PHYLOGENY AND FLORAL EVOLUTION OF THE SUBFAMILY GOMPHRENOIDEAE (AMARANTHACEAE)

BY

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ABSTRACT

PHYLOGENY AND FLORAL EVOLUTION OF THE SUBFAMILY GOMPHRENOIDEAE (AMARANTHACEAE)

by

Ivonne Sánchez-del Pino

Advisor: Dr. Timothy J. Motley

The subfamily Gomphrenoideae (Amaranthaceae) is characterized by having bilocular anthers. It includes 19 genera and 400 species predominantly from the Neotropics. The goals of this project were to test the monophyly of the subfamily Gomphrenoideae using chloroplast *trnL-F* regions and *rpl16* intron; to resolve the position of *Iresine*, which was inconclusive based on previous molecular studies in the family; to test the existence of a *Tidestromia-Alternanthera* clade; to establish the sister relationships of *Tidestromia* within the Gomphrenoideae; to reconstruct the phylogeny of *Alternanthera* using *trnL-F* and *rpl16*; to investigate subgeneric classifications proposed in *Alternanthera*; to determine the origin, colonization, and diversification of species of *Alternanthera* in the Galápagos Islands; to resolve the phylogenetic relationships within *Tidestromia* using *rpl16*, *trnL-F*, ITS, and morphology; to investigate the role of soils in the evolution of *Tidestromia*; and to explore evolutionary trends in anthers, pseudostaminodia, pollen, and gynoecium characters in the subfamily Gomphrenoideae.

This study resolved that subfamily Gomphrenoideae is monophyletic and includes *Iresine*. Three strongly supported clades were recovered in the consensus trees (Bayesian and Parsimony) distinguished by pollen types. The phylogeny suggested that there is not a *Tidestromia-Alternanthera* clade, but a *Tidestromia-Alternanthera-Pedersenia* clade. *Tidestromia* was resolved as sister to *Alternanthera* and *Pedersenia.*

The molecular phylogeny of *Alternanthera* supports Endlicher's subgeneric classification modified using the former sections *Bucholzia*, *Brandesia*, and *Mogiphanes*. In addition, *Alternanthera*, a large genus in the Galápagos, colonized the Archipelago at least three times with one clade radiating on the islands.

The monophyly of *Tidestromia* was supported in independent and combined analyses. The use of all sources of data provided resolution among taxa and identified a putative hybrid origin within the genus. Edaphic conditions play an important role in the evolution of *Tidestromia*.

The optimization of andrecium and gynoecium characters in the obtained phylogeny of the subfamily Gomphrenoideae suggested that there are not trends in evolution of pseudostaminodia as were suggested by Eliasson. Gynoecium characters were informative within the Gomphrenoideae to define generic circumscriptions. Bisporangiate anthers, metareticulate pollen, tecta on distal bands, and microspines distributed either around the apertures or in distal rows are derived characters in the subfamily Gomphrenoideae.

To the best mother in the world… my mother *Luz del Pino Arista* for all her love, cares, and support.

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GENERAL INTRODUCTION

1.1. AMARANTHACEAE

Amaranthaceae, a cosmopolitan family of herbs, shrubs, lianas, and trees includes some ornamental and edible species. *Amaranthus* L., is the most economically important genus in the subfamily Amaranthoideae. Seeds of *Amaranthus* species have been used as pseudo-cereal grains in many parts of the world. It was domesticated by American Indians and from that time three races have been developed in Central and South America (Robertson 1981). Nutritional analyses show the grain to be comparable to true cereals as a carbohydrate source and superior in protein and fat content (Sauer 1967). Among other uses, species of *Amaranthus*, *Alternanthera*, *Iresine*, *Gomphrena* and *Celosia* have either colorful foliage or showy inflorescences and are cultivated as ornamentals (Robertson 1981).

Recent studies indicate that Amaranthaceae and Chenopodiaceae form a monophyletic lineage within the Caryophyllales based on morphology and molecular data (Downie and Palmer 1994a, 1994b; Manhart and Retting 1994; Rodman 1990; Judd *et al*. 1999).This lineage has been referred to as the Amaranthaceae *s.l.* and was located within Eudicots according to APGII (2003). Amaranthaceae *s.l.* has 100 genera and 400 species (Robertson 1981; Eliasson 1988). It is characterized by exclusively having PIIIf plastide types and with seven-porate to polyporate pollen (Judd *et al*. 1999).

Alternatively, traditional systems of classification based strictly on morphology (Cronquist 1988), Amaranthaceae *s. s.* has been considered a separate family from Chenopodiaceae. Chenopodiaceae has been distinguished from Amaranthaceae by its free

stamen and greenish, membranous to fleshy tepals (Judd *et al.* 1999), while the Amaranthaceae has five stamen united at least at the base into a cup or tube of various lengths (Eliasson 1988) and the frequent presence of pseudostaminodia. Furthermore, tepals, bracts, and bracteoles are dry, scarious or chartaceous (Robertson 1981).

The Amaranthaceae *s. s.* comprises about 60 genera and 900 species. Approximately 25 genera are restricted to Africa, 13 genera are found throughout the Americas and in the Galapagos Islands, two genera are endemic to Madagascar, two to the Hawaiian Islands, and three to Australia (Robertson 1981). The Amaranthaceae *s. s.* is most abundant in the tropics, subtropics, and the warm-temperate regions. Numerous species occur in arid habitats. Representatives of the family are able to grow in harsh edaphic conditions such as sandy, calcareous, saline, gypseous, or serpentine soils (Lawrence 1951; Robertson 1981; Heywood 1993).

The pre-cladistic clasification that has been considered the most natural in the Amaranthaceae *s.s* recognizes two subfamilies: Amaranthoideae and Gomphrenoideae. The subfamily Gomphrenoideae includes 19 genera and ca. 400 species. Seven of these genera are monotypic, and *Gomphrena* and *Alternanthera* are the largest genera each cointaining 100 to 120 species. All the Gomphrenoideae genera are present in America with some species extending to Africa, Australia and Japan (Townsend 1993).

Studies based on macro and micromorphological characters in addition to sequence data from the internal transcribed spacer 2 of nuclear ribosomal DNA (ITS2) (McCauley 2002), palynology data (Borsch 1998), *rbcL* gene (Kadereit *et al.* 2003), and *mat*K/*trn*K (Müller and Borsch 2005) have indicated that there is not support for the recognition of intrafamily classification in the subfamily Gomphrenoideae as has been suggested in

traditional classifications. Data from *rbcL*, *mat*K/*trn*K, and a broad sample of Chenopodiaceae and Amaranthaceae species suggested that the core subfamily Gomphrenoideae includes the genera *Alternanthera*, *Blutaparon*, *Hebanthe*, *Froelichia*, *Gomphrena*, *Guilleminea*, *Pseudoplantago* and *Tidestromia* in a polytomy with *Iresine* and Amaranthoids I (Kadereit *et al.* 2003) or Achyranthoids (Müller and Borsch 2005). However, no extensive study has been made in order to further test the monophyly of the core subfamily Gomphrenoideae by increasing the sampling in number of taxa (larger than 20 species from a total of ca. 400 in the Gomphrenoideae) and including more data from molecular evidence. Furthermore, the monophyly of *Alternanthera* has never been tested and the placement of *Gossypianthus* within *Guilleminea* (Mears 1967) as a distinct genus (Henrickson 1987; Eliasson 1988; Pedersen 2000) has not been examined. Furthermore, *Gomphrena* which is apparently a paraphyletic lineage based on *rbcL* and ITS2 data that included a sampling of nine species from a total of 100-120 (Kadereit *et al.* 2003; McCauley 2002) have not been retested.

Gynoecium and androecium characteristics have been important in determining genera and subtribes in the subfamily Gomphrenoideae. Stigmas have not been studied in detail in the subfamily through the use of SEM. However, a taxonomic revision of *Tidestromia* and some related taxa indicated that distribution of the papillae in the stigmatic area was informative in a phylogenetic context (Sánchez-del Pino 2001). Furthermore, Eliasson (1988) described variation among pseudostaminodia in the genera *Alternanthera*, *Froelichiella*, *Froelichia*, *Gomphrena* and *Pseudogomphrena*. He suggested that long and laciniate pseudostaminodia present in *Alternanthera* are less advanced than the reduced and enter forms and probably the apical filament lobe in

Gomphrena is a homologous structure with the half pseudostaminodium in *Pseudogomphrena*. However, no formal study has examined floral evolution within the subfamily.

The third portion of this project is a continuation of the systematic revision of the genus *Tidestromia* which included a phylogenetic analysis using morphological characters. This is the only genus in the subfamily Gomphrenoideae that occurs with high incidence in calcareous, gypseous and salty soils (Sánchez-del Pino 2001). Results suggested that *Tidestromia* is a monophyletic genus and phylogenetic relationships among perennial species are unresolved; therefore, there is a need to include more characters, such as molecular data, to explore the relationships of the species in the *Tidestromia*, which can also help to examine the evolution of *Tidestromia* in gypsum and saline soils.

The fourth portion of this study includes the phylogeny of *Alternanthera* based on molecular data. Sections proposed in the past are tested and biogeography discussed considering that the genus has a high diversity of species in the Galápagos Islands (13 species).

Recent phylogenetic analysis conducted with morphological and molecular data of selected species of *Alternanthera* indicated that it is the sister group of *Tidestromia* (Sánchez-del Pino 2001; McCauley 2002; Kadereit *et al.* 2003). It suggested that there is evidence for the existence of an *Alternanthera*-*Tidestromia*-clade but statistical support is low and few members of *Alternathera* have been included in the sampling making it interesting to establish the sister group relationships of *Tidestromia* within the core Gomphrenoideae.

Eliasson (1988) hypothesized that *Tidestromia* has an insolated taxonomic position among the subfamily Gomphrenoideae. This idea agrees with Cavaco's classification (1962) who proposed the tribe Tidestromeae represented for the genus *Tidestromia* and there have been no studies to resolve this question.

1.2. BACKGROUND

1.2.1. Taxonomic history of subfamily Gomphrenoideae.

The first comprehensive classification of the Amaranthaceae *s. s.*, was proposed by Moquin-Tandon (1849) who divided the family into three tribes according to anther and ovules features (Tribes Celosieae, Achyrantheae and Gomphreneae). Bentham and Hooker (1883) followed Moquin-Tando's classification and renamed Tribe Achyrantheae as Amarantheae (Table1).

Schinz (1893, 1934) was the first to recognize subfamilies in the Amaranthaceae *s.s*. He divided the family into the subfamily Amaranthoideae (anthers 4-locular at maturity with two lines of dehiscence) and Gomphrenoideae (anthers 2-locular at maturity with a single line of dehiscence). Each subfamily was divided into two tribes and each tribe excluding tribe Celosieae was divided into two subtribes. Schinz's (1934) recognition of two subfamilies in the Amaranthaceae *s. s*. has been considered the best reflection of natural relationships in the group (Eliasson 1988; Townsend 1993). However, there was one objection to Schinz's proposal (1934) in the tribe Brayulineae within subfamily Gomphrenoideae. Eliasson (1988) considered Schinz's tribe Brayulineae to be artificial because *Brayulinea* Small (= *Guilleminea* Kunth) and *Tidestromia* Standl., the only two genera located in the tribe, have different basic floral and pollen characteristics.

TABLE 1. Classifications proposed in Amaranthaceae *s. s.* (Subf. = Subfamily; T = tribe; S = Subtribe)

MOQUIN-TANDON (1849)	BENTHAM AND HOOKER (1883)	SCHINZ (1934)	CAVACO (1962)	TOWNSEND (1993)
T. Celosieae	**T. Celosieae**	**Subf. Amaranthoideae**	**Subf. Amaranthoideae**	**Subf. Amaranthoideae**
T. Achyrantheae	**T. Amarantheae**	T. Celosieae	T. Amarantheae	T. Celosieae
S. Amarantheae	S. Euamarantaeae	T. Amarantheae	T. Achyrantheae	T. Amarantheae
S. Aerveae	S. Achyrantheae	S. Amaranthinae	**Subf. Celosioideae**	S. Amaranthinae
S. Desmochaeteae	**T. Gomphreneae**	S. Achyranthinae	**Subf. Gomphrenoideae**	S. Aervinae
S. Polycnemeae		**Subf. Gomphrenoideae**	T. Tidestromeae	**Subf. Gomphrenoideae**
T. Gomphreneae		T. Brayulineae	T. Gomphreneae	T. Pseudoiplantageae
		S. Brayulineinae	S. Froelichiinae	T. Gomphreneae
		S. Tidestromiinae	S. Gomphreninae	S. Froelichiinae
		T. Gomphreneae	**Subf. Brayulineoideae**	S. Gomphreninae
		S. Froelichiinae		
		S. Gomphreninae		

Instead, he considered *Guilleminea* to be more closely related to Schinz's tribe Gomphreneae, and he viewed the genus *Tidestromia* as most likely occupying a more isolated position within the subfamily Gomphrenoideae.

Cavaco's (1962) classification reflects artificial relationships within the Amaranthaceae *s.s.* He changed the status of tribes proposed by Schinz (1893, 1934) to subfamilies (Celosioideae, Amaranthoideae, Gomphrenoideae, and Brayulineoideae). He subdivided two of his four subfamilies. Subfamily Amaranthoideae was divided in three tribes (Amarantheae, Achyrantheae, and Digereae) and subfamily Gomphrenoideae in two tribes (Tidestromeae and Gomphreneae) based on sexual systems, inflorescences, trichomes and stigma form (Table 1).

Ignoring Cavaco's artificial system , Townsend (1993) modified Schinz's (1934) classification and proposed the most recent treatment for the Amaranthaceae *s.s*. Townsend (1993) eliminated Schinz's tribe Brayulineae and recognized the subtribes Gomphreninae and Froelichiinae in the subfamily Gomphrenoideae based on stigma and flower morphology. *Tidestromia* and *Guilleminea* originally located in Schinz's tribe Brayulineae were instead placed in the subtribe Froelichiinae.

Schinz's classification (1934) has been followed by many authors and it was recently emended by Borsch (1998). In the emended classification the subfamily Gomphrenoideae includes 19 genera.

TABLE 2. Changes in taxonomic categories and addition of genera in the most recent classifications proposed in the subfamily Gomphrenoideae.

Schinz's (1934) classification	Townsend's (1993) classification	Schinz's (1934) classification emended by Borsch (1998)
I. Subfamily Amaranthoideae 1. Tribe Celosieae 2. Tribe Amarantheae a. Subtribe Amaranthinae **II. Subfamily Gomphrenoideae** **1. Tribe Brayulineae** **a. Subtribe Brayulineinae** *Brayulineae* (=*Guilleminea*) **b. Subtribe Tidestromiinae** *Tidestromia* **2. Tribe Gomphreneae** **a. Subtribe Froelichiinae** *Gossypianthus* *Froelichia* *Froelichiella* *Pfaffia* *Alternanthera* **b. Subtribe Gomphreninae** *Woehleria* *Dicraurus* *Gomphrena* *Pseudogomphrena* *Iresine* *Lithophila*	I. Subfamily Amaranthoideae 1. Tribe Celosieae 2. Tribe Amarantheae a. Subtribe Amaranthinae **II. Subfamily Gomphrenoideae** **1. Tribe Pseudoplantageae** *Pseudoplantago* **2. Tribe Gomphreneae** **a. Subtribe Froelichiinae** ***Guilleminea*** *Gossypiatnuhs* *Tidestromia* *Froelichia* *Froelichiella* *Pfaffia* *Alternanthera* **b. Subtribe Gomphreninae** *Woehleria* *Gomphrena* *Pseudogomphrena* *Iresine* *Irenella* *Blutaparon* *Lithophila*	I. Subfamily Amaranthoideae 1. Tribe Celosieae 2. Tribe Amarantheae a.Subtribe Amaranthinae **II. Subfamily Gomphrenoideae** **1. Tribe Pseudoplantageae** *Pseudoplantago* **2. Tribe Gomphreneae** **a. Subtribe Froelichiinae** *Guilleminea* *Gossypiatnuhs* *Tidestromia* *Froelichia* *Froelichiella* *Pfaffia* *Quaternella* *Alternanthera* **b. Subtribe Gomphreninae** *Woehleria* *Gomphrena* *Xerosiphon* *Pedersenia* *Hebanthe* *Pseudogomphrena* *Iresine* *Irenella* *Blutaparon* *Lithophila*

1.2.2. Phylogenetic analyses in the subfamily Gomphrenoideae.

The monophyly of subfamily Gomphrenoideae was supported by pollen characters (Borsch 1998) and morphological and molecular evidence from ITS2 (McCauley 2002), however, results obtained from *rbcL* and *mat*K/*trn*Kdata indicate that subfamily Gomphrenoideae is polyphyletic (Kadereit *et al.* 2003; Müller and Borsch 2005).

Phylogenetic analysis from *rbcL* data based on small representative sample of members of the Amaranthaceae and Chenopodiaceae suggested that subfamily Gomphrenoideae includes the genus *Iresine* and a clade that is referred to as the "core subfamily Gomphrenoideae", that includes the genera *Alternathera*, *Tidestromia*, *Blutaparon*, *Gomphrena*, *Guilleminea*, *Froelichia*, *Hebanthe*, and *Pseudoplantago*. The core Gomphrenoideae clade is in a trichotomy with *Iresine* and the Amaranthoids I clade (Kadereit *et al.* 2003) or in a polytomy with Achyranthoids (Müller and Borsch 2005).

The genera located in the core Gomphrenoideae are representatives of each tribe and subtribe proposed by Schinz (1934). *Alternathera*, *Tidestromia, Guilleminea* and *Froelichia* were located in subtribe Froelichiinae and *Blutaparon* and *Hebanthe* in subtribe Gomphreninae within the tribe Gomphreneae and *Psedoplantago* as the sole genus of the tribe Pseudoplantageae. However, relationships in these groups are paraphyletic and therefore do not support Schinz's (1934) classification.

Among all the genera of the subfamily Gomphrenoideae, *Tidestromia* has been the only genus which monophyly has been tested using morphological characters. It is an interesting genus since it is the only one within the subfamily Gomphrenoideae with high occurrence in salty and gypseous soils and with many endemic taxa occurring in Coahuila, Mexico which is part of the Chihuahuan Desert (Sánchez-del Pino 2001). Recent data from molecular and morphological evidence has suggested the existence of the *Tidestromia*-*Alternanthera*-clade (Sánchez-del Pino 2001, Kadereit *et al.* 2002, and McCauley 2002) which will be examined by increasing sampling of the genus *Alternanthera* and adding molecular data.

1.2.3. Overview of subfamily Gomphrenoideae.

Subfamily Gomphrenoideae is characterized by having unilocular anthers and a single line of dehiscence at maturity (Schinz 1934; Townsed 1993; Eliasson 1988; Borsch 1998), ovary uniovulate and pendulous (Schinz 1934), campylotropous ovule on a recurvate funicule, the presence of sterile flowers, candelabriform or simple trichomes (Cavaco 1962) and *Gomphrena*- and *Pfaffia*-types of pollen (Borsch 1998).

The genera of Amaranthaceae are usually defined by androecium and gynoecium characters (Eliasson 1985; Eliasson 1986; Pedersen 1997). Characters traditionally employed to distinguish genera in subfamily Gomphrenoideae have been basically related to stigma forms; pseudostaminodia margin; fusion of the staminal tube with calyx; stamen number, position, fusion and margin; trichomes on tepals direction and size; tepals fusion and number; phyllotaxy; inflorescences type; involucre structure; sexual systems; bracteoles size; and habit. Recent generic concepts in the family Amaranthaceae include pollen features (e.g. *Hebanthe* by Borsch and Pedersen 1997).

The core of the subfamily Gomphrenoideae includes the monotypic genus *Pseudoplantago* Suesseng., the large genera *Alternanthera* Forss. (100), *Gomphrena* L. (±100-120), and *Froelichia* Moench (18). Genera with less than ten species include *Blutaparon* Raf. (4 sp.), *Guilleminea* H.B.K. (6 sp.), *Hebanthe* Mart. (8 sp.) and *Tidestromia* Standl. (6 sp.). The genera located in the core Gomphrenoideae display a broad diversity of morphological characters. They include woody lianas, shrubs, and annual or perennial herbs; inflorescences in dichasium, spikes or heads or branched racemose synflorescence; tepals free to base or fused; pseudostaminodia variable or absent and stigmas capitate or two liped (Table 3).

TABLE 3. Number of species and diagnostic features of the core Gomphrenoideae (Sn = species number).

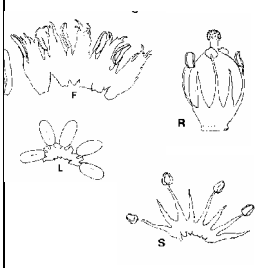
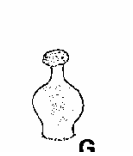
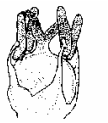
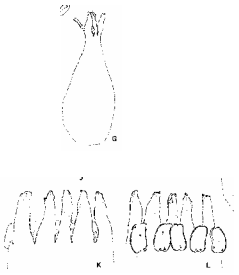
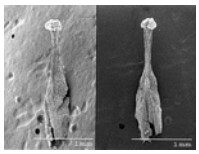
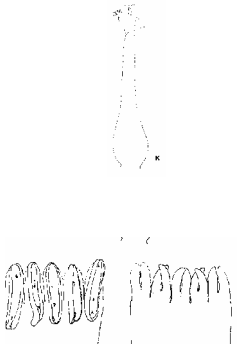
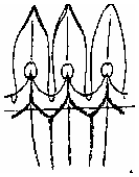
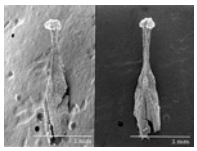
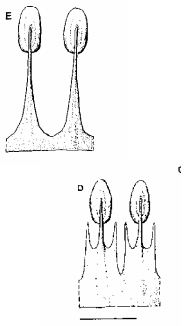
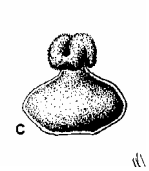
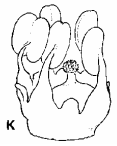
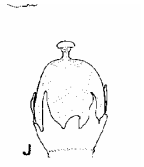
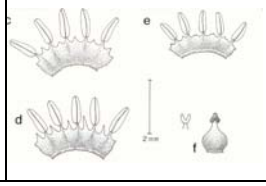
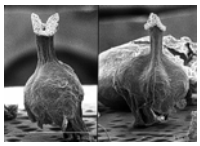
	Genus name	Sn	Diagnostic features	Staminal cups or tubes	Stigmas
CORE GOMPHRENOIDEAE	***Alternanthera***	100	Axillary spikes. Tepals free to base. Pseudostaminodia narrowly triangular to ligulate, and frequently laciniate. Stigma capitate.		
	Blutaparon	4	Axillary or terminal rounded to cylindrical spikes.		
	Froelichia	18	Tepals fused into a slightly two-lipped calyx. Filaments fused into a long apically, 5-lobed tube with anthers attached in the sinuses. Stigma capitate, sometimes fimbriate.		
	Gomphrena	±100-120	Filaments fused along all their length into a long tube with anthers attached in the sinuses between apical lobes. Flowers often variously pubescent. Stigma two-lipped or with two filiform lobes.		

TABLE 3. Number of species and diagnostic features of the core Gomphrenoideae (Sn = species number).

	Genus name	Sn	Diagnostic features	Staminal cups or tubes	Stigmas
CORE GOMPHRENOIDEAE	***Guilleminea***	6	Flowers single or in more or less dense axillary clusters. Stigmas two-lipped. Filament tube fused with calyx. Flowers perigynous.		
	Hebanthe	7	Perianth with conspicuous long and stiff trichomes. Androecium with glabrous filaments, gradually widening to the base and united into a shallow cup, and with or without two acute lateral appendages varying in size. Stigma bilobed.		
	Pseudoplantago	1	Flowers in spiciformly arranged reduced cymes. Stigma capitate, sometimes uneven with shallow rounded lobes.		
	Tidestromia	6	Alternate leaves, inflorescences in a dichasium and presence of an involucre.		

In Schinz's (1934) classification emended by Borsch, the subfamily Gomphrenoideae includes five additional monotypic genera (*Froelichiella* R. E. Fries, *Irenella* Suessenguth, *Pseudogomphrena* R. E. Fries, and *Woehleria* Grisebach). Five genera with two-eight species (*Gossypianthus* Hooker, *Lithophila* Swartz, *Pedersenia*

Holub, *Quaternella* Pedersen and *Xerosiphon* Turcz) and *Iresine* P. Browne with 40-70 species.

Three of the genera in the core Gomphrenoideae were subdivided by Schinz (1934) into infrageneric units: *Alternanthera*, *Gomphrena* and *Froelichia*. *Alternathera* was divided in two subgenera and each one in sections. Subgenus *Eualternanthera* contained the sections *Allaganthera* Forsk., and *Dassiera* Moq. Subgenus *Telanthera* R. Brown included the sections *Bucholzia* Lam., *Brandesia* Endl., and *Mogiphanes* Endl. (Table 4).

Gomphrena was divided in six sections, *Stachyanthus* Seub., *Gomphrena* Holzh. , *Gomphrenula* Seub., *Xerosiphon* Moq., *Chnoanthus* R.E.Fries (Holzhammer 1956) and *Pseudogomphrena* (R.E.Fries) J.C. Siqueira (Siqueira 1992) based on inflorescence types, staminal tube, pseudostaminodia, tepals, and bracts features. Recent changes in the taxonomy of the *Gomphrena* have indicated that *Xerosiphon* and *Pseudogomphrena* are valid genera (Pedersen 1990; 1997) (Table 4).

Froelichia was divided in the two sections: *Hoplotheca* Moq., and *Dilopha* Moq., based on type of inflorescences, style, stigma, and characteristics of persistent tepals in fruit morphology (Moquin-Tandon 1849) (Table 4).

Other Gomphrenoideae genera, not within the core Gomphrenoideae, have also been further subdivided. The genus *Iresine* contained the sections *Piloxerus* Endl., *Rosea* Mart., and *Iresinastrum* (Moquin-Tandon 1849). Schinz (1934) later suggested the section *Trommsdorfia* and he maintained the two other sections proposed in *Iresine*. Pedersen (1990, 1997) suggested elevating *Trommsdorfia* (= *Pedersenia*) to the generic level (Table 4).

TABLE 4. Infrageneric classification recognized for *Alternanthera*, *Froelichia*, *Guilleminea*, *Iresine* and *Pfaffia* in traditional systems of classification and recent taxonomic changes.

	GENUS NAME	MOQUIN-TANDOM (1849)	SCHINZ (1934)	STÜTZER (1935)	HOLZHAMMER (1956)	SIQUEIRA (1992)	RECENT TAXONOMY
CORE GOMPHRENOIDEAE	***Alternanthera***	**Genus *Alternanthera*** **Sections** *Trommsdorfia* Mart. (8 sp.) *Dassiera* Moq. (3 sp.) *Allaganthera* Forsk. (11 sp.) *Cladothrix* Nutt. (1 sp.)	**Subgenus *Eualternanthera*** **Sections** *Allaganthera* *Dassiera*				**Genus** *Pedersenia* (= *Trommsdorfia* Mart.) *Tidestromia* (=*Cladotrix*)
		Genus *Telanthera* R. Brown **Sections** *Bucholzia* Lam. (22 sp.) *Brandesia* Endl. (19 sp.) *Mogiphanes* Endl. (9sp.)	**Subgenus *Telanthera*** **Sections** *Bucholzia* *Brandesia* *Mogiphanes*				
	Froelichia	**Sections** *Hoploteca* Moq. (4 sp.) *Dilopha* Moq. (3 sp.)					
	Gomphrena	**Sections** *Seturnera* Endl. (5 sp.) *Hebanthe* Endl. (5 sp.) *Pfaffia* Endl. (10 sp.) *Wadapus* Moq. (58 sp.) *Xerosiphon* Moq. (2 sp.)			**Sections** *Stachyanthus* Seub. (1 sp.) *Gomphrena* Holzh. (41 sp.) *Gomphrenula* Seub. (44 sp.) *Xerosiphon* Moq. (3 sp.) *Chnoanthus* R.E.Fries (5 sp.)	**Sections** *Stachyanthus* *Xerosiphon* *Pseudogomphrena* J.C. Siqueira *Gomphrena* *Gomphrenula*	*Pseudogomphrena* J.C. Siqueira *Xerosiphon* Moq.
	Iresine	**Sections** *Philoxerus* Endl. (12sp.) *Rosea* Mart. (2sp.) *Iresinastrum* L. (13 sp.)	**Sections** *Trommsdorfia* *Rosea* *Iresinastrum*				*Pedersenia* (= *Trommsdorfia* Mart.) *Philoxerus*
	Pfaffia		**Sections** *Seturnera* *Hebanthe*	**Sections** *Eupfaffia* (20 sp.) *Seturnera* (30 sp.) *Hebanthe* (3 sp.) **Subsections** *Odontella* *Anodotella*			*Hebanthe*

Pfaffia was divided in sections *Eupfaffia*, *Seturnera*, and *Hebanthe*, the last section was divided in two subsections by Stützer (1935). Borsch and Pedersen (1997) recognized *Hebanthe* at the generic level based on branched racemose synflorescence type, woody lianas habit, and pollen grains with extremely narrow mesoporia with the tectum laterally reduced, resulting in large perforations close to the aperture (Table 4).

The sectional and subgeneric status in *Alternathera*, *Froelichia*, *Iresine*, *Gomphrena*, and *Pfaffia* requires revaluation. The monophyly of most of the genera are untested and relationships among them are unclear. For example, *Gossypianthus* was merged with *Guilleminea* by Mears (1967) but Henrickson (1987) recognized both as valid genera and circumscription of *Alternanthera* has never examined (Borsch pers. com.)

Among the 19 genera of the subfamily Gomphrenoidea, the monophyletic *Tidestromia* has been the only genus studied through a cladistic analysis including all the taxa recognized in the genus (Sánchez-del Pino 2001).

1.2.4. Geographical distribution of subfamily Gomphrenoideae.

Subfamily Gomphrenoideae has 19 genera and ca. 400 species. *Gomphrena*, *Alternanthera*, and *Iresine* are the largest genera with between 100 to 120 species (Townsend 1993). The core Gomphrenoideae includes two genera with restricted distribution: *Pseudoplantago* is known from Argentina and Venezuela (Fig. 1; Eliasson 1988; Townsend 1993) and *Tidestromia* restricted to the North American deserts (Fig. 1; Sánchez-del Pino 2001); two of them occur throughout the Americas. *Froelichia* occurs basically in temperate North America (Fig. 2C; McCauley 2002) and *Hebanthe*

distributed in South America, Mexico, and Central America (Fig. 1; Borsch and Pedersen 1997); and four of them are basically American but also occur in the old world: *Alternanthera* with ten species endemic to the Galapagos Islands (Fig. 2A), *Gomphrena* with less than 20 species indigenous to Australia (Fig. 2B; Eliasson 1988; Townsend 1993), *Guilleminea* with one species naturalized in southern Africa and northeastern Australia (Fig. 2D; Henrickson 1987; Eliasson 1988: Pedersen 2000). *Blutaparon* occurs from Florida to Mexico and Central America, the Antilles, West Africa and Micronesia. One species is endemic to the Galápagos Islands and one is endemic to Japan (Fig. 3; Mears 1982, Borsch 1998; Townsend 1993).

The remaining eleven genera of nineteen located in Schinz's (1934) classification emended by Borsch (1998) include eight genera with more restricted distribution of which four of them are monotypic, two genera are present throughout the Americas and one genus is widely distributed. The monotypic genera *Froelichiella* and *Pseudogomphrena* occur in Brazil (Eliasson 1988; Pedersen 1990; Townsend 1993), *Woehleria* is endemic to Cuba, and *Irenella* is known only from Ecuador (Eliasson 1988; Townsend 1993). The genera with restricted distributions are *Quaternella* with three species in Brazil and *Xeroshiphon* with two species reported only from Brazil (Pedersen 1990; Townsend 1993); *Lithophila* has a disjunct distribution, two species being endemic to the Galapagos Islands, and one restricted to the Caribbean (Eliasson 1985, 1988; Townsend 1993); *Pedersenia* has eight species present in Brazil and Paraguay (Pedersen 1997). These genera are widespread, *Gossypianthus* has two species distributed in the southern USA, Mexico, and some Caribbean Islands (Eliasson 1988) and *Pfaffia* has 35 species from southern Mexico to Argentina (Eliasson 1988; Borsch 1998; Townsend

1993), and *Iresine* is spread from the southern USA to South America and the Antilles and some representatives in Africa. (Fig. 3).

Fig. 1. Geographic distribution of members of core Gomphrenoideae. *Hebanthe* (◘), *Pseudoplantago* (☼), and *Tidestromia* (▲).

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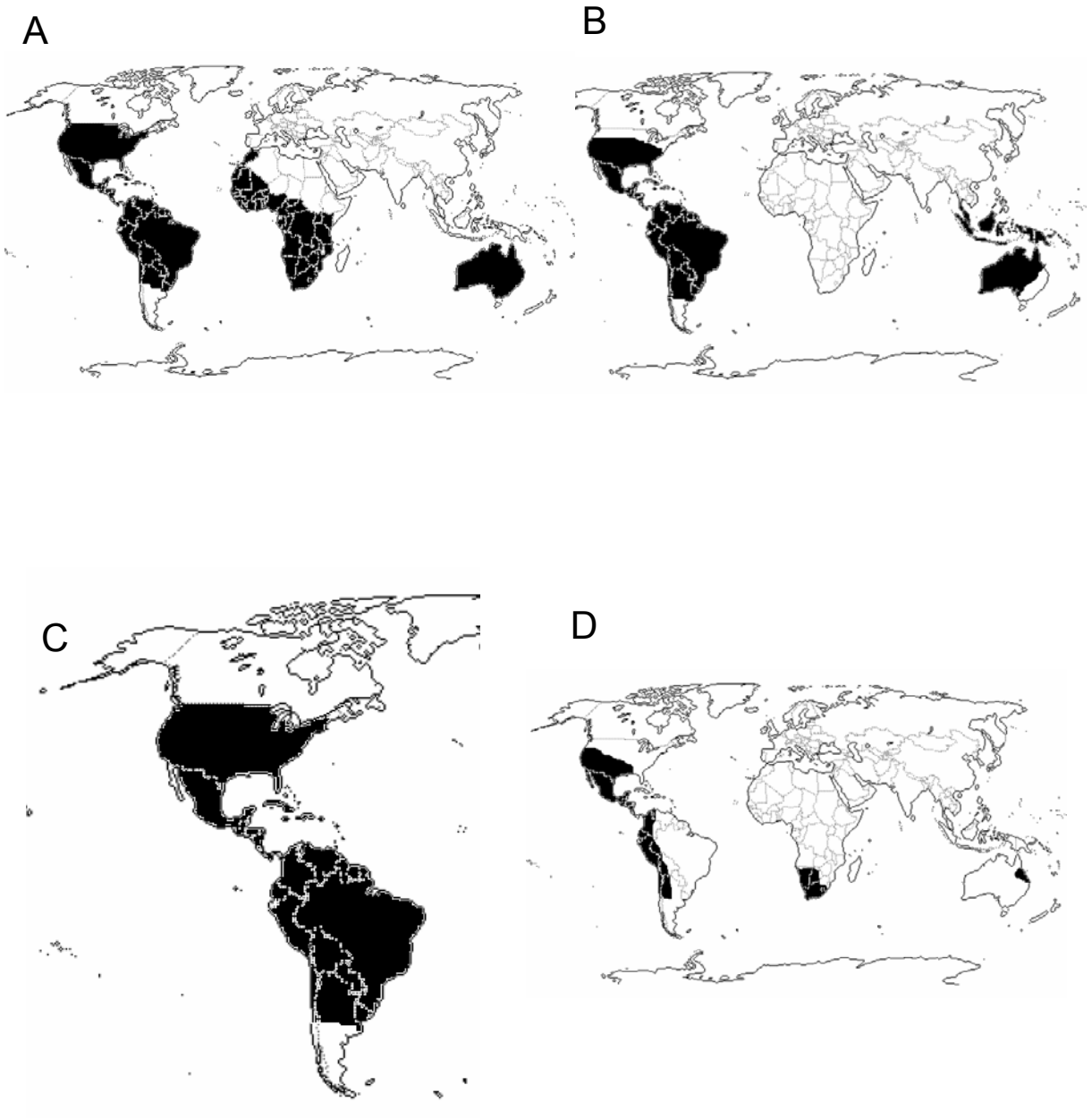

Fig. 2. Geographic distribution of members of core Gomphrenoideae.
A. *Alternanthera.* B. *Gomphrena.* C. *Froelichia.* D. *Guilleminea.*

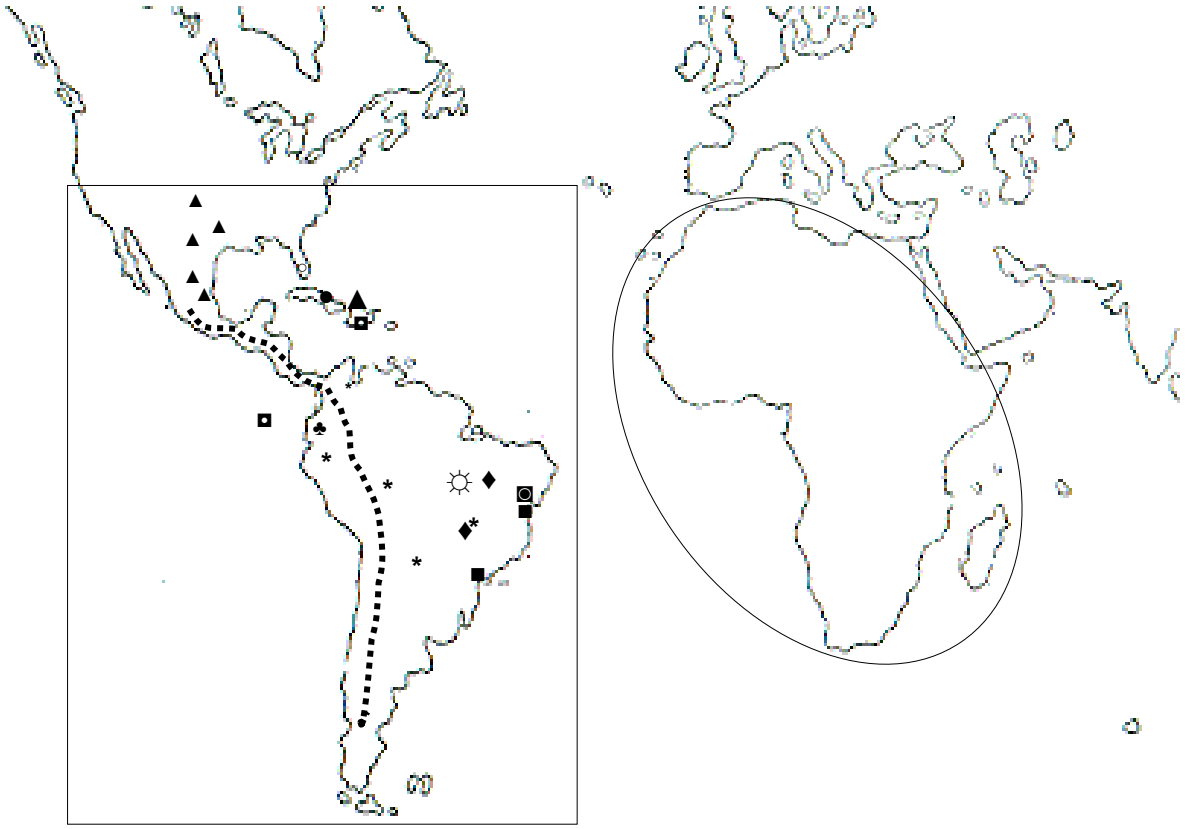

Fig. 3. Geographic distribution of the subfamily Gomphrenoideae. *Froelichiella* (☼), *Gossypianthus* (▲), *Iresine* (—), *Irenella* (♣), *Lithophila* (◘), *Pedersenia* (*), *Pfaffia* (▪▪▪▪▪▪▪▪), *Pseudogomphrena* (◙), *Quaternella* (■), *Woehleria* (●), and *Xerosiphon* (♦).

1.3. FLORAL VARIATION AND EVOLUTION

1.3.1. Pseudostaminodia.

Pseudostaminodia in the subfamily Gomphrenoideae can be conspicuous structures when they are well developed (Eliasson 1988). They range from entire, crenate, to lacinate (Fig. 4). Pseudostaminodia are present in many genera but are probably absent in *Guilleminea*, *Gossypianthus*, *Irenella* and *Woehleria* (Eliasson 1988).

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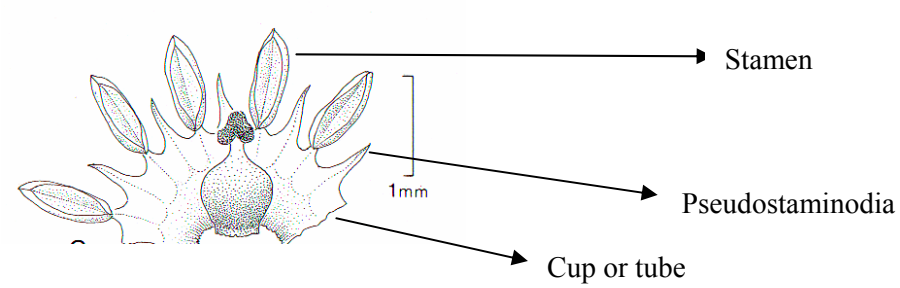

Fig. 4. Parts of the androecium in the Amaranthaceae (Source: Sánchez-del Pino, 2001).

Eliasson (1986; 1988) hypothesized two trends in evolution of pseudostaminodia in the Amaranthaceae *s. s.* In the first trend, he indicated that *Froelichiella* (Fig. 5-1B), *Froelichia* (Fig. 5-1C), and *Pseudogomphrena* (Fig. 5-1D) have a staminal tube derived from *Alternanthera* (Fig. 5-1A) which means that from a staminal tube with large filaments and large, laciniate pseudostaminodia there were reduction of filament length and fusion of pseudostaminodia with filaments in order to form staminal tubes with sessile anthers and large pseudostaminodia either entire or divided in two. Staminal tube in *Gomphrena* (Fig. 5-1E) could originate from a further decrease in distance between pseudostaminodia of the *Pseudogomphrena* type as well as a deeper forking of the

pseudostaminodia. Eliasson (1988) suggested that each apical filament lobe in *Gomphrena* would be homologous with half a pseudostaminodium in *Alternanthera* and *Froelichia*. He considered this hypothesis as a result of coalescence and splitting-up tendencies (Fig. 5-1A-E).

The second trend suggested that evolution of pseudostaminodia can be viewed in an opposite direction (Fig. 5-2). He indicated that a structure reminiscent of the pseudostaminodium in *Pseudogomphrena* (Fig. 5-2D) could result from the fusion of two adjacent filaments along most of their length (Fig. 5-2D), and the further fusion of them would lead to the entire or shallowly dentate pseudostaminodium of *Froelichia* (Fig. 5-2C).

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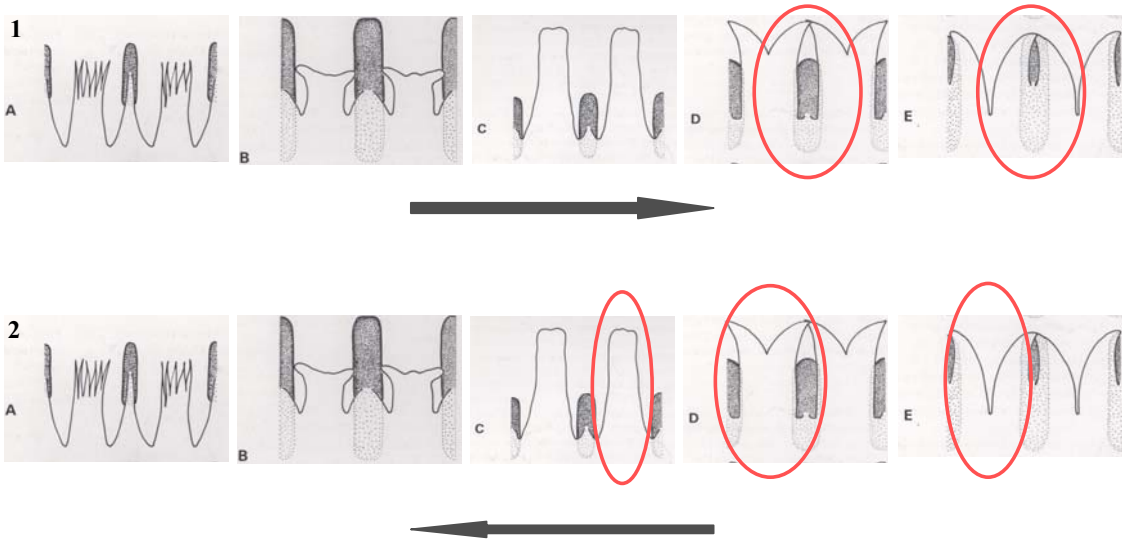

Fig. 5. Pseudostaminodia. *Alternanthera* (A), *Froelichiella* (B), *Froelichia* (C), *Pseudogomphrena*, and (D) *Gomphrena* (E). 1-2 Represent directions of possible hypothesis of pseudostaminodia evolution (Source: Eliasson 1988).

1.3.2. Gynoecium.

Stigma form was considered an important character in Schinz's (1934) classification of the Amaranthaceae *s.s*. He characterized the subtribe Froelichiinae for having capitate stigmas and subtribe Gomphreniinae for having bilobed stigmas.

Eliasson (1988) mentioned that stigmas features are probably not important in the overall taxonomy of the family but a complete SEM study of all genera of Amaranthaceae with emphasis in stigmas could be informative at the generic level.

The study of pistils using SEM in some taxa of *Froelichia*, *Guilleminea*, *Gossypianthus*, and *Tidestromia* revealed stigma lobing and additional gynoecium characters including nature of the glandular area of the style, folds in the stigma, and form and distribution of the papillae in the stigmatic surface are useful in diagnosis (Sánchez-del Pino 2001). Traditional descriptions of stigma forms can be changed when pistils are observed in the SEM, for example *Froelichia interrupta* and *Tidestromia* each have been described as capitate (Eliasson 1986, Robertson 1981), but SEM observations indicate that *Tidestromia* instead has deltoid stigmas.

Testing the evolutionary patterns of these characters will be the second part of this project.

1.4. *TIDESTROMIA* AND *ALTERNANTHERA* GENERA.

The genus *Alternanthera* inlcudes ca. 100 species (Townsend 1993). Eliasson, (1988) reported nine species endemic in the Galapagos Islands and Pedersen (1997, 2000) described 12 new species and 8 new varieties from Argentina, Paraguay and Brazil.

The genus *Alternanthera* has never been studied in a monograph including all the species however; Mears (1977) conducted the only study that includes nomenclature actualization and assignation of lectotypes of nine widespread species.

Schinz (1934) proposed sections and subgenera in *Alternathera*. In this work it is suggested to include representatives of each section of Subgenus *Eualternanthera* (Sections *Allaganthera*, *Dassiera*) and Subgenus *Telanthera* (Sections *Bucholzia, Brandesia, Mogiphanes*) within the genus *Alternanthera* as well as representatives of Galapagos Islands and South America.

Data and available material of the six species of *Tidestromia* has been obtained and they will be included in this study.

1.5. OBJECTIVES

1. To test the monophyly the subfamily Gomphrenoideae.
2. To clarify relationships within the subfamily Gomphrenoideae.
3. To test the existence of a *Tidestromia-Alternanthera* clade. This accounts to clarify the sister group relationships of *Tidestromia* within the subfamily Gomphrenoideae.
4. To explore evolutionary trends in anthers, pseudostaminodia, pollen and gynoecium characters in the subfamily Gomphrenenoideae.
5. To reconstruct the phylogeny of *Tidestromia* using molecular and morphological data.
6. To reconstruct the phylogeny of *Alternanthera* based on molecular data and to test the infrageneric relationships of *Alternathera*.

Chapter 2

Phylogenetic studies of the monophyletic subfamily Gomphrenoideae (Amaranthaceae): to examine the subfamilial classifications and evolutionary relationships of the genera.

2. 1. INTRODUCTION

The Amaranthaceae and Chenopodiaceae families (Takhtajan 1997; Cronquist 1988) in the Caryophyllales, were both included in the Amaranthaceae *s.l.* based on morphological, phytochemical, and molecular data (Downie and Palmer 1994a, 1994b; Manhart and Rettig 1994; Rodman 1990; Judd *et al*. 1999; Cuénoud *et al*. 2002; APG II 2003). The members of the Amaranthaceae *s.l.* were characterized by having P III f type plastids and seven-porate to polyporate pollen (Judd *et al*. 1999). However, after more thorough investigations (Kadereit *et al*. 2003; Müller and Borsch 2005) it became apparent that the relationships between Amaranthaceae and Chenopodiaceae are still unresolved. They are now collectively referred to as the "Chenopodiaceae-Amaranthaceae alliance" (Müller and Borsch 2005) and Amaranthaceae *s. str.* is considered a separate family from Chenopodiaceae (Judd *et al*. 1999; Müller and Borsch 2005). This study will follow the recent classifications based on molecular data and test the phylogenetic hypotheses at the infrafamilial level within the Amaranthanceae.

The Amaranthaceae *s. str.*, is a cosmopolitan family with about 70 genera and 800 species. The centers of diversity for the family are in the North American deserts, the Neotropics, tropical and southern Africa, and Australia (Müller and Borsch 2005). The

family includes *Amaranthus* as the most well known and economically important member, which is used as pseudo-cereal (Sauer 1967; Robertson 1981).

The infrafamilial classification of the Amaranthaceae has changed dramatically since it was first proposed (Table 5). Moquin-Tandom (1849) and Betham and Hooker (1883) divided the family into tribes and subtribes, whereas Schinz (1893, 1934) would later introduce subfamilies based on the number of anther locules. Schinz divided the Amaranthaceae into the subfamilies Amaranthoideae (4-locular) and Gomphrenoideae (2-locular). Each subfamily was divided into tribes and subtribes. Other authors (Cavaco 1962; Standley 1917) have proposed alternative classifications, but it was the classification of Schinz (1934) that Townsend (1993) followed when he proposed his treatment which maintained the two subfamily classification and is considered the best reflection of natural relationships in the family. It was Townsend's classification (1993) that Borsch (1998) used along with palynological data to describe pollen types which were used to define the genera described after Townsend's proposal (1993) in the Amaranthaceae *s. str.*

The subfamily Gomphrenoideae has 19 genera and ca. 400 species (Borsch 1998), mostly occurring in the New World (Müller and Borsch 2005). The Gomphrenoideae includes the largest and most widespread genera in the family such as *Gomphrena* (113 spp.), *Alternanthera* (100 spp.), and *Iresine* (40 spp.); five monotypic and narrowly endemic genera, *Froelichiella* (Brazil), *Hebanthodes* (Peru), *Irenella* (Ecuador), *Pseudogomphrena* (Brazil), and *Woehleria* (Cuba); two genera with disjunct distributions, *Lithophila* (the Galapagos and Caribbean islands), and *Blutaparon* (North and Central America, West Africa, Micronesia, and Japan); and the remaining nine

TABLE 5. Traditional classifications proposed in the family Amaranthaceae (Subf. = Subfamily; Subt.= Subtribe).

Moquin-Tandon (1849)	**Bentham and Hooker (1883)**	**Schinz (1893)**	**Standley (1917)**	**Schinz (1934)**	**Cavaco (1962)**	**Townsend (1993)**
Tribe Celosieae	Tribe Celosieae	Subf. Amaranthoideae	Tribe Celosieae	Subf. Amaranthoideae	Subf. Celosioideae	Subf. Amaranthoideae
Tribe Achyrantheae	Tribe Amarantheae	Tribe Celosieae	Tribe Amarantheae	Tribe Celosieae	Subf. Amaranthoideae	Tribe Celosieae
Subt. Amarantheae	Tribe Gomphreneae	Tribe Amarantheae	Tribe Centrostachydeae	Tribe Amarantheae	Tribe Amarantheae	Tribe Amarantheae
Subt. Aerveae		Subt. Amaranthinae	Tribe Brayulineae	Subt. Amaranthinae	Tribe Achyrantheae	Subt. Amaranthinae
Subt. Desmochaeteae		Subt. Achyranthinae	Tribe Froelichieae	Subt. Achyranthinae	Subf. Brayulineoideae	Subt. Aervinae
Subt. Polycnemeae		Subf. Gomphrenoideae	Tribe Gomphreneae	Subf. Gomphrenoideae	Subf. Gomphrenoideae	Subf. Gomphrenoideae
Tribe Gomphreneae		Tribe Gomphreneae		Tribe Brayulineae	Tribe Gomphreneae	Tribe Pseudoplantageae
		Subt. Froelichiinae		Subt. Brayulineinae	Subt. Froelichiinae	Tribe Gomphreneae
		Subt. Gomphreninae		Subt. Tidestromiinae	Subt. Gomphreninae	Suibt. Froleichiinae
		Tribe Guillemineae		Tribe Gomphreneae	Subt. Tidestromiinae	Subt. Gomphreninae
				Subt. Froelichiinae		
				Subt. Gomphreninae		

genera are distributed in various regions of the New World. *Tidestromia* and *Gossypianthus* are indigenous to Mexico and the southern United States. *Quaternella, Xeroshiphon,* and *Pedersenia* occur in Brazil with *Pedersenia* having a range that extends into Paraguay. *Pseudoplantago* is found in Argentina and Venezuela, and *Froelichia*, *Hebanthe*, and *Pfaffia* occur throughout the Americas.

Recent studies of these genera based on *rbcL* and *matk/trnk* sequence data with representative taxa sampling of Amaranthaceae and Chenopodiaceae (Kadereit *et al.* 2003; Müller and Borsch 2005) indicated the existence of a "core Gomphrenoideae" clade. This clade includes the genera *Alternathera*, *Tidestromia*, *Blutaparon*, *Gomphrena*, *Guilleminea*, *Froelichia*, *Hebanthe*, and *Pseudoplantago* and is supported by the synapomorphy of metareticullate pollen. The results were inconclusive regarding the placement of *Iresine* in either the core Gomphrenoideae or the Amaranthoids using both Parsimony and Bayesian analyses. Traditional classifications have placed *Iresine* in the subfamily Gomphrenoideae based on its 2-locular anthers (Schinz 1934).

Another significant finding in the Müller and Borsch (2005) study was that based on pollen characters and molecular data all previous infrafamily classifications appear to be artificial. However, Müller and Borsch's (2005) study was at the familial level and the sampling of the "core Gomphrenoideae" was limited to only 14 species and 9 genera.

This study will use molecular data and test the phylogenetic hypotheses at the infrafamilial level within the Amaranthanceae. The focuses of this paper are: (1) to test the monophyly of the subfamily Gomphrenoideae, and in particular, to clarify the position of the genus *Iresine* and other genera unsampled in the previous molecular studies; (2) to investigate relationships among genera in Gomphrenoideae based on a

thorough sampling of the subfamily by using previous subgeneric classifications to guide sampling of the larger genera; (3) to explore the phylogenetic utility of non-coding chloroplast sequence data from the *trnL-F* region and the *rpl*16 intron in the Amarthanaceae.

2. 2. MATERIALS AND METHODS

2.2.1. Taxon sampling. Eleven species were chosen as the outgroup taxa based on previous studies and the root was set to *Charpentiera* using the recent hypothesis that *Charpentiera* is the first branching in Amaranthaceae *s. str.* (Kadereit *et al.* 2003; Müller and Borsch 2005). The outgroup taxa belong to the subfamily Amaranthoideae and are *Achyropsis avicularis*, *Achyranthes aspera*, *Amaranthus greggii*, *Amaranthus spinosus*, *Centrostachys aquatica*, *Charpentiera obovata*, *Ch. tomentosa*, *Kyphocarpa angustifolia*, *Leucosphera bainesii*, *Pupalia lappacea*, and *Sericorema nemotiflora* (Table 6).

The ingroup contains 61 species (Table 6) from the subfamily Gomphrenoideae which represents 16 of 19 genera defined in Townsend's (1993) classification that was emended by Borsch (1998). The sampling of species was proportionate to the size of the genera and included representative taxa of the sections proposed in traditional classifications for big genera, such as *Gomphrena* and *Alternanthera*. The sampling strategy included genera that have not been sampled in other molecular analyses such as *Gossypianthus*, *Pfaffia*, *Woehleria*, *Xerosiphon*, *Pedersenia*, *Irenella* and *Lithophila*. An initial sampling of 33 species of *Alternanthera* was used to test the sections for the genus proposed by Moquin-Tandom (1849); however, once the monophylly of *Alternanthera* was confirmed, a subset of 13 species was selected across the subclades in the genus. The

complete sampling of *Alternanthera* species will be discussed in a subsequent study (Sánchez-del Pino, Chapter 3).

