahema Reveal & Hardham [b]
	(G) *Eriogonum* Michx.
	(G) *Gilmania* Coville
	Goodmania Reveal & Ertter [b]
	Hollisteria S. Watson [b]
	(G) *Johanneswellia crateriorum* Reveal[†]
	Lastarriaea J. Rémy [b]
	Mucronea Benth. [b]
	Oxytheca Nutt. [b]
	Stenogonum Nutt. [b]

	Systenotheca Reveal & Hardham [b]
	Pterostegieae Torr. & Gray
	Harfordia Greene & Parry [b]
	(G) *Pterostegia* Fisch. & C.A. Mey.

Polygonoideae Eaton	Polygonoideae
Triplareae Meisn.	Triplareae
Gymnopodium Rolfe	(na) *Gymnopodium*
Millspaughia Robins.	(na) *Millspaughia* in *Gymnopodium*
Leptogonum Benth.	(na) *Leptogonum*
Ruprechtia C. A. Mey.	(H) *Ruprechtia*
Triplaris Loefl. ex L.	(H) *Triplaris*
Symmeria Benth.	(na) *Symmeria*
Coccolobeae Dum. emend. Haraldson	Coccolobeae
Antigonon Endl.	(F) *Antigonon*
Brunnichia Banks ex Gaertn. (incl. *Afrobrunnichia* Hutch. & Dalziel)	(F) *Brunnichia* (incl. *Afrobrunnichia*)
Coccoloba P. Browne	(I) *Coccoloba*
Fallopia Adans.	(B) *Fallopia* in Polygoneae
Harpagocarpus Hutch. et Dandy [b]	(C) *Harpagocarpus* [b] in *Fagopyrum*

Muehlenbeckia Meisn. (incl. *Homalocladium* (F.J. Müll.) L.H. Bailey	(B) *Muehlenbeckia*. (incl. *Homalocladium*)
Neomillspaughia in *Podopterus*	(I) *Neomillspaughia* S.F. Blake
Podopterus Humb. & Bonpl.	(I) *Podopterus*
Reynoutria Houtt.	(B) *Reynoutria* in Polygoneae
Rumiceae Dum.	Rumiceae
Emex Neck. ex Campd.	(E) *Emex*
Oxyria Hill	(E) *Oxyria*
Rheum L.	(E) *Rheum*
Rumex L.	(E) *Rumex*
Polygoneae emend. Haraldson	Polygoneae
Atraphaxis L.	(B) *Atraphaxis*
Calligonum L.	(D) *Calligonum*
Fagopyrum Mill. (incl. *Pteroxygonum* Dammer & Diels)	(C) *Fagopyrum* in Persicareae
Fallopia in Coccolobeae	(B) *Fallopia*
Oxygonum Burch. ex Campd. [b]	(D) *Oxygonum*
Polygonum L. s. str.	(A, B) *Polygonum*
Polygonella Michx.	(B) *Polygonella*
Pteropyrum Jaub. & Spach	(D) *Pteropyrum*
Reynoutria in Coccolobeae	(B) *Reynoutria*
Persicarieae Dum.	Persicarieae

Aconogonon (Meisn.) Rchb.	(A) *Aconogonon* in *Persicaria*
Bistorta (L.) Scop.	(A) *Bistorta* in *Persicaria*
Fagopyrum in Polygoneae	(C) *Fagopyrum* (incl. (D) *Pteroxygonum*)
Koenigia L.	(A) *Koenigia*
Persicaria (L.) Mill.	(A) *Persicaria* (incl. (B) *Knorringia* (Czukav.) Tzvelev)

Note. Genera not mentioned in either classification include: *Antenoron* Raf., *Parapteropyrum* A.J. Li, *Rubrivena* M. Král and *Knorringia* (Czukav.) Tzvelev.
[a] Additional taxa named in Eriogonoideae include (Reveal 2005): *Acanthoscyphus* Small [b], *Johanneshowellia* Reveal, *Nemacaulis* Nutt [b], *Sidotheca* Reveal [b]
[b] Genera not sampled in this study. In right column upper case letters in parentheses indicate clade placement in this study. na = not placed with strong support or placement different in different analyses.

Figure. 2.1.

Topology resulting from maximum likelihood analysis of the combined chloroplast DNA data set using Garli (TVM + I + G) and RAxML. Bootstrap support values (≥ 50%) are indicated above or below the branches as Garli/RAxML/MP. Maximum parsimony (MP) recovered 60 most parsimonious trees (length = 3214, consistency index = 0.60, retention index = 0.72, rescaled consistency index = 0.43). When only one number is given, bootstrap support for all analyses (Garli, RAxML and MP) is the same. Differences between topologies obtained from the analyses are marked with stars.

Figure 2.1.

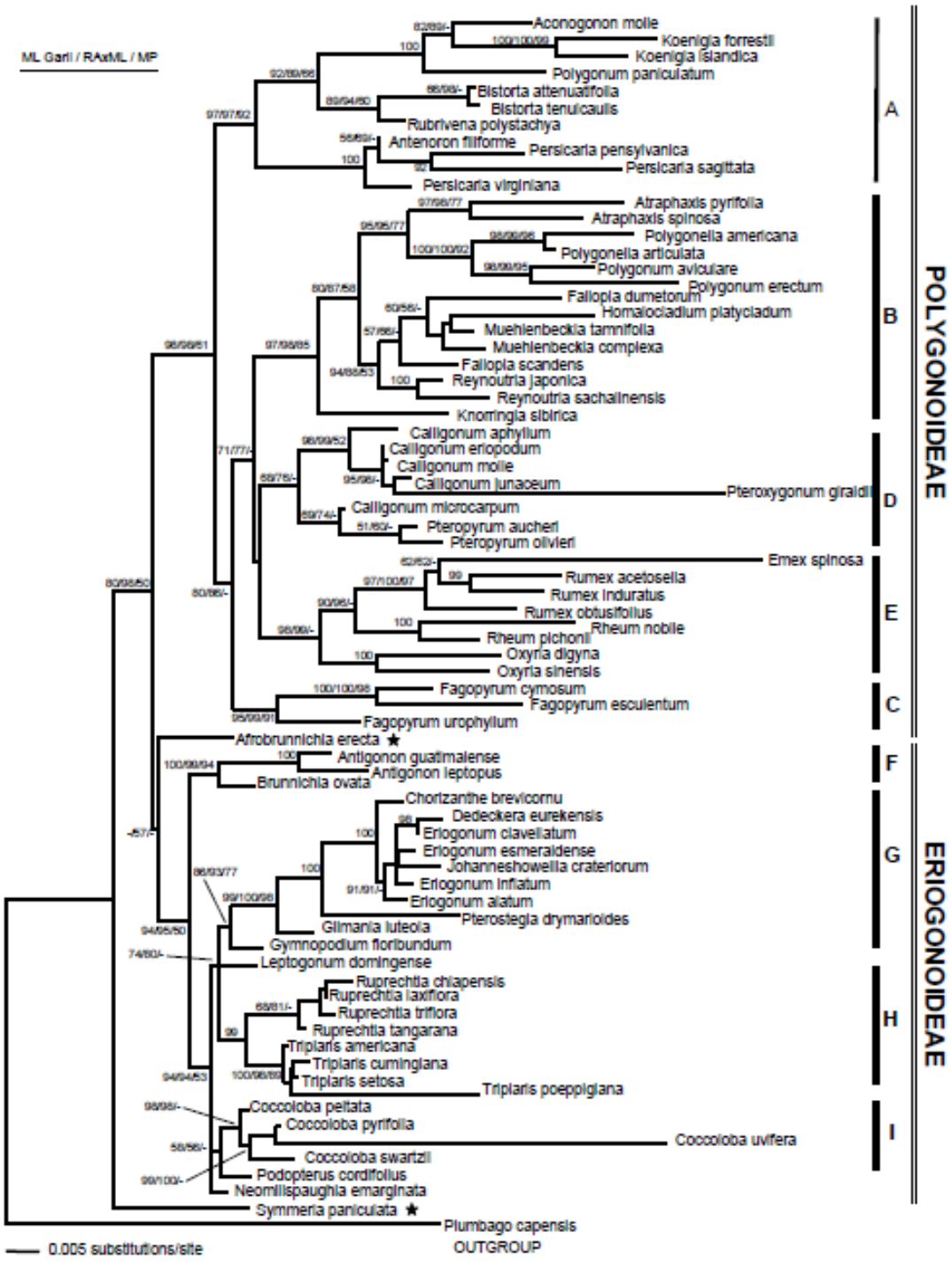
ML GarliRAxML / MP
Aconogonon molle
Koenigia forrestii
Koenigia islandica
Polygonum paniculatum
Bistorta attenuatifolia
Bistorta tenuicaulis
Rubrivena polystachya
Antenoron filiforme
Persicaria pensylvanica
Persicaria sagittata
Persicaria virginiana
Atraphaxis pyrifolia
Atraphaxis spinosa
Polygonella americana
Polygonella articulata
Polygonum aviculare
Polygonum erectum
Fallopia dumetorum
Homalocladium platycladum
Muehlenbeckia tamnifolia
Muehlenbeckia complexa
Fallopia scandens
Reynoutria japonica
Reynoutria sachalinensis
Knorringia sibirica
Calligonum aphyllum
Calligonum eriopodum
Calligonum molle
Calligonum junaceum
Pteroxygonum giraldii
Calligonum microcarpum
Pteropyrum aucheri
Pteropyrum olivieri
Emex spinosa
Rumex acetosella
Rumex induratus
Rumex obtusifolius
Rheum nobile
Rheum pichonii
Oxyria digyna
Oxyria sinensis
Fagopyrum cymosum
Fagopyrum esculentum
Fagopyrum urophyllum
Afrobrunnichia erecta
Antigonon guatimalense
Antigonon leptopus
Brunnichia ovata
Chorizanthe brevicornu
Dedeckera eurekensis
Eriogonum clavellatum
Eriogonum esmeraldense
Johanneshowellia crateriorum
Eriogonum inflatum
Eriogonum alatum
Pterostegia drymarioides
Gilmania luteola
Gymnopodium floribundum
Leptogonum domingense
Ruprechtia chiapensis
Ruprechtia laxiflora
Ruprechtia triflora
Ruprechtia tangarana
Triplaris americana
Triplaris cumingiana
Triplaris setosa
Triplaris poeppigiana
Coccoloba peltata
Coccoloba pyrifolia
Coccoloba uvifera
Coccoloba swartzii
Podopterus cordifolius
Neomillspaughia emarginata
Symmeria paniculata
Plumbago capensis
0.005 substitutions/site
OUTGROUP
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Figure 2.2.

Topology resulting from maximum likelihood analysis of ITS DNA using Garli (GTR + I + G) and RAxML. Bootstrap support values (>50%) are indicated above or below the branches as Garli/RAxML/MP. Maximum parsimony (MP), recovered 26 most parsimonious trees (length = 3412, consistency index = 0.36, retention index = 0.61, rescaled consistency index = 0.22). When only one number is provided, the bootstrap support for all analyses (Garli, RAxML, MP) is the same. Differences between topologies obtained from the analyses are marked with stars.

Figure 2.2.

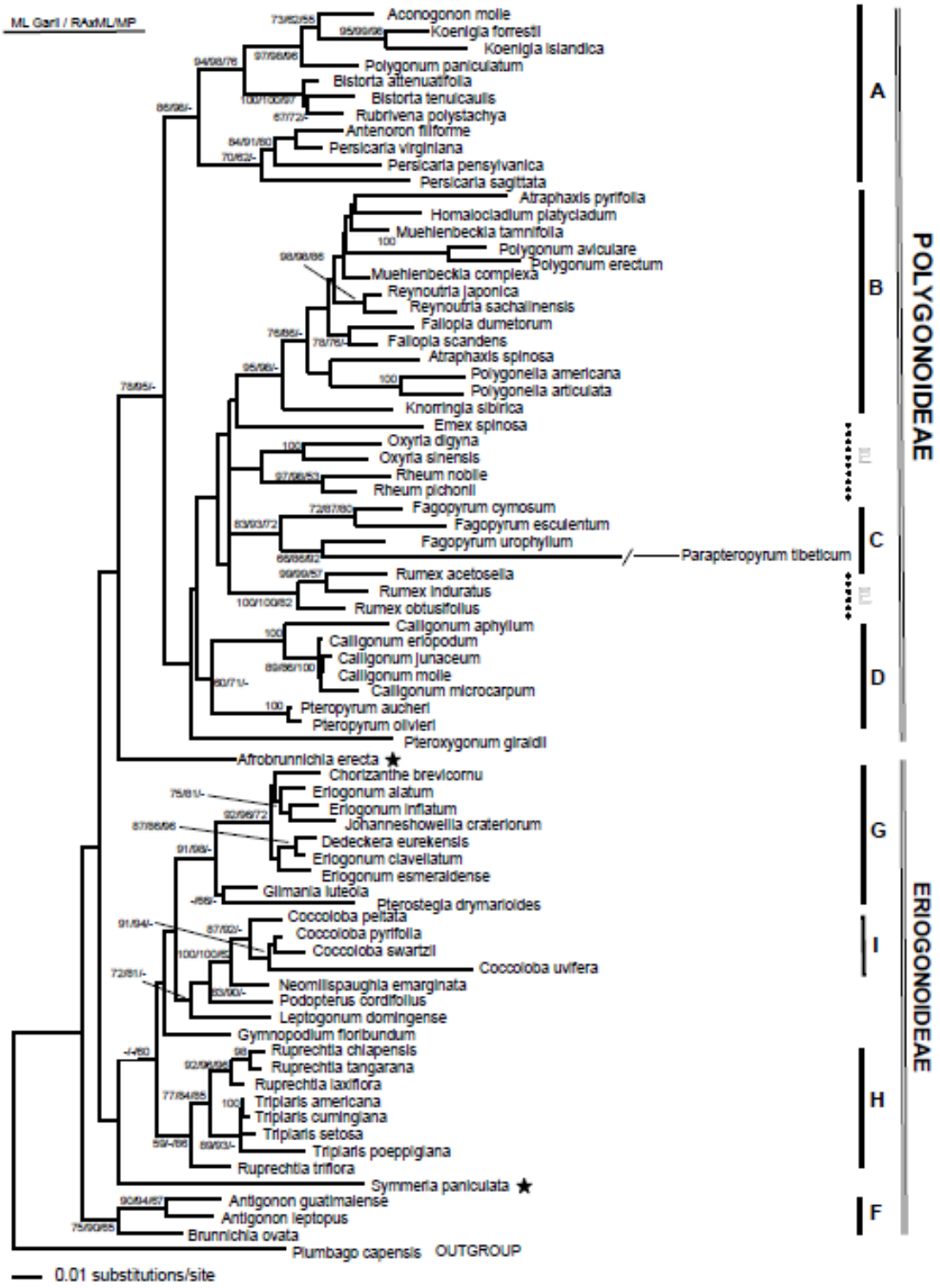
ML Garli / RAxML/MP
Aconogonon molle
Koenigia forrestii
Koenigia islandica
Polygonum paniculatum
Bistorta affinis
Bistorta tenuicaulis
Rubrivena polystachya
Antenoron filiforme
Persicaria virginiana
Persicaria pensylvanica
Persicaria sagittata
Atraphaxis pyrifolia
Homalocladium platycladum
Muehlenbeckia tamnifolia
Polygonum aviculare
Polygonum erectum
Muehlenbeckia complexa
Reynoutria japonica
Reynoutria sachalinensis
Fallopia dumetorum
Fallopia scandens
Atraphaxis spinosa
Polygonella americana
Polygonella articulata
Knorringia sibirica
Emex spinosa
Oxyria digyna
Oxyria sinensis
Rheum nobile
Rheum pichonii
Fagopyrum cymosum
Fagopyrum esculentum
Fagopyrum urophyllum
Parapteropyrum tibeticum
Rumex acetosella
Rumex induratus
Rumex obtusifolius
Calligonum aphyllum
Calligonum eriopodum
Calligonum junaceum
Calligonum mollle
Calligonum microcarpum
Pteropyrum aucheri
Pteropyrum olivieri
Pteroxygonum giraldii
Afrobrunnichia erecta
Chorizanthe brevicornu
Eriogonum alatum
Eriogonum inflatum
Johanneshowellia crateriorum
Dedeckera eurekensis
Eriogonum clavellatum
Eriogonum esmeraldense
Gilmania luteola
Pterostegia drymarioides
Coccoloba peltata
Coccoloba pyrifolia
Coccoloba swartzii
Coccoloba uvifera
Neomillspaughia emarginata
Podopterus cordifolius
Leptogonum domingense
Gymnopodium floribundum
Ruprechtia chiapensis
Ruprechtia tangarana
Ruprechtia laxiflora
Triplaris americana
Triplaris cumingiana
Triplaris setosa
Triplaris poeppigiana
Ruprechtia triflora
Symmeria paniculata
Antigonon guatimalense
Antigonon leptopus
Brunnichia ovata
Plumbago capensis OUTGROUP
0.01 substitutions/site
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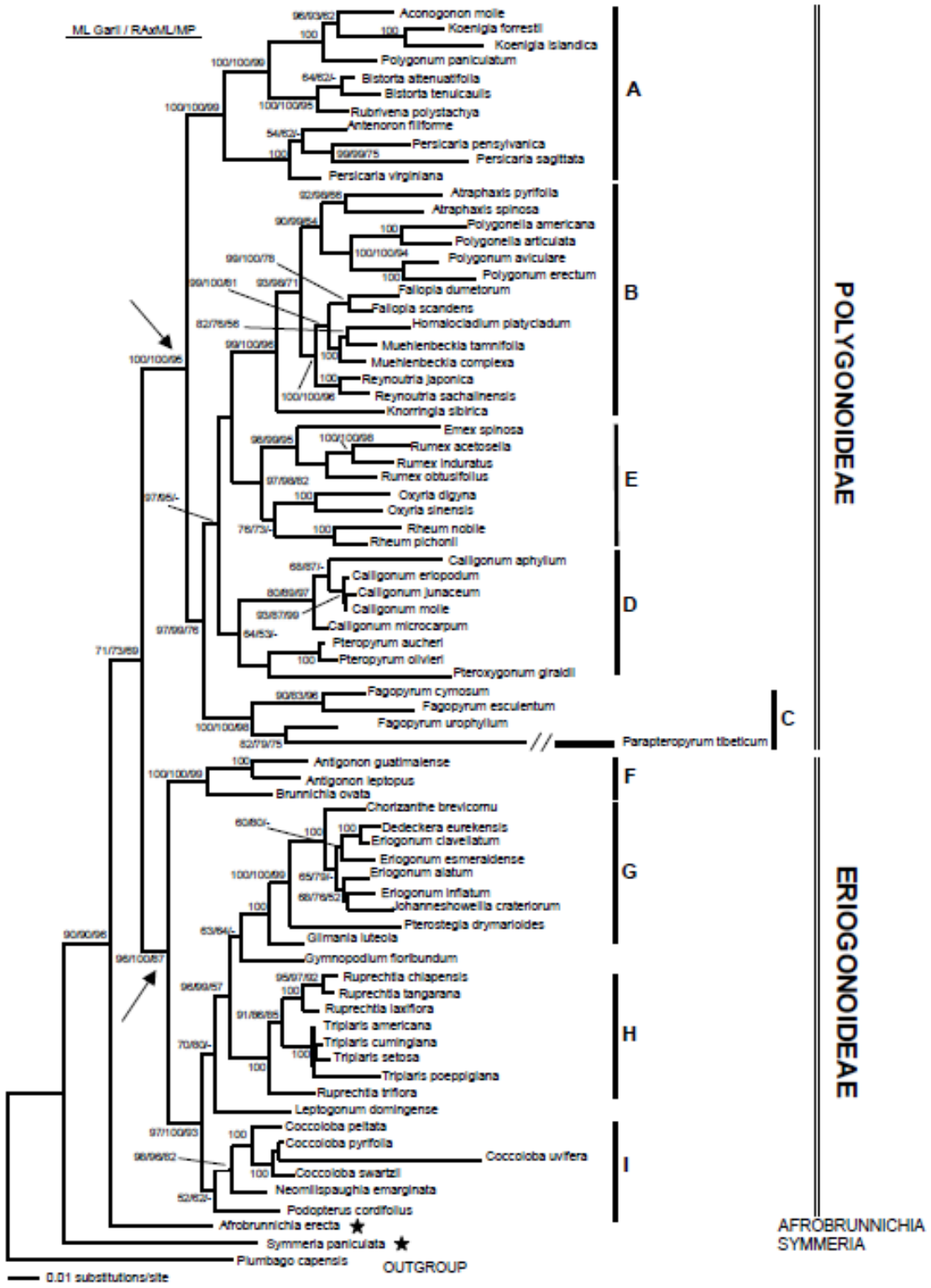
Figure 2.3.

Topology resulting from maximum likelihood analyses of the combined analysis of ITS and the chloroplast DNA data set using Garli (TVM + I + G) and RAxML. Bootstrap support values (>50%) are indicated above or below the branches as Garli/RAxML/MP. Maximum parsimony (MP), recovered 40 most parsimonious trees (length = 6696, consistency index = 0.48, retention index = 0.65, rescaled consistency index = 0.31). When only one number is given, the bootstrap support for all analyses (Garli, RAxML and MP) is the same. Arrows indicate nodes for Polygonoideae and Eriogonoideae. Stars indicate taxa with different placement in different analyses.

Figure 2.3.


ML Garli / RAxML/MP
Aconogonon molle
Koenigia forrestii
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Persicaria virginiana
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Atraphaxis spinosa
Polygonella americana
Polygonella articulata
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Polygonum erectum
Fallopia dumetorum
Fallopia scandens
Homalocladium platycladum
Muehlenbeckia tamnifolia
Muehlenbeckia complexa
Reynoutria japonica
Reynoutria sachalinensis
Knorringia sibirica
Emex spinosa
Rumex acetosella
Rumex induratus
Rumex obtusifolius
Oxyria digyna
Oxyria sinensis
Rheum nobile
Rheum pichonii
Calligonum aphyllum
Calligonum eriopodum
Calligonum junaceum
Calligonum mole
Calligonum microcarpum
Pteropyrum aucheri
Pteropyrum olivieri
Pteroxygonum giraldii
Fagopyrum cymosum
Fagopyrum esculentum
Fagopyrum urophylum
Parapteropyrum tibeticum
Antigonon guatimalense
Antigonon leptopus
Brunnichia ovata
Chorizanthe brevicornu
Dedeckera eurekensis
Eriogonum clavellatum
Eriogonum esmeraldense
Eriogonum alatum
Eriogonum inflatum
Johanneshowellia crateriorum
Pterostegia drymarioides
Gilmania luteola
Gymnopodium floribundum
Ruprechtia chiapensis
Ruprechtia tangarana
Ruprechtia laxiflora
Triplaris americana
Triplaris cumingiana
Triplaris setosa
Triplaris poeppigiana
Ruprechtia triflora
Leptogonum domingense
Coccoloba peltata
Coccoloba pyrifolia
Coccoloba uvifera
Coccoloba swartzii
Neomillspaughia emarginata
Podopterus cordifolius
Afrobrunnichia erecta
Symmeria paniculata
Plumbago capensis
0.01 substitutions/site
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AFROBRUNNICHIA
SYMMERIA

CHAPTER III

PLACING THE WOODY TROPICAL GENERA OF POLYGONACEAE: A HYPOTHESIS OF CHARACTER EVOLUTION AND PHYLOGENY

Janelle M. Burke, Adriana Sanchez, Kathleen Kron, and Melissa Luckow

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Abstract

Premise of the study: Taxonomic groups have often been recognized on the basis of geographic distinctions rather than accurately representing evolutionary relationships. This has been particularly true for temperate and tropical members from the same family. Polygonaceae exemplifies this problem, wherein the woody tropical genera were segregated from temperate members of the family and placed in the subfamily Polygonoideae as two tribes: Triplarideae and Coccolobeae. Modern phylogenetic studies, especially when inferred from many lines of evidence, can elucidate more probable hypotheses of relationships. This study builds on previous work in the family and aims to test the traditional classification of the tropical woody taxa, which have been understudied and undersampled compared to their temperate relatives.

Methods: A phylogenetic study was undertaken with expanded sampling of the tropical genera with data from five plastid markers (*psbA-trnH*, *psaI-accD*, *matK*, *ndhF*, and *rbcL)*, nuclear ribosomal DNA (ITS) and morphology.

Key results: Results support the placement of nine of 12 genera of the Triplarideae and Coccolobeae within Eriogonoideae, in which these genera form a paraphyletic assemblage giving rise to Eriogoneae. The remaining woody tropical genera excluded from Eriogonoideae occur in the paleotropics.

Conclusions: Traditional characters used to delimit Coccolobeae and Triplarideae are not useful for defining monophyletic groups. The six-tepal condition is derived from the five-tepal condition, and unisexual flowers have arisen multiple times in different sexual systems. The ruminate endosperm has arisen multiple times in the family, suggesting this character is highly plastic.

Introduction

Tropical taxa are often understudied in comparison to their temperate relatives, and in this regard the Polygonaceae are no exception. Many phylogenetic studies have shown that the circumscription of tropical vs. temperate groups may be artificial, with the tropical group giving rise to the temperate members or vice-versa, such as in Aceraceae/Sapindaceae (Buerki et al., 2009) or Salicaceae/Flacourtiaceae (Chase et al., 2002). Taxonomic problems are often exacerbated when workers repeatedly rely on a set of "key" taxonomic characters, without reassessing their utility for phylogenetics or their homology. Our study not only examines relationships among traditionally recognized tropical and temperate groups in the Polygonaceae, but also investigates key taxonomic characters in a phylogenetic context. Conclusions about the plasticity and evolution of these characters may have broader application to studies of other angiosperm families.

The Polygonaceae Juss. have long been recognized as a distinct, relatively isolated family (e.g., Meisner, 1856; Bentham and Hooker, 1880; Cronquist, 1981; Takhtajan, 1997), distinguished at least in part by the presence of an ochrea, a sheathing structure associated with leaf nodes (e.g., Bartling, 1830; Lindley, 1830; Endlicher, 1837). Molecular phylogenetic studies have supported this distinction; the Polygonaceae are a monophyletic group, sister to the Plumbaginaceae Juss. (Fay et al., 1997; Cuénoud et al., 2002). Much taxonomic work over the last several decades has focused on the temperate taxa within Polygonaceae, in particular, the generic circumscription of *Persicaria* Mill., *Polygonum* L., and *Eriogonum* Michx. (Haraldson, 1978; Ronse Decraene and Akeroyd, 1988; Reveal, 1989; Freeman and Reveal, 2005; Kim and Donoghue, 2008; Galasso et al., 2009). In contrast, the infrafamilial classification is still in flux.

Until recently, the Polygonaceae were divided into two subfamilies, Polygonoideae Eaton and Eriogonoideae Arn. (Haraldson, 1978; Brandbyge, 1993; Freeman and Reveal, 2005), largely based on a suite of diagnostic morphological characters, such as presence/absence of ochrea, monopodial/sympodial growth and presence/absence of an involucre. However molecular phylogenies with a broad sampling of genera (Sanchez and Kron, 2008; Sanchez et al., 2009) have supported a rearrangement of the two long-recognized subfamilies. Eriogonoideae s.s. is monophyletic but is nested in a clade among many of the woody, tropical genera (hereafter WTG) previously placed in Polygonoideae, rendering Polygonoideae paraphyletic.

The WTG consist of 11 or 12 genera of trees, shrubs, and lianas with a predominately neotropical distribution. Besides a woody habit, the WTG can be distinguished from the rest of the Polygonoideae by the presence of ruminate endosperm and greatly expanded tepals in fruit (Meisner, 1856; Jaretzky, 1925; Brandbyge, 1993). Several workers (Dammer, 1893; Gross, 1913) have even treated the WTG as a third subfamily, Coccoloboideae Luerss. Although generic circumscription is not controversial among these taxa, the relationships among them are not clear.

Most previous treatments have subdivided the WTG into two tribes within Polygonoideae based on tepal number and breeding system. Triplarideae C. A. Mey. includes mostly dioecious genera with six tepals, while Coccolobeae Dumort. includes trees, shrubs, and lianas with five tepals (Table 1). Among the WTG, *Coccoloba* P. Browne is the most species-rich with 120 – 150 species distributed in the Caribbean and the Amazon basin (Howard, 1960; Brandbyge, 1993). This genus is best known by the

widespread species *Coccoloba uvifera* L., or sea grape, which grows along beaches. *Antigonon* Endl. and *Brunnichia* Banks ex Gaertn. are lianas, as is *Afrobrunnichia* Hutch. & Dalziel (most recently recognized as distinct from *Brunnichia* by Sanchez and Kron, 2009); *Symmeria* Benth., *Ruprechtia* C. A. Mey., and *Triplaris* Loefl. are dioecious trees found mostly in South America. *Gymnopodium* Rolfe, *Podopterus* Humb. & Bonpl., and *Neomillspaughia* S. F. Blake are restricted to dry forests of Central America where they are used commonly in honey production in the Yucatán Peninsula (Ortiz, 1994). *Leptogonum* Benth. is a genus of small trees endemic to Hispaniola, and members of *Muehlenbeckia* Meisn. are small shrubs, often with adaptations to harsh environments (e.g., *M. vulcanica* Meisn., volcanic soils and high altitudes) across the southern hemisphere.

Table 2 details previous comprehensive infrafamilial treatments and the characters that have been used to distinguish either two traditional subfamilies, the WTG, or the tribes Coccolobeae and Triplarideae. This summary demonstrates that most treatments have focused on several key characters, namely, the presence of ochrea, tepal number, habit, sexual system, and endosperm type. The ochrea morphologies are quite variable across the tropical genera. In *Triplaris* and some species of *Coccoloba*, this structure is terminal, conical, and caducous. In *Antigonon* and *Brunnichia*, the stipule is a raised line with 1 – 2 mm of scarious tissue. This variability led Meisner (1856) to segregate genera such as *Brunnichia* and *Symmeria* in their own subfamilies (Brunnichioideae Meisn., Symmerioideae Meisn.) based on the absence or near absence of ochrea. Likewise, Roberty and Vautier (1964) recognized three subfamilies based on ochrea type, whether it was absent, scarious, caduceus or persistent and sheathing. In

general, the complete absence of ochrea in the Eriogonoideae s.s. was used to distinguish this group as a separate subfamily or tribe (as Eriogoneae Dumort.), though still included in the Polygonaceae (Meisner, 1856; Bentham and Hooker, 1880).

Sexual systems among the WTG are variable as well. Several genera are strictly dioecious (*Ruprechtia*, *Symmeria*, and *Triplaris*). Species of *Coccoloba* may also exhibit a dioecious condition, though after closer inspection they are often found to be polygamodioecious (Howard, 1960). In polygamodioecy, individual plants may have inflorescences with both bisexual flowers and unisexual flowers of only one sex. A sexual system such as this might be thought of as transitional between bisexual flowers and a strictly dioecious condition. Dioecy has often been used as a character in previous classifications (Meisner, 1856; Bentham and Hooker, 1880; Dammer, 1893), but the polygamodioecious condition is more recently documented (Howard, 1960; Brandbyge, 1993) and has not been widely used taxonomically. Polygamodioecy is found in a wide range of taxa throughout Polygonaceae including *Muehlenbeckia* and other temperate genera such as *Rumex* L., *Reynoutria* Houtt., and *Eriogonum.*

Tepal number has also been used to diagnose subfamilies and tribes (Meisner, 1856; Bentham and Hooker, 1880; Dammer, 1893; Gross, 1913; Jaretzky, 1925; Buchinger, 1957), even though there is a discrepancy in the older literature regarding tepal number for various genera (see Blake, 1921 for discussion). In addition to uncertainty regarding tepal number, there is a rich literature debating the fundamental polygonaceous flower plan and whether the six-tepal or five-tepal condition is derived (see Bentham, 1836 and Lamb-Frye and Kron, 2003 for contrasting theories), or whether the fundamental floral plan is spirally arranged or whorled (sensu Eichler, 1878).

Ruminate endosperm is found in at least 58 plant families (Bayer and Appel, 1996), and in the Polygonaceae, it is common among the woody, tropical genera. As mentioned, ruminate endosperm was used as a distinguishing morphological character for the WTG (Meisner, 1856; Dammer, 1893; Gross, 1913). The character is defined in Polygonaceae as invaginations of the seed coat into the endosperm (Fig. 1). Often this feature occurs in a late stage of development of the seed (Lindau, 1891a). In Polygonoideae, endosperm is present but not ruminate.

Given the apparent disagreement between the revised circumscription of subfamilies supported by recent molecular phylogenies (Sanchez and Kron, 2008; Sanchez et al., 2009) and traditional classifications based on morphology, we set out to study the incongruence between the two. This study incorporates increased sampling of taxa, especially of the WTG, a morphological data set focused on the characters most often used in infrafamilial classification, and more molecular data (five plastid regions and nuclear ribosomal ITS) to construct a phylogeny of the tribes Eriogoneae, Triplarideae, and Coccolobeae. In addition to testing the subfamilial classification, we examine the utility of traditional morphological characters to delimit natural groups and their effect on branch support and tree topology. We use the phylogeny to explore any incongruence between the different data sets, with the goal of assigning morphological synapomorphies to well supported natural groups. Proposed scenarios of character evolution, such as the intermediacy of polygamodioecy and the floral bauplan for the Polygonaceae are also tested.

Materials and Methods

Taxon sampling — Taxa were sampled intensively for the WTG, including representatives from all 12 genera in Coccolobeae and Triplarideae sensu Brandbyge (1993) for a total of 42 accessions. For the large genus *Coccoloba* (ca. 150 spp.), 15 species were sampled to encompass geographic range and diversity in growth habit. In addition, 10 accessions representing six genera from Eriogoneae and 39 accessions from 19 genera in Polygonoideae were included to test subfamilial limits.

Members of Plumbaginaceae were chosen as outgroups. *Plumbago auriculata* Lam. (= *Plumbago capensis* Thunb.), previously used by Sanchez and Kron (2008), was sequenced, and available GenBank sequences of *Ceratostigma minus* Stapf ex Prain, *Limonium dufourii* Kuntze, and *Limoniastrum monopetalum* Boiss. were also used (Appendix 1).

Molecular data collection — Total DNA was extracted from silica-dried leaf material when available. DNA extraction was completed with either a modified CTAB protocol (Doyle and Doyle, 1987) or with the Qiagen DNeasy Mini Plant Kit (Qiagen, Valencia, California, USA). Herbarium material was used for several species (see Appendix 1). For these species, plant material was manually ground and incubated for 18 h at 42 ° C with 600 μ L of an SDS-based buffer and 30 μ L of proteinase K before continuing with the protocol for the DNeasy kit, with a final elution of 100 μ L.

Data presented include nrITS and five plastid gene regions: three coding and two noncoding. Amplification for the regions ITS, *matK, ndhF,* and *rbcL* are described in Sanchez and Kron (2008). Primers for *psaI-accD* were from Shaw et al. (2007). Primers for *ycf6-psbM* were designed specific to Eriogonoideae to amplify a 600-bp region:

ycf6Fint (5 ′ -GAA GGG GAT ATG GAT GGT AAG-3 ′) and psbMRint (ATA GAA KAT ACA TAG GGY CCC). These regions were amplified with PCR conditions of a 25 μ L volume reaction with 5 μ L flexi buffer, 2 μ L MgCl 2, 1 μ L each primer [10 μ M], 0.13 μ L *Taq* polymerase. PCR cycling for *ycf6-psbM* was conducted at 52 ° C annealing. Products for *psaI-accD* were amplified using the "slow and cold" method of Shaw et al. (2005).