TABLE 6. Taxon sampling and voucher information

TAXON	COLLECTION/HERBARIUM
Achyranthes aspera L.	Sánchez-del Pino 105/ MEXU
Achyropsis avicularia	Venter 9671/ NY
Alternanthera altacruzensis Suesseng.	Nee & Vargas 43479/ NY
Alternanthera caracassana Kunth	Sánchez-del Pino *et al*. 20/ MEXU
Alternanthera crucis	Taylor 9531 & Lodge/NY
Alternanthera elongata (Willd.) Schinz	Beck 11078/ NY
Alternanthera flava (L.) Mears	Nee & Taylor 28763/ NY
Alternanthera flavescens Kunth	E. Martínez S. s.n/ NY
Alternanthera galapagensis (Stewart) Howell	Eliasson & Eliasson 726/ GB
Alternanthera halimifolia Standl.	FLSP2171/ NY
Alternanthera laguroides (Standl.) Standl.	Taylor 17394/ NY
Alternanthera olivacea Urb.	van Proosdij 1105/ NY
Alternanthera philoxeroides Griseb.	Thomas & Amason 142/ NY
Alternanthera pungens Kunth	Agra *et al.* 2084/ NY
Alternanthera tenella Colla	Nee 42581/ NY
Amaranthus greggii S. Watson	Sánchez -del Pino 106/ MEXU
Amaranthus spinosus L.	Sánchez -del Pino 98/ MEXU
Blutaparon vermiculare (L.) Mears	Liogier 34352/ NY
Centrostachys aquatica Wall	Venter 9733/ NY
Charpentiera obovata Gaudich.	10254/ HLA
Charpentiera tomentosa Sohmer	Project 98-244, LF(GJR-1119)/ HLA
Froelichia floridana (Nutt.) Moq.	Fryxell 1847/ NY
Froleichia interrupta Moq.	Nee 33212/ NY
Froleichia tomentosa Moq.	Casas & Molero FC4372/ NY
Gomphrena boliviana Moq.	Fortunato & Adelqui 5526/ NY
Gomphrena elegans Mart.	Nee 34257/ NY
Gomphrena flaccida R. Br.	Fryxell, Craven & Stewart 4748/ NY

TABLE 6. Taxon sampling and voucher information

TAXON	COLLECTION/HERBARIUM
Gomphrena globosa L.	Sánchez -del Pino 109/ MEXU
Gomphrena graminea Moq.	Pedersen 9570/ NY
Gomphrena haenkeana Mart.	Nee *et al*. 52178/ NY
Gomphrena macrocephala St. Hil.	A. Schinini & M. Dematteis 33318/NY
Gomphrena nitida Rothr.	Sánchez -del Pino 113 *et al*./ MEXU
Gomphrena serrata L.	Sánchez -del Pino 110/ MEXU
Gomphrena vaga Mart.	Estenssoro 608/ NY
Gossypianthus lanuginosus Moq.	Sánchez-del Pino 22/ MEXU
Guilleminea densa Moq.	Flores at al. HF 02-24/ MEXU
Guilleminea gracilis R. Fries	Nee *et al*. 51956/ NY
Hebanthe grandiflora (Hook.) T. Borsch & Pedersen	Ventura 3208/NY; Nee 47097/ NY
Hebanthe occidentalis (R. E. Fr.) T. Borsch & Pedersen	Nee & Saldias 47054/ NY
Hebanthe paniculada Mart.	Tressens 6346/NY; Queiroz 2831/ NY
Irenella chrysotricha Suess.	Asplund 16555/ NY
Iresine alternifolia S. Watson	Daniel 2340/ NY
Iresine arbuscula Uline & Bray	Sánchez -del Pino 100/ MEXU; Nee & Taylor 26505/NY
Iresine diffusa Humb. & Bonpl. ex Willd.	Sánchez -del Pino 118 and A. Campos/ MEXU
Iresine grandis Standl.	Rzedowski 29504/NY
Iresine heterophylla Standl.	Sánchez -del Pino 107 and G. Flores/ MEXU
Iresine leptoclada (Hook. f.) J. Henrickson & S. Sundberg	Chiang *et al*. 9644e/NY; Hinckley 2135/NY
Iresine palmeri Standl.	Nee & Taylor 29041/NY
Iresine sp.	Sánchez -del Pino 103 and A. Campos/ MEXU
Kyphocarpa angustifolia	Zietsman 3928/NY

TABLE 6. Taxon sampling and voucher information

TAXON	COLLECTION/HERBARIUM
Leucosphera bainesii	Zietsman 3944/NY
Lithophila muscoides Sw.	Correll 46472/ NY; Correll 43425/ NY
Pedersenia argentata (Mart.) J. Holub.	Nee 38784/NY
Pedersenia cardenasii (Standl.) J. Holub.	Borsch & Ortuño 3504/BONN
Pedersenia sp.	Borsch & Ibisch 3532/BONN
Pfaffia aff. iresinoides Sprenq.	Sánchez -del Pino 115/ MEXU
Pfaffia jubata Mart.	Uhlmann 88/NY
Pfaffia tuberosa (Moq. ex DC.) Hicker	Pedersen 1010/NY
Pseudoplantago friesii Suess.	Pedersen 15792/NY
Pupalia lappacea Juss.	Venter & Venter 9673/ NY
Sericorema nemotiflora	Venter & Venter 9713/NY
T. lanuginosa subsp. e*liassoniana* Sánch. Pino & Flores Olv.	Pinkava 9988/NY; Harrytate 1035/NY
T. lanuginosa (Nutt.) Standl. subsp. *lanuginosa*	Flores Olvera 02-18/MEXU; Flores Olvera 02-19/MEXU
T. rhizomatosa I. M. Johnst.	Flores Olvera 02-14/MEXU
T. suffruticosa var. *oblongifolia* (S. Watson) Sánch. Pino & Flores Olv.	Atwood 27875/ NY; Neese & Neese 10970/NY
T. suffruticosa (Torr.) Standl. var. *suffruticosa*	Flores Olvera 02-34/MEXU; Flores Olvera 02-16/MEXU
T. tenella I. M. Johnst.	Flores Olvera 02-25/MEXU
T. valdesiana Sánch. Pino & Flores Olv.	Flores Olvera 02-33/MEXU
Tidestromia carnosa (Steyerm.) I. M. Johnst.	Flores Olvera 02-22/MEXU
Woehleria serpyllifolia Griseb.	Britton 7356/NY
Xerosiphon angustiflorus (Mart.) Pedersen	Anderson 9006/NY
Xerosiphon aphyllus (Pohl ex Moq.) Pedersen	Fonseca 1303 *et al*./NY

2.2.2. DNA extraction, amplification and sequencing. Leaf samples were obtained from herbarium material or fresh tissue dried in silica gel. DNA extraction followed the Qiagen Plant DNeasy (Qiagen Inc., Valencia, California, USA) manufacturer's protocol and Fast Prep™ method (Qbiogen Inc., Carlsband, California, USA).The Lysis procedure was modified for herbarium material, it included, 30 µl of β–mercaptoethanol and 30 µl of highly purified proteinase K solution (Roche, Indianapolis, Indiana, USA) added to the recommended 400 ml of AP1 lysis buffer with constant mixing and incubation at 42°C for 12-24 hours.

PCR reactions were prepared in 25 µl reactions consisting of 10 µl of autoclaved and nanopure water, 2.5 µl of 10X buffer with $MgCl_2$, 2.5 µl dNTP, 2.5 µl BSA (bovine serum albumin), (sometimes using 1.25 µl DMSO (dimethyl sulfoxide) or 5 µl of betaine), 1µl of each of two primers in a 10 µmol/L concentration, 0.2 µl Taq polymerase (Qiagen), and 0.75 or 1 µl of DNA template. Ex Taq™ DNA Polymerase (hot-start version; Takara Mirus Bio, Madison, Wisconsin, USA) was used for some samples difficult to amplify, in this case PCR reactions of 35 µl included a mixture of reagents from Takara Ex Taq (HS) product consisting of 67 µl autoclaved and nanopure water, 10 µl of 10X Ex Taq Buffer, 8 µl dNTP mixture, 10 µl BSA, (sometimes 1.8 µl DMSO), 2 µl of each two primers with a 10 µmol/L concentration, 0.5 µl. Takara Ex Taq (HS), and 0.75 0r 1 µl of genomic DNA. In some cases 4% dilutions of PCR product yielded the best amplifications. All PCR and cycle sequencing reactions were run on a Gene Amp PCR system 9600 (Applied Biosystems, Foster City, California, USA). Amplification of *trnL-F* region used the primers reported by Taberlet *et al*. (1991) and the *rpl*16 intron was amplified using the primers designed by Asmussen (1999), as well as a primer designed

based on *rpl*16-584R reversal (TTC ATT GGG TGG GAT GGC GGAA). The PCR conditions for amplifications of the *trnL-F* region include: 1 cycle 97 ^{o}C for 2 min; 30 cycles of 94 ^{o}C for 1min, 48 ^{o}C for 2 min, 72 ^{o}C for 2 min; and 1 cycle 72 ^{o}C for 16 min, hold 4 ^{o}C whereas the PCR conditions for amplifications of the *rpl*16 intron are the next: 1 cycle 94 ^{o}C for 3 min; 30 cycles of 93 ^{o}C for 1min, 55 ^{o}C for 1 min, 72 ^{o}C for 1.5 min; and 1 cycle 72 ^{o}C for 5 min, hold 4 ^{o}C. PCR products were examined in 1 % agarose gels stained with ethidium bromide and visualized on a MultiGenius gel imager (Syngene, Synoptics, Ltd., Frederick, Maryland). Amplified products were purified with spin columns from QIAquick PCR Purification Kit (Qiagen) following manufactures protocols. Purified products were cycle sequenced with dye terminator ABI Prism Ready reaction mix (Applied Biosystems) using Big Dye v 3.1 (1/4 reaction). Sequencing products were separated on 5 % denaturing polyacrylamide gels on an ABI Prism 377XL DNA sequencher (Applied Biosystems).

2.2.3. Sequence alignment and indel coding. Sequences were edited in Sequencher version 4.1 for PC (Gene Codes, Ann Arbor, Michigan, USA). Edited sequences were automatically aligned using CLUSTALX v. 1.8 (Thompson *et al*. 1997) and defined parameters for gap cost and transitions/transversions values by default. Alignments were adjusted by eye using both BioEdit Sequence Alignment Editor v 7.0.0 (Hall 1999), and Quick Align (Müller and Müller 2003). The alignment proposed in this study followed the criteria for homology assessment suggested by Kelchner and Clark (1997), Kelchner (2000), Simmons and Ochoterena (2000), and Borsch *et al.* (2003). The guidelines for the alignment are: 1) Gaps were inserted only if they prevent the inclusion of more than two

substitutions among closely adjacent nucleotides. 2) Simple sequences repeats were recognized and their homology positions in the alignment were considered with higher priority when alternative gap placement was possible. 3) Some short repetitive motifs called microsatellites produced by DNA slippage occurs when DNA strands mispair during replication or recombination that the short stretches of sequence split against each other and they form loops when DNA is repaired, the result is in the loss or gain of motifs (Page and Holmes 1998). They have also been called slipped strand mispairing (SSM) and considered the major cause of length mutations. It has been considered that A/T rich regions are susceptible to SSM, but also it has been found to be rich in G and /or C repeats. Strings of mononucleotide repeats of A and T are more frequent within non-coding regions of the chloroplast. Homology assessments scored on length mutations can be impossible or questionable (Kelchner 2000). Regions of uncertain homology were referred as hotspots (Borsch *et al.* 2003). The hotspots were excluded in this analysis (Table 7). 4) Repeats with substitutions were excluded from the analysis by introducing ambiguity codes. The assumption suggests that substitutions can occur either in the template or in the inserted sequence during or after the replicate process and the inclusion of ambiguities is the most conservative approach since it is not possible to distinguish between the template and the inserted sequence.

Contiguous gaps were codified as binary character using the "simple gap coding" method proposed by Simmons and Ochoterena (2000) using the following guidelines: 1) all gaps having different 5' and/or 3' starting/ending positions are scored as separate presence/absence characters; 2) a taxon with a gap that entirely overlaps inclusive smaller gaps is scored as inapplicable for the smaller gaps; and 3) taxa with the smaller gap(s) are

scored as absent for the larger gap. The program SEQSTATE (Müller, 2005a) was used to score all the indels and the statistics values of the sequences included in the alignment (Table 8).

TABLE 7. Position of hotspots, and exons of the *trnL-F* and *rpl*16 and position of the domains of *rpL6*.

***trnL-F* intron**	***trnL-F* spacer**	***rpl*16 intron**
trnL 5' exon 1-17	H8. 992-1004 poly T	*rpl16* 5' exon 1-8
trnL intron	H9. 1249-1263 poly T	
H1. 78-87 poly A		H1. 228-247 poly T
H2. 156-161 poly A		H2. 306-349 poly A
H3. 209-227 poly A		H3. 384-398 poly A
H4. 338-343 poly A		H4. 799-803 poly A
H5. 411-418 poly A		H5. 905-925 poly T
H6. 481-498 poly T		H6. 1013-1055 poly A
H7. 629-651 poly A and T		H7. 1287-1312 poly A
trnL 3' exon 907-956		Domain I bp 9-698
		Domain II bp 699-758
		Domain III bp 759-894
		Domain IV bp 895-1382
		Domain V bp 1383-1422
		Domain VI bp 1422-1450

2.4. Phylogenetic analysis. The analysis included 72 taxa and DNA sequences of two chloroplast regions. The aligned *trnL-F* matrix was 1191 characters in length (143 gaps) with 252 informative characters. The *trnL* exon 5' (17bp), the *trnL* exon 3' (50 bp) with no informative characters and nine hotspots were excluded from maximum parsimony analysis and from the global alignment (Fig. 6; Table 7).

TABLE 8. Sequence statistics of the *trnL* intron, *trnL-F* spacer and *rpl*16 intron. S.D.= standard deviation of mean length; % divergence (range)= pairwise sequence distance in percent (uncorrected p distance, overall mean, lowest and highest values on brackets); ti/tv ratio= transitions/transversion ratios; % variable= percentage of variable positions; % informative= % of parsimony informative positions; % GC= GC content; NA= no applicable.

	trnL-F region	*trnL* intron	*trnL-F* spacer	*rpl*16 region	*rpl*16 intron	*rpl*16 spacer
Position in the alignment	1-1191	1-799	800-1191	1-1394	1-1222	1223-1394
Length range including hotspots	769-1103	509-668	144-375	1614-1632	871-1209	NA
Length range excluding hotspots	663-952	474-599	135-355	987-1200	815-1028	172
Mean length including hotspots	998.9	610.7	322	1630.7	1023.6	NA
Mean length excluding hotspots	865.2	560.2	305	1077.3	905.3	172
S.D. including hotspots	85.1	26.8	81.4	3.3	43.8	NA
S.D. excluding hotspots	77.2	21.1	78.1	26.9	26.8	0.0
Number of characters, excluding hotspots (=length of alignment)	1191	799	392	1394	1394	172
% divergence	4.6 (0.0-13.0)	4.2 (0.0-11.9)	5.6 (0.0-14.5)	5.5 (0.0-12.3)	5.9 (0.0-12.9)	3.3 (0.0-10.6)
% variable characters	27.6	24.9	33.9	31.6	32.7	23.3
Number of parsimony informative characters	252	147	105	329	301	28
% informative characters	21.0	18.3	26.8	23.5	24.6	16.3
Number of indels	143	112	31	193	193	NA
Number of parsimony informative indels	82	64	18	122	122	NA
% G/C	32.2	29.7	36.6	32.9	30.9	43.4
ti:tv ratio	0.9 (0.0-8.0)	0.7 (0.0-4.0)	1.4 (0.0-8.0)	0.8 (0.0-7.0)	0.8 (0.0-6.0)	1.7 (0.0-11.0)

The aligned *rpl*16 matrix (including the intron and the exon 3') was 1394 characters in length. The *rpl*16 intron had 1222 characters (193 gaps) of which 302 were informative whereas the *rpl*16 exon 3' had 72 characters and 29 were informative. The seven hotspots (Fig. 7; Table 7) found in the *rpl*16 region were removed from the analysis and the alignment.

Maximum Parsimony.

Uninformative characters were deactivated. Heuristic parsimony analyses were conducted using *Nona* (Goloboff 1993) spawned by *Winclada* (Nixon 1999-2002). TBR swapping on Wagner trees were conducted from 10,000 random taxon addition sequences with 10 trees held in memory for each of the replicate initiations expanding the memory to 100 000 to do further TBR (h 100 000, mult* 10 000, ho/10).

Data sets were analyzed independently and then combined and analyzed using simultaneous analysis approach (Nixon and Carpenter, 1996). Data were run both including and excluding gaps. Jackknife branching support was calculated by *Nona* using *Winclada* with 1 000 replications with 100 search replications and 10 tree hold in memory with the next parameters (mult*100; ho/10; max*). Jackknife percentage are described as high (85-100%), moderate (75-84%) and low (>50-74%).

Bayesian analysis.

Modeltest 3.06 (Posada and Crandall 1998) was conducted to select the best fit model of molecular evolution for the present data. The model selected was GTR + Γ +I based on both model selection strategy such as the hierarchical likelihood ratio tests

(hLRT4) and Akaike Information Criterion (AIC) (Posada and Crandall, 2001; Posada and Buckley 2004). Bayesian analysis was conducted using the program "Mr. Bayes" (Huelsenbeck and Ronquist, 2001) burning 1, 500, 000 generations. Posterior probabilities distribution of trees and branch lengths were obtained using Markov Chain Monte Carlo method (MCMC; Metropolis *et al*. 1953; Hastings 1970). Four chains were run with temperature of 0.2 as defined by default in the program. Chains were sampled every 100 generations and trees were saved. The starting trees for the chain were randomly selected. This procedure was repeated two times and probabilities converged around generation 250,000. A consensus tree was obtained based on the two samples burned.

2.3. RESULTS

2.3.1. Patterns of variation in *trnL-F* region.

The *trnL-F* region is located in the large single-copy region of the chloroplast genome around 18 kb downstream of *rbcL*. The *trnL* intron, which is part of the Group I intron (Shaw *et al*. 2005), and *trnL-F* spacer have become some the most widely used chloroplast markers for phylogenetic analyses in vascular plants (Soltis and Soltis 1998; Quandt *et al.* 2004) because the region is conservative enough to align unit genera in a family and variable enough to provide some resolution among species within a genus. This chloroplast region has been used for addressing phylogenetic relationships at species and generic levels (Apocynaceae, Potgieter and Albert 2001; Lamiaceae, Bunsawat *et al.* 2004; Rubiaceae, Motley *et al*. 2005; Euphorbiaceae, Wurdack *et al*. 2005) in many groups of plants. The *trnL-F* marker was considered a good candidate region to resolve

phylogenetic relationships within subfamily Gomphrenoideae. In addition, there are no previous studies using these gene regions in the systematics of the Amaranthaceae, therefore this study will also test these regions for their utility in this lineage.

The 72 aligned sequences of the *trnL-F* region included 1191 characters. Nine hotspots (bp. 78-87; 156-161; 209-227; 338-343; 411-418; 481-498; 629-651; 992-1004; 1249-1263; Table 7) and the exons were excluded (Fig. 6). Seven hotspots were found in the *trnL* intron and two in the *trnL-F* spacer (Fig. 6; Table 7). The *trnL* intron had an aligned length of 799 bp and 18.3 % parsimony informative characters which means that it contains 42 more informative characters, but is nearly twice the length of the spacer. The *trnL*-spacer ranges a length of 391 bp and 26.8 % parsimony informative characters (see Table 8). In general the *trnL* intron contains less point mutations but more structural variation than the spacer. This is supported by the greater occurrence of hotspots regions in the intron than the spacer, 7 versus 2, respectively (Fig.6, Table 7). Therefore, the *trnL-F* intron is highly conserved part of the region and the spacer is more variable with respect to length and substitutions (Borsch *et al.* 2003; Quand *et al.* 2004; Lönne and Borsch 2004) and thus more informative than the intron (Shaw *et al.* 2005) as we found in this study. Transition/transversion ratio is greater in the *trnL-F* spacer (1.4) than the *trnL* intron (0.7). In addition, base composition of the region revealed that in the Gomphrenoideae the GC content was 7% higger in the spacer (36.6 %) than the intron (29.7 %). Quand *et al.* (2004), in a study of the *trnL-F* regions across land plants found that the introns are always more conserved than the spacer with regard to sequence length and substitution rates. They also found that the levels GC content while varying greatly

across the sequences of land plants was consistently higher in the spacer regions. Based on our data this trend holds true also in the Amaranthaceae.

2.3.2. Patterns of variation in *rpl*16 intron.

The intron in the chloroplast gene encoding ribosomal protein 16 is called the *rpl*16 intron. It is located in the large single-copy region of the chloroplast (Kelchner and Clark 1997; Small *et al*. 1998). The *rpl*16 intron forms part of the Group II introns and it has been found to resolve phylogenetic relationships at the infrageneric and familial levels (Kelchner 2002; Shaw *et al.* 2005). It has been pointed out that *rpl*16 region has high sequence variation in flowering plants (Shaw *et al.* 2005). Therefore, it was considered promising for this study and this genomic region has not been used for phylogenetic studies in the family Amaranthaceae.

The aligned *rpl*16 sequences had a total length of 1632 bp. Eight hotspots (bp. 321-347; 578-620; 708-728; 830-834; 1236-1250; 1285-1328; 1387-1406; 1570-1608; Table 7) were excluded from the analysis. Sequences ranged from 987 to 1200 bp (excluding hotspots) among taxa. The alignment after the excision of hotspots was 1394 bp. The *rpl*16 intron was 1222 bps of which 25 % were informative characters (301 bp) and the spacer is 172 bp of with a total of 16 % informative characters (28 bp).

Analysis of the *rpl*16 intron data set yielded a transition/transversion ratio that was close to one (0.8). The base composition of GC content was lower than the spacer (31 % versus 43.4 %). Additional sequence statistics for *rpl*16 intron are included in Table 8.

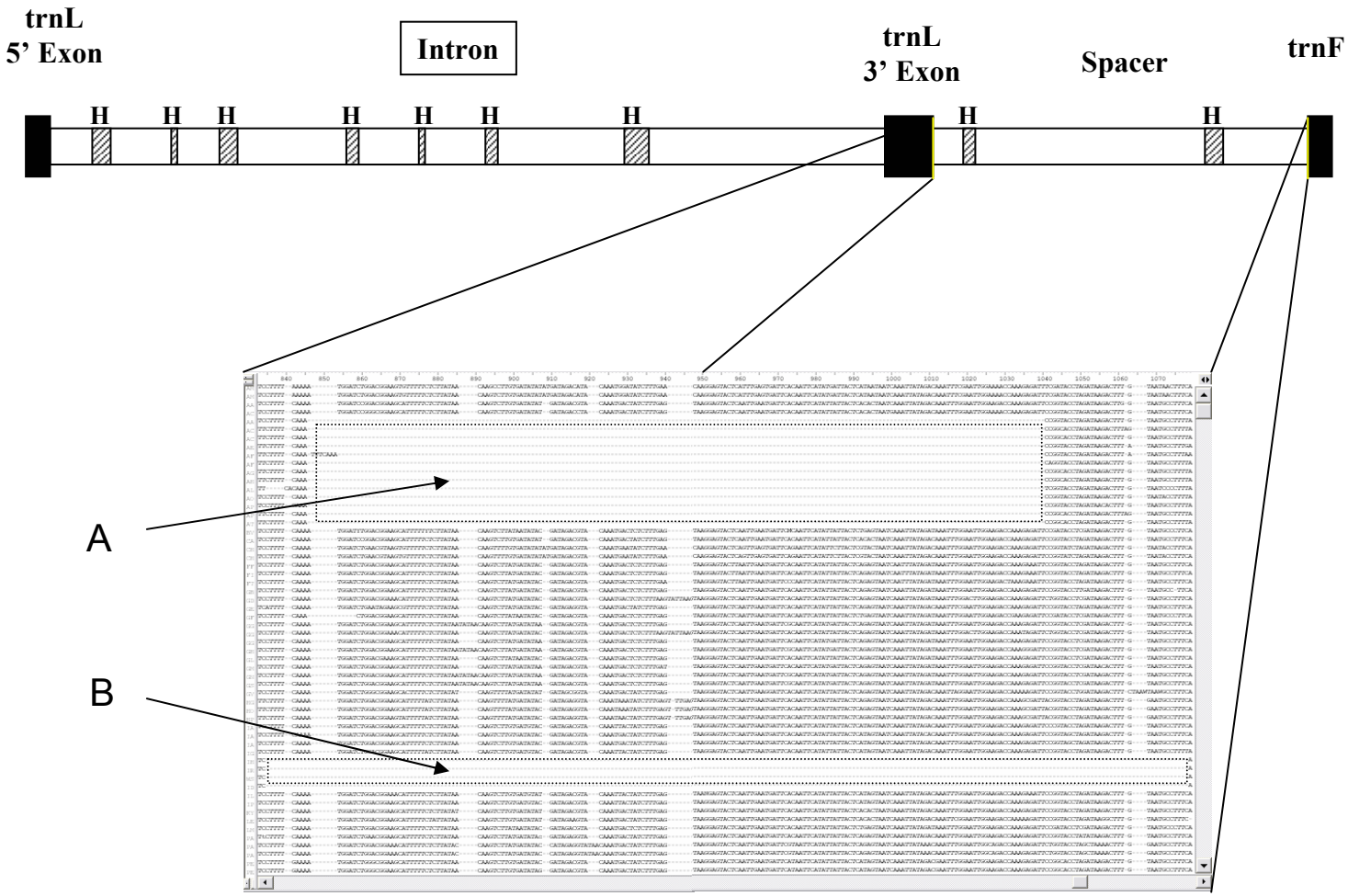

Fig. 6. Structure of the *trnL-F* in the Amaranthaceae used in the present study. The spacer and intron are illustrated by an empty bar with mutational hotspots in grey. The two large deletions in the spacer are indicated letters A-B. **A.** 187 bp deletion and **B.** 244 bp deletion.

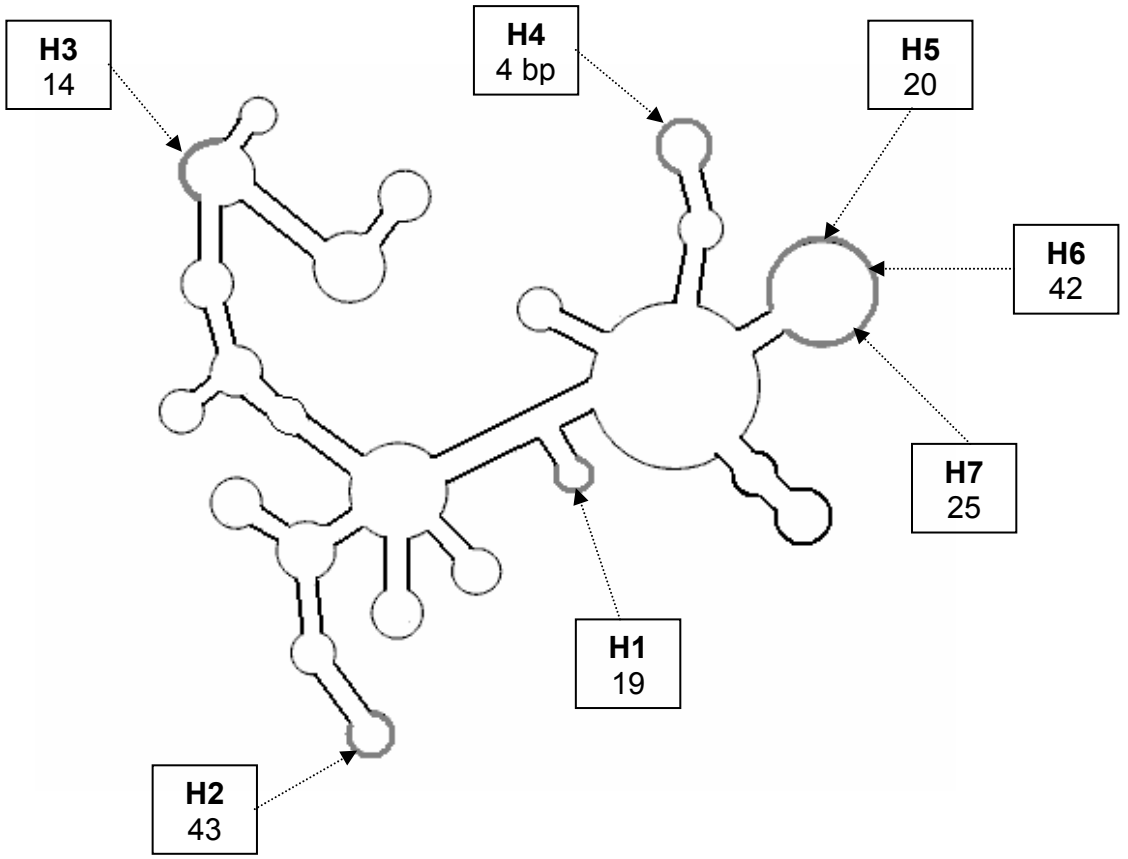

Fig. 7. Secondary structure model of group II introns (following Michel *et al.* 1989). Visual identification of reverse complement. In the *rpl16* data was based on this model. Position of the seven hotspots in *rpl16* intron is illustrated by gray stripes. Hotspots number and size are indicated in boxes.

Michel *et al.*, (1989) provided a secondary structure model for Group II introns and their model has six main domains and multiple subdomains. The primary function of Group II intron is to self-direct its extrication from gene transcriptions prior to translation of the mRNA into a protein and requires two autocatalytic reactions called splicing reactions. The failure to precisely remove efficiently the intron affects the protein synthesis and this has significant information for phylogenetic analyses. Domain I is the most complex, with many structurally important subhelices and it usually is the biggest domain which makes up more than a half of the intron, Domain II and III are shorter and seem to contribute to tertiary structure and splicing efficiency, Domain IV can be a large region and in some introns locates the maturase Open Reading Frame (ORF), Domain V is the most conserved regions due to its fundamental role in ribozyme folding, and Domain VI varies in length, and has nucleotides that initiate splicing reactions. Furthermore, high A/T content has been reported for Domains I-IV and Domain VI based on presence of loops, bulges, and interhelical sequences whereas Domain V is conserved in the content of G/C (Kelchner 2002).

The domains in the *rpl*16 intron (Table 7; Fig. 7) were found following Kelchner (2002) and this task was our focus, in order to bring information about the gene in the family, due to presence of seven hotspots in the intron. We found Domain I contains 43 % of the indels, Domain II included 14% and Domain IV had the remaining 43 %. High percentages of indels were found in Domains I and IV in the *rpl*16 intron. Similar results were reported in *petD*, another marker that form part of the Group II introns (Löne and Borsch 2004).

2.3.3. Insertions and Deletions.

More informative indels were present in the *trnL* intron (64) than in the *trnL-F* spacer (18; Table 7). Most of the indels are simple sequence repeats of 4-15 bps in length. These simple repeat indels were also commonly observed in the *rpl*16 region. Twenty five percent (standard deviation (SD) is < 5 %) of sequence length in the *trnL-F* spacer is made up of indels. This large percentage of indels is due to the presence of two large deletions. The two deletions are located just prior to the promoter (α-type 35). The α-type 35 promoter has been located in the land plants 45 bp upstream of the $trnF_{GAA}$ gene. One deletion is a synapomorphy for the *Alternanthera* clade and it has a length of 187 bps (positions 854-1040; Figs. 6, 10). The other large indel has a length of 244 bps (835-1078; Fig. 6) and is a synapomorphy for the subclade C (*Irenella chrysotricha*, *Iresine diffusa*, *Iresine heterophylla*, and *Woehleria serpillyfolia*) within the Iresinoids (Fig. 10). Big deletions in the spacer have also reported in other plant families (Motley *et al.* 2005; Wurdack *et al.* 2005)

2.3.4. Phylogeny reconstruction.

The phylogenetic analysis of the *trnL-F* data resulted in 28 most parsimonious trees (MPT) of 643 steps in length (CI = 0.64, RI = 0.85) and the *rpl*16 data yielded 33 MPT with a length of 939 steps (CI = 0.62, RI = 0.85) (Fig. 8).

The strict consensus trees for the two analyses were congruent for the major clades. There were three areas of incongruence between the two data sets. The first area of incongruence concerns the genus *Gossypianthus lanuginosus*. The *rpl*16 analysis resolved it sister to *Gomphrena boliviana* and both taxa formed a weakly supported clade

(54% JK). Alternatively, the *trnL-F* analysis resolved *Gossypianthus lanuginosus* as a genus in a polytomy that include *Blutaparon*, *Lithophila*, and *Gomphrena* species (clade A, subclade a). The second area of incongruence is the alternative position of *Gomphrena vaga* as either sister to the monophyletic *Hebanthe* (28 JK; PP) as it is shown in the *trnL-F* analysis or in a polytomy with *Hebanthe*, *Pfaffia*, and *Gomphrena elegans* as indicated in the *rpl*16 analysis (clade A, subclade b). The third area of incongruence is a result of the relationship of *Alternanthera* with its sister group therefore, *Pedersenia* sister to the clade *Alternanthera* + *Tidestromia* (*trnL-F*) is weakly supported (52% JK) versus the highly supported relationship of *Tidestromia* as the sister to the clade *Alternanthera* + *Pedersenia* (*rpl*16; 79 % JK). The *rpl*16 data provided highly resolution at species level and the *trnL-F* analysis came out with frequent polytomic resolutions.

The two data sets were congruent and were used in a combined analysis taking the total evidence approach. The combination of both genomic regions yielded eight MPT (L = 1587 steps, CI = 0.63, RI = 0.85; Fig. 10) and it includes higher branch support than either of the independent analyses. No significant topology differences were found when gaps were not included in the analyses. Branch support was higher when gaps were included and some polytomies were resolved. The consensus trees for the combined analyses using maximum parsimony and Bayesian analysis (Fig. 11) did show major topology differences. They produced almost identical tree topologies and major clades that were significantly supported by Bayesian posterior probabilities. Because these two methods gave similar results, we will refer mostly to the parsimony analysis of the combined data. In addition, the length branch results are shown in Fig. 12.

The consensus tree obtained from combined analysis indicated that Gomphrenoideae is monophyletic with high branching support (99 % JK; Fig. 10). Three major clades can be defined within the Gomphrenoideae named in this work the Gomphrenoid, Alternanthoid, and Iresinoid clades.

The Gomphrenoid clade (Fig. 10, clade A) is strongly supported (87 % JK). It includes *Pseudoplantago friessii* as the sister group of the rest of the clade with high support (95 % JK). The remaining major clade includes the polyphyletic *Gomphrena* with taxa in two different subclades (a and b). Subclade a is well supported (100% JK) and resulted in a trichotomy. The trichotomy includes a clade with *Gomphrena flaccida* closely related to *Lithophila muscoides* and *Blutaparon vermiculare* (99% JK), a second clade that includes six *Gomphrena* species (99 % JK), and the third clade includes *Gomphrena boliviana* sister to *Gossypianthus lanuginosus* (54% JK). The subclade b (99% JK) consists of two weakly supported monophyletic groups. The first clade is the paraphyletic genus *Pfaffia* and is a sister group to *Gomphrena elegans* (47% JK) and the second clade *Gomphrena vaga* as sister to the monophyletic *Hebanthe* (55% JK). The remaining three subclades belong to the monophyletic genera *Froelichia*, *Xerosiphon*, and *Guilleminea* which were each highly supported (100 % JK).

The Alternanthoid clade (Fig. 10, clade B) has 93 % jackknife support and it is composed of three monophyletic genera with high branching support: *Alternanthera* (99% JK), *Pedersenia* (100% JK), and *Tidestromia* (100% JK). The relationships among these genera resolved *Tidestromia* as sister to an *Alternanthera* and *Pedersenia* clade (71 % JK).

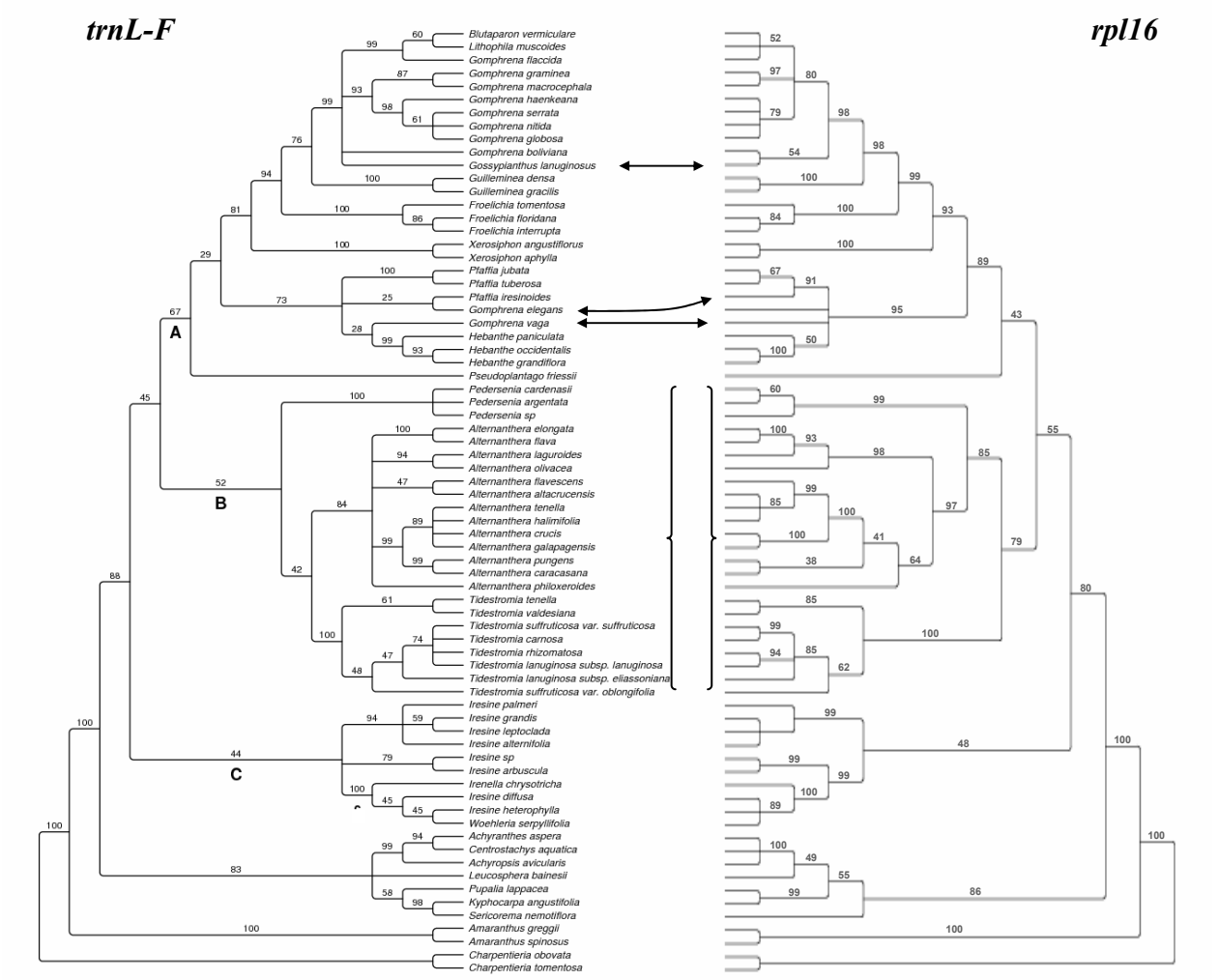

Fig. 8. The strict consensus trees from the two independent analyses. The tree of the left is the strict consensus resulted in 28 MPT (L= 643 steps, CI= 0.64, RI= 0.85) obtained *trnL-F* data and the *rpl16* data, on the rigth, yielded 33 MPT (L= 939 steps, CI= 0.62, RI= 0.85). Numbers above the branches are jackknife values.

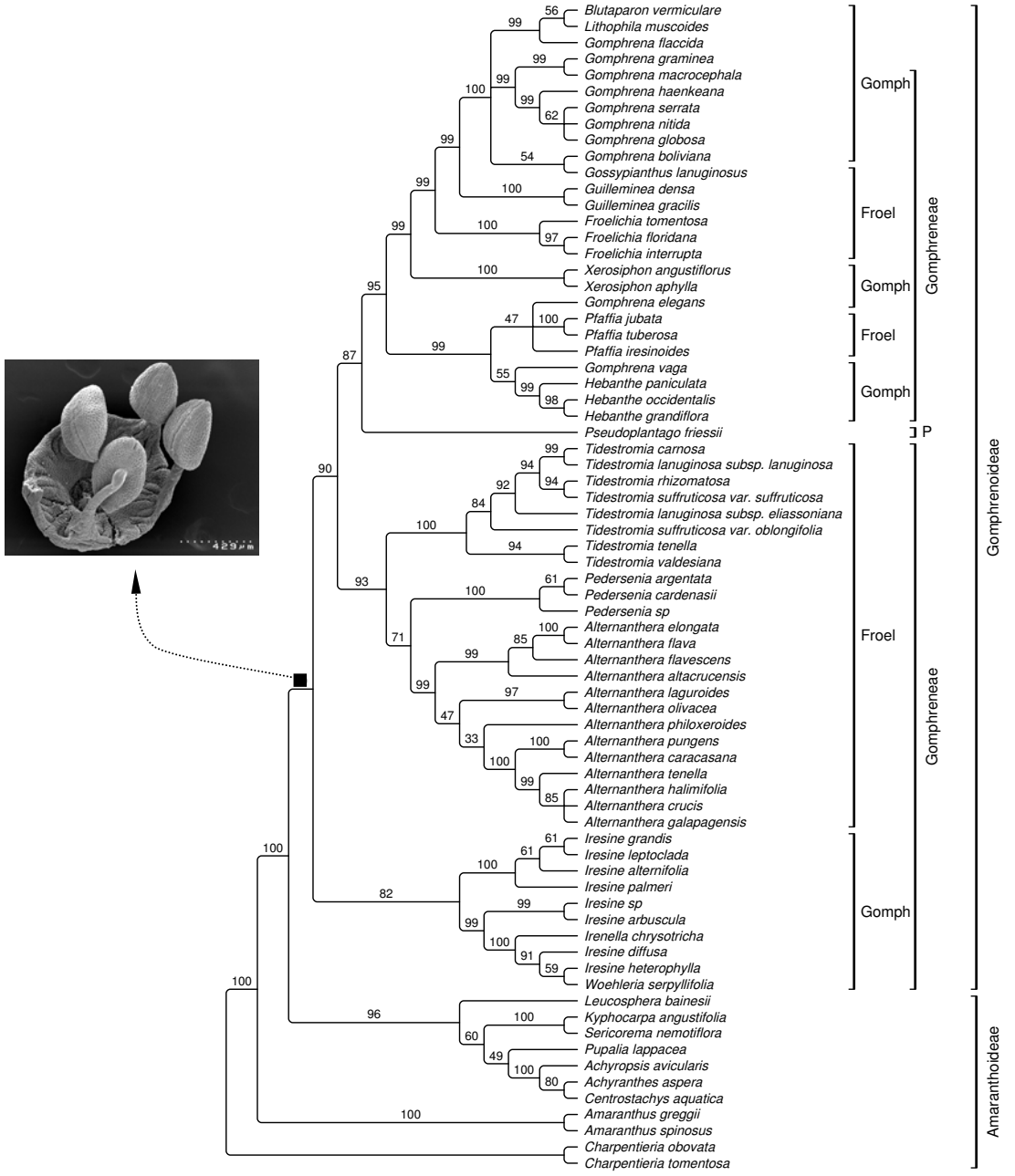

Fig. 9. The strict consensus from the combined analysis using *trnL-F* and *rpl16* yielded eight MPT (L= 1587 steps, CI= 0.63, RI= 0.85). The figure represents the synapomorphy Jackknife values above branches.Townsend's classification (1993) is represented in this phylogeny. From inner to outer brackets subtribes, tribes, and subfamily are represented. Froel = Froelichiinae; Gomph = Gomphreninae; P = Pseudoplantageae. Picture and black bar show the bilocular anthers.

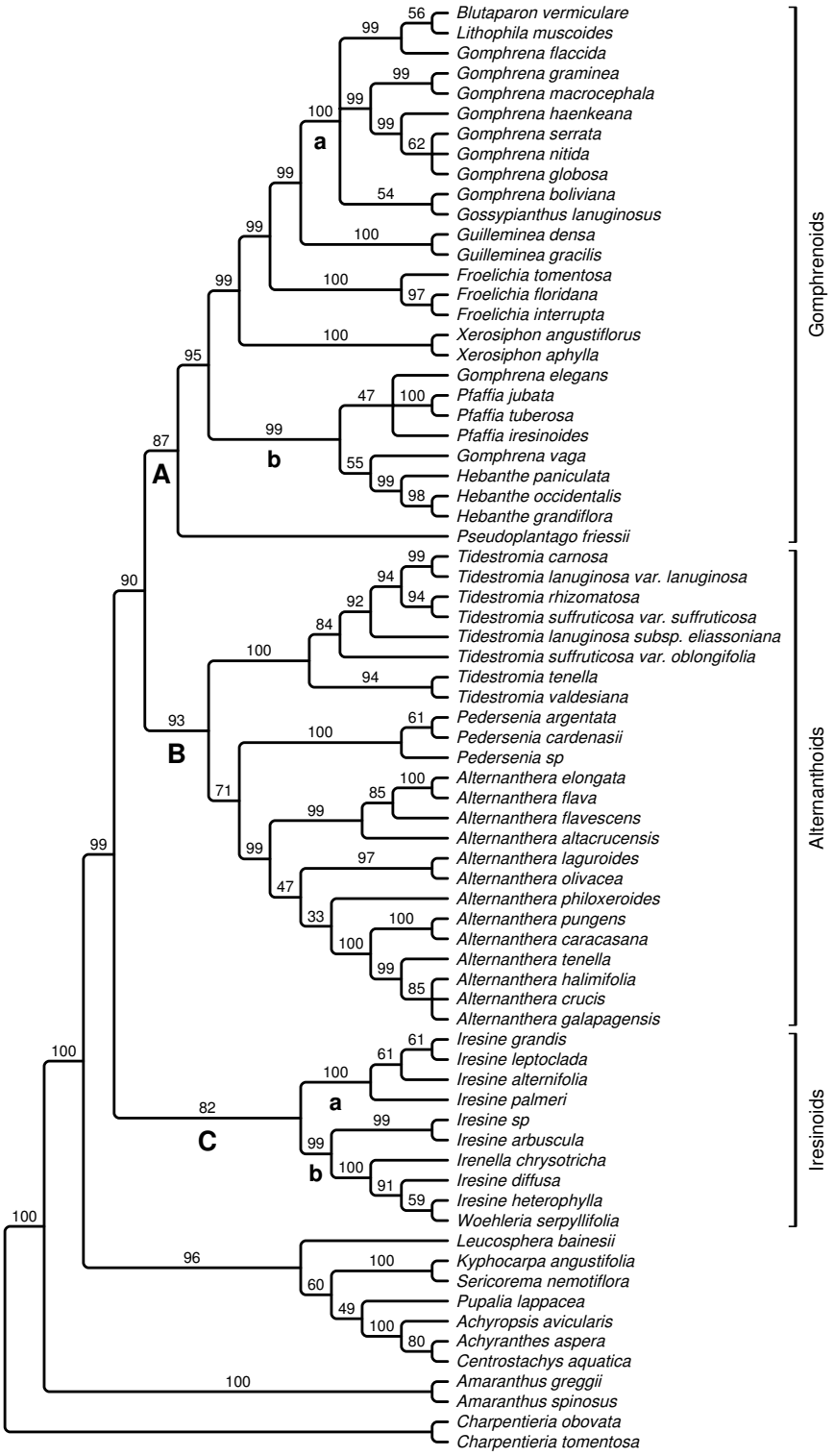

Fig. 10. The strict consensus tree generated from eight MPT yielded in the combined analysis using *trnL-F* and *rpl16* (L= 1587 steps, CI= 0.63, RI= 0.85). Jackknife values above branches. Major groups recognized in the Subfamily Gomphrenoideae.

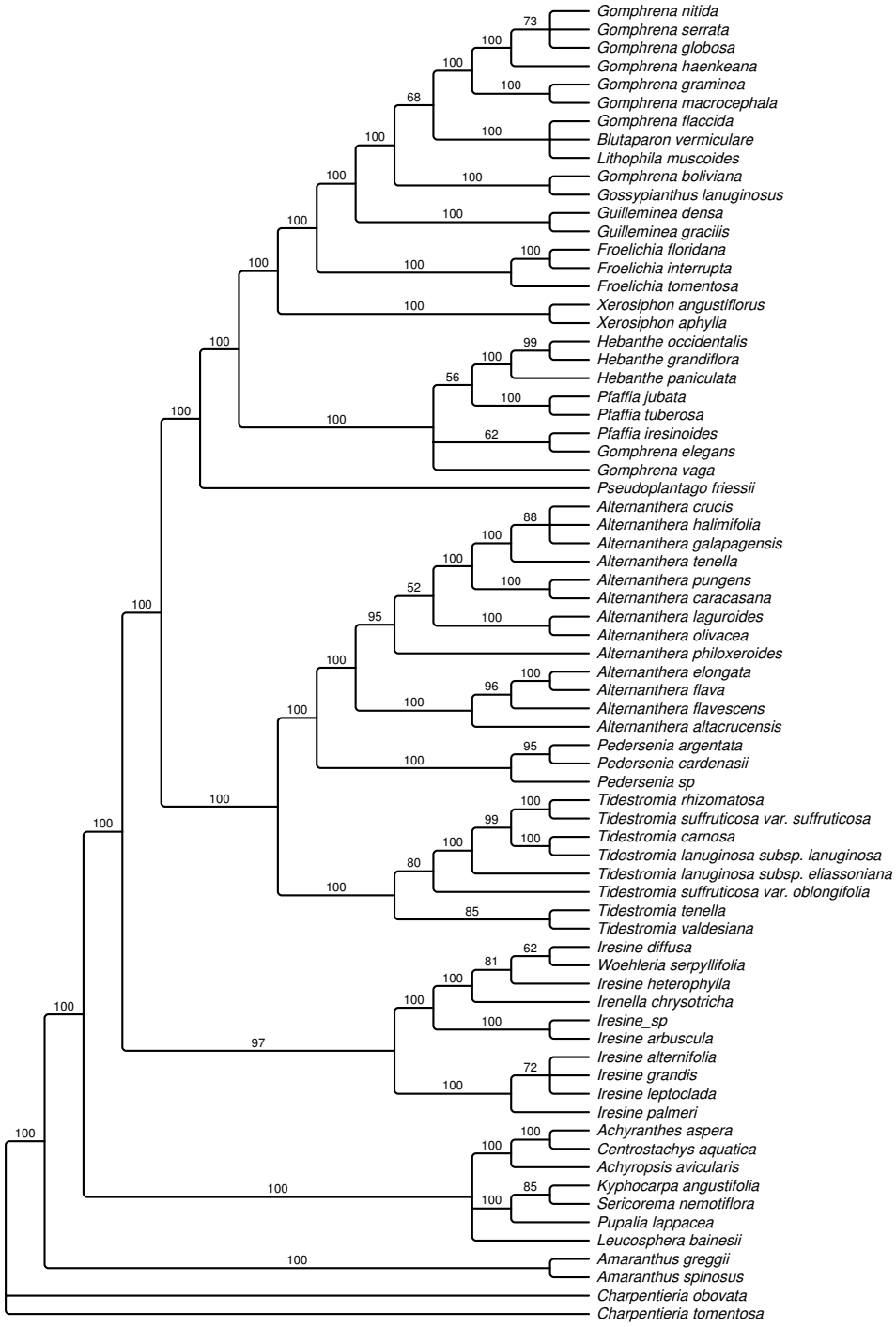

Fig. 11. Consensus tree obtained from the Bayesian analysis. Posterior probabilities above branches.

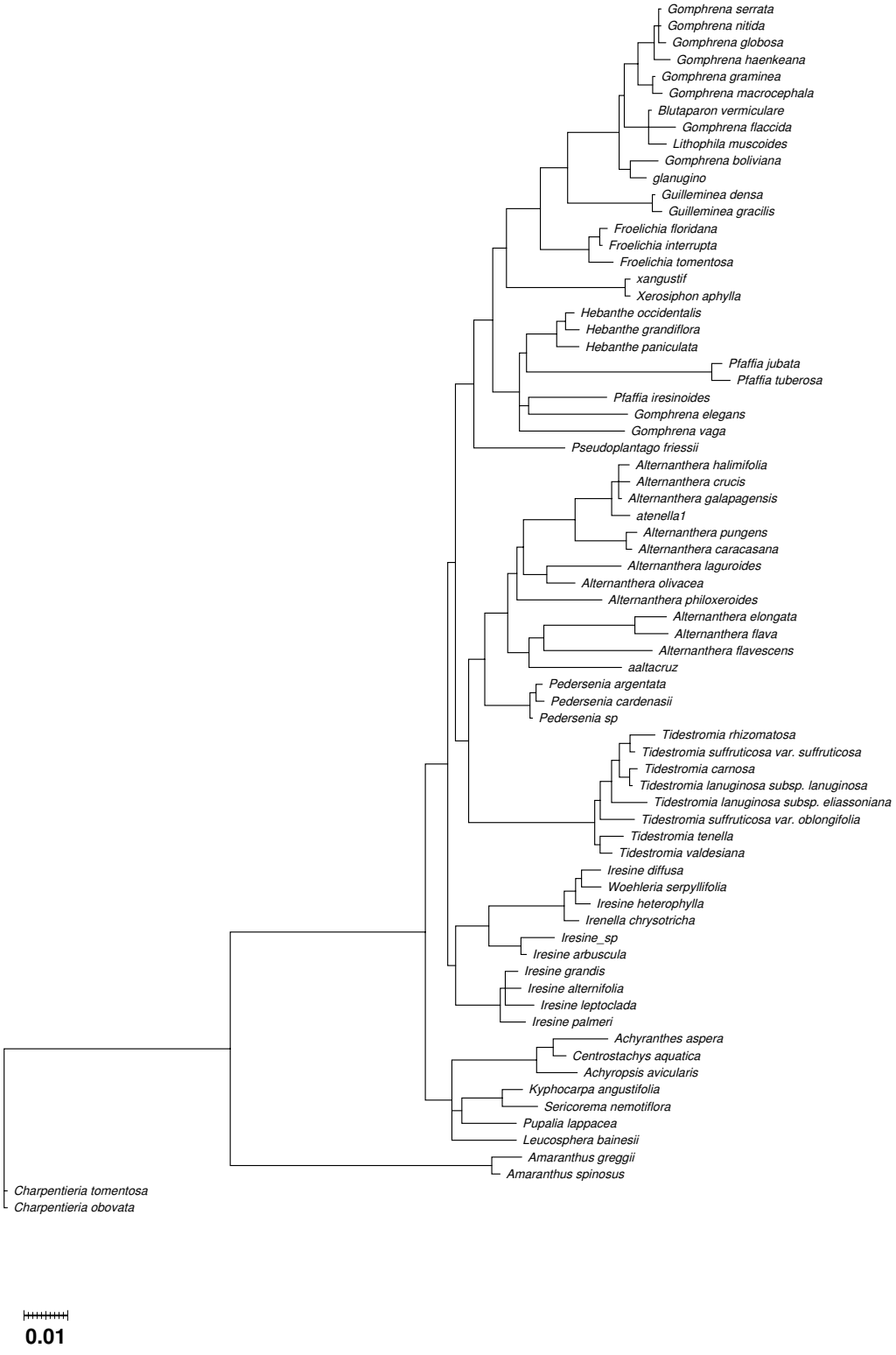

Fig. 12. Phylogram derived from the Bayesian analysis that illustrates branch lengths.

The Iresinoid clade (Fig. 10, clade C) is moderately supported (82 % JK). It includes the monotypic genera *Woehleria* and *Irenella* nested with *Iresine*.

2.4. DISCUSSION

2.4.1. Molecular evolution and phylogenetic utility of the *trnL-F* region and the *rpl*16 intron.

Comparing *trnL-F* and *rpl*16 markers.

Data obtained from the chloroplast genome of *trnL* intron and *rpl*16 intron allowed for comparisons of group I and II introns. The group I *trnL* intron, the *trnF-L* intergenic spacer, and the group II intron in the *rpl*16 have been considered rapidly evolving regions of the chloroplast genome (Soltis and Soltis 1988). A meaningful comparison of three genomic regions indicated that among them the *trnL-F* spacer has the highest percentage of parsimony informative characters with regard to sequence length (26.8 %) compared with the two introns (18.3 % in *trnL* intron and 24.6 % in the *rpl*16 intron). However the *rpl*16 intron included more informative gaps (122) than the entire *trnL-F* region (64 for *trnL* intron and 18 for *trnL-F* spacer). The *rpl*16 provided more phylogenetic information than *trnL-F* data sets which corresponds to results from other studies that described *rpl*16 intron as the fastest evolving sequence regions in the plastome (Small *et al.* 1998; Kelchner 2002) and more useful in providing resolution at the generic and species level than the *trnL-F* region (Shaw *et al.* 2005).

Noncoding versus coding markers in the Amarathaceae

Previous molecular analyses in the family Amaranthaceae have employed two protein-coding markers: *rbc*L (Kadereit *et al.* 2003) and *mat*K; and the non-coding *trn*K

intron including parts of the flanking 5' and 3' of the intron parts (Müller and Borsch 2005). These markers and the two non-coding genes used in this study (*trnL-F* and *rpl*16) are located in the large single copy (LSC) region of the chloroplast genome. For a meaningful discussion of sequence variability, the percentage of parsimony informative characters and length of each region (*mat*K, *trn*K intron, *rbc*L, *trnL-F* region and *rpl*16 intron) were compared using the values reported by Kadereit *et al.* (2003), Müller and Borsch (2005), and those generated in this study. The most informative region was *mat*K with 44.7 % variability and a length of 1503 to 1539 bp. The *trnK* intron was the most variable among the introns with 38.8 % of the characters being parsimoniously informative and was the largest region (2378-2644 bp). The 5' end of the *trnK* intron ranged from 625 to 739 bp. It had 34.7% of parsimony informative characters making it the third most variable of the non-coding regions. The next most variable regions were the *trnL-F* spacer (26.8 %) with a length range of 135-355 bp, then *rbc*L (26.5 %) with 1343 bp, *rpl*16 intron (24.6%) ranging from 815-1028 bp, the *trnK* intron 3'end (24.5%) with a length range of 213-379 bp, and *trnL* intron (18.3%) having around 474-599 bp.

Our data is in contrast to what Shaw *et al.* (2005) found regarding to the variability of introns. He found the *trnK* intron provides fewer parsimony informative characters compared to *rpl*16 and *trnL* introns; however, it seems that in the family Amaranthaceae, the *trnK* intron, which has the largest size, is more informative that the *rpl*16 and *trnL* introns; the *trnL-F* spacer; and the *trnK* intron 5' end. In the Amaranthaceae, the *matK* protein-coding region is more phylogenetically informative than the non-coding *trnK* intron and this confirms that it has been considered one of the most rapidly evolving protein-coding regions (Soltis and Soltis 1998).

2.4.2. Monophyly of Gomphrenoideae.

Previous phylogenetic studies of the Amaranthaceae or the Amaranthaceae-Chenopodiaceae clade (Kadereit *et al.* 2003; Müller and Borsch 2005) have used *rbc*L and *mat*K/*trn*K data and a broad sampling strategy selecting only a few individuals of each genus in the lineage. These studies identified a well supported clade named the core Gomphrenoideae (Kadereit *et al*. 2003) which did not include the genus *Iresine*. The genus *Iresine* has traditionally been placed in the subfamily Gomphrenoideae based on the presence of 2-locular anthers. Parsimony analysis using *rbc*L (Kadereit *et al*. 2003) resulted in a polytomy that included *Iresine*, the core Gomphrenoideae, and an unresolved clade called the Amaranthoids II. The *trnK*/*matK* (Müller and Borsh 2005) left the position of *Iresine* unresolved in a trichotomy with the core Gomphrenoideae and the Achyranthoids. Alternatively, Bayesian and likelihood analyses of the same data sets resolved *Iresine* as paraphyletic with respect to the core Gomphrenoideae and the Achyranthoid clade (Müller and Borsch 2005). The groups Amaranthoids II and Achyranthoids include genera that were traditionally placed in the subfamily Amaranthoideae, which is the other subfamily in the family Amaranthaceae. These first studies, however, were based on a relatively sparse sampling of the subfamily Gomphrenoideae (10 genera, 14 species). Therefore, the monophyly of the Gomphrenoideae was still in question because of the uncertain position of *Iresine*

In order to test the monophyly of the subfamily Gomphrenoideae it was prudent to include a larger sample size than those used in previous studies and to explore additional gene regions. This study included a more comprehensive (16 genera and 61 species) sampling of the Gomphrenoideae. The results indicate that Gomphrenoideae is

monophyletic and includes the genus *Iresine* and the genera *Gossypianthus*, *Irenella*, *Lithophila*, *Pedersenia*, *Pfaffia*, and *Woeheleria*, which were not sampled in previous studies. Therefore the Gomphrenoideae clade contains 16 genera (see Table 6) although the inclusion and phylogenetic placement of *Hebanthoides*, *Pseudogomphrena*, and *Quaternella* remain untested. The *trnL-F* and *rpl*16 data were analyzed independently and combined using both parsimony and Bayesian criteria. In each case the Gomphrenoideae clade was supported as a monophyletic lineage sister to the Achyranthoids clade [recognized by Müller and Borsh (2005) and Müller (2005b)]. Results of this study are mostly congruent with those obtained with *rbc*L and *matK*/*trnK* data with *trnL-F* and *rpl*16 data. The major difference is the inclusion of *Iresine* in the Gomphrenoideae. The Gomphrenoideae clade is highly supported (80-100 % JK; 100 PP; Figs. 8-10) and the presence of 2-locular anthers gives cohesion to the subfamily circumscription (Fig. 9) as it was originally circumscribed by Schinz (1934). The Gomphrenoideae is the only monophyletic subfamily in the Amaranthaceae, because the subfamily Amaranthoideae has been shown to be paraphyletic (Kadereit *et al.* 2003; Müller and Borsch 2005; Müller 2005b). The monophyly of Gomphrenoideae seems well supported despite of the absence of three genera in this study. However, the three unsampled genera have two locular anthers and morphologically *Hebanthodes* appears similar to *Hebanthe* and *Pseudogomphrena* and *Quaternella* are likely closely related to *Gomphrena*. Although the generic sampling is not complete, the present *trnL-F* and *rpl*16 results strongly support a monophyletic Gomphrenoideae that has three well supported clades.

2.4.3. Three major clades versus traditional classification.

Townsend's (1993) classification recognized two tribes in the subfamily Gomphrenoideae (Table 5). The tribe Pseudoplantageae is monotypic and the tribe Gomphreneae was divided into two subtribes based on stigmas (capitate vs. bilobed) and flower morphology (never compressed vs. distinctly compressed). The subtribe Froelichiinae with the capitate stigmas and uncompressed flowers is represented in this study by the genera *Alternanthera*, *Froelichia*, *Guilleminea*, *Pfaffia*, and *Tidestromia*, and the subtribe Gomphreninae is represented by the genera *Blutaparon*, *Hebanthe*, *Lithophila*, *Gomphrena*, *Irenella*, *Iresine*, *Pedersenia*, *Xerosiphon*, and *Woehleria*. The tribes and subtribes are paraphyletic based on the *trnL-F* and *rpl*16 data. These results confirm those of Kadereit *et al.* (2003) and Müller and Borsch (2005) that Townsend's classification was artificial.

Kadereit *et al.* (2003) and Müller and Borsch (2005) recognized a single clade called the core Gomphrenoideae represented by *Alternanthera*, *Blutaparon*, *Froelichia*, *Gomphrena*, *Guilleminea*, *Hebanthe*, *Tidestromia*, and *Pseudoplantago.* Müller and Borsch (2005) defined this group of eight genera by having the synopamorphy of *Gomphrena*-type pollen (Fig. 13B-D). Alternatively the consensus tree using *trnL-F* and *rpl*16 and additional sampling of the Gomphrenoideae resolved three well supported clades, we refer to as the Gomphrenoids, Alternanthoids, and Iresinoids (Fig. 10). This informal classification is also retrieved in the Bayesian phylogeny (Fig. 11). The genera in each group are supported by palynological synapomorphies based on Amaranthaceae pollen types described in earlier studies (Borsch 1998).

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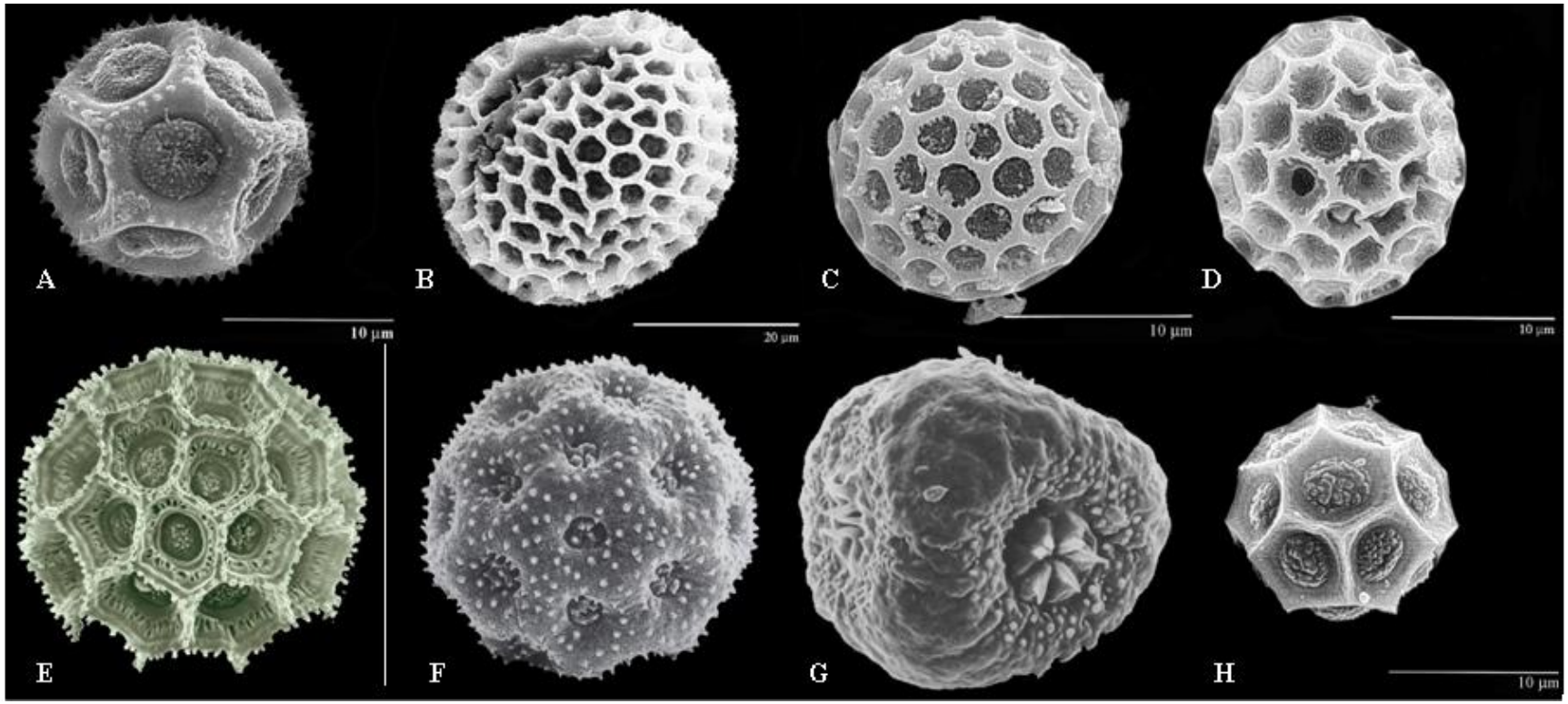

Fig. 13. Pollen types in selected representatives of Gomphrenoideae. *Pfaffia*-type with dodecahedric form: **A** *Alternanthera*; *Gomphrena*-type: **B** *Froelichia*; **C** *Gossypianthus*; **D** *Guilleminea*; *Pfaffia*-type with spheroidal form: **E** *Hebanthe*; *Iresine*-type: **F** *Iresine*; *Pseudoplantago*-type: **G** *Pseudoplantago*; *Tidestromia*-type: **H** *Tidestromia*. Scale bars: 10 µm in all except B, 20 µm.

The Gomphrenoid clade includes eight of the genera sampled by Kadereit *et al.* (2003) and Müller and Borsch (2005) when they defined the core Gomphrenoideae with the addition of *Gossypianthus*, *Lithophila*, *Pfaffia*, and *Xerosiphon*. The Gomphrenoids are characterized as having *Gomphrena*-type pollen (Fig. 13B-D). *Pseudoplantago* is the exception; it has unique pollen called the *Pseudoplantago*-type (Fig. 13G; Borsch, 1998). In this study, *Pseudoplantago* is the basally branching sister genus to the other Gomphrenoid genera. This is contrary to the results of Kadereit *et al.* (2003) and Müller and Borsch (2005) who resolved the genus as nested within Gomphrenoideae. Based on our results and the distinctive pollen of *Pseudoplantago* one could consider it a distinctive group at a different taxonomic level within the Gomphrenoids.

The genera *Alternanthera*, *Tidestromia*, and *Pedersenia* form the Alternanthoid clade. Two genera (*Alternanthera* and *Tidestromia*) were part of the core Gomphrenoideae (Kadereit *et al*. 2003; Müller and Borsch 2005). In this study, *Tidestromia* is sister to *Alternanthera* and *Pedersenia*. The members of the Alternanthoid clade have either *Tidestromia*-type (Fig. 13H) or the dodecahedric grains of *Pfaffia*-type pollen (Fig. 13A).

The Iresinoid clade includes the genus *Iresine* and the monotypic genera *Irenella* and *Woeheleria*. These genera all have *Iresine*-type pollen. The Iresinoids are sister to the Gomphrenoids and Alternanthoids.

2.4.4. The Gomphrenoids clade (Fig. 10, clade A).

The Gomphrenoid clade in all analyses includes ten genera (Figs. 8-11). Most genera are monophyletic with the exception of *Gomphrena* and *Pfaffia.* Gomphrenoid

genera have basically two spherical types of pollen, the *Gomphrena*-type (Fig. 13B-D) and the *Pfaffia*-type (Fig. 13A, E). The only member of the Gomphrenoids that does not conform to this palynological trend is the genus *Pseudoplantago* characterized for having cubic pollen with stellate ornamentation called *Pseudoplantago*–type pollen (Fig. 13G; Borsch, 1998). Within the Gomphrenoid clade (Fig. 10, clade A) it is possible to recognize two subclades based on pollen. There is a highly supported subclade (99 % JK; 100 PP) that has *Gomphrena*-type pollen (*Blutaparon*, *Froelichia*, *Gomphrena*, *Gossypianthus*, *Guilleminea*, *Hebanthe*, *Lithophila*, *Pfaffia*, and *Xerosiphon*). A similar clade was already identified by Müller and Borsch (2005) with four genera (*Gomphrena* s.str., *Blutaparon*, *Guilleminea*, and *Froelichia*) supported using *rbc*L and *trn*K markers within the core Gomphrenoids. Our study used a much broader sampling of the genus *Gompherna* (not only *Gomphrena* s.str.) which may account for these significant results.

A second well supported subclade (Fig. 10 subclade b) includes *Hebanthe*, *Pfaffia*, *Gomphrena elegans* and *Gomphrena vaga*, which was referred as Pfaffioid clade (*atpB*-*rbcL* data; Müller, 2005). They share the presence of the spherical *Pfaffia*-type pollen, although the pollen of *Hebanthe* is transitional between the *Gomphrena*-type and *Pfaffia*-type (Borsch 1998; Müller 2005b; Borsch *et al.* in prep[1].).

The polyphyletic *Gomphrena.*

Gomphrena has a disjunctive distribution with around 80 species in the Americas (Holzhammer 1956), which includes the Neotropics (mainly in Bolivia and Brazil; Ortuño and Borsch 2005), and 30 species endemic to Australia (Palmer 1998).

The genus *Gomphrena* as originally described (Linnaeus 1753) was much broader than today. The genus *Gomphrena* was located within the tribe Gomphreneae (Endlicher 1837; Moquin-Tandom 1849; Bentham-Hooker 1883; Standley 1916) and has been dived into as many as ten sections (*Serturnera*, *Hebanthe*, *Pfaffia*, *Wadapus*, *Xerosiphon; Stachyanthus, Gomphrena, Gomphrenula; Chnoanthus*; and *Pseudogomphrena*) depending on the author (Moquin-Tandom 1849; Holzhammer 1955; Siqueira 1992). Recent changes in the taxonomy of the *Gomphrena* have now elevated *Blutaparon*, *Froelichia*, *Alternanthera*, *Hebanthe*, *Pfaffia*, *Pseudogomphrena*, and *Xerosiphon* to the generic level and with *Gomphrena* are placed together in the subtribe Gomphreninae, tribe Gomphreneae, and subfamily Gomphrenoideae (Schinz 1934; Townsend 1993).

Both *trnL-F* and *rpl*16 data in the combined and independent analyses defined a polyphyletic *Gomphrena* which agrees with results of previous studies (Kadereit *et al.* 2003; Müller and Borsch 2005; Ortuño and Borsch 2005). The generic sections (Holzhammer 1956; Siqueira 1992) are not natural. *Gomphrena* species are dispersed throughout the Gomphrendiod clade (Fig. 10). Most species are in the *Gomphrena* clade sensu stricto (99% JK; 100 PP); however, *Gomphrena flaccida* is sister to *Blutaparon vermiculare* and *Lithophila muscoides* (99% JK; 100 PP); *G. boliviana* is sister to *Gossypianthus lanuginosus* (54% JK; 100 PP); and *G. vaga* and *G. elegans* are resolved within the *Hebanthe* and *Pffafia* clade (99% JK; 100 PP). The result, with species of *Gomphrena* in five subclades of the Gomphrenoids, indicates that the entire genus requires further re-evaluation and circumscription. Studies using a more exhaustive sampling of species of *Gomphrena* will be needed to redefine the genus.

The *Gomphrena flaccida*, *Blutaparon vermiculare*, and *Lithophila muscoides* clade (Fig. 10, clade A, subclade a). This clade contains the only Australian exemplar of *Gomphrena*, *Blutaparon vermiculare* which occurs in the Americas as well as Africa, and *Lithophila muscoides* from the Caribbean islands. The American species of *Gomphrena* have flowers with typically a longer staminal tube than pistil. In contrast, the Australian species usually have staminal tubes (fusion of filaments) shorter than the pistil (Mears 1982; Townsend 1993). The relationships of this clade make sense morphologically as *Gomphrena*, *Blutaparon*, and *Lithophila* share similarities with regard to flower shape and pubescence, stamen number, staminal tube length, and filament shape (Mears 1982).

The morphological distinctiveness of Australian species of *Gomphrena* is not a new concept. Brown (1810) originally proposed the genus *Philoxerus* for the Australian species and Mears (1982) agreed with this circumscription, but several other authors (e.g. Schinz 1934) have preferred a broader *Gomphrena* concept. Most recently, Palmer (1998) revised the Australian taxa, and treated *Philoxerous* and *Gomphrena* as congenerics, because he considered that staminal tube length did not warrant generic segregation. However, our results (*trnL-F* and *rpl*16) with the placement of *Gomphrena flaccida* with *Blutaparon vermiculare* and *Lithophila muscoides* instead of with the *Gomphrena* clade suggests that the Australia *Gomphrena-Philoxerus* question still exists.

The relationship of the Australian species *Gomphrena flaccida* with *Blutaparon* and *Lithophila* still represents a biogeographic disjunction. *Blutaparon* is from the Americas, West Africa, Micronesia, and Japan, and *Lithophila* occurs in the Galapagos and Caribbean islands. However, the sister relationship between these two genera was suggested by Eliasson (1988) who suspected that the genera may have arisen from a

common ancestor. Other authors have placed *Lithophila* within *Alternanthera* (Moquin-Tandom 1849) or treated it as distinct section of *Alternanthera* (Bentham and Hooker 1883). The molecular data supports Eliasson's common ancestor hypotheses and lends no support to any relationship with *Alternathera*.

The *Gomphrena* clade (Fig. 10, clade A, subclade a; second trichotomy). It is represented by six species in this study. All the species except *G. graminea* belong to the section *Gomphrena* (Holzhammer 1955; Siqueira 1992) which is defined by having globoses or capitate inflorescences and erect staminal tubes. *Gomphrena graminea* is a representative of the section *Stachyanthus*, whose members have spike-like inflorescences and curved staminal tubes (Holzhammer 1955; Siqueira 1992). The six species of *Gomphrena* included in this clade have hermaphrodite flowers, staminal tube longer than pistil, with pseudostaminodia, and two linear stigmas, which are considered diagnostic characters for the genus *Gomphrena*.

The *Gomphrena boliviana* and *Gossypianthus lanuginosus* clade (Fig 10, clade A, subclade a; third trichotomy). The morphology between *Gomphrena boliviana* and *Gossypianthus lanuginosus* is extremely different. *Gossypianthus lanuginosus* is a decumbent herb with flowers in glomerate spikes, filaments united into a staminal cup, pseudostaminodia absent, and two short stout stigmatic lobes (Henrickson 1987) and *G. boliviana* is an erect herb with flowers in capitate inflorescences, filaments united in a staminal tube, pseudostaminodia present, and two long, linear stigmatic areas (Moquin-Tandom 1849). This two species only share an affinity to arid or semi-arid regions and

the *Gomphrena*-type pollen. This ecological affinity was already mentioned by Pedersen (1997) who suggested that *Gomphrena boliviana*, *G. martiana* and *G. platycephala* form a natural group based on habit, indumentum, inflorescences, and floral characteristics. These three species occur mainly in arid and semi-arid regions of central South America from just north of the Tropic of Capricorn to northern Patagonia. While it may have been suspected in the past that the shared morphological characteristics of these species were due to ecology, it now seems that a shared common ancestry can not be ruled out.

The *Gomphrena elegans* / *Pfaffia* and *Gomphrena vaga* /*Hebanthe* clade (Fig. 10, clade A, subclade b). Historicaly *Gomphrena* has been thought to be closely related to *Pfaffia* and some species of *Pfaffia* have been treated by some authors as *Gomphrena* (Moquin-Tandom 1849; Seubert 1875). The bifid stigma of *Gomphrena* is the most reliable diagnostic character to distinguish it from *Pfaffia* and *Hebanthe* which have shallowly lobed or slightly emarginated stigmas (Pedersen 1990). Recently, the Pfaffiod clade with *Gomphrena vaga*, *Hebanthe* (2 sp.) and *Pfaffia* (1 sp.) was defined based on spherical *Pffafia*-type pollen and *atpB-rbcL* molecular data (Müller 2005b). Our results confirm the presence of this clade but expand to include *Gomphrena elegans* which also has the same *Pfaffia*-type pollen. Based on earlier subgeneric treatments of *Gomphrena* (Fries 1919) and these molecular results, the phylogenetic placement of *G. holosericea*, *G. pilosa*, and *G. mandonii* should be tested as likely candidates as members of the Paffioid clade.

The paraphyletic *Pfaffia* (Fig. 10 clade A, subclade b). *Pfaffia* with approximately 35 species was divided into three sections *Pfaffia*, *Seturnera*, and *Hebanthe* (Kuntze 1891; Stützer 1935; Cuadrado 1988; Pedersen 1990) until Borsch and Pedersen (1997) elevated *Hebanthe* to the generic rank. The phylogenetic placement of *Pfaffia* has never been tested using molecular data. Of the three species included in this study, our data (*trnL-F* and *rpl*16*)* indicates that *Pfaffia* is not a monophyletic genus. *Pfaffia jubata* and *P. tuberosa* are in section *Pfaffia* Mart. and *Pfaffia iresinoides* is in section *Serturnera* Mart. R.E. Fries (Stüger 1935). Section *Seturnera* was merged into *Pfaffia* and it has been accepted by many authors (Standey 1917; Eliasson 1988; Townsend 1993, Borsch and Pedersden 1997). Although the species used in this study are morphologically similar in appearance and share the same pollen type, the molecular data does not support this result. The two sections of *Pffafia* seem distinct with *P. iresiodes* being in an unresolved trichotomy with *Pffafia* section *Pffafia* and *Gomphrena elegans* (MP analyses; Fig. 10) or sister to *G. elegans* and *Hebanthe* (Bayesian; Fig. 11). Since pollen morphology has been important to characterize genera in the family probably it can help in the taxonomy of *Pffafia* allowing us to reconsider the infrageneric classification of the genus *Pfaffia*.

The *Hebanthe* clade (Fig. 10, clade A, subclade b). The genus *Hebanthe* as circumscribed contains seven species occurring from Mexico to South America. Our data indicated that *Hebanthe* is monophyletic (Figs. 8-11) and distinct from *Pfaffia*. This supports its recognition as a distinct genus by Borsch and Pedersen (1997). This change was further supported by palynological data (Cuadrado, 1988) and the lack of fimbriate filaments (Pedersen 1990; Borsch and Pedersen 1997).

Phylogenetic relationships of *Hebanthe* within the Gomphrenoideae using *rbc*L showed a sister relationship between *Hebanthe* and *Pseudoplantago* (Kadereit *et al.* 2003). In a latter study (*mat*K/*trn*K), *Hebanthe* was resolved as sister to a clade that includes *Gomphrena*, *Froelichia*, *Guilleminea*, and *Blutaparon* (Müller and Borsch, 2005). Our molecular data suggests that *Gomphrena vaga* is weakly supported as sister to *Hebanthe* (JK 35 %) or in the Bayesian analyses as sister to *Pffafia* section *Pfaffia* (100 PP).

***Gossypianthus* and *Guilleminea* clade** (Fig. 10 clade A). The circumscriptions of *Gossypianthus* and *Guilleminea* have been controversial. Some authors (Mears 1967; Townsend 1993) have treated *Guilleminea* as subgenus of *Gossypianthus*. Others (Henrickson 1987; Eliasson 1988; Pedersen 2000) have considered this relationship artificial and recognized them as distinct genera. The two genera are clearly in the Gomphrenoid clade (Fig. 10; also see discussion of *Gomphrena boliviana-Gossypianthus* clade) as they were traditionally located (subfamily Gomphrenoideae, tribe Gomphreneae, Fig. 9) based on the presence of 2-locular anthers. Furthermore, based on our molecular results it appears taxonomically valid to recognize both *Gossypianthus* and *Guilleminea* as separate genera.

Gossypianthus and *Guilleminea* share many morphological characters and are basically distinguished on the grade of adnation of the stamens to the tepals. *Gossypianthus* species have flowers with free tepals and free stamens, whereas *Guilleminea* has tepals fused at the base and stamen adnate to the perianth (Henrickson 1987; Eliasson 1988; Pedersen 2000)

Guilleminea was the monotypic member of the subtribe Brayulineinae (Schinz 1934), tribe Brayulineae (Standley 1917; Schinz 1934), or subfamily Brayulineoideae (Cavaco 1962) depending on the treatment (Table 5). This was because of the unique perigynous staminal position. Eliasson (1987) however, questioned the delimitation of the Brayulineae, he suggested that tribe *Guilleminea* seemed to be more closely related to the members of the tribe Gomphreneae based on inflorescence structures and pollen morphology. Later, Townsend (1993) did not recognize the tribe Brayulineae as distinct from the tribe Gomphreneae and classified *Guilleminea* within the subtribe Froelichiinae. Our consensus trees (Fig. 10 and 11) indicates that *Guilleminea* is more closely related to a clade that includes most of the *Gomphrena* species. This result agrees with Eliasson's (1998) suggestion and other molecular studies [*rbcL* (Kadereit *et al.* 2003) and *mat*K/*trn*K (Müller and Borsch 2005)] that *Guilleminea* is well supported as a member of the Gomphreneae.

***Froelichia* and *Xerosiphon* clades** (Fig. 10 clade A). A close relationship between *Froelichia* and *Xerosiphon* has been previously suggested (McCauley 2002). Both genera share the characteristic of the tepals that become endurated when fruit develops which occurs in no other genera of the lineage. Traditional systematists once placed *Xerosiphon* as a subgeneric section of *Gomphrena* (Moquin-Tandon 1849). There appears to be little support for treating *Xerosiphon* as part of *Gomphrena* as *Froelichia* is more closely related to *Gomphrena* than *Xerosiphon*. Our data suggested that *Xerosiphon* is a monophyletic genus as is *Froelichia*. *Xerosiphon* is a genus of two species from Brazil (Pedersen 1990) whereas *Froelichia* distributes in America (McCauley 2002 2004).

Pseudoplatago (Fig. 10 clade A)**.** The genus *Pseudoplantago* was located within the subfamily Gomphrenoideae because it has bilocular anthers and was the monotypic member of the tribe Pseudoplantageae in Townsend's (1993) tribal classification. It has a peculiar cuboidal type of pollen and unique floral features that make it appear more closely associated with the subfamily Amaranthoideae (Eliasson 1988). Borsch (1998) considered these features as the result of parallel evolution. He confirmed this when *Pseudoplantago* was found nested within the Gomphrenoideae in their *rbcL* and *mat*K/*trn*K study (Kadereit *et al.* 2003; Müller and Borsch 2005). However, the placement of the genus remained dubious because in subsequent molecular studies its placement varied. In the *rbcL* analysis it was sister to *Hebanthe* (Kadereit *et al.* 2003), but in the *mat*K/*trn*K analysis it was part of a grade within the core Gomphrenoideae with *Alternanthera*, and *Tidestromia* (Müller and Borsch 2005).

In this study, *Pseudoplantago* is the basally branching taxon in the Gomphrenoid clade and due to this placement and the unique characteristic *Pseudoplantago*-type pollen with stellate ornamentation which differentiates from all other Gomphrenoids it could be treated as a monotypic clade discrete from the Gomphrenoids; however, here we are taking the conservative approach and including it with the Gomphrenoids.

2.4.5. The Alternantheroid clade (Fig. 10 clade B).

The parsimony and Bayesian analyses using *trnL-F* and *rpl*16 molecular data indicated that the Alternantheroid clade is strongly supported (93%; 100 PP) and includes the monophyletic genera *Alternanthera* (99 %; 100 PP), *Pedersenia* (100%; 100 PP), and *Tidestromia* (100%; 100 PP). Similar results were observed by Müller and Borsch (2005)

when they defined a grade within the core Gomphrenoideae of *Alternanthera*, *Pseudoplantago*, and *Tidestromia* as a basally branching unresolved clade.

The *Alternanthera* clade (Fig. 10 clade B). *Alternanthera* is one of the biggest genera in the Gomphrenoideae. *Alternanthera* has an estimated 100 species (Townsend 1993) and it has never been monographed. The only study that recognizes taxonomic groups in *Alternanthera* is Moquin-Tandom's (1849) treatment where he recognized four sections in *Alternanthera*. Although our combined analysis indicated that sections recognized by Moquin-Tandom (1849) are not supported, it is possible to characterize some clades defined in the consensus trees base on inflorescences and habit form. There were three strongly supported subclades in the Alternanthoid clade.

Phylogeny appears to reflect morphology and biogeographic patterns in the genus *Alternanthera*. However, without more exhaustive sampling of this large genus it is difficult to say much at this time. A subgeneric phylogenetic study is underway (Sánchez-del Pino *et al*.; see Chapter 3).

The *Pedersenia* clade (Fig. 10 clade B). It includes seven species occurring in South and Central America (Holub 1998) and it was recently restored to the generic level based on morphology and pollen characters (Pedersen 1997). Species of *Pedersenia* have merged with *Hebanthe*, *Pffaffia* and *Iresine* but pollen morphology clearly indicates differences among them (Holub 1998). Molecular studies have found *Pedersenia* as a monophyletic genus in the *atpb-rbcL* study (Müller 2005b) and in our analyses.

The phylogenetic relationships of *Pedersenia* within the core Gomphrenoidea were not resolved in the *atpb-rbcL* (Müller 2005b) study due to limited sampling. The genus was sister to a clade that includes *Pseudoplantago*, *Tidestromia*, *Froelichia*, *Guilleminea*, *Gomphrena*, and *Blutaparon*. However, the more extensive sampling in the *trnL-F* and *rpl*16 study indicated that *Pedersenia* is sister to *Alternanthera*. This finding supports Moquin-Tandom's (1849) hypotheses that considered *Pedersenia* is closely related to *Alternanthera*.

The *Tidestromia* clade (Fig. 10 clade B). *Tidestromia* is a genus of six species that is distributed throughout the North American deserts with high levels of endemism in the Chihuahuan desert, particularly in Coahuila Mexico. The genus is recently characterized by dichasial inflorescences, alternate phylotaxy, and pollen features (ornamentation and mesoporia vaultation; Sánchez-del Pino and Flores Olvera in press). *Tidestromia* as well as *Pedesrenia* were treated as two of four sections in *Alternanthera* in Moquin-Tandom's (1849) classification. *Tidestromia* was considered closely related to *Gossypianthus* and *Achyranthes* (= *Alternanthera* in part; Standley 1915) based on morphology. Eliasson (1988) considered *Tidestromia* as a very distinctive genus among the Gomphrenoideae with probably a more isolated taxonomic position within the subfamily based on pollen and inflorescences. Cavaco (1962) placed the genus in the tribe Tidestromeae, but Townsend (1993) moved the genus into the subtribe Froelichiinae. Recently, molecular data indicated that *Alternanthera* is the closely related sister to *Tidestromia* but branch support was low (56 % bootstrap; Kadereit *et al.* 2003).

Molecular results support the hypothesis of the close relationship of *Pedersenia* to *Alternanthera* (Fig. 10) as was suspected by Moquin-Tandom (1849) and rejects the hypothesis of a sister relationship between *Tidestromia* and *Alternanthera*. *Tidestromia* is sister to the clade *Pedersenia* + *Alternanthera* (Fig. 10). Pollen morphology corroborates this relationship. *Alternanthera* and *Pedersenia* have similar pollen types, dodecaedric grains of *Pffafia*-type (Fig. 13A), and *Tidestromia* has a unique pollen morphology the *Tidestromia*-type (Fig. 13H). This study corroborates that *Tidestromia* is not closely related to *Guilleminea* as was suggested by Schinz (1934) and lends some credence to Eliasson's (1988) observation about the isolated position of *Tidestromia*.

Morphological phylogenetics within *Tidestromia* species was unable to resolve phylogenetic relationships among the species and subspecies; however, some phylogenetic structure was observed among the annual species of the genus (Sánchez-del Pino and Flores Olvera in press). Molecular data and the addition of subspecific taxa of *Tidestromia* may resolve the interspecific relationships among species (see Sánchez-del Pino *et al*. see Chapter 4).

2.4.6 The Iresinoid clade (Fig. 10 clade C).

The Iresinoid clade is comprised of *Iresine* and the monotypic genera *Irenella* and *Woehleria*. The species of *Iresine* sampled in this study have a similar pollen type (*Iresine*-type) (Borsch 1998; Borsch *et al* in prep[2].). Eliasson (1986; 1988) suggested a close relationship among *Iresine, Irenella,* and *Woehleria.* This hypothesis was strengthened when it was discovered that all the genera share the *Iresine*-type pollen (Borsch 1998). This trigeneric, monoplyletic clade was moderately supported in all our

analyses; however, *Iresine* is paraphyletic. In every analysis *I. diffusa* and *I. heterophylla* form a strongly supported clade with the monotypic genera *Irenella* and *Woehleria* nested inside of *Iresine* (Fig. 8-11). In fact these four species share a 200 bp deletion in the *trnL-F* spacer (Fig. 7). This raises the question of whether *Irenella* and *Woehleria* should remain recognized at the generic level or transferred to *Iresine*. Alternatively, perhaps *Irenella*, *Woehleria*, and the *Iresine* species which share this large deletion represent a distinct subgenus.

Presently, *Iresine* is a genus of 70 species restricted to North and South America and *Irenella* and *Woehleria* are monotypic, the former genus is endemic to Ecuador and the latter to Cuba (Eliasson 1986; 1987). The genus *Iresine* shares most of the morphological characters with these monotypic genera. The main difference is stamen number but this character seems too minor to distinguish genera. Pollen characters seem to be informative, but a complete monograph is need to deal with high morphological variation in inflorescences, habit, sexuality (bisexual, unisexual, and polygamous) that occurs among the species of *Iresine*.

In order to test former treatments of *Iresine* we included species formally assigned to the genus *Dicraurus* (*Dicraurus alternifolius* (=*Iresine alternifolia*), *D. leptocladus* (=*I. leptoclada*). The generic circumscription of *Dicraurus* was tested in this study. Species of *Dicraurus* were defined by having sessile stigmas, subglobose seeds, broad, concave cotyledons, and alternate leaves (Eliasson 1988). The phylotaxy was the most useful character employed by several authors (Schinz 1893; Standley 1917; Reed 1979) to distinguish the genera; *Dicraurus* with alternate leaves and *Iresine* with opposite leaves. The generic recognition of *Dicraurus* remained dubious since the two species of

the genus seem more similar to species of *Iresine* than to one another (Henrickson and Sundberg 1986). However, some authors remained reluctant to make the change with insufficient data noting the unique female flowers structure in the *Dicraurus* species (Eliasson 1986). This result does not support the recognition of *Dicraurus* and our data although not complete does not support the other segregate monotypic genera (*Irenella* and *Woehelria*), but rather a large diverse *Iresine*.

2.5. CONCLUSIONS

The Subfamily Gomphrenoideae is monophyletic based on a combined analysis using *rpl*16 and *trnL-F* molecular data.

Three main clades with high statistical support resolved in consensus trees (Bayesian and Parsimony) named Gomphrenoid, Alternanthoid, and Iresinoid clades. They are distinguished by pollen types as previously described by Borsch (1998).

Many genera are monophyletic (*Alteranthera*, *Froelichia*, *Gossypianthus*, *Guilleminea*, *Hebanthe*, *Pedersenia*, *Pseudoplantago*, *Tidestromia*, and *Xerosiphon*). Several genera are not monophyletic (*Gomphrena* is polyphyletic whereas *Pfaffia* and *Iresine* are paraphyletic). These genera and some others (*Blutaparon* and *Lithophila*) need further exhaustive sampling.

For future work it will be interesting to study those genera that resolved as not monophyletic and to produce generic (at species level) phylogenies which are necessary in the family Amaranthaceae.

Chapter 3

Phylogeny and subgeneric classification of the genus *Alternanthera* (Amaranthaceae, Gomphrenoideae) and its multiple colonizations of Galápagos Islands

3.1. INTRODUCTION

Alternanthera Forssk. is the largest genus in the subfamily Gomphrenoideae. The genus is principally from the New World, but a few species are native in the Old World (Eliasson 1990). *Alternanthera* contains 100 species (Townsend 1993), but some species estimates have been as high as 200 (Robertson 1981; Eliasson 1987, 1990; Siqueira 2004). The genus is distributed in the New World tropics and subtropics (Townsend 1993) with most species diversity occurring in South America (Mears 1977). Recently, twelve new species and 8 new varieties were described from Argentina, Paraguay and Brazil (Pedersen 1997; 2000). Thirteen species occur in the Galápagos Islands (Eliasson 1988; 2004) and a few species extended to Africa, Asia, and Australia (Robertson 1981). Most species of *Alternanthera* are annual or perennial herbs, rarely shrubs or small trees (Robertson 1981). The genus is characterized as having inflorescences in heads or short spikes, flowers with stamens united in a cup, with long and laciniate to small or rarely obsolete pseudostaminodia, and capitate stigmas (Townsend 1993). *Alternanthera* has not been recently monographed. Because the genus has only been revised regionally, it is necessary to update the synonymy and taxonomy. Mears (1977) made one attempt to clarify typification and proposed various lectotypes, but his *Alternanthera* treatment included only nine widespread species. *Alternathera* is commonly used as an ornamental for its colorful foliage (e. g., *A. bettzichiana* (Regel) G. Nicholson; Robertson 1981;

Eliasson 1987) or used in aquariums (e. g., *A. ficoidea* (L.) P. Beauv. or *A. reineckii* Briq.). A few species are used in folk medicine. *Alternathera tenella* Colla is used as an anti-inflammatory remedy in Brazil (Guerra *et al.* 2003) and *A. repens* (L.) Link (= *A. pungens* Kunth) is used as a cure for gastrointestinal infections in Mexico because of its antiprotozoal activity (Tapia *et al.* 2003). Furthermore, some species are reported as invasive weeds in different parts of the world (e.g. *A. caracasana* Kunth, *A. sessilis* (L.) DC., *A pungens* Kunth, *A. paronychioides* St-Hil.; Robertson 1981; Eliasson 1987).

The circumscription of the genus was much broader in the past (Table 9). Recent changes in the taxonomy of *Alternanthera* have now elevated *Lithophila* Swartz, *Pedersenia* (=*Trommsdorfia*) Holub, and *Tidestromia* (= *Cladothrix*) Standl., to the generic level. In addition, some species placed in *Alternanthera* were transferred to *Iresine* P. Browne (Endlicher 1837; Moquin-Tandom 1849; Bentham and Hooker 1883; Schinz 1893, 1934; Townsend 1993).

The phylogenetic position of *Alternanthera* as belonging to the subfamily Gomphrenoideae has been determined using molecular data. The analysis of *rbcL* DNA sequences resolved *Tidestromia* as sister to *Alternanthera* with weak branch support (Kadereit *et al.* 2004). Müller and Borsch (2005) found a polytomy of four clades within the core Gomphrenoideae: this is consisting of *Alternanthera*, *Pseudoplantago* Suesseng., and *Tidestromia* and the remainder of genera appearing in a monophyletic lineage. Analyses using *trnL-F* and *rpl*16 sequence data for the subfamily Gomphrenoideae produced a well supported clade (83%; 100 PP) termed the “Alternantoid clade”. This clade included the monophyletic genus *Alternanthera* (99 %; 100 PP) sister to the monophyletic *Pedersenia* and *Tidestromia* clades (Sánchez-del Pino, Chapter 2).

TABLE 9. Traditional classifications proposed for the genus *Alternanthera* (Sec. = Section; Subg = Subgenus).

Endlicher (1837)	**Moquin-Tandon (1849)**	**Bentham and Hooker (1883)**	**Schinz (1893)**	**Schinz (1934)**	**Townsend (1993)**
Alternanthera	*Alternanthera*	*Alternanthera*	*Alternanthera*	*Alternanthera*	*Alternanthera*
Teleianthera	Sec. *Trommsdorffia*	Sec. *Allaganthera*	*Lithophila*	Subg. *Eualternanthera*	*Lithophila*
Sec. *Bucholzia*	Sec. *Dassiera*	Sec. *Lithophila*	*Cladothrix*	Sec. *Allaganthera*	*Pedersenia* (= *Trommsdorffia*)
Sec. *Brandesia*	Sec. *Allaganthera*		*Iresine*	Sec. *Dassiera*	*Tidestromia* (=*Cladothrix*)
Sec. *Mogiphanes*	Sec. *Cladothrix*	*Telanthera*	(including		*Iresine* (narrower concept)
		Sec. *Bucholzia*	members of	Subg. *Telanthera*	
	Telanthera	Sec. *Brandesia*	*Trommsdorffia*)	Sec. *Bucholzia*	
	Sec. *Bucholzia*			Sec. *Brandesia*	
	Sec. *Brandesia*	*Mogiphanes*		Sec. *Mogiphanes*	
	Sec. *Mogiphanes*				
		Cladotrix			

Throughout the taxonomic history of the genus three main problems have arisen. One problem is when the genus *Alternanthera* was proposed by Forsskål (1775) in the *Flora Aegyptiaco Arabica*, the author did not accurately indicate the specific ephithet of the *Alternanthera* type specimen. Later, Melville (1958) indicated that some authors such as Christensen in 1922, Hiern in 1900, and Vahl in 1790 had tried to identify the type species of *Alternanthera*; however, there was some confusion with the Linnean species. Therefore, specimens from the Forsskål and the Linnean herbaria were compared and after an exhaustive evaluation, the type species of *Alternathera* was assigned to *A. sessilis*, which has the basionym *Gomphrena sessilis* L. (Melville 1958).

The second problem is the circumscription of *Alternanthera*, which has varied (Table 9) over time among authors. Endlicher (1837) recognized two genera (*Alternanthera* and *Teleianthera* R. Brown) based on the degree of stamen connation (shallow cup versus long tube) and pseudostaminodia form (toothed, entire, or trifid versus ligulate and fimbriate). Moquin-Tandom (1849) also recognized these two genera, but with a slight difference. It seems that Endlicher misspelled the name *Telanthera* because he indicated R. Brown as the authority of that name. However, Moquin-Tandom may have realized this mistake and he recognized the genus *Telanthera* R. Brown instead of *Teleianthera* Endl. Bentham and Hooker (1883) followed Moquin-Tandom's circumscription. Schinz (1934) recognized *Alternanthera* with two subgenera: the subgenus *Eualternanthera* and the subgenus *Telanthera*.

The third problem is related to an incorrect lectotypification of the type species of *Achyranthes* L., made by Standley (1915). He included most of the species of *Alternanthera* within *Achyranthes* and species placed in *Achyranthes* were transferred to

Centrostachys Wallich. (Bullock 1957; Mears 1977; Melville 1958; Robertson 2003). Standley's (1915) argument was abandoned and the circumscription of *Alternathera* and *Achyranthes* went back to the pre-1915 classification. Traditional classifications placed *Alternanthera* in the subfamily Gomphrenoideae and *Achyranthes* in the subfamily Amaranthoideae (Schinz 1934; Townsend 1993). Molecular studies have supported *Achyranthes* in the group called Achyranthoid. It has no close phylogenetic relationships with *Alternanthera*, and *Alternanthera* was part of the core Gomphrenoids (Kadereit *et al* 2004; Müller and Borsch 2005; see Chapter 2)

Oceanic islands are natural laboratories for the study of plant evolution. Because of there small size and many ecological habitats islands can reveal evolutionary events particularly through studies of their endemic taxa (Stuessy *et al.* 1998). The Galápagos Islands, are well known as a center for evolution (Darwin 1859; Perry 1984). The archipelago is 3 to 4 million years old (McMullen 1987) and 900 km away from the nearest continental mainland (Grehan 2001). The family Amaranthaceae is well represented with 38 taxa (including subspecies) of which 27 are likely endemics (Eliasson 1990). Eliasson (2004) in a recent revision indicated that diversification and speciation have occurred in the Galápagos Islands in the subfamily Gomphrenoideae in three genera. Among them, *Alternanthera* has the most island endemics. *Alternanthera* includes nine endemic species from a total of 13 species indigenous to the Galápagos. *Alternanthera* represents a biogeographic case study in these islands. Carlquist (1974) and Eliasson (2004) indicated that the endemic species of *Alternanthera* can be traced back to two or possibly more successful colonizers.

The objectives of this study are: 1) to test the subgeneric classifications proposed in *Alternanthera* but more importantly to analyze relationships within the genus, and 2) to determine the origin, colonization, and diversification of species of *Alternanthera* in the Galápagos Islands.

3.2. MATERIALS AND METHODS

3.2.1. Taxon sampling. The outgroup taxa include *Gomphrena globosa* L., *Pedersenia cardenasii* (Standl.) Holu, *Pseudoplantago friesii* Suess., and *Tidestromia lanuginosa*(Nutt.) Standl. These species were selected based on previous subfamily studies (Kadereit *et al* 2004; Müller and Borsch 2005; Sánchez-del Pino, Chapter 2). Following the outgroup selection criteria of Nixon and Carpenter (1993) the root was set as *Pseudoplantago friesii* because it shares the fewest synapomorphies with *Alternanthera*.

The ingroup contains 32 species of *Alternanthera* (Table 10) from a total of ca. 100 described species (Eliasson 1987; Townsend 1993). Nineteen new species were added in this study, which have never before been included in a phylogenetic study. The sampling of species was selected to best represent the sections described by previous authors (Table 9) and was limited only by the availability of plant material.

3.2.2. DNA extraction, amplification and sequencing. Leaf samples were obtained from herbarium material or fresh tissue dried in silica gel. DNA extraction followed the Qiagen Plant DNeasy (Qiagen Inc., Valencia, California, USA) manufacturer's protocol and Fast Prep^{TM} method (Qbiogen Inc., Carlsband, California, USA).The Lysis procedure

was modified for herbarium material, it included, 30 µl of β–mercaptoethanol and 30 µl of highly purified proteinase K solution (Roche, Indianapolis, Indiana, USA) added to the recommended 400 ml of AP1 lysis buffer with constant mixing and incubation at 42°C for 12-24 hours.

TABLE 10. Taxon sampling and voucher information

TAXON	COLLECTION/HERBARIUM
Alternanthera altacruzensis Suesseng.	Nee & Vargas 43479/ NY
Alternanthera brasiliana Kuntze	Gonzales 147/ NY
Alternanthera caracassana Kunth	Sánchez-del Pino *et al*. 20/ MEXU
Alternanthera chacoënsis Moroni ex Moroni & Britton	Nee & Coimbra 40161/ NY
Alternanthera echinocephala (Hook. f.) Christoph.	van der Werff 931/ NY
Alternanthera elongata (Willd.) Schinz	Beck 11078/ NY
Alternanthera filifolia (Hook. f.) Howell	Eliasson & Eliasson 1668/ GB
Alternanthera flava (L.) Mears	Nee & Taylor 28763/ NY
Alternanthera flavescens Kunth	Martínez 30202/ NY
Alternanthera flavicoma (Anderss.) Howell	Eliasson & Eliasson 1888/ GB
Alternanthera galapagensis (Stewart) Howell	Eliasson & Eliasson 726/ GB
Alternanthera geniculata Urb.	Alain & Liogier 26490/ NY
Alternanthera halimifolia (Lam.) Standl.	FLSP2171/ NY
Alternanthera hirtula (Mart.) R. E. Fries	Zardini & Aquino 28437/ NY
Alternanthera laguroides (Standl.) Standl.	Taylor 17394/ NY
Alternanthera maritima (Mart.) St.-Hil.	Correll 45459/ NY
Alternanthera macbridei Standl.	Cowan *et al*. 4276/ NY
Alternanthera mexicana Moq.	Barringer *et al*. 2270/NY
Alternanthera nesiotes I. M. Johnst.	Eliasson & Eliasson 2057/ GB
Alternanthera obovata Millsp.	Ventura 1314/ NY
Alternanthera olivacea Urb.	Van Proosdij 1105/ NY
Alternanthera paranychioides St.-Hil.	Thomas 114, 1179/ NY

TABLE 10. Taxon sampling and voucher information

TAXON	COLLECTION/HERBARIUM
Alternanthera philoxeroides (Mart.) Griseb.	Thomas & Amason 142, 585/ NY
Alternanthera porrigens (Jacq.) Kuntze	Weigend *et al*. 544/ NY
Alternanthera pubiflora (Benth.) Kuntze	Burch *et al*. 1176/ NY
Alternanthera pungens Kunth	Agra *et al.* 2084/ NY
Alternanthera pycnantha (Benth.) Standl.	
Alternanthera serpyllifolia Urb.	Alain & Liogier 11185/ NY
Alternanthera sessilis (L.) DC.	Patel *et al*. 4183/ NY
Alternanthera snodgrassii (B. L. Rob.) Howell	Eliasson & Eliasson 1810/ GB
Alternanthera tenella Colla	Nee 42581/ NY
Alternanthera vestita (Anderss.) Howell	Eliasson & Eliasson 1912/ GB
Gomphrena globosa L.	Sánchez-del Pino 109/ MEXU
Pedersenia cardenasii (Standl.) J. Holub.	Borsch & Ortuño 3504/ Bonn
Pseudoplantago friesii Suess.	Pedersen 15792/NY
Tidestromia lanuginosa (Nutt.) Standl.	Flores *et al*. HF 02-19/ MEXU

PCR reactions were prepared in 25 µl reactions consisting of 10 µl of autoclaved and nanopure water, 2.5 µl of 10X buffer with $MgCl_2$, 2.5 µl dNTP, 2.5 µl BSA (bovine serum albumin), (sometimes using 1.25 µl DMSO (dimethyl sulfoxide) or 5 µl of betaine), 1µl of each of two primers in a 10 µmol/L concentration, 0.2 µl Taq polymerase (Qiagen), and 0.75 or 1 µl of DNA template. Ex Taq™ DNA Polymerase (hot-start version; Takara Mirus Bio, Madison, Wisconsin, USA) was used for some samples difficult to amplify, in this case PCR reactions of 35 µl included a mixture of reagents from Takara Ex Taq (HS) product consisting of 67 µl autoclaved and nanopure water, 10 µl of 10X Ex Taq Buffer, 8 µl dNTP mixture, 10 µl BSA, (sometimes 1.8 µl DMSO), 2 µl of each two primers with a 10 µmol/L concentration, 0.5 µl. Takara Ex Taq (HS), and

0.75 0r 1 µl of genomic DNA. In some cases 4% dilutions of PCR product yielded the best amplifications. All PCR and cycle sequencing reactions were run on a Gene Amp PCR system 9600 (Applied Biosystems, Foster City, California, USA). Amplification of *trnL-F* region used the primers reported by Taberlet *et al*. (1991) and the *rpl*16 intron was amplified using the primers designed by Asmussen (1999), as well as a primer designed based on *rpl*16-584R reversal (TTC ATT GGG TGG GAT GGC GGAA). The PCR conditions for amplifications of the *trnL-F* region include: 1 cycle 97 ^{o}C for 2 min; 30 cycles of 94 ^{o}C for 1min, 48 ^{o}C for 2 min, 72 ^{o}C for 2 min; and 1 cycle 72 ^{o}C for 16 min, hold 4 ^{o}C whereas the PCR conditions for amplifications of the *rpl*16 intron are the next: 1 cycle 94 ^{o}C for 3 min; 30 cycles of 93 ^{o}C for 1min, 55 ^{o}C for 1 min, 72 ^{o}C for 1.5 min; and 1 cycle 72 ^{o}C for 5 min, hold 4 ^{o}C. PCR products were examined in 1 % agarose gels stained with ethidium bromide and visualized on a MultiGenius gel imager (Syngene, Synoptics, Ltd., Frederick, Maryland). Amplified products were purified with spin columns from QIAquick PCR Purification Kit (Qiagen) following manufactures protocols. Purified products were cycle sequenced with dye terminator ABI Prism Ready reaction mix (Applied Biosystems) using Big Dye v 3.1 (1/4 reaction). Sequencing products were separated on 5 % denaturing polyacrylamide gels on an ABI 377XL DNA sequencher (Applied Biosystems).

3.2.3. Sequence alignment and indel coding. Sequences were edited in Sequencher version 4.1 for PC (Gene Codes, Ann Arbor, Michigan, USA). Edited sequences were automatically aligned using CLUSTALX v. 1.8 (Thompson *et al.* 1997) and defined parameters for gap cost and transitions/transversions values by default. Alignments were

adjusted by eye using both BioEdit Sequence Alignment Editor v 7.0.0 (Hall 1999), and Quick Align (Müller and Müller 2003). The alignment proposed in this study followed the criteria for homology assessment suggested by Kelchner and Clark (1997), Kelchner (2000), Simmons and Ochoterena (2000), and Borsch *et al.* (2003). The guidelines for the alignment are: 1) Gaps were inserted only if they prevent the inclusion of more than two substitutions among closely adjacent nucleotides. 2) Simple sequences repeats were recognized and their homology positions in the alignment were considered with higher priority when alternative gaps placement were possible. 3) Some short repetitive motifs called microsatellites produced by DNA slippage occurs when DNA strands mispair during replication or recombination that the short stretches of sequence split against each other and they form loops that when DNA is repaired, the result is in the loss or gain of motifs (Page and Holmes 1998). They have also been called slipped strand mispairing (SSM) and considered the major cause of length mutations. It has been considered that A/T rich regions are susceptible to SSM, but also it has been found to be rich in G and /or C repeats. Strings of mononucleotide repeats of A and T are more frequent within non-coding regions of the chloroplast. Homology assessments scored on length mutations can be impossible or questionable (Kelchner 2000). Regions of uncertain homology were referred as hotspots (Borsch *et al.* 2003). The hotspots were excluded in this analysis (Table 11). 4) Repeats with substitutions were excluded from the analysis by introducing ambiguity codes. The assumption suggests that substitutions can occur either in the template or in the inserted sequence during or after the replicate process and the inclusion of ambiguities is the most conservative approach since it is not possible to distinguish between the template and the inserted sequence.

Contiguous gaps were codified as binary character using the "simple gap coding" method proposed by Simmons and Ochoterena (2000) using the following guidelines: 1) all gaps having different 5' and/or 3' starting/ending positions are scored as separate presence/absence characters; 2) a taxon with a gap that entirely overlaps inclusive smaller gaps is scored as inapplicable for the smaller gaps; and 3) taxa with the smaller gap(s) are scored as absent for the larger gap. The program SEQSTATE (Müller 2005a) was used to score all the indels.

3.2.4. Phylogenetic analysis. Uninformative characters were deactivated. Heuristic parsimony analyses were conducted using *Nona* (Goloboff, 1993) spawned by *Winclada* (Nixon 1999-2002). TBR swapping on Wagner trees were conducted from 10,000 random taxon addition sequences with 10 trees held in memory for each of the replicate initiations expanding the memory to 100 000 to do further TBR (h 100 000, mult* 10 000, ho/10).

Data sets were analyzed independently and then combined and analyzed using simultaneous analysis approach (Nixon and Carpenter 1996). Jackknife branching support was calculated by *Nona* using *Winclada* with 10 000 replications with 100 search replications and 10 tree hold in memory with the next parameters (mult*100; ho/10; max*). Jackknife percentage are described as high (85-100%), moderate (75-84%) and low (>50-74%).

3.3. RESULTS

The analysis included 36 taxa and DNA sequences of two chloroplast regions. The molecular matrix consisted of the *trnL-F* intron and spacer sequences that yielded a total of 1190 characters (82 gaps) with 115 informative characters. The *trnL* exon 5' (17bp), the *trnL* exon 3' (50 bp) with no informative characters and nine hotspots were excluded from maximum parsimony analysis and from the global alignment (Table 11). The alignment of the *rpl*16 region included a total of 1434 characters (138 gaps) with 172 informative characters. Five hotspot regions (Table 11) were removed from the alignment and the analysis.

TABLE 11. Position of hotspots, and exons in *trnL-F* and *rpl*16.

***trnL-F* region**	***rpl*16 region**
trnL 5' exon 1-17	*rpl*16 5' exon 1-8
trnL intron	deleted from 1-54
H1. 78-84 poly A	H1. 217-236 poly T and G
H2. 127-131 poly A	H2. 274-315 poly A
H3. 159-176 poly A	H3. 350-364 poly A
H4. 278-281 poly A	H4. 808-823 poly T
H5. 349-352 poly A	H5. 1136-1176 poly T and A
H6. 405-422 poly T	
H7. 515-532 poly A and T	
trnL 3' exon 778-827	
H8. 863-877 poly T	
H9. 1106-1117 poly T	

The phylogenetic analysis of the *trnL-F* data resulted in 2 most parsimonious trees (MPT) of 214 steps in length (CI = 0.64, RI = 0.85) and the *rpl*16 data yielded 6 MPT with a length of 366 steps (CI = 0.56, RI = 0.80) (Figs. 14 and 15, respectively).