PCR products were run on a 1% agarose gel stained with ethidium bromide. Because of preferential primer binding, plastid intergenic spacers were cleaned with enzymes Exonuclease I and Antarctic Phosphotase (New England BioLabs, Ipswich, Massachusetts, USA) to remove residual PCR primers before adding one sequencing primer. All other regions were cleaned with Qiagen QIAquick PCR purification columns. Cleaned products were sequenced either at the Cornell Biotechnology Resource Center on an Applied Biosystems (Foster City, California, USA) 3730 DNA analyzer or on an ABI 377 DNA sequencer at Wake Forest University's Automated Sequencing Facility.

Several samples from herbarium specimens, for which there was no other material, were sequenced several times to acquire the best quality sequence. These included *Leptogonum* (ITS, *psaI-accD, ycf6-psbM*) and *Symmeria* (ITS). A complete list of taxa and vouchers, along with GenBank numbers, can be found in Appendix 1.

Morphological data — Twenty-two morphological characters were scored for 41 species in Polygonaceae and four species in Plumbaginaceae as outgroups (Appendix S1, see Supplemental Data at http://www.amjbot.org/cgi/content/full/ajb.1000022/DC1). Additional species not in the molecular data set were scored either because they represented morphological diversity for large genera or because ample herbarium

material was available for study (*Coccoloba barbadensis* Jacq., *C. densifrons* Mart. ex Meisn., *C. latifolia* Lam., and *C. lehmannii* Lindau). Characters selected were those traditionally used to define tribes Coccolobeae and Triplarideae (Meisner, 1856; Dammer, 1893; Gross, 1913; Blake, 1921; Jaretzky, 1925; Vautier, 1949; Roberty and Vautier, 1964; Haraldson, 1978; Brandbyge, 1993) in addition to novel characters. In the character list below, traditional characters are in lightface, and novel characters are in boldface.

1. Woodiness. Herbaceous (0), Suffrutescent (1), Woody (2)

2. Habit. Erect (0), Climbing (1)

3. **Stem pith.** Hollow (0), Solid (1). This character was coded as inapplicable for nonwoody taxa.

4. **Presence of salt-secreting glands on leaves.** Absent (0), Present (1)

5. Presence of ochrea. Absent (0), Present (1). Here, ochrea was defined as a sheathing structure with at least 1 mm of tissue above a circular scar at the leaf base. Can be caducous or persistent.

6. **Ochrea persistence.** Caducous (0), Persistent (1). Taxa distally caduceus were coded as caducous.

7. Sexual system. Bisexual flowers (synoecious) (0), Dioecious (1), Polygamodioecious (2).

8. **Floral stipe winged.** Absent (0), Present (1). All genera of tropical Polygonaceae have an articulation between the distal stipe and proximal pedicel of the flower; hence, a distinction is made between which structure is winged.

9. **Presence of distinct perianth whorls.** Absent (0), Present (1). This character distinguishes the sepals and petals (found in Plumbaginaceae) from tepals (Polygonaceae).

Subequal or dimorphic tepals in Polygonaceae were not coded as distinct, due to the cyclic nature of the perianth.

10. Tepal (perianth lobe) number. 4 (0), 5 (1), 6 (2). Because the Polygonaceae do not have a differentiated calyx and corolla, the perianth lobes are traditionally termed tepals.

11. **Perianth accrescent in fruit.** Absent (0), Present (1). A perianth was scored as accrescent if there was a lengthening of perianth between anthesis and fruiting stage, and the perianth completely enclosed the fruit at maturity.

12. Part of accrescent perianth enclosing fruit. Hypanthium (0), Inner tepals (1), Outer tepals (2), Tepal lobes (3), Whole perianth (4). Taxa without accrescent tepals were coded as inapplicable. Most Polygonaceae flowers have a well-developed hypanthium, though this term is not often used in the literature. Howard, in his papers on *Coccoloba* (1960) and for the flora of Nicaragua (Howard, 2001), drew attention to the hypanthium as a structure because it was useful to distinguish the part of the perianth enclosing the fruit: hypanthium (proximal) or tepal lobes (distal). We followed Howard and applied the term hypanthium to accurately describe the fusion of tepal and staminal tissue at the base of the flower.

13. Perianth texture in fruit. Hyaline-chartaceous (0), Coriaceous (1), Fleshy-succulent (2). This character is independent of whether the tepals are accrescent as all taxa had some part of the perianth persistent in fruit.

14. Stamen number. 3 (0), 5 (1), 6 (2), 7 (3), 8 (4), 9 (5), greater than 20 (6).

15. **Filament morphology.** Filiform or fl attened (0), Dilated (1), Subulate (2). The filament morphology was based on the proximal half of the stamen. Dilated state was

scored when the width of the stamen was wider at the base than the apex. Subulate is similar to dilated, except the base is also recurved and not lying in one plane.

16. **Stamen fusion.** Free until fused to short hypanthium (0), Adnate to perianth for most of length (1), Connate (2).

17. Carpel number. 2 (0), 3 (1), 4 (2), 5 (3).

18. **Style fusion.** Absent (0), Present (1). The difference between style and stigma was discerned based on the presence of papillae or ornamentations on the stigmatic surface. Partially fused styles were coded as present.

19. Stigma morphology. Capitate (0), Peltate (1), Fimbriate (2), Decurrent along adaxial surface (3), Penicillate (4), Bifi d (5).

20. Fruit type. Achene (0), Capsule (1), Utricle (2).

21. **Achene shape.** Shape of the achene was coded based on 3-dimensional form and shape in cross section. Trigonous: cross section bluntly 3-lobed, sides indented (0), Terete: cross section round, oblong (1), Globose to subglobose: cross section circular, spherical; (2), Pyramidal: cross section triangular (3), Lenticular: cross section ellipsoid (4). Members of Plumbaginaceae were coded as inapplicable.

22. Endosperm type. Uniform (0), Ruminate (1). See Fig. 1 for explanation.

Five to 10 specimens of each species were coded. Missing data were coded as ambiguous in a parsimony analysis. All characters are unordered. Dissections were made from herbarium specimens and rehydrated with boiling water with 1 – 5% detergent. Pictures of dissected material (Fig. 1) have not been altered, except the background has been lightened to increase contrast and remove shadows.

Sequence alignment and phylogenetic analyses — Alignment was conducted with the programs MUSCLE (Edgar, 2004) or Mafft (Katoh et al., 2005) and subsequently adjusted by hand. The alignment of coding plastid regions was unambiguous. Sequences of *ycf6-psbM* and *psaI-accD* for *Persicaria virginiana* (L.) Gaertn. were excluded because they were too divergent to align. An expanded sampling for Polygonoideae, to test subfamily delimitations, was not included for the two intergenic spaces due to length variation and ambiguity of alignment. For the region *psaI-accD* two short regions (totaling 71 bp) were excluded due to variation in length of homopolymer repeats. Each gene region was initially partitioned and analyzed separately, then concatenated for a combined molecular data set. Statistics for the molecular matrix are shown in Table 3. Insertions/deletions were not coded as characters in this analysis.

Alignment was straightforward for ITS across representatives of Polygonaceae in the conserved regions (e.g., 5.8S) but the spacers showed high levels of variability. A total of 114 ambiguous base pairs were excluded due to dubious alignment. Because ITS alignment was variable, a sensitivity analysis was conducted, including several alignments with Mafft, MUSCLE, and DIALIGN (Morgenstern, 2004) used to test the effects of alignment on the recovered topology (available from the fi rst author upon request). Different alignments produced highly congruent topologies, indicating that the phylogenetic results are robust and not highly sensitive to changes in alignment. The aligned matrices of molecular data are available at http://purl.org/phylo/treebase/phylows/study/TB2:S10420, study number 10420. Maximum parsimony analyses were conducted in the program TNT (Goloboff et al., 2008). Matrices were mopped to contain only informative characters and first analyzed

with TBR and xmult with 10 starting trees and 10 search replicates per search with 1000 replicates. Trees from each run were then analyzed with at least 600 iterations of the ratchet, 100 replicates of sectorial searches, 100 replicates of tree fusion, and 100 replicates of tree drift. This search was repeated several times until no additional MP trees were recovered.

Maximum likelihood (ML) analyses were performed using the program GARLI (Zwickl, 2006). Before likelihood analyses, each data set was analyzed with the program ModelTest (Posada and Crandall, 1998) in the program PAUP* (Swofford, 2002) for the best model of evolution for the data set. The model GTR+I+G was selected as the optimal model for each data set analyzed and was implemented in GARLI. Configuration settings were kept at default in GARLI except the setting attachments per taxon was changed to more than two times the total taxa analyzed (200 for the combined data set) and "genthreshfortopoterm" was changed to 50 000, with two search replicates per generation.

Support values were calculated with a statistical bootstrap. Parametric bootstraps were conducted in the program TNT with 10 starting trees and 10 search replicates, saving the best tree, for 1000 replicates. Nonparametric bootstrap values for ML were calculated based on 200 replicates with only one search replicate per bootstrap replicate due to the inordinate amount of time required to add additional search replicates.

Before analysis, matrices from different data sets were tested for incongruence using the incongruence length difference (ILD) test (Farris et al., 1994). We decided to combine data sets if they were found to be not significantly incongruent. In cases of moderate significance of incongruence ($P = 0.03 – 0.05$), we also assessed support for

conflicting topologies of the analysis before deciding whether to combine. We questioned results with moderate significance due to some documented issues with the ILD test such as: (1) possibility of increased type I error for this test (Cunningham, 1997) and (2) ability for the test to reject congruence due to variation in rate of character evolution (Dolphin et al., 2000).

Results

Phylogenetic relationships — *Molecular* — Table 3 presents a complete list of gene regions and combined data matrix statistics. This study generated 174 new sequences (Appendix 1). The remaining sequences were taken from GenBank. Of the six gene regions sampled, ITS was the most variable region (69.7%) and the most parsimony informative (45.7%), and *ndhF* was the least parsimony informative (20.0%). The two intergenic spacers had the lowest portion of parsimony-informative characters, but taxon sampling was mostly limited to the Eriogonoideae so the numbers are not directly comparable. The combined molecular data set had 19.9% missing data in terms of gene region sampling for ingroup taxa mostly due to the limited number of genera and species sampled from Polygonoideae for the two intergenic spacers, as compared to the other molecular regions. The combined data set for all regions was 5984 bp long and contained 24.7% parsimony informative characters. The combined plastid data set was 5131 bp long and contained 21.3% parsimony informative characters.

ITS and combined plastid data sets were found to be incongruent with moderate significance ($P = 0.03$). The decision was made to combine the molecular data sets, based on the lack of support for conflicting topologies in the ITS tree. These topological incongruences are discussed below. The ILD test also rejected congruence with high significance ($P = 0.0196$) for the combined molecular and morphological data sets. In this case, we did not combine. Instead we present a tree generated from molecular data, which had higher support values and resolution, and mapped morphological characters on it (Fig. 3), although we also discuss some of the results from the total evidence analysis.

Phylogenetic trees reconstructed from ITS and combined plastid regions were largely concordant (results not shown) under a maximum parsimony criterion. Plastid and ITS trees supported the same members of the major clades, with only one topological incongruence regarding the WTG taxa. *Afrobrunnichia* was placed sister to Polygonoideae based on the plastid data set (44% bootstrap) and was at the base of the tree with evidence from ITS (72% bootstrap). Under the maximum likelihood criterion, the ITS data set recovered a most likely tree where *Symmeria* and *Brunnichia* + *Antigonon* were sister to Polygonoideae, though with little support (11%/22% bootstrap, respectively). The placement of *Symmeria* and *Afrobrunnichia* for the plastid ML analysis was different from MP as well. For ML, *Symmeria* is nested within the six tepal clade, and *Afrobrunnichia* is sister to this same clade (results not shown).

Results from the maximum parsimony analysis (MP) for the combined molecular data set recovered nine trees (L = 5742, CI = 0.42, RI = 0.71). The tree with the lowest likelihood score from maximum likelihood analyses (ML) was Ln = − 46 868.69 (Appendix S2, see online Supplemental Data). Results from combined molecular analyses for both MP and ML recovered with 89/88% bootstrap scores, respectively, for a broad Eriogonoideae (sensu Sanchez and Kron, 2008), excluding *Afrobrunnichia*, *Symmeria,* and *Muehlenbeckia* (Fig. 2). *Symmeria* and *Afrobrunnichia* are at the base of the tree with 100% and 93% parsimony bootstrap support, respectively. In the ML analysis, *Symmeria* is at the base of the tree and *Afrobrunnichia* is sister to the rest of Polygonoideae with very little bootstrap support (28%, Fig. 2, Appendix S2). *Muehlenbeckia* is nested within Polygonoideae, consistent with previous analyses (Lamb-Frye and Kron, 2003; Won et al., 2007; Galasso et al., 2009; Sanchez et al., 2009).

The first diverging lineage in Eriogonoideae is a strongly supported clade of *Antigonon* + *Brunnichia* (100% bootstrap support). The next two major clades distinguish taxa with five tepals (*Coccoloba, Neomillspaughia)* from those with six tepals, with the exception of *Podopterus,* which is sister to the six-tepal clade (30% and 61% for MP and ML). The second clade includes the six-tepaled taxa: *Leptogonum, Triplaris, Ruprechtia, Gymnopodium,* and the genera of Eriogoneae. *Leptogonum* is sister to the other genera with moderate support (80% and 87% for ML and MP, respectively). Two subclades are strongly supported as monophyletic within the six-tepaled taxa: a clade of *Triplaris* and *Ruprechtia* (Fig. 2) and Eriogoneae (monophyletic with 100% bootstrap support). *Gymnopodium* is placed as sister to Eriogoneae (100/88% bootstrap) in both analyses.

The WTG of Polygonaceae are not monophyletic in the recovered tree nor are the tribes Coccolobeae or Triplarideae (compare Fig. 2 and Table 2). *Afrobrunnichia* and *Muehlenbeckia* are in the Polygonoideae clade, and the remaining genera of the Coccolobeae (*Antigonon, Brunnichia, Coccoloba, Neomillspaughia,* and *Podopterus)* form a paraphyletic assemblage, giving rise to the six-tepaled taxa. In turn, the genera of Triplarideae (*Gymnopodium, Leptogonum, Ruprechtia,* and *Triplaris),* excluding *Symmeria,* form a paraphyletic group giving rise to Eriogoneae.

Morphology — The strict consensus tree from parsimony analysis of morphological characters (L = 102, N = > 10 000) was largely unresolved (results not shown). The Polygonaceae were recovered as strongly monophyletic. In contrast to molecular results, the genera of Eriogoneae make up the basal lineages of the tree, with the tropical genera nested within, although none of these relationships are well supported (< 50% bootstrap).

Combined morphology and molecular — A combined parsimony analysis of both data sets for a subset of taxa (59) produced 312 most parsimonious trees (L = 3597) with a topology almost identical to the molecular tree (Fig. 2), with the exception of *Gymnopodium*, which is placed, with low support, as sister to *Ruprechtia* and *Triplaris* (results not shown). The bootstrap value for Eriogonoideae was 71%; support for other internal clades was also lower than with molecules alone, with the exception of Eriogoneae, which was recovered with 100% support and the *Brunnichia + Antigonon* clade with 99% support.

Of the 22 morphological characters examined, none provided an unreversed synapomorphy for Eriogonoideae, neither when analyzed together with molecular data nor when mapped on a tree generated from molecular data. However, several characters added support to clades within Eriogonoideae such as tepal number, supporting a six-tepaled clade, and absence of ochrea, in Eriogoneae (Fig. 3). Many of the traditional taxonomic characters were homoplasious. The least consistent characters within Polygonaceae were endosperm type (no. steps = 7, ci = 0.14, Fig. 3), stipe wings (no. steps = 6, ci = 0.16), ochreae persistence (no. steps = 7, ci = 0.14), stem pith (no. steps = 6, ci = 0.16), and stamen fusion (no. steps = 12, ci = 0.16). Characters with higher consistency indices were stamen number (no. steps = 8, ci = 0.62), gynoecium merosity (no. steps = 4, ci = 0.50), achene shape (no. steps = 6, ci = 0.66), tepal number (no. steps = 5, ci = 0.40, Fig. 3), and ochrea presence (no. steps = 2, ci = 0.50, Fig. 3).

Discussion

Monophyly and relationships among genera — Based on many taxonomic treatments, the WTG were thought of as either representing a separate subfamily or derived from within

Polygonoideae (Dammer, 1893; Gross, 1913; Roberty and Vautier, 1964). Heintze (1927) departed from the common evolutionary scenario when he postulated that both Polygonoideae and Eriogonoideae were derived from a more primitive Coccoloboideae (synonymous here with WTG). Reveal (1978) considered the WTG and Eriogoneae to be monophyletic groups derived from the same Polygonaceous tropical ancestor. None of these evolutionary hypotheses is supported by our data. Instead, this study corroborates previous molecular phylogenetic studies in the family (Sanchez and Kron, 2008; Sanchez et al., 2009). Eriogonoideae is a monophyletic subfamily containing most members of WTG, excluding the genera *Afrobrunnichia, Muehlenbeckia,* and *Symmeria* (Figs. 2, 3). Even with increased sampling of gene regions and outgroups compared to previous studies, the position of *Symmeria* and *Afrobrunnichia* remains at the base of the tree (as in Sanchez and Kron, 2009; Sanchez et al., 2009). We are confident that *Symmeria* is not closely related to the rest of Eriogonoideae; however, the position of *Afrobrunnichia* changes depending on the gene region used as data; hence, we are not confident about its position and have excluded it from Eriogonoideae until additional supporting evidence is discovered.

A number of other relationships have strong support based on molecular data. The clade of *Antigonon* + *Brunnichia* is monophyletic (Figs. 2, 3), with morphological synapomorphies such as the climbing habit and short, caducous ochrea. Our analyses also

clarify the position of *Neomillspaughia emarginata* S. F. Blake as sister to *Coccoloba.* Previous to its segregation as a new genus by Blake (1921), *Neomillspaughia* had been placed either in *Campderia* Benth. (Donnell Smith, 1899) or *Podopterus* (Gross, 1913). *Podopterus* and *Neomillspaughia* were placed together taxonomically based on their similar habit (dry forest shrubs) and presence of membranaceous tepals surrounding the fruit (Gross, 1913). Although *Neomillspaughia* lacks the fleshy tepals in fruit and globose achene found in most species of *Coccoloba,* the two genera share numerous vegetative characters, including coriaceous leaves and similar ochrea morphologies. Given the affinity of *Neomillspaughia* to *Coccoloba* in our analyses, it appears that membranaceous tepals in fruit are plesiomorphic, and fleshy tepals have been derived in *Coccoloba.*

There have not been many subgeneric classifications of *Coccoloba. Campderia* was segregated as a genus by Bentham and Hooker (1880) and Donnell Smith (1899) but was subsequently recognized as a section of *Coccoloba* by Lindau (1891b). The characters distinguishing sect. *Campderia* from the other *Coccoloba* were the accrescent tepal lobes (instead of a hypanthium) in fruit and a perianth not adherent to the achene (Bentham and Hinds, 1844; Lindau, 1891b). For our morphological analyses, species that were previously placed in *Campderia* are *Coccoloba lehmannii, C. venosa* L., and *C. tenuifolia* L.; these do not form a monophyletic group in either the molecular or combined analysis. *Coccoloba venosa* and *C. tenuifolia* are in different subclades based on the molecular data, and *C. lehmannii* is placed as sister to the rest of *Coccoloba* based on morphological data alone. The latter differs from the other *Coccoloba* species sampled in the presence of coriaceous instead of fleshy tepals in fruit. Fleshy tepals seem to be a synapomorphy for the rest of the genus, but the combined analysis samples only 18 of ca.

150 species, so a more thorough assessment of variation across the genus is needed to determine the diagnostic value of this character.

Our results are ambiguous about the affinities of *Podopterus* relative to other genera (Figs. 2, 3). The genus consists of three species, two of which are sampled here and were recovered as sister with strong support (100%). This relationship is upheld by floral morphology, although they have different growth habits. *Podopterus mexicanus* Humb. & Bonpl. is a shrub of dry forests in southeastern Mexico, with white fl owers and the terminal buds on branches modified into spines. *Podopterus cordifolius* Rose & Standl. is a remarkable species with only a few populations documented so far in Mexico: Oaxaca and the type locality in Colima. They are large lianas, often with numerous stems and foliar growth in the dry forest canopy. Flowers appear in February and April, after the first rains, and are lilac, a unique character in Polygonaceae. There are several morphological similarities that suggest *Podopterus* is related to *Neomillspaughia,* such as membranaceous tepals with dorsal wings (Blake, 1921; Roberty and Vautier, 1964), but our study finds no convincing molecular evidence for such a relationship. In Sanchez et al. (2009) a relationship of *Podopterus* to *Neomillspaughia* and *Coccoloba* was supported by a bootstrap of 52 – 62%, but the addition of more molecular data and increased taxon sampling has changed this hypothesized relationship.

The six-tepaled clade is supported as monophyletic (Figs. 2, 3), with *Leptogonum* at the base of the clade. *Leptogonum* is a monospecific genus, rare and endemic to the serpentine soils on Hispaniola (J. Salazar, Universidad Autónoma de Santo Domingo, personal communication). Although *Leptogonum* is nested within Eriogonoideae and is sister to the remaining genera of the six-tepaled clade, it appears to have retained

ancestral characters. It is distinguished by leaves that are clustered apically on the shoots and by the absence of an accrescent perianth (Brandbyge, 1990), unlike other tropical members of Eriogonoideae. Brandbyge (1990) attributed the limited evolutionary success of the genus, as assessed by its limited range, to a lack of adaptations to dispersal, and he interpreted this feature as an ancient condition. To the contrary, it could be argued that *Leptogonum* has evolved to become exclusively adapted to serpentine soils and hence cannot expand its range beyond this soil type.

The South American, dioecious tree genera *Triplaris* and *Ruprechtia* form a clade with high support (95/99% bootstrap; Fig. 2) in which *Triplaris* is monophyletic but *Ruprechtia* is not. *Ruprechtia triflora* Griseb. is at the base of the clade but without strong support. Because of the morphological gradation between many characters, generic circumscription has been a taxonomic issue for these genera (Cocucci, 1961; Roberty and Vautier, 1964; Brandbyge and Øllgard, 1984; Pendry, 2004). One of the diagnostic characters that has been used is the extent to which the accrescent hypanthium is fused to cover the achene (Cocucci, 1961), a quantitative character. *Ruprechtia triflora* exhibits only 1 mm of hypanthium fusion, though this elongates in fruit. From the phylogenetic trees (Figs. 2, 3), this lack of fusion seems to be the ancestral trait, with more fusion (as it occurs in *Triplaris)* derived. More sampling of species from both genera is needed to test the monophyly of these genera and whether the generic circumscriptions hold.

Our analyses place *Gymnopodium* as the sister group to Eriogoneae (100/88% bootstrap), which is also supported by some morphological characters. *Gymnopodium* shares floral traits with this clade, such as filiform fi laments having relatively small

anthers. In addition, the ochreae exhibited by *Gymnopodium* are scarious and minute (1 – 2 mm), a possible intermediate state before the loss of ochrea observed in Eriogoneae.

Afrobrunnichia, Muehlenbeckia, and *Symmeria* were previously placed in Eriogonoideae but are not closely related to them in the present analysis. *Muehlenbeckia* is sister to *Fallopia,* where it shares characters such as vine habit and presence of extrafloral nectaries (Haraldson, 1978; Brandbyge, 1993). The genus *Symmeria* is monospecific, morphologically variable, and has a disjunct distribution with individuals occurring throughout the Amazon Basin (though restricted to Igapó forests) and West Africa (Brandbyge, 1988; Aymard and Howard, 2004). It also has many autapomorphic characters unique to Polygonaceae, such as dilated petiole wings covering the apical meristem (instead of a true ochrea), pyramidal achenes with three tepals adnate to the fruit wall, and male flowers with more than 20 stamens. All these characters suggest that *Symmeria* is highly derived. The other tropical genus excluded from Eriogonoideae is *Afrobrunnichia* (Sanchez and Kron, 2009), which differs from *Brunnichia ovata* (Walter) Shinners in its West African distribution and the presence of ruminate endosperm (absent in *Brunnichia,* Fig. 1). A more thorough discussion of these genera can be found in Sanchez and Kron (2009). The original placement of these genera among the other WTG genera likely was based on convergence of morphological characters such as habit, endosperm type, or fleshy tepals in fruit. Some of these characters are discussed later.

Character evolution — The ochrea, or sheathing structure surrounding leaf nodes, is observed in most members of Polygonaceae and is often thought of as a distinctive vegetative character for the family (Zomlefer, 1994; Simpson, 2005). This term was introduced into botanical usage and defined by Willdenow (1799, p. 440), but the

character had been used to recognize the group previously (e.g., Jussieu, 1789). Most species in Polygonaceae have outgrowths associated with leaf bases, but they may not be "ochrea-like", i.e., sheathing, persistent, and hyaline. In the phylogenetic hypothesis presented here (Fig. 3), the ochrea is a synapomorphy for Polygonaceae (with the possible exception of *Symmeria),* with one reversal in the Eriogoneae clade (depending on the placement and coding of some species of *Chorizanthe* R. Br. ex Benth., which may have a vestigial stipular structure; see Freeman and Reveal, 2005). The absence of ochrea led early workers, such as Bentham (1836) to treat Eriogoneae as a separate tribe, a taxonomic division we accept in this study.

In Polygonoideae, ochrea morphology is relatively uniform, with long ochreae having hyaline texture, though reduced ochreae are still common (e.g., *Calligonum, Koenigia*). Ochreae in this group are often persistent, though commonly split distally with age. Among the Eriogonoideae, we see a wide range in ochrea morphologies. In *Coccoloba,* as well as *Neomillspaughia* and *Triplaris,* the ochrea functions to enclose the emerging leaves and apical meristem and can be large, though it does not persist and soon abscises to leave a circular scar. In *Coccoloba,* ochreae are foliaceous and often distally caducous. In *Triplaris,* the shape of the stipule is conical and soon deciduous. Genera such as *Antigonon, Brunnichia,* and some species of *Ruprechtia* have reduced ochreae consisting of several millimeters of scarious tissue above a stipular line. And in *Podopterus cordifolius,* the ochreae are long, sheathing, and hyaline, more similar to those found in Polygonoideae. Overall, the leaf node structure as seen in Eriogonoideae may not be ochrea-like in the strict sense. Though not quantified here, it seems that the

ochrea is more variable in subfamily Eriogonoideae than in Polygonoideae and has not become "fixed" on one morphology.

Tepal number and floral bauplan in the progenitors to the family Polygonaceae have been debated for centuries. The most common condition in Polygonaceae is five or six tepals, with occasional reduction to three or four in such genera as *Koenigia* L., *Oxyria* Hill, and *Persicaria* (L.) Mill. The debate regarding the perianth of Polygonaceae has focused on ancestral merosity (five or six) and fundamental arrangement (spiral or whorled). Early on, Bentham (1836) postulated trimerous flowers with six tepals in two whorls as the primitive state. Later, Eichler (1878) published his ideas about floral arrangement in studies across the plant kingdom. He divided perianth arrangements into either cyclic (spirally arranged) or acyclic (whorled). This division was adopted by Dammer (1893) in his treatment of Polygonaceae. He placed the five-tepal taxa into the cyclic category, and the six tepals were treated as acyclic, thereby not limiting the floral bauplan in Polygonaceae to one condition.

Toward the 1920s, the theory shifted toward the tepals being spirally arranged, instead of in separate whorls (sensu Eichler), and the debate also focused on the ancestral condition for the family. Bauer (1922) conducted ontogenetic studies and concluded that the five-tepal, spirally arranged condition is fundamental, and four or six tepals represent the derived state, with the four or six tepal condition the result of variability in nutrient availability to the developing flower. Lundbald (1922), also using ontogenetic studies, concluded the opposite: the six tepal, spirally arranged condition is primitive, and the transitional arrangement in a five tepal flower is a "double tepal" and not homologous to the others. Figure 4 depicts the position of this transitional tepal. According to Lundbald'

s hypothesis, it is formed from the fusion of the adjacent tepals in the spiral arrangement, thereby reducing the tepal number from six to fi ve. Vautier (1949) also investigated the evolution of tepal number through careful anatomical studies. She maintained the view of a transitional tepal, but distinguished whether this tepal was inserted in a clockwise or counterclockwise fashion. Based on his own anatomical studies and a synthesis of previous literature, Laubengayer (1937) supported the six tepal condition as primitive. However, he found contradictory evidence: the tepal primordia were arranged in a spiral sequence, while the vasculature was arranged in whorls. Our results support the five-tepal condition as ancestral and the six-tepal condition as derived within the Eriogonoideae (Fig. 3). Our study also refutes the "transitional tepal" theory, in which the joined tepal is formed by reduction. The transition to six tepals is likely formed from an addition of a primordium in a spiral arrangement as opposed to a switch from a fundamental floral plan from spiral to whorled.

Within the Eriogonoideae, there are a number of different sexual systems. Strict dioecy appears as a synapomorphy for one clade: *Ruprechtia* and *Triplaris.* Sexual systems in species of *Coccoloba* have not been well documented and it is often hard to assess functionality of sexual organs based on herbarium specimens. Nevertheless, it is clear that both dioecy and polygamodioecy are found in the genus (Howard, 1960). We hypothesized that a "leaky" system such as polygamodioecy would be transitional between plants with bisexual flowers (ancestral) and strict dioecy (derived). However, this does not appear to be the case; the strictly dioecious genera (*Triplaris, Ruprechtia)* are in a separate clade from *Coccoloba* (Fig. 3), suggesting that these systems have evolved independently. In fact, polygamodioecy has evolved multiple times in

Polygonaceae: the condition also occurs in some species of *Eriogonum, Muehlenbeckia, Oxygonum* Burch., *Persicaria, Reynoutria,* and *Rumex.* Dioecy also occurs outside of Eriogonoideae, as in *Rheum* and *Symmeria* (Brandbyge, 1993; Freeman and Reveal, 2005).

The presence of ruminate endosperm was previously used as a character to distinguish subfamily Coccoloboideae consisting of all the WTG (Dammer, 1893; Gross, 1913). As mapped on our tree, this character appears to have evolved multiple times in Eriogonoideae (Fig. 3). Optimization is ambiguous at the base of Polygonaceae, so it is unclear if the presence of ruminate endosperm is plesiomorphic. In Fig. 3, it is optimized favoring parallelisms (DELTRAN). We chose this optimization because ruminate endosperm has evolved in parallel many times in the angiosperms (Bayer and Appel, 1996), and we lack evidence that this is a synapomorphy for Polygonaceae. We also discovered that ruminate endosperm varies among genera (Fig. 1). The amount of seed coat invagination is variable by stage of development and was only apparent in mature seeds, making it difficult to quantify different types of ruminate endosperm in seeds unless the seeds are all at the same stage of maturity. In some cases, what appear to be seed coat invaginations into the endosperm is actually a deeply lobed seed, as in *Brunnichia* (Fig. 1). Lack of homology in this character further supports the separation of *Brunnichia* from *Afrobrunnichia* because the latter has true ruminate endosperm. In *Coccoloba,* the one species investigated that did not appear to have ruminate endosperm was *C. lehmannii.* This species is placed as sister to the rest of *Coccoloba* in a morphological analysis (not shown). Combined with the lack of a fleshy hypanthium in

fruit, this supports its position as a basal species without some of the derived characters exhibited by the rest of the *Coccoloba* species sampled.

Future work will include a search for additional morphological characters with the potential to support natural groups. From his morphological studies, Galle (1977) concluded that the relationship of the flower to the stem and the ochreolae (fused bracteoles subtending the primary inflorescence) were the most phylogenetically informative characters. From this, he assumed an affinity between some tropical genera and Eriogoneae. Other characters worthy of investigation in Eriogonoideae may be palynological. Even though pollen morphology does not vary much within Eriogoneae, there is variability in organization of colpi, pores, and ornamentation among the other genera of Eriogonoideae (Nowicke and Skvarla, 1977; Mondal, 1997). Pollen characters have previously been used to posit an affinity between Polygonaceae and Plumbaginaceae (Nowicke and Skvarla, 1977) or to place Polygonaceae as a transitional family within Caryophyllales (Wodehouse, 1931).

Conclusions — This study is the first to address thoroughly the relationships among taxa in Eriogonoideae using morphological and molecular characters. The subfamily Eriogonoideae is supported as monophyletic if one excludes *Afrobrunnichia* and *Symmeria.* Coccolobeae and Triplarideae are not monophyletic, although there is strong support for a six-tepaled clade comprising Eriogoneae, *Gymnopodium, Leptogonum, Ruprechtia,* and *Triplaris.* We recommend that the circumscription of Coccolobeae be modified to include *Coccoloba, Neomillspaughia,* and possibly *Podopterus.* Triplarideae, to remain a monophyletic group, should only include the genera *Ruprechtia* and *Triplaris.* These tribes can easily be distinguished based on morphological synapomorphies. A

more comprehensive tribal treatment of the family is forthcoming (J. Burke and A. Sanchez, unpublished manuscript).

Morphological work did not elucidate synapomorphies for the subfamily Eriogonoideae, but we discovered that several characters traditionally used to delimit subfamilies and tribes are homoplasious. The ochrea is a highly variable character in Eriogonoideae, and its presence, in the strict morphological sense, may be restricted to the subfamily Polygonoideae. The six-tepaled condition is derived from five tepals and likely results from additional primordium to the floral plan. Polygamodioecy has evolved multiple times in Polygonaceae, and endosperm rumination is a variable character, sometimes confounded with a deeply lobed seed coat.

Our findings indicate that the woody, tropical genera of Polygonaceae have given rise to the temperate Eriogoneae, a tribe which is strongly supported as monophyletic. The latter is extremely diverse in the western North America, with some 325 species. Often tropical genera are treated as their own family or subfamily. Our study supports the origin of a diverse temperate group from a heterogeneous tropical assemblage, which was not previously hypothesized before the advent of molecular phylogenetics. This phylogenetic arrangement elicits broader evolutionary questions about dispersal from a tropical region, and rapid radiation in a new habitat.

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Appendix 3.1.

Voucher information for DNA extractions used in this study. Sequences obtained from GenBank are given with their respective site specific numbers. New sequences generated for this study provide the following information: taxon, collector(s), collection number (in italics), and GenBank accession numbers. Specimens are deposited at WFU (Wake Forest University) unless another herbarium is given. Herbarium acronyms follow Index Herbariorum, K = Royal Botanic Gardens Kew, E = Royal Botanic Gardens Edinburgh, BH= Cornell University, MO = Missouri Botanical Garden, NCU = University of North Carolina. Gene abbreviations are as follows: R = *rbcL* , M = *matK* , N = *ndhF*, P = *psbM-ycf6* , A = *psaI-accD* and I = ITS. NA= not used in this study.

(**1) Sequences obtained from GenBank.** ***Aconogonon molle*** (D. Don) Hara, R-EF653764, M-GQ206190, N-GQ206271, P-NA, Y-NA, I-EF653687; ***Afrobrunnichia erecta*** Hutch. & Dalziel, R-FJ154447, M-FJ154489, N-FJ154501, I-FJ154459; ***Antenoron filiforme*** (Thunb.) Roberty & Vautier (***Polygonum filiforme*** Thunb. ***subsp. neofiliforme*** (Nakai) Kitam.), R-GQ206211, M-NA, N-GQ206272, P-NA, Y-NA, I-GQ206237; ***Antigonon guatimalense*** Meisn., R-FJ154449, M-FJ154491, N-FJ154503, I-FJ154461; ***Antigonon leptopus*** Hook. & Arn., R-AF297146, M-EF437988, N-EF438027, I-FJ154462; ***Atraphaxis pyrifolia*** Bunge., R-GQ206212, M-GQ206191, N-GQ206273, P-NA, Y-NA, I-NA; ***Atraphaxis spinosa*** L., R-AF297123, M-EF437989, N-EF438028, I-FJ154463; ***Bistorta attenuatifolia*** Miyam. & H. Ohba, R-GQ206213, M-NA, N-NA, P-NA, Y-NA, I-GQ206239; ***Bistorta tenuicaulis*** Petrov, R-GQ206214, M-NA, N-GQ206274, P-NA, Y-NA, I-GQ206240; ***Brunnichia ovata*** (Walter) Shinners, R-FJ154451, M-EF437990, N-EF438029 I-FJ154465; ***Calligonum aphyllum*** (Pall.) G ü rke,

R-GQ206215, M-GQ206192, N-GQ206275, P-NA, Y-NA, I-GQ206241; ***Calligonum eriopodum*** Bunge, R-GQ206216, M-GQ206193, N-GQ206276, P-NA, Y-NA, I-GQ206242; ***Calligonum microcarpum*** Borszcz., R-GQ206218, M-GQ206195, N-GQ206278, P-NA, Y-NA, I-GQ206244; ***Chorizanthe brevicornu*** Torr. var. ***brevicornu*** , R-EF437974, M-EF437991, N-EF438030, I-FJ154466; ***Coccoloba swartzii*** Meisn., R-AF297150, M-EF437995, N-EF438034, I-FJ154469; ***Coccoloba uvifera*** (L.) L., R-AF206753, M-EF437996, N-NA, I-GQ206246; ***Dedeckera eurekensis*** Reveal & J. T. Howell, R-EF437976, M-EF437997, N-EF438036, I-FJ154470; ***Emex spinosa*** (L.) Campd., R-AF297142, M-AY042582, N-EF438037, P-NA, Y-NA, I-FJ154471; ***Eriogonum alatum*** Torr. var. ***alatum*** , R-EF437977, M-EF437998, N-EF438038, I-FJ154472; ***Eriogonum clavellatum*** Small, R-EF437979, M-EF438000, N-EF438040, I-GQ206247; ***Eriogonum esmeraldense*** S. Watson var. ***toiyabense*** J. T. Howell, R-EF437981, M-EF438003, N-EF438043, I-GQ206248; ***Eriogonum inflatum*** Torr. & Frém., R-EF437984, M-EF438006, N-EF438046, I-GQ206249; ***Fagopyrum cymosum*** Meisn., R-D86286, M-EF438008, N-GQ206280, P-NA, Y-NA, I-AB000329; ***Fagopyrum esculentum*** Moench, R-D86285, M-AB093087, N-EU254477, P-NA, Y-NA, I-AB000331; ***Fagopyrum urophyllum*** (Bureau & Franch.) H. Gross, R-D86288, M-AB026332, N-NA, P-NA, Y-NA, I-AB000342; ***Fallopia dumetorum*** (L.) Holub, R-FM883613, M-AM503813, N-AM503835, P-NA, Y-NA, I-AF040068; ***Fallopia scandens*** (L.) Holub, R-EF653785, M-NA, N-NA, P-NA, Y-NA, I-AF040069; ***Gilmania luteola*** (Coville) Coville, M-EF438010, N-EF438049, I-GQ206250; ***Gymnopodium floribundum*** Rolfe, R-GQ206220, M-GQ206197, N-GQ206282, I-GQ206251; ***Johanneshowellia crateriorum*** Reveal, R-EF437986, M-EF438011, N-EF438050, I-

GQ206252; ***Knorringia sibirica*** (Laxm.) S.P. Hong (= ***Polygonum sibiricum*** Laxm.), R-GQ206222, M-NA, N-GQ206284, P-NA, Y-NA, I-GQ206253; ***Koenigia forrestii*** (Diels) M ě sí č ek and Soják, R-AF297144, M-EF438012, N-EF438051, P-NA, Y-NA, I-GQ206254; ***Koenigia islandica*** L., R-EF653789, M-NA, N-EU840371, P-NA, Y-NA, I-EF653686; ***Leptogonum domingense*** Benth, R-GQ206223, M-GQ206199, N-GQ206285, I-GQ206256; ***Neomillspaughia emarginata*** (H. Gross) S.F. Blake, R-GQ206225, M-GQ206201, N-GQ206287, I-GQ206257; ***Muehlenbeckia complexa*** (A. Cunn.) Meisn., R-GQ206224, M-GQ206200, N-GQ206286, P-NA, Y-NA, I-AF040076; ***Muehlenbeckia platycladum*** (F.J. M ü ll.) L.H. Bailey, R-GQ206221, M-GQ206198, N-GQ206283, P-NA, Y-NA, I-AF189738; ***Muehlenbeckia tamnifolia*** Meisn. R-FJ154453, M-FJ154499, N-FJ154511, I-FJ154473; ***Oxyria digyna*** Hill, R-FJ154454, M-FJ154500, N-FJ154512, P-NA, Y-NA, I-FJ154474; ***Oxyria sinensis*** Hill, R-AF297148, M- F438013, N-EF438053, P-NA, Y-NA, I-GQ206258; ***Persicaria pensylvanica*** (L.) M. Gómez, R-AF297133, M-EF438017, N-EF438056, P-NA, Y-NA, I-FJ154475; ***Persicaria sagittata*** (L.) H. Gross, R-AF287141, M-EF438018, N-GQ206288, P-NA, Y-NA, I-FJ154476; ***Persicaria virginiana*** (L.) Gaertn., R-AF297135, M-EF438019, N-EF438058, I-FJ154477; ***Polygonella americana*** (Fisch. & C.A. Mey.) Small, R-GQ206226, M-GQ206202, N-GQ206289, P-NA, Y-NA, I-GQ206259; ***Polygonella articulata*** (L.) Meisn., R-EF653760, M-NA; N-GQ206290, P-NA, Y-NA, I-EF653683; ***Polygonum aviculare*** L., R-AF297127, M-EF438020, N-EF438059, P-NA, Y-NA, I-FJ154478; ***Polygonum erectum*** L. R-AF297128, M-GQ206203, N-NA, P-NA, Y-NA, I-GQ206260; ***Polygonum paniculatum*** Blume, R-AF297129, M-GQ206204, N-GQ206291, P-NA, Y-NA, I-GQ206255; ***Pteropyrum aucheri*** Jaub. & Spach, R-GQ206227, M-GQ206205, N-

GQ206292, P-NA, Y-NA, I-GQ206261; ***Pteropyrum olivieri*** Jaub. & Spach, R-GQ206228, M-NA, N-GQ206293, P-NA, Y-NA, I-GQ206262; ***Pterostegia drymarioides*** Fisch. & C.A. Mey., R-GQ206229, M-GQ206206, N-GQ206294, P-NA, Y-NA, I-GQ206263; ***Reynoutria japonica*** Houtt., R-AF297131, M-AY042586, N-EF438048, P-NA, Y-NA, I-AF189734; ***Reynoutria sachalinensis*** F. Schmidt Petrop., R-AF297125, M-EF438009, N-GQ206281, P-NA, Y-NA, I-AF189737; ***Rheum nobile*** Hook. & Thomson, R-AF297147, M-EF438021, N-EF438060, P-NA, Y-NA, I-GQ206264; ***Rheum pichonii*** Pierre ex F.B.Forbes & Hemnsl., R-GQ206231, M-GQ206208, N-GQ206296, P-NA, Y-NA, I-GQ206265; ***Rubrivena polystachya*** (Wall. ex Meisn.) M. Kr á l ***(=Polygonum polystachyum*** Wall. ex Meisn.), R-GQ206232, M-NA, N-GQ206297, P-NA, Y-NA, I-GQ206266; ***Rumex acetosella*** L., R-D86290, M-EF438022, N-GQ206298, P-NA, Y-NA, I-AF189730; ***Rumex induratus*** Boiss. & Reut., R-AF297122, M-AY042647, N-EF438061, P-NA, Y-NA, I-FJ154480; ***Rumex obtusifolius*** L., R-AF297126, M-EF438023, N-EF438062, I-FJ154481; ***Ruprechtia chiapensis*** Lundell ex Standl. & Steyerm. R-FJ154456, M-FJ154495, N-FJ154506, I-FJ154482; ***Ruprechtia laxiflora*** Meisn., R-EF437987, M-EF438024, N-EF438063, P-NA, Y-NA, I-FJ154484; ***Ruprechtia tangarana*** Standl ., R-GQ206233, M-EF438025, N-EF438064, I-FJ154485; ***Ruprechtia triflora*** Griseb., *Pendry* (E), R-GQ206234, N-GQ206299, P-NA, Y-NA, I-GQ206267; ***Symmeria paniculata*** Benth., R-GQ206235, M-GQ206209, N-GQ206300, P-NA, Y-NA, I-GQ206268; ***Triplaris americana*** L., R-Y16910, M-AY042668, N-FJ154508; I-FJ154486; ***Triplaris cumingiana*** Fisch. & C.A. Mey. ex C.A. Mey., R-GQ206236, M-GQ206210, N-GQ206301, I-GQ206269; ***Triplaris poeppigiana*** Wedd., R-AF297137 M-FJ154497, N-FJ154509, I-FJ154487; ***Triplaris setosa*** Rusby, R-

FJ154458, M-FJ154498, N-FJ154510, I-FJ154488; ***Ceratostigma minus*** Stapf ex Prain, R-Z97641, M-AY042566, N-NA, P-NA, Y-NA, I-NA; ***Limonium dufourii*** Kuntze, R-AJ286363, M-NA, N-NA, P-NA, Y-NA, I-AJ222840; ***Limoniastrum monopetalum*** Boiss., R-Z97642, M-AY042609, N-NA, P-NA, Y-NA, I-NA; ***Plumbago auriculata*** Lam., R-M77701, M-EF438026, N-EF438065, P-NA, Y-NA, I-GQ206270.

(2) Sequences generated in this study. Materials obtained from herbarium specimens are indicated with an asterisk (*) after the collection number. ***Afrobrunnichia erecta*** Hutch. & Dalziel, *Stone J., G. Walters, T. Nzabi & T. Mboumbore 3272* (MO), P-HM137447, A-HM137493; ***Antigonon cinerascens*** M.Martens & Galeotti, *Burke 8* (BH), R-HM137363, M-HM137385, N-HM137406, P-NA, A-HM137494, I-HM137427; ***Antigonon guatimalense*** Meisn., *Luckow 4634* (BH), P-HM137448, A-HM137495; ***Antigonon leptopus*** Hook. & Arn., *Luckow 4630* (BH), P-HM137449, A-HM137496; ***Atraphaxis spinosa*** L., *Anon. s.n.* (E), P-NA, A-HM137497; ***Brunnichia ovata*** (Walter) Shinners, *Alford 3851* (USMS), P-HM137450, A-HM137498; ***Chorizanthe brevicornu*** Torr. var. ***brevicornu*** , *Reveal 8462* , P-HM137451, A-HM137499; ***Coccoloba acapulcensis*** Standl., *Bruke 61* (BH), R-HM137364, M-HM137386, N-HM137407, P-HM137452, A-HM137500, I-HM137428; ***Coccoloba belizensis*** Standl., *Burke 72* (BH), R-HM137365, M-HM137387, N-HM137408, P-HM137453, A-HM137501, I-HM137429; ***Coccoloba brasiliensis*** Nees & Mart., *Harley 27120** (A), R-HM137366, M-HM137388, N-HM137409, P-HM137454, A-HM137502, I-HM137430; ***Coccoloba diversifolia*** Jacq., *Sanchez 102* , R-HM137367, M-HM137389, N-HM137410, P-HM137455, A-HM137503, I-HM137431; ***Coccoloba krugii*** Lindau, *Sanchez 108*, R-HM137368, M-HM137390, N-HM137411, P-HM137456, A-HM137504, I-NA;

Coccoloba latifolia Lam., *Sanchez 106,* R-HM137369, M-HM137391, N-HM137412, P-HM137457, A-HM137505, I-HM137432; ***Coccoloba northropiae*** Britton, *Sanchez 110,* R-HM137370, M-HM137392, N-HM137413, P-HM137458, A-HM137506, I-HM137433; ***Coccoloba pallida*** C. Wright ex Griseb., *Sanchez 112,* R-HM137371, M-HM137393, N-HM137414, P-HM137459, A-HM137507, I-NA; ***Coccoloba pubescens*** L., *Sanchez 103,* R-HM137372, M-HM137394, N-HM137415, P-HM137460, A-HM137508, I-HM137434; ***Coccoloba rugosa*** Desf., *Sanchez 101,* R-HM137373, M-HM137395, N-HM137416, P-HM137461, A-HM137509, I-HM137435; ***Coccoloba spicata*** Lundell, *Burke 54* (BH), R-HM137374, M-HM137396, N-HM137417, P-HM137462, A-HM137510, I-HM137436; ***Coccoloba swartzii*** Meisn., *Sanchez 109,* P-HM137463, A-HM137511; ***Coccoloba tenuifolia*** L., *Sanchez 111* , R-HM137375, M-HM137397, N-HM137418, P-HM137464, A-HM137512, I-HM137437; ***Coccoloba uvifera*** (L.) L., *Burke s.n.* (BH), P-HM137465, A-NA; ***Coccoloba venosa*** L., *Burke 126* (BH), R-NA, M-NA, N-NA, P-HM137466, A-HM137513, I-NA; ***Dedeckera eurekensis*** Reveal & J. T. Howell, *Reveal 8456* , P-HM137467, A-NA; ***Eriogonum alatum*** Torr. var. ***alatum*** , *Reveal 8515,* P-NA, A-HM137514; ***Eriogonum clavellatum*** Small, *Reveal & Broome 8478* , P-HM137468, A-HM137515; ***Eriogonum esmeraldense*** S. Watson var. ***toiyabense*** , *Tiehm 14537* , P-HM137469, A-HM137516; ***Eriogonum inflatum*** Torr. & Frém., *Reveal 8458* , P-HM137470, A-NA; ***Eriogonum umbellatum*** Torr., *Reveal 8526* , R-HM137376, M-NA, N-HM137419, P-HM137471, A-HM137517, I-HM137438; ***Fallopia aubertii*** (L. Henry) Holub, *Burke s.n.* (BH), R-HM137377, M-HM137398, N-HM137420, P-NA, A-HM137518; ***Gilmania luteola*** (Coville) Coville, *Reveal 8465* R-HM137378, P-HM137473, A-HM137520; ***Gymnopodium floribundum*** Rolfe, *Burke 48*

(BH), P-HM137474, A-HM137521; ***Gymnopodium fl oribundum*** Rolfe, *Burke 70* (BH), R-HM137379, M-HM137399, N-HM137421, P-HM137475, A-HM137522, I-HM137440; ***Johanneshowellia crateriorum*** Reveal, *Reveal 8469,* P-HM137476, A-HM137523; ***Leptogonum domingense*** Benth, *Gustafson 3077** (RSA), P-HM137477, A-HM137524; ***Muehlenbeckia tamnifolia*** Meisn., *Burke 18* (BH), P-HM137478, A-HM137525; ***Neomillspaughia emarginata*** (H. Gross) S.F. Blake, *Burke 66* (BH), P-HM137479, A-HM137526; ***Persicaria virginiana*** (L.) Gaertn., *Burke s.n.* (BH), P-NA, A-HM137527; ***Podopterus cordifolius*** Rose & Standl., *Burke 30* (BH), P-HM137480, A-HM137528; ***Podopterus mexicanus*** Bonpl., *Burke 27* (BH), R-HM137380, M-HM137400, N-HM137422, P-HM137481, A-NA, I-HM137441; ***Polygonum aviculare*** L., *Kron s.n.,* P-NA, A-HM137529; ***Reynoutria japonica*** Houtt., *Burke s.n.* , P-HM137472, A-HM137519; ***Rumex obtusifolius*** L., *Burke s.n.* , P-HM137482, A-HM137530; ***Ruprechtia chiapensis*** Lundell ex Standl. & Steyerm. *Burke s.n.* (BH), P-HM137483, A-HM137531; ***Ruprechtia coriacea*** (H. Karst.) S.F. Blake, *Sanchez 104,* R-HM137381, M-HM137401, N-HM137423, P-HM137484, A-HM137532, I-HM137442; ***Ruprechtia cruegeri*** Griseb. ex Lindau, *Luckow 4587* (BH), R-HM137382, M-HM137402, N-HM137424, P-HM137485, A-HM137533, I-HM137443; ***Ruprechtia pallida*** Standl., *Burke 129* (BH), R-HM137383, M-HM137403, N-HM137425, P-HM137486, A-HM137534, I-HM137445; ***Ruprechtia tangarana*** Standl ., *Silman s.n.** (WFU), P-HM137487, A-HM137535; ***Triplaris americana*** L., Luckow 4635 (BH), P-HM137489, A-HM137537; ***Triplaris cumingiana*** Fisch. & C.A. Mey. ex C.A. Mey., *Sanchez 100* , P-HM137490, A-HM137538; ***Triplaris poeppigiana*** Wedd., *Sanchez 89,* P-HM137491, A-HM137539; ***Triplaris setosa*** Rusby, *Fuentes 5351* (MO), P-HM137492, A-HM137540;

Triplaris weigeltiana (Rchb.) Kuntze, *Michelangeli s.n.*, R-HM137384, M-HM137405, N-HM137426, P-HM137488, A-HM137536, I-HM137446.

Table 3.1.

Traditional tribal circumscription of Coccolobeae and Triplarideae, based on Brandbyge (1993).

Tribe Coccolobeae Dumort.	Tribe Triplarideae C. A. Mey.
Afrobrunnichia Hutch. & Dalziel	*Gymnopodium* Rolfe
Antigonon Endl.	*Leptogonum* Benth.
Brunnichia Banks ex Gaertn.	*Ruprechtia* C. A. Mey.
Coccoloba P. Browne	*Symmeria* Benth.
Muehlenbeckia Meisn.	*Triplaris* Loefl.
Podopterus Humb. & Bonpl.	

Table 3.2.

Table depicting characters used by various workers to either segregate woody tropical genera from the rest of Polygonaceae or to distinguish tribes from each other. Tepal number and ochrea presence were the morphological characters mostly commonly used.

Treatment	**Characters used**						
	Sexual system	Tepal number	Habit	Presence of ochrea	Endosperm type	Stigma type	Perianth texture in fruit
Meisner 1856	**X**	**X**	**X**	**X**	**X**		**X**
Bentham & Hooker 1880	**X**	**X**		**X**			
Dammer 1893	**X**	**X**			**X**		
Gross 1913		**X**		**X**	**X**		
Jaretzky 1925		**X**	**X**	**X**			
Roberty & Vautier 1964			**X**	**X**		**X**	
Haraldson 1978		**X**		**X**		**X**	**X**
Brandbyge 1993		**X**	**X**	**X**	**X**		

Table 3.3.

Statistics for gene regions used in the phylogenetic analysis. Ingroup is defined as Polygonaceae taxa. Missing values for the two intergenic spacers are mostly from Polygonoideae.

Statistic/Partition	*matK*	*ndHF*	*rbcL*	*accD-psaI*	*psbM-ycf6*	ITS	Combined plastid	Combined molecular
Aligned length	876	1235	1315	957	748	853	5131	5984
% GC content range by taxon	27.3–34.3	27.8–39.0	40.7–45.8	25.7–29.1	33.9–37.2	45.6–77.9	—	—
Variable sites (%)	490 (55.9)	406 (32.9)	568 (43.2)	335 (35.0)	207 (27.7)	595 (69.7)	2006 (39,1)	2601 (43.5)
Parsimony informative characters (%)	335 (38.2)	247 (20.0)	265 (20.2)	144 (15.0)	100 (13.4)	390 (45.7)	1091 (21.3)	1481 (24.7)
Missing ingroup taxa (%)	11 (12.1)	5 (5.49)	1 (1.10)	43 (47.3)	45 (49.5)	4 (4.40)	105 (23.1)	109 (19.9)
CI	0.50	0.54	0.51	0.67	0.71	0.34	0.50	0.42
RI	0.77	0.80	0.75	0.84	0.87	0.66	0.75	0.71

Figure 3.1.

Diversity of ruminate endosperm in Polygonaceae achenes. (A) *Podopterus mexicanus,* Newman 63, US. (B). *Symmeria paniculata,* unknown specimen, GH. (C) *Brunnichia ovata,* Ford 2027, BH. (D) *Afrobrunnichia erecta*, FH1-16715, BH. (E) *Coccoloba latifolia*, Steyermark 114961, A. (F) *Antigonon leptopus,* Britton 3107, NY. Of the species shown, only *Brunnichia ovata* was scored as not ruminate. As seen, there are no invaginations of the seed coat into the endosperm, instead, the whole seed is lobed. All sections are cross sections except *A. leptopus,* which is tangential. Scale bar = 1mm.

Figure 3.1.

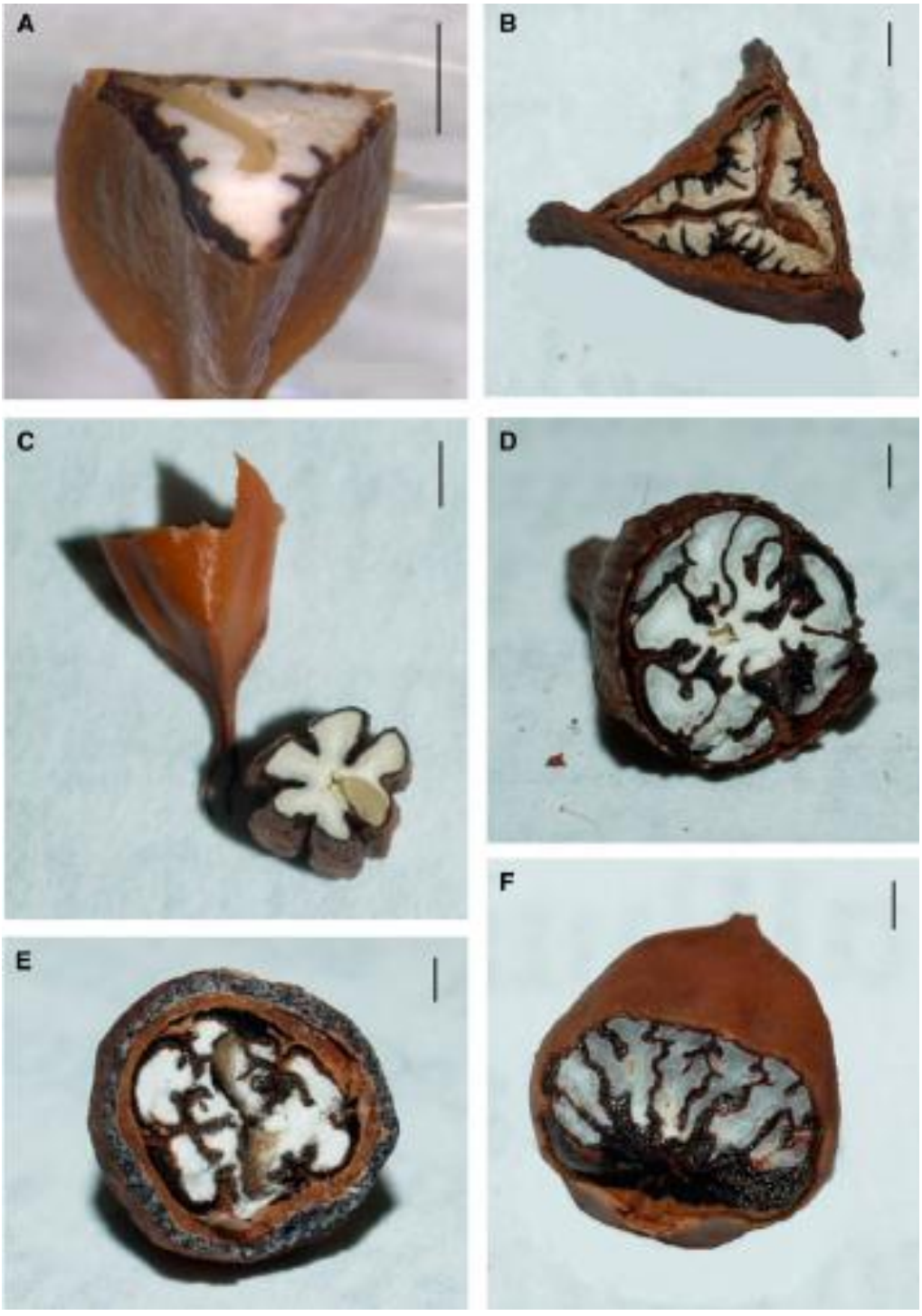

Figure 3.2.

Cladogram obtained from the strict consensus of the maximum parsimony (MP) analysis of the combined molecular data. The maximum likelihood (ML) phylogram can be found in online Appendix S2. Numbers below branches indicate bootstrap values for MP/ML. A hyphen (-) indicates a topology not supported by the ML tree. If only one number is present, both ML and MP had the same values, denoted by an asterisk (*).The dashed-line branch accents the lack of support for the phylogenetic placement of *Podopterus.* This position varies depending on the gene region and taxon sampling used in the analysis. Boldfaced taxa indicate members of the woody tropical genera. Recommended subfamily classification is depicted on right where *Symmeria* and *Afrobrunnichia* are incertae sedis (*).

Figure 3.2.

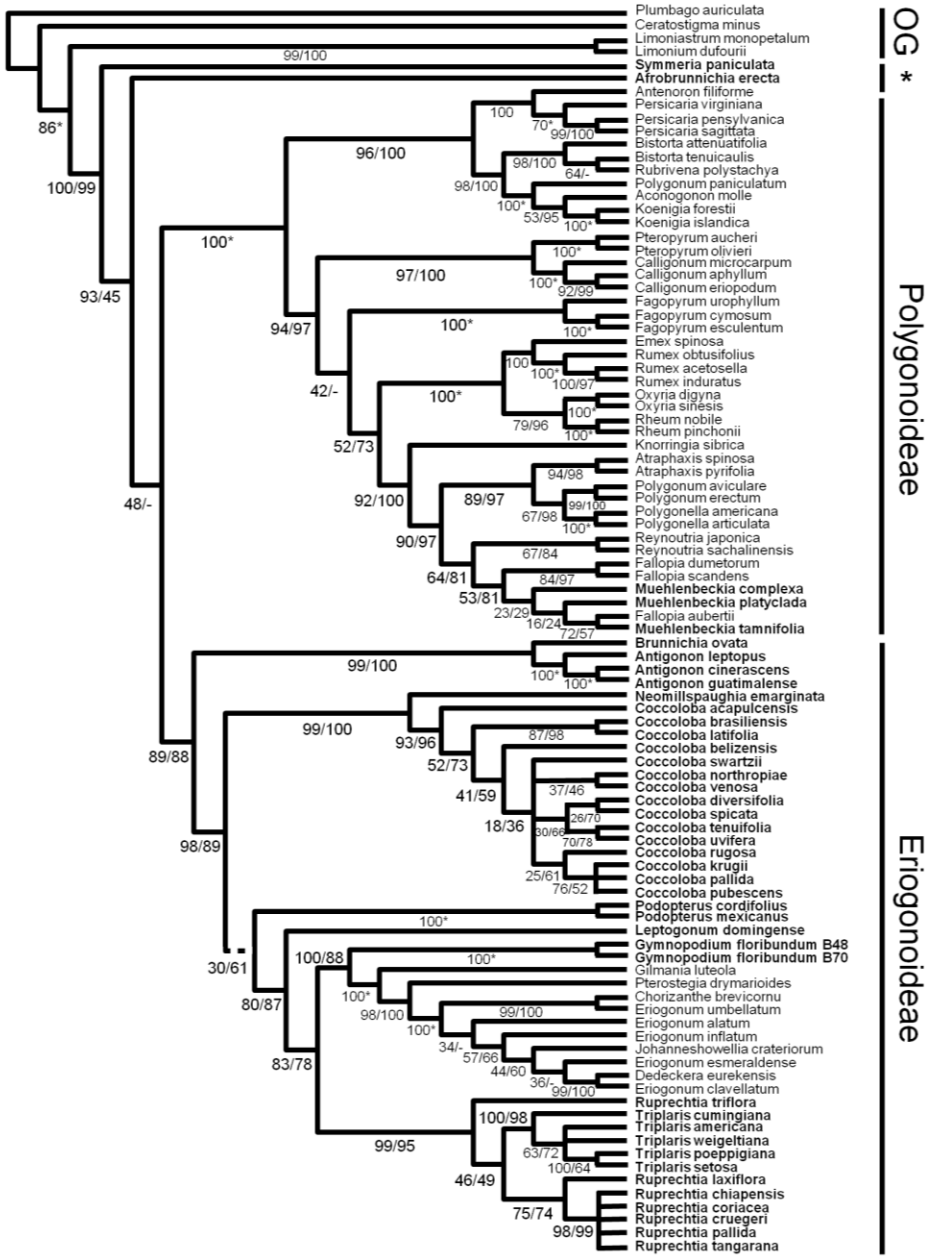
Plumbago auriculata
Ceratostigma minus
Limoniastrum monopetalum
Limonium dufourii
Symmeria paniculata
Afrobrunnichia erecta
Antenoron filiforme
Persicaria virginiana
Persicaria pensylvanica
Persicaria sagittata
Bistorta attenuatifolia
Bistorta tenuicaulis
Rubrivena polystachya
Polygonum paniculatum
Aconogonon molle
Koenigia forestii
Koenigia islandica
Pteropyrum aucheri
Pteropyrum olivieri
Calligonum microcarpum
Calligonum aphyllum
Calligonum eriopodum
Fagopyrum urophyllum
Fagopyrum cymosum
Fagopyrum esculentum
Emex spinosa
Rumex obtusifolius
Rumex acetosella
Rumex induratus
Oxyria digyna
Oxyria sinesis
Rheum nobile
Rheum pinchonii
Knorringia sibrica
Atraphaxis spinosa
Atraphaxis pyrifolia
Polygonum aviculare
Polygonum erectum
Polygonella americana
Polygonella articulata
Reynoutria japonica
Reynoutria sachalinensis
Fallopia dumetorum
Fallopia scandens
Muehlenbeckia complexa
Muehlenbeckia platyclada
Fallopia aubertii
Muehlenbeckia tamnifolia
Brunnichia ovata
Antigonon leptopus
Antigonon cinerascens
Antigonon guatimalense
Neomillspaughia emarginata
Coccoloba acapulcensis
Coccoloba brasiliensis
Coccoloba latifolia
Coccoloba belizensis
Coccoloba swartzii
Coccoloba northropiae
Coccoloba venosa
Coccoloba diversifolia
Coccoloba spicata
Coccoloba tenuifolia
Coccoloba uvifera
Coccoloba rugosa
Coccoloba krugii
Coccoloba pallida
Coccoloba pubescens
Podopterus cordifolius
Podopterus mexicanus
Leptogonum domingense
Gymnopodium floribundum B48
Gymnopodium floribundum B70
Gilmania luteola
Pterostegia drymarioides
Chorizanthe brevicornu
Eriogonum umbellatum
Eriogonum alatum
Eriogonum inflatum
Johanneshowellia crateriorum
Eriogonum esmeraldense
Dedeckera eurekensis
Eriogonum clavellatum
Ruprechtia triflora
Triplaris cumingiana
Triplaris americana
Triplaris weigeltiana
Triplaris poeppigiana
Triplaris setosa
Ruprechtia laxiflora
Ruprechtia chiapensis
Ruprechtia coriacea
Ruprechtia cruegeri
Ruprechtia pallida
Ruprechtia tangarana
OG *
Polygonoideae
Eriogonoideae

Figure 3.3.

One most parsimonious tree recovered from the molecular data set, with most Polygonoideae taxa pruned. When morphology is included in the analysis, *Gymnopodium* is placed sister to *Ruprechtia* + *Triplaris*. Characters are mapped, and optimization is unambiguous, except in the character of ruminate endosperm. Both five tepals and presence of ochrea were reconstructed as ancestral for Polygonaceae, while ruminate endosperm was ambiguous, here mapped as DELTRAN. The traditional tribal designations are shown on the tree with dashed lines to indicate they are not monophyletic. T = Triplarideae, C = Coccolobeae. WTG taxa are indicated with thick branches.

Figure 3.3.

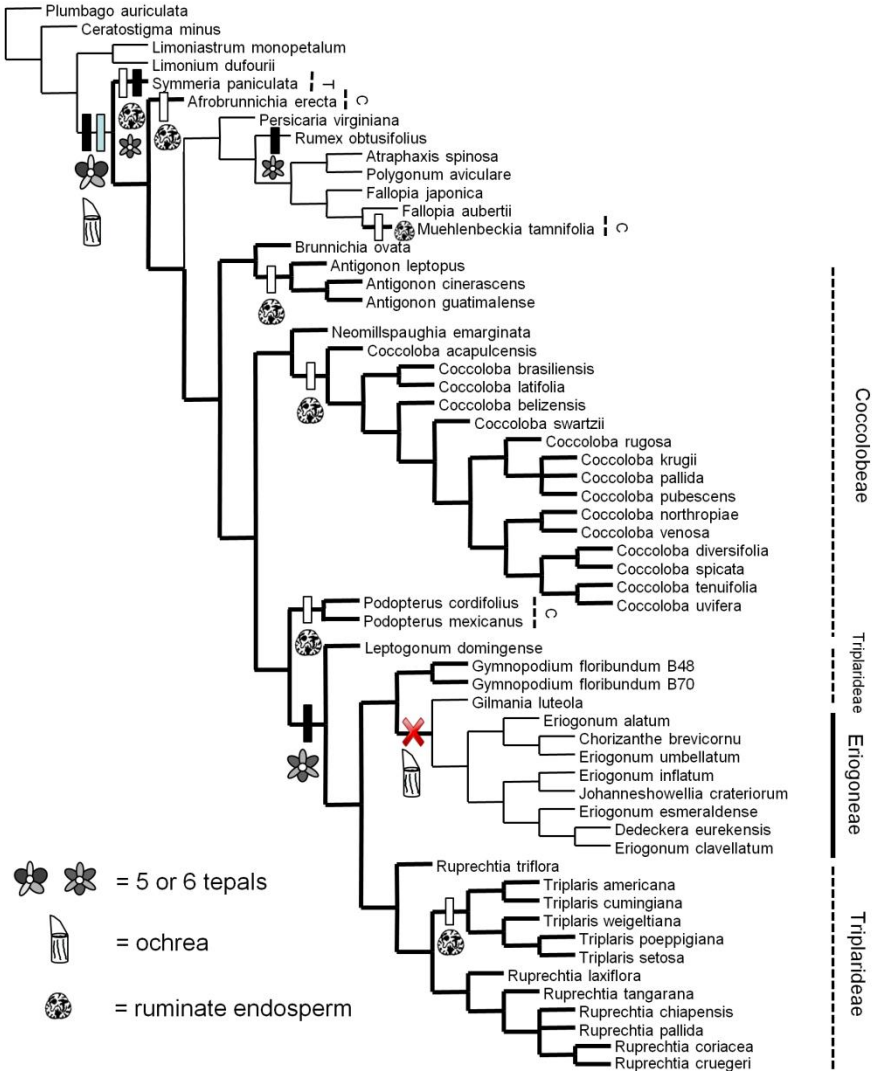
Plumbago auriculata
Ceratostigma minus
Limoniastrum monopetalum
Limonium dufourii
Symmeria paniculata
Afrobrunnichia erecta
Persicaria virginiana
Rumex obtusifolius
Atraphaxis spinosa
Polygonum aviculare
Fallopia japonica
Fallopia aubertii
Muehlenbeckia tamnifolia
Brunnichia ovata
Antigonon leptopus
Antigonon cinerascens
Antigonon guatimalense
Neomillspaughia emarginata
Coccoloba acapulcensis
Coccoloba brasiliensis
Coccoloba latifolia
Coccoloba belizensis
Coccoloba swartzii
Coccoloba rugosa
Coccoloba krugii
Coccoloba pallida
Coccoloba pubescens
Coccoloba northropiae
Coccoloba venosa
Coccoloba diversifolia
Coccoloba spicata
Coccoloba tenuifolia
Coccoloba uvifera
Podopterus cordifolius
Podopterus mexicanus
Leptogonum domingense
Gymnopodium floribundum B48
Gymnopodium floribundum B70
Gilmania luteola
Eriogonum alatum
Chorizanthe brevicornu
Eriogonum umbellatum
Eriogonum inflatum
Johanneshowellia crateriorum
Eriogonum esmeraldense
Dedeckera eurekensis
Eriogonum clavellatum
Ruprechtia triflora
Triplaris americana
Triplaris cumingiana
Triplaris weigeltiana
Triplaris poeppigiana
Triplaris setosa
Ruprechtia laxiflora
Ruprechtia tangarana
Ruprechtia chiapensis
Ruprechtia pallida
Ruprechtia coriacea
Ruprechtia cruegeri
= 5 or 6 tepals
= ochrea
= ruminate endosperm
Coccolobeae
Triplarideae
Eriogoneae
Triplarideae

Figure 3.4.