The strict consensus trees for the two analyses were congruent with respect to the monophyly of the genus *Alternanthera*. However, there were five areas of inconsistency between the two data sets. The first area of inconsistency concerns the main clade *Alternanthera*. This clade is resolved in two subclades in the *rpl*16 tree, whereas a polytomy of three subclades was obtained in the *trnL-F* tree. The second area of inconsistency was *A. porrigens*, which was closely related to *A. pubiflora* and *A. mexicana* and sister to *A. macbridei* in the *rpl*16 tree (clade B; Fig. 14). However, the phylogenetic relationships of *A. pubiflora* and *A. macbridei* and their sister taxa are unresolved in the *trnL-F* tree (clade B; Fig. 15). A third area of inconsistency is the alternative position of *A. laguroides*. The *rpl*16 data resolved *A. laguroides* in a polytomy with three subclades (clade A; Fig. 14), but the *trnL-F* data suggested that *A. laguroides* forms a polytomy together with *A. serpyllifolia* and a clade that includes the sister species *A. olivacea* and *A. geniculata* (clade C; Fig. 15). The fourth area of inconsistency is the position of *Alternanthera maritima*. The *rpl*16 data suggested that *A. maritima* is sister to a clade that includes *A. nesiotes*, *A. chacoënsis*, *A. paranychioides*, *A. caracasana*, and *A. pungens* (clade A; Fig. 14), but the *trnL-F* data resolved *A. nesiotes* as sister to *A. maritima* and the other taxa (*A. caracasana*, *A. chacoënsis*, *A. paranychioides*, and *A. pungens*; clade A; Fig. 14). The fifth area of inconsistency is in a clade that includes *Alternanthera tenella*, *A. galapagensis*, *A. halimifolia*, *A. snodgrassii*, *A. flavicoma*, *A.*

filifolia, and *A. vestita* that is well resolved using *rpl*16 data (clade A; Fig. 14), but were unresolved in a polytomy in the *trnL-F* tree (clade A; Fig. 15)

The two data sets were combined taking a total evidence analysis approach and the strict consensus tree obtained is congruent with *rpl*16 tree. The combination of both regions yielded four MPT (L = 586 steps, CI = 0.59, RI = 0.81; Fig. 16) and branch support is higher than that obtained in either of the independent analyses.

The *Alternanthera* clade is strongly supported as monophyletic (99 % JK; Fig. 16). This clade contains two subclades. Subclade A is weakly supported and includes most of the species sampled in this study arranged in four subclades. The first subclade A1 (100 % JK) includes *Alternanthera flavicoma*, *A. filifolia*, *A. vestita*, *A. snodgrassii*, *A. halimifolia*, *A. galapagensis*, and *A. tenella*. A second subclade A2 (100 % JK) consists of *Alternanthera nesiotes*, *A. maritima*, *A. caracasana*, *A. pungens*, *A. chacoënsis*, and *A. paranychioides*. These two well supported subclades are sister clades and a third subclade A3 (100 % JK) is sister to the former subclades. Subclade A3 contains *Alternanthera philoxeroides*, *A. sessilis*, and *A. obovata*. The fourth subclade A4 (98 % JK) is sister to the latter three subclades and includes *Alternanthera laguroides*, *A. serpyllifolia*, *A. olivacea*, and *A. geniculata*. Subclade B is strongly supported (99 % JK) and contains 12 species. *Alternanthera altacruzensis* is sister to the remaining species of the subclades B1 and B2. Subclade B1 (100 % JK) includes *Alternanthera brasiliana*, *A. hirtula*, and *A. flavescens* and is sister to the second well supported subclade B2 (99 % JK) that contains *A. pycnantha*, *A. echinocephala*, *A. flava*, *A. macbridei*, *A. mexicana*, *A. elongata*, *A. porrigens*, and *A. pubiflora*.

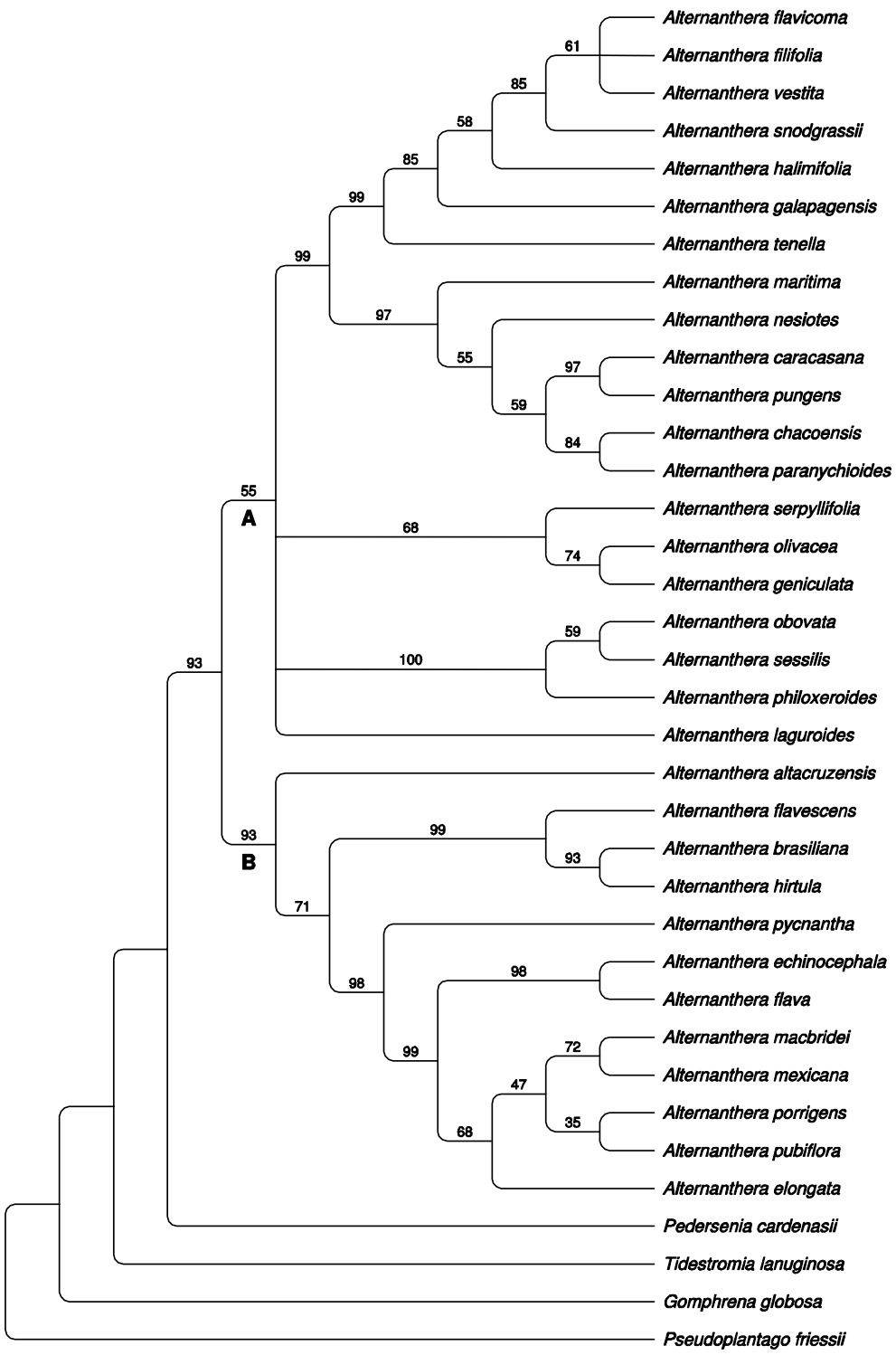

Fig. 14. The strict consensus tree from 6 MPT (L= 366 steps, CI= 0.56, RI= 0.80) obtained using *rpl*16 data. Numbers above the branches are jackknife values. Letters below branches indicate two subclades.

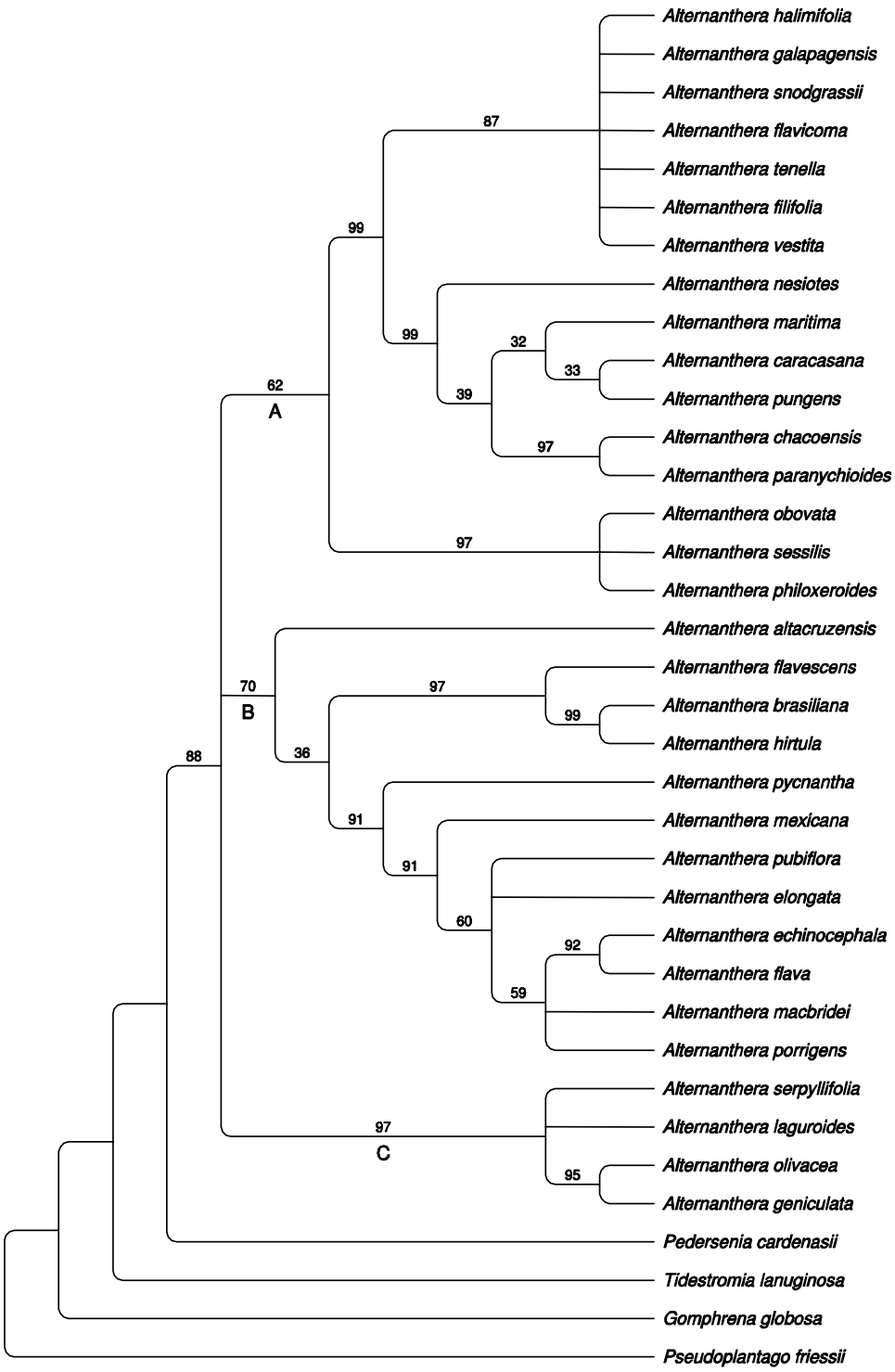

Fig. 15. The strict consensus tree from 2 MPT (L= 214 steps, CI= 0.64, RI= 0.85) obtained using *trnL-F* data. Numbers above the branches are jackknife values.

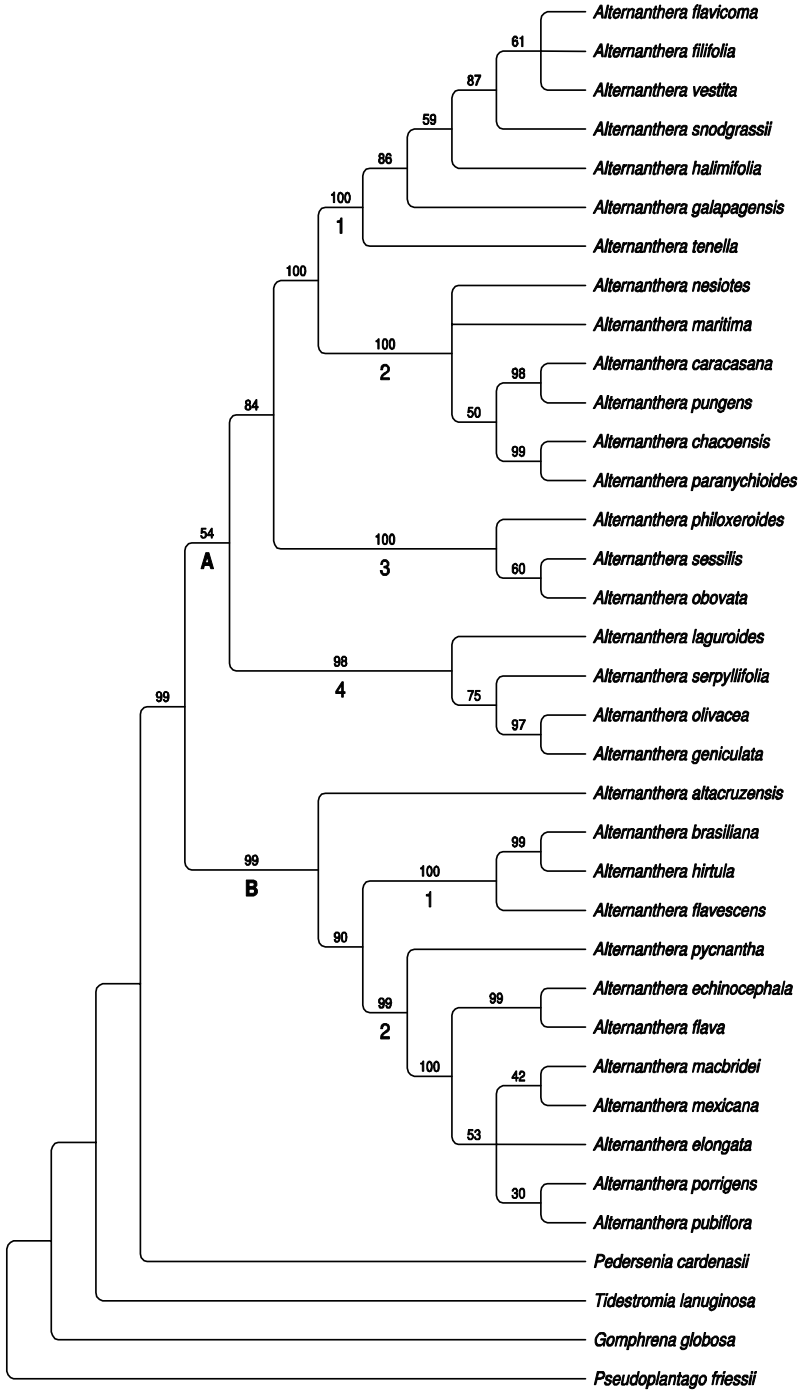

Fig. 16. The strict consensus tree from 4 MPT (L= 586 steps, CI= 0.59, RI= 0.81) from the combined *trnL-F* and *rpl16* analysis. Jackknife values are above branches. Letters below branches indicate two subclades.

3.4. DISCUSSION

3.4.1. Monophyly of *Alternanthera*.

The major taxonomic question that has surrounded the genus *Alternanthera* is its generic circumscription (Table 9). As presently circumscribed *Alternanthera* encompasses the former genus *Telanthera* (Endlicher 1837; Moquin-Tandon 1849; Bentham and Hooker 1883). *Telanthera* was distinguished from *Alternanthera* by longer styles, fusion of stamens into a tube, and ligulate pseudostaminodia (Moquin-Tandon 1849). These morphological features plus the relative tepal length and tendency of flowers to shed at maturity are all variable characters according to Eliasson (1987) who did not recognize the genus. *Lithophila*, *Pedersenia*, and *Tidestromia* which were originally treated as sections of *Alternanthera* (Moquin-Tandom 1849) and have now been elevated to the generic level and some former species of *Alternanthera* have been transferred to *Iresine* (see Townsend 1993; Table 9).

Eliasson (1987) who indicated that despite of the wide morphological variation in *Alternanthera* from small herbs to tall trees, the genus seems to be a natural taxon characterized by a combination of floral characteristics: stamen filaments alternating with triangular or ligulate pseudostaminodia, and capitate stigma. Also, he indicated that pollen was the same type as that of *Pfaffa*, which was later, confirmed and characterized as a dodecahedric grain of the *Pfaffia*-type pollen by Borsch (1998). The results in this study found that inflorescences and life form are important characteristics in the taxonomy of the genus.

3.4.2. Phylogenetic relationships within *Alternanthera*.

The taxonomic history of *Alternanthera* and *Telanthera* include several modifications in the subgeneric classification. Endlicher (1837; Table 9) suggested the first subgeneric classification of *Telanthera*. He recognized the sections *Bucholzia* Mart., *Brandesia* Mart., and *Mogiphanes* Mart. based on inflorescence form, flower pedicels (present or absent), flower pedicels size and surface (when present), and pseudostaminodia shape. Moquin-Tandom (1849) accepted Endlicher's (1837) subgeneric classification of *Telanthera* and proposed four sections in *Alternanthera* (*Trommsdorfia* Mart., *Dassiera* Moq., *Allaganthera* Mart., and *Cladothrix* Nutt.) based on sexual expression, stem habit, inflorescence type, stamen number, stigma shape, and tepal features. Bentham and Hooker's (1883) classification differed in that they recognized only two of the three sections in *Telanthera* (*Bucholzia* and *Brandesia*) and two in *Alternanthera* (*Allaganthera* already proposed by Moquin-Tandom and a new section *Lithophila*; Table 9). *Mogiphanes* and *Cladothrix* (= *Tidestromia*) were elevated to the generic rank. Later, Schinz (1893) merged *Mogiphanes* with *Alternanthera*. He did not recognize the genus *Telanthera* and excluded *Lithophila* from *Alternanthera*. Schinz (1934) recognized two subgenera within *Alternanthera*: *Eualternanthera* and *Telanthera*. The subgenus *Eualternanthera* was characterized for having stamen filaments elongated and small, with dentate or ligulate pseudostaminodia [these same characters were used to distinguish *Alternanthera* from *Telanthera* by Moquin-Tandom (1849)]. The subgenus *Eualternanthera* included the sections *Allaganthera* and *Dassiera*. Schinz (1934) did not change the diagnosis of the subgenus *Telanthera* and he maintained the three sections

proposed by Endlicher (1837). The last classification recognized *Alternanthera*, but not the sections and subgenera proposed in the past (Townsend 1993).

The combined analysis using *trnL-F* and *rpl*16 indicated that subgenera *Eualternanthera* and *Telanthera* proposed within *Alternanthera* are artificial, but the sections originally recognized by Endlicher (1837) are monophyletic lineages. Endlicher (1837) described the diagnostic characters of three sections (*Mogiphanes*, *Brandesia*, and *Bucholzia*) but it was Moquin-Tandom (1849) who published an extensive list of species of *Alternanthera* in the Endlicher sections. It is important to emphasize that species described after Moquin-Tandom's (1849) classification are included in this study and have no sectional designation in Figure 17. The results of our study supports the monophyly of section *Mogiphanes* represented by *Alternanthera brasiliana*, *A. flavescens*, and *A. hirtula* (clade B, subclade 1; Fig. 17). These species have the diagnostic characters of the section, long pedunculate inflorescences, stipitate flowers, and sulcate pedicels. Most of the species placed in section *Brandesia* form a clade (clade B, subclade 2; Fig. 17), with the exception of *A. serpyllifolia*. This species forms a clade with species not classified by earlier researchers (clade A, subclade 4; Fig. 17). After an evaluation of the characters traditionally used in the diagnosis of *Brandesia*, only two of these are diagnostic for this section: long pedunculate inflorescences and flowers with small pedicels. The species located in section *Bucholzia* are in a well supported clade (84% JK) that includes subclades A1-3. However, members of section *Allaganthera* are nested within *Bucholzia*. Section *Allaganthera* (Moquin-Tandom 1849) is represented by the species *A. paranychioides*, and *A. sessilis*. *Alternanthera pungens* and *A. tenella* have been variously treated in either section *Allaganthera* or *Bucholzia* (Fig. 17) and *A.*

philoxeroides was placed in section *Bucholzia* (Moquin-Tandom 1849), but later moved to section *Allagantera* by Schinz (1934; Fig. 18). Sectional limits between both sections have not been clear. In fact, section *Bucholzia* and *Allaganthera* share many features in common. The most important is the presence of sessile flowers within sessile, axillary inflorescences. Pseudostaminodia and stigma shape are variable and cannot be considered diagnostic characteristics to distinguish these sections. In this study, data suggests that section *Allaganthera* should be sunk into section *Bucholzia*. By making this synonymization, *Bucholzia* would be monophyletic based on *trnL-F* and *rpl*16 molecular data (clade A, subclade 1-3; Fig. 17). The last section to discuss is section *Dassiera* (Moquin-Tandom 1849; Schinz 1934). Section *Dassiera* is not recognized because subsequent to the Schinz (1934) taxonomic realignment of the genus *Alternanthera* and species located in the section *Dassiera* are now treated in the genera *Lithophila* and *Gomphrena*.

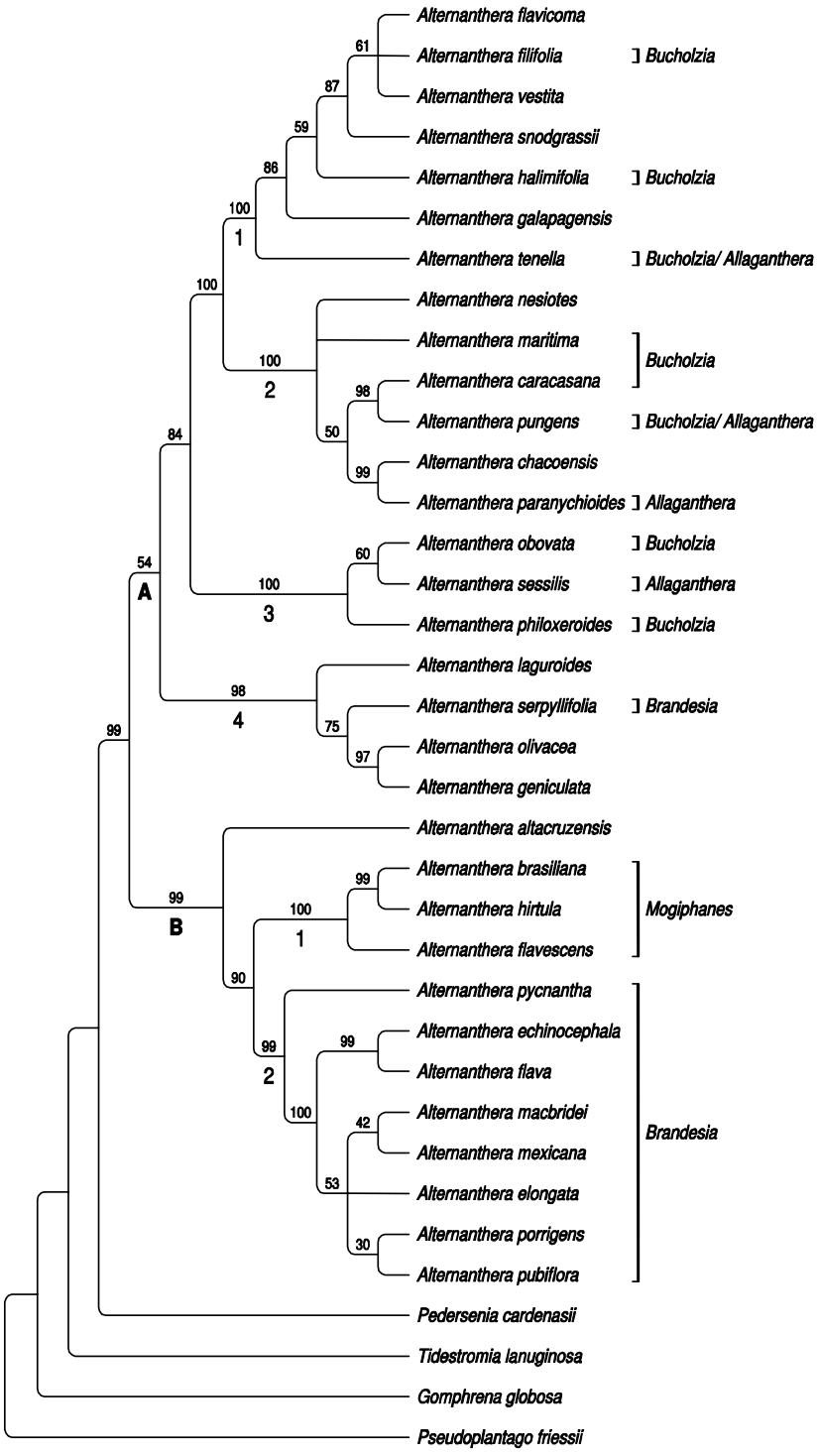

Fig. 17. The strict consensus tree from the combined analysis using *trnL-F* and *rpl16 data* indicating sections proposed for *Alternanthera* by Moquin-Tandom (1849).

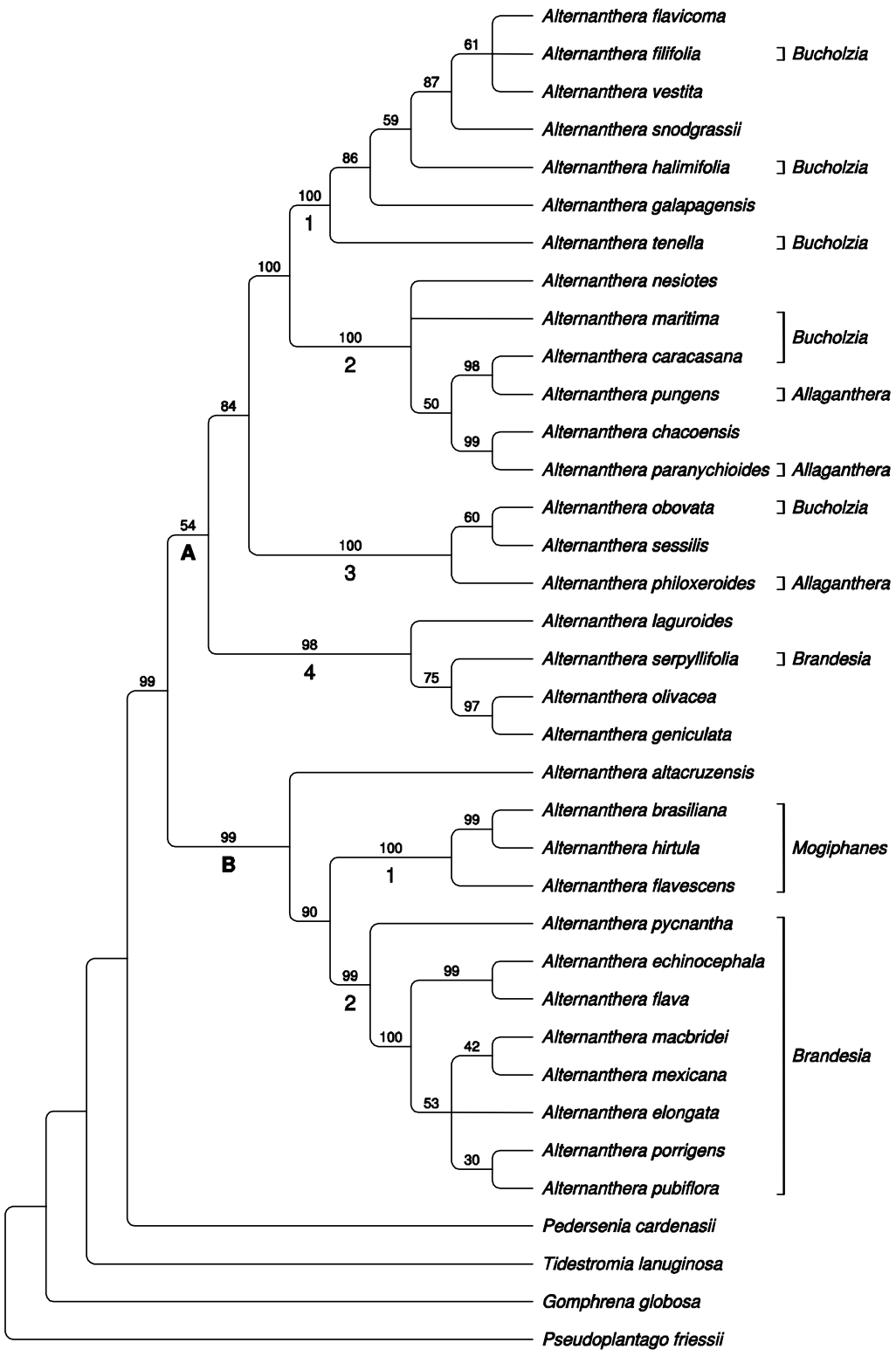

Fig. 18. The strict consensus tree from the combined analysis using *trnL-F* and *rpl16* indicating sections proposed for *Alternanthera* by Schinz (1934).

3.4.3. Major groups recognized in *Alternanthera* based on inflorescences.

It is possible to characterize two major groups based on inflorescence type in the combined (*trnL-F*, *rpl*16) strict consensus tree (Fig. 19). One group (clade A, subclades 1-3 and *A. macbrideii*) have sessile, axillary inflorescences. Inflorescences can be solitary or grouped (2-5) in spikes. The second group (clade A, subclade 4 and clade B) includes species with long pedunculate inflorescences. Inflorescences in this group can be simple or compound.

The subclades recognized and numbered in the strict consensus tree (Fig. 19) reflect morphological patterns related to habit, stem habit, and flower pedicel size and surface (if present).

The typically shrubby and sessile inflorescences clade (subclade A, group 1; Fig. 19).

This highly supported clade (100 % JK) includes species that have axillary, sessile flowers and inflorescences that are in simple heads. All these species are typically shrubs except the problematic *A. tenella*. Originally, *A. tenella* was located, depending on the treatment, in both sections *Allaganthera* and *Bucholzia* and *Alternanthera halimifolia* and *A. filifolia* were located in section *Bucholzia* by Moquin-Tandom (1849; Fig. 17). After an evaluation of morphology, taxonomic diagnoses, and the results *trnL-F* and *rpl*16 combined consensus tree, these species are correctly placed in *Bucholzia* (which includes section *Allaganthera*).

Alternanthera flavicoma, *A. filifolia*, and *A. vestita* form a trichotomy (61 % JK). These three species are shrubs and they are quite similar morphologically. Howell (1933) made reference to this close relationship between *A. filifolia* and *A. flavicoma*. He said

that the former might be considered a pubescent variant of *A. flavicoma* and noted it was not easy to distinguish between the two species. In addition, Mears's annotations on herbarium specimens show that he considered *A. flavicoma* a subspecies or a form of *A. filifolia*, but this nomenclatural change was never published. Eliasson (1971) indicated that leaf shape is a character that helps to distinguish *A. vestita* from *A. flavicoma* and *A. filifolia*. The former has elliptic-lanceolate, oblanceolate or narrowly obovate leaves and the two latter species have narrowly linear to linear lanceolate leaves. Later, Eliasson (1990) mentioned that some species closely related to *A. filifolia* such as *A. flavicoma* represent branches of the same evolutionary tree and could perhaps be accommodated as subspecies within *A. filifolia*. In this study, the results suggest that it is possible to recognize only one species, but it is not prudent since our phylogenetic results are based on chloroplast genes which are not appropriate for resolving fast evolving insular taxa. The sister species to the three unresolved is *A. snodgrassii*, which shares their shrubby habit. *Alternanthera snodgrassii*, according to Howell (1933) and Eliasson (1971) is closely related to *A. vestita*. The species differ mainly in trichome type. *Alternanthera snodgrassii* has simple trichomes and *A. vestita* has stellate trichomes. The phylogeny obtained in this study could not confirm this hypothesis because of the lack of resolution among *A. vestita* and its closely related sister taxa. *Alternanthera halimifolia* is sister to the four shrubby species. This is a trailing or a bushy perennial plant with stems either spreading and rooting at the nodes or ascending and forming bushes up to 1 m in height (Eliasson 1971). The sister species of all these shrubs and bushy species is *A. galapagensis*, a suffrutescent species that measures 3 dm in height (Eliasson 1971). *Alternanthera tenella* differs from all these species in the clade by being a perennial herb

with prostrate stems. This clade perhaps is an example of the transition to insular woodiness as suggested by Carlquist as a common evolutionary event on islands (Carlquist, 1974).

The prostrate perennial herbs and sessile inflorescences clade (subclade A, group 2; Fig. 19).

This clade is strongly supported (100 % JK) and consists of six species. The species have inflorescences are solitary heads or of 2-5 heads grouped in the leaf axils. Species in this clade have been placed in both sections *Bucholzia* and *Allaganthera*.

The clade includes *A. nesiotes* which is an herb or subshrub (Jorgensen 1999) *A. maritima* another prostrate perennial with succulent, ovoid leaves, and pseudostaminodia regularly fimbriate apically (Mears 1977), and a subclade of four additional species. Among the four species in the subclade, *A. caracasana* and *A. pungens* form sister species. These two species are perennial herbs. They share many morphological characteristics and are commonly confused. The close relationship between these species was indicated by several authors (e.g. Eliasson 1987; Standley 1917). Eliasson (1987) distinguished *A. caracasana* from *A. pungens* by its shorter tepals with almost non-pungent tips, and proportionally narrower leaves. He indicated that these features help to hold each taxon at the species level. After further evaluation of morphological characters, it was determined that the character that distinguishes the species is the apex of the tepals and bracteoles, and tepal and leaf size (Sánchez-del Pino *et al.* 1999). *Alternanthera pungens* has a longer bracteole midrib and the tepals have long pungent tips, whereas *A. caracasana* has bracteoles and tepals with acute to pointleted apices. Other sister species

in the subclade (99 % JK) include *A. paranychioide*s and *A. chacoënsis*. Pedersen (1967) described six varieties of *Alternanthera paranychioides*. *Alternanthera paranychioides* var. *chacoënsis* differs in having tepals sub-chartaceous and opaque, leaves mostly lanceolate or narrowly ovate and usually acute while *A. paranychioides* var. *paranychioides* has tepals scariosus and more or less shiny and leaves not mucronate. Mears (1977) placed *A. paranychiodes* var. *chacoënsis* and *A. chacoënsis* (basonym) as synonyms of *A. paranychioides*. Later, Pedersen (1990) reestablished the taxon at species level as *A. chacoënsis.* However, a detailed analysis of *rpl*16 and *trnL-F* data and morphology showed that *A. chacoënsis* and *A. paranychioides* are closely related and share so many characters in common that they should represent a single species.

The procumbent perennial herbs and sessile inflorescence clade (subcalde A, group 3; Fig. 19).

This clade is well supported (100 % JK) and includes perennial plants with obovate leaves and mostly sessile, globose or cylindric inflorescences. Among the three species included in this clade *A. philoxeroides* and *A. sessilis* were formerly placed in either section *Bucholzia* (Moquin-Tandom 1849) or section *Allaganthera* (Schinz 1934). *Alternanthera philoxeroides* is an aquatic or subaquatic (Mears 1977), not stoloniferous, perennial herb with ascending or decumbent stems. It is sister to a weakly supported clade (60 % JK), which includes a suffrutescent plant (*A. obovata*) and an annual or perennial plant with procumbent or erect stems (*A. sessilis*).

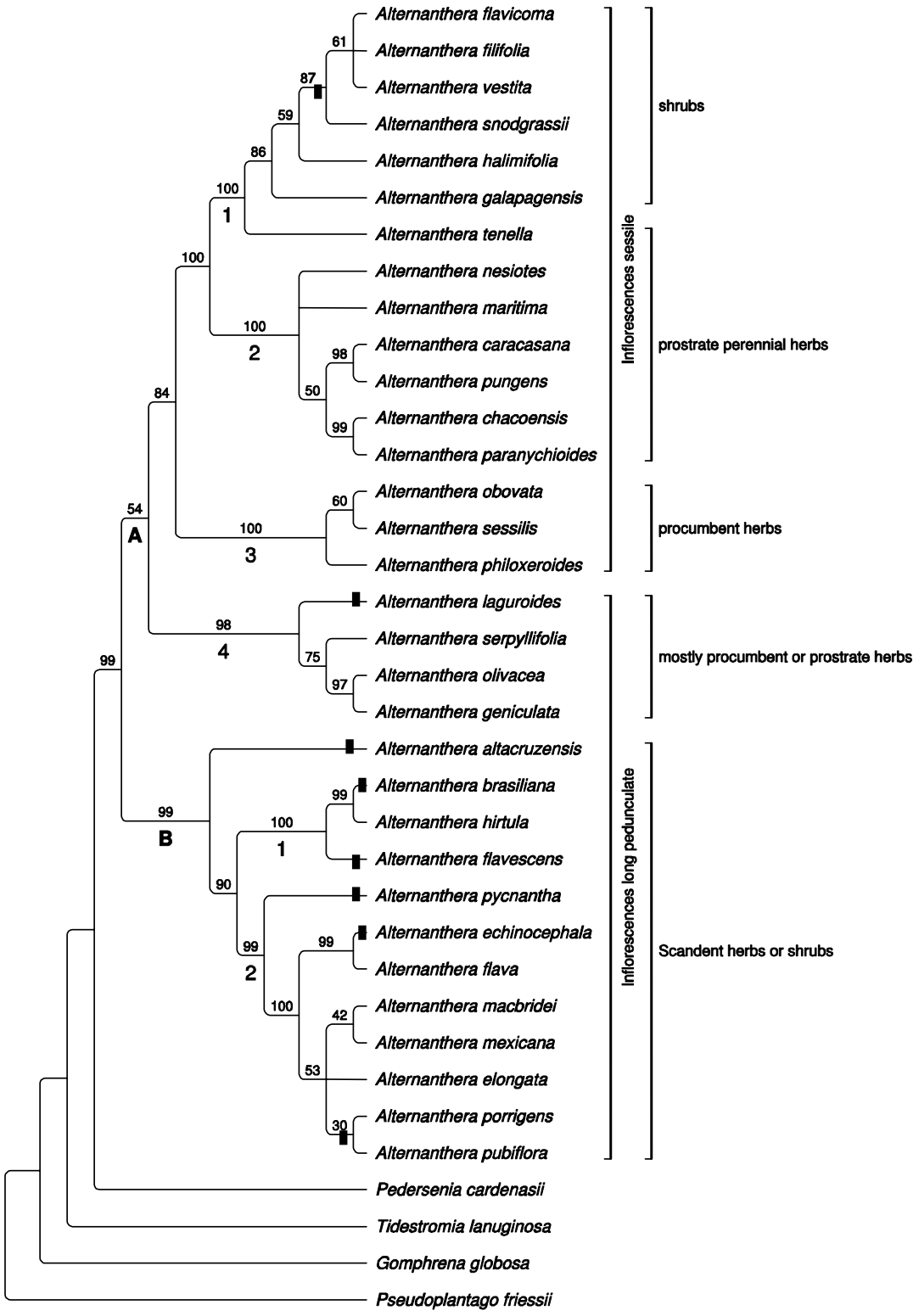

Fig. 19. The strict consensus tree from the combined *trnL-F* and *rpl16* analysis mapping morphology patterns in the genus *Alternanthera*. Solid black bars indicate shrubby character on the tree.

The procumbent or prostrate perennial herbs and long pedunculate inflorescence clade (subclade A, group 4; Fig. 19).

This clade includes four species and among them only *A. serpyllifolia* was located formerly in the section *Brandesia* (Moquin-Tandom 1849). *Alternathera serpyllifolia*, *A. olivacea*, *A. geniculata*, and *A. costaricensis* (the latter species not included in this study) were considered a different genus by Mears, but his proposal remained unpublished. Some specimens from herbarium material were identified by Mears under the name *Jamesbondia*. Molecular data of *rpl*16 and *trnL-F* resulted in a moderately supported *Jamesbondia* clade (75 % JK) sister to *A. laguroides*. The Jamesbondias are suffrutescent procumbent or prostrate perennials (Standley 1917) and share the presence of inflorescences in simple spikes with flowers arranged along a rachis. The flowers never give the appearance of originating from a common point like a globose or cylindrical inflorescence that characterizes all the other species in the genus *Alternanthera*. Among these "Jamesbondia" species *A. geniculata* has stipitate flowers, whereas *A. olivacea* has sessile flowers. Both have evident long styles, small stigmas, and ligulate, laciniate pseudostaminodia. Their closely related sister species, *A. serpyllifolia*, differs by having bracts, bracteoles, and tepals with very thick midnerves, pistils with short styles, and pseudostaminodia absent. *Alternanthera laguroides* is sister to these three species. *Alternanthera laguroides* includes erect or clambering herbs or subshrubs, with inflorescences in long pedunculate heads arranged in thyrsoides, and ligulate, laciniate pseudostaminodia (Burger 1983; Borsch 1998). The four species included in this clade share a common biogeographic area (The Caribbean Islands), which will be further discussed later.

The procumbent or climbing, simple to compound inflorescence clade (subclade B; Fig. 19).

This clade includes 12 species. Moquin-Tandom (1849) placed *A. brasiliana*, *A. flavescens* and *A. hirtula* in section *Mogiphanes*. The strict consensus based on *rpl*16 and *trnL-F* data supported the section *Mogiphanes* as monophyletic (clade B, subclade 1; Fig. 17-18) with strong support (100% JK). Therefore, the section *Mogiphanes* as it was originally considered by Endlicher (1837) with long pedunculate inflorescences and stipitate flowers with sulcate pedicels should be maintained as a natural lineage. The clade *Mogiphanes* includes perennial herbs (*A. brasiliana*) to suffrutescent herbs or shrubs (*A. flavescens*). Inflorescences range from long pedunculate, rarely sessile heads (*A. brasiliana*) to paniculate compound inflorescences (*A. flavescens*).

The remaining species are included in a well supported clade (99% JK) of species located in section *Brandesia* according to Moquin-Tandom (clade B, subclade 2; Fig. 17-18). The *Brandesia* clade includes prostrate herbs (*Alternanthera macbridei*), clambering suffruticose plants (*A. flava*) to shrubs (*A. echinocephala*). Inflorescences can be arranged in globose, sessile heads (*A. macbridei*) or long pedunculate panicles (*A. mexicana*).

Alternanthera altacruzensis is sister to the species located in sections *Mogiphanes* and *Brandesia*. It shares morphological characters with both clades and differs mainly in its inflorescence type. It has three long pedunculate heads originating from a single point, whereas all other species in the clade have simple or compound panicles.

3.4.4. Habit.

The members of the Amaranthaceae develop anomalous secondary thickening in stems (Joshi 1937; Rajput and Rao 2000; Carlquist 2003). The vascular cambium (or wood producing layer) ceases to function almost as soon as it is formed. Therefore, wood is produced by second thickening through a succession of secondary cambia (Joshi, 1937; Carlquist 1965). Although, there has been studies of anatomy wood in the Amaranthaceae (Carlquist 2003) still much more research is required. In this study, woodiness is used to explain a possible trend in habit evolution in the genus *Alternanthera*. The genus *Alternanthera* shares the perennial habit with *Pedersenia*, which is its closest sister group. The genus *Pedersenia* consists of scandent shrubs or small liana species (Borsch and Pedersen 1997) and based on the *trnL-F* and *rpl*16 phylogeny the genus *Alternanthera* (clade B) includes many scandent herbs or shrubs (Fig. 19). It appears based on the phylogeny that scandent shrubs or small lianas habits are the plesiomorphic condition in *Alternanthera*. In addition, the *trnL-F* and *rpl*16 data show that it is possible to recognize tree groups based on habit: scandent herbs or shrubs, procumbent or prostrate perennial herbs, and suffrutescent plants or shrubs. It is possible to hypothesize that the woody condition in *Alternanthera* arose several times in the genus (Fig. 19).

The suffrutescent herbs and shrub species of *Alternanthera* that are woody climbers form a monophyletic group (excluding *A. echinocephala*, *A. macbrideii*, and *A. pycnantha*; clade B; Fig. 20). Although there is no a study of secondary xylem in species of *Alternanthera* with climbing habit, the morphology suggests a similarity to the genus *Bosea*. The wood anatomy of *Bosea* L., was recently studied by Carlquist (2003). Carlquist (2003) characterized *Bosea* (3 spp.; Townsend 1993) as shrubs of dry areas

with relatively little climbing activity rather than a true vine with only one species being a vine and the rest are shrubs. The climbing habit is characterized by having notably elongate, libriform fibers, which are correlated to the presence of elongate branches. These fibers are hypothesized to be for self-support of branches until they reach branches of other shrubs or objects that can be used for support. The presence of wide multiseriate rays and wide bands of conjunctive tissue, composed of thin walled parenchyma suggest flexibility in *Bosea* stems, a feature often found in lianas. It is hypothesized that climbing species of *Alternanthera* probably share these anatomical characters and clade B in *Alternanthera* would be a perfect lineage to study this adaptive shift to the climbing habit.

A second question about habit adaptation exists in a lineage that includes prostrate or procumbent perennial herbs (clade A; subclade 2-4; Fig. 20). The question is weather they have a woody or herbaceous ancestry. Raylessness xylem and reduced cambial activity has been documented in several species of *Alternanthera* (*A. pungens*, *A. sessilis*, and *A. triandra*; Rajput and Rao 2000). The lack of rays has often been associated with phylesis from herbaceousness to increased woodiness (Carlquist 1995). However, Carlquist (2003) considered that none of those species studied by Rajput and Rao (2000) are rayless. A conclusive study of these species to determine the absence of rays (composed of erect cells and relatively long vessel elements) will support an interpretation for an herbaceous ancestry (Carlquist 1974).

A second group to study this phenomenon would be in the Galápagos Islands. Howell's (1933) observations on the Galápagos species of *Alternanthera* indicated habit variation in the genus that was dependent on edaphic and climatic factors. Carlquist

(1974) also mentioned moderate climate favored increased woodiness. The bushy *A. halimifolia* grows in the rain forest on the windward side of the higher islands. It becomes 1-1.5 m tall and is abundant in that zone. This same species assumes a trailing habit, growing along the ground or clambering among low shrubs and herbs above the rain forest in the more arid grasslands of the island summits. Eliasson (1982) indicated that *Alternanthera nesiotes* is an inconspicuous plant with leaves often of the same color as the lava. It grows on lava gravel at Black Beach on Floreana Island.

It is possible to hypothesize an herbaceous origin that evolved into woody herbs or shrubs in the Galápagos. The *trnL-F* and *rpl*16 phylogeny support this assertion. Woodiness in the family Amaranthaceae has evolved in the Galápagos Islands through a unique type of secondary growth (Eliasson 2004) seen in other islands species. Woods with anomalous secondary xylem [e.g. *Charpentiera* Gaudich., *Bosea*, *Nototrichium* (A. Gray) Hillebr.] have increased parenchymatization related to successive cambium formation. Genera characterized for having this type of secondary growth are good examples of insular woodiness because the anomalous secondary thickening seen in annual representatives of Amaranthaceae and others families (Cactaceae, Chenopodiaceae, Nictaginaceae) can be continued indefinitely in situations where climatic moderations permits (Carlquist 1974). Conversion of herbs to shrubs and trees is not unexpected on islands as it occurs in the Hawaiian members of the Amaranthaceae (*Charpentiera* and *Nototrichium*;Carlquist 1965). The distinction between woody and non-woody conditions of course is not absolute, and certain island plants may exhibit a series of species with all transition stages. However, secondary growth seems to be a

derived condition in *Alternanthera* and the Galápagos endemics are classic examples of insular woodiness (Eliasson 2004).

3.4.5. Biogeography.

The subclades in *Alternathera* reflect biogeographic patterns in the Galápagos Islands, Caribbean Islands, Central America, and South America

The Galápagos Islands.

The Galápagos Islands are an extremely dry island environment. The flora includes a total of 550 taxa (species and varieties) of which 250 are endemic. The Galápagos flora is considered to have a low number of endemics (compared to other islands, e.g. Hawaiian, Canary, and Society Islands) because of its recent age (3-4 million years old) and the reduced isolation from continental ancestors. The closest relatives of Galápagos flora appear to have come from South America, with fewer from Mexico and Central America, and even fewer from the Caribbean Islands (Carlquist 1965). Porter (1984) confirmed this and pinpointed many of the closest relatives of the original introductions to South America and in particular the Andean region. He indicated for flowering plants that birds are the main source of dispersal followed by humans, oceanic drift, and wind.

The estimated age for the Amaranthaceae ranges from 83 million year before present (MYBP; Magallón *et al*. 1999) to 104-111 MYBP (Wikström *et al*. 2001). The family is older than the Galápagos Islands which are 3-4 million years old (McMullen 1987). The Amaranthaceae is the sixth largest family of vascular plants in the Galápagos

Islands represented by thirty-three species, varieties, and forms or 29 species in seven genera (Stewart 1911; Eliasson 1990). This suggests that rate of speciation in the group was fast or there were multiple introductions to the Archipelago.

Amaranthaceae includes many weedy species well adapted for colonization in habitats on the Galápagos Islands. Carlquist (1965) mentioned that organisms best adapted for long-distance dispersal are weedy plants. Many colonizers in the Galápagos flora appear to be weedy species which could succeed in the islands by inhabiting disturbed environments. Several weedy species of *Alternanthera* have been reported in the Galápagos Islands (*A. caracasana*, *A. mexicana*, and *A. sessilis*; Eliasson 1990) and nine are considered endemic to the Galápagos (Jorgensen 1999; Eliasson 2004).

Adaptive radiation likely occured in species of *Alternanthera* in the Galápagos Islands following colonization. *Alternanthera flavicoma*, *A. filifolia*, and *A. vestita* are shrubs 0.5-2 m high with linear or linear-lanceolate leaves (Eliasson 1971) that are adapted to develop xerophitic environments. *Alternanthera snodgrassii*, a shrub up to 6 dm in height and *A. galapagensis* a suffrutescent plant 3 dm in height (Eliasson 1971; 1990) occur in arid regions. However, the smallest endemic to the Galápagos, *Alternanthera nesiotes*, has the most extreme adaptation to the habitat. It is an inconspicuous prostrate species, with ovate to suborbicular leaves 4-9 mm wide; the leaves have the same color as the lava gravel where it grows (Eliasson 1982). *Alternanthera halimifolia* is a very variable species. In the rain forest on the windward side of the higher islands this species is abundant and becomes bushy and 1-1.5 m tall and bears large ovate or ovate-lanceolate leaves whereas in the more arid grasslands of the islands, this species assumes a trailing habit, growing along the ground or clambering

among low shrubs and bushy herbs. The greatest reduction in leaf-size occurs in the lowland deserts. This variation is dependent on edaphic and climatic factors (Howell 1933).

In this study, *trnL-F* and *rpl*16 data resolved a highly supported clade (100 % JK; clade A, subclade 1; Fig. 20) that includes six species that are either endemic or indigenous (native, but also occur in other localities) to the Galápagos Islands, the Galápagos endemic *A. nesiotes* in clade 2, and *A. enchinocephala*, a indigenous species that also occurs in South America, in Clade B2.

The most parsimonious hypothesis for the origin of the Galápagos species of *Alternanthera* based on our data suggests three introductions to the Galápagos Islands and two back migrations to the mainland. Alternatively, at least 5 migration events, including several separate migrations for sister species and subsequent radiations, would be required to explain the present *Alternanthera* distribution. Carlquist (1974) indicated that the Galápagos species of *Alternanthera* most likely represented two or possibly more introductions. Eliasson (2004) proposed that based on morphological features the endemic species can be traced back to two or possibly three successful colonizers.

In this study, the combined phylogeny using *trnL-F* and *rpl*16 data suggests that it is possible to hypothesize that the A1 clade is considered a single introduction from an *A. tenella*-like ancestor (clade A, subclade 1; Fig. 20). *Alternanthera tenella* occurs from southern Mexico through Central America and the Caribbean Islands to Bolivia and southern Brazil (Burger 1983). This lineage gave rise to *A. flavicoma*, *A. filifolia*, *A. galapagensis*, and *A. snodgrassi* which are endemics to the Galápagos, and to the more widespread species in the clade, *A. halimifolia*, and *A. vestita*. *Alternanthera halimifolia*

occurs in Chile, the Galápagos (Eliasson 1971) the Guianas, Peru (DeFilipps and Maina 2003), Guadeloupe, and Martinique (Fournet 2002) and *Alternanthera vestita* occurs in the Galápagos and Chile (Eliasson 1971). Therefore, if *A. halimifolia* and *A. vestita* are derived from these Galápagos endemics it suggests that there have been two back migrations to the mainland (Fig. 20). The alternative is that *A. halimifolia* and *A. vestita* each represent separate introductions with A. *halimifolia* giving rise to *A. snodgrassi* and *A. vestita* radiating into *A. flavicoma* and *A. filifolia*. This would require three radiation events in the Galápagos rather than a single radiation in the clade (clade A, subclade 1; Fig. 20).

The second introduction is an *A. nesiotes* ancestor, a species endemic to the Galápagos Islands (Jorgensen 1999) but not in the clade with the other endemics. Because *A. nesiotes* is endemic to the Galápagos Islands (Jorgensen 1999) in a clade that consists of species that are distributed throughout the Americas (clade A, subclade 2; Fig. 20) it must be a separate distinct introduction to the Archipelago. The third introduction relates to *A. echinocephala*, which is a common coastal plant in the Galápagos Islands and Peru (Eliasson 1971) with close affinities to *A. flava* from Mexico. *Alternanthera echinocephala* is resolved in a clade that includes species mostly from Central America and South America (clade B, subclade 2; Fig. 20). Thus, this is another distinct colonization event.

This study suggests that it might be possible that species of *Alternanthera* have affinities to Chile, Peru, and Mexico. Eliasson (1985; 1990; 2004) already mentioned that two or three Galápagos *Alternanthera* species are more morphologically similar to plants from Chile and southern Peru than to species from the geographically closer Ecuador.

The Galapagos species of *Alternanthera* must be the result of long-distance dispersal because the islands are volcanic and have never been in contact with the continent. Eliasson (2004) suggested that subfamily Gomphrenoideae is strongly established on the South American mainland from where species may have spread to the Galápagos, most likely internally or externally by birds. Those researchers that follow vicariance biogeography refused the existence of long-distance dispersal and instead they explained orderly migrations as the result of tectonic events (Nelson and Platnick 1981). A theory suggest a Carnegie Ridge system as a possible explanation of conecction between Galápagos spreading centers with lower Central America probably Ecuador. The Carnegie Ridge was brought about by a late Oligocene or early Miocene collision at the final closing-off of the Caribbean plate, a vicariance model of Caribbean biogeography (Rosen 1976). These vicarience arguments have little support because the age suggested for the appearance of a Carnegie Ridge system and the age of the Galápagos does not match. In addition, many recent studies show that long distance dispersal events to islands are the most common and parsimonious explanations (e.g. Jorgensen 1999; Ballard and Sytsma 2000; Motley *et al.* 2005). Panbeogeography offers other theories, but these are not commonly accepted (Grehan 2001).