Photomicrograph of *Antigonon cinerascens* (Burke 34) with tepals labeled as acyclic, with a transitional or "double tepal" sensu Eichler or Lundbald. O = outer tepal, I = inner tepal, T = transitional. The transitional tepal has been interpreted as a fusion of one tepal from each whorl, a hypothesis contradicted by our analysis.

CHAPTER IV

PHYLOGENETIC RELATIONSHIPS OF *TRIPLARIS* AND *RUPRECHTIA*: RE-DELIMITATION OF THE RECOGNIZED GENERA AND TWO NEW GENERA FOR TRIBE TRIPLARIDEAE (POLYGONACEAE)

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Abstract

Recent phylogenetic work has increased our knowledge of the relationships within Polygonaceae. However, few studies have explored the generic relationships within Eriogonoideae. Two understudied genera are *Triplaris* and *Ruprechtia* (tribe Triplarideae), a group of approximately 55 Neotropical species of trees, shrubs and lianas. The generic classification of *Triplaris* and *Ruprechtia* has been unstable mostly due to different taxonomic interpretations and the difficulty of characterizing each genus morphologically. Although some studies have proposed diagnostic morphological characters for each, most have exceptions. In this study, we explored the phylogenetic relationships of 32 species of *Triplaris* and *Ruprechtia* using four chloroplast (*matK, ndhF, rps16-trnK, ndhC-trnV*), and two nuclear regions (ITS, second intron of *Leafy*). Results confirm the monophyly of *Triplaris,* but *Ruprechtia* is polyphyletic. To maintain monophyletic genera, two new names are proposed: ***Magoniella*** and ***Salta***. The two new genera are formally described and morphological synapomorphies are proposed for the four genera of Triplaridae.

Introduction

Several molecular phylogenetic studies have recently been carried out on Polygonaceae Juss. (Lamb-Frye and Kron 2003; Sanchez and Kron 2008; Sanchez et al. 2009; Burke et al. 2010). However, most work has focused on large-scale relationships at the family or generic level within Polygonoideae Arn. (e.g., Ohsako and Ohnishi 2000; Navajas-Pérez et al. 2005; Wang et al. 2005; Sanchez et al. 2009; Galasso et al. 2009). Eriogonoideae Arn., on the other hand, has remained largely unexplored. Only one recent study (Burke et al. 2010) focused on the relationships at the subfamily level by incorporating several molecular regions and morphological characters, but there are few studies at the generic level (Sanchez and Kron 2009). *Triplaris* Loefl. and *Ruprechtia* C.A. Mey. represent examples of genera largely understudied in Eriogonoideae.

Triplaris and *Ruprechtia* are woody plants (trees and shrubs, and in few instances, lianas) that share characters such as a terminal, conical ochrea enclosing the developing shoot and leaf, three-winged fruits, flowers with six tepals and six stamens, and trigonous achenes. *Triplaris* was first published by Loefling in 1758 (type: *T. americana* L.), while *Ruprechtia* was established as a new genus in 1840 by Meyer (lectotype: *R. ramiflora* (Jacq.) C.A. Mey.). *Ruprechtia* was segregated from *Triplaris* on the basis of its pyramidate, trisulcate, and semilocular achenes.

Triplaris and *Ruprechtia* were placed in tribe Triplarideae by Endlicher (1842) along with *Podopterus* Bonpl. Triplarideae was later reduced to a subtribe (within the nomenclaturally inadmissible “tribe Apterocarpae” of Polygonoideae) by Meisner (1856), while Bentham (1880) retained it as a tribe, but included other genera such as *Leptogonum* Benth. and *Symmeria* Benth. Dammer (1893) excluded *Podopterus* from

Triplarideae, and Gross (1913) maintained Dammer's circumscription adding the newly proposed genera *Gymnopodium* Rolfe and *Millspaugia* B.L. Rob. (=*Gymnopodium*). Roberty and Vautier (1964) maintained Triplarideae as circumscribed by Dammer (e.g., *Leptogonum*, *Ruprechtia*, *Symmeria* and *Triplaris*), but more recent treatments (Haraldson 1978; Brandbyge 1993) included *Gymnopodium*. Brandbyge's (1993) Triplarideae was characterized as dioecious trees and shrubs with a perianth in two whorls of three, and the outer tepals enlarged in the fruit.

Recent molecular work (Burke et al. 2010) found that there is no support for Triplarideae as previously recognized; as a result, a new circumscription has been proposed (Burke and Sanchez in press) by redefining the tribe to include only *Triplaris* and *Ruprechtia*. This new circumscription comprises approximately 55 species, distributed from northern Mexico to Argentina (except Chile), and the Antilles.

Although several authors have maintained *Ruprechtia* as distinct from *Triplaris* (i.e., Meisner 1856; Bentham 1880; Dammer 1893; Brandbyge and Øllgard 1984; Brandbyge 1986; Pendry 2004), others have expressed different views on the circumscription of both genera. Endlicher (1847) divided *Triplaris* into two sections: sect. *Triplaris* (as "*Eutriplaris*") with triquetrous subulate angled achenes, and sect. *Ruprechtia* (C.A. Mey.) Endl. with pyramidal trisulcate achenes. Kuntze (1891) transferred the 25 species of *Ruprechtia* named at his time to *Magonia* (a genus established by Vellozo in 1825). However, he soon realized that *Magonia* Vell. was a later homonym of *Magonia* A. St-Hil. (Sapindaceae), and then assigned the 25 names to *Triplaris* without further discussion (Kuntze 1898). Herzog (1922) created the monospecific genus *Enneatypus* (*E. nordenskjoeldii* Herzog = *Ruprechtia laxiflora*

Meisn.] and published *R. bolivensis* Herzog (= *R. apetala* Wedd.) simultaneously. Herzog linked *Enneatypus* to *Coccoloba* P. Browne, but did not discuss its possible relationship to *Ruprechtia*.

Cocucci (1957) changed the circumscription of *Triplaris scandens* (Vell.) Cocucci, a nomenclaturally superfluous name (non *T. scandens* Schott ex Meisn.) based on *Magonia scandens* Vell. and thus not legitimate, who included as synonymy *T. laurifolia* Cham. & Schltdl., *T. macrocalyx* Casar., *Ruprechtia lundii* Meisn., *R. obidensis* Huber, *R. macrocalyx* Huber, and *R. scandens* Rusby. This circumscription was disputed by Howard (1985) and Brandbyge (1989) who concluded that there were more than one species, and that those species were better placed in *Ruprechtia*.

Roberty and Vautier (1964) divided *Ruprechtia* into two genera: *Enneatypus* (with free and not auriculate sepals) and *Ruprechtia* (with fused and articulate sepals). However, their treatment is self-contradictory, as the lectotype of *Enneatypus*, *E. ramiflorus*, has fused sepals.

Ruprechtia currently comprises 37 species (Pendry 2004), and includes species previously assigned to *Enneatypus* and *Magonia*. As now defined, the genus is found in all Latin American countries (except Chile) with the highest diversity in Brazil (17 species), followed by Venezuela (eight species). The majority of the species are found in seasonally dry forests, although some grow in seasonally inundated and gallery forests. *Triplaris* includes 19 species (Brandbyge 1986, 1990) and it is found mostly in the Amazon Basin, with few species in Central America, the Antilles, and northern South America. The highest diversity occurs in Peru (11 species), followed by Brazil (eight species) and Colombia (seven species). *Triplaris* is considered a pioneer plant that grows

in seasonally inundated forests, along rivers, or in disturbed areas; however, there are some species found in dry forests in northern Colombia and in San Martin, Peru (Sanchez, pers. obs.).

Several morphological characters have been used to distinguish *Triplaris* from *Ruprechtia*, and are discussed in detailed in Cocucci (1961) and by Brandbyge and Øllgard (1984) (Table 1); however it is evident that some of these characters might not be useful for delimiting the genera. For instance, a one-flowered partial female inflorescence is shared by *Triplaris* and *R. triflora* Griseb., and a scar at the base of the fruits is a character shared between *Triplaris* and *R. obidensis* and *R. laurifolia* (Schltdl. & Cham.) C.A. Mey. (Table 1). Since no study has examined the validity of the characters for segregating *Triplaris* and *Ruprechtia* using a cladistic analysis, the hypothesis of this segregation remains untested. The only study that has included phylogenetic work is found in Pendry's (2004) monograph of *Ruprechtia.* In his work, he included an ITS analysis for 19 species, but rooted his tree with three species of *Triplaris*; therefore, he did not test for the monophyly of *Ruprechtia*. He considered that characters such as perianth tube shorter than achene, tubular bracteoles, pedicellate male flowers, and male perianth segments connate for one-third their length, constituted valid synapomorphies for the genus.

Since no previous study has tested the circumscription of *Triplaris* and *Ruprechtia*, this study aims to address that circumscription by using several molecular regions (four chloroplast genes, ITS and the second intron of *Leafy* [*lfy2i*]) and multiple outgroups from Eriogonoideae.

Materials and Methods

Taxon Sampling

For this study, a total of 32 putative species were sampled (Appendix 1). We included nine from *Triplaris* (of the 18 described [Brandbyge 1986, 1990]) and 19 from *Ruprechtia* (of the 37 currently recognized [Pendry 2004]), as well as four outgroups: *Antigonon leptopus* Hook. & Arn., *Coccoloba swartzii* Meisn., *Eriogonum alatum* Torr., and *Gymnopodium floribundum*.

DNA Extraction, Amplification, and Sequencing

Total DNA was extracted from silica-dried leaf material when available, or from herbarium material, using the Qiagen DNeasy Mini Plant Kit (Qiagen, Valencia, California, USA). In the case of herbarium samples (Appendix 1), plant material was manually ground and incubated for 18 h with 30 µL of BME (beta-mercapto-ethanol) and 30 µL of proteinase K before continuing with the protocol for the DNeasy kit, with a final elution of 100 µL. In some instances, we obtained extracted DNA aliquots through the generosity of Toby Pennington (Royal Botanical Gardens at Edinburgh), and Janelle Burke (Cornell University).

Data for this study includes the nrITS, four chloroplast regions: two coding (*matK, ndhF*) and two noncoding spacers (*trnV-ndhC, rps16-trnK*), and the second intron of the low-copy nuclear region *Leafy*. Protocols for standard polymerase chain reaction (PCR) followed those of Sanchez and Kron (2009). Primers for *trnV-ndhC* and *rps16-trnK* were derived from Shaw et al. (2007). Two different sets of primers for *lfy2i* were used. Multiple sequences were obtained with degenerate primers for the *lfy2i* from Frohlich and Meyerowitz (1997), but several others were amplified with Eriogonoideae specific

primers. The latter were designed by Janelle Burke (Cornell University) and amplified a region variable in size (from 750 to 1500bp): 1R (CCT GCC GAC ATA NTG GCG CAT CTT GGG CTT) and 3F (TGC AAG GGG TAA GAA GAA CGG CCT TGA).

PCR products were cleaned using Qiagen QIAquick PCR purification columns (Valencia, California). All sequences were run on an ABI 377 Automated DNA Sequencer (Ramsey, Minnesota) at Wake Forest University's Automated DNA Sequencing Facility. Sequences were edited with Sequencher v.3.1.1 (Gene Codes, Ann Arbor, Michigan). In the case of ITS and chloroplast DNA regions, PCR product purification was followed by direct sequencing. For *lfy2i*, purification was followed by cloning using the Invitrogen TOPO TA Cloning kit Carlsbad, California). Cloning products were cleaned with ExoSAP-IT (Affymetrix Inc, California) and three clones per sample were screened to evaluate sequence heterogeneity.

Sequence Alignment and Phylogenetic Analyses

Sequences were aligned using Mafft (Katoh et al. 2005) and subsequently adjusted by hand in MacClade version 4.0 (Maddison and Maddison 2002), as needed. All matrices are available in TreeBASE (study number XXX) and all sequences were deposited in GenBank (Appendix 1). The number of taxa sampled for each region was 32 for each nrITS, *matK*, *ndhF* and *rps16-trnK*, 29 for *ndhC-trnV* and 24 for *Leafy* (two sequences excluded in the analysis, see below). Total missing data for this study was 5.7% (see Appendix 1).

The alignment of all regions, except *lfy2i*, was relatively unambiguous. For *lfy2i* the alignment of outgroups was problematic (sequences are too divergent to align), and therefore we excluded *Antigonon leptopus* and *Eriogonum alatum* from the analyses of

this region. Each gene region was initially partitioned and analyzed separately, then concatenated for a combined molecular data set. Statistics for the molecular matrix are shown in Table 3. Insertions/deletions were not coded as characters in this analysis.

Maximum parsimony (MP) analyses of the combined chloroplast, ITS-only, *lfy2i*-only, and total combined (chloroplast, ITS, and *lfy2i*) data sets were conducted using TNT (Goloboff et al. 2008), under the traditional search option (or heuristic search), with one random seed, tree-bisection reconnection branch swapping, 10 random sequence additions, and saving 10 trees per replication. Maximum Likelihood (ML) analyses were performed for the same data sets as MP, in the program GARLI (Zwickl 2006). The model of evolution was estimated in the program and for each analysis corresponded to a GTR+I+G.

Bootstrap analysis was used for evaluating node support (Felsenstein 1985). Bootstrap supports for MP were conducted in the program TNT with 10 starting trees and 10 search replicates, saving the best tree, for 1000 replicates. Bootstrap values for ML were calculated in GARLI, based on 200 replicates with only one search replicate per bootstrap replicate.

Before the analyses, the data sets matrices (chloroplast, ITS, and *lfy2i*) were tested for incongruence, using the incongruence length difference (ILD) test as implemented in PAUP* version 4.0b1.0 (Swofford 1998). All ILD tests used default parameters and 1000 replicates. The ILD test is often used to test if different molecular regions are congruent or not, although it is known to be sensitive to between-partition differences in evolutionary rates and extremes of rate heterogeneity among sites within the data (Dolphin et al. 2000; Yoder et al. 2001; Barker and Lutzoni 2002; Dowton and Austin

2002). Therefore, many studies have currently suggested that even if the ILD test shows significant incongruence, concatenating datasets from different regions may not result in misleading phylogenies (e.g., Yoder et al. 2001; Barker and Lutzoni 2002; Dowton and Austin 2002). In our case, when there was significant incongruence ($p = 0.01$) between regions, we assessed support for conflicting topologies of the analysis, before deciding whether to combine.

Results

Phylogenetic Relationships

Table 2 presents a complete list of gene regions and combined data matrix statistics. This study generated 127 new sequences (Appendix 1); the 53 remaining sequences were previously published in Lamb-Frye and Kron 2003, Sanchez and Kron 2008, Sanchez et al. 2009, and Burke et al. 2010. Of the six gene regions sampled, *lfy2i* was the most parsimony informative (24%) region while *ndhF* was the least parsimony informative (1.7 %). The combined data set for all regions was 7379 bp long and contained 9.15% parsimony informative characters for MP and 18.6% for ML. The combined plastid data set was 4576 bp long and contained 5% parsimony informative characters for MP and 11.5% for ML.

The combined chloroplast data set and *lfy2i* were found to be congruent (p=0.2) for the ILD test; however, ITS was incongruent with both the chloroplast data set and *lfy2i* (p=0.01). Most of the incongruent regions (described below) received low bootstrap support (<60%) and the ones that were supported, such as the conflict in the relationships of *Triplaris americana, T. cumingiana* Fisch. & Mey. ex C.A. Mey. and *T. melaenodendron* (Bertol.) Standl. & Steyerm., and the position of *Ruprechtia cruegeri* Griseb. ex Lindau, were only supported by ML. The decision was to combine data sets for several reasons: 1) the incongruence of data sets was due to few conflicts, most of them with low support or only supported in ML; and 2) there were no conflicts in the reconstruction of the backbone topology of the tree. Since the scope of this study is not the interspecific relationships of *Triplaris* or *Ruprechtia,* but the relationships between both genera, these conflicts did not affect any of the conclusions reached in this study.

Even more, by combining different regions, there was stronger support for the backbone topology of the tree, which allowed us to identify the circumscription problems in the genera.

CHLOROPLAST (CP), ITS, AND LFY2I DATA SETS– The least variable data set corresponded to the combined cp regions, followed by ITS and *lfy2i* (Table 2). Phylogenetic trees reconstructed from each data set are topologically congruent on the backbone (Fig. 1), where *Ruprechtia triflora* and *Ruprechtia obidensis* are placed as sisters to a *Ruprechtia* s.str. + *Triplaris*, and in the monophyly of *Triplaris* (99 / 95% ML/MP for ITS; 95% ML/MP for cp; 90 / 83% ML/MP for *lfy2i*).

There are some areas of incongruence between ITS vs cp and *lfy2i*, found in the interspecific relationships within *Ruprechtia* s.str. and within *Triplaris.* However, the different topologies are mostly not supported (bootstraps < 60%), or are only supported by one analysis (usually ML). In the case of the cp data set, *R. cruegeri* is sister to *R. coriacea* Benth. & Hook. (71% only for ML; *R. cruegeri* was not amplified for *lfy2i*). For ITS, *R. cruegeri* is placed as sister to *R. tangarana* Standl. with moderate bootstrap support (87 / 82% for ML/MP), and these two species are sister to *R. coriacea* (66% bootstrap support, only for ML). Other minor incongruences between data sets in *Ruprechtia* correspond to the placement of *R. lundii* Meisn. and *R. latifunda* Pendry. In ITS both species are strongly supported as sister (92 / 95%), while in *lfy2i, R. lundii* is sister to *R. aperta* Pendry + *R. obovata* Pendry with only 53% ML bootstrap support (no support for MP; there is no support for any of these branches in the cp data set) (Fig. 1).

An area of congruence for *lfy2i* and ITS, is in the sister relationships of *Ruprechtia aperta* + *R. obovata*, where both data sets moderately support it (66% ML [no support in MP] for *lfy2i*, 78 / 71% for ITS; no support in the cp data set) (Fig. 1).

For the interspecific relationships of *Triplaris* (Fig. 1), ITS and cp support the sister relationships of *Triplaris americana* and *T. cumingiana* with low support (ITS only for ML 66%; 52% ML for cp). *Triplaris melaenodendron* is supported as sister to *T. purdiei* Meisn. for ITS (77% only for ML), but it is supported as sister to *T. americana* + *T. cumingiana* in the cp data set (56% only for ML). The *lfy2i* analysis places *T. cumingiana* and *T. melaenodendron* as sister with high bootstrap support (98 / 80%). Another conflict supported only in ML, is in the position of *T. setosa* Rusby. For ITS, this species is placed as sister to *T. longifolia* Huber + *T. peruviana* Fisch. & Mey. ex C.A. Mey. (75% ML), while in cp *T. setosa* is placed as sister to *T. poeppigiana* Wedd. (73% ML). There is no support for any placement in the *lfy2i* data set. All data sets are congruent in the sister relationships between *T. longifolia* and *T. peruviana* (83 / 63% for ITS; 86 / 63% for cp; 66% only ML for *lfy2i*) (Fig. 1).

TOTAL COMBINED– Results from the MP analysis for the combined molecular data set recovered 62 trees (L = 2924; Table 2). The tree with the lowest likelihood score from ML was Ln = –25833.25. There were no topological incongruences between the strict consensus tree of MP and the results of the ML analysis. As mentioned above, most of the incongruences between data sets are in the placement of some species in *Triplaris* and in *Ruprechtia*. However, the individual data sets support the monophyly of *Triplaris* and a clade of *Ruprechtia* s.str. (Fig. 2) that excludes *R. triflora* and *R. obidensis*.

In the total combined analysis, there is strong support for the monophyly of Triplarideae (100% ML and MP), and the position of *R. triflora* at the base of the tribe (Fig. 2; 99 / 96%). *Ruprechtia obidensis* is the following diverging species, supported as sister to *Ruprechtia* s.str. and *Triplaris* (Fig. 2; 86 / 78%). *Triplaris* is strongly supported as monophyletic (100% for ML/MP) as well as *Ruprechtia* s.str (100 / 99%). There is not much support for interspecific relationships within *Triplaris* and *Ruprechtia* s.str. (Fig. 2), although there is a strongly supported clade within *Ruprechtia* that includes *R. tangarana* Standl., *R. costata* Meisn., *R. nicaraguensis* Pendry and *R. laevigata* Pendry (Fig. 2).

The most relevant result from our combined analysis is the strongly supported monophyly of *Triplaris* whereas *Ruprechtia*, as currently circumscribed (Pendry, 2004), is polyphyletic. Since *R. obidensis* and *R. triflora* are not included within *Ruprechtia*, a new circumscription is needed to maintain monophyletic groups.

Discussion

This is the first phylogenetic study exploring the relationships of *Triplaris* and *Ruprechtia* simultaneously, and it includes a broad sampling of both genera, several molecular regions, and outgroups from within Eriogonoideae. Based on our results, we propose a new generic delimitation within Triplarideae which results in two new genera (*Magoniella* and *Salta*) and a new circumscription of *Ruprechtia* (see below).

Monophyly and relationships among genera

Our results indicate that *Ruprechtia,* as recognized by Pendry (2004), is not monophyletic (Fig. 2). The analysis of all independent genes as well as the total combined data, support the same hypothesis: *Ruprechtia triflora* and *Ruprechtia obidensis* are not included in the same clade with the remaining species of *Ruprechtia* sampled (Fig. 1 and 2). Rather, there is support for their placement as sister to the remaining species of Triplarideae, where *R. triflora* is the first diverging member, followed by *R. obidensis*. Previous studies had recovered a similar pattern where *R. triflora* was not placed in the same clade as other *Ruprechtia* (Sanchez et al. 2009; Burke et al. 2010; Burke and Sanchez, in press.); our more inclusive sampling (for both genes and species in *Triplaris* and *Ruprechtia*) supports the same placement of this species as the first diverging clade to remaining *Ruprechtia* and *Triplaris* sampled.

However, it was not unanticipated that the circumscription of *Triplaris* and *Ruprechtia* might be problematic. The flux of different taxonomic treatments (see above), is evidence that the delimitation of both genera has been difficult. While some authors merged *Ruprechtia* under *Triplaris* (e.g., Endlicher 1847; Kuntze 1898), others segregated some species of *Ruprechtia* into new *Enneatypus* (Herzog 1922; Roberty and

Vautier 1964) or *Magonia* (Kuntze 1891). However, changes in the circumscriptions of the genera are not always clear and most authors did not address the differences between them (e.g., Kuntze 1891, 1898; Herzog 1922).

The only study that incorporated a phylogenetic approach, using the ribosomal ITS region, was by Pendry (2004) in his monograph of *Ruprechtia*. He sampled 19 species of *Ruprechtia* and three species of *Triplaris* as outgroups. *Ruprechtia* was strongly supported as monophyletic, but there was no test for its monophyly, since the tree was rooted with *Triplaris*. Interestingly, *R. triflora* was placed at the base of the remaining species, with a long diverging branch (Fig. 4 in Pendry). Pendry (2004) recognized that *R. triflora* was a distinctive species, based on pronounced development of brachyblasts (described as short, leafy side branches), short inflorescences, and a single-flowered partial female inflorescence (other *Ruprechtia* are two- or three-flowered in this character).

Although it is evident that the morphological characters used to differentiate *Ruprechtia* and *Triplaris* do not hold true for all taxa described (Table 1), the monophyly of both genera had not been tested using morphological and/or molecular characters in a cladistic approach. From the characters proposed by Cocucci (1961) and by Brandbyge and Øllgaard (1984), the only one that has a consistent pattern is habit: only one of our sampled species (*R. obidensis*) is a strict liana. In *Ruprechtia* there is only one other species, *R. laurifolia*, described as a strict liana. Since it is likely that *R. laurifolia* is closely related to *R. obidensis* (see below), the lianacious habit is considered as a synapomorphy for these two species.

Other characters discussed by Brandbyge and Øllgaard (1984) that could be relevant in maintaining and delimiting the genera were leaf size and habitat (Table 1). According to these authors, the two genera followed two different evolutionary trends with *Triplaris* representing a line of large-leaved, fast-growing trees colonizing wet, open habitats, and *Ruprechtia* being a line of smaller-leaved, shrubby inhabitants of drier habitats. Although there is such tendency, there are several exceptions; for example, *T. peruviana* and *T. poeppigiana* can be found in dry environments in Peru (Sanchez, pers. obs.); *R. cruegerii* inhabits dry forests as well as seasonally inundated; *R. obidensis* is found in lowland rain forests; and, *R. tangarana* has large leaves and inhabits lowland rainforests.

Hollow stems inhabited by ants of the genus *Pseudomyrmex* Lund is a character often used to identify *Triplaris* (Cocucci 1961; Brandbyge 1989). Although seven species of *Ruprechtia* have hollow stems (they are occasionally seen in species such as *R. cruegeri* and *R. tangarana*; Table 1), only *R. tangarana* has been reported as inhabited by *Pseudomyrmex* ants (Brandbyge and Øllgaard 1984; Brandbyge 1989). Ants have also been discovered in *R. lundii, R. latifunda* and *R. cruegeri* (Sanchez and Burke pers. obs.). In these plants the associated ants are not *Pseudomyrmex* but mostly members of *Crematogaster* Lund (Sanchez and Ward, pers. obs.). Opportunistically associated ants might be found in any plant with hollow cavities, and the hollow twigs of *Ruprechtia* are no exception. However, obligate relationships with some ant species of the *Pseudomyrmex viduus* (Smith) group might be exclusive to *Triplaris* (Ward 1999). More ant collections are necessary to establish if *Ruprechtia* is obligately mutualistic with *Pseudomyrmex*.

TAXONOMIC TREATMENT

As circumscribed by Burke and Sanchez (in press), Triplarideae included only two genera, *Ruprechtia* and *Triplaris*. However, based on the present results, it is clear that to maintain monophyletic genera, two new genera require description. It was evident from previous work (Sanchez et al. 2009; Burke et al. 2010; Burke and Sanchez, in press) that *Ruprechtia* was not monophyletic, since *R. triflora* was not placed with other species of the genus. In those studies sampling was too sparse to propose a new generic delimitation with confidence. This study uses more species and additional data to analyze relationships within Triplaridae and we are confident that our sampling is broad enough to propose the two new genera. We had the option to merge all species in a *Triplaris* s.l. and name subgenera or sections, but we chose not to since there are differences among the genera that would be better acknowledged if their status is maintained.

Genera are listed according to their position on the evolutionary tree (Fig. 2), from base to apex. For each treatment diagnostic characters are in bold.

Key to the genera in Triplarideae

1a. Base of the fruit produced into a short stalk ……. *Ruprechtia*

1b. Base of the fruit abruptly terminated by scar from abscised pedicel…… 2

2a. Lianas ……..*Magoniella*

2b. Trees or shrubs ….…….. 3

3a. Leaves clustered on short side shoots (brachyblasts). Fused base of sepals not fully enclosing achene ……...…… *Salta*

3b. Leaves more or less evenly spaced along the twig, brachyblasts not developed. Fused based of sepals fully enclosing achene ……...…*Triplaris*

Salta Adr. Sanchez, **gen. nov.**

— TYPE: *Ruprechtia triflora* Grisebach [= *Salta triflora* (Grisebach) Adr. Sanchez]

Prominens evolutus de brachyblasti (caulis brevis em folia forma). Inflorescentiae cum pedunculus brevissimus et a brachyblastis productae. Fructus cum constrictio angustus ad basim.

The name *Salta* refers to the province of Salta in Argentina, where a type collection for *Ruprechtia triflora* was made. This new monotypic genus is confined to southern South America in Argentina, Bolivia, and Paraguay. The genus is characterized by the **pronounced development of brachyblasts** (leafy short side branches), the short axis of the inflorescences (less than 0.2 cm) borne on a short shoot, the base of the sepals fused but not enclosing the achene, and a narrow constriction at the base of the fruits.

Salta triflora (Griseb.) Adr. Sanchez, comb. nov., basionym *Ruprechtia triflora* Griseb. in Abh. Königl. Ges. Wiss. Göttingen 24: 89, 1879. — TYPE: ARGENTINA. Salta: Gran Chaco, Dragones, Aug. 1873, *Lorentz & Hieronymus 599* (isotype: B, photo).

Salta triflora is a common plant that grows in Chaco forests and thickets and occasionally, in seasonally inundated forests, between 200-1500 m. It flowers and fruits in the dry season (while the plant is leafless). The species is described in detail by Pendry (2004) as *R. triflora*.

Magoniella Adr. Sanchez, **gen. nov**.

— TYPE: *Ruprechtia obidensis* Huber [= *Magoniella obidensis* (Huber) Adr. Sanchez]

Perianthia cum cicatrice ad fructus basim *Triplaris* similis, flores masculi pedicellatus cum perianthia libera *Ruprechtia* similis, ad ambobus liane cum caul cavus differt.

The name *Magoniella* is based on Vellozo's *Magonia* (*Magonia scandens* = *Ruprechtia laurifolia* = *Magoniella laurifolia*). *Magonia* means a "mystic cloud realm", which the author might have used to refer to the liana habit of the genus. *Magoniella*, a lesser *Magonia*, maintains a historical reference to Vellozo and the growth form of the genus. This genus comprises two species distributed in Brazil, Bolivia and Venezuela, and it is defined by the **strict lianaceous habit**. *Magoniella* possess hollow stems and the fruits are green with red sepals. It shares with *Triplaris* the presence of a scar at the base of the perianth in the fruit (resulting from the abscission from the pedicel), and with *Ruprechtia* s.s. such characters as two-three-flowered female partial inflorescences, free male perianth segments, pedicellate male flowers, and a perianth tube that is shorter than the achene. Two species are recognized:

Magoniella laurifolia (Cham. & Schltdl) Adr. Sanchez, comb. nov., basionym *Magonia scandens* Vell. in Fl. Flum.: 165. 1825. — TYPE: Vellozo's illustration, Flora Fluminensis icones 4: t. 60 (lectotype designated by Pendry, 2004).

Confined to southeastern Brazil in mâta atlântica and in restingas, between 0-500 m.

Magoniella obidensis (Huber) Adr. Sanchez, comb. nov., basionym *Ruprechtia obidensis* Huber in Bol. Mus. Paraense "Emilio Goeldi" 5: 344. 1909. — TYPE: BOLIVIA. Huachi: head of Beni river, 18 Aug. 1921, Rusby 972 (isotype: K, photo).

Known from Brazil, Bolivia, and Venezuela where it occurs in secondary forests and on margins of lowland rain forests, between 100-900 m.

Both species are amply described in Pendry (2004) under *Ruprechtia*. Although in the present study we did not include an accession of *M. laurifolia* in our molecular analysis, we are confident of its placement due to their morphological similarity, as the two only differ by the presence of a yellow reticulation on the underside of the leaf of *M. laurifolia* when the plants are male or sterile, or if the plants are female and have fruits, then the pedicels of *M. obidensis* are longer than the bracts, and are noticeable after the flowers have fallen. *Magoniella laurifolia* also tends to possess a less dense indumentum in female and male inflorescences (Pendry 2004).

TRIPLARIS– The genus comprises 18 species as recognized by Brandbyge (1986). *Triplaris* is distributed from Southern Mexico (in Oaxaca) to Southern Brazil (State of Parana) and is defined by morphological synapomorphies such as **ruminate endosperm**, **sessile male flowers**, and **microreticulate pollen**.

In the present study we included nine of the 18 species recognized. However, work is currently under way for a more comprehensive study of the interspecific relationships of the genus (Sanchez, in prep.). Molecular data strongly supports *Triplaris* as monophyletic as do morphological synapomorphies.

RUPRECHTIA– As circumscribed in this study, the genus includes 34 of the 37 species accepted by Pendry (2004). Three species, *R. laurifolia*, *R. obidensis*, and *R.*

triflora are not considered part of *Ruprechtia* based on molecular and morphological characters (see above). *Ruprechtia* is distributed from Mexico to Argentina (excluding Chile) and it is characterized by the presence of a **short stalk at the base of the fruit**, which is an extension of the calyx.

Of the 34 species we recognize, 17 were included in our analysis. We also consider that seven species (*R. albida* Pendry, *R. apurensis* Pendry, *R. brachysepala, R. carina* Pendry, *R. curranii* S.F. Blake, *R. ramiflora,* and *R. tenuiflora* Benth.) sampled in Pendry's ITS analysis, are part of *Ruprechtia* since they are nested within the genus, and are sister to species sampled for this study (see Fig. 4 in Pendry 2004, and Fig. 2 in this study). Although we did not include a sample of *R. ramiflora* (the lectotype of *Ruprechtia*), it is important to mention that this species is placed in Pendry's study close to *R. carina, R. cruegeri, R. tangarana,* and *R. tenuiflora* (Fig. 3 and 4 in Pendry 2004).

The placement and relationships of the 10 species that remain to be sampled (*R. brachystachya* Benth., *R. crenata* (Casar.) R.A. Howard, *R. exploraticis* Sandwith, *R. glauca* Meisn., *R. jamesonii* Meisn., *R. maracensis* Brandbyge, *R. paranensis* Pendry, *R. peruviana* Pendry, *R. salicifolia* (Cham. & Schltdl.) C.A. Mey., and *R. standleyana* Cocucci) are not clear. Most probably all will be placed within *Ruprechtia* (as recognized in this study), but a comprehensive phylogenetic study needs to be done to assess interspecific relationships. Work is also needed to establish additional morphological synapomorphies that apply to our circumscription of the genus.

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Appendix 4.1. Voucher information for DNA extractions used in this study. Sequences obtained from Genbank are given with their respective site specific numbers. The following information is provided for new sequences generated by this study: Taxon, collector(s), collection number (in italics), and Genbank accession numbers. Herbarium acronyms follow Index Herbariorum (Holmgren et al., http://sciweb.nybg.org/science2/IndexHerbariorum.asp). Gene abbreviations are as follows: M = *matK*, N = *ndhF*, D= *ndhC-trnV*, K= *rps16-trnK*, I = ITS, L = *lfy2i*. NA= not used in this study.

1. Sequences obtained from Genbank. ***Antigonon leptopus*** Hook. & Arn., M-EF437988, N- EF438027, I- FJ154462, L- EF442788; ***Coccoloba swartzii*** Meisn., M-EF437995, N- EF438034, I- FJ154469, L- EF442787; ***Eriogonum alatum*** Torr. var. ***alatum***, M- EF437998, N- EF438038, I- FJ154472, L- EF438068; ***Gymnopodium floribundum*** Rolfe, M-GQ206197, N-GQ206282, I- GQ206251; ***Ruprechtia chiapensis*** Lundell ex Standl. & Steyerm. M- FJ154495, N- FJ154506, I- FJ154482; ***Ruprechtia coriacea*** (H. Karst.) S.F. Blake, *Sanchez 104*, M- HM137401, N- HM137423, I-HM137442; ***Ruprechtia cruegeri*** Griseb. ex Lindau, M- HM137402, N- HM137424, I-HM137443; ***Ruprechtia fusca*** Fernald, M- FJ154496, N- FJ154507, I- FJ154483; ***Ruprechtia laxiflora*** Meisn., M- EF438024, N- EF438063, I- FJ154484; ***Ruprechtia pallida*** Standl., M- HM137403, N- HM137425, I- HM137445; ***Ruprechtia tangarana*** Standl., M- EF438025, N- EF438064, I- FJ154485; ***Triplaris americana*** L., M-AY042668, N- FJ154508; I- FJ154486; ***Triplaris cumingiana*** Fisch. & C.A. Mey. ex C.A. Mey., M-GQ206210, N-GQ206301, I- GQ206269; ***Triplaris poeppigiana*** Wedd., M- FJ154497, N- FJ154509, I- FJ154487; ***Triplaris setosa*** Rusby, M- FJ154498, N-

FJ154510, I- FJ154488; ***Triplaris weigeltiana*** (Rchb.) Kuntze, M- HM137405, N-HM137426, I- HM137446; ***Salta triflora*** (Griseb.) Adr. Sanchez, N-GQ206299, I-GQ206267.

2. Sequences generated by this study. Materials obtained from herbarium specimens are indicated with a * after the collection number. ***Antigonon leptopus*** Hook. & Arn., *Luckow 4630* (BH), D- HQ693163, K- HQ693194; ***Coccoloba swartzii*** Meisn., *Sanchez 109*, D- HQ693164, K- HQ693195; ***Eriogonum alatum*** Torr. var. ***alatum***, *Reveal 8515* (MARY), D- HQ693165, K- HQ693196; ***Gymnopodium floribundum*** Rolfe, *Burke 48* (BH), D- HQ693166, K- HQ693197, L- HQ693138; ***Magoniella obidensis*** (Huber) Adr. Sanchez, *Cayola et al. 107** (MO), M- HQ693198, N- HQ693214, D- HQ693151, K-HQ693167, I- HQ693103, L- HQ693137; ***Ruprechtia aperta*** Pendry, *Sarkinen et al. 2192** (FHO), M- HQ693199, N- HQ693215, D- HQ693139, K- HQ693169, I-HQ693104, L- HQ693118; ***Ruprechtia apetala*** Wedd., *Nee & Flores 54796** (NY), M-HQ693200, N- HQ693216, D- HQ693140, K- HQ693170, I- HQ693105, L- NA; ***Ruprechtia chiapensis*** Lundell ex Standl. & Steyerm. *Burke s.n.* (BH), D- HQ693141, K- HQ693171, L- HQ693119; ***Ruprechtia coriacea*** (H. Karst.) S.F. Blake, *Sanchez 104* (WFU), D- HQ693142, K- HQ693172, L- HQ693120; ***Ruprechtia costaricensis*** Pendry, *Sanchez 400* (WFU), M- HQ693201, N- HQ693217, D- HQ693143, K- HQ693173, I-HQ693106, L- HQ693121; ***Ruprechtia costata*** Meisn., *Castillo 2119** (MO), M-HQ693202, N- HQ693218, D- NA, K- HQ693174, I- HQ693107, L- NA; ***Ruprechtia cruegeri*** Griseb. ex Lindau, *Luckow 4587* (BH), D- HQ693144, K- HQ693175, L- NA; ***Ruprechtia fagifolia*** Meisn., *W. Thomas et al. 9638** (NY), M- HQ693203, N-HQ693219, D- HQ693145, K- HQ693176, I- HQ693108, L- NA; ***Ruprechtia fusca***

Fernald, *Pendry 868* (E), D- HQ693146, K- HQ693177, L- HQ693122; ***Ruprechtia laevigata*** Pendry, *Torres et al. 9084** (MO), M- HQ693204, N- HQ693220, D- HQ693147, K- HQ693178, I- HQ693109, L- NA; ***Ruprechtia latifunda*** Pendry, *Sanchez 164* (WFU), M- HQ693205, N- NA, D- NA, K- NA, I- HQ693110, L- HQ693123; ***Ruprechtia laxiflora*** Meisn., *Prado s.n.* (E), D- HQ693148, K- HQ693179, L- HQ693124; ***Ruprechtia lundii*** Meisn., *Sanchez 160* (WFU), M- HQ693206, N- HQ693221, D- HQ693149, K- HQ693180, I- HQ693111, L- HQ693125; ***Ruprechtia nicaraguensis*** Pendry, *Rueda et al. 2444** (MO), M- HQ693207, N- HQ693222, D- HQ693150, K- HQ693181, I- HQ693112, L- HQ693126; ***Ruprechtia obovata*** Pendry, *Sarkinen et al. 2221** (FHO), M- HQ693208, N- HQ693223, D- HQ693152, K- HQ693182, I- HQ693113, L- HQ693127; ***Ruprechtia pallida*** Standl., *Burke 129* (BH), D- NA, K- HQ693183, L- NA; ***Ruprechtia tangarana*** Standl., *Silman s.n.* (WFU), D- HQ693153, K- HQ693184, L- HQ693128; ***Salta triflora*** (Griseb.) Adr. Sanchez *Nee et al. 53657** (MO), M- HQ693213, D- HQ693154, K- HQ693168, L- NA; ***Triplaris americana*** L., *Luckow 4635* (BH), D- HQ693155, K- HQ693185, L- HQ693129; ***Triplaris cumingiana*** Fisch. & C.A. Mey. ex C.A. Mey., *Sanchez 100* (WFU), D- HQ693156, K- HQ693186, L- HQ693130; ***Triplaris longifolia*** Huber, *Sanchez* 188 (WFU), M- HQ693209, N- HQ693224, D- NA, K- HQ693187, I- HQ693114, L- HQ693131; ***Triplaris melaenodendron*** (Bertol.) Standl. & Steyerm., *Sanchez 405* (WFU), M- HQ693210, N- HQ693225, D- HQ693157, K- HQ693188, I- HQ693115, L- HQ693132; ***Triplaris peruviana*** Fisch. & Meyer ex C.A. Meyer, *Sanchez 171* (WFU), M- HQ693211, N- HQ693226, D- HQ693158, K- HQ693189, I- HQ693116, L- HQ693133; ***Triplaris poeppigiana*** Wedd., *Sanchez 89* (WFU), D- HQ693159, K-

HQ693190, L- HQ693134; ***Triplaris purdiei*** Meissn. in Mart., *Sanchez 100* (WFU), M-HQ693212, N- HQ693227, D- HQ693160, K- HQ693191, I- HQ693117, L- NA; ***Triplaris setosa*** Rusby, *Fuentes 5351* (MO), D- HQ693161, K- HQ693192, L-HQ693135; ***Triplaris weigeltiana*** (Rchb.) Kuntze, *Michelangeli s.n.* (WFU), D-HQ693162, K- HQ693193, L- HQ693136.

Table 4.1.

Circumscription of tribe Triplarideae through history.

Author	Genera recognized
This study	*Magoniella* Adr. Sanchez, *Ruprechtia* C.A. Mey., *Salta* Adr. Sanchez, *Triplaris* Loefl.
Brandbyge 1993	*Gymnopodium* Rolfe, *Leptogonum* Benth., *Ruprechtia*, *Symmeria* Benth., *Triplaris*
Haraldson 1978	*Gymnopodium, Leptogonum, Millspaughia* B.L. Rob, *Ruprechtia, Symmeria, Triplaris*
Roberty and Vautier 1964	*Enneatypus* Herzog, *Leptogonum, Ruprechtia, Symmeria, Triplaris*
Gross 1913	*Gymnopodium, Leptogonum, Millspaughia, Ruprechtia, Symmeria, Triplaris*
Dammer 1893	*Leptogonum, Ruprechtia, Symmeria, Triplaris*
Bentham, 1880	*Leptogonum, Podopterus* Bonpl., *Ruprechtia, Symmeria, Triplaris*
Meisner 1856	(as subtribe) *Podopterus, Ruprechtia, Triplaris*

Table 4.2.

Morphological characters used by Cocucci (1961) and by Brandbyge and Øllgaard (1984) to segregate *Triplaris* and *Ruprechtia*. * *Ruprechtia cruegeri, R. maracensis, R. latifunda, R. laurifolia, R. lundii, R. obidensis, R. tangarana*

Character	***Triplaris***	***Ruprechtia***
According to Cocucci		
Twigs	Hollow	Solid (except for **seven** species*)
Perianth tube	More than half as long as the achene	Up to half as long as the achene
Achenes	Three-angled in cross section	Trilobe in cross section
Ochreas	Persistent (only in youngest shoots)	Caducous
Chromosome number	X= 11	X = 14
According to Brandbyge and Øllgaard		
Female partial inflorescences	1-flowered	2-3 flowered (except for *R. triflora*)
Perianth tube	Longer than the achene	¾ as long as the achene
Base of perianth tube	Not extended into a stalk; fruits with a scar at the base	Extending into a stalk; fruits with short stalk at the base (modifications in *R. triflora, R. obidensis* and *R. laurifolia*)
Bracteoles	Fissured abaxially	Tubular
Male flowers	Sessile	Pedicellate
Male perianth segments	Connate for more than half of their length	Free
Pollen	Microreticulate or punctuate-microreticulate	Perforate-rugulose
Other potential, relevant characters (*Brandbyge and Øllgaard*)		
Leaves	Large	Small
Habitat	Wet and open	Drier

Table 4.3.

Statistics for gene regions used in the Maximum Parsimony (MP) analysis. PIC = parsimony informative characters, CI = consistency index, RI = retention index.

Statistic	*matK*	*ndhF*	*ndhC-trnV*	*rps16-trnK*	Combined cp	ITS	*lfy2i*	Total combined
Aligned length	868	1240	945	1528	4576	842	1961	7379
Variable sites (%)	120 (13.8)	76 (6.1)	223 (23.6)	386 (25.3)	803 (17.5)	361 (42.9)	784 (40)	1947 (26.4)
PIC (%)	44 (5.1)	21 (1.7)	93 (9.8)	113 (7.4)	228 (5)	176 (20.9)	286 (24)	675 (9.15)
Missing taxa (%)	0	0	3 (9.4)	0	3 (2.3)	0	8 (25)	11 (5.7)
CI	1.075	1.098	1.036	0.869	0.822	0.862	0.840	0.806
RI	1.139	1.229	1.138	0.706	0.647	0.830	0.765	0.701

Figure 4.1 A-C.

Cladograms obtained for the analysis of each individual dataset. Numbers above or below the branches indicate bootstrap support values for maximum likelihood (ML) followed by maximum parsimony (MP). Only bootstrap values > 50% are shown; a hyphen (-) indicates a topology with support < 50%. An asterisk (*) denotes a topology that received 100% bootstrap support for ML and MP. A = ML results for the chloroplast dataset; B = ML results for ITS; C = MP results for *lfy2i*.

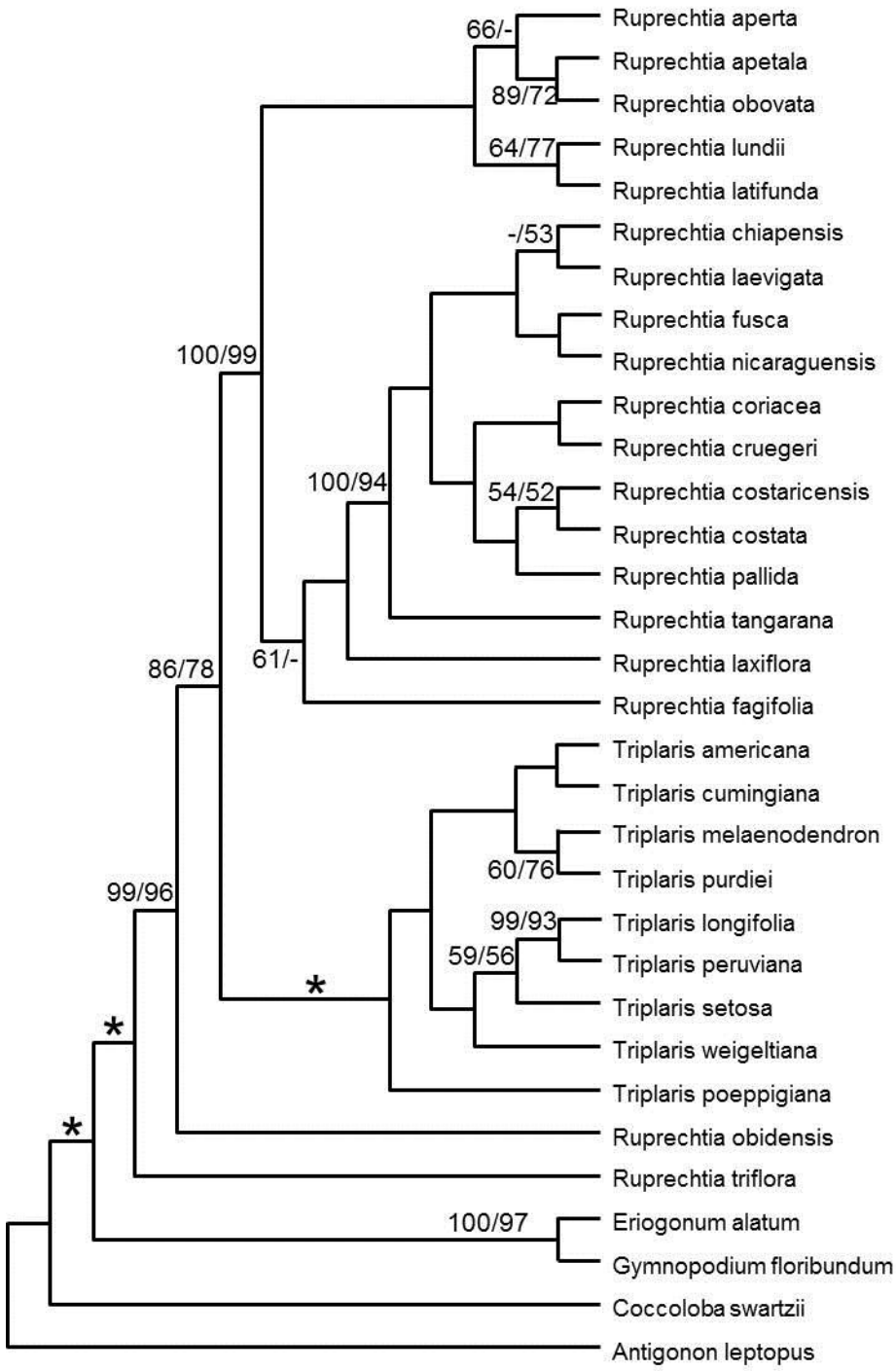
Fig. 4.1.


A
Ruprechtia aperta
Ruprechtia fagifolia
Ruprechtia lundii
Ruprechtia laxiflora
Ruprechtia chiapensis
Ruprechtia laevigata
Ruprechtia coriacea
Ruprechtia cruegeri
Ruprechtia costaricensis
Ruprechtia costata
Ruprechtia fusca
Ruprechtia pallida
Ruprechtia nicaraguensis
Ruprechtia apetala
Ruprechtia tangarana
Ruprechtia obovata
Ruprechtia latifunda
Triplaris americana
Triplaris cumingiana
Triplaris melaenodendron
Triplaris poeppigiana
Triplaris setosa
Triplaris longifolia
Triplaris peruviana
Triplaris purdiei
Triplaris weigeltiana
Ruprechtia obidensis
Ruprechtia triflora
Eriogonum alatum
Gymnopodium floribundum
Coccoloba swartzii
Antigonon leptopus
71/-
87/57
52/-
56/-
85/-
73/-
60/100
86/63
95
70/-
100/98
*
*
B
78/71
Ruprechtia aperta
Ruprechtia obovata
Ruprechtia apetala
Ruprechtia chiapensis
Ruprechtia costata
Ruprechtia nicaraguensis
Ruprechtia pallida
Ruprechtia fusca
Ruprechtia laevigata
Ruprechtia coriacea
Ruprechtia cruegeri
Ruprechtia tangarana
Ruprechtia costaricensis
Ruprechtia laxiflora
Ruprechtia fagifolia
Ruprechtia lundii
Ruprechtia latifunda
Triplaris americana
Triplaris cumingiana
Triplaris melaenodendron
Triplaris purdiei
Triplaris poeppigiana
Triplaris weigeltiana
Triplaris longifolia
Triplaris peruviana
Triplaris setosa
Ruprechtia obidensis
Ruprechtia triflora
Gymnopodium floribundum
Eriogonum alatum
Coccoloba swartzii
Antigonon leptopus
66/-
87/82
-/51
92/95
66/-
83/85
77/-
99/95
77/98
83/63
75/-
77/83
C
-/53
Ruprechtia aperta
Ruprechtia lundii
Ruprechtia obovata
-/53
Ruprechtia chiapensis
Ruprechtia nicaraguensis
-/76
-/96
Ruprechtia coriacea
-/50
Ruprechtia costaricensis
Ruprechtia fusca
Ruprechtia tangarana
Ruprechtia laxiflora
Ruprechtia latifunda
Triplaris americana
Triplaris cumingiana
Triplaris melaenodendron
65/-
98/80
*
Triplaris longifolia
Triplaris peruviana
66/-
90/83
Triplaris setosa
Triplaris weigeltiana
Triplaris poeppigiana
Ruprechtia obidensis
Gymnopodium floribundum
Coccoloba swartzii

Figure 4.2.

Cladogram obtained from the maximum likelihood (ML) analysis for the total combined dataset of four chloroplast regions (*matK, ndhF, rps16-trnK, ndhC-trnV*), ITS and *lfy2i*. Numbers above the branches indicate bootstrap support values for ML followed by maximum parsimony (MP). A hyphen (-) indicates that a topology was not supported. An asterisk (*) denotes a topology that received 100% bootstrap support for ML and MP.

Fig. 4.2.


Ruprechtia aperta
Ruprechtia apetala
Ruprechtia obovata
Ruprechtia lundii
Ruprechtia latifunda
Ruprechtia chiapensis
Ruprechtia laevigata
Ruprechtia fusca
Ruprechtia nicaraguensis
Ruprechtia coriacea
Ruprechtia cruegeri
Ruprechtia costaricensis
Ruprechtia costata
Ruprechtia pallida
Ruprechtia tangarana
Ruprechtia laxiflora
Ruprechtia fagifolia
Triplaris americana
Triplaris cumingiana
Triplaris melaenodendron
Triplaris purdiei
Triplaris longifolia
Triplaris peruviana
Triplaris setosa
Triplaris weigeltiana
Triplaris poeppigiana
Ruprechtia obidensis
Ruprechtia triflora
Eriogonum alatum
Gymnopodium floribundum
Coccoloba swartzii
Antigonon leptopus
66/-
89/72
64/77
-/53
100/99
100/94
54/52
61/-
86/78
60/76
99/96
99/93
59/56
100/97
*
*
*

CHAPTER V

MUTUALISM BETWEEN *TRIPLARIS* AND *PSEUDOMYRMEX*: PHYLOGENY AND GEOGRAPHICAL DISTRIBUTION

Adriana Sanchez, Francois Lutzoni and Kathleen A. Kron

The following manuscript will be submitted for review to *Molecular Phylogenetics and Evolution* after corrections. Stylistic variations are due to the requirements of the journal. A. Sanchez performed the data collection, data analysis and prepared the manuscript. F. Lutzoni provided logistical support and guidance. K.A. Kron provided logistical support, guidance and editorial assistance.

Abstract

The association between the myrmecophyte *Triplaris* and ants of the genus *Pseudomyrmex* is an often reported example of mutualism, albeit poorly studied. In order to increase our knowledge of this symbiosis, we reconstruct the intraspecific relationships of *Triplaris* using five molecular markers (two choloroplast and three nuclear), and the relationships of the associated *Pseudomyrmex* using two molecular regions (one mitochondrial and one nuclear). We compare the phylogeny of the ants to that of the plants following two approaches: a single individual per species and a multiple individual per species phylogeny for the plants. Using a multiple individual phylogeny can provide a finer resolution to understand the patterns of association between the two organisms on the individual basis. We also compiled a data set with all the collections of plant hosts and resident ants known, and mapped them on GIS. The pattern of distribution of both organisms reveals that one species of the *Pseudomyrmex triplarinus* subgroup is more specific to its host than others.

Introduction

Triplaris Loefl. (Eriogonoideae, Polygonaceae) is a genus that includes 18 dioecious species of trees with a Neotropical distribution from Southern Mexico to Southern Brazil. *Triplaris* occurs in lowland habitats from sea level to 2000 m in altitude, with most species growing at elevations lower than 1000 m. Most species naturally grow in clearings, seasonally inundated forests, and along rivers and roads, but there are some that can grow in dry thickets and dry deciduous forests. All species are considered pioneer plants and they are important components in all stages of secondary successions (Brandbyge, 1986).

Even though *Triplaris* is an important component of the flora in the Neotropical lowlands, much confusion still exists regarding the taxonomy of the species within the group. Until 1986, 73 names were described, but Brandbyge reduced the number of species to 18 (Brandbyge, 1986, 1990). According to Brandbyge, many of the names previously recognized (e.g. *T. guayaquilensis, T. macombii, T. surinamensis, T. pyrimidalis*) could be merged into some of the widespread species (e.g. *T. americana, T. cumingiana, T. melaenodendron, T. weigeltiana*). Prior to Brandbyge's work, the genus was treated on a local scale (e.g. floras) and as a consequence, the same species received several different names in different areas of their distribution. However, species such as *T. americana* are still great sources of confusion and it is common to encounter misidentified species in herbaria and botanical gardens.

A conspicuous feature of all species in *Triplaris* are the hollow stems that harbor associate ants. Although no food bodies and/or extrafloral nectaries are produced by the plants, *Triplaris* offers nesting-space (or domatia) to the ant partners. Rewards to the ants

are provided by a third symbiont - scale insects (Coccidea, Hemiptera) in the form of honeydew. Although few ecological experiments have been done in this particular symbiosis, it is thought that in exchange for the nesting sites, the ant partners protect their host plants against herbivore damage (Sanchez, pers. obs.), they prune vegetation around their host (Davidson et al., 1988) and might maintain the plants free of pathogens.

Triplaris is mainly colonized by a group of ant partners belonging to the large and worldwide distributed genus *Pseudomyrmex* Lund. (300 species; Pseudomyrmecinae). They are characterized by possessing large eyes and stingers (Ward, 1990). Five species of *Pseudomyrmex* in the *viduus* group have been recognized as obligate and specific mutualists to *Triplaris* (specialized ants have not been found nesting outside their plants; Ward, 1999): *P. dendroicus, P. mordax, P. triplaridis, P. triplarinus,* and *P. ultrix*. These species are distributed from Southern Panama to Southern Brazil.

Although *Triplaris* tends to associate specifically with these five species of *Pseudomyrmex,* several different ant genera can opportunistically colonize these plants. It is not uncommon to find species of *Triplaris* colonized by non-specialist ant genera such as *Azteca, Camponotus, Cephalotes, Crematogaster, Dolychoderus, Pheidole,* and *Pseudomyrmex elongatus, P. fortis, P. gebelli, P. gracilis, P. ita, P. longior, P. rubiginosus,* and *P. viduus* (Longino, 1996; Wheeler, 1942; Ward, 1999; A. Sanchez pers. obs.).

Myrmecophytism, or plants that associate with ants, is a pervasive case of symbiosis, especially common in the Neotropics (more than 200 species; Benson, 1985). There are several well-known cases of these associations, such as the interaction between *Acacia-Pseudomyrmex, Cecropia-Azteca, Macaranga-Crematogaster, Neonauclea-*

Cladomyrma. Most studies on these interactions focus on ecological aspects such as defense against herbivores and the effect of different ant genera or species on the hosts (e.g., Dejean et al., 2006; Frederickson, 2005; Frederickson and Gordon, 2009; Gaume et al., 2005; Heil et al., 2001); however, fewer studies have addressed phylogenetic aspects of the interactions.

Phylogenetic studies on ant-plant relationships have shown that there is no support for parallel evolution; myrmecophytism has often evolved several times or the associate ants to a given host do not form a monophyletic group (Ayala et al., 1996; Bänfer et al., 2004; Blattner et al., 2001; Chenuil and McKey, 1996; Davis et al., 2001; Gómez-Acevedo et al., 2010; Quek et al., 2004; Razafimandimbison et al., 2005; Ward, 1991). In many cases myrmecophytism has evolved multiple times in a single genus (e.g., *Macaranga*; Davis et al., 2001) and in other cases it has evolved in just one clade (i.e. *Acacia*; Gómez-Acevedo et al., 2010). However, the system is always promiscuous since a host species can associate with different obligate ant species, and ant species can colonize different hosts.

However, existing phylogenetic studies addressing evolution of ant-plant interactions, in general, sample one or very few genes from only one or few representative individuals per plant and/or ant species. A more comprehensive understanding of the interaction requires extensive sampling of multiple loci for multiple individuals within the plants and the ants. Phylogenetic work can also be linked to the geographical distribution of the individuals in order to have a more complete picture of the association. Multiple collections of individual plants and their associate ants across

the range of distribution can shed light on the specificity of the interaction and will help us understand if some ant species discriminate among host species or not.

Although the *Triplaris-Pseudomyrmex* interaction is a well-known case of myrmecophytism in the Neotropics, there are few ecological studies on the interaction (Davidson et al., 1988; Oliveira et al., 1987; Larrea-Alcazar and Simonetti, 2007; Longino, 1996). There has been some work done on the relationships between the *Pseudomyrmex* species associated with *Triplaris* (Ward, 1999), but there has been no attempt to compare these two organisms using a phylogenetic framework.

This study represents the first step towards elucidating the evolution of ant-plant associations in *Triplaris*. In order to understand this association in a comprehensive way, we explored three main points: 1. The intraspecific relationships of *Triplaris* using five molecular markers (two chloroplast and three nuclear), and those of the obligate *Pseudomyrmex* ants' with two markers (one mitochondrial and one nuclear); 2. Comparison of the phylogeny of both organisms to understand the pattern of association; 3 Mapped in ArcGIS the collections from multiple individuals of the plant species with their associate ant colony, to understand patterns of association at a geographical level. We also show how sampling and the understanding of the species distribution and monophyly, can affect our hypothesis of relationship between these organisms.

Materials and Methods

1. *Triplaris*

Taxon sampling– 12 species of *Triplaris* were sampled (of the 18 described [Brandbyge, 1986, 1990]; Appendix 1), for a total of 32 individuals. We included several individuals per species, especially for those that have wide ranges of distribution. We only included 12 of the 18 described species of *Triplaris* since several species are highly restricted to geographical areas (i.e. *T. efistulifera*, *T. matogrossensis*, *T. moyobambensis* and *T. physocalyx*), and therefore are not easily collected in the field. Herbarium material was used in order to amplify the six species, but we could only amplify –if anything- ITS. Missing data affected our analyses so we chose to exclude those species. For our analysis we included five outgroups: *Coccoloba swartzii, Ruprechtia chiapensis, Ruprechtia fusca*, *Ruprechtia tangarana* and *Salta triflora*.

DNA Extraction, Amplification, and Sequencing– Total DNA was extracted from silica-dried leaf material when available, or from herbarium material, using the Qiagen DNeasy Mini Plant Kit (Qiagen, Valencia, California, USA). In the case of herbarium samples (Appendix 1), plant material was manually ground and incubated for 18 h with 30 µL of BME (beta-mercapto-ethanol) and 30 µL of proteinase K before continuing with the protocol for the DNeasy kit, with a final elution of 100 µL. In some instances, we obtained extracted DNA aliquots through the generosity of Toby Pennington (Royal Botanical Gardens at Edinburgh), and Janelle Burke (Cornell University).

Data for the reconstruction of the plant phylogeny includes two non-coding chloroplast regions: *psbA-trnH, rps16-trnK*, and three nuclear regions: nrITS, the second intron of the low-copy nuclear region *Leafy* (*lfy2i*) and the third intron of the nitrate

reductase gene NIA (NIA3i). Protocols for standard polymerase chain reaction (PCR) followed those of Sanchez and Kron (2009). Primers for *psbA-trnH* and *rps16-trnK* were derived from Shaw et al. (2007). Primers for *lfy2i* used are described in Sanchez and Kron (in rev.), and primers for NIA3i were derived from Howarth and Baum (2002).

PCR products were cleaned using Qiagen QIAquick PCR purification columns (Valencia, California). All sequences were run on an ABI 377 Automated DNA Sequencer (Ramsey, Minnesota) at Wake Forest University's Automated DNA Sequencing Facility. Sequences were edited with Sequencher v.3.1.1 (Gene Codes, Ann Arbor, Michigan). In the case of ITS and chloroplast DNA regions, PCR product purification was followed by direct sequencing. For *Leafy* and NIA, purification was followed by cloning using the Invitrogen TOPO TA Cloning kit Carlsbad, California). Cloning products were cleaned with ExoSAP-IT (Affymetrix Inc, California) and three clones per sample were screened to evaluate sequence heterogeneity.

2. *Pseudomyrmex*

Taxon sampling– A total of 22 species of *Pseudomyrmex* were sampled for this study. Most of the sequences derive from GenBank (Appendix 2), except for those belonging to the *Pseudomyrmex* associated with *Triplaris*. A total of eight species and 15 individuals were included to represent the *P. viduus* group as well as other species of *Pseudomyrmex* that can colonize *Triplaris* (Appendix 2). Of the eight ant species collected in *Triplaris*, four are considered specialists (*P. dendroicus, P. mordax, P. triplaridis,* and *P. triplarinus*) and four are considered generalists (i.e., can inhabit other plant species; *P. elongatus*, *P. gebelli*, *P. longior*, and *P. viduus*).

DNA Extraction, Amplification, and Sequencing– Total DNA was extracted from ants preserved in 90% ethanol, using the Qiagen DNeasy Tissue Kit (Qiagen, Valencia, California, USA) and following the instructions by the manufacturer. Protocols for PCR followed those of Kautz et al. (2009). Data for the phylogenetic reconstruction of the ant phylogeny includes a fragment covering most of the mitochondrial cytochrome oxidase one gene, COI, and the nuclear long-wavelength rhodopsin gene (LR). Primers for LR were derived from Ward and Downie (2005) and for COI two sets of primers (COI-LCO and COI-HCO; COI-Jerry and COI-Pat) were used following those published by Kautz et al. (2009).

PCR products were cleaned using the Macherey-Nagle NucleoFast 96-well plate (Macherey-Nagle, Bethlehem, PA), and sequenced using Big Dye chemistry with an ABI 3730 automated sequencer (PE Applied Biosystems, Foster City, CA) at Duke University.

3. *Sequence Alignment and Phylogenetic Analyses*

All sequences were aligned using Mafft (Katoh et al., 2005) and subsequently adjusted by hand in MacClade version 4.0 (Maddison and Maddison, 2002), as needed. All matrices are available in TreeBASE (study number XXX) and all sequences were deposited in GenBank (Appendix 1 and 2). For *Triplaris* total missing data was between 6.3 – 9.7% (see Appendix 1; Table 1) and for *Pseudomyrmex,* 11.5 – 12.1% (Appendix 2; Table 3). Each gene region for both, plants and ants, was initially partitioned and analyzed separately, then concatenated for a combined molecular data set. Insertions/deletions were not coded as characters in this analysis.

Maximum Likelihood (ML) analyses were conducted on the matrices of the plants (*Triplaris*) and the ants (*Pseudomyrmex*). For the plants, we reconstructed phylogenies

using Garli (Zwickl, 2006) for the combined chloroplast, ITS-only, *lfy2i*-only, NIA3i-only, and total combined (chloroplast and nuclear) data sets. We also analyzed the total combined data set in two different ways: 1. including only one "representative" individual per species for a total of 16 taxa (including outgroups), and 2. multiple individuals per species (total of 36 taxa). The model of evolution was estimated in Garli and for each analysis corresponded to a GTR+I+G.

For the ants, we analyzed COI and LR separately and combined, under the same parameters as the plants (single- and multiple-individuals). Bootstrap analysis was used for evaluating node support (Felsenstein, 1985). Bootstrap supports for ML were calculated in Garli, based on 200 replicates with only one search replicate per bootstrap replicate.

Before the analyses, the dataset matrices for each organism were tested for incongruence, using the incongruence length difference (ILD) test as implemented in PAUP* version 4.0b1.0 (Swofford, 1998). All ILD tests used default parameters and 1000 replicates. The ILD test is often used to test if different molecular regions are or not congruent, although it is known to be sensitive to between-partition differences in evolutionary rates and extremes of rate heterogeneity among sites within the data (Dolphin et al., 2000; Yoder et al., 2001; Barker and Lutzoni, 2002; Dowton and Austin, 2002). Therefore, many studies have currently suggested that even if the ILD test shows significant incongruence, concatenating datasets from different regions may not result in misleading phylogenies (e.g., Yoder et al., 2001; Barker and Lutzoni, 2002; Dowton and Austin, 2002). In our case, when there was significant incongruence ($p = 0.01$) between regions, we assessed support for conflicting topologies of the analysis, before deciding

whether to combine.

4. Quantifying the degree of monophyly for *Triplaris spp.*

In cases where species do not appear monophyletic, we wanted to quantify how closely accessions or individuals from each morphological *Triplaris* species approach monophyly. We used the genealogical sorting index (GSI) to detect if taxon clustering exists in the data (Cummings et al., 2008). Each branch tip in each gene genealogy and in the total combined dataset was assigned to a class representing one of the seven species with more than two accessions (Appendix 1). The GSI quantifies the relative degree of exclusive ancestry of a group on a rooted tree topology. It is essentially the ratio of the minimum number of branches required to make a group (in this case, the species) monophyletic to the observed amount of exclusivity (smallest clade that includes all members of that group). The significance of the GSI is evaluated by permuting tip labels and determining the frequency of the values that are equal or greater than the observed GSI. It is a useful estimate since it is not affected by polytomies and can detect clustering even if the designated group is not monophyletic. The GSI provides a value between 0 and 1, where 1 indicates monophyly and 0 polyphyly (absence of exclusive ancestry). When GSI is below 1 it indicates paraphyletic groups and provides an estimate of genealogical differentiation.

The GSI and associated probability (p-value) were calculated using 10.000 permutations at http://www.genealogicalsorting.org/. We calculated the values across the trees for all four loci and for a total combined, with the null hypothesis that the degree of exclusive ancestry of branch tips observed is random (all individuals come from the same undifferentiated gene pool).

5. Ant-plant interactions

For studying the interaction between plants and ants we followed two approaches: 1. We compared the total combined, single individual phylogenies and 2. Compared the multiple-individuals phylogeny of plants to the ants associated.

For the first approach we compared phylogenies and drew a tanglegram between the taxa that associate. A tanglegram is a visual way to compare two phylogenetic trees; they are often used to compare evolutionary histories of host and parasite species and to analyze genes of species in the same geographical area (Venkatachalam et al., 2009). For the second approach we used GSI. For GSI we labeled the tips of the plant phylogeny with the names of the ant species that is associated with a given plant individual.