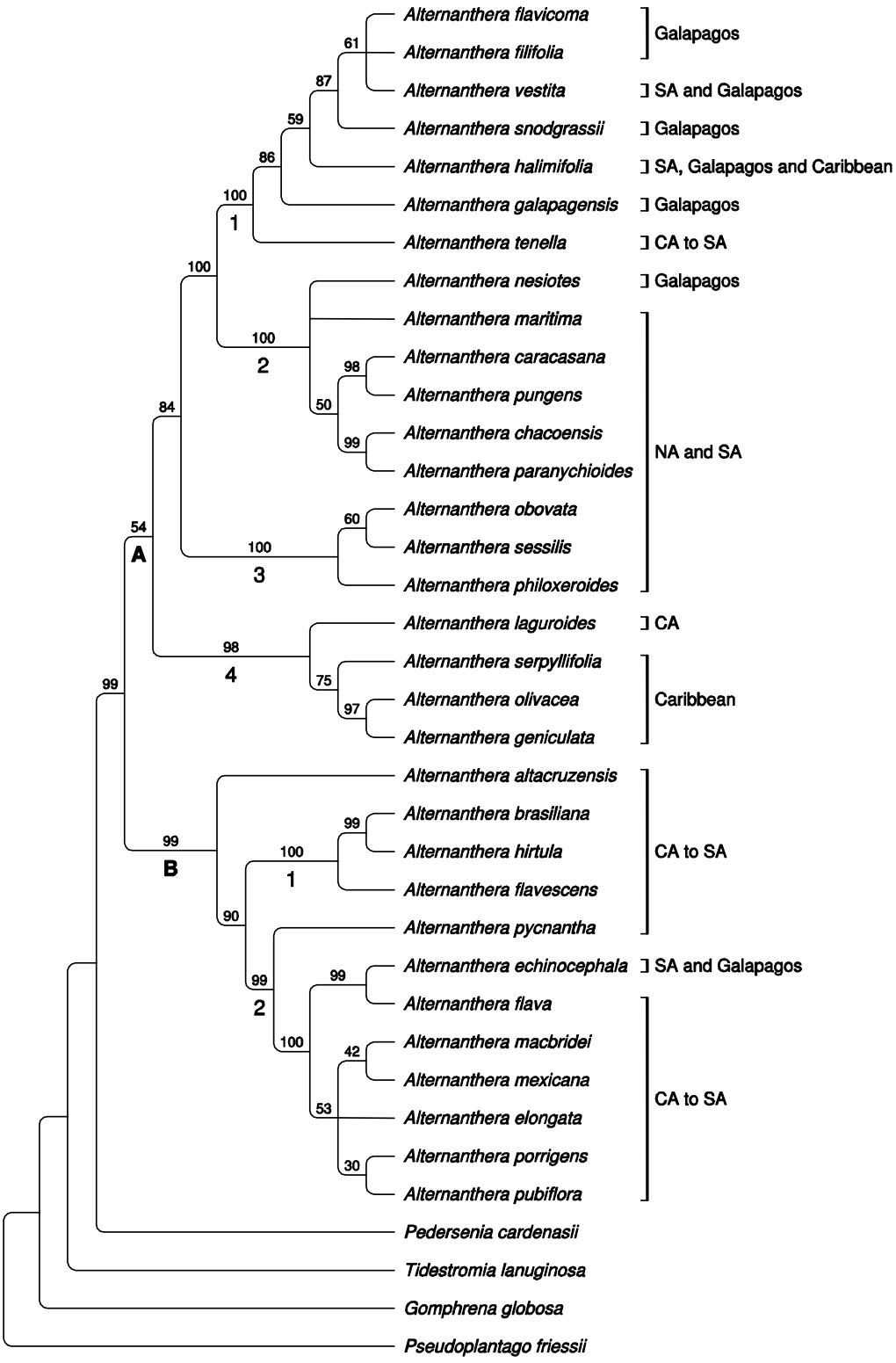

Fig. 20. The strict consensus tree from the combined *trnL-F* and *rpl16* analysis indicating geographic distributions of species of *Alternanthera*. NA = North America, SA= South America, and CA= Central America.

The Invasive Americas clade.

Several of the species in this clade [clade A, subclades 2 (excluding *A. nesiotes*) and 3; Fig. 20) are weeds and are widespread. Recently, they have been introduced to other continents areas as invasives (Eliasson 1987). *Alternanthera maritima* occurs along the Atlantic coast of Africa and South America (Pedersen 1990). *Alternanthera pungens* is a widespread species that occurs throughout the Americas and has spread to the Old World. It is a weed or invasive in the United States, southern Europe and in some regions of Africa, Asia, and Australia. *Alternanthera caracasana* is a widespread weed in the Americas and is spreading in SW Europe (Eliasson 1987). *Alternanthera paranychioides* is a species that occurs in the Americas, as well as Asia, Africa, and the Pacific (Eliasson 1987), and separate varieties (with the exception of var. *paranychioides*) were each described for a geographic range (Pedersen 1967). *Alternanthera paranychioides* var. *chacoënsis* occurs in the Chaco region of Argentina and Paraguay. *Alternanthera philoxeroides* is common in eastern South America and in the southeastern United States, Central America, and the Caribbean. It is introduced in New Zealand, Indonesia, and Thailand (Mears 1977). *Alternanthera sessilis* is the type species of *Alternanthera* and the most widespread species of the genus. It occurs from Central America to the Caribbean Islands, southern Peru as well as tropical and subtropical regions of Africa, Asia and Australia (Eliasson 1987). It is not surprising that a clade of invasive species has reached the Galápagos (e.g. *A. nesiotes*).

The species Caribbean and Central America clade (clade A, subclade 4; Fig. 20).

The islands of the West Indies extend 200 km south of North America (Florida) east of Central America and South America to Venezuela (Fritsch and McDowell 2003). Although all these areas are close to the Caribbean Islands they were not connected to the continents when Caribbean floras and faunas were being established (Carlquist 1974). Long distance dispersal is the predominant biogeographical explanation for groups (e.g. Rubiaceae) in the Caribbean Islands (Fritsch and McDowell 2003). *Alternanthera laguroides* from Central America is sister to the Caribbean species. *Alternathera serpyllifolia*, *A. olivacea*, *A. geniculata* occur in the Caribbean Islands (Standley 1917) and only *A. olivacea* has been collected outside the islands in Brazil, Venezuela, Costa Rica, Nicaragua, and Panama (Burger 1983). It seems likely to hypothesize one long-distance dispersal introduction to the Caribbean Islands with affinities to Central America followed by radiation and dispersal throughout the Caribbean. Since *Alternanthera olivacea* is nested among the Caribbean species, its existence in Central and South America is likely a later back migration to the continent.

The Central America and South America clade (clade B, subclade 5; Fig. 20). The species of this clade grow in tropical and subtropical areas of Mexico (*Alternanthera flava*, *A. mexicana*, *A. pycnantha*), Central America (*A. mexicana*, *A. pubiflora*) and South America (*A. altacrucensis*, *A. flavescens, A. hirtula, A. macbridei, A. mexicana, A. porrigens*, and *A. pubiflora*). Some species are strictly Andean (*A. flavescens*, *A. porrigens*, and probably *A. macbridei* and *A. pubiflora*) and others Amazonian (*Alternanthera elongata*). Beacuse not all the species of *Alternanthera* were sampled in this study it is difficult to characterize a biogeographic pattern for this lineage. However,

Alternanthera has many species in South America with only 20-30 species in Brazil (Siqueira 2004). It is suggested that it has a wide pantropical distribution because most of the species occur in tropical and subtropical regions of the world. The factors that favor this distribution are by air and human transportation, specially those species with medicine, ornamental, and food properties (Siqueira 1994), as well as a large number of species adapted to a diversity of environments (Siqueira 2004).

3.5. CONCLUSIONS

Many early classifications recognized segregate genera (Endlicher 1837; Moquin-Tandom 1849; Bentham and Hooker 1883) others treated these groups as subgenera (Schinz 1934), but in the most recent circumscription *Alternanthera* includes the former genus *Telanthera* and several sections once included in *Alternanthera* are elevated to genera (see Table 9). This circumscription is supported by the phylogeny. The results of the molecular phylogeny do not support the subgeneric classification of either Moquin-Tandom (1849) or Schinz (1934). However, perhaps there can be a modified subgeneric classification of Endlicher (1837) using *Bucholzia*, *Brandesia*, and *Mogiphanes* sections. Each was a distinct clade considering habit, inflorescences and flowers either stipitate or sessile.

The recognition of a segregate genus "*Jamesbondia*" within *Alternanthera* as was informally suggested by Mears, but was never formally made. The species he suggested do share some common morphological characters (inflorescences in simple spikes with flowers arranged along a rachis but never in a globose or cylindrical inflorescence), a geographic region, and were found to be monophyletic. However, they also share the generic characteristics with *Alternanthera* and are nested with the genus. So while the

clade may be recognized as a phylogenetic unit it does not warrant a new generic designation. The number of species in *Alternanthera* is still unknown and a monograph of the genus is needed. Most species diversity of the genus occur in South America, therefore it can be possible to hypothesize that the genus *Alternanthera* originated in South America. Also, it can be considered that the plesiomorphic condition for the genus was scandent herbs and climbering shrubs, which occurs in many South American representatives species. From South America the genus has radiated to the Galápagos, the Caribbean, Central America, and North America. The results presented here indicate that clades of *Alternanthera* reflect biogeography with clades representing Caribbean, New World, and Galápagos lineages. Within the islands of the New World, *Alternanthera* is a large genus in the Galápagos with at least 3 introductions with one clade radiating in the islands. A similar radiation also occurred in the Caribbean.

Alternanthera species have weedy characteristics and disperse easily that they are excellent colonizers or good candidates for establishment in open ecological niches such as islands and in dry open disturbed habitats. Some morphological character such as shrubby habit has evolved several times in several lineages.

In conclusion, *Alternanthera* as proposed by Townsend (1993) is a monophyletic group which has been an important genus for colonizing various habitats in Caribbean, New World, and Galápagos Islands. Unfortunately this means many species are also invasive species elsewhere in the world. Understanding the evolution of the lineages of these species will aid in better understanding this highly adaptive lineage.

Chapter 4

Phylogenetics of *Tidestromia* (Amaranthaceae) and evidence of hybrid speciation origin

4.1. INTRODUCTION

Tidestromia is a genus of six species, two varieties and two subspecies (Sánchez-del Pino 2001) placed in the subtribe Froelichiinae, subfamily Gomphrenoideae (Schinz 1934; Townsend 1993) of the Amaranthaceae. The genus had been considered isolated in the lineage with uncertain affinities within the subfamily (Eliasson 1988). A molecular study using *rpl*16 and *trnL-F* to study the subfamily Gomphrenoideae indicated that the subtribe Froelichiinae is artificial and *Tidestromia* is monophyletic and closely related to *Alternanthera* and *Pedersenia* (Chapter 1). *Tidestromia* is part of the Alternanthoid clade together with *Alternanthera* and *Pedersenia*.

A cladistic analysis using morphological characters yielded low resolution among taxa (Sánchez-del Pino and Flores Olvera in press), but the genus was strongly supported as monophyletic based on four synapomorphies: inflorescence in dichasium; alternate phyllotaxy, psilate pollen, and narrow mesoporia vaultation. In the *rpl*16 and *trnL-F* Gomphrenoideae study the molecular data and the subspecific classification was in conflict (Chapter 2). The authors suggested that it may be prudent to raise the infraspecific taxa of *Tidestromia* to the specific level. Thus, *Tidestromia* would include eight species. The distribution of the species of *Tidestromia* is from the southwestern United States to Mexico and the Dominican Republic. It is the only genus in the family Amaranthaceae which occurs in the North American deserts: Mojave Desert, Sonoran Desert and Chihuahuan Desert. Each of the three major hot deserts is generally

characterized by a composition of factors (climate, soil, altitude; Macmahon and Wagner, 1985) that have influenced speciation in *Tidestromia*. Most of the species are endemic to the Chihuahuan desert and have various edaphic preferences. Coahuila, Mexico was proposed as the center of diversity for the genus (Sánchez-del Pino 2001; Sánchez-del Pino and Flores Olvera in press).

Since neither the morphological phylogeny nor the *trnL-F* and *rpl*16 study could fully resolve the interspecific relationships in *Tidestromia* (Sánchez-del Pino 2001; Chapter 2) the integration of new data was attempted to provide a more resolved phylogenetic hypothesis for *Tidestromia*. The addition of the nuclear ribosomal DNA internal transcribed spacer region (ITS) to the two chloroplast genes (*rpl*16 and *trnL-F*) will likely provide additional resolution and information from a biparentally inherited genome. The ITS region has been broadly used to provide resolution within genera (Soltis and Soltis 1998). Recently, it has become a necessity to increase the use of nuclear gene regions in order to complement chloroplast DNA information, to help in the resolution of strong conflict between chloroplast DNA trees and relationships obtained from other data sources, often explained by interspecific hybridization, or lineage sorting, and insufficient phylogenetic resolution in chloroplast DNA trees (Baldwin *et al.* 1995). Furthermore, adding morphological data to the molecular data sets may be useful to resolve the infraspecific questions.

The objectives of this study are a) to resolve the phylogenetic relationships within the genus *Tidestromia* using both molecular and morphological data. A combined analysis will test the infraspecific classification of the genus *Tidestromia* and; b) to investigate the role of soils in the evolution of the genus. A well-resolved phylogeny will

clarify patterns of speciation and adaptive evolution in respect to edaphic factors of the desert environments.

4.2. MATERIALS AND METHODS

4.2.1. Taxon sampling. Four species were chosen as the outgroup taxa based on previous studies and the root was set to *Alternanthera* (Chapter 2). The outgroup taxa are *Alternanthera laguroides*, *A. tenella*, *Pedersenia cardenasiii*, and *P. argentata* members from the sister genera in the Alternanthoids clade (Table 12). The ingroup contains the eight taxa (Table 12). Six species, two varieties and two subspecies described in *Tidestromia* (Sánchez-del Pino, 2001).

TABLE 12. Taxon sampling and voucher information.

TAXON	COLLECTION/HERBARIUM
Alternanthera laguroides (Standl.) Standl.	Taylor 17394/ NY
Alternanthera tenella Colla	Nee 42581/ NY
Alternanthera vestita (Anderss.) Howell	Eliasson & Eliasson 1912/ GB
Pedersenia argentata (Mart.) J. Holub.	Nee 38784/NY
Pedersenia cardenasii (Standl.) J. Holub.	Borsch & Ortuño 3504/ Bonn
Tidestromia carnosa (Steyerm.) I. M. Johnst.	Flores Olvera 02-22/MEXU
Tidestromia lanuginosa subsp. e*liassoniana* Sánch. Pino & Flores Olv.	Pinkava 9988/NY; Harrytate 1035/NY
Tidestromia lanuginosa (Nutt.) Standl. subs. l*anuginosa*	Flores *et al*. HF 02-19/ MEXU
Tidestromia rhizomatosa I. M. Johnst.	Flores *et al*. HF 02-14/ MEXU
Tidestromia suffruticosa var. *oblongifolia* (S. Watson) Sánch. Pino & Flores Olv.	Atwood 27875/ NY; Neese & Neese 10970/NY
Tidestromia suffruticosa (Torr.) Standl. var. *suffruticosa*	Flores Olvera 02-34/MEXU; Flores Olvera 02-16/MEXU

TABLE 12. Taxon sampling and voucher information.

TAXON	COLLECTION/HERBARIUM
Tidestromia tenella I. M. Johnst.	Flores Olvera 02-25/MEXU
Tidestromia valdesiana Sánch. Pino & Flores Olv.	Flores Olvera 02-33/MEXU

4.2.2. Morphological data. The data matrix consists of 12 characters of 19 used in a cladistic analysis by Sánchez-del Pino and Flores Olvera (in press). The remaining seven characters were excluded because they were uninformative in this study. While scoring outgroup taxa, one additional character was included. Therefore, a total of 13 morphological characters were used in this analysis. All characters were treated as unordered (non-additive) and equally weighted. Justification for selection and coding of morphological characters, along with the complete data matrix is presented in Appendix 2.

4.2.3. DNA extraction, amplification and sequencing. Leaf samples were obtained from herbarium material or fresh tissue dried in silica gel. DNA extraction followed the Qiagen Plant DNeasy (Qiagen Inc., Valencia, California, USA) manufacturer's protocol and Fast Prep^{TM} method (Qbiogen Inc., Carlsband, California, USA).The Lysis procedure was modified for herbarium material, it included, 30 µl of β–mercaptoethanol and 30 µl of highly purified proteinase K solution (Roche, Indianapolis, Indiana, USA) added to the recommended 400 ml of AP1 lysis buffer with constant mixing and incubation at 42^{o}C for 12-24 hours.

PCR reactions were prepared in 25 µl reactions consisting of 10 µl of autoclaved and nanopure water, 2.5 µl of 10X buffer with $MgCl_2$, 2.5 µl dNTP, 2.5 µl BSA (bovine serum albumin), (sometimes using 1.25 µl DMSO (dimethyl sulfoxide) or 5 µl of betaine), 1µl of each of two primers in a 10 µmol/L concentration, 0.2 µl Taq polymerase (Qiagen), and 0.75 or 1 µl of DNA template. Ex Taq™ DNA Polymerase (hot-start version; Takara Mirus Bio, Madison, Wisconsin, USA) was used for some samples difficult to amplify, in this case PCR reactions of 35 µl included a mixture of reagents from Takara Ex Taq (HS) product consisting of 67 µl autoclaved and nanopure water, 10 µl of 10X Ex Taq Buffer, 8 µl dNTP mixture, 10 µl BSA, (sometimes 1.8 µl DMSO), 2 µl of each two primers with a 10 µmol/L concentration, 0.5 µl. Takara Ex Taq (HS), and 0.75 0r 1 µl of genomic DNA. In some cases 4% dilutions of PCR product yielded the best amplifications. All PCR and cycle sequencing reactions were run on a Gene Amp PCR system 9600 (Applied Biosystems, Foster City, California, USA). The ITS region was amplified using forward (5'-CCTTATCATTAGAGGAAGGAG-3') and reverse (5'-TATGCTTAAAYTCAGCGGGT-3') (modified from White *et al.* 1990; Baldwin *et al.* 1995).

Amplification of *trnL-F* region used the primers reported by Taberlet *et al*. (1991) and the *rpl*16 intron was amplified using the primers designed by Asmussen (1999), as well as a primer designed based on *rp1*16-584R reversal (TTC ATT GGG TGG GAT GGC GGAA). The PCR conditions for amplifications of the ITS region were: 1 cycle 97°C for 50 s; 30 cycles of 97 °C for 50 s, 53 °C for 50 s, 72°C for 1 min 50 s; and 1 cycle 72 °C for 7 min, hold 10 °C. The *trnL-F* region include: 1 cycle 97 °C for 2 min; 30 cycles of 94 °C for 1min, 48 °C for 2 min, 72 °C for 2 min; and 1 cycle 72 °C for 16 min,

hold 4 ^{o}C whereas the PCR conditions for amplifications of the *rpl*16 intron are the next: 1 cycle 94 ^{o}C for 3 min; 30 cycles of 93 ^{o}C for 1min, 55 ^{o}C for 1 min, 72 ^{o}C for 1.5 min; and 1 cycle 72 ^{o}C for 5 min, hold 4 ^{o}C. PCR products were examined in 1 % agarose gels stained with ethidium bromide and visualized on a MultiGenius gel imager (Syngene, Synoptics, Ltd., Frederick, Maryland). Amplified products were purified with spin columns from QIAquick PCR Purification Kit (Qiagen) following manufactures protocols. Purified products were cycle sequenced with dye terminator ABI Prism Ready reaction mix (Applied Biosystems) using Big Dye v 3.1 (1/4 reaction). Sequencing products were separated on 5 % denaturing polyacrylamide gels on an ABI Prism 377XL DNA sequencher (Applied Biosystems).

4.2.4. Sequence alignment and indel coding. Sequences were edited in Sequencher version 4.1 for PC (Gene Codes, Ann Arbor, Michigan, USA). Edited sequences were automatically aligned using CLUSTALX v. 1.8 (Thompson *et al.* 1997) and defined parameters for gap cost and transitions/transversions values by default. Alignments were adjusted by eye using both BioEdit Sequence Alignment Editor v 7.0.0 (Hall 1999), and Quick Align (Müller and Müller, 2003). The alignment proposed in this study followed the criteria for homology assessment suggested by Kelchner and Clark (1997), Kelchner (2000), Simmons and Ochoterena (2000), and Borsch *et al*. (2003). The guidelines for the alignment are: 1) Gaps were inserted only if they prevent the inclusion of more than two substitutions among closely adjacent nucleotides. 2) Simple sequences repeats were recognized and their homology positions in the alignment were considered with higher priority when alternative gaps placement were possible. 3) Some short repetitive motifs

called microsatellites produced by DNA slippage occurs when DNA strands mispair during replication or recombination that the short stretches of sequence split against each other and they form loops that when DNA is repaired, the result is in the loss or gain of motifs (Page and Holmes 1998). They have also been called slipped strand mispairing (SSM) and considered the major cause of length mutations. It has been considered that A/T rich regions are susceptible to SSM, but also it has been found to be rich in G and /or C repeats. Strings of mononucleotide repeats of A and T are more frequent within non-coding regions of the chloroplast. Homology assessments scored on length mutations can be impossible or questionable (Kelchner, 2000). Regions of uncertain homology were referred as hot spots (Borsch *et al*. 2003). The hot spots were excluded in this analysis (Table 13). 4) Repeats with substitutions were excluded from the analysis by introducing ambiguity codes. The assumption suggests that substitutions can occur either in the template or in the inserted sequence during or after the replicate process and the inclusion of ambiguities is the most conservative approach since it is not possible to distinguish between the template and the inserted sequence.

Contiguous gaps were codified as binary character using the "simple gap coding" method proposed by Simmons and Ochoterena (2000) using the following guidelines: 1) all gaps having different 5' and/or 3' starting/ending positions are scored as separate presence/absence characters; 2) a taxon with a gap that entirely overlaps inclusive smaller gaps is scored as inapplicable for the smaller gaps; and 3) taxa with the smaller gap(s) are scored as absent for the larger gap. The program SEQSTATE (Müller 2005a) was used to score all the indels.

TABLE 13. Position of hot spots, and exons of the *trnL-F* and *rpl16*.

***trnL-F* region**	***rpl*16 region**
trnL 5' exon 1-11	*rpl*16 5' exon 1-8
trnL intron	
H1. 79-85 poly A	H1. 38-45 poly A
H2. 128-132 poly A	H2. 205-217 poly T
H3. 155-170 poly A	H3. 247-281 poly A
H4. 294-296 poly A	H4. 316-328 poly A
H5. 348-365 poly T	H5. 737-752 poly T
H6. 442-460 poly A and T	H6. 809-851 poly A and T
trnL 3' exon 680-729	H7. 1008-1034 poly A and T
H7. 764-776 poly T	
H8. 941-1003 poly T	

4.2.5. Phylogenetic analysis. Uninformative characters were deactivated. Heuristic parsimony analyses were conducted using *Nona* (Goloboff 1993) spawned by *Winclada* (Nixon 1999-2002). TBR swapping on Wagner trees were conducted from 10,000 random taxon addition sequences with 10 trees held in memory for each of the replicate initiations expanding the memory to 100 000 to do further TBR (h 100 000, mult* 10 000, ho/10).

Data sets were analyzed combined using simultaneous analysis approach (Nixon and Carpenter 1996) in two modalities, one using all the data sets and the second using chloroplast DNA data sets. Only nuclear DNA of ITS data set was analyzed separately. Data were run including gaps. Jackknife branching support was calculated by *Nona* using *Winclada* with 1 000 replications with 100 search replications and 10 tree hold in memory with the next parameters (mult*100; ho/10; max*). Jackknife percentage are described as high (85-100%), moderate (75-84%) and low (>50-74%).

4.3. RESULTS

4.3.1. Phylogeny reconstruction.

Thirteen morphological characters were parsimony informative and used in the phylogenetic analysis of this study (Appendix 1). The ITS data set had a length of 744 nucleotides (52 gaps), of which 180 were parsimony informative. The aligned *trnL-F* matrix was 1017 characters in length (20 gaps) with 47 informative characters. The *trnL* exon 5' (18bp), the *trnL* exon 3' (50 bp) with no informative characters and eight hotspots were excluded from maximum parsimony analysis and from the global alignment (Table 13). The aligned *rpl*16 matrix (including the intron and the exon 3') was 1147 characters in length (30 gaps), of which 70 are informative. The seven hot spots (Table 13) found in the *rpl*16 region were removed from the analysis and the alignment.

The phylogenetic analysis of the nuclear ITS data resulted in a single parsimonious tree of 302 steps in length (CI = 0.79, RI = 0.83; Fig. 21) and the combined chloroplast *rpl*16 and *trnL-F* data yielded one tree with a length of 139 steps (CI = 0.89, RI = 0.93; Fig. 22).

The trees for the two analyses were congruent for the major clades. There were three areas of incongruence between the nuclear and chloroplast data sets. The first area of incongruence concerns *Tidestromia valdesiana*. In the chloroplast tree *T. valdesiana* is sister to *T. tenella* in a highly supported monophyletic clade and in the nuclear tree *T. valdesiana* is resolved as sister to the rest of the *Tidestromia* taxa. The second area of incongruence is *T. lanuginosa* subsp. *eliassoniana* and *T. suffruticosa* var. *oblongifolia* sister to one another in a strongly supported clade based on chloroplast DNA or fully resolved based on the nuclear ITS DNA. The third area of incongruence includes the

species *T. rhizomatosa* and *T. suffruticosa* var. *suffruticosa* as sister in a monophyletic clade in the chloroplast tree or fully resolved in the ITS tree.

Simultaneous analysis of *rpl*16, *trnL-F*, *ITS* and morphological data resulted in one single most parsimonious tree of 476 steps (CI= 0.80, RI= 0.85; Fig. 23). *Tidestromia* is highly supported (100% JK) as monophyletic. *Tidestromia valdesiana* is sister to the remaining species that form a highly supported (85 % JK) monophyletic group. Within this clade, *T. tenella* is very weakly supported (43 % JK) as sister to the rest of the members of the clade. This clade consists of a *Tidestromia lanuginosa* subsp. *eliassoniana* and *T. suffruticosa* var. *oblongifolia* clade sister to a clade with two subclades. *Tidestromia lanuginosa* subsp. *lanuginosa* and *T. carnosa* are in one highly supported subclade (99 % JK) sister and *T. rhizomatosa* and *T. suffruticosa* var. *suffruticosa* in the other moderately supported subclade (69 % JK).

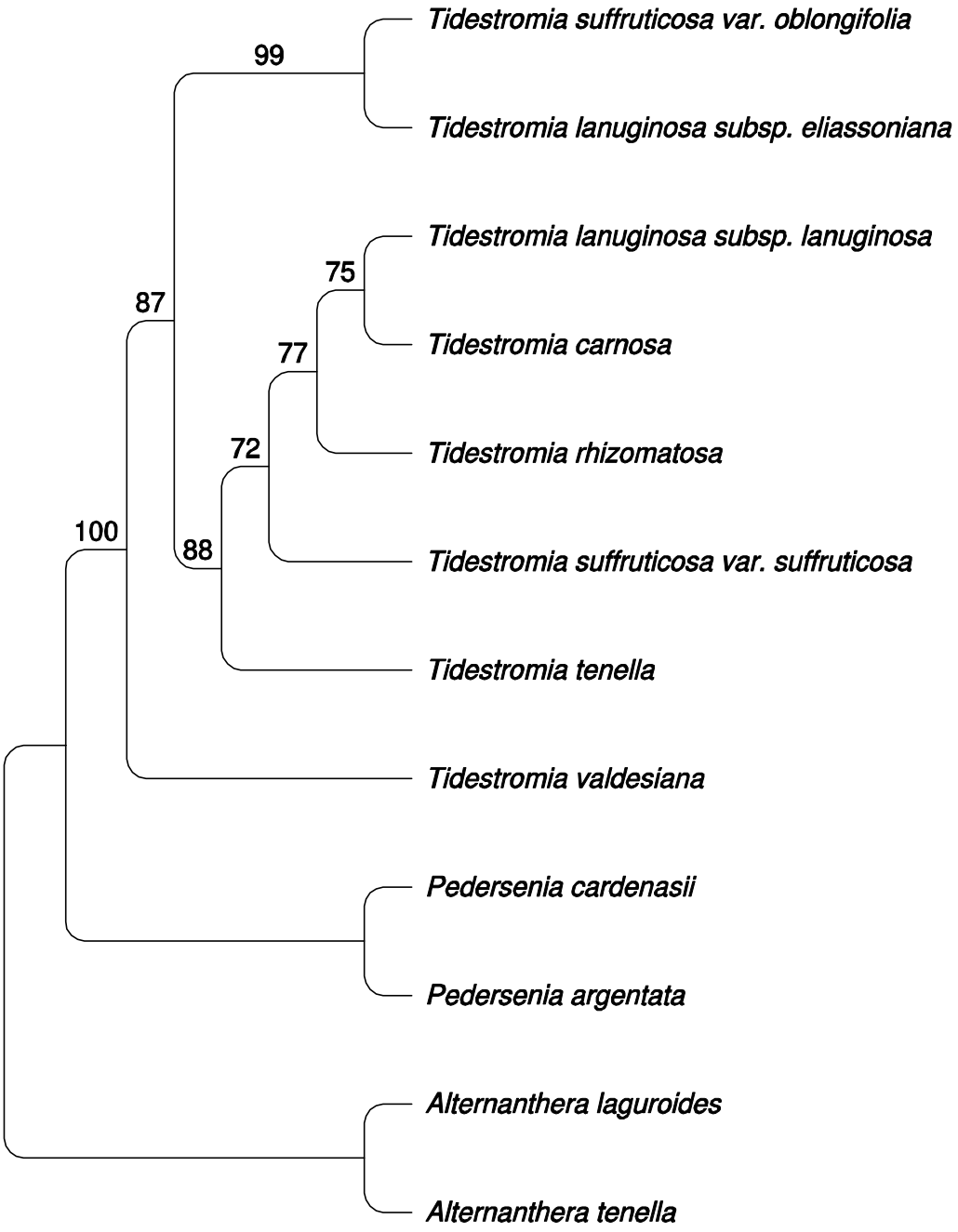

Fig. 21. The single most parsimonious tree yielded from the analysis of the data set ITS (L= 302 steps, CI= 0.79, RI= 0.83). Jackknife values are given above

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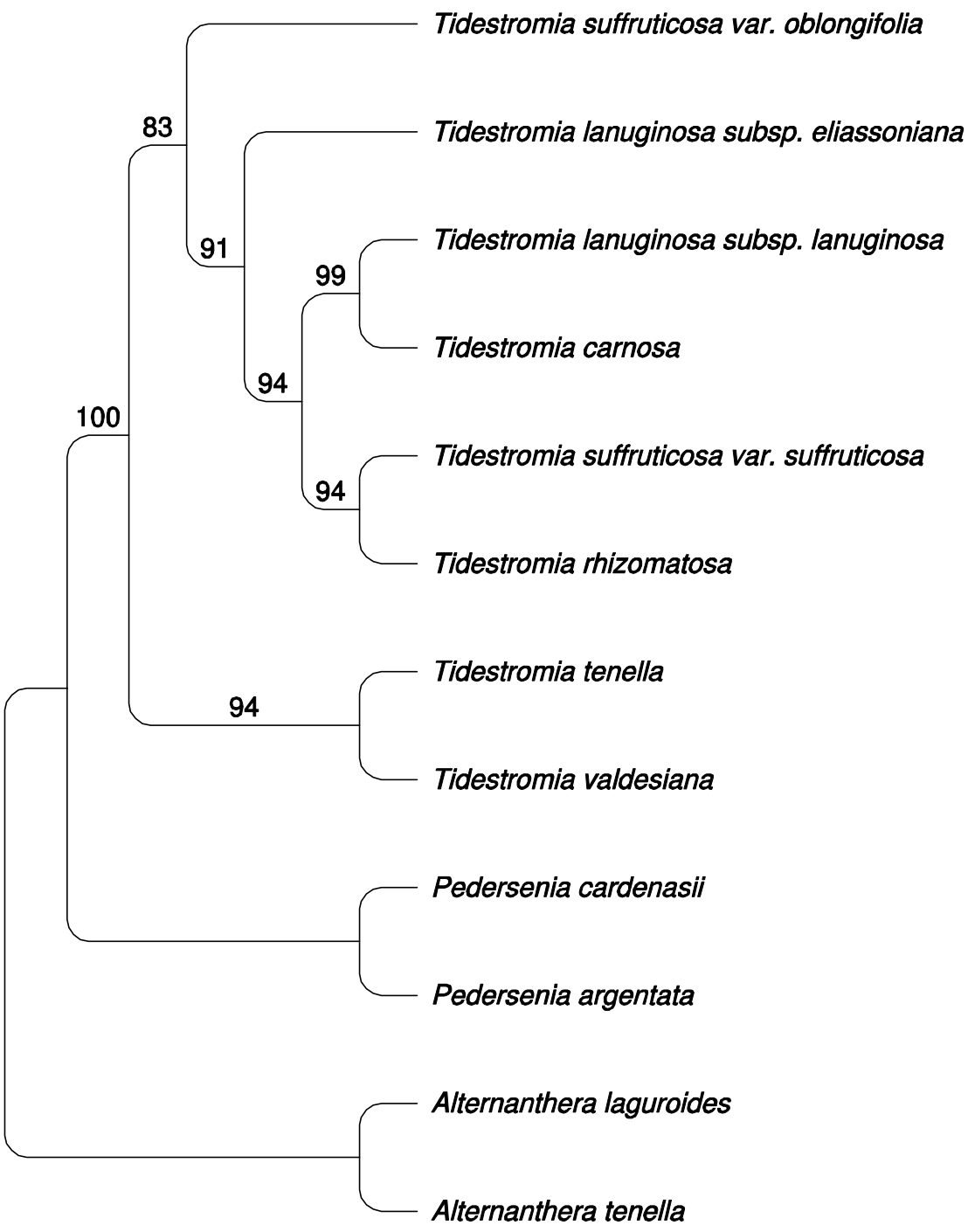

Fig. 22. The single most parsimonious tree yielded from the combined *trnL-F* and *rpl16* analysis (L= 139 steps, CI= 0.89, RI= 0.93). Jackknife values are given above branches.

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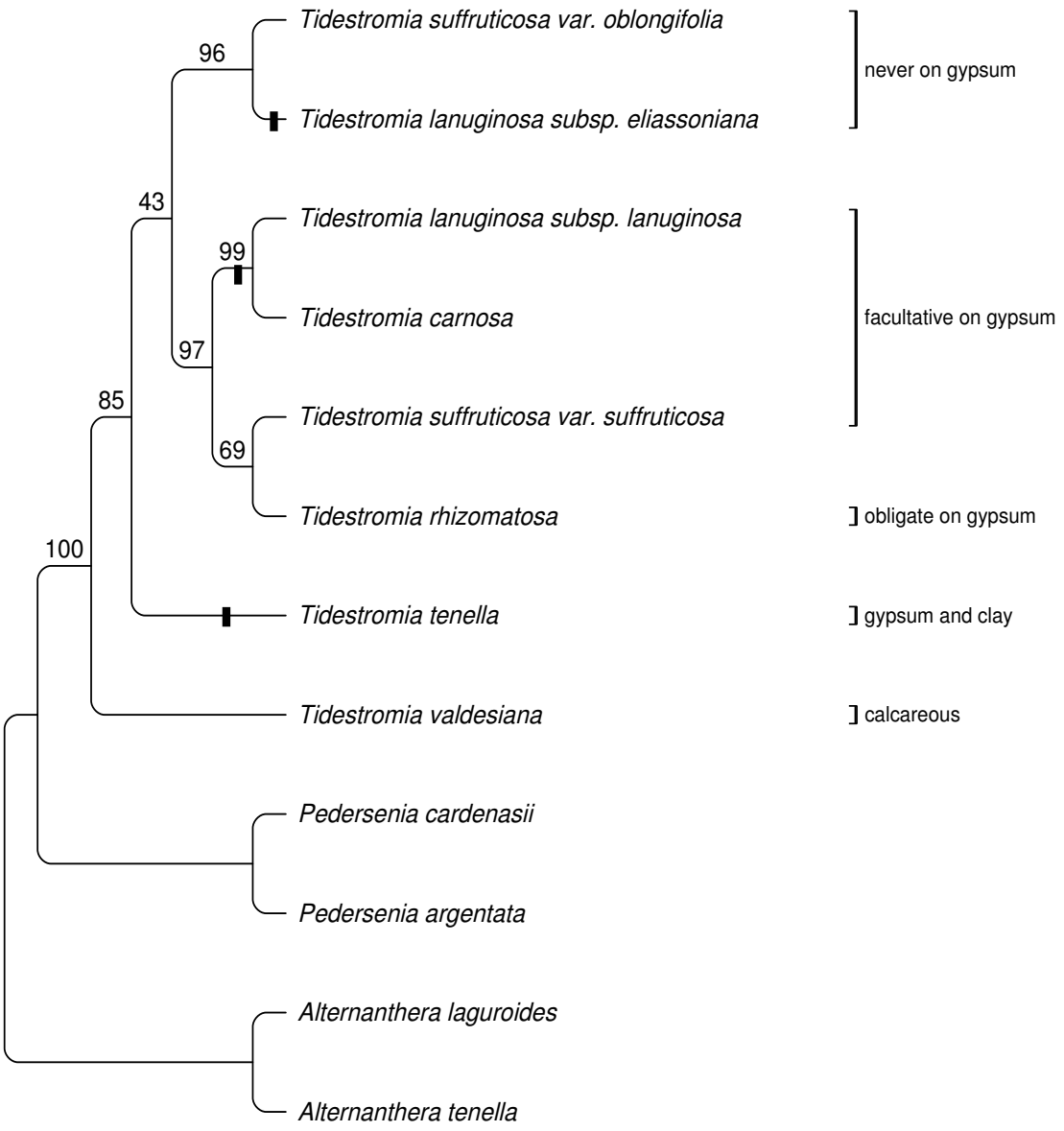

Fig. 23. The single most parsimonious tree from the combined analysis using morphology, ITS, *trnL-F,* and *rpl16.* (L= 476 steps, CI= 0.80, RI= 0.85). Jackknife values are given above branches. Solid black bars indicate annual habit character on the tree.

4.3.2. Infraspecific Tidestromia taxa.

The independent and combined analyses resolved that infraspecific taxa recognized in *Tidestromia* based on morphology (Sánchez-del Pino 2001) is incorrect. The phylogeny using chloroplast data indicated that infraspecific taxa should be elevated to species level (Fig. 22). This result was corroborated using nuclear ITS molecular data (Fig. 21). The comparison between the previous phylogeny of *Tidestromia* based on morphology and the molecular phylogenies show opposite results. The former shows that varieties and subspecies are closely related, whereas the latter suggests that infraspecific taxa are not related. The infraspecific taxa were elevated to species level (Appendix 1).

4.3.3. Hybrid origin.

The relationships of *Tidestromia tenella* changes using both chloroplast DNA and ITS nuclear DNA independently. The phylogeny obtained using chloroplast data suggested that *T. tenella* is sister to *T. valdesiana*, whereas the phylogeny based on nuclear data suggested that *T. tenella* is sister to the remaining *Tidestromia* taxa and *T. valdesiana* is the basal first branching of the genus. The variable position of *Tidestromia tenella* on these phylogenetic trees suggest that *T. tenella* has a hybrid origin considering the fact that chloroplast DNA is maternally inherited and nuclear DNA is paternally inherited.

4.4. Discussion

The genus *Tidestromia* is monophyletic and relationships within the genus were resolved including molecular data. However, results suggest that infraspecific taxa

recognized before based on morphology are incorrect. Therefore, varieties and subspecies recognized in the genus will be elevated to species level. In addition, results from independent analyses based on chloroplast DNA and nuclear DNA suggest hybridization within *Tidestromia*.

4.4.1. Phylogenetic relationships within *Tidestromia* and comparison of morphological and simultaneous analyses. Molecular and morphological data together provide a well-resolved phylogenetic hypothesis for *Tidestromia* (with perhaps the exception of the weak support for the *T. tenella* branch). The genus remains monophyletic based on morphology (Sánchez-del Pino and Flores in press; Fig. 24), a combined analysis based on chloroplast DNA from *rpl*16 and *trnL-F* (Fig. 22), an analysis based on nuclear DNA as ITS (Fig. 21), and a simultaneous analysis using morphology, *rpl*16, *trnL-F* and ITS (Fig. 23). The results from the simultaneous analysis are better resolved compared to the previous morphological analysis and supports most major clades which are supported by the same morphological synapomorphies. The monophyly of *Tidestromia* is supported by the four morphological synapomorphies already indicated by Sánchez-del Pino and Flores Olvera (in press) and also by an additional character related to stamen size (longer than pistil; character 10 [0]) and by a large number of synapomorphic nucleotide substitutions (103).

The phylogenetic relationships within *Tidestromia* were not resolved using morphology (Fig. 24), but they produced a monophyletic clade that consists of four taxa with an annual habit (*Tidestromia carnosa*, *T. lanuginosa* subsp. *eliassoniana*, *T. lanuginosa* subsp. *lanuginosa* and *T. tenella*; Fig. 24). The topology obtained using three

molecular data sets and morphology helped to discern among the annuals and perennials of *Tidestromia* however; simultaneous analysis suggested that changes from an annual and perennial habit have occurred several times in the history of the genus (Fig. 23). Therefore the ability of members of *Tidestromia* to produce secondary stem growth seems common in the genus and is perhaps a response to longer life spans in dry areas (Carlquist 2003).

Tidestromia valdesiana is a perennial species that does not develop involucres. It is sister to the rest of the *Tidestromia* species, which are supported by the presence of involucres and by nine synapomorphic nucleotide substitutions. This result is similar to that obtained from morphological data by Sánchez-del Pino and Flores Olvera (in press). *Tidestromia tenella*, its sister species, is an annual that is morphologically extremely different from the perennial species *T. valdesiana*, but these two species share a morphological character: presence of barbed trichomes on tepals. The remaining species of *Tidestromia* form a monophyletic clade supported by a character reversal, the presence of dendritic trichomes on tepals (character 3 [1]) and three nucleotide substitutions. The phylogenetic relationships within this clade were resolved based on synapomorphic nucleotide substitutions rather than by morphology. The annuals *T. carnosa* and *T. lanuginosa* subsp. *lanuginosa* were considered closely related based on morphology (Steyermark 1932) however, differences in leaf and stem texture, color, and pubescence as well as fruit involucres are useful for distinguishing the different taxa. The clade that includes *T. rhizomatosa* and *T. suffruticosa* shares the perennial habit and pubescence type; however, they can be distinguished based on stem type, leaf form and texture, and

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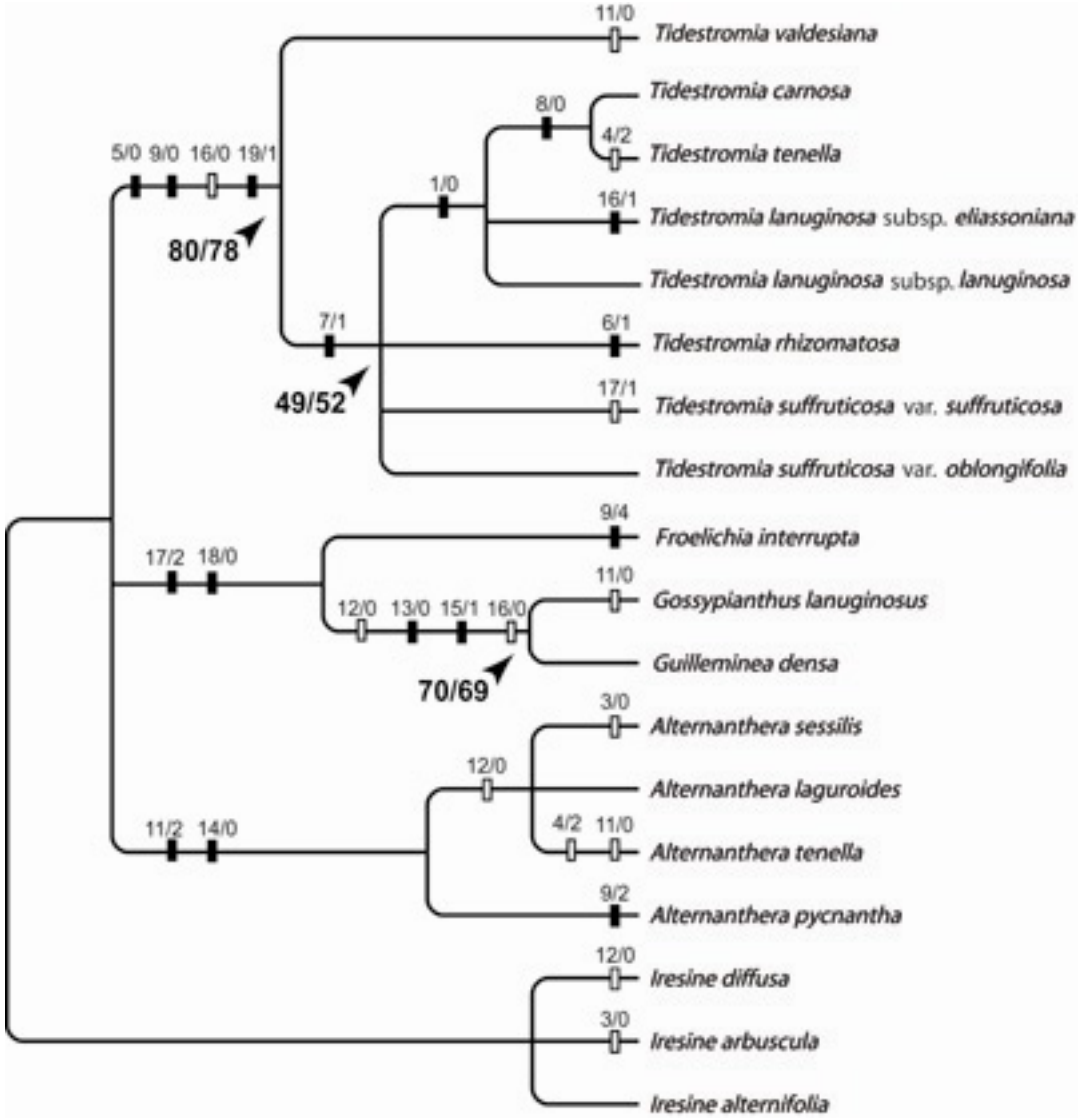

Fig. 24. The single most parsimonious tree from the combined analysis using morphology (L= 37 steps, CI= 0.70, RI= 0.84). Jackknife values are given above branches.

pseudostaminodia type and size. The only sister relationship that does not have shared morphological synapomorphies is *T. lanuginosa* subsp. *eliassoniana* and *T. suffruticosa* var. *oblongifolia*. The former is mostly glabrous annual with typically reddish thin stems, and narrow, lanceolate, small leaves. *Tidestromia suffruticosa* var. *oblongifolia* is densely pubescent perennial with thicker and grayish stems, and long, obovate leaves. The main autapomorphy is the presence of ornamented pollen in the former taxon and unornamented in the latter. However, these sister taxa do share the same geographic distribution, which includes both the Sonora and Mojave Deserts (Fig. 27).

4.4.2. Hybrid origin within *Tidestromia*.

Comparisons between the three single most parsimonious trees obtained in this study show a conflict resolution of *Tidestromia tenella* position between chloroplast and nuclear trees (Figs. 21, 22). Additional analyses were conducted excluding *T. tenella* in order to corroborate that it was causing the incongruence in tree topologies and a low branch support in the combined tree. These results indicated that excluding *T. tenella* increased the branch support throughout the topology (76 to 100 % JK). Several studies in different group of plants [e.g. *Dubautia* (Asteraceae); *Heuchera* group (Saxifragaceae)] have demostrated similar results. It was demonstrated that together with other evidence, chloroplast results are, in part, incongruent with nuclear DNA component of species relationships, often explained as probably a result of hybridization (Balwin *et al*. 1995). As well as in *Draba* (Brassicaceae, Widmer and Baltisberger 1999); *Gilia* (Polemoniaceae, Morrell and Rieseberg 1998), and *Populus* (Salicaceae, Hamzeh and Dayanandan 2004)

The conflict with *Tidestromia tenella* in the molecular data sets suggests that it is of hybrid origin. Morphologically *T. tenella* is very similar to *T. carnosa* and certain populations of *T. lanuginosa* subsp. *lanuginosa*. These three taxa share habit, succulence, and a characteristic green-yellowish color of the whole plant. However, the results suggested that *Tidestromia rhizomatosa* and *T. suffruticosa* var. *suffruticosa* are the putative parents based on the phylogeny resulted from the combined analysis, distribution, and soil preferences. *Tidestromia rhizomatosa* and *T. suffruticosa* var. *suffruticosa* are the closer sisters to *T. tenella*. These species share soil preferences; *T. tenella* occurs on gypsum and clay soils, *T. rhizomatosa* is obligate on gypsum, and *T. suffruticosa* var. *suffruticosa* is facultative on gypsum. In addition, the range distribution of these three species is shared in Coahuila, which is part of the Chihuahuan desert. *Tidestromia tenella* does not appear to be an F_1 hybrid but rather a species of recent hybrid origin.

A previous cytogenetic study (Sánchez-del Pino 2001 unpub. data) in *Tidestromia* corroborates a possible hybrid origin of *T. tenella*. Chromosome counts obtained from meiotic cells of two taxa. *Tidestromia lanuginosa* subsp. *lanuginosa* (x = 12) and *T. tenella* (x = 11) showed variation in the genus. These values differed from the count reported by Goldblatt (1988) for *T. lanuginosa* subsp. *lanuginosa* as x = 10. These values suggest that either a mistake in the determination of number of chromosomes exists or there is more that one set of chromosome number within *Tidestromia*. In addition, certain abnormalities were observed such as irregular chromosome segregation in the meiosis divisions (Fig. 25c) leading to micronuclei or microcytes (Fig. 25d) formation in the

tetrad (Sánchez-del Pino 2001). These abnormalities are often explained as a result of the loss of chromosome homology caused by regions that inhibit a chromosome match or

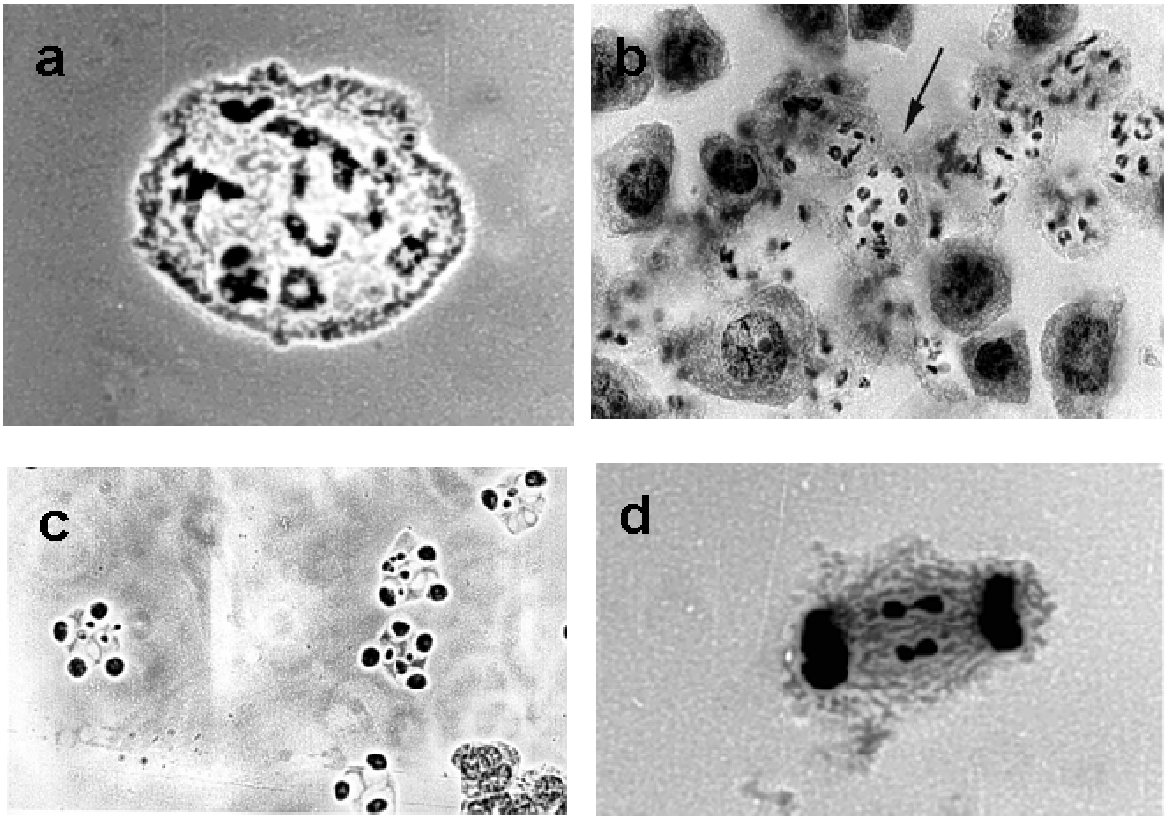

Fig. 25. Chromosomes. a. *Tidestromia lanuginosa* subsp. *lanuginosa* with x = 12II. **b.** *T. tenella* with x = 11II. **c.** Micronuclei of *T. suffruticosa* var. *suffruticosa*. **d.** Chromosome segregation in *T. tenella*.

presence of extra chromosomes due to a hybrid origin (Singh 1993). Turner (1994) mentioned that although two-thirds of the genera of the Amaranthaceae remain to be counted, that polyploidy is very common in the family. He mentioned that diploid counts with a base number of $\underline{x}$ = 8 or 9 are commonly found in the family along with $\underline{x}$ = 17. He considered these counts to be ancestral amphidiploids (8 + 9).

Other genera have base chromosome number of $\underline{x}$ = 10 and 11, but these appear to be mostly higher dysploid derivatives from the fairly widespread base number of $\underline{x}$ = 9 (Turner 1994). The array of diploid counts on a base number of $\underline{x}$ = 7, 8, 9, 10 and 11 suggest that both dysploidy (7 ← 8 → 9, 10, 11) and amphiploidy (17 = 8 + 9) have been a factor both in the evolution of the Amaranthaceae and in the order Caryophyllales (Turner 1994).

Grant (1959) obtained the chromosome counts of x = 16 and x = 17 in four species of *Amaranthus*. He mentioned that haploid numbers of both 16 and 17 are found in both monoecious and dioecious species, this suggest that perhaps the aneuploid condition arose early in *Amaranthus*.

4.4.3. Molecular evidence support new combinations in *Tidestromia* (Amaranthaceae).

The characters used to distinguish species within the genus *Tidestromia* include habit, type of pubescence, type of trichomes on tepals, fruiting involucres, leaf texture, and pseudostaminodia size (Sánchez-del Pino 2001; Sánchez-del Pino and Clemants 2003). Infraspecific categories were based on pollen ornamentation and trichomes that grow on stems and leaves, leaf shape, stems node length, and number of pollen pores

(Sánchez-del Pino 2001; Sánchez-del Pino and Flores Olvera 2002). These characters were not considered important at species level. Distribution seems to be an important factor in the circumscription of taxa of *Tidestromia*.

Morphological characters did not help to resolve phylogenetic relationships within *Tidestromia* and recent molecular evidence using *trnL-F* and *rpl*16 chloroplast genes used to find phylogenetic relationships within the subfamily Gomphrenoideae (Sánchez-del Pino *et al*. in progress) suggested that subspecies and varieties proposed in *Tidestromia* should be elevated to species level. The ITS data presented in this paper support the taxonomic changes as well. Here are some informal suggestions on how these taxa are planed to be treated in a future publication to better reflect the phylogeny of *Tidestromia*.

First change: *Tidestromia lanuginosa* subsp. *eliassoniana* to *Tidestromia eliassoniana*:

Tidestromia eliassoniana (Sánchez-del Pino and Flores Olvera) Sánchez-del Pino **comb. nov.**

Tidestromia lanuginosa subsp. *eliassoniana* Sánchez-del Pino and Flores Olvera. Novon 12: 401 (2002).

The original description of this taxon did not consider pollen grain characters for a systematic evaluation. Molecular evidence suggests this taxon should be elevated to the species level. It has unique pollen characters which separate it from *Tidestromia lanuginosa*. This species would have a distribution from the southwestern United States to northwestern Mexico.

Second change: *Tidestromia suffruticosa* var. *oblongifolia* should be changed back to *Tidestromia oblongifolia*:

Tidestromia oblongifolia (S. Watson) Standl. J. Wash. Acad. Arts 17: 376. 1916.

Cladothrix oblongifolia S. Watson Proc. Amer. Acad. Arts 17: 376. 1882.

Tidestromia oblongifolia subsp. *cryptantha* (S. Watson) Wiggins Contr. Dudley Herb. 4: 16. 1950.

Tidestromia suffruticosa var. *oblongifolia* (S. Watson) Sánchez-del Pino and Flores Olvera Novon 12: 406 (2002)

Tidestromia oblongifolia was originally described as a species by Watson (1882) and Standley (1916). However, an evaluation of the diagnosis suggested that there was no evidence to distinguish it from *Tidestromia suffruticosa* and instead it was considered a variety of *Tidestromia suffruticosa* (Sánchez-del Pino and Flores Olvera 2002).

DNA data suggested that *T. oblongifolia* should be maintained at the species level, but with a change in the diagnosis. It was not resolved sister to *Tidestromia suffruticosa* var. *suffruticosa*. The number of pores in pollen, leaf shape, and its distribution can be used to diagnose this species.

Tidestromia suffruticosa is not conspecific to *T. oblongifolia*. *Tidestromia suffruticosa* occurs from soutwestern United States to northwestern of Mexico. *T. oblongifolia* occurs in the southern Nevada in the Great Basin Desert and the transition to the Mojave and Sonoran deserts.

4.4.4. Distribution and habitat.

Tidestromia is distributed in the North American deserts. Five species occur in the Chihuahuan desert of which three of them are endemic to Coahuila, Mexico; one species occurs in the Sonora Desert; one in the Mojave and the northwestern Sonora Desert, and one is widespread in North America with a disjunct distribution in Dominican Republic. The species of *Tidestromia* mostly grow in xerophytic, shrubland vegetation with some species occurring in different vegetation types. *Tidestromia lanuginosa* subsp. *eliassoniana* occurs in thornscrub, coastal scrub, subtropical rainforest, riparian vegetation, tropical rainforest, and grasslands. *Tidestromia lanuginosa* subsp. *lanuginosa* is found in sand dunes, mangroves, halophytic grasslands, tropical rainforest, conifer forest, and riparian vegetation. *Tidestromia suffruticosa* var. *oblongifolia* grows in grasslands and subtropical rainforest. *Tidestromia suffruticosa* var. *suffruticosa* and *T. rhizomatosa* are halophytic, and *T. lanuginosa* subsp. *lanuginosa* and *T. suffruticosa* var. *suffruticosa* grow in disturbed areas such as roadsides or other perturbed areas, and cultivated fields.

Shreve (1925) indicated that in every desert it is the moisture conditions, which are critical to the physiological life history of the individual plant species. The variation in the moisture of various habitats is what influences the differences in vegetation. Moisture conditions, which are maintained by geography, shading, or texture of the soil effect local distributions. Rzewdowski (1955) indicated that endemic plant communities associated with particular geologic substrates are a relatively common feature of the vegetation of semiarid regions. *Tidestromia* is a good example of a assemblage of taxa whose distribution is influenced by geologic substrates.

Chihuahuan Desert

The Chihuahuan Desert includes southern New Mexico, and and southwestern Texas but 80% of its extent is in the Mexican states of Chihuahua, Coahuila, Nuevo Leon, Durango, Zacatecas, San Luis Potosí, Tamaulipas and extreme northern Guanajuato (Macmahon and Wagner 1985). The Chihuahuan Desert is one of the highest American deserts. Geologically, it is a high plateau between the two great mountain ranges of Mexico, the Sierra Madre Occidental and the Sierra Madre Oriental, both of which attain elevations in excess of 3048 m. The arid central plateau of northern Mexico maintains its basal plains or bolsónes at elevations of 914 to 2134 m (Wells, 1977). The Chihuahuan Desert receives its moisture largely during summer from the Gulf of Mexico to the east and southeast (Macmahon and Wagner 1985). Vegetation within the Chihuahuan Desert is affected by many physical factors of which elevation, climate, and edaphic factors are the most important (Henrickson 1974). Gypsum exposures are found throughout the Chihuahuan Desert region (Powell and Turner 1977) as well as white sands in New Mexico (Macmahon and Wagner 1985). The gypsum exposures, although small in size, always support communities of plant species, which are characteristic of gypsum soils (Powell and Turner 1977).

The genus *Tidestromia* has three endemic species in Coahuila, Mexico with different edaphic affinities. One endemic is *T. valdesiana*, it grows in calcareous rocky soils on the top of hills and mesas. It is restricted to this environment and it is sympatric with *T. suffruticosa* var. *suffruticosa*. The second endemic species is *Tidestromia tenella*, it was collected for the time in Laguna del Rey, Coahuila and is abundant on a silty desert flats (Johnston, 1939). It was considered as gypsophilous (Johnston 1943; Robertson

1998), but this species also occurs in reddish clay flats mixed with gypsum outcrops. The third endemic is *Tidestromia rhizomatosa*, which is a halophytic, gypsophilous species. The other species that occur in the Chihuahuan Desert are *T. carnosa* which grows in gypsum, limestone, and sandy soils in Texas and Chihuahua and *T. suffruticosa* var. *suffruticosa* that occurs in Arizona, New Mexico, Texas, and the Mexican states of Chihuahua, Coahuila, and Durango in a variety of soils including sandy, calcareous, limestone, gypsum, salty, clay, and granite (Fig. 23, 26-27).

The species that occur in the Chihuahuan Desert bloom from June to November. The rainig season and the specific affinity for certain type of soils would be the possible factors in the speciation of these *Tidestromia* taxa.

The Mojave and Sonoran Deserts

The Mojave and Colorado Desert are recent in origin 2-3 million years. Their origin was due to the Pliocene-Pleistocene uplift of the Sierra Nevada, Transverse, and Peninsular ranges in southern California that created a huge rain shadow to the east. Most of the moisture gathered from the Pacific Ocean by the prevailing westerly winds precipitated on the western slopes of the ranges (Thorne 1986). The Mojave Desert extends from extreme southwestern Utah, southern Nevada to California; and the botanically diverse Sonoran Desert covers Arizona, California and the Mexican states of Sonora, Baja California, and Baja California Sur (Macmahon and Wagner, 1985). Arid California has been divided into the Mojave Desert and Colorado Desert, a delineation established based on geographical division rather than a biological division. The aridity of California desert is due to the high mountains, which lie between it and the sea. The

principal rainfall on the desert is simultaneous with the heaviest precipitation on the coast in the winter and early spring. The adjacent deserts to the east receive a large percentage of their annual precipitation in the summer months (Shreve 1925). The Mojave Desert differs from the adjacent desert areas for having the principal part of its rainfall in the late winter. The late winter rain is heavy enough to assure a relatively moist soil for several weeks after the temperatures have begun to be favorable for growth (Shreve 1925).

The unique Sonoran vegetation depends on patterns in rainfall, temperature, and elevation (Cohn 1996). Biseasonal rainfall is characteristic of the northern part of the Sonoran Desert, but further south the winter precipitation becomes more and more uncertain (Shreve 1937). In the upper Sonoran desert in central Arizona, the winter storms are the primary stimulus of productivity much of it in the form of winter ephemeral growth (Patter 1978). The torrential summer rains make them less effective for replenishing soil moisture than the more prolonged winter rains. The copious fall rains, however, spread over a period of three months, give the vegetation available moisture which remains available in the soil long enough for the entire life cycle of herbaceous plants (Shreve 1937).

Tidestromia lanuginosa subsp. *eliassoniana* and *T. suffruticosa* var. *oblongiolia* grow in the southwestern United States and northwestern Mexico. They experience the influence of different climatic and edaphic conditions. *Tidestromia lanuginosa* subsp. *eliassoniana* occurs in Utah, Arizona, New Mexico, and Chihuahua, Baja California Sur, Sonora, and Sinaloa. This species never occurs on gypsum soils, it grows in sandy, clay, salty, or igneous soils (Fig. 23, 26-27). Gypsum is a common evaporite in semiarid or arid climates throughout the world (Turner and Powell, 1979) composed of hydrous

calcium sulfate (Powell and Turner, 1974) that makes it different from other type of soils. *Tidestromia suffruticosa* var. *oblongifolia* occurs in California, Nevada, Utah, Arizona, Texas, Baja California, and Sonora. It grows in sandy, rocky basalt, clay, gypsum, salty, and gravel.

These two species bloom throughout the year. *Tidestromia suffruticosa* var. *oblongifolia* occurs in the southern Nevada in the Great Basin Desert and the transition to the Mojave and Sonoran deserts. Beatley (1975) mentioned that plant communities in the transition zone between the Mojave and Great Basin deserts of southern Nevada are primarily influenced by climatic variables. Rainfall increases and temperature decreases according to altitude. Within the basins, the climates and vegetation patterns are primarily controlled by air circulation and nocturnal cold air accumulation and secondarily of edaphic factors. It might be possible that *Tidestromia suffruticosa* var. *oblongifolia* and *T. lanuginosa* subsp. *eliassoniana* have preferences for the raining season that occurs in these deserts and isolated them from those taxa of *Tidestromia* that occurs in the Chihuahuan desert with a different raining season.

North America

Tidestromia lanuginosa subsp. *lanuginosa* is the most widespread species of the genus that occurs from the southeastern United States through Mexico with a disjunct population in the Dominican Republic. This species occurs in gypsum, salty, calcareous, limestone, sandy, granite, and clay (Fig. 23, 26-27). It is sympatric with *T. suffruticosa* var. *suffruticosa* in Coahuila. This species seems more adapted to different environments probably due to its weedy condition.

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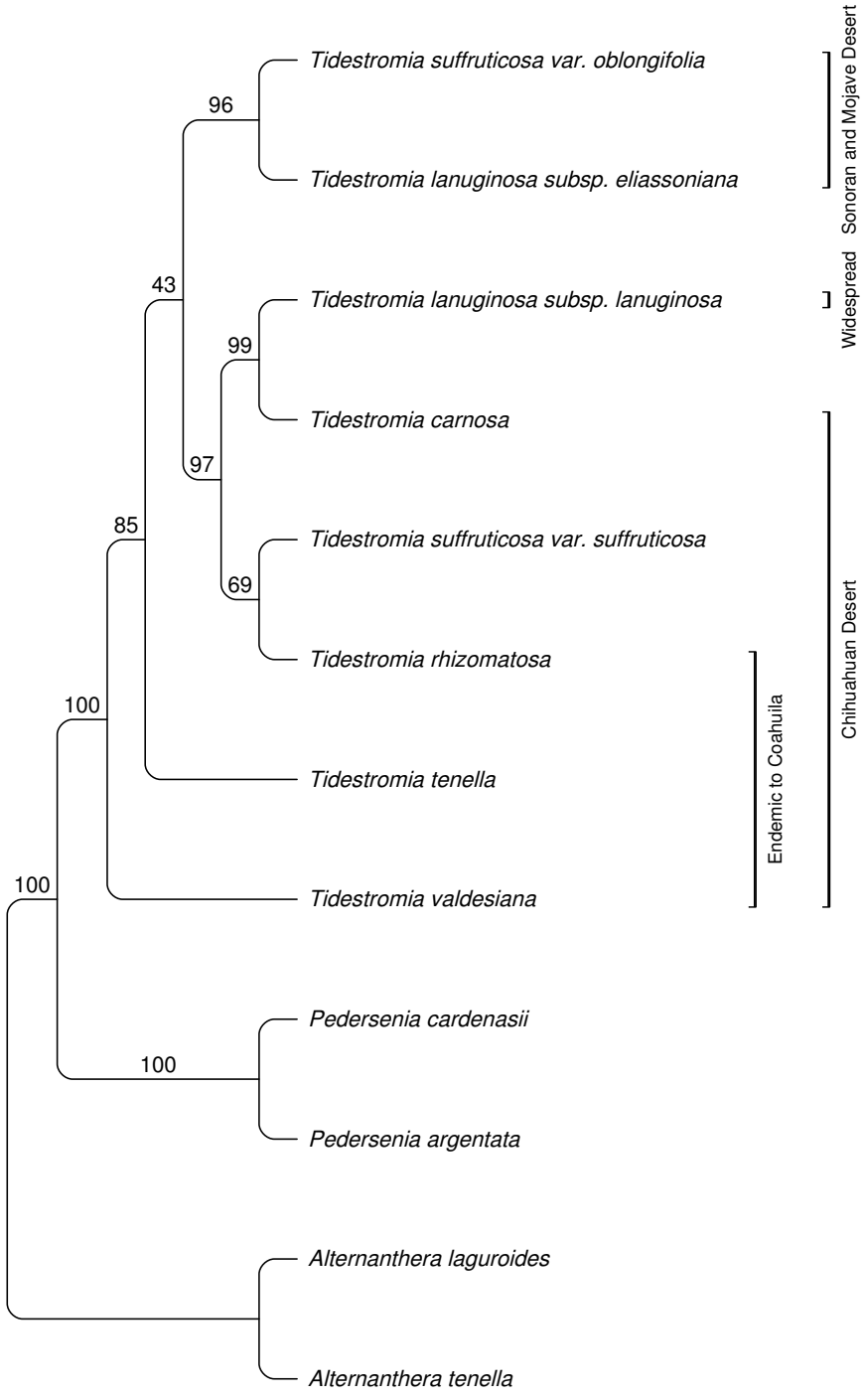

Fig. 26. The single tree yielded from the combined analysis using morphology, ITS, *trnL-F,* and *rpl16* (L= 476 steps, CI= 0.80, RI= 0.85). Brackets indicate distribution. Jackknife values above branches.

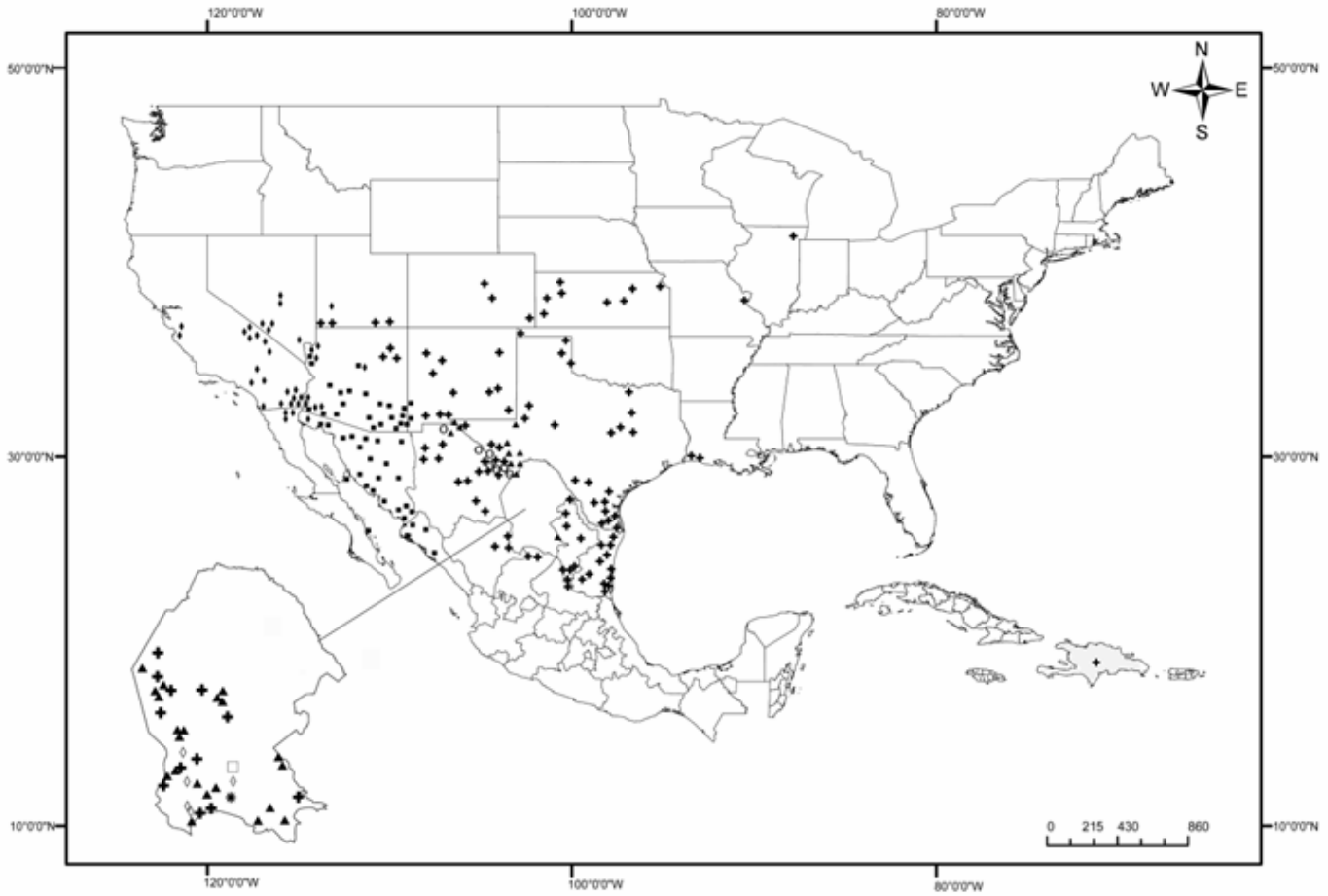

Fig. 27. Geographic distribution of *Tidestromia.* *Tidestromia carnosa* (○), *Tidestromia lanuginoa* subsp. *lanuginosa* (+), *Tidestromia lanuginosa* subsp. *eliassoniana* (■), *Tidestromia rhizomatosa* (□), *Tidestromia suffruticosa* var. *suffruticosa* (▲), *Tidestromia suffruticosa* var. *oblongifolia* (♦), *Tidestromia tenella* (◊), *Tidestromia valdesiana* (*). The inset shows the distributions of *Tidestromia* in Coahuila, Mexico.

4.5. CONCLUSIONS

The monophyly of the genus *Tidestromia* was documented in a phylogeny of the subtribe (Chapter 2) and has been again supported here in independent and combined analysis using nuclear as well as chloroplast gene regions. The use of all sources of data helped to find resolution within the genus and reveal a possible hybrid origin within the genus. This result helped corroborate early karyological studies on the genus (Sánchez-del Pino 2001). It is possible to suggest that speciation in *Tidestromia* perhaps resulted by either aneuploidy as Grant (1959) suggested in *Amaranthus* or dysploidy and amphiploidy as suggested by Turner (1994) in the family. The based chromosome numbers n= 10, 11 and 12 suggested for *Tidestromia* make it is possible to infer ascendant disploidy derived from chromosome number n= 9 following Turner (1994).

The results based on independent and combined analysis suggested that infraspecific categories recognized in *Tidestromia* based on morphology is artificial. *Tidestromia suffruticosa* var. *oblongifolia* will be elevated back at species level and *T. lanuginosa* var. *eliassoniana* will be raised to species level. Therefore, the species *T. suffruticosa* and *T. lanuginosa* have not infraspecies taxa.

Soil moisture plays an important role in the dissimilarity and morphology of the vegetation in desert areas where the maintenance of a balance between the water income and the water expenditure of the plants influence habit, pubescences, secondary growth (annuals versus perennials), and phenology (Shreve 1925). Gypsum content of the soil may also have a major influence on water relations under desert conditions and in *Tidestromia* (Meyer 1986). Species of *Tidestromia* show specific adaptations to certain type of soils. It is the presence of calcareous, gypsum, and a mix of reddish clays with

gypsum of species that occurs in Coauhila, Mexico which made this region of center of *Tidestromia* speciation with individual species adapting to unique soil types. Edaphic conditions and moisture conditions play an important role in the evolution of the species of *Tidestromia*. The basally branching sister species to the other members of the genus, *T. valdesiana*, occurs in calcareous soils. There is a lineage of species that occurs in gypsum soils either as obligate (*T. rhizomatosa*) or facultative (*T. carnosa, T. suffruticosa and T. lanuginosa subsp. lanuginosa*) gypsophiles. These gypsophiles are sister to a lineage that contains *T. suffruticosa* var. *oblongifolia* and *T. lanuginosa* subsp. *eliassoniana*, which have never been collected in gypsum environments. These two lineages are sister to *T. tenella* which has edaphic preferences for gypsum and clay. It appears that *Tidestromia* radiated from the center of diversity in Coahuila, Mexico (with six species) to SW of United States on the Sonoran and Mojave deserts. Soil type appears have been a major influence in the species radiation. Allowing geographic isolation due to habitat preferences.

Chapter 5

Floral variation and evolution in the subfamily Gomphrenoideae

5.1. INTRODUCTION

The family Amaranthaceae has its highest diversity of species in the Neotropics, subtropics, southern Africa and Australia (Townsend 1993). According to the most recent report, the family has approximately 77 genera and 840 species. The three largest genera are *Gomphrena*, *Alternanthera*, and *Ptilotus* (Müller and Borsch 2005). Two-subfamily classification (Schinz 1934) within the Amaranthaceae has been followed by most authors (e.g. Eliasson 1988; Townsend 1993; Borsch 1998). Schinz (1934) recognized the subfamilies Amaranthoideae and Gomphrenoideae based on anther characters. Other floral characters have also been important for the classification of the family. Pseudostaminodia and stigmas were used in traditional classifications. Martius (1825) was the first to use androecium and gynoecium characters in his generic classification of the Amaranthaceae *s.s*. Later, Endlicher (1837) used other floral characters and the presence or absence of pseudostaminodia, position of psuedostaminodia, and fruit dehiscence to distinguish genera within his tribal classification. Stigma form was considered an important character in Schinz's (1934) classification of the Amaranthaceae *s.s*. He characterized the subtribe Froelichiinae for having capitate stigmas and subtribe Gomphreniinae for having bilobed stigmas. Recent molecular studies based on *rbcL* (Kadereit *et al.* 2003) *matK*/*trnK* (Müller and Borsch 2005) and *trnL-F* and *rpl*16 (Chapter 2) support the monophyly of subfamily Gomphrenoideae, but studies of stigma and pseudostaminodia evolution within the Gomphrenoideae are minimal. Eliasson (1988) mentioned that stigmatic features are likely not important in the overall taxonomy

of the family, but a complete SEM study of all genera of Amaranthaceae with emphasis on stigma morphology could be informative at the generic level. The study of pistils using SEM in some taxa of *Froelichia*, *Guilleminea*, *Gossypianthus*, and *Tidestromia* revealed that stigmatic lobing and gynoecium characters (e.g., the nature of the glandular area of the style, folds in the stigma, and form and distribution of the papillae on the stigmatic surface) are useful in generic diagnosis (Sánchez-del Pino 2001). Traditional descriptions of stigma morphology can be incorrect. When pistils are observed with Scanning Electron Microscopy (SEM), *Tidestromia* described to have capitate stigmas (Eliasson 1986; Robertson 1981), but SEM observations indicate that *Tidestromia* has deltoid stigmas (Sánchez-del Pino 2001).

Pseudostaminodia in the subfamily Gomphrenoideae can be well developed conspicuous structures (Eliasson 1988). They range from entire, crenate, to lacinate. Pseudostaminodia are present in many genera, but appear absent in *Guilleminea*, *Gossypianthus*, *Irenella*, and *Woehleria* (Eliasson 1988). Eliasson (1986; 1988) hypothesized two evolutionary trends in pseudostaminodia development in the Amaranthaceae *s.s.* In the first trend, he hypothesized that *Froelichiella*, *Froelichia*, and *Pseudogomphrena* have a staminal tube derived from an *Alternanthera*-type staminal tube. This means that from a staminal tube with large filaments and large, laciniate pseudostaminodia there was a reduction of filament length and fusion of pseudostaminodia with filaments to form a staminal tube with sessile anthers and large pseudostaminodia either entire or divided and bifid. The staminal tube in *Gomphrena* could then originate from a further decrease in distance between pseudostaminodia of the *Pseudogomphrena*-type staminal tube as well as a deeper bifurcation of the

pseudostaminodia. Eliasson (1988) suggested that each apical filament lobe in *Gomphrena* could be homologous to half a pseudostaminodium in *Alternanthera* and *Froelichia*. The second evolutionary trend he hypothesized was that the development of pseudostaminodia can be derived in the opposite direction. He indicated that a structure reminiscent of a pseudostaminodium in *Pseudogomphrena* could result from the fusion of two adjacent filaments in *Gomphrena*-type staminal tube. This fusion would occur along most of their length, and the continued fusion of the filament could lead to the entire or shallowly dentate pseudostaminodium present in *Froelichia*.

Interpretation of floral morphology in the Amaranthaceae was considered difficult for use to define taxonomic groups and to trace character evolution in the family. More recently, pollen has been explored and it has been very informative to define clades reconstructed in phylogenetic studies of the Amaranthaceae (Müller and Borsch 2005). Erdtman (1960) was the first to distinguish two types of pollen in the Amaranthaceae: *Amaranthus*-type and the *Gomphrena*-type. The former type is present in genera classified in the subfamily Amaranthoideae in Schinz's classification (1934) and the latter type is representative of the genera in the subfamily Gomphernoideae. Subsequent studies of pollen in the family (e.g. Vishnu-Mittre 1963; Livingstone *et al.* 1973; Zandonella and Lecocq 1977; Nowicke and Skvarla 1979; Eliasson 1988; Borsch and Barthlott 1998; Borsch 1998) enriched the knowledge of palynological characters in the Amaranthaceae and some other authors (e.g. Vishnu-Mittre 1963; Zandonella and Lecocq 1977; Borsch 1998) distinguished additional pollen types. In a recent study, Borsch (1998) described 17 types of pollen in the Amaranthaceae. He offered a diagnosis for each pollen type. In a subsequent study Müller and Borsch (2005) defined the subfamily

Gomphrenoideae by the number of locules in the anthers and two palinologycal characters: metareticulate pollen and stellate pore ornamentation, which were optimized in a phylogenetic context in a combined analysis using both morphological and molecular data. The present study will explore trends in evolution among floral characters within the Gomphrenoideae.

The goals of this study are: (1) to examine evolution of anthers, (2) to test the evolutionary hypotheses about pseudostaminodia development proposed by Eliasson, (3) to document variation and evolution of gynoecium characters, and (4) to reexamine pollen characters defined by Borsch (1998) within the Gomphrenoideae with more comprehensive sampling among genera.

5.2. Materials and Methods

5.2.1. Taxon Sampling. The taxon sampling for DNA sequence data is the same as in Chapter 2 (Table14). Eleven species were chosen as the outgroup taxa based on previous studies and the root was set to *Charpentiera* using the recent hypothesis that *Charpentiera* is the sister genus to the rest of the Amaranthaceae *s. str.* (Kadereit *et al.* 2003; Müller and Borsch 2005). The outgroup taxa belong to the subfamily Amaranthoideae and are *Achyropsis avicularis*, *Achyranthes aspera*, *Amaranthus greggii*, *Amaranthus spinosus*, *Centrostachys aquatica*, *Charpentiera obovata*, *Ch. tomentosa*, *Kyphocarpa angustifolia*, *Leucosphera bainesii*, *Pupalia lappacea*, and *Sericorema nemotiflora* (Table 14). The ingroup contains 60 species of the subfamily Gomphrenoideae which represents 16 of 19 genera defined in Townsend's (1993) classification as emended by Borsch (1998).

TABLE 14. Taxon sampling and voucher information

TAXON	COLLECTION/HERBARIUM	DATA TYPE
Achyranthes aspera L.	I. Sánchez-del Pino 105 & A. Campos/ MEXU	DNA, flowers, pollen
Achyropsis avicularia	Venter 9671/ NY	DNA
Alternanthera altacruzensis Suesseng.	Nee & Vargas 43479/ NY	DNA
Alternanthera caracassana Kunth	Sánchez-del Pino *et al.* 20/ MEXU	DNA
Alternanthera crucis	Taylor 9531 & Lodge/NY	DNA
Alternanthera echinocephala	Eliasson 160/ GH	Flowers, pollen
Alternanthera elongata (Willd.) Schinz	Beck 11078/ NY	DNA
Alternanthera flava (L.) Mears	Nee & Taylor 29116/ NY	Flowers, pollen
	Nee & Taylor 28763/ NY	DNA
Alternanthera flavescens Kunth	E. Martínez S. s.n/ NY	DNA
Alternanthera galapagensis (Stewart) Howell	Eliasson & Eliasson 726/ GB	DNA
Alternanthera halimifolia Standl.	FLSP2171/ NY	DNA
Alternanthera laguroides (Standl.) Standl.	Taylor 17394/ NY	DNA
Alternanthera olivacea Urb.	Harward 10881/ GH	Flowers, pollen
	van Proosdij 1105/ NY	DNA
Alternanthera philoxeroides Griseb.	Thomas & Amason 142/ NY	DNA
Alternanthera pungens Kunth	Agra *et al.* 2084/ NY	DNA
Alternanthera snodgrassii	Eliasson 1810/ GH	Flowers, pollen
Alternanthera tenella Colla	Nee 42581/ NY	DNA
Alternanthera sp.	T. Borsch 3565/ BONN	Flowers
Alternanthera sp.	T. Borsch 3525/ BONN	Flowers
Alternanthera sp.	I. Sánchez-del Pino 119 & G. Flores/ MEXU	Flowers, pollen
Amaranthus greggii S. Watson	I. Sánchez-del Pino 106 & A. Campos/ MEXU	DNA, flowers

TABLE 14. Taxon sampling and voucher information

TAXON	COLLECTION/HERBARIUM	DATA TYPE
Amaranthus spinosus L.	Sánchez -del Pino 98/ MEXU	DNA
Blutaparon vermiculare (L.) Mears	T. Borsch 3444/ BONN	Flowers
	Liogier 34352/ NY	DNA
Centrostachys aquatica Wall	Venter 9733/ NY	DNA, flowers, pollen
Charpentiera obovata Gaudich.	Wood 4224 & Perlman/ NY; Wood 4082 & Perlman/ NY	Flowers
	10254/HLA	DNA
Charpentiera tomentosa Sohmer	Project 98-244, LF(GJR-1119)/HLA	DNA
Froelichia floridana (Nutt.) Moq.	Fryxell 1847/ NY	DNA
Froelichia interrupta Moq.	Correll & Johnston 17834/ NY	Flowers
	Nee 33212/ NY	DNA
Froelichia tomentosa Moq.	Casas & Molero FC4372/ NY	DNA
Gomphrena boliviana Moq.	Fortunato & Adelqui 5526/ NY	DNA, flowers, pollen
Gomphrena elegans Mart.	Davis 1734 *et al.*/ NY	Flowers, pollen
	Nee 34257/ NY	DNA
Gomphrena flaccida R. Br.	Fryxell, Craven & Stewart 4748/ NY	DNA, flowers, pollen
Gomphrena globosa L.	Sánchez -del Pino 109/ MEXU	DNA
Gomphrena graminea Moq.	Pedersen 9570/ NY	DNA, flowers, pollen
Gomphrena haenkeana Mart.	T. Borsch 3585/ BONN	Flowers, pollen
	Nee *et al.* 52178/ NY	DNA
Gomphrena macrocephala St. Hil.	A. Schinini & M. Dematteis 33318/NY	DNA
Gomphrena nitida Rothr.	Sánchez -del Pino 113 *et al.*/ MEXU	DNA
Gomphrena serrata L.	I. Sánchez-del Pino 110 & G. Flores/ MEXU	DNA, flowers, pollen

TABLE 14. Taxon sampling and voucher information

TAXON	COLLECTION/HERBARIUM	DATA TYPE
Gomphrena vaga Mart.	T. Borsch 3503/ BONN	Flowers, pollen
	Estenssoro 608/ NY	DNA
Gomphrena sp.	T. Borsch 3523/ BONN	Flowers
Gomphrena sp.	T. Borsch 3443/ BONN	Flowers
Gossypianthus lanuginosus Moq.	Fryxell 1866/ NY	Flowers
	Sánchez-del Pino 22/ MEXU	DNA
Guilleminea densa Moq.	Flores at al. HF 02-24/ MEXU	DNA
Guilleminea gracilis R. Fries	Nee *et al.* 51956/ NY	DNA
Guilleminea sp.	T. Borsch 3434/ BONN	Flowers
Hebanthe grandiflora (Hook.) T. Borsch & Pedersen	Ventura 3208/NY; Nee 47097/ NY	DNA
Hebanthe occidentalis (R. E. Fr.) T. Borsch & Pedersen	Nee & Saldias 47054/ NY	DNA
Hebanthe paniculada Mart.	Tressens 6346/NY; Queiroz 2831/ NY	DNA
Hebanthe sp.	T. Borsch 3512/ BONN	Flowers, pollen
Irenella chrysotricha Suess.	Asplund 16555/ NY	DNA, flowers, pollen
Iresine alternifolia S. Watson	Daniel 2340/ NY	DNA, flowers, pollen
Iresine arbuscula Uline & Bray	Sánchez -del Pino 100/ MEXU; Nee & Taylor 26505/NY	DNA
Iresine difusa Humb. & Bonpl. ex Willd.	I. Sánchez-del Pino 118 & A. Campos/ MEXU	DNA, flowers
Iresine grandis Standl.	Standley 60761/ NY	Pollen
	Rzedowski 29504/ NY	DNA
Iresine heterophylla Standl.	I. Sánchez-del Pino 107 & G. Flores/ MEXU	DNA, flowers
Iresine leptoclada (Hook. f.) J. Henrickson & S. Sundberg	Johnston 9644/ NY	Pollen
	Chiang *et al.* 9644e/ NY; Hinckley 2135/ NY	DNA
Iresine palmeri Standl.	Nee & Taylor 29041/ NY	DNA

TABLE 14. Taxon sampling and voucher information

TAXON	COLLECTION/HERBARIUM	DATA TYPE
Iresine sp.	I. Sánchez- del Pino 103 & A. Campos/ MEXU	DNA, flowers, pollen
Kyphocarpa angustifolia	Zietsman 3928/ NY	DNA
Leucosphera bainesii	Zietsman 3944/ NY	DNA
Lithophila muscoides Sw.	Correll 43423/ NY	Flowers, pollen
	Correll 46472/ NY; Correll 43425/ NY	DNA
Pedersenia argentata (Mart.) J. Holub.	T. Borsch 3532/ BONN	Flowers, pollen
	Nee 38784/ NY	DNA
Pedersenia cardenasii (Standl.) J. Holub.	T. Borsch & Ortuño 3504/ BONN	DNA, flowers
	Kuntze s.n/ NY	Pollen
Pedersenia sp.	T. Borsch & Ibisch 3532/ BONN	DNA
Pedersenia sp.	T. Borsch 3537/ BONN	Flowers
Pedersenia sp.	T. Borsch 3537/ BONN	Flowers
Pfaffia fruticulosa	T. Borsch 3522/ BONN	Flowers
Pfaffia aff. iresinoides Sprenq.	Sánchez -del Pino 115/ MEXU	DNA
Pfaffia jubata Mart.	Uhlmann 88/ NY	DNA
Pfaffia tuberosa (Moq. ex DC.) Hicker	Pedersen 1010/ NY	DNA
Pfaffia sp.	T. Borsch 3576/ BONN	Flowers
Pfaffia sp.	T. Borsch 3533/ BONN	Flowers, pollen
Pseudoplantago friessii Suess.	Pedersen 15792/ NY	DNA, flowers, pollen
Pupalia lappacea Juss.	Venter & Venter 9673/ NY	DNA, flowers, pollen
Sericorema nemotiflora	Venter & Venter 9713/ NY	DNA, flowers, pollen
Tidestromia carnosa I. M. Johnst.	Flores Olvera 02-22/ MEXU	DNA
T. lanuginosa subsp. e*liassoniana* Sánch. Pino & Flores Olv.	Pinkava 9988/NY; Harrytate 1035/ NY	DNA

TABLE 14. Taxon sampling and voucher information

TAXON	COLLECTION/HERBARIUM	DATA TYPE
T. lanuginosa (Nutt.) Standl. subsp. *lanuginosa*	Flores Olvera 02-18/MEXU; Flores Olvera 02-19/ MEXU	DNA
T. rhizomatosa I. M. Johnst.	Flores Olvera 02-14/ MEXU	DNA
T. suffruticosa var. *oblongifolia* (S. Watson) Sánch. Pino & Flores Olv.	Atwood 27875/ NY; Neese & Neese 10970/ NY	DNA
T. suffruticosa (Torr.) Standl. var. *suffruticosa*	Flores Olvera 02-34/ MEXU; Flores Olvera 02-16/ MEXU	DNA
T. tenella I. M. Johnst.	Flores Olvera 02-25/ MEXU	DNA
T. valdesiana Sánch. Pino & Flores Olv.	Flores Olvera 02-33/ MEXU	DNA
Woehleria serpyllifolia Griseb.	Britton 7356 *et al.*/ NY	DNA, flowers, pollen
Xerosiphon angustifolius (Mart.) Pedersen	Anderson 9066/ NY	DNA, flowers, pollen
Xerosiphon aphyllus (Pohl ex Moq.) Pedersen	Fonseca 1303 *et al.*/ NY	DNA

5.2.2. Character analyses. The morphological coding of the 16 genera sampled in this study was based on herbarium specimens from GH, MEXU, MO, NY (acronyms following Holmgren *et al.* 1990) on loan at NY; from material collected during fieldwork in Mexico; or obtained on loan from colleagues. A list of species, sources of material, and voucher are listed in Table 1. Eighteen characters from pseudostaminodia, stigmas, and pollen were defined as primary hypotheses of homology (sensu De Pinna 1991) to be used in the cladistic analysis. A complete list of characters and discussion of states of character is given in Appendix 3.

Flowers were fixed in 70% ethanol and those of *Alternanthera echinocephala*, *A. flava*, *A. olivacea*, *A. snodgrassii*, *Charpentiera obovata*, *Froelichia interrupta*, *Gomphrena boliviana*, *G. elegans*, *G. flaccida*, *G. graminea*, *Gossypianthus lanuginosus*, *Irenella chrysotricha*, *Iresine alternifolia*, *Lithophila muscoides*, *Pseudoplantago friessii*, *Pupalia lappacea*, *Sericorema nemotiflora*, *Woehleria serpyllifolia*, and *Xerosiphon angustifolius* were fixed in 3% Glutaraldehyde for 24-72 hrs. The fixation process was stopped with Na-K Phosphate Buffer (0.1 M; pH 7.2).

In order to analyze the micromorphology of pseudostaminodia, stigmas, and pollen, these structures were dehydrated in ethanol series (10 %, 30 %, 50 %, 70 %, 90 %, 100 %, 100 %, 100 %) for 10 minutes each step. Then, samples were dried in a Critical Point Dryer (CPD; Tousimis Samdri-790, Tousimis Research Corporation Rockville, MD). The material was mounted on aluminium stubs and coated using a Sputter Coater (Anatech Hummer, Systems Division-Anatech, Hayward, CA). The samples were observed with a Scanning Electron Microscope (SEM; Hitachi S-700, Hitachi High Technologies America, Electron Microscope Division, Pleasanton, CA) at Lehman College, City University of New York (CUNY).

Most of the palynological characters of the family Amaranthaceae were previously reported (Skvarla and Nowicke 1976; Zandonella and Lecocq 1977; Eliasson 1988; Borsch 1998; Sánchez-del Pino 2001). Therefore, pollen grains of one representative sample of *Achyranthes aspera*, *Alternanthera echinocephala*, *A. flava*, *A. olivacea*, *A. snodgrassii*, *Alternanthera* sp., *Centrostachys aquatica*, *Gomphrena boliviana G. elegans*, *G. flaccida*, *G. graminea*, *G. haenkeana*, *G. serrata*, *G. vaga*, *Hebanthe* sp., *Irenella chrysotricha*, *Iresine alternifolius*, *I. grandis*, *I. leptoclada*, *Iresine*

sp., *Lithophila muscoides*, *Pedersenia* aff. *argentata*, *P. cardenasii*, *Pfaffia* sp., *Pseudoplantago friessii*, *Pupalia lappacea*, *Sericorema nemotiflora*, *Woehleria serpyllifolia*, and *Xerosiphon angustifolius* were observed directly with SEM to complete the sampling information necessary to code characters.

5.2.3. DNA extraction, amplification, and sequencing. DNA extraction, amplification, and sequencing followed the protocols provided in Chapter 2. The DNA sequences used in this study are the same that were used to generate the phylogeny of the subfamily Gomphrenoideae using *rpl16* and *trnL-F* regions.

5.2.4. Phylogenetic analysis. Uninformative characters were deactivated. Heuristic parsimony analyses were conducted using *Nona* (Goloboff, 1993) spawned by *Winclada* (Nixon, 1999-2002). TBR swapping on Wagner trees were conducted from 10,000 random taxon addition sequences with 10 trees held in memory for each of the replicate initiations expanding the memory to 100 000 to do further TBR (h 100 000, mult* 10 000, ho/10).

Data sets were analyzed combined using simultaneous analysis approach (Nixon and Carpenter 1996) in two modalities, one using all the data sets (morphology and molecular) and the second using only morphological characters. Data were run including gaps. Jackknife (JK) branching support was calculated by *Nona* using *Winclada* with 1 000 replications with 100 search replications and 10 tree hold in memory with the next parameters (mult*100; ho/10; max*). Jackknife percentage are described as high (85-100%), moderate (75-84%) and low (>50-74%).

5.2.5. Tracing the evolution of morphological characters. The eighteen morphological characters listed in Appendix 3 were traced onto the consensus combined tree using *Winclada*. It was constructed both binary and multistage codings for some characters. Inapplicable characters lead to conflicting reconstructions.

All morphological characters were phylogenetically informative. However, character 6, related to adnation of pseudostaminodia to a second layer (present vs. absent) was not phylogenetically informative for the subfamily Gomphrenoideae. Therefore, character 6 was not represented in the combined tree in this study.

5.3. RESULTS

5.3.1. Phylogeny reconstruction.

The analysis included 71 taxa, DNA sequences of two chloroplast regions: *trnL-F* and *rpL16* (see Chapter 2) and 18 informative morphological characters (Appendix 3). The combined phylogenetic analysis resulted in 36 most parsimonious trees (MPT) of 1670 steps in length (CI = 0.61, RI = 0.84; Figs. 29-30) and the morphological data yielded 10, 000 MPT and stopped to completion at 10, 000 with a length of 62 steps (CI = 0.48, RI = 0.88) (Fig. 28).

The strict consensus trees for the two analyses were incongruent. The phylogeny obtained using morphology resulted in some clades, but branch support was less than 50% jackknife (Fig. 28), whereas a more resolved and statistically supported phylogeny was obtained using simultaneous analysis (Figs. 29, 30). In addition, the phylogenetic relationships among the species of the clades based on morphology were incongruent with the consensus tree obtained using combined data sets. The consensus tree obtained

from morphology supported the monophyly of the subfamily Gomphrenoideae as did the consensus tree using combined data. Three major clades Iresinoid, Alternanthoid, and Gomphrenoid (described in Chapter 2; Fig. 31) were resolved using the combined data, but these collapsed in the morphology tree (Fig. 28). The areas of incongruence between the morphology tree and the combined analyses are: one, *Lithophila muscoides* was sister to *Guilleminea* and *Gossypianthus* whereas in the combined analysis *Lithophila* was sister to *Blutaparon*; second, *Iresine* was not grouped with *Irenella* and *Woehleria*, but in the combined analysis was in a clade that included *Iresine*, *Irenella*, and *Woehleria*; third, *Gomphrena elegans* was sister to *Xerosiphon*, *Froelichia*, and *Gomphrena* (excluding *G. vaga*) whereas the consensus tree using combined data sets resolved *Gomphrena elegans* sister to *G. vaga*, *Pfaffia*, and *Hebanthe*; and fourth, *Froelichia*, *Gomphrena*, and *Xerosiphon* formed a clade based on morphology; however, it was in a large clade (with *Guilleminea*, *Gossypianthus*, *Blutaparon*, and *Lithophila*) in the combined analysis.

The comparison between the consensus tree obtained with molecular data (Chapter 2; Fig. 31) and the one obtained with morphology plus molecular data were mostly congruent with the exception of four areas of incongruence discussed above (Fig. 30, 31). The first area of incongruence concerns the clade that includes *Gomphrena boliviana* and *Gossypianthus lanuginosus* that are well resolved based on molecular data alone. However, these two species are unresolved with *Gomphrena*, *Blutaparon*, and *Lithophila* using combined data. The second area of incongruence concerns the *Gomphrena elegans*, *G. vaga*, *Pfaffia*, *Hebanthe* clade. This clade was resolved in two subclades in the molecular tree, whereas a polytomy was obtained with the addition of the morphological characters. The third area of incongruence is *Iresine diffusa*, which

was closely related to a clade with *I. heterophylla* and *Woehleria serpyllifolia* in the molecular tree. However, phylogenetic relationships of these three species were unresolved in the tree in the simultaneous analysis. A fourth area of incongruence concerns the genus *Leucosphera* which was sister to the *Kyphocarpa angustifolia*-*Sericorema nemotiflora* subclade and the subclade that contains the genera *Puppalia*, *Achyropsis*, *Achyranthes* and *Centrostachys*. The simultaneous analysis produced an unresolved relationship of *Leucosphera* to these subclades. It appears that the morphological data (CI= 0.48, RI= 0.88; Fig. 28) has introduced much homoplasy to the molecular data set [simultaneous analysis (CI= 0.61, RI= 0.84; Fig. 29-30) vs. molecular (CI= 0.63, RI= 0.85; Fig. 31)], which caused many nodes to collapse.

The strict consensus tree obtained with morphology and molecular data shows that morphological synapomorphies are basically of two types: homologies resulting from pollen and anthers characters and homoplasies base on pseudostaminodia and stigmatic characters (Fig. 29). The strict consensus tree with combined data sets resolved that the subfamily Gomphrenoideae (99 % JK; Fig. 30) was supported by bisporangiate anthers (character 1 [1]) and the homoplasious character lanceolate pseudostaminodia (character 3 [2]). Lanceolate pseudostaminodia is a character shared with the outgroup taxon *Centrostachys aquatica*. The Gomphrenoid (90 % JK; Fig. 30) and Alternanthoid clades (96 % JK; Fig. 30) consist of two synapomorphies: metareticulate pollen (character 13 [0]) and microspines distributed in the distal row of the metareticula (character 17 [0]). The Alternanthoid clade is defined by the homoplasious character dodecahedric pollen (character 12 [1]), which is shared with *Kyphocarpa angustifolia*.

Within the Alternanthoids there are three monophyletic subclades. The first subclade includes the genus *Tidestromia* (100 % JK; Fig. 30). It is supported by two homopasious synapomorphies: lobed pseudostaminodia (character 3 [4]) and psilate pollen (character 16 [0]). The latter character also supports subclade D within the Gomphrenoids. Subclade D includes *Blutaparon*, *Guilleminea*, *Gossypianthus*, *Lithophila*, and the majority of *Gomphrena* species (100 % JK; Fig. 30). The second subclade within Alternanthoids includes the genus *Pedersenia* (100 % JK; Fig. 30) defined by cordate pseudostaminodia (character 3 [3]). The genus *Alternanthera* (100 % JK; Fig. 30) is the third monophyletic subclade within the Alternanthoids. It consists of three homoplasious synapomorphies: presence of pseudostaminodia longer than filaments (character 4 [1]) shared by several genera within the Gomphrenoids (subclade C; 99 % JK); fimbriate pseudostaminodia (character 5[2]); and entire stigma (character 9[0]), which also occurs in *Pseudoplantago friessii* (within subfamily Gomphrenoideae) and in the *Achyranthes*/ *Achyropsis*/ *Centrostachys*/ *Kyphocarpa*/ *Leucosphera*/ *Pupalia*/ *Sericorema* clade (99 % JK; Fig. 30).

The Gomphrenoid clade was supported by presence of pseudostaminodia 1/3 longer than filaments (character 4[3]), which also occurs in *Centrostachys aquatica*. Among the Gomphrenoids, *Pseudoplantago friessii*, the first branching species, was defined by five synapomorphies. These are cubic or tetrahedral pollen (character 12[2]), microspines arranged around the apertures (character 17[1]), and three homoplasious characters: entire stigma (character 9[0]); long style (character 11[2]), which also supported the monophyly of *Froelichia*; and pollen with stellate ornamentation (character 18[1] also present in *Pupalia lappacea*). Subclade B, within the Gomphrenoid clade,

includes *Pfaffia*, *Hebanthe*, *Gomphrena elegans*, and *G. vaga* (98 % JK; Fig. 30) that share of the homoplasious character sessile stigmas (character 11[0]). Sessile stigmas also supported *Guilleminea*, *Gossypianthus lanuginosus*, and the monophyletic *Amaranthus* clade. The Gomphrenoid clade, subclade C (99% JK; Fig. 30) is defined by the presence of tectum only in distal bands (character 14[1]) plus two homoplasious characters, pseudostaminodia longer than filaments (character 4[1]) and filaments in a staminal tube (character 8[1]). This subclade includes the monophyletic *Guilleminea* (100 % JK; Fig. 30) with four homoplasious synapomorphies: filaments in a staminal cup (character 8[0]), papillae distributed in inner sides of stigmas (character 10[1]), sessile stigma (character 11[0]), and pollen with mesoporia acute vaulted (character 15[2]). All these characters are shared with *Gossypianthus lanuginosus* and only two of these (characters 8[0], 15[2]) support the sister relationship between *Blutaparon vermiculare* and *Lithophila muscoides* (66 % JK; Fig. 30). The *Gomphrena graminea*/ *G. macrocephala*/ *G. haenkeana*/ *G. nitida/ G. serrata/ G. globosa* clade (99 % JK; Fig. 30) is defined by the homoplasious character filaments enclosed by pseudostaminodia (character 7[1]), which they share with *G. boliviana*.

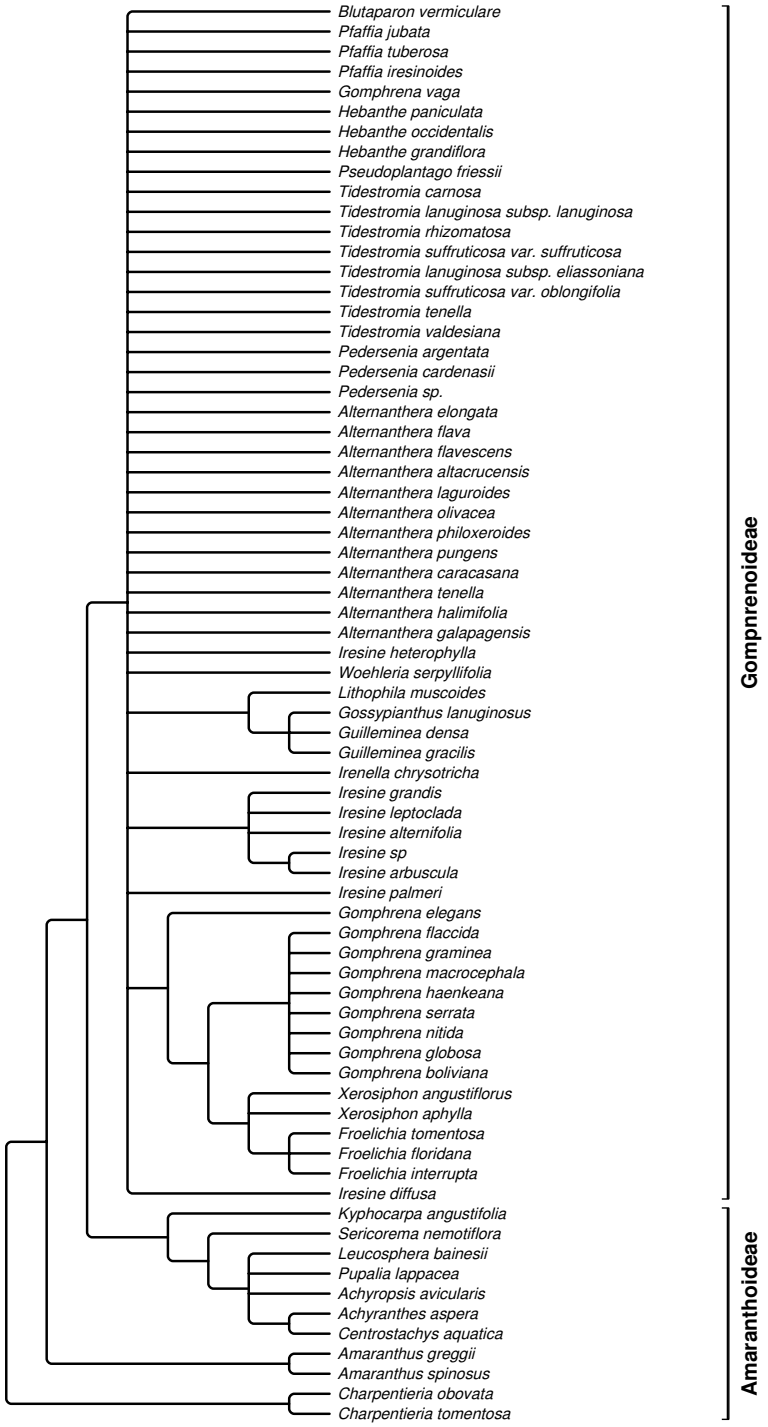

Fig. 28. The strict consensus tree created from 10, 000 MPT (L= 62 CI= 0.48 RI= 0.88) yielded from the morphological data set. Brackets indicate subfamily circumscriptions.

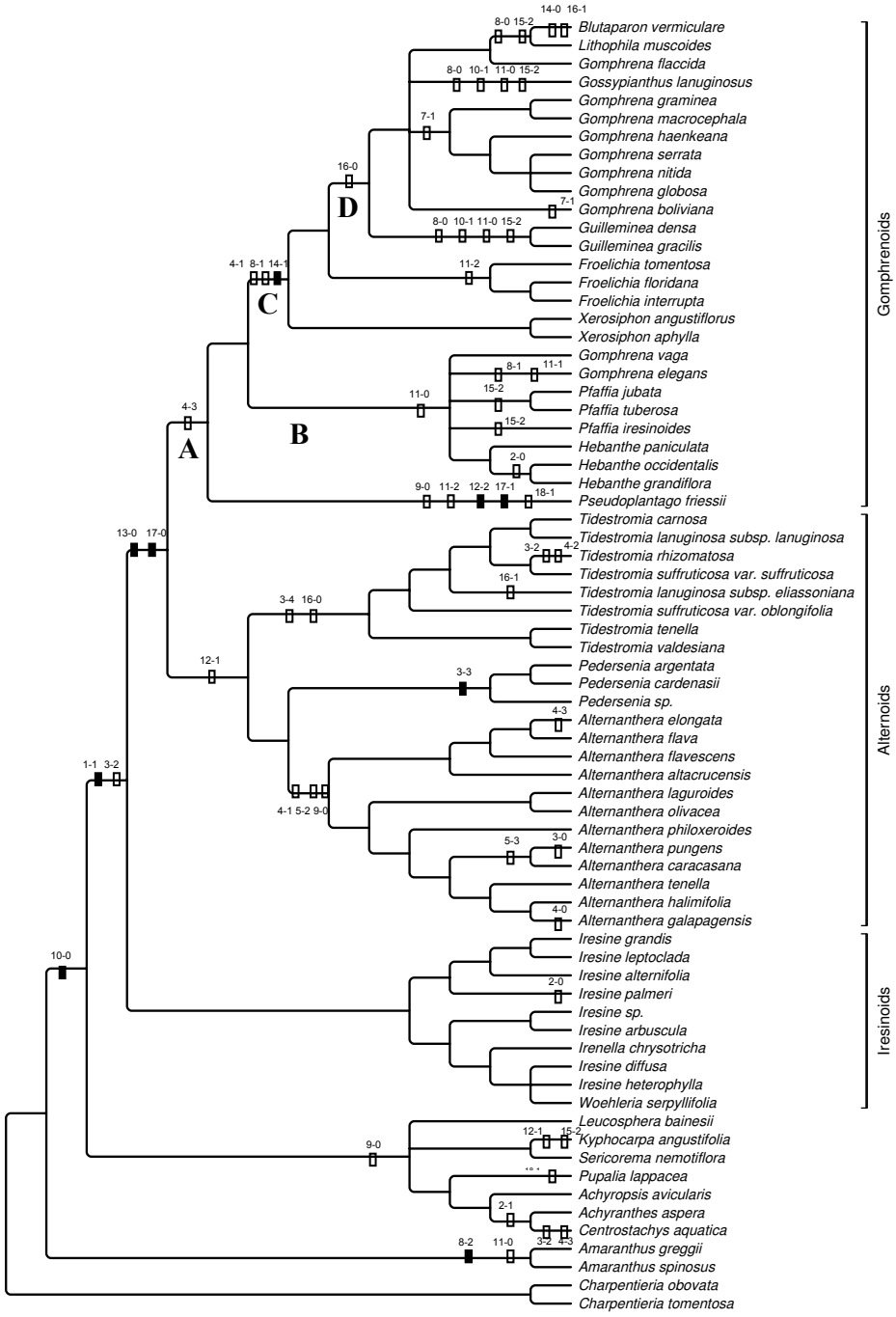

Fig. 29. The strict consensus tree of resulting 36 MPT from the combined analysis using *trnL-F*, *rpl16*, and morphology (L= 1670 CI= 0.61 RI= 0.84). Solid black bars represent homologies and white bars represent homoplasious characters. The numbers above each character transformation refers to the character and character state given in Appendix 1. Brackets indicate the three major clades.

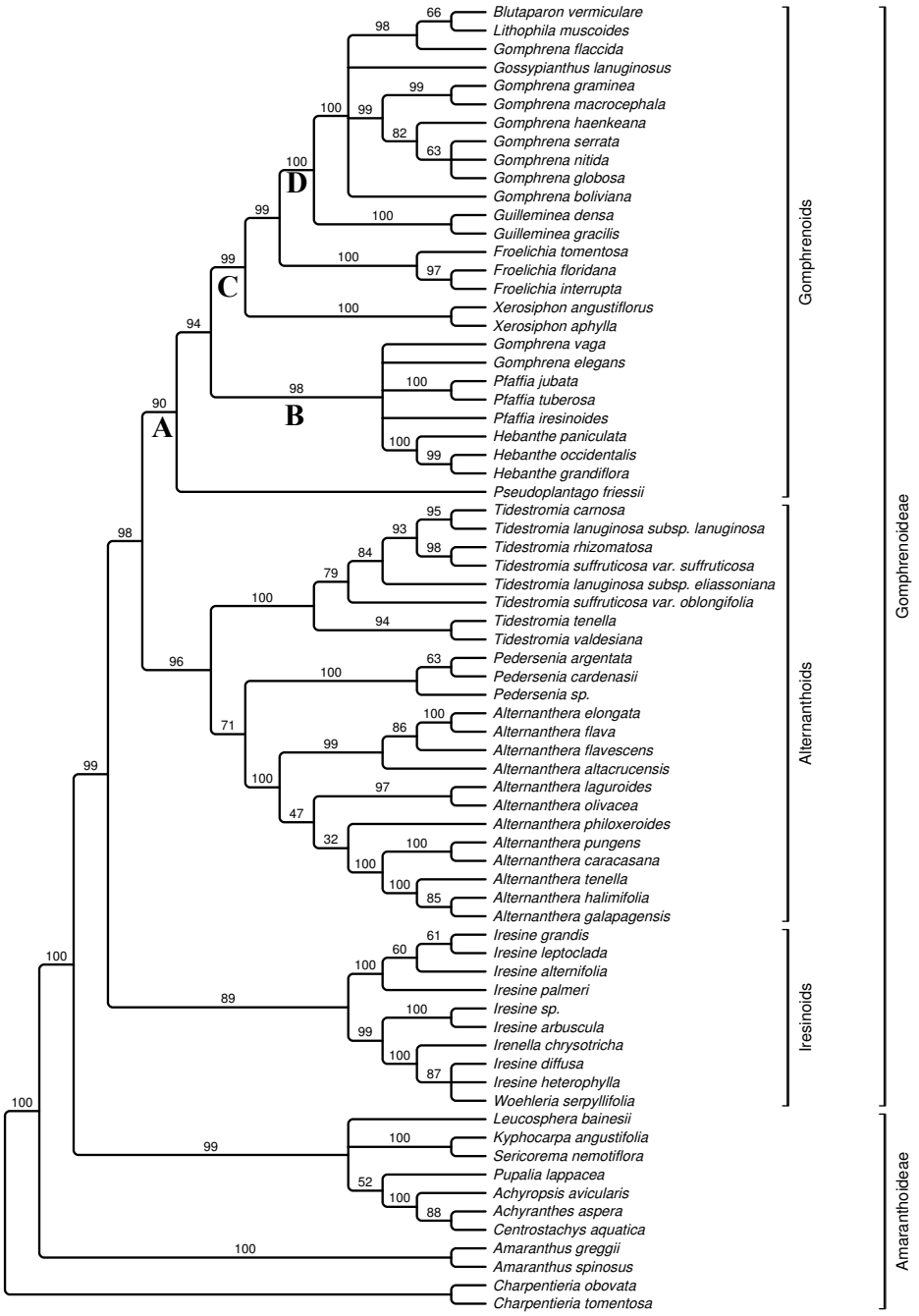

Fig. 30. The strict consensus tree generated from 36 MPT yielded in the combined analysis using *trnL-F*, *rpl16*, and morphology data (L= 1670 CI= 0.61 RI= 0.84). The numbers below the branches are jackknife support values, and brackets define major clades and subfamilies.