6. Geographical distributions

We also mapped in ArcGIS (Esri, Redlands, CA) all the available data collections from Sanchez and Ward (Appendix 3) and those provided in the literature by Davidson et al. (1988), Ule (1906), and Wheeler (1942). We compared the distributions of the ants and plants in order to understand the pattern of association at a geographical scale.

Results

1. *Triplaris*

Table 1 presents a complete list of gene regions and combined data matrix statistics for the analyses of the single individual and multiple individual phylogenies. This study generated 166 new sequences (Appendix 1).

One individual per species. Of the four data sets analyzed (cp, ITS, *lfy2i*, NIA3i), NIA3i was the most parsimony informative (17.7%) region, while *lfy2i* was the least parsimony informative (12.6 %). NIA3i, *lfy2i*, and ITS were congruent for the ILD test ($p > 0.1$); however, cp was not congruent with any of the nuclear genes ($p = 0.01$). Although there were some general differences in the reconstructions of the intraspecific relationships of *Triplaris*, none of the differences were supported. The bootstrap for the cp data set only recovered two clades with less than 75% support. One of the clades was congruent with NIA3i and the other clade was only partially incongruent with *lfy2i* in the reconstruction of one species, *Triplaris punctata* (in *lfy2i* this species is placed in a polytomy). The incongruence that was strongly supported between cp and the nuclear data sets was the relationship of *Ruprechtia* to *Triplaris*. In the cp data set, *Salta triflora* is placed as sister to the rest of *Ruprechtia* while in the nuclear data sets *S. triflora* is sister to *Triplaris* + *Ruprechtia*. We decided to combine all data sets, since there was no strong incongruence in the reconstructions of the intraspecific relationships of *Triplaris*.

The combined data set for all regions was 5774 bp long and contained 18% parsimony informative characters (pic). In the total combined (as well as the individual data sets) there is strong support for *Triplaris* as monophyletic and sister to *Ruprechtia*. *Salta triflora* is supported as sister to both genera (95% bootstrap; Fig. 1A). Within

Triplaris, there is no support for the monophyly of *T. melaenodendron*, since the accessions from Colombia (S110) and Costa Rica (S405) are not more closely related. The accession from Colombia is supported as sister to *T. purdiei* (Fig. 1A; 77%) and this clade is in turn sister to *T. cumingiana* (85%); the accession from Costa Rica is sister to this three species, although the support is low (58%). *Triplaris peruviana* and *T. longifolia* are strongly supported as sister (Fig. 1A; 98%) and *T. americana* is placed in a clade with *T. weigeltiana* and *T. poeppigiana* (Fig 1A; 88% support).

Multiple individual per species. For the analyses we included 32 individuals in 12 species of *Triplaris*. We included several accessions for species that have a wide geographic distribution: nine accessions of *T. americana* (the most widespread species), five individuals of both *T. melaenodendron* and *T. weigeltiana*, and two of each, *T. cumingiana, T. dugandii, T. peruviana*, and *T. poeppigiana* (Appendix 1). For all other species we only included one accession, since those species have a more restricted distribution.

The most variable region was NIA3i (21.2% pic) and the least *lfy2i* (16% pic; Table 1). All regions were incongruent according to ILD ($p = 0.01$), except NIA3i and ITS ($p = 0.4$). When bootstraps supports between regions are compared, again, few relationships are moderately or highly supported (> 70%). For the intraspecific relationships of *Triplaris*, only 3 clades for the chloroplast data set were recovered, two of which received less than 55% bootstrap support. For ITS there were only five clades with support over 70%, seven for *lfy2i*, and four for NIA3i. There were few supported incongruences: 1. *Triplaris purdiei* was supported as sister to one accession of *T. cumingiana* L4623 for cp (94% bootstrap), while NIA3i placed it as sister to another

accession of *T. cumingiana* (S122; 80% support); in the *lfy2i* data set, *T. cumingiana* L4623 was sister to *T. cumingiana* S122 (99%; but *T. purdiei* was not amplified for *lfy2i*). 2. *Triplaris weigeltiana* Z18 was placed as sister to *T. longifolia* in *lfy2i* (74% support), while ITS and NIA3i placed the former with other individuals of *T. weigeltiana* (78% for ITS; 73% for NIA3i). As with the one representative/species data sets, another source of incongruence was the reconstruction of relationships within *Ruprechtia* and the placement of *Salta.*

The GSI test for each individual molecular region found instances of significant clustering based on taxonomy (Table 2). A total of 13 of the 28 test conducted yielded p-values of less than 0.05 (Table 2). *Triplaris melaenodendron* was significantly clustered in all the different data sets ($p < 0.02$; Table 2); other species, such as *T. americana*, *T. cumingiana* (including *T. purdiei*), and *T. weigeltiana* showed a significant clustering in at least two data sets, while the clustering of *T. peruviana* was only supported in *lfy2i*. *Triplaris dugandii* and *T. poeppigiana* were not supported as clustered (Table 2).

Since the incongruences between data sets were minor, and most data sets supported similar clustering of species using GSI, we decided to combine data sets. The total combined matrix had 21.5% pic (Table 1). *Triplaris* received strong support as monophyletic (100%, Fig. 1B), although few of the intraspecific relationships were supported. Three clades that received some support correspond to currently recognized species: *T. melaenodendron* (65% support), *T. peruviana* (56%), and *T. weigeltiana* (78%).

Other species such as *T. americana*, *T. cumingiana*, *T. dugandii*, and *T. poeppigiana*, were not recovered as monophyletic; for example, the nine accessions of *T.*

americana were placed in four different places in the cladogram (Fig. 1B). However, when the individual accessions of *T. americana, T. dugandii*, and *T. poeppigiana* are examined closely, there is evidence for some geographical structure (Fig 1B and 1C). Some clades correspond to accessions that were collected in sympatry, such as the case of *T. poeppigiana* S134 and *T. americana* S129 (75% bootstrap support), or *T. dugandii* S58 and *T. poeppigiana* S209 (74% bootstrap support; Fig. 1B and 1C). Geographical structure is also evident in the accessions of *T. americana* collected in Colombia (S97 and S121) and those collected in Southeastern Peru, Northern Bolivia, and Western Brazil (S140 to S139; Fig 1B). The group comprising *T. punctata* to *T. longifolia* is composed of individuals collected in San Martin, Peru (see insert on Fig. 1C).

Triplaris purdiei is nested within *T. cumingiana* and the clade is moderately supported (76%), since *T. purdiei* is more closely related to an accession of *T. cumingiana* from Colombia (60% support). For the remaining species sampled (i.e., *T. gardneriana, T. longifolia, T. punctata,* and *T. setosa*), we only included one individual; therefore at present we cannot assess their monophyly.

Using the GSI test to examine the clustering of species, we recovered four clades that are considered monophyletic (GSI = 1; Table 2) and correspond to the three strongly supported clades mentioned above plus *T. cumingiana* (including *T. purdiei*). *Triplaris americana* shows significant clustering although it is paraphyletic (GSI = 0.57; $p < 0.001$). The accessions of *T. dugandii* and *T. poeppigiana* are not significantly clustered in either case (Table 2).

2. <u>*Pseudomyrmex*</u>

When the matrices were analyzed separately for the single- and multiple-individual phylogeny, COI was more parsimony informative than LR (Table 3). Both regions were incongruent according to ILD ($p = 0.01$); however, the reconstructions differed in the intraspecific relationships of *Pseudomyrmex* not associated with *Triplaris* and the relationships of the outgroups (*Tetraponera*). The eight species of *Pseudomyrmex* collected from *Triplaris* did not present moderately or strongly supported incongruences between data sets. Since we are only interested in recovering the relationships of the *Pseudomyrmex* associated with *Triplaris*, and not in the details of the intraspecific relationships of the species of ants sampled, we decided to combine our data sets. As a result, our total combined matrix had 2125 bp and 33.5% pic.

Using one individual per species of the associate ants to *Triplaris*, or more (two or three individuals) did not make any difference in the reconstruction since the species were strongly supported as monophyletic (> 80% bootstrap support; Fig. 2A and B). Five of the eight ant species collected in *Triplaris* were recovered in a strongly supported clade (Fig. 2; 98% bootstrap), four of which are considered obligate symbionts (*P. viduus* is not considered a specialist). *Pseudomyrmex dendroicus* and *P. triplarinus* are strongly supported as sister (100% bootstrap) and they are in turn sister to *P. mordax* (55% bootstrap). The former two species are considered to have pruning behavior. The placement of *P. viduus* and *P. triplaridis* within the *P. triplarinus* subgroup (sensu Ward) is not resolved (< 50%).

Pseudomyrmex elongatus, *P. gebelli* and *P. longior* are placed in a same clade (97%; Fig. 2B) and are placed as sister to a clade containing species such as *P. gracilis* and *P. nigropilosus*.

3. Ant-plant interactions

Overall the relationships between *Triplaris* and *Pseudomyrmex* depicted in Fig. 3 are highly promiscuous; for most species there is no consistent pattern of specialization since one species of plant can associate with multiple species of ants and vice versa. Plants with wide geographical distributions, such as *T. americana* are inhabited by the four obligate species of *Pseudomyrmex*. Also, the ants that have a greater distribution, such as *P. triplaridis* and *P. triplarinus*, associate with more than five species of *Triplaris* that overlap their range of distribution. Species that have restricted distributions, such as *P. mordax*, associate with fewer species. However, in the case of *T. poeppigiana*, we found no association to *Pseudomyrmex*, but with *Azteca* (Fig. 3).

An approach that considers the relationships at the individual level (using multiple individuals of the same species) shows in detail that species such as *P. triplaridis* and *P. triplarinus* associate with multiple host individuals from different species, without consideration of phylogenetic history (Fig. 4). When the ant associates are used as labels for the multiple individual phylogeny of *Triplaris* using GSI, it is evident that some ant species show more structure than others (Table 4). For example, *P. dendroicus* is only associated with individuals of *T. americana* from a particular clade; the same pattern occurs with *P. mordax* (Fig. 4). *Pseudomyrmex triplaridis* is also significantly clustered ($p = 0.025$), a pattern that is evidenced clearly in the accessions of *T. weigeltiana* (Fig. 4).

Since we only included a reduced number of individuals for our phylogeny, we mapped the data from more collections (Appendix 3), using GIS. Using a more extensive

data set it is clear that *P. mordax* associates with three species of *Triplaris* (*T. americana*, *T. cumingiana*, and *T. purdiei*) that overlap their geographical range of distribution (Fig. 5; Appendix 3). *Pseudomyrmex dendroicus* associates only with *T. americana* even when other species of *Triplaris* are found in sympatry (Fig. 5; Appendix 3). *Pseudomyrmex triplaridis* and *P. triplarinus* are associated with multiple host plants growing in sympatry, but *Triplaris weigeltiana*, when associated with an obligate ant species, tends to be found with *P. triplaridis* (Appendix 3).

Discussion

In this study we included a broad sampling of ants and plants, several molecular regions from different genomes, and data from multiple collections across the geographical range of distribution. We integrated the phylogenies of both organisms as well as geographical data, in order to have a broad perspective of this case of ant-plant interaction. This is the first phylogenetic study exploring the intraspecific relationships of *Triplaris*, the intraspecific relationships of the *Pseudomyrmex* associated with *Triplaris*, and to study the interaction using molecular and geographical data.

1. *Triplaris*

Triplaris comprises 18 species of trees distributed from Southern Mexico to Southern Brazil, but the diversity is highest (15 species) in the Amazon Basin. Some species have widespread distributions (i.e., *T. americana, T. cumingiana*, *T. melaenodendron*, and *T. weigeltiana*); however most species (*T. caracassana, T. efistulifera, T. longifolia, T. peruviana, T. physocalyx, T. punctata, T. purdiei, T. matogrossensis*, and *T. moyobambensis*) have restricted distributions. These species have been collected in few localities and not much is known about their ecology.

Identifying *Triplaris* at the species level can be difficult, since most species are very similar when sterile and the characters for reliably discriminating species are based on fruits (the genus is dioecious; Brandbyge, 1986). Fruit morphology can be highly variable, with most species displaying long-winged fruits, except one, *T. physocalyx*, which has very short wings (that could potentially affect their dispersal). Although for the present study we included collections from herbaria (collections that could be reliably identified), and field collections of mostly female plants, more morphological work is

needed in order to provide more characters for discriminating the species. Identifying the species correctly is a relevant issue that can affect how we understand their ecology and their interaction with organisms such as ants.

From the molecular standpoint, our understanding of the intraspecific relationships of *Triplaris* change as we include more individuals per species. When we sample a "representative" individual for each species, we are considering the history of that particular individual and we are assuming that the species is monophyletic. However, when more individuals per species are included, the reconstructed gene trees for each molecular region and for the total combined analysis do not support several species as monophyletic (Fig. 1B).

A different way to approach monophyly versus nonmonophyly is to use the recently published GSI test (Cummings et al., 2008). From a phylogenetic point of view, the interpretation of paraphyly is only associated with the absence of phylogenetic support for a given taxon (in our case, a species). However, the transition from polyphyly to monophyly is continuous and daughter lines accumulate a nonrandom distribution of alleles before reaching reciprocal monophyly (Cummings et al., 2008). The GSI index provides a metric for estimating the accumulation of genetic ancestry before reaching monophyly and has the potential to bridge population studies to phylogenetics, since it gives an index and a statistical value for patterns of clustering (Cummings et al. 2008).

In the case of *Triplaris* the clustering was significant for species such as *T. melaenodendron* in all different gene regions, while others were significantly clustered in only two or three gene regions, and two species were not clustered in any region (*T. dugandii* and *T. poeppigiana*; Table 2). Since each individual gene region did not provide

enough variability to recover the intraspecific relationships of *Triplaris*, especially at the backbone, GSI proved once again to be useful for detecting species clustering based on taxonomy, when data sets are combined. The pattern was consistent with what was found in the individual gene regions, where two species showed no significant clustering, while the other five did (Table 2).

Accessions of *T. americana* were significantly clustered although the species is not monophyletic (GSI < 1). In the case of species with widespread geographical ranges, such as *T. americana*, that had yet not achieved reciprocal monophyly, there may be clades that represent reciprocal gene flow due to geographic proximity. As shown in Fig. 1C, the two accessions of *T. americana* from Colombia are more closely related, as are the four accessions from Southeastern Peru, Western Brazil and Bolivia. This result indicates that this species could be approaching taxonomic exclusivity, a pattern that is supported by all molecular data sets except ITS (Table 2). However, we could also suggest that there might be some clades that could be differentiating from each other due to restricted gene flow, or there might be cryptic species.

In the cases where there is no significant clustering (*T. dugandii* and *T. poeppigiana*), hybridization or incomplete lineage sorting might be rendering the species as polyphyletic. However, there could be cryptic species as well.

2. <u>*Pseudomyrmex*</u>

Ward (1999) studied the relationships of 12 *Pseudomyrmex* species from the *P. viduus* group in a cladistic analysis, using 72 morphological characters and three outgroups. In his analysis he recovered two strongly supported clades he denominated the

P. triplarinus and *P. concolor* subgroups (Fig. 169 in Ward, 1999). Each of these subgroups is characterized by their specificity to a host plant: *Triplaris* and *Tachigali* (Fabaceae) respectively.

The *P. triplarinus* subgroup is confined to South America and Panama with the exception of *P. viduus*, which has a wide spread distribution, ranging from Mexico to Bolivia and Brazil. Within the subgroup (95% bootstrap support), Ward (1999) recovered two well-supported clades, one comprising *P. triplaridis*, *P. viduus*, and *P. vitabilis* (96% support in Ward, 1999) and the second one comprising four species: *P. ultrix*, *P. mordax*, *P. dendroicus*, and *P. triplarinus* (89% support).

Although in our single- and multiple-individual analyses we did not include two species from the *P. triplarinus* subgroup (*P. vitabilis* and *P. ultrix*), the subgroup is recovered with strong support (>90% bootstrap; Fig. 2A and B), corroborating Ward's analysis. Our multiple-individual analysis also suggested that the five species within the subgroup are monophyletic. Within the subgroup, there is low support (55 and 56%, Fig. 2A and B) for the sister relationship of *P. mordax* to *P. triplarinus* and *P. dendroicus*; however, when *P. ultrix* is included, the clade has stronger support (89%; Fig. 169 in Ward, 1999). Our main difference with Ward's cladogram is that we do not recover a sister relationship between *P. triplaridis* and *P. viduus*. Instead, *P triplaridis* is placed at the base of the clade followed by *P. viduus*, although there is no support (< 50%; Fig. 2A and B).

Pseudomyrmex viduus is the most widespread species within this clade and it is the least host-specific species, since it can colonize several different plant genera (e.g. *Cordia*, *Pseudobombax*, *Sapium*) in addition to *Triplaris* (Ward, 1999). According to

Ward, the generalist habit of *P. viduus* would represent a shift from an ancestral *Triplaris* host plant to an expanded host plant use. However, in our phylogeny there is no support for the placement of *P. triplaridis* and *P. viduus* (Fig. 2A and B). Therefore we have two hypotheses: if the reconstruction supports the first divergent species to be *P. viduus*, then the specialist behavior to a *Triplaris* host would have evolved from a generalist ant species only once. The association to multiple host plants would be the precursor for a specialization to one of those plant genera. If *P. triplaridis* is at the base of the clade, *P. viduus* would represent a reversal to a generalist behavior from a specialized ancestor. More studies using additional gene regions could clarify the position of these two species and therefore discriminate between our two hypotheses.

A common characteristic to the ant species in the *P. triplarinus* subgroup is the highly aggressive behavior, where they patrol their hosts constantly and attack herbivores. However, pruning behavior, where the ants clear the vegetation around the host tree, has evolved only once in this subgroup: in the clade of the sister species *P. triplarinus* and *P. dendroicus* (Fig. 2A and B; Ward, 1999). This behavior, displayed in other species of *Pseudomyrmex* associated with *Acacia*, has been thought to favor the plant host by reducing the competing vegetation and therefore increasing the availability of nutrients (Janzen, 1966, 1972). However, Davidson et al. (1988) demonstrated that pruning is meant to reduce the invasion by other dominant ant genera (i.e., *Crematogaster*). However, it is unclear why only these two species of the *P. triplarinus* subgroup display the behavior.

Several other species of *Pseudomyrmex* can also colonize the hollow stems of *Triplaris*, although, not all display aggressive behaviors (Ward, 1999; Sanchez pers. obs.).

In our analyses we included collections from *P. elongatus*, *P. gebelli*, and *P. longior*, but other species, such as those from the *P. sericeus* group sensu Ward (e.g., *P. fortis*, *P. ita*, and *P. rubiginosus*), have also been reported to colonize *Triplaris.* These associations have evolved independently from the *P. triplarinus* subgroup (Fig. 2A and B; Ward, 1991) and all of these species are considered generalists, since they inhabit many different plant genera.

3. Ant-plant interactions

Most coevolutionary studies using molecular phylogenetics use one or very few genes and usually include only one representative individual per species (e.g., Machado et al., 2001; Weiblen and Bush, 2002; Quek et al., 2004; Weiblen, 2004; Gómez-Acevedo et al., 2010). This type of sampling takes into account the history of a particular gene and individual, and might emphasize ancient processes, bias the interpretation of coevolution or arrive to misleading conclusions (Jackson et al., 2008). Even more, when only one individual is sampled the monophyly of a species is assumed, but what do the patterns of association mean, if the species are not monophyletic? And what would our conclusions imply, if the intraspecific relationships change as we add more data?

Our study represents the most comprehensive phylogenetic study of an ant-plant interaction. We analyzed our multiple loci datasets using the single individual per species approach, but we also included multiple individuals per species, especially of those with wide range of distribution. In general, there is no coevolutionary pattern between *Triplaris* and *Pseudomyrmex*, however, there is some specificity and geographical sorting. This symbiosis probably evolved from a generalist ant ancestor that later specialized on

Triplaris and with time some ant species became even more specific to a particular host (i.e., *P. dendroicus*).

3.1. *Phylogenetic patterns.* Few cases of symbioses involve a significant pattern of coevolution between organisms, and most of the known cases involve parasites and their hosts (Hafner et al., 1994; Hafner and Page, 1995; Banks et al., 2006; Light and Hafner, 2007; Hammer et al., 2010). Some other cases that were thought to follow co-cladogenesis, such as the case of figs and their pollinators, have been demonstrated to be less specific than once thought (Machado et al., 2001; Cook and Rasplus, 2003; Jackson et al., 2008). The ant-plant interactions are no exception. The few studies that have addressed this type of association by comparing the phylogenies of both organisms have arrived at the same conclusion: there is no strict coevolution between the organisms (e.g., Chenuil and McKey, 1996; Quek et al., 2004; Gómez-Acevedo et al., 2010). In the association between *Acacia* and *Pseudomyrmex*, there is a clade of ants that is specialized on a clade of myrmecophyte *Acacia* (Gómez-Acevedo et al., 2010); but within those clades, the interactions are promiscuous, with several host species being colonized by multiple associates. The case of *Triplaris* and *Pseudomyrmex* follows a similar scenario. *Triplaris* and the ants of the *P. triplarinus* subgroup are strongly supported as monophyletic (Fig. 1 and 2). All species of *Triplaris* known to date are myrmecophytes and the ants of the subgroup (with the exception of *P. viduus*) are specialized on *Triplaris*.

Studying the *Triplaris-Pseudomyrmex* association using one individual per species is in accordance with the results of Gómez-Acevedo et al. (2010): the relationships are diffuse (Fig. 3). There is absence of congruence between phylogenies, which might reflect a recurrent change on hosts depending on availability. Species such

as *T. americana*, with ample ranges of distribution, tend to associate with the four obligate species of *Pseudomyrmex*. Other species with restricted distributions such as *T. purdiei*, are known to associate with only one species of obligate *Pseudomyrmex* (*P. mordax*). Another interesting pattern is that displayed by *T. poeppigiana*; although this species does not have a restricted range of distribution (Brandbyge, 1986) and occurs in sympatry with several species of *Triplaris* and three of the four obligate ant species, the species has only been recorded to associate with *Azteca* in different localities of Northern and Southern Peru (Appendix 3).

This one individual per species pattern provides a generalized picture of the mutualism between *Triplaris* and *Pseudomyrmex*. From Fig. 1A, we could infer that the association with *Pseudomyrmex* ants was present in all species of *Triplaris*, and that *T. poeppigiana* represents a shift on associate. We could also hypothesize that since most species of *Triplaris* have restricted ranges of distribution and *T. americana* is the most widespread species, the latter could represent an expansion in range that generated opportunities for more ant species to colonize thus creating the association pattern in Fig. 3. Since the pattern described in Fig. 1A and 3 reflects the history of that one particular individual, caution must be exercised when drawing conclusions on the evolution of the association.

As we include more individuals per species for reconstructing the phylogeny of *Triplaris*, we recover a tree with less structure (the backbone is not supported), and some species are not monophyletic (Fig. 1B). Although it is clear that the association with ants is no more specific than when a single individual per species is used, there are some patterns that are only evident when more individuals per species are included in an

analysis. There are three groups of *T. americana* that are associated with the obligate *Pseudomyrmex* (Fig. 4). One clade corresponds to the accessions from Colombia and it is associated with *P. mordax*, while another is associated with *P. dendroicus* (Fig. 4). Both of these associations are significantly clustered using GSI (Table 4). However, the individuals of *T. americana* that associate with *P. triplarinus* are not placed in a same clade (Fig. 4). This pattern is consistent with what is known about *P. triplarinus*: although an obligate species to *Triplaris* it colonizes several different hosts. As expected, this ant species is the only species from the obligate ants, that is not significantly clustered (Table 4). *Pseudomyrmex triplaridis* also tends to associate with several different species of *Triplaris* (three different species in our phylogeny; Fig. 4) and it is therefore a paraphyletic associate (Table 4); however, it has a very specific pattern of association with *T. weigeltiana*, which allows GSI to detect a non-random clustering (Table 4).

Other species of *Triplaris*, such as *T. melaenodendron* are not known to associate with any of the four obligate ant species (Fig. 3 and 4). *Triplaris melaenodendron* is distributed in Central America and Central Colombia (Brandbyge, 1986). The four obligate ant species pertaining to the *Pseudomyrmex triplarinus* subgroup are distributed from Panama to Southern Brazil and they do not overlap in their range of distribution with the Central American *T. melaenodendron*; however, this plant species is associated with *P. viduus* and several other *Pseudomyrmex* species, mainly from the *P. sericeus* group (Ward, 1999). In Colombia, *T. melaenodendron* was also not associated with any obligate plant-*Pseudomyrmex*, although *P. mordax* overlaps their range of distribution.

This might be a sampling effect or could reflect a lack of specialization by this host species.

3.2. *Geographical patterns*. In almost all myrmecophytes, multiple ant queens often colonize different modules of a same plant, which can lead to strong intra- and interspecific competition (Davidson and McKey, 1993). Strong competition can potentially be the major factor driving specialization in mutualisms (Federle and Rheindt, 2005) and if competitive interactions among ants are sufficiently strong and constant, ecological sorting could produce predictable patterns of ant-plant associations. Plants and ant lineages may have evolved in concert but some factors such as host-switching, secondary colonization and ecological replacement can modify the associations (Davidson and McKey, 1993). However, several other reasons can also cause a lack of host specificity in a symbiotic relationship. Ants may be more sensitive to habitat than to taxonomic differences among symbiotic partners (Longino, 1996; Yu and Davidson, 1997), and/or they could be affected by the demographic and life-history characteristics of the plant and ant populations (Beattie, 1985). It is likely that ecological processes that require no strict coevolution can therefore maintain the symbiosis. In the case of *Macaranga* it has been found that the association can be sensitive to processes such as geographic or biotic isolation, climatic change and fragmentation that cause local extinctions (Fiala et al., 1989).

The symbiosis between *Triplaris* and *Pseudomyrmex* probably follows a geographical mosaic, where species associate with others that overlap their range of distribution. Several species of plants and ants occur in sympatry: in San Martin, Peru seven species of plants were collected in proximity (Fig. 5B). Of the *Triplaris*-associate

ants, three species have restricted ranges of distribution (*P. mordax*, *P. vitabilis*, and *P. ultrix*), while the other three species (*P. dendroicus*, *P. triplaridis*, and *P. triplarinus*) occur in sympatry and overlap extensively in the Amazon basin (Ward, 1999).

Species of ants such as *P. mordax* and *P. triplarinus* do not seem to discriminate between host species. *Pseudomyrmex mordax* colonizes the three species of *Triplaris* that overlap its range of distribution (Fig. 5A; Appendix 3) and *Pseudomyrmex triplarinus* has been frequently collected in five of the 12 host plants we sampled (all in sympatry; Appendix 3). However, other species display more specificity. The 44 collection records for *P. dendroicus* (Appendix 3) show the same pattern: they were collected in a *T. americana* host. Although the monophyly of *T. americana* is disputable (Fig. 1B but see Table 2), the two accessions that were associated to *P. dendroicus* were placed in a same clade (Fig. 4). Even more, despite the fact that more species of *Triplaris* overlap the ant's range of distribution, *P. dendroicus* has not been collected with any other host. What can cause this pattern? Although we cannot conclude at present the main cause, some explanations can be based on distribution: *Triplaris americana* is the only host species that overlaps all of the ant's range of distribution. This could have caused, over time, a more specialized recognition by *P. dendroicus*. Another explanation could be based on habitat. The ants might choose *T. americana* because they establish and grow in places that are suitable for the ants. A third explanation can be based on competition. *Triplaris americana* is the most widespread and abundant plant species; if *P. dendroicus* is a weaker competitor it could be that the abundance determines the chance to encounter a host and successfully establishing a colony. In this scenario, a behavior registered for this ant species (Sanchez, unpub. data) could corroborate this hypothesis: *P. dendroicus* can

colonize several individuals of *Triplaris* by moving their colonies horizontally (the workers disperse eggs, larvae, pupae and even coccids between host plants). The workers can travel for more than five meters and establish colonies in other individuals without the presence of a queen. Although more experiments are needed in order to understand why this association is so specific, there might be a suite of explanations from historical to ecological processes. *Triplaris weigeltiana* also displays a level of specificity. Although this species has been found with several non-obligate ant species and genera (Fig 5; Appendix 3), the obligate associate is *P. triplaridis*. This specificity could be caused by a preference of *P. triplaridis* to *T. weigeltiana* based on factors such as habitat and geographical distribution.

4. Future directions

Since several host and ant species occur sympatrically, the question of when these species started diversifying arises. Some studies on Amazonian taxa (Bush, 1994; Patton et al., 1997), as well as the case of *Acacia-Pseudomyrmex* (Gómez-Acevedo et al., 2010) have been estimated to diversify before the Pleistocene. According to Ward (1999), the well-resolved phylogeny of the *Pseudomyrmex viduus* group (Fig. 2; Fig. 169 in Ward, 1999) and the extensive sympatry of the species suggests that ant diversification occurred before the Pleistocene, around the Tertiary. In the case of *Triplaris*, the only known fossil belongs to *Ruprechtia* (the sister genus), and the origin of the latter is estimated at approximately 8 mya (Burnham and Graham, 1999). It is possible that both, ants and plants, began diversifying at similar times in the Tertiary; however more studies are needed in order to estimate accurately the phylogenetic relationships of *Triplaris* and to try to elucidate the pattern of association with its ants.

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Appendix 5.1. Voucher information for plant DNA extractions used in this study. Sequences obtained from Genbank are given with their respective site specific numbers. New sequences generated for this study provide the following information: Taxon, collector(s), collection number (in italics), and Genbank accession numbers. Specimens are deposited at WFU (Wake Forest University) unless another herbarium is given. Herbarium acronyms follow Index Herbariorum, E = Royal Botanic Gardens Edinburgh, NY = New York Botanical Garden, BH= Cornell University, MO = Missouri Botanical Garden, WFU = Wake Forest University. Gene abbreviations are as follows: K= *rps16-trnK*, P= *psbA-trnH*, I = ITS, L = *lfy2i*, N = NIA. NA= not used in this study.

1. Sequences obtained from previous studies. ***Coccoloba swartzii*** Meisn., K- XX, I- FJ154469, L- EF442787; ***Ruprechtia chiapensis*** Lundell ex Standl. & Steyerm. K- XX, I- FJ154482, L- XX; ***Ruprechtia fusca*** Fernald, K- XX, I- FJ154483, L-XX; ***Ruprechtia tangarana*** Standl., K- XX, I- FJ154485, L- XX; ***Salta triflora*** (Griseb.) Adr. Sanchez, K- XX, I- GQ206267; ***Triplaris americana*** L., *Luckow 4635* (BH), K- XX, I- FJ154486, L- XX; ***Triplaris longifolia*** Huber, *Sanchez* 188, K- XX, I- XX, L- XX; ***Triplaris setosa*** Rusby, *Fuentes 5351* (MO), K- XX, I- FJ154488, L- XX; ***Triplaris weigeltiana*** (Rchb.) Kuntze, *Michelangeli s.n.*, K- XX, I- HM137446, L- XX.

2. Sequences generated in this study. Materials obtained from herbarium specimens are indicated with a * after the collection number.

Coccoloba swartzii Meisn., P- XX, N- XX; ***Ruprechtia chiapensis*** Lundell ex Standl. & Steyerm., *Burke s.n.* (BH), P- XX, N- NA; ***Ruprechtia fusca*** Fernald, *Pendry 868* (E), P- XX, N- XX; ***Ruprechtia tangarana*** Standl., P- XX, N- NA; ***Salta triflora*** (Griseb.) Adr. Sanchez, P- XX, L- NA, N- XX; ***Triplaris americana*** L., *Gutierrez et al. 865** (MO); K=

XX, P= XX, I = XX, L = NA, N = XX; ***Triplaris americana*** L., *Luckow 4635* (BH); P- XX, N- XX; ***Triplaris americana*** L., *Sanchez 77* (WFU); K= XX, P= NA, I = XX, L = XX, N = XX; ***Triplaris americana*** L., *Sanchez 97* (WFU); K= XX, P= XX, I = XX, L = XX, N = XX; ***Triplaris americana*** L., *Sanchez 121* (WFU); K= XX, P= XX, I = XX, L = XX, N = XX; ***Triplaris americana*** L., *Sanchez 129* (WFU); K= XX, P= XX, I = XX, L = XX, N = XX; ***Triplaris americana*** L., *Sanchez 139* (WFU); K= XX, P= XX, I = XX, L = XX, N = NA; ***Triplaris americana*** L., *Sanchez 140* (WFU); K= XX, P= XX, I = XX, L = XX, N = XX; ***Triplaris americana*** L., *Sanchez 176* (WFU); K= XX, P= XX, I = XX, L = XX, N = XX; ***Triplaris cumingiana*** Fisch. & C.A. Mey. ex C.A. Mey., *Luckow 4623* (BH); K= XX, P= XX, I = XX, L = XX, N = XX; ***Triplaris cumingiana*** Fisch. & C.A. Mey. ex C.A. Mey., *Sanchez 123* (WFU); K= XX, P= XX, I = XX, L = XX, N = XX; ***Triplaris dugandii*** Brandbyge, *Sanchez 58* (WFU); K= XX, P= XX, I = XX, L = NA, N = XX; ***Triplaris dugandii*** Brandbyge, *Sanchez 203* (WFU); K= XX, P= XX, I = XX, L = XX, N = NA; ***Triplaris gardneriana*** Wedd., *Rodal 426** (NY) K= NA, P= XX, I = XX, L = NA, N = XX; ***Triplaris longifolia*** Huber, *Sanchez 188* (WFU), K- XX, I- XX, L- XX; ***Triplaris melaenodendron*** (Bertol.) Standl. & Steyerm. ***subsp. colombiana*** (Meisn.) Brandbyge, *Sanchez 110* (WFU); K= XX, P= XX, I = XX, L = XX, N = NA; ***Triplaris melaenodendron*** (Bertol.) Standl. & Steyerm. ***subsp. colombiana*** (Meisn.) Brandbyge, *Sanchez 119* (WFU); K= XX, P= XX, I = XX, L = XX, N = XX; ***Triplaris melaenodendron*** (Bertol.) Standl. & Steyerm., *Sanchez 405* (WFU); K= NA, P= XX, I = NA, L = XX, N = XX; ***Triplaris melaenodendron*** (Bertol.) Standl. & Steyerm., *Sanchez 407* (WFU); K= XX, P= XX, I = XX, L = XX, N = XX; ***Triplaris melaenodendron*** (Bertol.) Standl. & Steyerm., *Sanchez 411* (WFU); K= XX, P= XX, I = XX, L = XX, N =

XX; ***Triplaris peruviana*** Fisch. & Meyer ex C.A. Meyer, *Sanchez 171* (WFU); K= XX, P= XX, I = XX, L = XX, N = NA; ***Triplaris peruviana*** Fisch. & Meyer ex C.A. Meyer, *Sanchez 173* (WFU); K= XX, P= XX, I = NA, L = XX, N = XX; ***Triplaris poeppigiana*** Wedd., *Sanchez 134* (WFU); K= XX, P= XX, I = XX, L = NA, N = XX; ***Triplaris poeppigiana*** Wedd., *Sanchez 209* (WFU); K= XX, P= XX, I = XX, L = XX, N = NA; ***Triplaris punctata*** Standl., *Sanchez 205* (WFU); K= XX, P= XX, I = XX, L = XX, N = NA; ***Triplaris purdiei*** Meisnn. in Mart., *Sanchez 100* (WFU); K= XX, P= XX, I = XX, L = NA, N = XX; ***Triplaris setosa*** Rusby, *Fuentes 5351* (MO), P= XX, N = NA; ***Triplaris weigeltiana*** (Rchb.) Kuntze, *Michelangeli s.n.* (WFU); K- XX, I- HM137446, L- XX; ***Triplaris weigeltiana*** (Rchb.) Kuntze, *Ramirez s.n.* (ANDES); K= XX, P= XX, I = XX, L = XX, N = XX; ***Triplaris weigeltiana*** (Rchb.) Kuntze, *Sanchez 44* (WFU); K= XX, P= XX, I = XX, L = NA, N = XX; ***Triplaris weigeltiana*** (Rchb.) Kuntze, *Zambrano 8* (ANDES); K= XX, P= XX, I = XX, L = NA, N = XX; ***Triplaris weigeltiana*** (Rchb.) Kuntze, *Zambrano 18* (ANDES); K= XX, P= XX, I = XX, L = XX, N = XX.