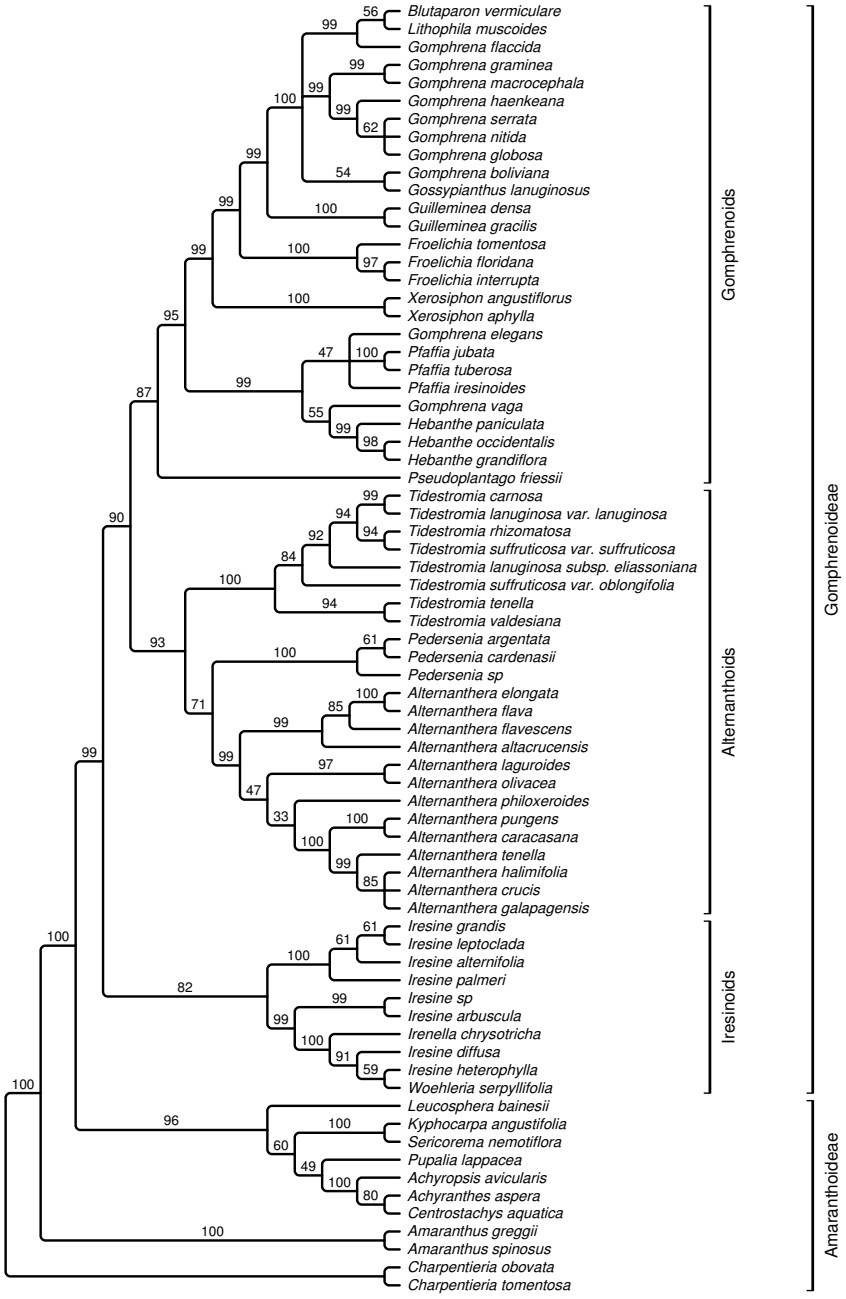

Fig. 31. The strict consensus tree generated eight MPT yielded in the combined analysis using *trnL-F* and *rpl16* data (L= 1587 steps, CI= 0.63, RI= 0.85). Jackknife values above branches. Brackets indicate major groups recognized in the Subfamily Gomphrenoideae.

The *Achyranthes/ Achyropsis/ Centrostachys/ Kyphocarpa/ Leucosphera/ Pupalia/ Sericorema* subclade (99 % JK; Fig. 30), within the subfamily Amaranthoideae clade (the outgroup taxa), and the monophyletic subfamily Gomphrenoideae clade were supported by the presence of papillae widespread over the stigmatic surface (character 10[0]). Finally, the monophyly of *Amaranthus* (100 % JK; Fig. 30) was supported by the presence of filaments free to the base (character 8[2]) and the homoplasious character sessile stigma (character 11[0]).

5.3.2. Morphological variation.

5.3.2.1. Pseudostaminodia.

The androecia of the family Amaranthaceae typically include five stamens and five pseudostaminodia fused at the base. When pseudostaminodia are present, filaments and pseudostaminodia can be fused along their length in two forms: a) more than a half of the filament length to form a staminal tube as happens in *Gomphrena* (Fig. 34a-d), *Froelichia* (Fig. 33h), and *Xerosiphon*; or b) the adnation of filaments and pseudostaminodia can fuse half or less than a half of the filament length and then form a staminal cup as happens in *Achyropsis*, *Achyranthes* (Fig. 33d), *Alternanthera* (Fig. 33f, g), *Blutaparon* (Fig. 32b), *Centrostachys* (Fig. 33e), *Charpentiera* (Fig. 32e), *Gossypianthus*, *Guilleminea*, *Hebanthe* (Fig. 32d), *Iresine* (Fig. 32f-h), *Irenella* (Fig. 32c), *Kyphocarpa*, *Lithophila*, *Pedersenia*, *Pfaffia*, *Pseudoplantago*, *Pupalia*, *Sericorema*, and *Tidestromia*. The pseudostaminodia in the taxa sampled come from a single layer of tissue (Fig. 32-34). However, an additional layer that covers the base of

the staminal cup was found only in *Achyropsis*, *Achyranthes*, and *Centrostachys* (Fig. 33d, e). Some genus such as *Amaranthus* lack pseudostaminodia and have filaments free to base (Fig. 32a).

The SEM shows that pseudostaminodia are present in *Alternanthera*, *Achyranthes*, *Centrostachys*, *Charpentiera*, *Froelichia*, *Gomphrena* (excluding *G. flaccida*; Fig. 34e), *Hebanthe paniculata*, *Xerosiphon*, *Pedersenia*, *Pseudoplantago*, *Tidestromia*, *Woehleria* (Fig. 33c), and most of the *Iresine* species. Five different forms of pseudostaminodia were found in this study: triangular, oblong, lanceolate, cordate, and lobed. These forms are defined based on Stearn's (1995) terminology. Triangular pseudostaminodia are those which have a triangular shape. These are present in *Alternanthera pungens*, *Hebanthe paniculata*, *Iresine diffusa* (Fig. 32f, g), *I. heterophylla*, and some infraspecific taxa of *Tidestromia*. Oblong pseudostaminodia are those which have an elliptical shape or are obtuse at each end as in *Achyranthes*, *Achyranthopsis*, *Iresine* species (e.g. *I. arbuscula*, Fig. 32h). Lanceolate pseudostaminodia are narrowly elliptical, tapering equally to each end. Lanceolate pseudostaminodia are present in *Centrostachys* (Fig. 33e), *Froelichia*, *Iresine alternifolia*, *I. grandis*, *I. leptoclada*, *Pseudoplantago*, *Tidestromia rhizomatosa*, *Woehleria* (Fig. 33c), *Xerosiphon*, and most species of *Alternanthera* (Fig. 33f, g). Cordate pseudostaminodia have two equally rounded lobes or are heart-shaped. *Pedersenia* exclusively has cordate pseudostaminodia (Fig. 33a, b). Lobed pseudostaminodia have rounded or obtuse apices and are present in *Charpentiera* (Fig. 32e), *Hebanthe paniculata*, *Iresine heterophylla*, *Kyphocarpa*, and *Tidestromia*.

Four different lengths of pseudostaminodia were found in the sampling: a) pseudostaminodia 2/3 longer or more than filaments in *Froelichia* (Fig. 33h), *Gomphrena* (Fig. 34a, b), *Xerosiphon*, and most of the species of *Alternanthera* (Fig. 33f, g); b) pseudostaminodia equal to filaments in *Alternanthera caracasana*, *A. laguroides*, *A. olivacea*, *A. tenella*, and *Tidestromia rhizomatosa*; c) pseudostaminodia 1/3 longer than filaments are in *A. caracasana*, *A. elongata*, *Gomphrena elegans* (Fig. 34d), *G. vaga* (Fig. 34c), *Pseudoplantago*, and *Centrostachys*; and d) pseudostaminodia shorter than filaments in *Alternanthera pungens*, *A. galapagensis*, *Achyranthes*, *Achyranthopsis*, *Charpentiera* (Fig. 32e), *Hebanthe paniculata*, *Iresine* (Fig. 32f-h), *Kyphocarpa*, *Pedersenia*(Fig. 33a, b), and *Woehleria* (Fig. 33c).

The pseudostaminodia margins are morphologically variable. Four different forms occurred in this study: entire, ciliate, fimbriate, crenate. The entire margin characterize by absence of marginal division. These are present in *Alternanthera caracasana*, *Charpentiera*, *Froelichia, Gomphrena, Hebanthe paniculata*, *Iresine*, *Kyphocarpa*, *Pedersenia*, *Pseudoplantago*, *Tidestromia*, *Woehleria*, and *Xerosiphon*. Ciliate margins are characterized as having filiform processes thinner than hairs. The genus *Iresine* has ciliate pseudostaminodia (*Achyranthes* on lateral sides; Fig. 33d). Fimbriate margins have borders with long filiform processes thicker than hairs. *Achyropsis*, *Achyranthes*, *Centrostachys*, and *Alternanthera* have fimbriate pseudostaminodia. Crenate margins have convex teeth. *Iresine arbuscula*, *Gomphrena*, *Hebanthe paniculata*, *Xerosiphon*, some taxa of *Tidestromia*, *Alternanthera caracasana* and *A. pungens* have crenate pseudostaminodia.

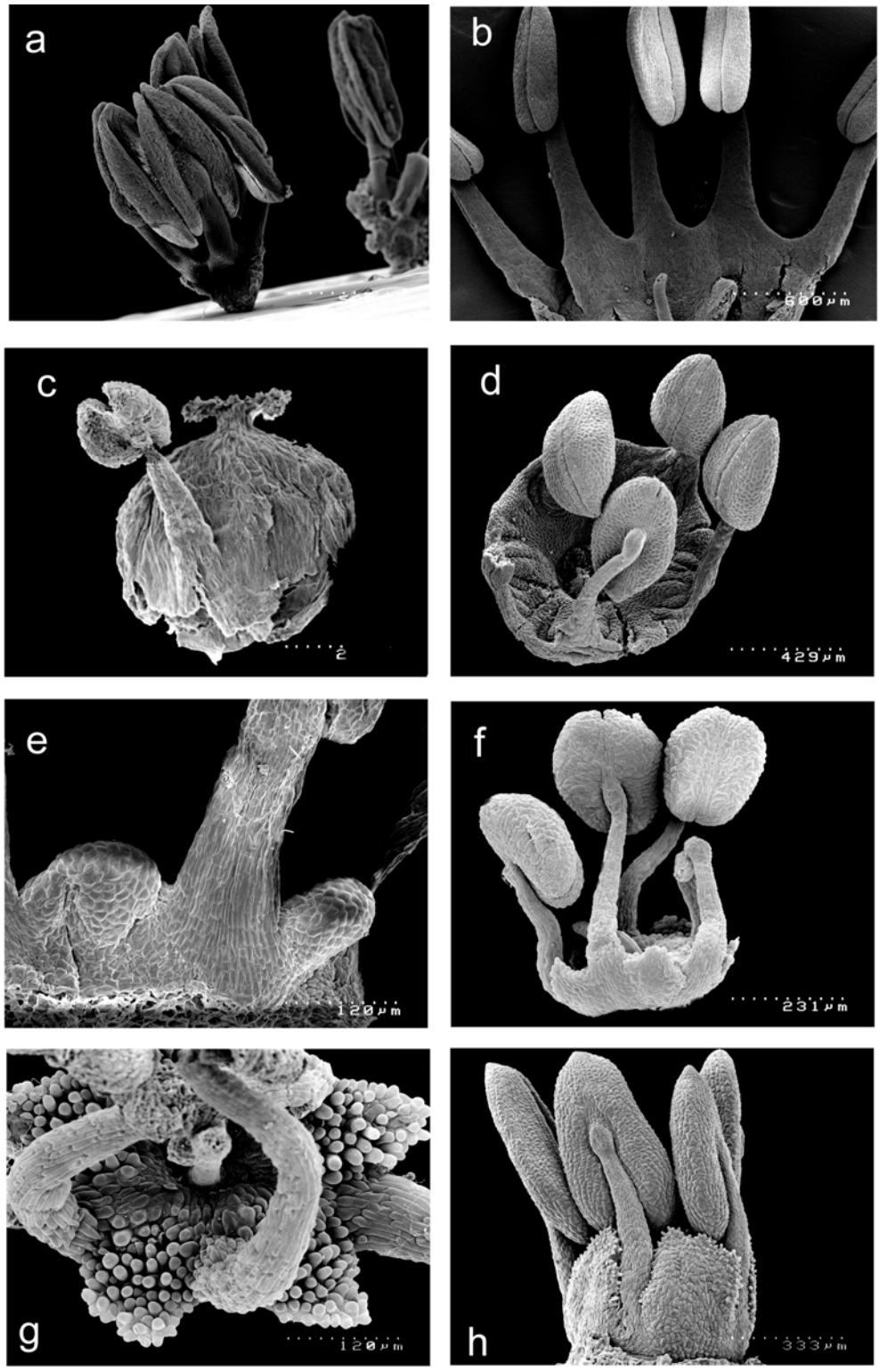

Fig. 32. Pseudostaminodia morphology. Absence of pseudostaminodia in selected representatives of Amaranthaceae: a. *Amaranthus*, b. *Blutaparon*, c. *Irenella*, d. *Hebanthe*. **Lobed:** e. *Charpentiera*. **Triangular:** f-g. *Iresine diffusa*. **Oblong:** h. *Iresine arbuscula*.

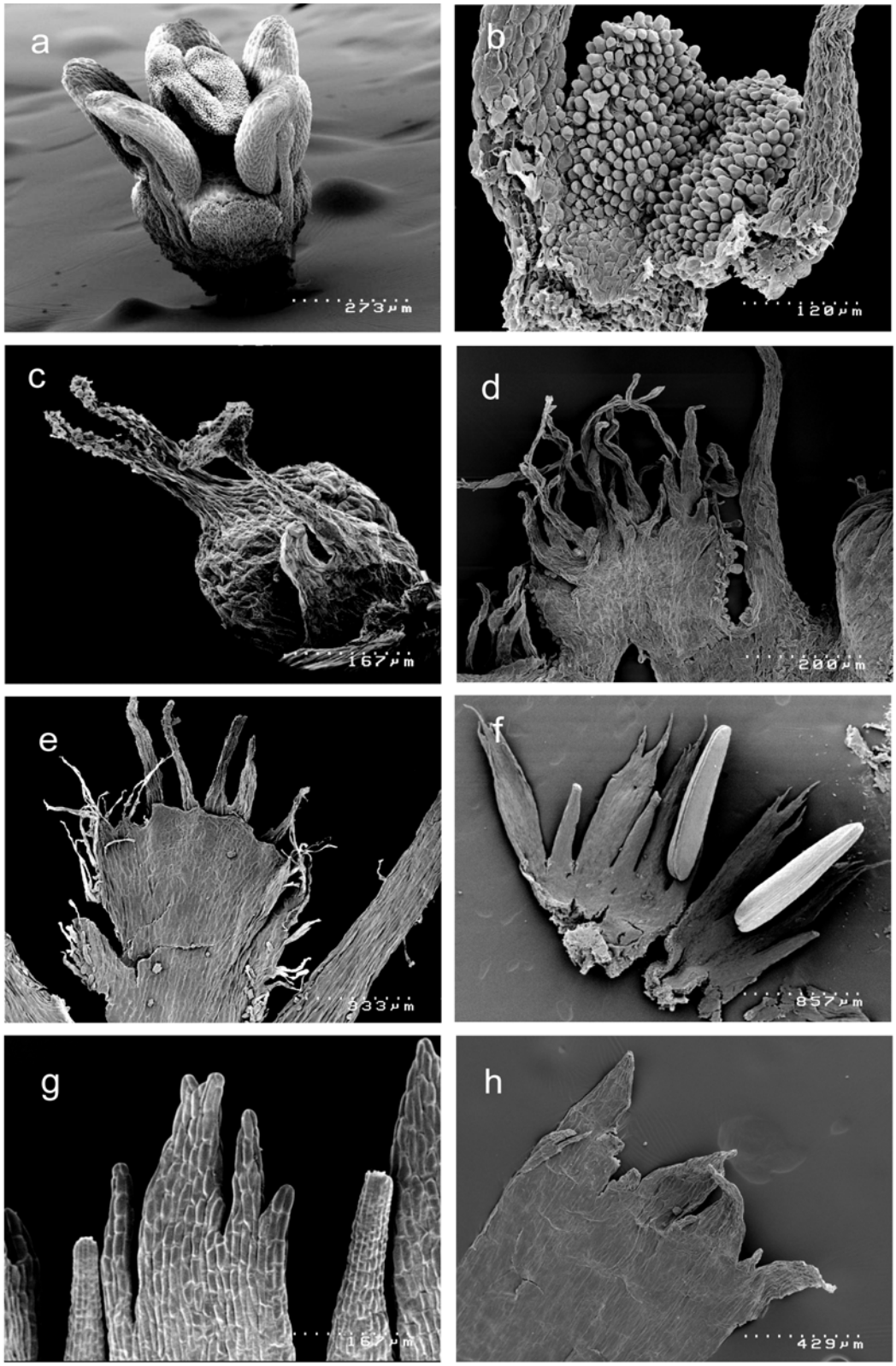

Fig. 33. Pseudostaminodia morphology. Cordate: a-b. *Pedersenia*. **Lanceolate:** c. *Woehleria*. **Laciniate:** d. *Achyranthes*, e. *Centrostachys*, f-g. *Alternanthera*. **Fused in a satminal tube:** h. *Froelichia*.

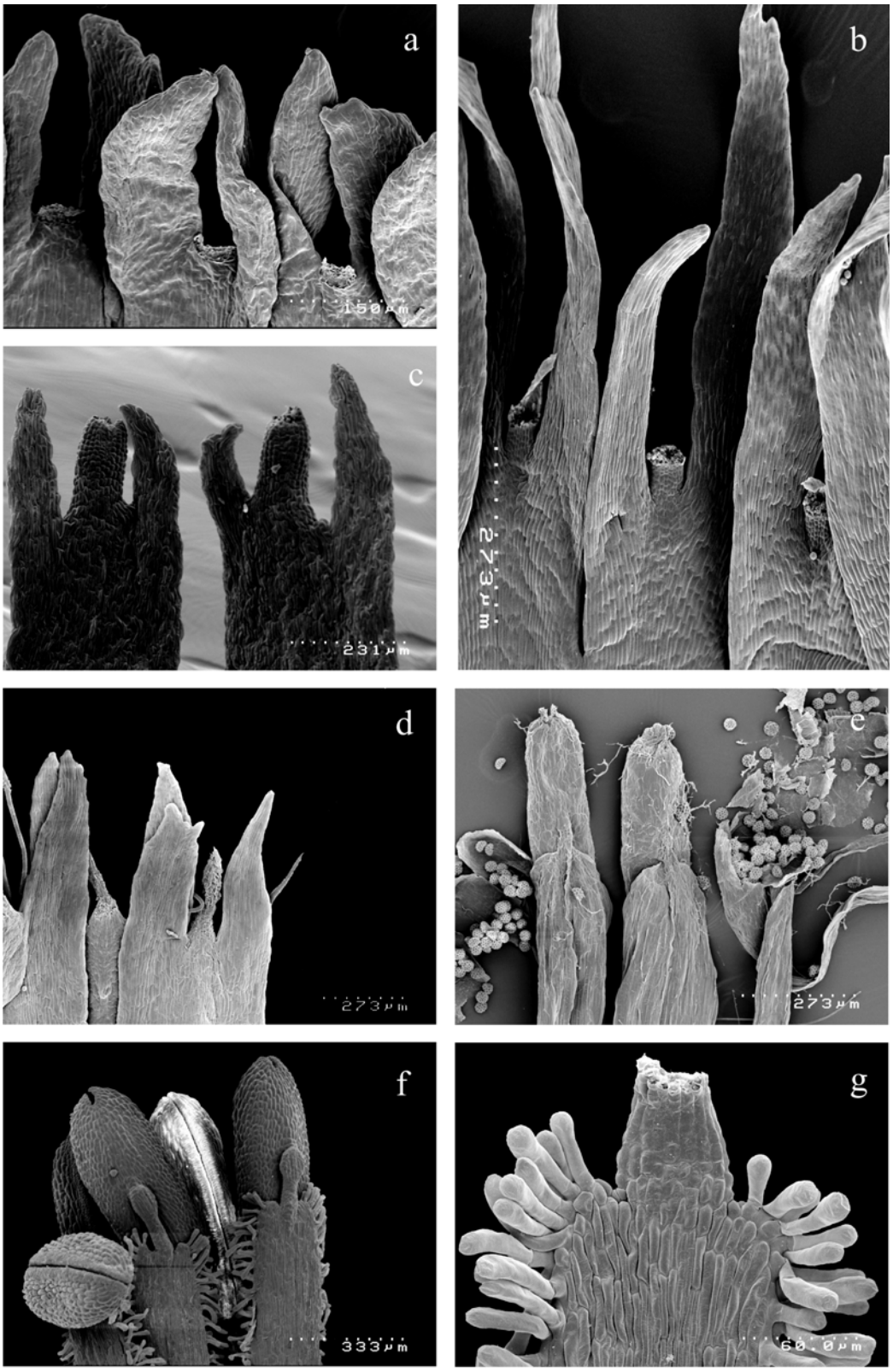

Fig 34. Pseudostaminodia morphology. Filaments fused in a staminal tube: a. *Gomphrena*, b. *G. haenkeana*, c. *G. vaga*, d. *G. elegans*. **Fusion of filaments and pseudostaminodia:** e. *Gomphrena flaccida*, f-g. *Pfaffia*.

In this study, it was found that pseudostaminodia present in *Gomphrena* are not homologous with pseudostaminodia within the Gomphrenoideae. The pseudostaminodia of *Gomphrena* enclose the filament and this does not occur in any other genera (Fig. 34a-d) making it a novel autapomorphy for *Gomphrena*.

5.3.2.2. Gynoecium.

Three different forms of stigmas were found in this study: entire, bilobed and trilobed. *Achyropsis*, *Achyranthes* (Fig. 35a), *Alternanthera* (Fig. 35c), *Centrostachys* (Fig. 35b), *Kyphocarpa*, *Leucosphera*, *Pseudoplantago* (Fig. 35d), *Pupalia* (Fig. 35e), and *Sericorema* have entire stigmas, which have undivided stigmatic surfaces. Bilobed stigmas are present in *Amaranthus*, *Blutaparon* (Fig. 37d), *Charpentiera* (Fig. 37a), *Froelichia* (Fig. 36a), *Gomphrena* (Fig. 36c, 137e, f), *Gossypianthus* (Fig. 36g), *Guilleminea* (Fig. 36b), *Hebanthe*(Fig. 36d, e), *Iresine* (Fig. 37j), *Irenella* (Fig. 37b), *Lithophila* (Fig. 37c), *Pedersenia* (Fig. 37g, h), *Pfaffia* (Fig. 36f, h), *Tidestromia* (Fig. 37i), *Woehleria*, and *Xerosiphon*. The taxa *Amaranthus* (Fig. 36i) and *Tidestromia suffruticosa* var. *oblongifolia* also have trilobed stigmas in addition to bilobed stigmas.

Observations of the stigmas using SEM revealed two positions of the stigmatic papillae. Most of the taxa have papillae widely distributed along the stigma lobes, whereas *Amaranthus* (Fig. 36i), *Charpentiera* (Fig. 37a), *Gossypianthus* (Fig. 36g) and *Guilleminea* (Fig. 36b) have papillae restricted to the inner areas of the stigmatic lobules. Length of the style was another distinct difference. *Achyropsis*, *Achyranthes*, *Leucosphera*, *Pupalia*, *Pseudoplantago* and *Froelichia* have long styles; *Amaranthus*, *Pfaffia*, *Gomphrena vaga*, *Hebanthe*, *Guilleminea* and *Gossypianthus* have sessile

stigmas; and *Alternanthera*, *Blutaparon*, *Charpentiera*, *Gomphrena*, *Iresine*, *Irenella*, *Lithophila*, *Pedersenia*, *Sericorema*, *Tidestromia*, *Woehleria*, and *Xerosiphon* have short styles.

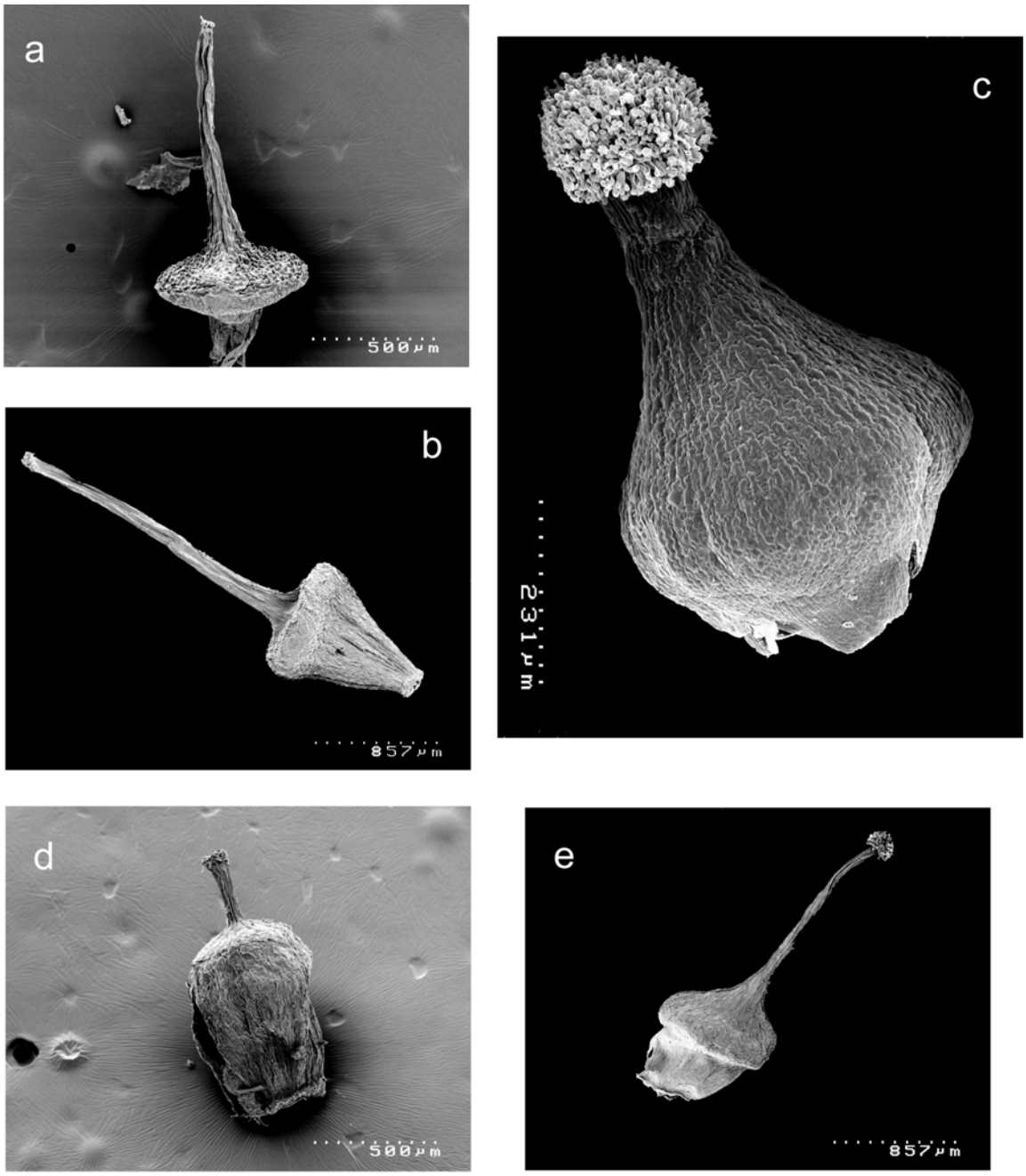

Fig. 35. Gynoecium morphology. Entire stigmas: a. *Achyranthes*, b. *Centrostachys*, c. *Alternanthera*, d. *Pseudoplantago*, and e. *Pupalia*.

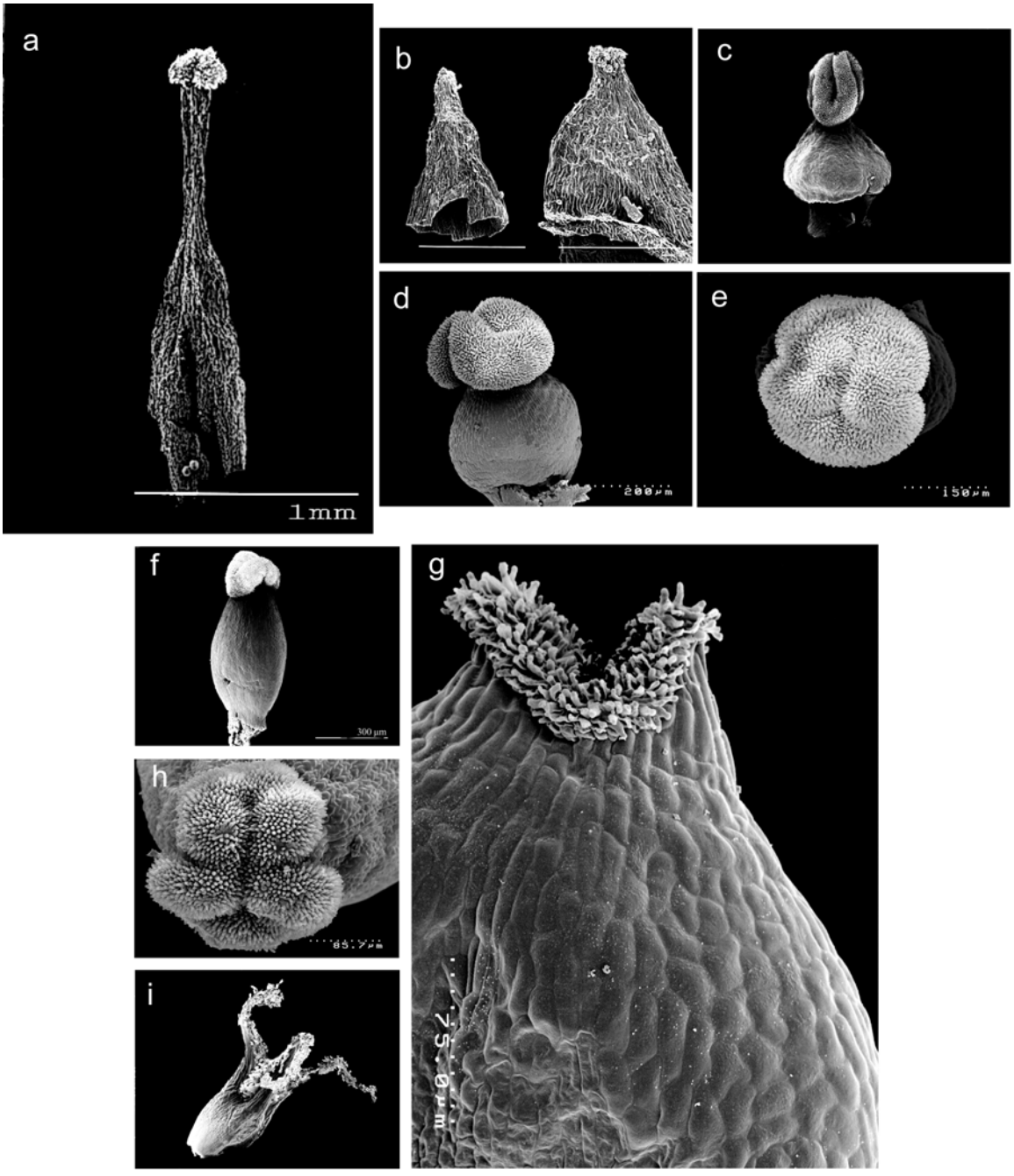

Fig. 36. Gynoecium morphology. Stigmas bilobed and sessile: a. *Froelichia*, b. *Guilleminea*, c. *Gomphrena vaga*, d-e. *Hebanthe*, f. *Pfaffia*, g. *Gossypianthus,* h. *Pfaffia*. **Stigmas trilobed and sessile:** i. *Amaranthus*.

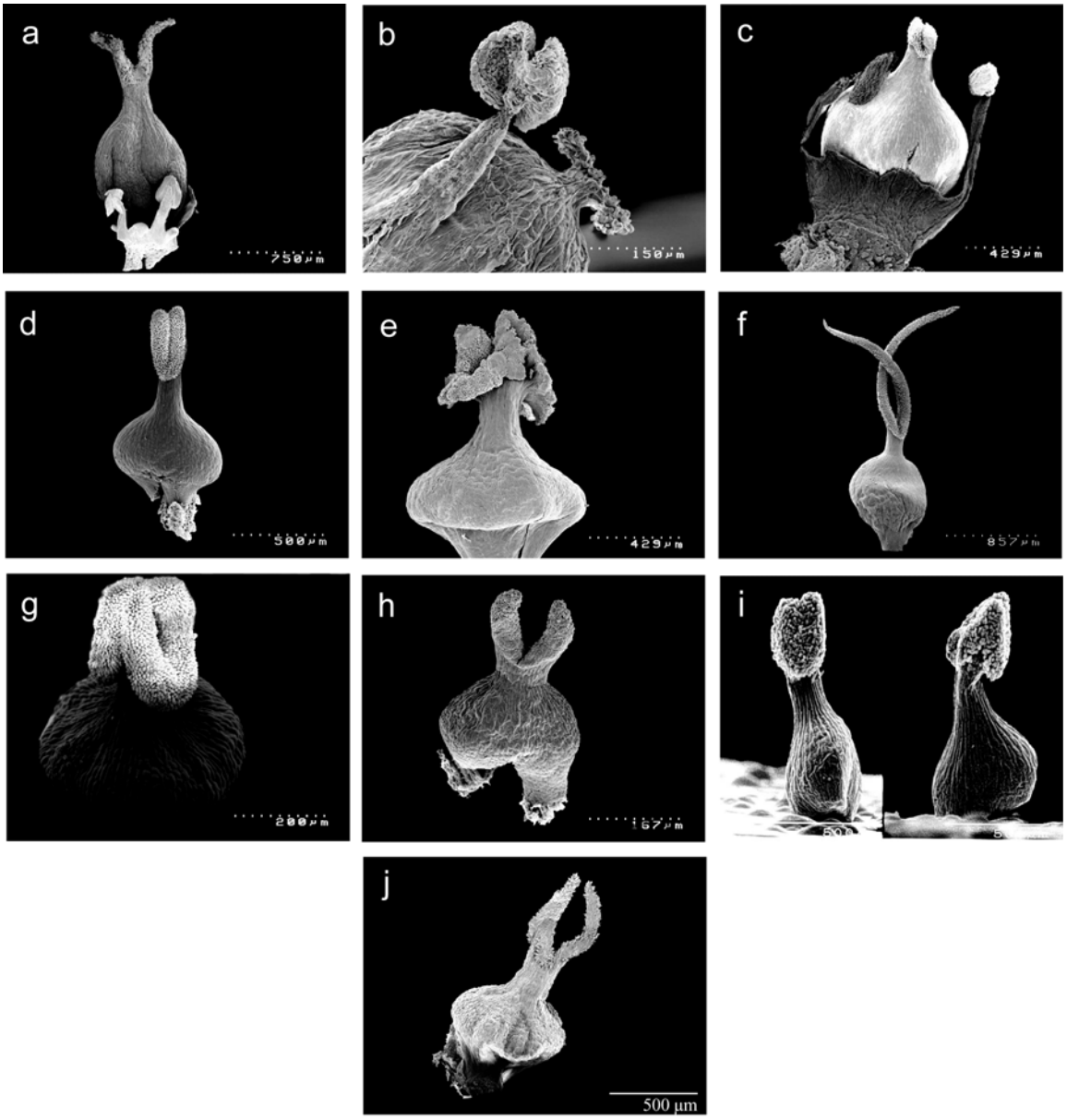

Fig. 37. Gynoecium morphology. Stigma bilobed with short style: a. *Charpentiera*, b. *Irenella*, c. *Lithophila*, d. *Blutaparon*, e. *Gomphrena elegans*, f. *G. haenkeana*, g-h. *Pedersenia*, i. *Tidestromia*, j. *Iresine*.

5.3.2.3. Pollen.

Pollen morphology variation was evaluated using bibliographic sources and completed with observations using the SEM. Three basic pollen forms were found in the sampling: dodecahedric, spheroidal, and cubic. *Alternanthera* (Fig. 39a, b), *Pedersenia* (Fig. 39c), *Tidestromia* (Fig. 39d), and *Kyphocarpa* have dodecahedric pollen. *Achyropsis*, *Achyranthes*, *Amaranthus*, *Blutaparon*, *Centrostachys* (Fig. 38a), *Charpentiera*, *Froelichia* (Fig. 40a), *Gomphrena* (Fig. 40b, c), *Gossypianthus* (Fig. 40d), *Guilleminea* (Fig. 40e), *Hebanthe* (Fig. 40f), *Iresine* (Fig. 40c), *Irenella*, *Leucosphera*, *Lithophila* (Fig. 40g), *Pfaffia* (Fig. 41a), *Pupalia* (Fig. 41e), *Sericorema* (Fig. 38b), *Tidestromia suffruticosa* var. *suffruticosa*, *Woehleria* (Fig. 38d), and *Xerosiphon* (Fig. 38h) have spheroidal pollen. *Pseudoplantago* has cubic pollen (Fig. 41d).

Amaranthaceae pollen can be either metareticulate or not metareticulate. Borsch and Barthlott (1998) proposed the term metareticulate to describe the type of pollen in the Amaranthaceae, considering that a reticulate tectum is not homologous to a metareticulate structure. Metareticulate pollen was interpreted as reticulate in the past (e.g., Nowicke and Skavarla 1979; Eliasson 1988). However, metareticulate pollen has one porate aperture in each of the meshes (lumina) (Fig. 39, 40, 41a-c) whereas reticulate pollen has less number of apertures than meshes and majority of lumina do not posses apertures. Reticulate refers to ornamentation in the exine (Borsch and Barthlott 1998). *Pseudoplantago* was considered as not metareticulate (Müller and Borsch 2005), but this might be an oversight of the unusual cubic pollen (Fig. 41d) and needs further investigation. *Achyropsis*, *Achyranthes*, *Amaranthus*, *Centrostachys*, *Charpentiera*, *Kyphocarpa*, *Leucosphera*, *Pupalia*, *Sericorema* (the outgroup taxa) and *Iresine* do not

have metareticulate pollen (Fig. 38) whereas the rest of the sampling taxa have metareticulate pollen. Metareticulate pollen is formed by vaulted mesoporia and sunken pores. Mesoporia seems to be an informative character because genera in the family have different vaultation. According to Borsch (1998) pollen with flat mesoporia occur in the genera *Achyropsis*, *Achyranthes*, *Amaranthus*, *Centrostachys*, *Leucosphera*, *Pupalia* and *Sericorema*; broadly vaulted mesoporia occurs in *Achyranthes*, *Achyropsis*, *Amaranthus*, *Centrostachys*, *Charpentiera*, *Iresine*, *Irenella*, and *Woehleria*. Pollen with acute vaulted mesoporia are present in the genera: *Alternanthera*, *Blutaparon*, *Charpentiera*, *Gossypianthus*, *Guilleminea*, *Iresine*, *Irenella*, *Kyphocarpa*, *Lithophila*, *Pedersenia*, *Pfaffia*, *Pseudoplantago*, and *Woehleria*. Pollen with extremely narrow mesoporia occur in *Froelichia*, *Gomphrena*, *Hebanthe*, *Tidestromia*, and *Xerosiphon*.

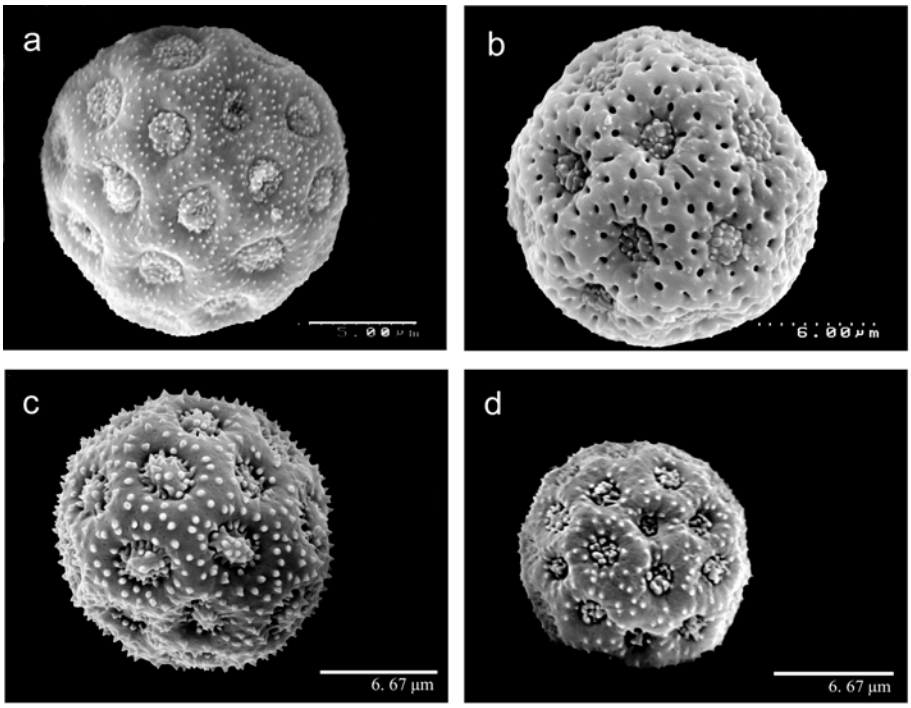

Fig. 38. Pollen types. *Iresine*-**type:** a. *Centrostachys*, b. *Sericorema*, c. *Iresine*, d. *Woehleria*.

The completeness of the tectum layer on the pollen grain, tectum ornamentation, and distribution of that ornamentation on tecta are variable characters in this study. *Achyranthes*, *Achyropsis*, *Alternanthera*, *Blutaparon vermiculare*, *Centrostachys*, *Gomphrena elegans*, *G. vaga*, *Hebanthe*, *Irenella*, *Iresine*, *Kyphocarpa*, *Leucosphera*, *Pedersenia*, *Pfaffia*, *Pseudoplantago*, *Pupalia*, *Sericorema*, *Tidestromia*, and *Woehleria* have continuous tectum on the lateral sides of the mesoporia, and no columellae can be seen from outside. *Froelichia*, *Gomphrena*, *Gossypianthus*, *Guilleminea*, *Lithophila*, and *Xerosiphon* have pollen grains with the tectum only distally present, and thus exposed columellae. Tecta ornamentation can be of two types: psilate or ornamented with microspines. *Gomphrena* [excluding *Gomphrena elegans* (Fig. 41b) and *G. vaga* (Fig. 41c)], *Gossypianthus*, *Guilleminea*, *Lithophila*, and *Tidestromia* (except for *Tidestromia lanuginosa* subsp. *eliassoniana*) have pollen unornamented. The rest of the species sampled have pollen ornamented with microspines. Tecta can be covered by microspines which can be either conical or have convex or concave flanks and with different lengths (Borsch 1998). The microspines can be arranged in: a) rows at the distal part of the mesoporia, which is the most common condition (Fig. 39a-c, 41a-c), b) arranged around the pores (Fig. 41d) or c) evenly over the total tectum surface (Fig. 38, 41e). *Pseudoplantago* has microspines distributed around the apertures; *Irenella*, *Iresine*, *Woehleria* and all the outgroup taxa have microspines distributed evenly over the total tectum surface and the remaining taxa have microspines distributed in rows at the distal region of the mesoporia. Finally, stellate pore ornamentation was defined as flecks of ektexine (Skvarla and Nowicke 1976) or ektexinous bodies that project in hooks (Borsch

1998) and cover the pore membrane (Fig. 41d, e). This character is exclusively found in the genus *Pseudoplantago* and the outgroup taxon *Pupalia* in the Gomphrenoideae.

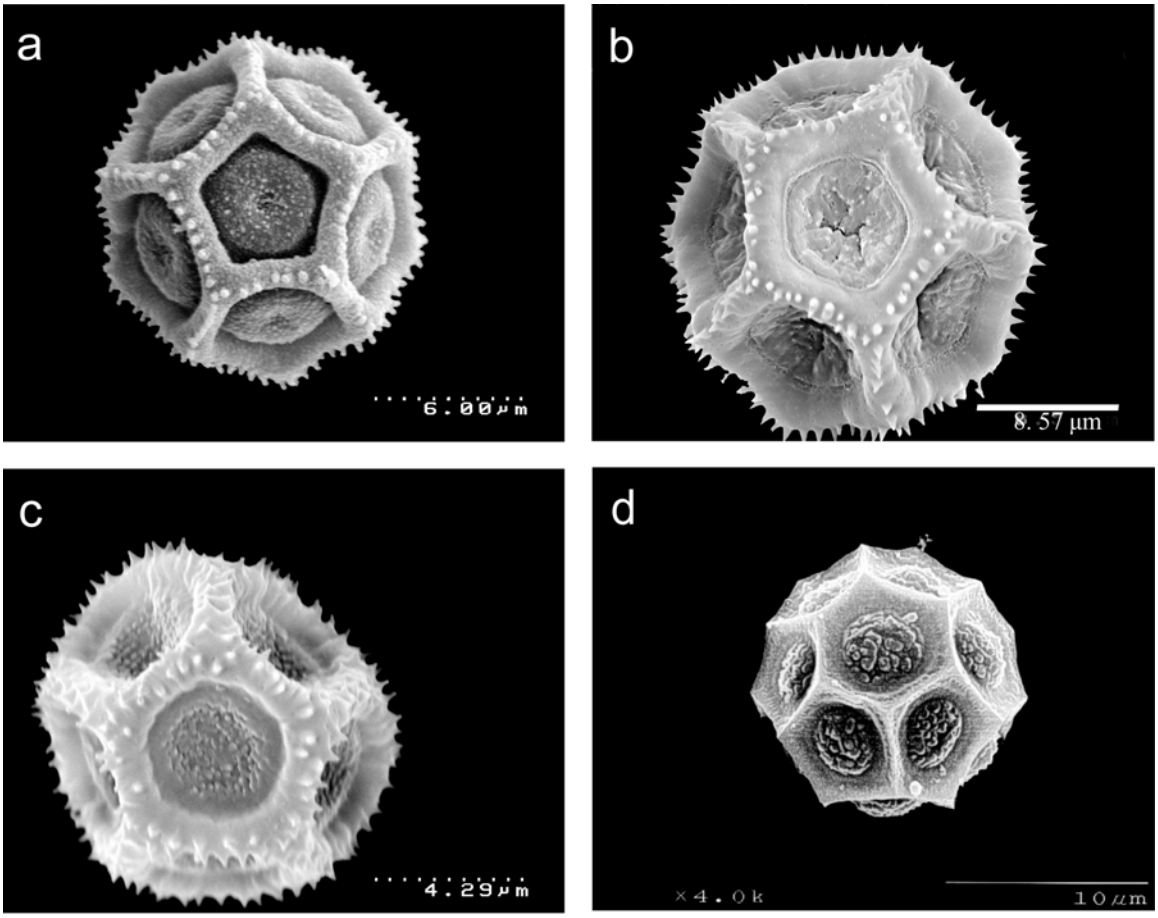

Fig. 39. Pollen types. *Pfaffia*-**type with dodecahedric form:** a. *Alternanthera* sp., b. *Alternanthera olivacea*, c. *Pedersenia*, d. *Tidestromia*.

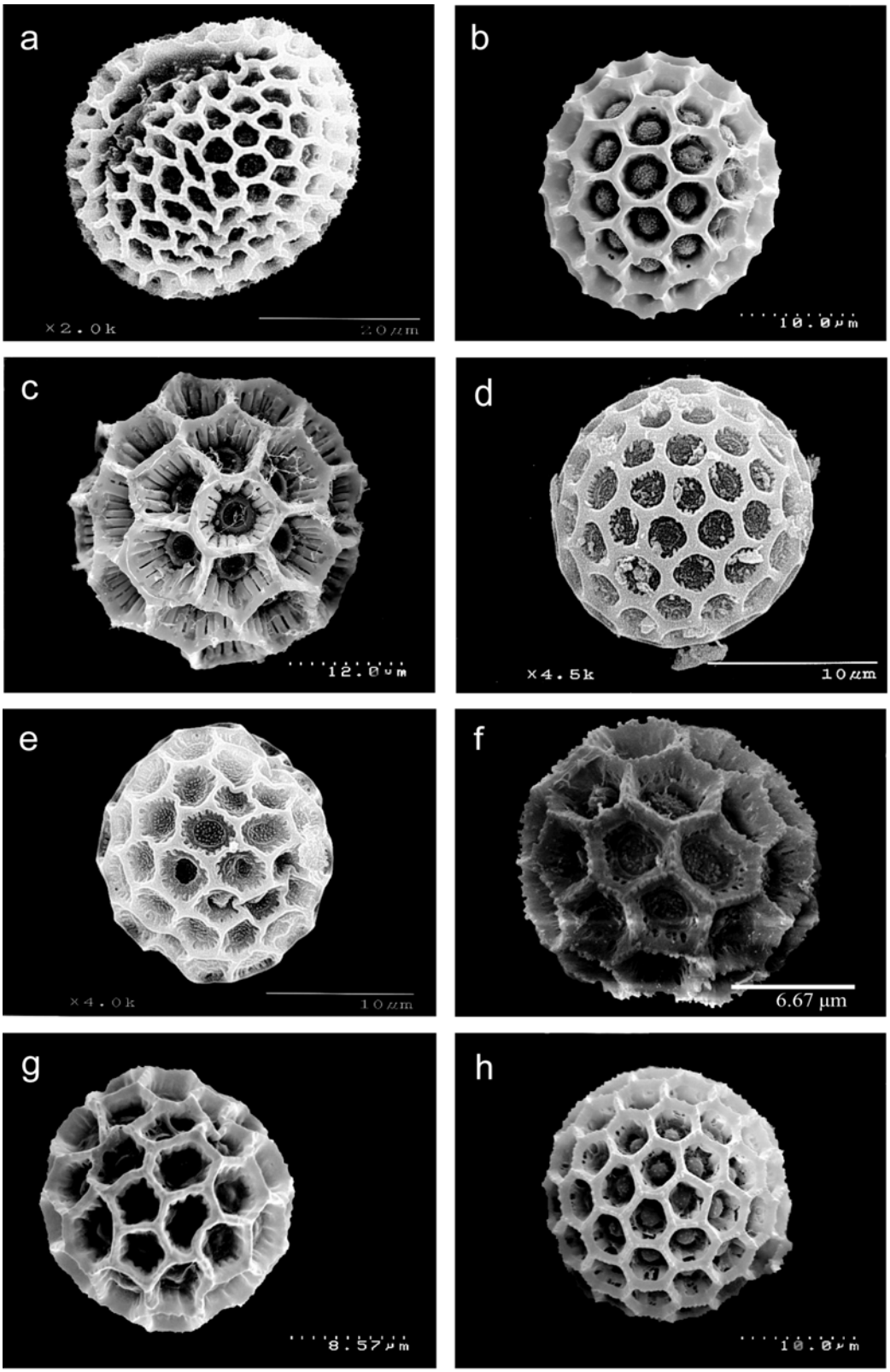

Fig. 40. Pollen types. *Gomphrena*-**type:** a. *Froelichia*, b. *Gomphrena graminea*, c. *Gomphrena flaccida*, d. *Gossypianthus*, e. *Guilleminea*, f. *Hebanthe*, g. *Lithophila*, h. *Xerosiphon*.

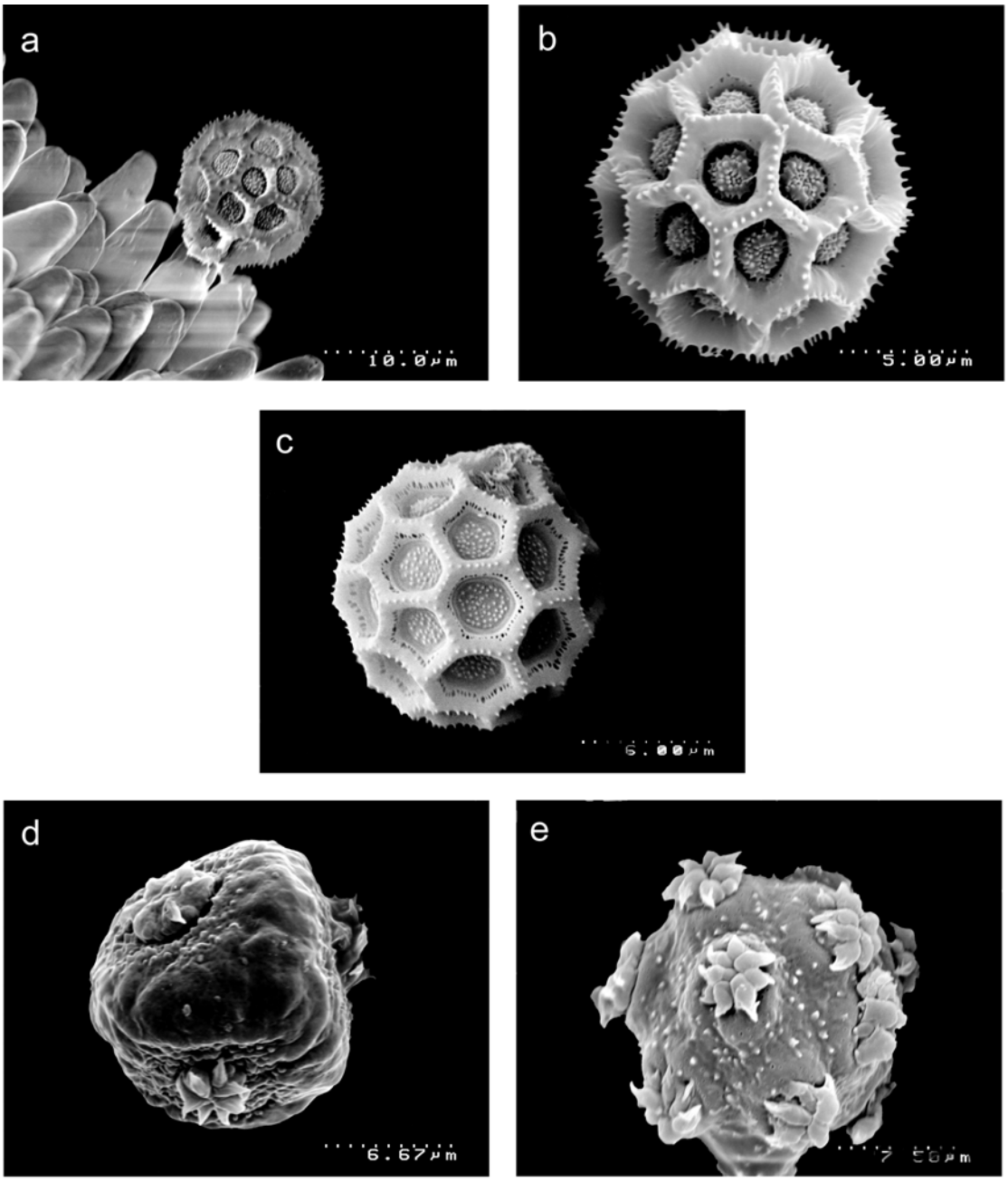

Fig. 41. Pollen types. *Pfaffia*-**type with spherical form:** a. *Pfaffia*, b. *Gomphrena elegans*, c. *Gompherna vaga*. **Stellate pore ornamentation:** *Pseudoplantago*-**type:** d. *Pseudoplantago*. *Psilotrichum*-**type:** e. *Pupalia*.