Appendix 5.2. Voucher information for ant DNA extractions used in this study. Sequences obtained from Genbank are given with their respective site specific numbers. New sequences generated for this study provide the following information: Taxon, collector(s), collection number (in italics), and Genbank accession numbers. Specimens are part of Sanchez collection with duplicates in UCD (P. Ward collection). Gene abbreviations are as follows: C= COI, L = LR. NA= not used in this study.

1. Sequences obtained from Genbank. ***Myrcidris epicharis*** Ward, C- NA, L- AY703785; ***Pseudomyrmex ferrugineus*** F. Smith, C- FJ436818, L- HM020792; ***Pseudomyrmex flavicornis*** F. Smith, C- FJ436819, L- AY703795; ***Pseudomyrmex***

gracilis Fabricius, C- FJ436825, L- AY703797; ***Pseudomyrmex godmani*** Forel, C- FJ436820, L- AY703796; ***Pseudomyrmex haytianus*** (Forel) Wheeler, C- FJ436826, L- AY703798; ***Pseudomyrmex major*** (Forel) Ward, C- FJ436827, L- FJ436878; ***Pseudomyrmex mixtecus*** Ward, C- FJ436829, L- HM020793; ***Pseudomyrmex nigrocinctus*** Emery, C- FJ436830, L- AY703802; ***Pseudomyrmex nigropilosus*** Emery, C- FJ436833, L- AY703803; ***Pseudomyrmex peperi*** Forel, C- FJ436836, L- HM020794; ***Pseudomyrmex perboscii*** F. Smith C- FJ436837, L- FJ436886; ***Pseudomyrmex satanicus*** Wheeler, C- FJ436840, L- FJ436889; ***Pseudomyrmex spinicola*** Emery, C- FJ436841, L- FJ436890; ***Pseudomyrmex tachigaliae*** Forel, C- NA, L- AY703814; ***Pseudomyrmex viduus*** F. Smith C- NA, L- AY703818; ***Tetraponera ambigua*** Emery, C- NA, L- AY703772; ***Tetraponera punctulata*** Smith, C- DQ373001, L- AY703782; ***Tetraponera rufonigra*** Jerdon, C- FJ436846, L- AY703783.

2. Sequences generated in this study. ***Pseudomyrmex dendroicus*** Forel, *Sanchez 31*; C- XX, L- XX; ***Pseudomyrmex dendroicus*** Forel, *Sanchez 38*; C- XX, L- XX; ***Pseudomyrmex dendroicus*** Forel, *Sanchez 40*; C- XX, L- NA; ***Pseudomyrmex elongatus*** Mayr, *Sanchez 55*; C- XX, L- XX; ***Pseudomyrmex gebellii*** Forel, *Sanchez 68*; C- XX, L- XX; ***Pseudomyrmex longior*** Forel, *Sanchez 67*; C- XX, L- XX; ***Pseudomyrmex mordax*** Warming, *Sanchez 51*; C- XX, L- XX; ***Pseudomyrmex mordax*** Warming, *Sanchez 58*; C- XX, L- XX; ***Pseudomyrmex mordax*** Warming, *Sanchez 70*; C- XX, L- XX; ***Pseudomyrmex triplaridis*** Forel, *Sanchez 1*; C- XX, L- XX; ***Pseudomyrmex triplaridis*** Forel, *Sanchez 20*; C- XX, L- XX; ***Pseudomyrmex triplarinus*** Weddell, *Sanchez 9*; C- XX, L- NA; ***Pseudomyrmex triplarinus*** Weddell, *Sanchez 18*; C- XX, L- NA; ***Pseudomyrmex viduus*** F. Smith, *Sanchez 19*; C- XX, L- XX.

Appendix 5.3. Collections of *Triplaris* and associate ants known to date.

Triplaris							Ants		
Species	**#**	**Collector**	**Country**	**State**	**Lat.**	**Long.**	**#**	**Species**	**NOTES**
T. americana	S69	A. Sanchez	Peru	Madre de Dios	-12.84	-69.29	S29	Pseudomyrmex dendroicus	
T. americana	S70	A. Sanchez	Peru	Madre de Dios	-12.84	-69.29		Pseudomyrmex dendroicus	
T. americana	S74	A. Sanchez	Peru	Madre de Dios	-12.84	-69.29	S31	Pseudomyrmex dendroicus	
T. americana	S75	A. Sanchez	Peru	Madre de Dios	-12.84	-69.29	S32	Pseudomyrmex dendroicus	
T. americana	S76	A. Sanchez	Peru	Madre de Dios	-12.84	-69.29	S33	Pseudomyrmex dendroicus	
T. americana	S77	A. Sanchez	Peru	Madre de Dios	-12.84	-69.28	S34	Pseudomyrmex dendroicus	
T. americana	S78	A. Sanchez	Peru	Madre de Dios	-12.84	-69.29	S35	Pseudomyrmex dendroicus	
T. americana	S79	A. Sanchez	Peru	Madre de Dios	-12.84	-69.29	S36	Pseudomyrmex dendroicus	
T. americana	S80	A. Sanchez	Peru	Madre de Dios	-12.57	-70.09	S37	Pseudomyrmex dendroicus	
T. americana	S81	A. Sanchez	Peru	Madre de Dios	-12.57	-70.09		Pseudomyrmex dendroicus	
T. americana	S82	A. Sanchez	Peru	Madre de Dios	-12.57	-70.09	S38	Pseudomyrmex dendroicus	
T. americana	S84	A. Sanchez	Peru	Madre de Dios	-12.57	-70.10	S40	Pseudomyrmex dendroicus	
T. americana	S85	A. Sanchez	Peru	Madre de Dios	-12.56	-70.09	S41	Pseudomyrmex dendroicus	
T. americana	S86	A. Sanchez	Peru	Madre de Dios	-12.56	-70.09	S42	Pseudomyrmex dendroicus	
T. americana	S87	A. Sanchez	Peru	Madre de Dios	-12.57	-70.09		Pseudomyrmex dendroicus	
T. americana	S91A	A. Sanchez	Peru	Madre de Dios	-12.57	-70.09	S46A	Pseudomyrmex dendroicus	
T. americana	S93	A. Sanchez	Colombia	Santander	6.52	-74.08	S48	Crematogaster sp.	
T. americana	S94	A. Sanchez	Colombia	Santander	6.86	-73.75	S49	Azteca sp.	
T. americana	S95	A. Sanchez	Colombia	Santander	6.86	-73.75	S50	Pseudomyrmex mordax	
T. americana	S96	A. Sanchez	Colombia	Santander	7.60	-73.55	S51	Pseudomyrmex mordax	
T. americana	S97	A. Sanchez	Colombia	Cesar	8.62	-73.68	S52	Pseudomyrmex mordax	
T. americana	S98	A. Sanchez	Colombia	Cesar	9.14	-73.66	S53	Pseudomyrmex mordax	
T. americana	S103	A. Sanchez	Colombia	Bolivar	10.14	-75.04	S58	Pseudomyrmex mordax	
T. americana	S104	A. Sanchez	Colombia	Sucre	9.58	-75.19	S59	Pseudomyrmex mordax	
T. americana	S105	A. Sanchez	Colombia	Sucre	9.49	-75.23	S60	Pseudomyrmex elongatus	
T. americana	S106	A. Sanchez	Colombia	Sucre	9.41	-75.09	S61	Pseudomyrmex mordax	
T. americana	S107	A. Sanchez	Colombia	Cordoba	9.30	-75.88	S62	Pseudomyrmex mordax	
T. americana	S108	A. Sanchez	Colombia	Cordoba	9.30	-75.85	S63	Crematogaster sp.	
T. americana	S109	A. Sanchez	Colombia	Cordoba	8.59	-75.73	S64	Pseudomyrmex mordax	

Triplaris							Ants		
Species	**#**	**Collector**	**Country**	**State**	**Lat.**	**Long.**	**#**	**Species**	**NOTES**
T. americana	S120	A. Sanchez	Colombia	Tolima	4.07	-74.94	S69	Crematogaster sp.	
T. americana	S121	A. Sanchez	Colombia	Tolima	4.07	-74.94	S70	Pseudomyrmex mordax	
T. americana	S123	A. Sanchez	Brazil	Acre	-9.98	-66.80	S72	Pseudomyrmex triplarinus	
T. americana	S125	A. Sanchez	Brazil	Acre	-9.98	-66.77	S74	Pseudomyrmex triplarinus	
T. americana	S126	A. Sanchez	Brazil	Acre	-9.98	-66.77	S75	Pseudomyrmex triplarinus	
T. americana	S127	A. Sanchez	Brazil	Acre	-10.01	-66.77	S76	Pseudomyrmex triplarinus	
T. americana	S128	A. Sanchez	Brazil	Acre	-10.02	-66.77	S77	Pseudomyrmex triplarinus	
T. americana	S136	A. Sanchez	Brazil	Acre	-10.08	-67.55	S84	Pseudomyrmex dendroicus	
T. americana	S140	A. Sanchez	Brazil	Acre	-9.75	-67.67	S88	Pseudomyrmex triplarinus	
T. americana	S155	A. Sanchez	Brazil	Acre	-9.95	-67.81	S99	Pseudomyrmex dendroicus	
T. americana	S156	A. Sanchez	Brazil	Acre	-9.93	-67.89	S100	Pseudomyrmex dendroicus	
T. americana	S157	A. Sanchez	Brazil	Acre	-10.02	-67.84		Pseudomyrmex dendroicus	
T. americana	S158	A. Sanchez	Brazil	Acre	-10.02	-67.84		Pseudomyrmex dendroicus	
T. americana	S159	A. Sanchez	Brazil	Acre	-10.06	-67.86		Pseudomyrmex dendroicus	
T. americana	S175	A. Sanchez	Peru	San Martín	-6.72	-76.21	S106	Pseudomyrmex triplarinus	
T. americana	S176	A. Sanchez	Peru	San Martín	-6.72	-76.21	S107	Pseudomyrmex triplarinus	
T. americana	S184	A. Sanchez	Peru	San Martín	-7.35	-76.68	S115	Pseudomyrmex triplaridis	
T. americana	S186	A. Sanchez	Peru	San Martín	-6.37	-76.54	S117	Pseudomyrmex triplarinus	
T. americana	S198	A. Sanchez	Peru	San Martín	-5.98	-77.22	S126	Pseudomyrmex dendroicus	
T. americana	S208	A. Sanchez	Peru	Loreto	-5.12	-75.73	S135	Pseudomyrmex triplaridis	
T. americana	S210	A. Sanchez	Peru	Loreto	-5.12	-75.73	S136	Pseudomyrmex dendroicus	
T. americana		D.W. Davidson	Peru	Madre de Dios	-11.85	-71.32		Pseudomyrmex dendroicus	
T. americana		P.S. Ward	Bolivia	Beni	-14.80	-66.38	PSW9076	Pseudomyrmex dendroicus	
T. americana		P. Bettella	Bolivia	Santa Cruz	-14.73	-62.80		Pseudomyrmex dendroicus	
T. americana		P.S. Ward	Ecuador	Napo	-1.07	-77.62	PSW11323	Pseudomyrmex dendroicus	
T. americana	B33476	J. Brandbyge	Ecuador	Sucumbios	-0.25	-76.35		Pseudomyrmex dendroicus	
T. americana		N.L. Toft	Peru	Huanuco	-9.58	-74.80		Pseudomyrmex dendroicus	
T. americana			Peru	Tarapoto				Pseudomyrmex dendroicus	Ule, 1906; Ward, 1999
T. americana			Colombia	Villavicencio	4.28	-74.05		Pseudomyrmex dendroicus	Schremmer, 1984; Ward, 1999
T. americana			Venezuela	Miranda	10.27	-66.33		Pseudomyrmex mordax	Jaffe et al., 1986
T. americana			Colombia	Since	8.75	-66.27		Pseudomyrmex mordax	Schremmer, 1984; Ward, 1999
T. americana		P.S. Ward	Bolivia	Beni	-14.80	-66.38	PSW9075	Pseudomyrmex triplarinus	
T. americana		P. Bettella	Bolivia	Santa Cruz	-17.85	-63.17		Pseudomyrmex triplarinus	
T. americana		Unknown	Brazil	Rondonia	-8.75	-63.47		Pseudomyrmex triplarinus	

Triplaris Species	#	Collector	Country	State	Lat.	Long.	Ants #	Ants Species	NOTES
T. americana		N.L. Toft	Peru	Huanuco	-9.58	-74.80		Pseudomyrmex triplarinus	
T. americana			Brazil	Mato Grosso	-17.27	-56.98		Pseudomyrmex triplarinus	Oliveira et al., 1987
T. americana	L4635	M. Luckow	Venezuela	Caracas	10.48	-66.90		Pseudomyrmex viduus	
. cf. americana	S129	A. Sanchez	Brazil	Acre	-10.02	-66.78	S78	Pseudomyrmex triplarinus	
. cf. americana	S130	A. Sanchez	Brazil	Acre	-10.05	-66.81	S79	Pseudomyrmex triplarinus	
. cf. americana	S131	A. Sanchez	Brazil	Acre	-10.08	-66.84	S80	Pseudomyrmex triplarinus	
. cf. americana	S132	A. Sanchez	Brazil	Acre	-10.08	-66.86	S81	Pseudomyrmex triplarinus	
. cf. americana	S133	A. Sanchez	Brazil	Acre	-10.01	-66.78	S82	Pseudomyrmex triplarinus	
. cf. americana	S138	A. Sanchez	Brazil	Acre	-10.02	-67.56	S86	Pseudomyrmex dendroicus	
. cf. americana	S139	A. Sanchez	Brazil	Acre	-9.95	-67.86	S87	Pseudomyrmex dendroicus	
. cf. americana	S141	A. Sanchez	Brazil	Acre	-9.75	-67.67	S89	Pseudomyrmex dendroicus	
. cf. americana	S143	A. Sanchez	Brazil	Acre	-9.76	-67.67	S89B	Pseudomyrmex triplarinus	
. cf. americana	S145	A. Sanchez	Brazil	Acre	-9.75	-67.67	S90	Pseudomyrmex dendroicus	
. cf. americana	S146	A. Sanchez	Brazil	Acre	-9.71	-68.09	S91	Pseudomyrmex dendroicus	
. cf. americana	S147	A. Sanchez	Brazil	Acre	-9.71	-68.09	S92	Pseudomyrmex dendroicus	
. cf. americana	S148	A. Sanchez	Brazil	Acre	-9.71	-68.11	S93	Pseudomyrmex dendroicus	
. cf. americana	S149	A. Sanchez	Brazil	Acre	-9.61	-68.25	S94	Pseudomyrmex dendroicus	
. cf. americana	S150	A. Sanchez	Brazil	Acre	-9.57	-68.28	S95	Pseudomyrmex dendroicus	
. cf. americana	S151	A. Sanchez	Brazil	Acre	-9.49	-68.35	S96	Pseudomyrmex dendroicus	
. cf. americana	S152	A. Sanchez	Brazil	Acre	-9.49	-68.35		Pseudomyrmex dendroicus	
. cf. americana	S153	A. Sanchez	Brazil	Acre	-9.46	-68.38	S97	Pseudomyrmex dendroicus	
Γ. cumingiana	S90B	A. Sanchez	Colombia	Cundinamarca	5.18	-74.66		No ants	
Γ. cumingiana	S91B	A. Sanchez	Colombia	Cundinamarca	5.18	-74.66		No ants	
Γ. cumingiana	S92	A. Sanchez	Colombia	Cundinamarca	5.19	-74.70		No ants	
Γ. cumingiana	S122	A. Sanchez	Colombia	Cundinamarca	4.49	-74.61	S71	Crematogaster sp.	
Γ. cumingiana			Panama	Gatun Lake	8.95	-79.8		Pseudomyrmex mordax	Wheeler, 1942
T. dugandii	S49	A. Sanchez	Peru	Loreto	-3.63	-73.34	S09	Pseudomyrmex triplarinus	
T. dugandii	S58	A. Sanchez	Peru	Loreto	-3.54	-73.08	S18	Pseudomyrmex triplarinus	
T. dugandii	S66	A. Sanchez	Peru	Loreto	-4.48	-73.51	S26	Pseudomyrmex triplarinus	
T. dugandii	S124	A. Sanchez	Brazil	Acre	-9.98	-66.80	S73	Pseudomyrmex triplarinus	
T. dugandii	S142	A. Sanchez	Brazil	Acre	-9.75	-67.67		No ants	
T. dugandii	S144	A. Sanchez	Brazil	Acre	-9.76	-67.67		No ants	
T. dugandii	S181	A. Sanchez	Peru	San Martín	-6.72	-76.25	S112	Pseudomyrmex triplaridis	
T. dugandii	S190	A. Sanchez	Peru	San Martín	-6.57	-76.13	S121	Pseudomyrmex triplaridis	

Triplaris							Ants	
Species	**#**	**Collector**	**Country**	**State**	**Lat.**	**Long.**	**#**	**Species**
T. dugandii	S200	A. Sanchez	Peru	San Martín	-6.06	-77.26	S128	Pseudomyrmex triplaridis
T. dugandii	S201	A. Sanchez	Peru	San Martín	-6.05	-77.26		Pseudomyrmex triplaridis
T. dugandii	S203	A. Sanchez	Peru	San Martín	-6.22	-76.82	S130	Pseudomyrmex triplaridis
T. dugandii	B36208	J. Brandbyge	Ecuador	Sucumbios	-0.25	-76.35		Pseudomyrmex triplaridis
T. dugandii		P.S. Ward	Ecuador	Napo	-0.80	-77.78	PSW11393	Pseudomyrmex ultrix
T. longifolia	S189	A. Sanchez	Peru	San Martín	-6.62	-76.17	S120	Pseudomyrmex triplarinus
T. longifolia	S192	A. Sanchez	Peru	San Martín	-6.60	-76.15	S123	Pseudomyrmex triplarinus
T. cf. longifolia	S188	A. Sanchez	Peru	San Martín	-6.46	-76.35	S119	Pseudomyrmex triplarinus
T. cf. longifolia	S191	A. Sanchez	Peru	San Martín	-6.60	-76.15	S122	Pseudomyrmex triplarinus
T. cf. longifolia	S193	A. Sanchez	Peru	San Martín	-6.61	-76.16		Pseudomyrmex triplarinus
Γ. melaenodendron	S110	A. Sanchez	Colombia	Antioquia	6.27	-75.57		No ants
Γ. melaenodendron	S111	A. Sanchez	Colombia	Antioquia	6.27	-75.57		No ants
Γ. melaenodendron	S112	A. Sanchez	Colombia	Antioquia	6.49	-75.79		No ants
Γ. melaenodendron	S113	A. Sanchez	Colombia	Antioquia	6.49	-75.79		No ants
Γ. melaenodendron	S114	A. Sanchez	Colombia	Antioquia	6.56	-75.84		No ants
Γ. melaenodendron	S115	A. Sanchez	Colombia	Antioquia	6.56	-75.84		No ants
Γ. melaenodendron	S116	A. Sanchez	Colombia	Antioquia	6.59	-75.85	S65	Crematogaster sp.
Γ. melaenodendron	S117	A. Sanchez	Colombia	Antioquia	6.59	-75.85	S66	Crematogaster sp.
Γ. melaenodendron	S118	A. Sanchez	Colombia	Antioquia	6.54	-75.77	S67	Pseudomyrmex longior
Γ. melaenodendron	S119	A. Sanchez	Colombia	Valle del Cauca	4.01	-77.23	S68	Pseudomyrmex gebellii
Γ. melaenodendron	S402	A. Sanchez	Costa Rica	Guanacaste	10.34	-85.27	S140	Pseudomyrmex viduus
Γ. melaenodendron	S403	A. Sanchez	Costa Rica	Guanacaste	10.17	-85.59	S141	Pseudomyrmex viduus
Γ. melaenodendron	S404	A. Sanchez	Costa Rica	Guanacaste	10.80	-85.65	S142	Pseudomyrmex viduus
Γ. melaenodendron	S405	A. Sanchez	Costa Rica	Guanacaste	10.80	-85.65		Crematogaster sp.
Γ. melaenodendron	S406	A. Sanchez	Costa Rica	Guanacaste	10.97	-85.69		Pseudomyrmex viduus
Γ. melaenodendron	S407	A. Sanchez	Costa Rica	Puntarenas	9.79	-84.61	S143	Crematogaster sp.
Γ. melaenodendron	S408	A. Sanchez	Costa Rica	Puntarenas	8.53	-83.39	S144	Crematogaster sp.
Γ. melaenodendron	S409	A. Sanchez	Costa Rica	Puntarenas	8.53	-83.39		No ants
Γ. melaenodendron	S410	A. Sanchez	Costa Rica	Puntarenas	8.99	-83.26		Crematogaster sp.
Γ. melaenodendron	S411	A. Sanchez	Costa Rica	Puntarenas	8.48	-83.60		No ants
Γ. melaenodendron		D.H. Janzen	Costa Rica	Guanacaste	10.33	-85.20		Pseudomyrmex viduus
Γ. melaenodendron		J. Longino	Costa Rica	Guanacaste	10.35	-85.35		Pseudomyrmex viduus
T. peruviana	S171	A. Sanchez	Peru	San Martín	-6.55	-76.34	S102	Pseudomyrmex triplaridis
T. peruviana	S172	A. Sanchez	Peru	San Martín	-6.55	-76.34	S103	Pseudomyrmex triplaridis

Triplaris Species	#	Collector	Country	State	Lat.	Long.	Ants #	Ants Species
T. peruviana	S173	A. Sanchez	Peru	San Martín	-6.59	-76.31	S104	Pseudomyrmex triplaridis
T. peruviana	S174	A. Sanchez	Peru	San Martín	-6.59	-76.31	S105	Pseudomyrmex triplarinus
T. peruviana	S179	A. Sanchez	Peru	San Martín	-6.73	-76.25	S110	Azteca sp.
T. peruviana	S180	A. Sanchez	Peru	San Martín	-6.73	-76.25	S111	Pseudomyrmex triplarinus
T. peruviana	S183	A. Sanchez	Peru	San Martín	-6.92	-76.38	S114	Pseudomyrmex triplaridis
T. peruviana	S185	A. Sanchez	Peru	San Martín	-7.12	-76.69	S116	Pseudomyrmex triplaridis
T. peruviana	S187	A. Sanchez	Peru	San Martín	-6.44	-76.50	S118	Pseudomyrmex triplarinus
T. poeppigiana	S71	A. Sanchez	Peru	Madre de Dios	-12.83	-69.29		Azteca sp.
T. poeppigiana	S72	A. Sanchez	Peru	Madre de Dios	-12.83	-69.29		Azteca sp.
T. poeppigiana	S73	A. Sanchez	Peru	Madre de Dios	-12.84	-69.29	S30	Azteca sp.
T. poeppigiana	S83	A. Sanchez	Peru	Madre de Dios	-12.57	-70.09	S39	Azteca sp.
T. poeppigiana	S89	A. Sanchez	Peru	Madre de Dios	-12.57	-70.09	S44	Azteca sp.
T. poeppigiana	S90A	A. Sanchez	Peru	Madre de Dios	-12.57	-70.09	S45A	Azteca sp.
T. poeppigiana	S134	A. Sanchez	Brazil	Acre	-10.01	-66.78		No ants
T. poeppigiana	S182	A. Sanchez	Peru	San Martín	-6.86	-76.36	S113	Azteca sp.
T. poeppigiana	S209	A. Sanchez	Peru	Loreto	-5.12	-75.73		Azteca sp.
T. punctata	S205	A. Sanchez	Peru	San Martín	-6.47	-76.32	S132	Pseudomyrmex triplarinus
T. purdiei	S99	A. Sanchez	Colombia	Cesar	9.23	-73.52	S54	Pseudomyrmex mordax
T. purdiei	S100	A. Sanchez	Colombia	Magdalena	9.57	-73.90	S55	Pseudomyrmex elongatus
T. purdiei	S101	A. Sanchez	Colombia	Magdalena	9.57	-73.90	S56	Crematogaster sp.
T. purdiei	S102	A. Sanchez	Colombia	Atlantico	10.71	-74.75	S57	Crematogaster sp.
T. setosa	S199	A. Sanchez	Peru	San Martín	-6.06	-77.26	S127	Pseudomyrmex sp.
T. setosa	S204	A. Sanchez	Peru	San Martín	-6.41	-76.62	S131	Pseudomyrmex triplaridis
T. sp.	S178	A. Sanchez	Peru	San Martín	-6.71	-76.21	S109	Pseudomyrmex triplaridis
T. sp.	S207	A. Sanchez	Peru	Loreto	-5.12	-75.69	S134	Pseudomyrmex triplaridis
T. weigeltiana	S40	A. Sanchez	Peru	Loreto	-3.74	-73.25	S01	Pseudomyrmex triplaridis
T. weigeltiana	S41	A. Sanchez	Peru	Loreto	-3.83	-73.38	S03	Pseudomyrmex triplaridis
T. weigeltiana	S42	A. Sanchez	Peru	Loreto	-3.84	-73.41		No ants
T. weigeltiana	S43	A. Sanchez	Peru	Loreto	-3.85	-73.43	S04	Pseudomyrmex triplaridis
T. weigeltiana	S44	A. Sanchez	Peru	Loreto	-3.85	-73.45	S05	Azteca sp.
T. weigeltiana	S45	A. Sanchez	Peru	Loreto	-3.84	-73.40	S06	Pseudomyrmex triplaridis
T. weigeltiana	S46	A. Sanchez	Peru	Loreto	-3.83	-73.39	S07	Dolychoderus bidens
T. weigeltiana	S47	A. Sanchez	Peru	Loreto	-3.63	-73.34	S07b	Pseudomyrmex triplaridis
T. weigeltiana	S48	A. Sanchez	Peru	Loreto	-3.63	-73.34	S08	Pseudomyrmex triplaridis

Triplaris							Ants		
Species	**#**	**Collector**	**Country**	**State**	**Lat.**	**Long.**	**#**	**Species**	**NOTES**
Γ. weigeltiana	S50	A. Sanchez	Peru	Loreto	-3.94	-73.32	S10	Azteca sp.	
Γ. weigeltiana	S51	A. Sanchez	Peru	Loreto	-3.92	-73.32	S11	Pseudomyrmex triplaridis	
Γ. weigeltiana	S52	A. Sanchez	Peru	Loreto	-3.90	-73.32	S12	Azteca sp.	
Γ. weigeltiana	S53	A. Sanchez	Peru	Loreto	-3.90	-73.32	S13	Cephalotes ramiphilus	
Γ. weigeltiana	S54	A. Sanchez	Peru	Loreto	-3.89	-73.32	S14	Crematogaster sp.	
Γ. weigeltiana	S55	A. Sanchez	Peru	Loreto	-3.79	-73.26	S15	Azteca sp.	
Γ. weigeltiana	S56	A. Sanchez	Peru	Loreto	-3.59	-73.12	S16	Pseudomyrmex triplaridis	
Γ. weigeltiana	S57	A. Sanchez	Peru	Loreto	-3.58	-73.11	S17	Camponotus sexguttatus	
Γ. weigeltiana	S59	A. Sanchez	Peru	Loreto	-3.54	-73.08	S19	Pseudomyrmex viduus	
Γ. weigeltiana	S60	A. Sanchez	Peru	Loreto	-3.49	-73.09	S20	Pseudomyrmex triplaridis	
Γ. weigeltiana	S61	A. Sanchez	Peru	Loreto	-3.48	-73.08	S21	Pseudomyrmex triplaridis	
Γ. weigeltiana	S62	A. Sanchez	Peru	Loreto	-3.43	-73.04	S22	Pseudomyrmex triplaridis	
Γ. weigeltiana	S63	A. Sanchez	Peru	Loreto	-3.42	-73.02	S23	Azteca sp.	
Γ. weigeltiana	S64	A. Sanchez	Peru	Loreto	-3.40	-73.00	S24	Pseudomyrmex triplaridis	
Γ. weigeltiana	S65	A. Sanchez	Peru	Loreto	-3.41	-73.01	S25	Azteca sp.	
Γ. weigeltiana	S67	A. Sanchez	Peru	Loreto	-4.46	-73.49	S27	Crematogaster sp.	
Γ. weigeltiana	S68	A. Sanchez	Peru	Loreto	-4.46	-73.44	S28	Pseudomyrmex triplaridis	
Γ. weigeltiana	S177	A. Sanchez	Peru	San Martín	-6.71	-76.21	S108	Pseudomyrmex triplaridis	
Γ. weigeltiana	S206	A. Sanchez	Peru	Loreto	-5.12	-75.69	S133	Pseudomyrmex triplaridis	
Γ. weigeltiana		J. Brandbyge	Ecuador	Sucumbios	-0.27	-76.33		Pseudomyrmex triplaridis	
Γ. weigeltiana	B36192	J. Brandbyge	Ecuador	Sucumbios	-0.25	-76.35		Pseudomyrmex triplaridis	
Γ. weigeltiana		H.E. Box	Guyana	Mahaica	6.25	-57.55		Pseudomyrmex triplaridis	
Γ. weigeltiana			Guyana	Camaria	6.65	-59.57		Pseudomyrmex triplaridis	Wheeler, 1942
Γ. weigeltiana			Peru	Iquitos				Pseudomyrmex triplaridis	Ule, 1906; Ward, 1999
Γ. weigeltiana		L. Gillespie	Guyana	Cuyuni	6.80	-59.75		Pseudomyrmex viduus	
Γ. weigeltiana		H.E. Box	Guyana	Mahaica	6.25	-57.55		Pseudomyrmex viduus	
cf. weigeltiana	S135	A. Sanchez	Brazil	Acre	-10.08	-67.54	S83	Pseudomyrmex triplaridis	
cf. weigeltiana	S137	A. Sanchez	Brazil	Acre	-10.09	-67.55	S85	Pseudomyrmex triplaridis	
cf. weigeltiana	S154	A. Sanchez	Brazil	Acre	-9.23	-68.53	S98	Pseudomyrmex triplarinus	
cf. weigeltiana	S194	A. Sanchez	Peru	San Martín	-6.04	-77.12	S124	Pseudomyrmex triplaridis	
cf. weigeltiana	S195	A. Sanchez	Peru	San Martín	-6.04	-77.12		No ants	
cf. weigeltiana	S196	A. Sanchez	Peru	San Martín	-5.98	-77.21		Pseudomyrmex triplaridis	
cf. weigeltiana	S197	A. Sanchez	Peru	San Martín	-5.98	-77.22	S125	Azteca sp.	
cf. weigeltiana	S202	A. Sanchez	Peru	San Martín	-6.05	-77.16	S129	Azteca sp.	

Table 5.1.

Statistics for the gene regions used for *Triplaris* under Maximum Likelihood (ML). PIC = parsimony informative characters.

Analysis	Statistic	cp	ITS	*lfy2i*	NIA3i	Total combined
One individual	Aligned length	1943	780	1657	1388	5768
	Variable sites (%)	509 (26.2)	229 (29.4)	406 (24.5)	528 (38)	1663 (28.8)
	PIC (%)	298 (15.3)	137 (17.6)	208 (12.6)	246 (17.7)	1040 (18)
	Missing taxa (%)	0 (0)	0 (0)	2 (12.5)	3 (18.9)	5 (6.3)
Multiple individuals	Aligned length	2059	780	1659	1397	5895
	Variable sites (%)	602 (29.2)	246 (31.5)	449 (27.1)	592 (42.4)	1872 (31.8)
	PIC (%)	425 (20.6)	152 (19.5)	264 (16)	296 (21.2)	1268 (21.5)
	Missing taxa (%)	0 (0)	2 (5.4)	8 (21.6)	8 (21.6)	18 (9.7)

Table 5.2.

Genealogical sorting index (GSI) using taxonomic species as labels, in the multiple-individual phylogenies of *Triplaris*.

	cp		ITS		*lfy2i*		NIA3i		Combined	
Triplaris	GSI	p-value	GSI	p-value	GSI	p-value	GSI	p-value	GSI	p-value
americana	0.23	0.098	0.31	0.02*	0.33	0.016*	0.62	0.000*	0.57	0.000*
cumingiana + *purdiei*	0.47	0.009*	0.20	0.15	1	0.02*	0.55	0.002*	1	0.001*
dugandii	0.07	0.77	0.08	0.636	-	-	-	-	0.07	0.787
melaenodendron	1	0.000*	0.32	0.027*	0.77	0.000*	0.55	0.002*	1	0.000*
peruviana	0.31	0.09	-	-	1	0.03*	-	-	1	0.014*
poeppigiana	0.23	0.14	0.18	0.21	-	-	-	-	0.14	0.297
weigeltiana	0.20	0.17	1	0.000*	0.11	0.47	1	0.000*	1	0.000*

Table 5.3.

Statistics for the gene regions used for *Pseudomyrmex* under Maximum Likelihood (ML). PIC = parsimony informative characters.

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Analysis	Statistic	COI	LR	Total combined
One individual	Aligned length	1527	595	2122
	Variable sites (%)	601 (39.4)	167 (28)	770 (36.3)
	PIC (%)	530 (34.7)	100 (16.8)	694 (32.7)
	Missing taxa (%)	3 (15.4)	2 (7.7)	6 (11.5)
Multiple individuals	Aligned length	1528	597	2125
	Variable sites (%)	621 (40.6)	167 (27.8)	788 (37.1)
	PIC (%)	572 (37.4)	101 (16.9)	712 (33.5)
	Missing taxa (%)	4 (12.1)	4 (12.1)	8 (12.1)

Table 5.4.

Genealogical sorting index (GSI) using ants as labels on the total combined, multiple-individual phylogeny of *Triplaris*.

Ant species	GSI	p-value
P. dendroicus	0.49	**0.048**
P. mordax	1	**0.016**
P. triplaridis	0.29	**0.025**
P. triplarinus	0.25	0.057
P. viduus	0.12	0.381
Azteca	0.14	0.296

Figure 5.1 A-C.

Intraspecific relationships of *Triplaris* and geographical distribution of the collections used for the cladograms. For the phylogenies, numbers above or below the branches indicate bootstrap support values for maximum likelihood (ML); only bootstrap values > 50% are shown. A = ML results for the total combined dataset using one individual per species; B = ML results for the total combined dataset using multiple individual per species. C = Geographical distribution of the accessions used for the phylogenies; arrows with collection numbers indicate cases in which sympatric individuals are most closely related on the cladogram. Insert on the left is a zoom to the collections in San Martin, Peru. Colors denote different species, see legend insert.

Figure 5.1 A-C.

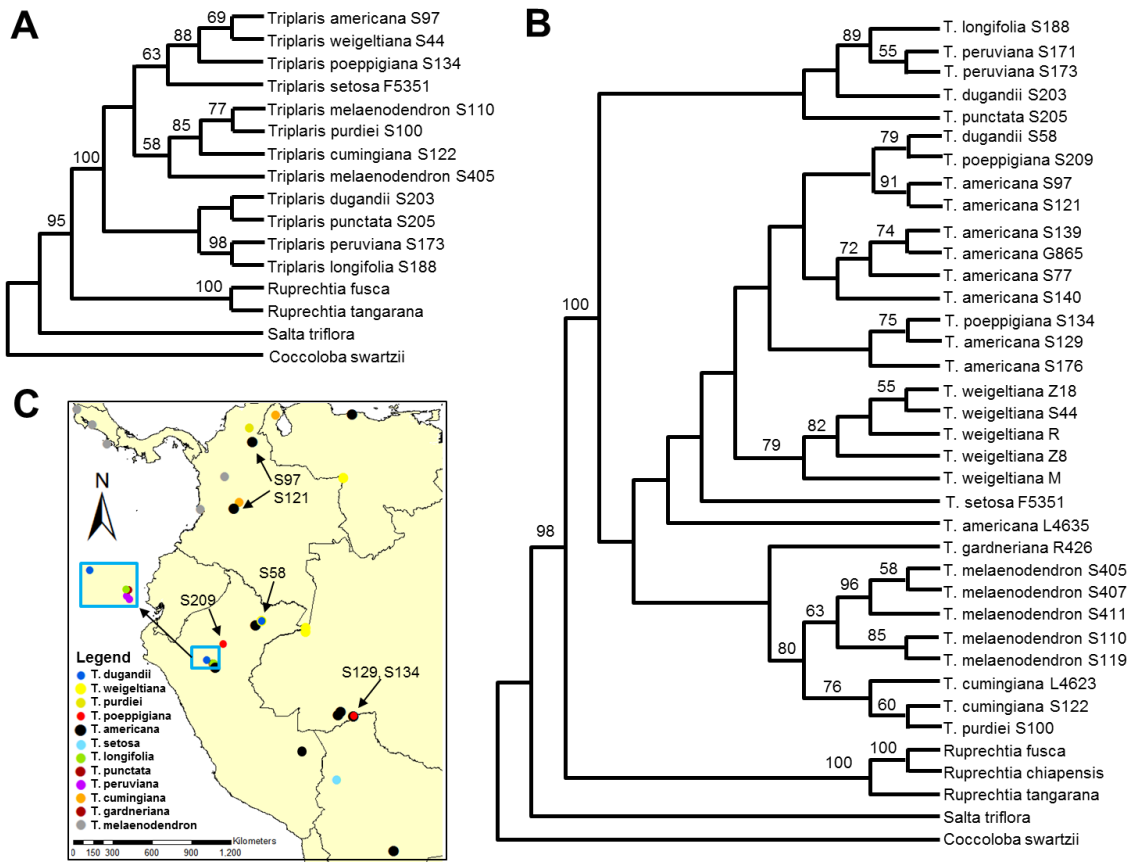

A
Triplaris americana S97
Triplaris weigeltiana S44
Triplaris poeppigiana S134
Triplaris setosa F5351
Triplaris melaenodendron S110
Triplaris purdiei S100
Triplaris cumingiana S122
Triplaris melaenodendron S405
Triplaris dugandii S203
Triplaris punctata S205
Triplaris peruviana S173
Triplaris longifolia S188
Ruprechtia fusca
Ruprechtia tangarana
Salta triflora
Coccoloba swartzii
B
T. longifolia S188
T. peruviana S171
T. peruviana S173
T. dugandii S203
T. punctata S205
T. dugandii S58
T. poeppigiana S209
T. americana S97
T. americana S121
T. americana S139
T. americana G865
T. americana S77
T. americana S140
T. poeppigiana S134
T. americana S129
T. americana S176
T. weigeltiana Z18
T. weigeltiana S44
T. weigeltiana R
T. weigeltiana Z8
T. weigeltiana M
T. setosa F5351
T. americana L4635
T. gardneriana R426
T. melaenodendron S405
T. melaenodendron S407
T. melaenodendron S411
T. melaenodendron S110
T. melaenodendron S119
T. cumingiana L4623
T. cumingiana S122
T. purdiei S100
Ruprechtia fusca
Ruprechtia chiapensis
Ruprechtia tangarana
Salta triflora
Coccoloba swartzii
C
N
S97
S121
S58
S209
S129, S134
Legend
T. dugandii
T. weigeltiana
T. purdiei
T. poeppigiana
T. americana
T. setosa
T. longifolia
T. punctata
T. peruviana
T. cumingiana
T. gardneriana
T. melaenodendron
Kilometers
0 150 300 600 900 1,200

Figure 5.2 A-B.

Cladograms obtained for the intraspecific relationships of *Pseudomyrmex* with emphasis on the *P. triplarinus* subgroup. In blue are the four obligate symbiont species; dashed lines indicate topologies that are not supported and might change our understanding of the evolution of ant specialists to *Triplaris*. Numbers above or below the branches indicate bootstrap support values for maximum likelihood (ML). Only bootstrap values > 50% are shown. A = ML results for the total combined dataset using one individual per species; B = ML results for the total combined dataset using multiple individual per species.

Figure 5.2 A-B.

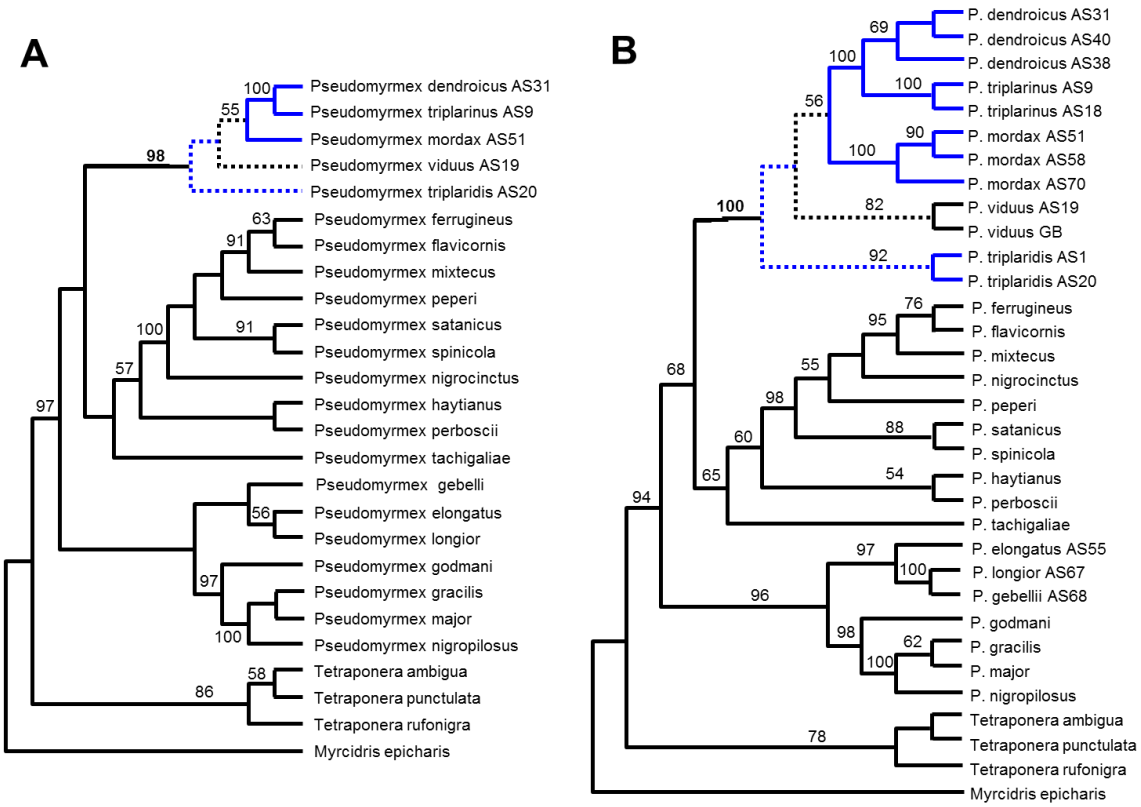

A
100
55
98
Pseudomyrmex dendroicus AS31
Pseudomyrmex triplarinus AS9
Pseudomyrmex mordax AS51
Pseudomyrmex viduus AS19
Pseudomyrmex triplaridis AS20
63
91
Pseudomyrmex ferrugineus
Pseudomyrmex flavicornis
Pseudomyrmex mixtecus
Pseudomyrmex peperi
100
91
Pseudomyrmex satanicus
Pseudomyrmex spinicola
57
Pseudomyrmex nigrocinctus
97
Pseudomyrmex haytianus
Pseudomyrmex perboscii
Pseudomyrmex tachigaliae
Pseudomyrmex gebelli
56
Pseudomyrmex elongatus
Pseudomyrmex longior
97
Pseudomyrmex godmani
Pseudomyrmex gracilis
100
Pseudomyrmex major
Pseudomyrmex nigropilosus
58
86
Tetraponera ambigua
Tetraponera punctulata
Tetraponera rufonigra
Myrcidris epicharis
B
69
100
100
56
90
100
P. dendroicus AS31
P. dendroicus AS40
P. dendroicus AS38
P. triplarinus AS9
P. triplarinus AS18
P. mordax AS51
P. mordax AS58
P. mordax AS70
100
82
P. viduus AS19
P. viduus GB
92
P. triplaridis AS1
P. triplaridis AS20
76
95
P. ferrugineus
P. flavicornis
P. mixtecus
55
P. nigrocinctus
68
98
P. peperi
88
P. satanicus
60
P. spinicola
65
54
P. haytianus
94
P. perboscii
P. tachigaliae
97
P. elongatus AS55
100
P. longior AS67
96
P. gebellii AS68
98
P. godmani
62
P. gracilis
100
P. major
P. nigropilosus
Tetraponera ambigua
78
Tetraponera punctulata
Tetraponera rufonigra
Myrcidris epicharis

Figure 5.3.

Tanglegram comparing the relationships between *Triplaris* and the *Pseudomyrmex* associated, based on their single individual per species maximum likelihood trees. The lines between trees indicate the associations reported in the literature and the information derived from Sanchez and Ward collections (Appendix 3). Dashed lines indicate associations with generalist ants (in grey); note that *T. melaenodendron* only associates with generalist ants. *Triplaris poeppigiana* was only found in association with ants of the genus *Azteca*.

Figure 5.3.

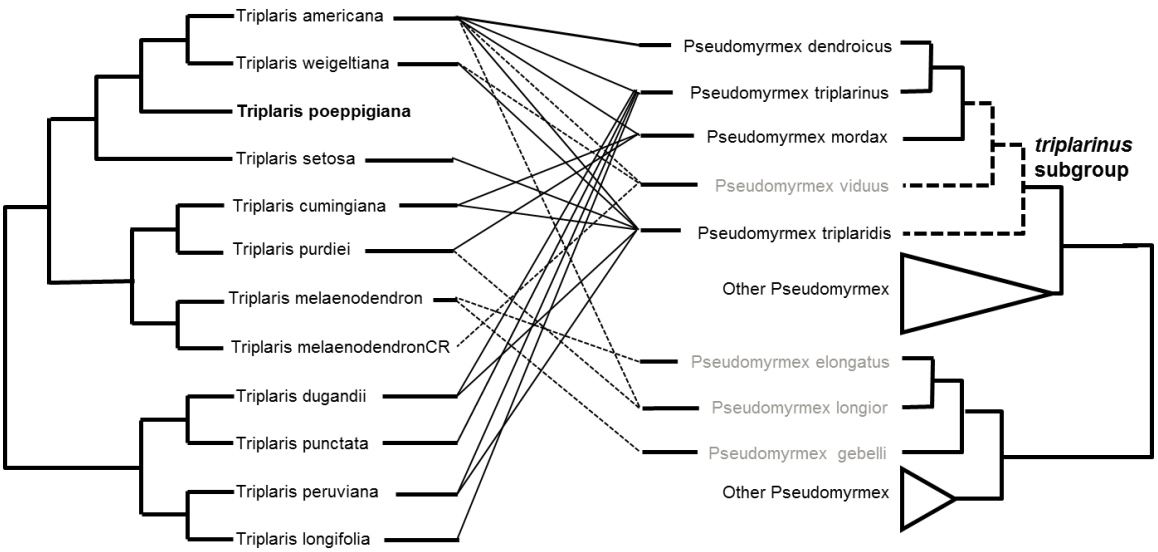

Triplaris americana
Triplaris weigeltiana
Triplaris poeppigiana
Triplaris setosa
Triplaris cumingiana
Triplaris purdiei
Triplaris melaenodendron
Triplaris melaenodendronCR
Triplaris dugandii
Triplaris punctata
Triplaris peruviana
Triplaris longifolia
Pseudomyrmex dendroicus
Pseudomyrmex triplarinus
Pseudomyrmex mordax
Pseudomyrmex viduus
Pseudomyrmex triplaridis
Other Pseudomyrmex
Pseudomyrmex elongatus
Pseudomyrmex longior
Pseudomyrmex gebelli
Other Pseudomyrmex
triplarinus
subgroup

Figure 5.4.

Colored key comparison between the multiple individual per species phylogey of *Triplaris* and a reduced phylogey of the *Pseudomyrmex triplarinus* subgroup. In grey are individual accessions that were not associated with ants from this subgroup, did not have information of the ants associated, or had any ants at all (see Appendix 3). Colors match plant host and ant associate.

Figure 5.4.

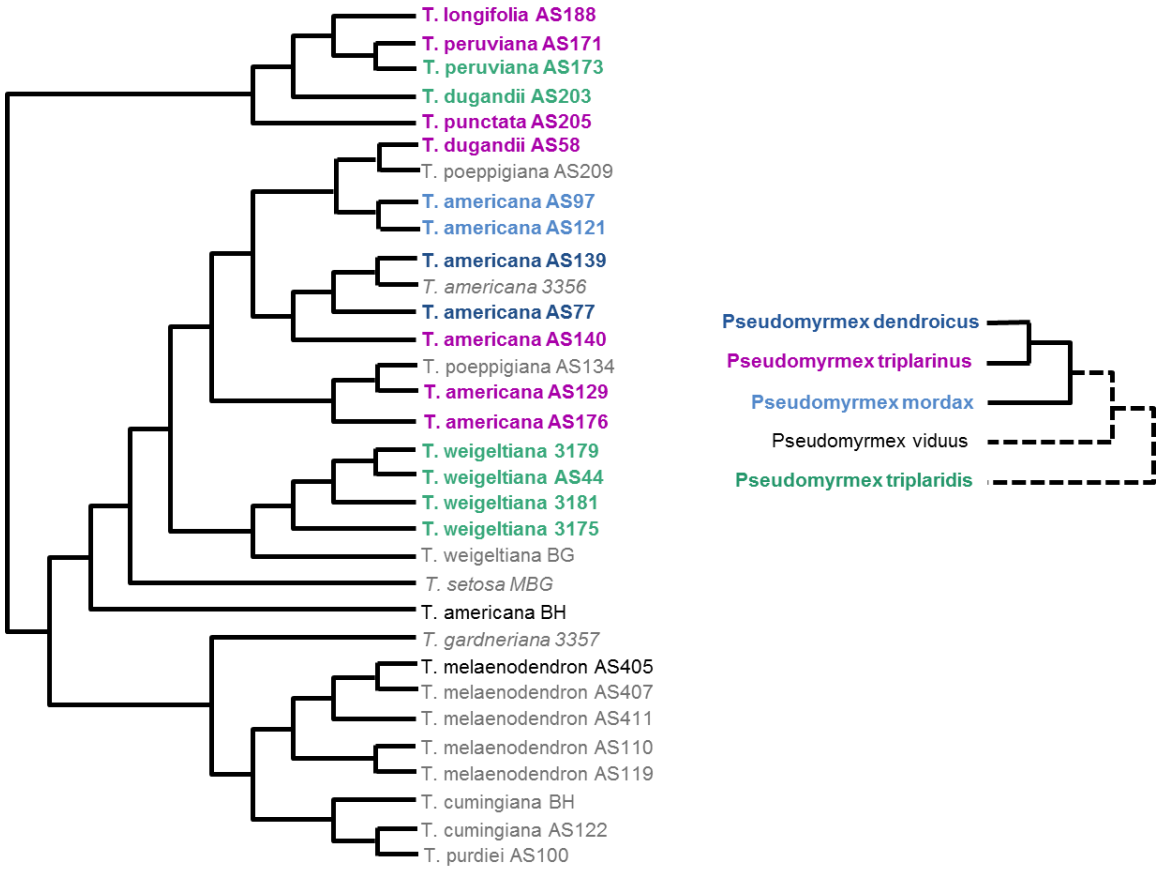
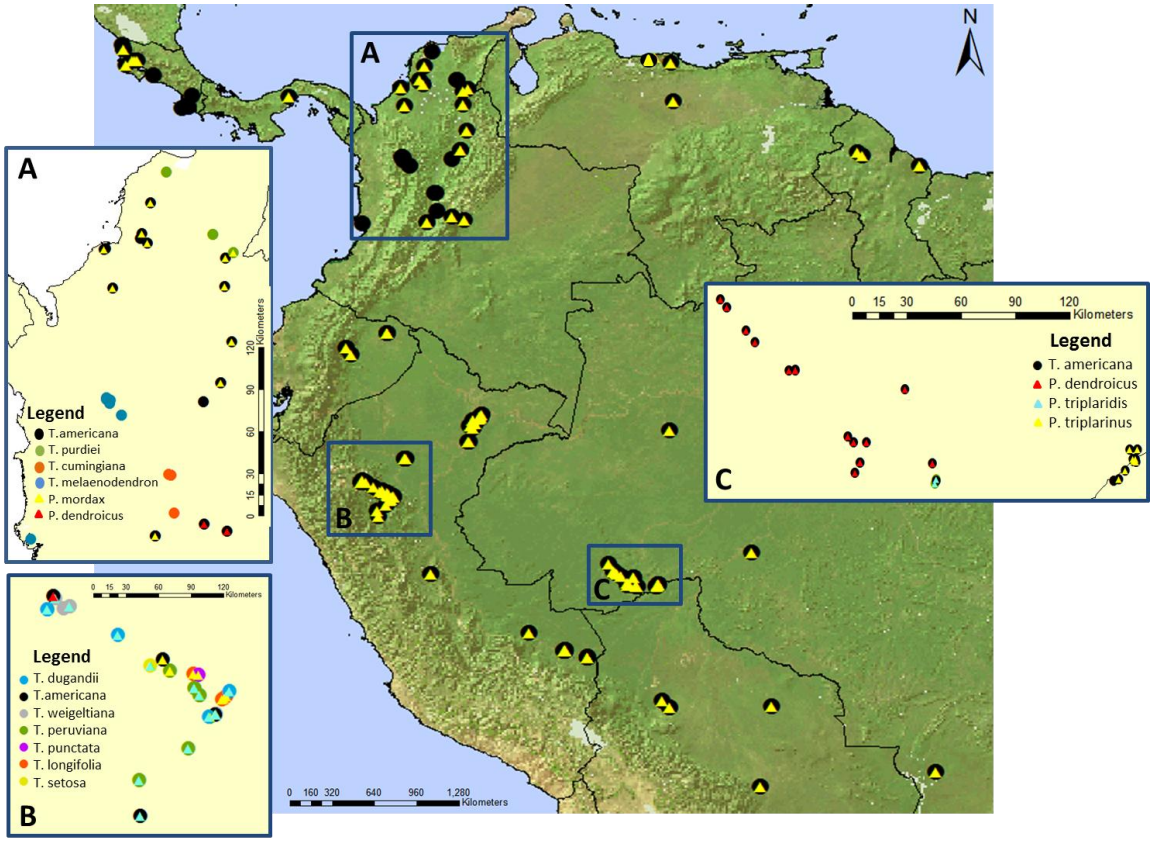

T. longifolia AS188
T. peruviana AS171
T. peruviana AS173
T. dugandii AS203
T. punctata AS205
T. dugandii AS58
T. poeppigiana AS209
T. americana AS97
T. americana AS121
T. americana AS139
T. americana 3356
T. americana AS77
T. americana AS140
T. poeppigiana AS134
T. americana AS129
T. americana AS176
T. weigeltiana 3179
T. weigeltiana AS44
T. weigeltiana 3181
T. weigeltiana 3175
T. weigeltiana BG
T. setosa MBG
T. americana BH
T. gardneriana 3357
T. melaenodendron AS405
T. melaenodendron AS407
T. melaenodendron AS411
T. melaenodendron AS110
T. melaenodendron AS119
T. cumingiana BH
T. cumingiana AS122
T. purdiei AS100
Pseudomyrmex dendroicus
Pseudomyrmex triplarinus
Pseudomyrmex mordax
Pseudomyrmex viduus
Pseudomyrmex triplaridis

Figure 5.5.

Geographical distribution of all *Triplaris* - obligate *Pseudomyrmex* ant collections known to date (Appendix 3). Circles represent plant species and triangles ant species. Inserts A to C represent zoom-in areas, where several species occur in sympatry. Colors represent different species, see legend inserts. B and C share the same ant species color key.

Figure 5.5.


A
B
C
N
Legend
T.americana
T. purdiei
T. cumingiana
T. melaenodendron
P. mordax
P. dendroicus
Legend
T. dugandii
T.americana
T. weigeltiana
T. peruviana
T. punctata
T. longifolia
T. setosa
Legend
T. americana
P. dendroicus
P. triplaridis
P. triplarinus
0 15 30 60 90 120 Kilometers
0 160 320 640 960 1,280 Kilometers

CHAPTER VI

CONCLUSIONS AND FUTURE DIRECTIONS

Before this study began, the monophyly of Polygonaceae had been supported with DNA sequence data (Chase et al., 1993; Lledo et al., 1998; Cuénoud et al., 2002; Lamb-Frye and Kron, 2003); however, the circumscription of groups within the family had not been addressed using molecular data. Two subfamilies, Eriogonoideae and Polygonoideae, were traditionally recognized (Reveal, 1989; Brandbyge, 1993; Freeman and Reveal, 2005), but their monophyly had not been tested. Sanchez and Kron (2008) presented the first attempt to test the subfamily circumscription in Polygonaceae, by using three chloroplast genes (*rbcL*, *matK*, and *ndhF*) and by sampling several species in Eriogoneae. Since their results did not support the traditional delimitation of both subfamilies, they proposed a new circumscription: Eriogonoideae was expanded to include the woody, tropical genera previously placed in Polygonoideae (e.g., *Antigonon, Coccoloba, Triplaris*).

The study by Sanchez and Kron (2008) represented the first step towards elucidating the relationships within the family, however, their taxon sampling was limited and only chloroplast genes were used. The large scale phylogeny in Chapter II included an increased taxon sampling (75 species in 40 recognized genera) and molecular characters from two genomes (chloroplast and nuclear). The results strongly support the monophyly of Eriogonoideae and Polygonoideae sensu Sanchez and Kron; however, two genera, *Afrobrunnichia* and *Symmeria*, were not placed in any subfamily, but were recovered at the base of the tree (Fig. 2.3). This chapter also provided a hypothesis of relationships between genera and a phylogenetic based circumscription of tribes since none of the traditionally recognized were monophyletic (except Rumiceae).

The results from the large scale phylogeny provided a basis for more in depth studies in both subfamilies. In particular, it provided a framework for understanding the phylogenetic relationships and the evolution of certain morphological characters in the genera of Eriogonoideae (Chapter III).

Chapter III was the first study to address thoroughly the relationships among taxa in Eriogonoideae using morphological (22 characters) and molecular (five chloroplast regions and ITS) characters. The analyses indicated that Coccolobeae and Triplarideae are not monophyletic, although there is strong support for a six-tepaled clade comprising Eriogoneae, *Gymnopodium*, *Leptogonum*, *Ruprechtia,* and *Triplaris.* The findings suggest that the woody, tropical genera of Polygonaceae have given rise to the temperate Eriogoneae, a tribe that is strongly supported as monophyletic. The latter is extremely diverse in the western North America, with some 325 species. Although tropical genera are often treated in their own family or subfamily, this study supports the origin of a diverse temperate group from a heterogeneous tropical assemblage, which was not previously hypothesized before the advent of molecular analyses. This phylogenetic arrangement elicits broader evolutionary questions about dispersal from a tropical region, and rapid radiation in a new habitat.

In Chapter II and III, two genera do not fall neatly into either Polygonoideae or Eriogonoideae: *Afrobrunnichia* and *Symmeria.* These two genera were thought to be more closely related to genera such as *Brunnichia* or genera in Triplarideae (Table 3.1). The position of *Afrobrunnichia* is ambiguous: it either is placed sister to Polygonaceae or with *Brunnichia + Antigonon* depending on the gene region used to reconstruct relationships (see data set published in Chapter III). *Symmeria* consistently falls as sister

to the rest of Polygonaceae, even with increased outgroup sampling in Plumbaginaceae Juss. Both studies suggest the recognition of a third subfamily, Symmerioideae (Burke and Sanchez, in rev.).

Analysis of the morphological data found that traditional characters used for previous classifications of subfamilies and tribes in Polygonaceae are not synapomorphies since several characters are homoplasious. For example, the six-tepal condition is derived from the five-tepal condition (probably as a result from an additional primordium in the floral plan), and unisexual flowers have arisen multiple times in different sexual systems (Fig. 3.3). The ruminate endosperm has arisen multiple times in the family, although this character is sometimes confounded with a deeply lobed seed coat (Fig. 3.1). The ocreae is a highly variable character in Eriogonoideae, and its presence, in the strict morphological sense, may be restricted to the subfamily Polygonoideae.

Two genera in Eriogonoideae, *Triplaris* and *Ruprechtia*, were previously placed in tribe Triplarideae (Brandbyge, 1993; Dammer, 1893; Gross, 1913; Haraldson, 1978; Roberty and Vautier, 1964) along with other six-tepaled genera (e.g., *Gymnopodium*, *Leptogonum*; Table 3.1). However, the phylogenetic study on the subfamily clarified the position of *Triplaris* and *Ruprechtia*, and demonstrated that they do not form a clade with the other six-tepaled genera, since *Gymnopodium* is most closely related to Eriogoneae (Fig. 3.2). It was therefore proposed that tribe Triplarideae should be restricted to include only *Triplaris* and *Ruprechtia* (Burke and Sanchez, in rev.). However, when *Triplaris* and *Ruprechtia* are studied in more detail (Chapter IV), it is evident that the story is more complicated.

The delimitation of *Triplaris* and *Ruprechtia* has been difficult through history and it is evidenced by the flux of taxonomic treatments. While some authors merged *Ruprechtia* under *Triplaris* (e.g., Endlicher 1847; Kuntze 1898), others segregated some species of *Ruprechtia* into new genera such as *Enneatypus* (Herzog 1922; Roberty and Vautier 1964) or *Magonia* (Kuntze 1891).

The investigation of the relationships between both genera using multiple species of each genus (nine of *Triplaris* and 19 of *Ruprechtia*) and several molecular regions (five regions: *matK, ndhF, trnV-ndhC, rps16-trnK*, nrITS, and *lfy2i*), revealed that *Ruprechtia* is not monophyletic. Two species, *R. triflora* and *R. obidensis*, were placed as sister to a reduced clade of *Ruprechtia* + *Triplaris* (Fig. 4.2). In order to recognize monophyletic groups, the circumscription of the genera was changed and two new genera in Triplarideae were recognized: *Salta* (for *R. triflora*) and *Magoniella* (for *R. obidensis* and *R. laurifolia*).

Magoniella is characterized by the liana habit. They are the only two species of Triplarideae to be strictly lianas. *Ruprechtia* is characterized by a short stalk at the base of the fruit, while *Triplaris* has ruminate endosperm, sessile male flowers, and microreticulate pollen. In *Salta*, there is a pronounced development of brachyblasts, which is unique to this new genus.

Once the monophyly of *Triplaris* was established (Fig. 4.2), the intraspecific relationships of 12 of the 18 recognized species (Brandbyge, 1986; 1990) were addressed, using five molecular markers (two chloroplast and three nuclear). Since all *Triplaris* species are known to associate with ants (myrmecophytes), the intraspecific relationship

of the main ant associate *Pseudomyrmex* was explored, using two molecular regions (one mitochondrial and one nuclear).

Two types of sampling for the plant phylogeny were used: a single individual per species and a multiple individuals per species. These were analyzed separately and the ant phylogeny compared to each. When a single individual per species for *Triplaris* was analyzed a strongly resolved pattern of intraspecific relationships was recovered. However, as more accessions (a total of 32 individuals) were included resolution was lost and several species were not recovered as monophyletic (*T. americana, T. cumingiana, T. dugandii,* and *T. poeppigiana*; Fig. 5.1). In the case of the ants, a single and multiple individual analyses recovered the same pattern of intraspecific relationships: all species were supported as monophyletic (Fig. 5.2).

Using a multiple individual phylogeny also provided a finer resolution to understand the patterns of association between the ants and plants (Fig. 5.4). The analysis showed that some ant species tend to be more specific to a given host (i.e., *P. dendroicus*), while others associate with all hosts that overlap their range of distribution (e.g., *P. triplarinus*; Fig. 5.4). These findings were also corroborated with a compiled data set of all the collections of plant hosts and resident ants known. The pattern of distribution of both organisms (Fig. 5.5) reveals that *P. dendroicus* is more specific to its host than others.

There is still much work to be done in the plant family Polygonaceae. Although there is a well-resolved pattern of evolutionary relationships (Chapter II), the placement of some genera (*Afrobrunnichia*, *Oxygonum*, and *Pteroxygonum*) is still not known and

some other genera need extensive revisions (e.g., *Calligonum*, *Coccoloba*, *Polygonum s.s.*). The biogeography of the family and most of its genera has not been studied. For example, *Symmeria* is the only genus with an amphi-Atlantic distribution; however, it is not clear if the genus is monospecific or if the West African populations represent a different species (discussed in Sanchez and Kron, 2009)

Regarding *Triplaris*, an analysis including all species and a morphological revision of the genus is needed. *Triplaris* plays an important role in the ecosystem (they are pioneer plants) and is involved in symbiotic relationships with ants and Hemipterans (Wheeler, 1942; Ward, 1999). These plants present an opportunity to carry out ecological studies on the ant-plant interactions as well as other studies involving diversification times of the ant-plant interaction.

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EDUCATION

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M. S. Biology, 2005, Universidad de los Andes, Bogotá, Colombia
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TEACHING

Laboratory Classes Taught:

Molecular Biology (2001): Universidad de los Andes
Biology of Organisms (2002 – 2003); Universidad de los Andes
Biological Principles (2005 – 2007); Wake Forest University
Biology and the Human Condition (2009); Wake Forest University

Guest Lecturer:

"Evolución y Ecología en Polygonaceae, con énfasis en *Triplaris* y sus relaciones con hormigas" (2009); Universidade Federal do Acre, Brazil

High & Middle School:

Biology Teacher 5th to 8th grade (2002 – 2003); Gimnasio La Arboleda, Bogotá, Colombia

Mentoring:

Advised an undergraduate student; Universidad de los Andes (2009-2010)
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RESEARCH

Publications:

Sanchez A and KA Kron. 2008. Phylogenetics of Polygonaceae emphasizing on the evolution of Eriogonoideae. *Systematic Botany* 33: 87-96.

Sanchez A and KA Kron. 2009. Phylogenetic relationships of *Afrobrunnichia* Hutch. & Dalziel (Polygonaceae) based on three chloroplast genes and ITS. *Taxon* 58: 781-792.

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Sanchez A, TM Schuster, JM Burke, and KA Kron. 2011. Taxonomy of Polygonoideae (Polygonaceae): a new tribal classification. *Taxon* 60: 151-160.

Manuscripts in Press:

Burke JM and A Sanchez. A revised subfamily classification for Polygonaceae, with a tribal classification for Eriogonoideae. *Brittonia*.

Sanchez A and KA Kron. Phylogenetic relationships of *Triplaris* and *Ruprechtia:* re-delimitation of the recognized genera and two new genera for tribe Triplarideae (Polygonaceae). *Systematic Botany*.

Manuscripts in Preparation:

Sanchez A., F Lutzoni, and KA Kron. Mutualism between *Triplaris* and *Pseudomyrmex*: phylogeny and geographical distribution.

Oral Presentations at Meetings:

Sanchez A and K Kron: "Phylogenetic relationships of *Eriogonum* and relatives using the second intron of LEAFY" (Chico, CA 2006).

Sanchez A and K Kron: "Preliminary phylogenetic relationships of *Calligonum* L. (Polygonaceae) using two chloroplast genes" (Vancouver, Canada 2008).

Sanchez A and K Kron: "Phylogenetic relationships of *Afrobrunnichia* Hutch. & Dalziel (Polygonaceae) based on three chloroplast genes and ITS" (Vancouver, Canada 2008).

Sanchez A: "Evolución de la familia de plantas Polygonaceae con énfasis en Triplaris su relación con hormigas". Simposio ColEvol, (Bogotá, Colombia 2008).

Burke JM, A Sanchez, KA Kron and M Luckow: "Placing the woody tropical genera of Polygonaceae: A hypothesis of character evolution and phylogeny" (Snowbird, UT 2009**).**

Sanchez A, TM Schuster and KA Kron: "A large scale phylogeny of Polygonaceae based on molecular data" (Snowbird, UT 2009).

Sanchez A and KA Kron: "Phylogenetic relationships of *Triplaris* Loefl. and *Ruprechtia* C. A. Mey. With emphasis on *Triplaris* (Polygonaceae) and its associations with *Pseudomyrmex* ants" (Providence, RI 2010).

Bush C, PW Fritsch, BC Cruz, A Sanchez and KA Kron: "The phylogeny, morphology and biogeography of the Brazilian *Gaultheria* species" (Providence, RI 2010).

Invited Seminar Speaker:

Sanchez A: "Relaciones filogenéticas de Eriogonoideae (Polygonaceae) con énfasis en el género *Triplaris* y su relación con hormigas". Museo de Historia Natural de la Universidad San Marcos (Lima, Peru 2007).

Sanchez A: "Molecular Phylogenetics of an Andean Adaptive Radiation: New Insights from Chloroplast DNA". Rancho Santa Ana Botanic Garden (Claremont, CA 2007).

Sanchez A and KA Kron: "Phylogenetic relationships of *Afrobrunnichia* Hutch. & Dalziel (Polygonaceae) based on three chloroplast genes and ITS". Missouri Botanical Garden (St. Louis, MO 2008).

Sanchez A: "Evolución de la familia de plantas Polygonaceae con énfasis en Triplaris y su relación con hormigas". Universidad de los Andes (Bogotá, Colombia 2009).

Sanchez A: "From evolutionary relationships to ecological interactions in the plant family Polygonaceae". Duke University (Durham, NC 2009).

Sanchez A: "From evolutionary relationships to ecological interactions in the plant family Polygonaceae". Universidade de São Paulo, (São Paulo, Brazil 2009).

Field Research:

Colombia: Sierra Nevada de Santa Marta. Beetle collections. 1998 – 1999.
Colombia: Tinigua National Park. Frugivory, Phenology and Primate behavioral observations. 2001.
Ecuador: Carchi and Quito. Ecology of *Espeletia pycnophylla*. 2002.
Colombia: Boyaca, Cundinamarca, Norte de Santander, Santander. Phylogenetics of the tribe Espeletiinae. 2003 – 2004.
Peru: San Martin, Loreto and Madre de Dios. *Triplaris* collections. 2007 – 2009.
Uzbekistan. *Calligonum* collections. 2008.
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Brazil: Acre, Rio de Janeiro. *Triplaris* and *Ruprechtia* collections. 2009
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Awards:

Third place, Best Presentation
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Grants and Funding:

Idea Wild, Grant for Field Equipment (2006)
Vecellio Award: Wake Forest University (2006, 2007, 2008, 2009, 2010)
Amazon Conservation Association (2007)
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Elthon C. Cocke Travel Award: Wake Forest University (2006, 2007, 2008, 2009, 2010)
Environmental Center: Wake Forest University (2009)
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Professional Affiliations:

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Languages:

Spanish and English: spoken and written fluently
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