5.3.3. Character evolution.

The optimization of 18 morphological characters on the consensus tree is described on the next paragraphs.

Anthers (Fig. 42)

Two conditions of anthers characterize the family Amaranthaceae, tetrasporangiate and bisporangiate. In a phylogenetic analysis where these two states were coded, bisporangiate anthers are derived in the subfamily Gomphrenoideae and tetrasporangiate anthers are the plesiomorphic condition.

Pseudostaminodia (Fig. 43)

Mapping pseudostaminodia (present vs. absent) on the combined tree indicates that presence of pseudostaminodia is plesiomorphic in the subfamily Gomphrenoideae. Since sampling is limited in the subfamily Amaranthoideae, the evolution of this character is ambiguous in the family. It does appear that the loss of pseudostaminodia has independently occurred at least six times in the subfamily Gomphrenoideae (two times within the Iresinoid clade and four within the Gomphrenoids). *Iresine heterophylla* and *Tidestromia* are dimorphic for this character.

Pseudostaminodia form (Fig. 44)

Optimization of pseudostaminodia morphology (triangular, oblong, lanceolate, cordate, and lobed) suggests that the lobed condition is plesiomorphic. The monophyletic *Tidestromia* is the only clade supported by the lobed condition. However, *Tidestromia rhizomatosa* has lanceolate pseudostaminodia and the infraspecific taxa are polymorphic (lobed and triangular) for this character. The cordate pseudostaminodia are derived from lanceolate pseudostaminodia and this character is a synapomorphy for *Pedersenia*. The lanceolate pseudostaminodia condition is the most common within the Gomphrenoideae. Oblong pseudostaminodia present in *Iresine* sp./ *Iresine arbuscula* subclade are derived. In general, cordate and oblong pseudostaminodia are derived only a single time, whereas

the rest of the types of pseudostaminodia originated several times within Gomphrenodieae (triangular two times, and lanceolate five times).

Pseudostaminodia size (Fig. 45)

Optimization of pseudostaminodia size (shorter than filaments, 2/3 longer or more than filaments, equal to filaments, and 1/3 longer than filaments) revealed that the plesiomorphic condition within the Gomphrenoideae is shorter than filaments. Pseudostaminodia 1/3 longer than filaments and pseudostaminodia 2/3 longer or more than filaments occurred at least two times each in the Gomphrenoideae, once in *Alternanthera*, and once in the Gomphrenoids. Pseudostaminodia as long as filaments is derived in *Alternanthera olivacea* and *Tidestromia rhizomatosa*. Some species of *Alternanthera* are polymorphic (2/3 longer or more, equal to or 1/3 longer than filaments) for pseudostaminodia size.

Pseudostaminodia margin (Fig. 46)

Optimization of margin types of pseudostaminodia (entire, ciliate, laciniate, and crenate) has occurred several times in the evolution of Gomphrenoideae. Entire pseudostaminodia is the plesiomorphic condition and occurs in Iresinoids, Alternantheroids and in most Gomphrenoids. The switch to laciniate pseudostaminodia occurs in *Alternanthera*. Ciliate pseudostaminodia are derived in some species of *Iresine*. Crenate pseudostaminodia evolved twice, once in *Alternanthera* and a second time in *Iresine*. *Alternanthera caracasana* is polymorphic (entire and crenate) for the margin condition.

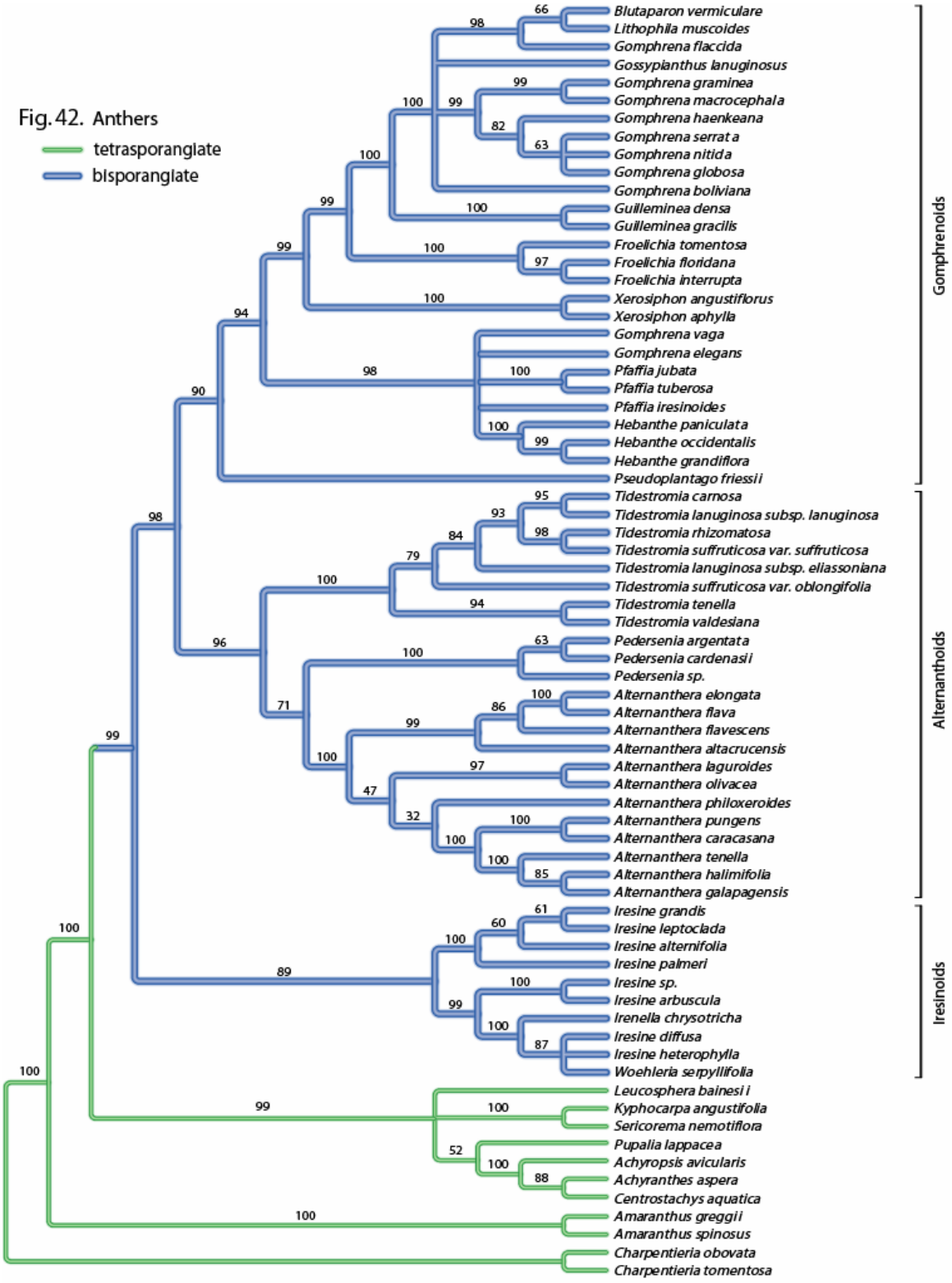

Fig. 42. Binary reconstruction of anthers dehiscence

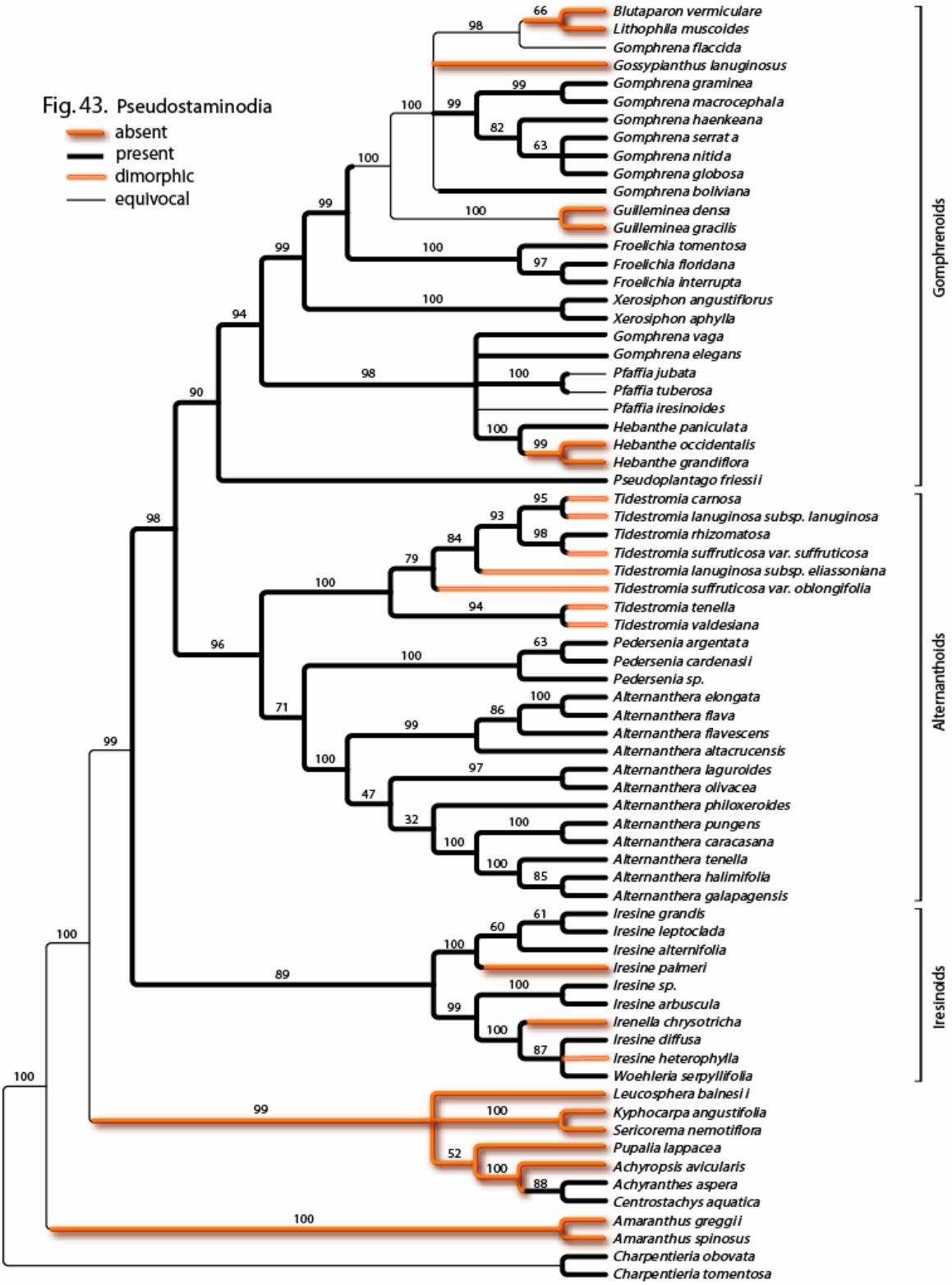

Fig. 43. Binary reconstruction of pseudostaminodia

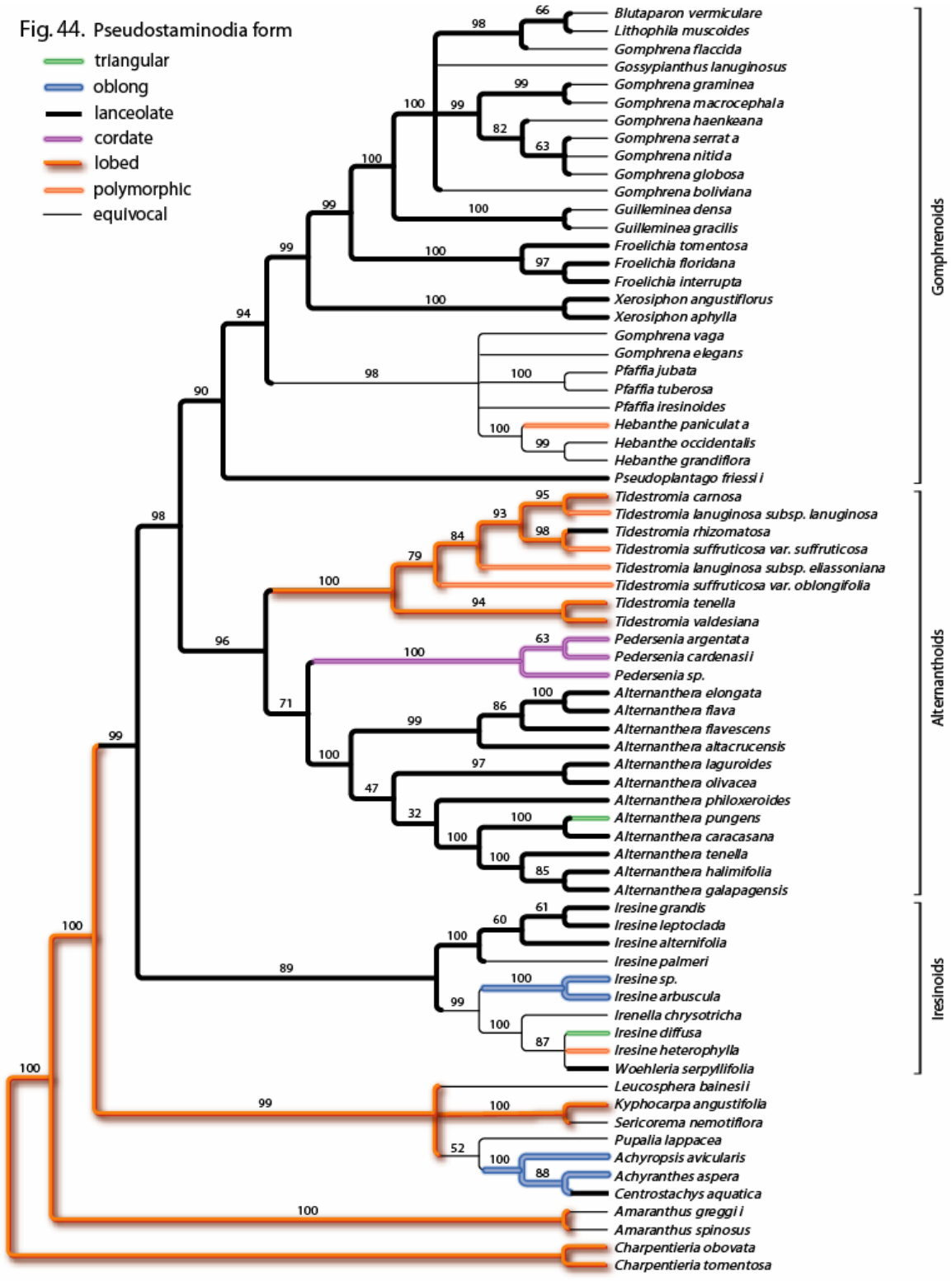

Fig. 44. Multistate reconstruction of pseudostaminodia form

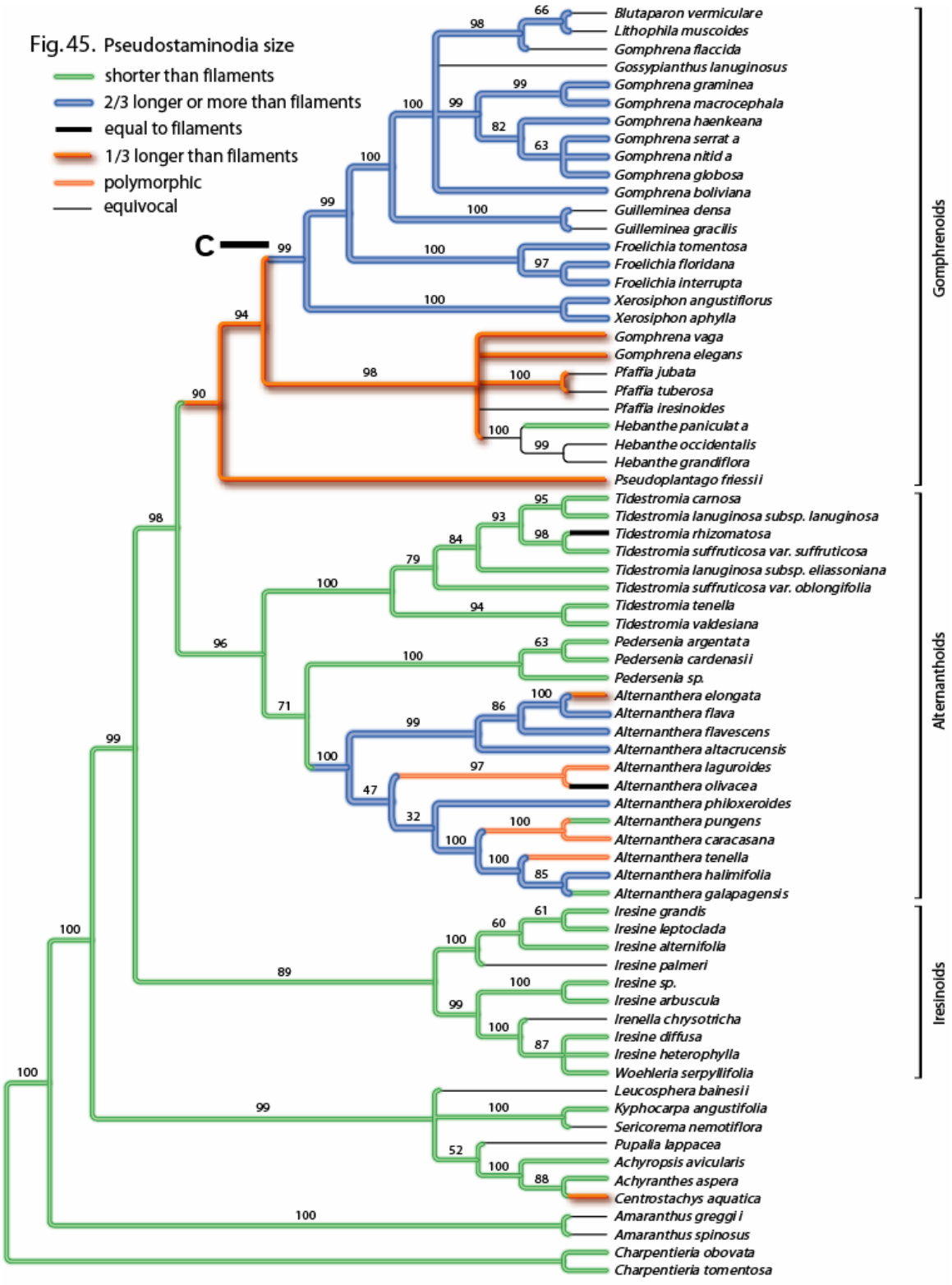

Fig. 45. Multistate reconstruction of pseudostaminodia size

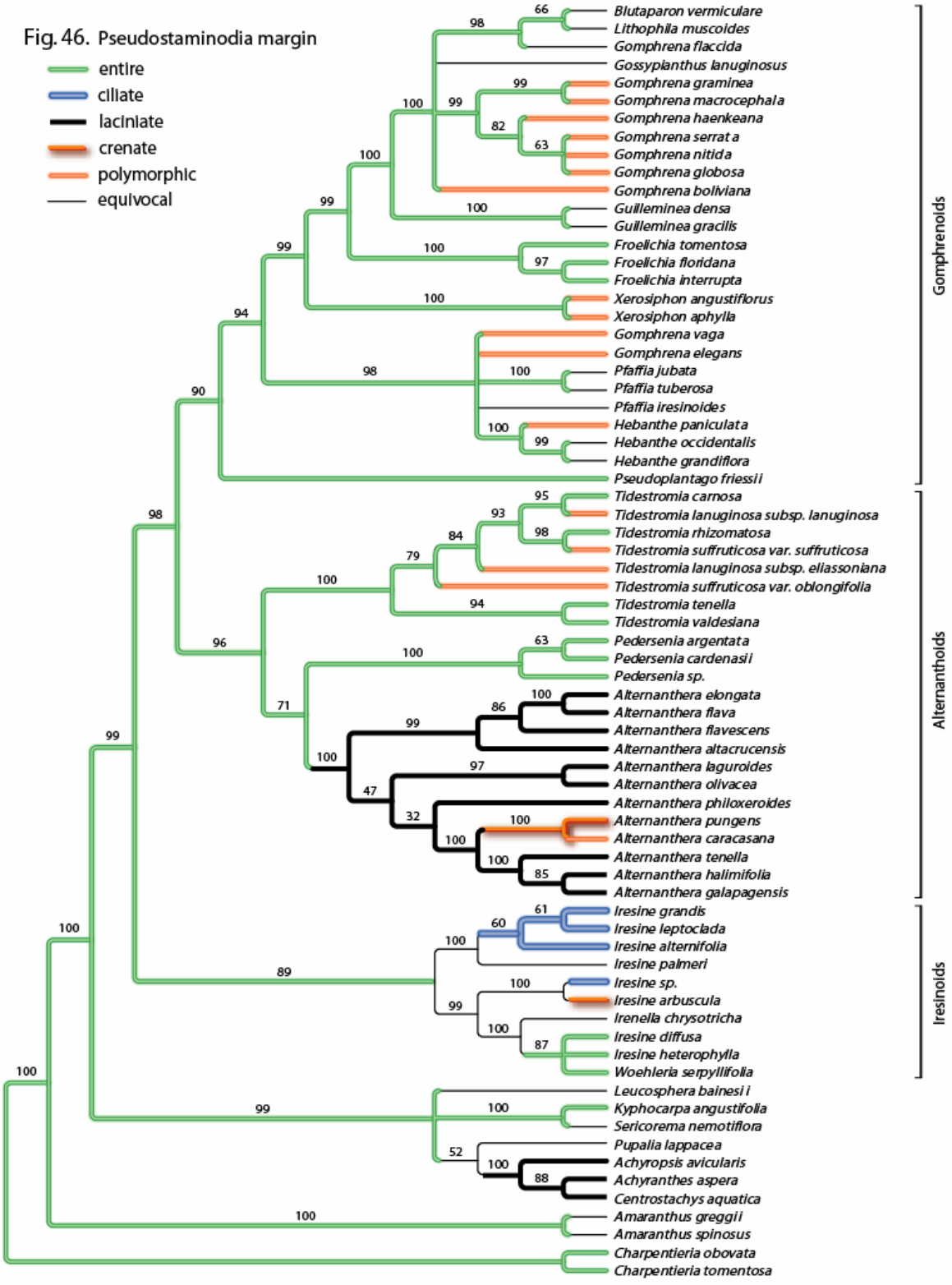

Fig. 46. Multistate reconstruction of pseudostaminodia margin

Filaments enclosed by pseudostaminodia (Fig. 47)

This study suggests that filaments enclosed by pseudostaminodia (absent vs. present) are exclusive of the genus *Gomphrena* which has several modifications in this structure. Also, it is suggested that the character filaments enclosed by pseudostaminodia is distributed among multiple species of the genus *Gomphrena*. Therefore, filaments enclosed by pseudostaminodia may have originated repeatedly within this genus. However, because it does not occur in any other clade, it is a derived condition within the Gomphrenoideae.

Filaments and pseudostaminodia length adnation (Fig. 48)

Optimization of filaments free or adnated to pseudostaminodia (in a staminal cup, staminal tube, and free to base) suggests that evolution of staminal cups is the plesiomorphic condition and staminal tubes are derived at least two times in the Gomphrenoid clade (subclade C and *Gomphrena elegnas*). Three reversals to the plesiomorphic condition occurred in the Gomphrenoids. The genus *Amaranthus* has filaments free to base.

Stigma form (Fig. 49)

Optimization of stigma shape (entire, bilobed, and trilobed) revealed the bilobed condition as the plesiomorphic condition and entire stigmas as the derived condition. The derived condition occurred two times within the Gomphrenoideae, once in *Alternanthera* and a second time in *Pseudoplantago friessii*. The loss of the plesiomorphic condition occurred in the Gomphrenoids, Iresinoids, *Tidestromia*, and *Pedersenia*. The taxon *Tidestromia suffruticosa* var. *oblongifolia* has a dimorphic condition (lobed and trilobed) stigma condition, which also occurs in the outgroup genus *Amaranthus*.

Papillae distribution (Fig. 50)

Optimization of the character papillae distribution along the stigmatic surface (throughout the stigma and inner sides of the stigma) indicates that the plesiomorphic condition was papillae distributed on the inner sides of the stigma, whereas papillae distributed throughout the stigma is the derived condition. However, the plesiomorphic condition reappears within the Gomphrenoideae in *Guilleminea* and *Gossypianthus*.

Style size (Fig. 51)

Optimization of style size is considered relevant to the Gomphrenoids (sessile, short, and long). Small styles are the most common condition and considered plesiomoprhic. The derived conditions sessile evolved three times in the Gomphrenoideae, within the Gomphrenoids. The long styles derived from short styles occur twice in the Gomphrenoids, once in *Pseudoplantago*, and second in *Froelichia*.

Pollen form (Fig. 52)

Optimization of pollen shape (spheroidal, dodecahedric, and cubic) described in Gomphrenoideae suggest that spheroidal pollen is plesiomorphic in the subfamily and two changes occurred in the group, one in the genus *Pseudoplantago*, which has the derived cubic pollen, and the second in the Alternanthoid clade with the derived condition dodecahedric pollen.

Pollen structure (Fig. 53)

Optimization of pollen structure (metareticulate and non-metareticulate) resolved in this is study that the plesiomorphic condition is non-metareticulate pollen. This condition appears in the Iresinoid clade whereas the metareticulate pollen is a derived condition for the Alternanthoid and Gomphrenoid clades.

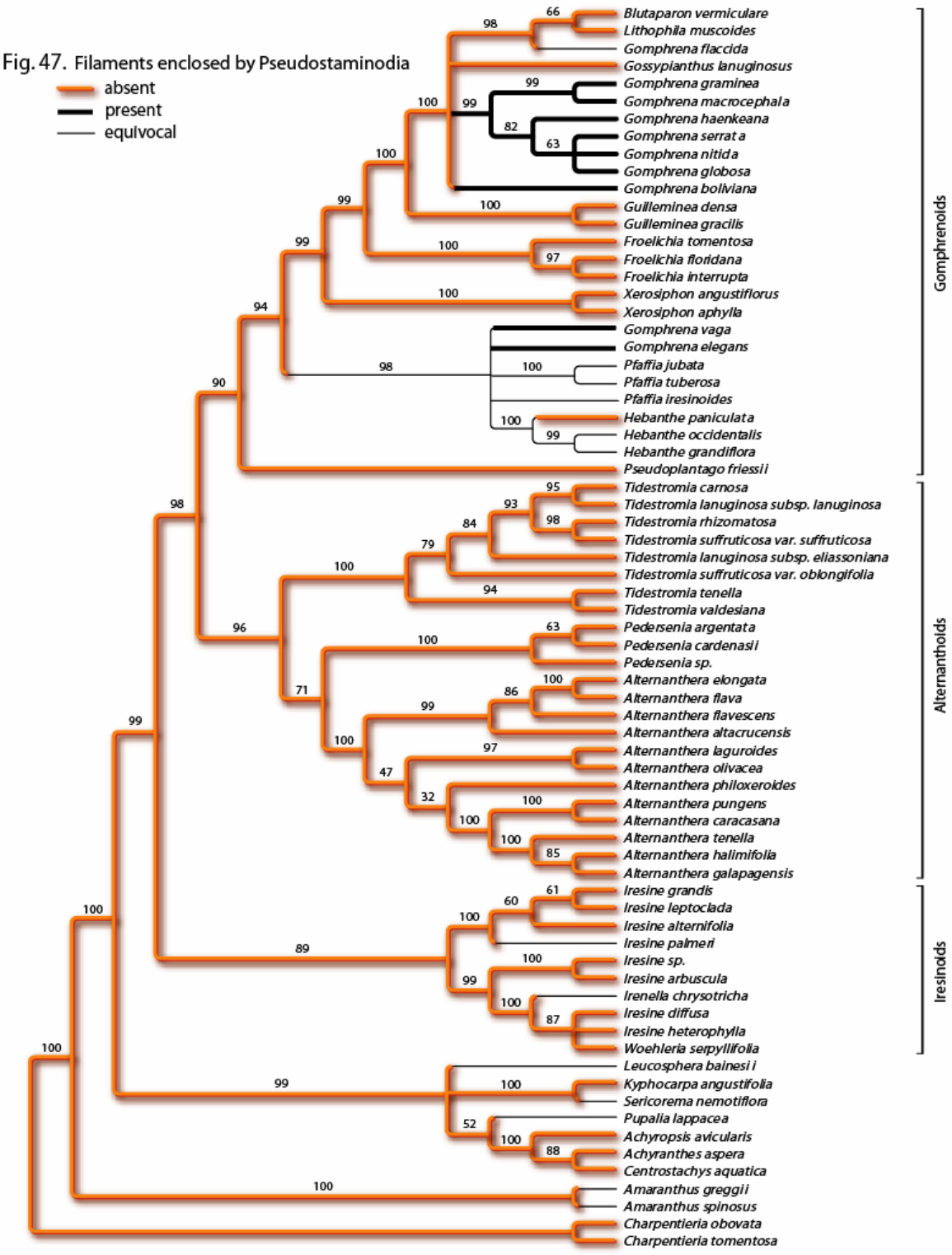

Fig. 47. Multistate reconstruction of filaments enclosed by pseudostaminodia

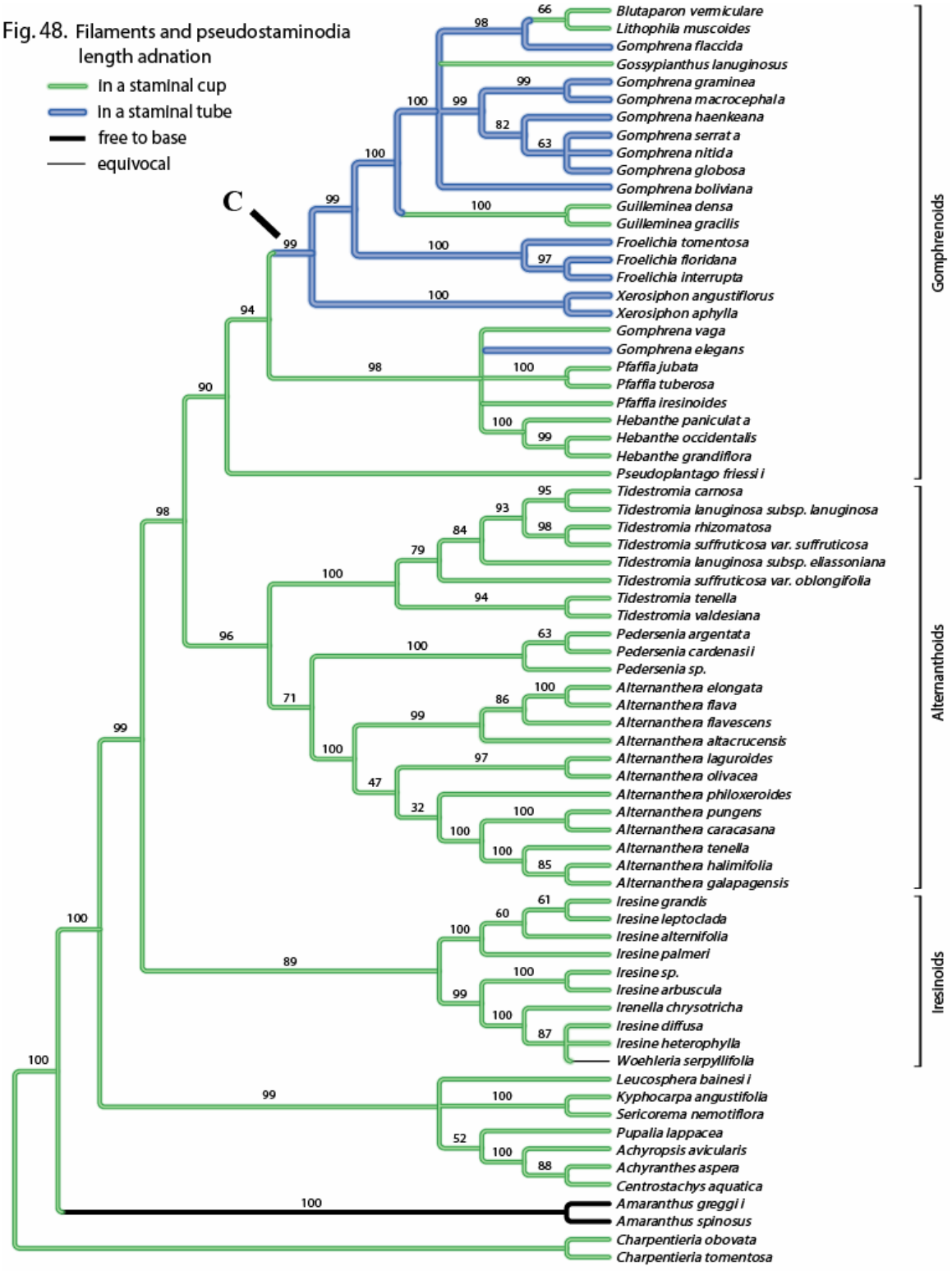

Fig. 48. Multistate reconstructionof filaments and pseudostaminodialength adnation

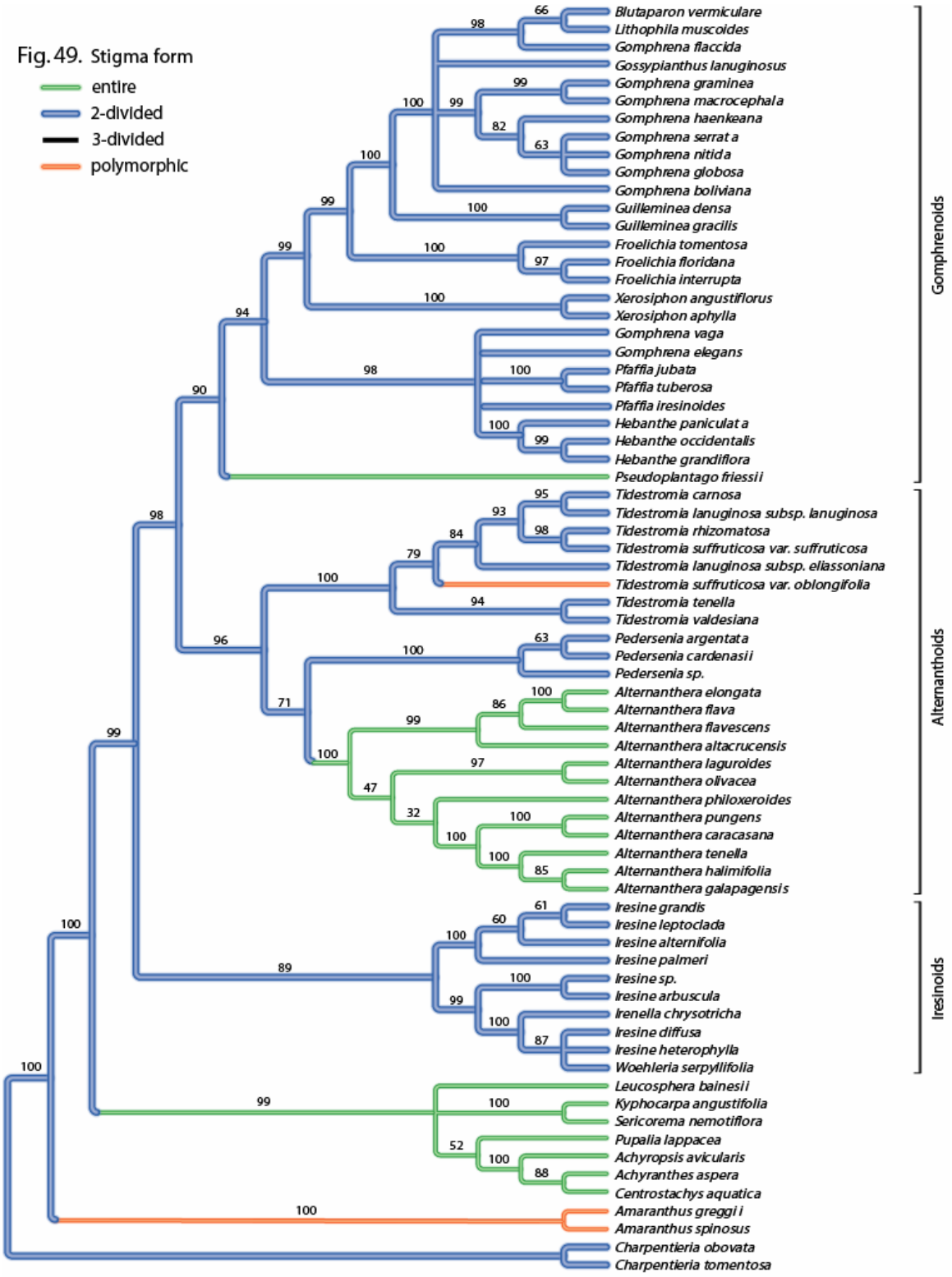

Fig. 49. Multistate reconstruction of stigma form

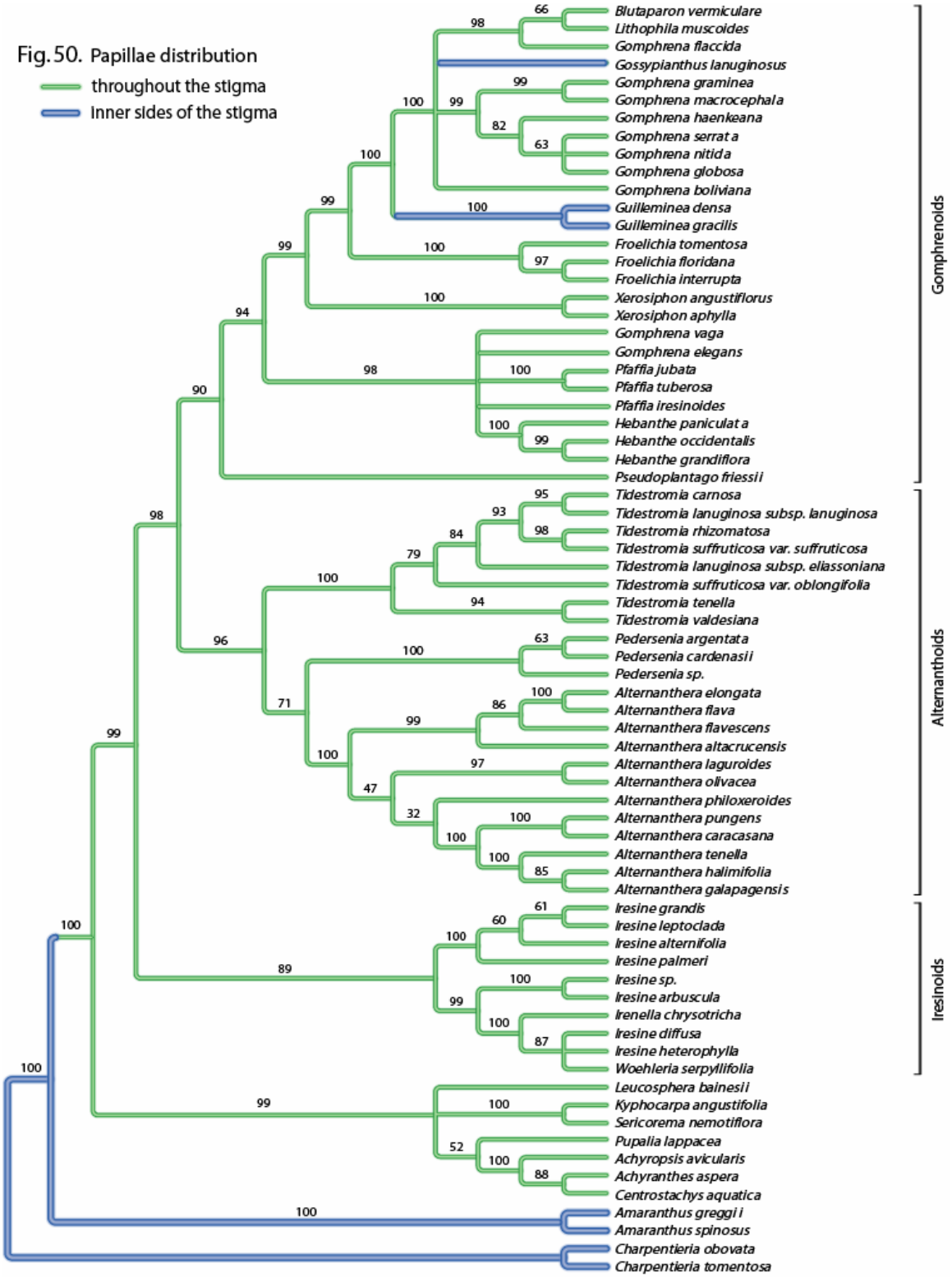

Fig. 50. Binary reconstruction of papillae distribution

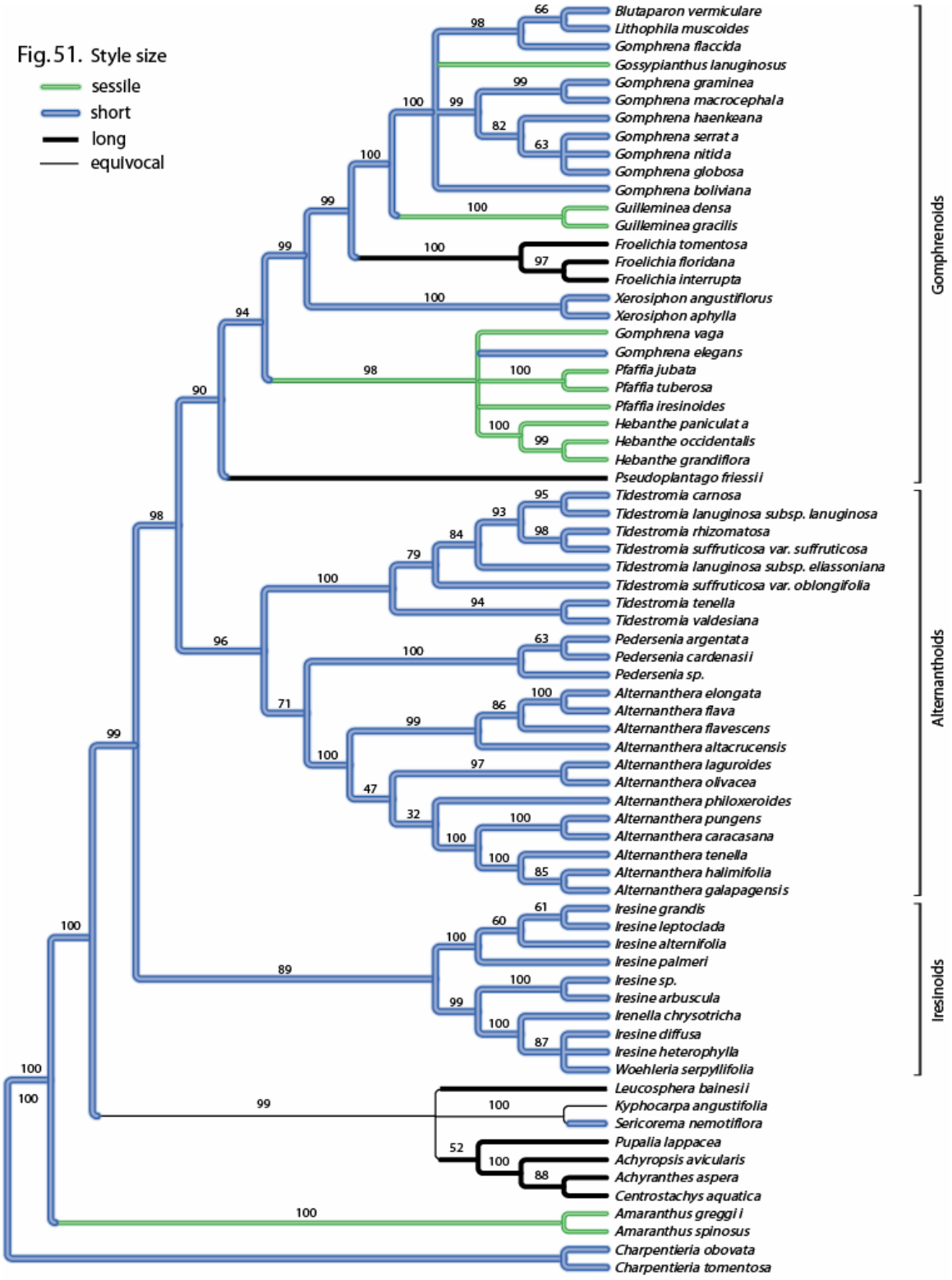

Fig. 51. Multistate reconstruction of style size

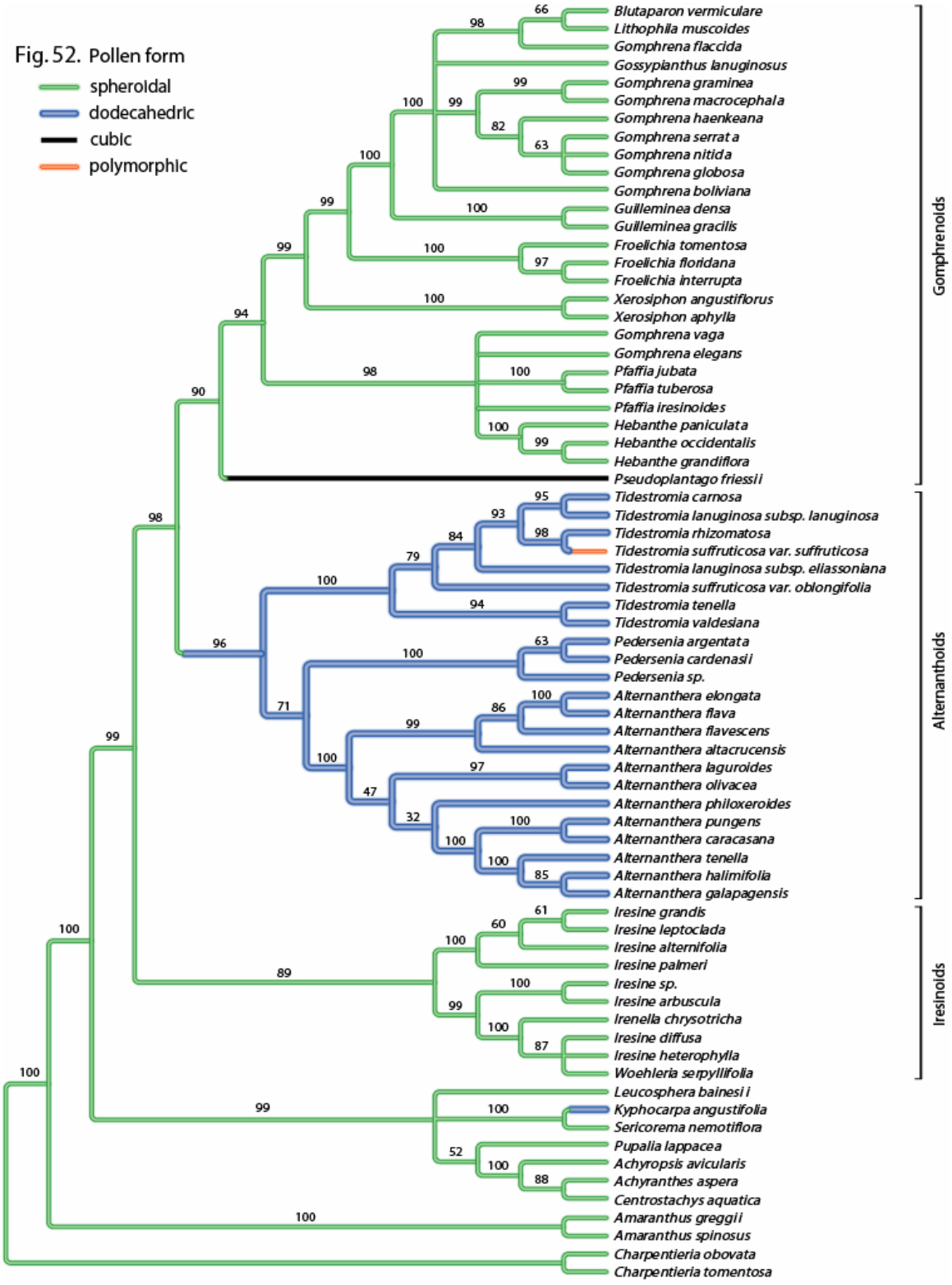

Fig. 52. Multistate reconstruction of pollen form

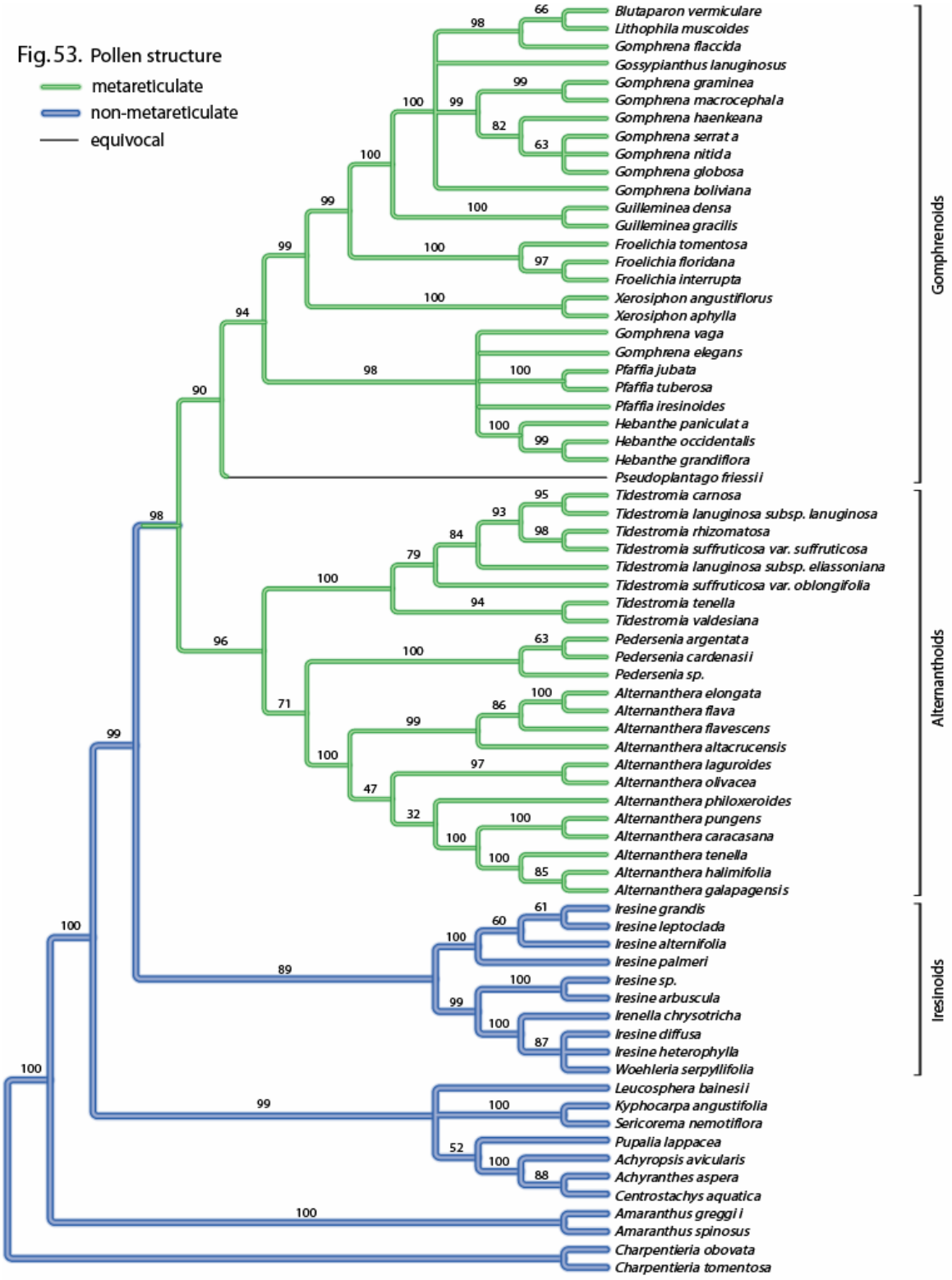

Fig. 53. Binary reconstruction of pollen structure

Tectum characters (Fig. 54)

In this study tecta were evaluated as complete or present only in distal bands. Character optimization suggests that the plesiomorphic condition is tecta complete, whereas tecta in distal bands is the derived condition that occurs in the subclade C within the Gomphrenoid clade. The plesiomorphic condition occurs in *Blutaparon vermiculare* within the subclade C.

Pollen ornamentation (Fig. 55)

In the optimization of tectum layer considered either psilate or ornamented. The psilate pollen evolved independently in two clades: the *Tidestromia* clade and the subclade D within the Gomphrenoideae. However, within the *Tidestromia* clade the reversal to plesiomorphic condition occurred in *T. lanuginosa* subsp. *eliassoniana* (with ornamented pollen) and in *Blutaparon vermiculare* within the subclade D.

Microspines distribution (Fig. 56)

Tecta can be covered by microspines arranged in different patterns (distal row, around the apertures, and evenly spread). In the phylogenetic optimization of this character, the plesiomorphic condition is microspines evenly distributed. The derived condition, the distribution of microspines in rows at the distal part of the mesoporia occurred in both clades Alternanthoid and Gomphrenoid, and microspines distributed around the apertures only occurred in *Pseudoplantago*.

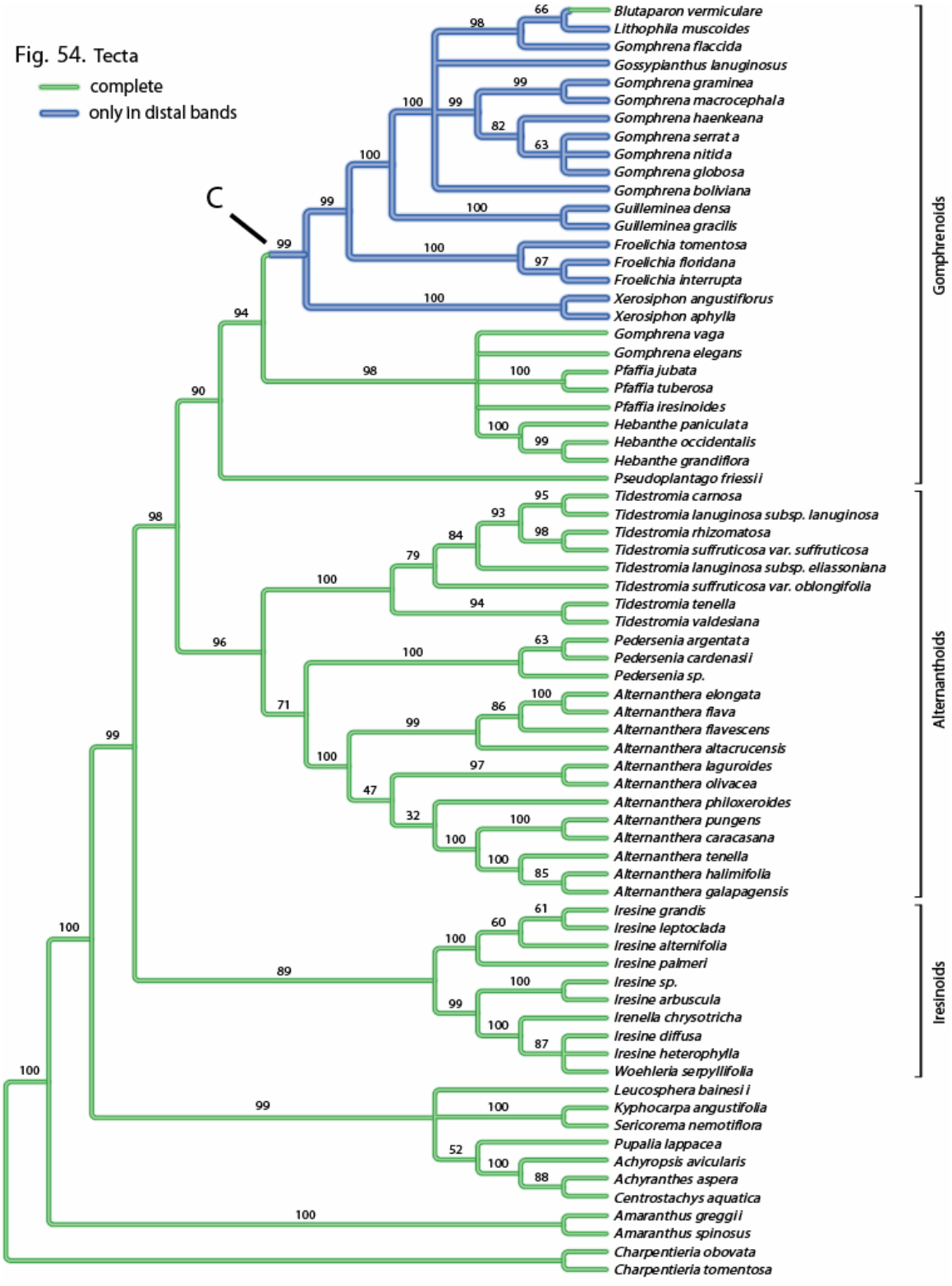

Fig. 54. Binary reconstruction of tectum

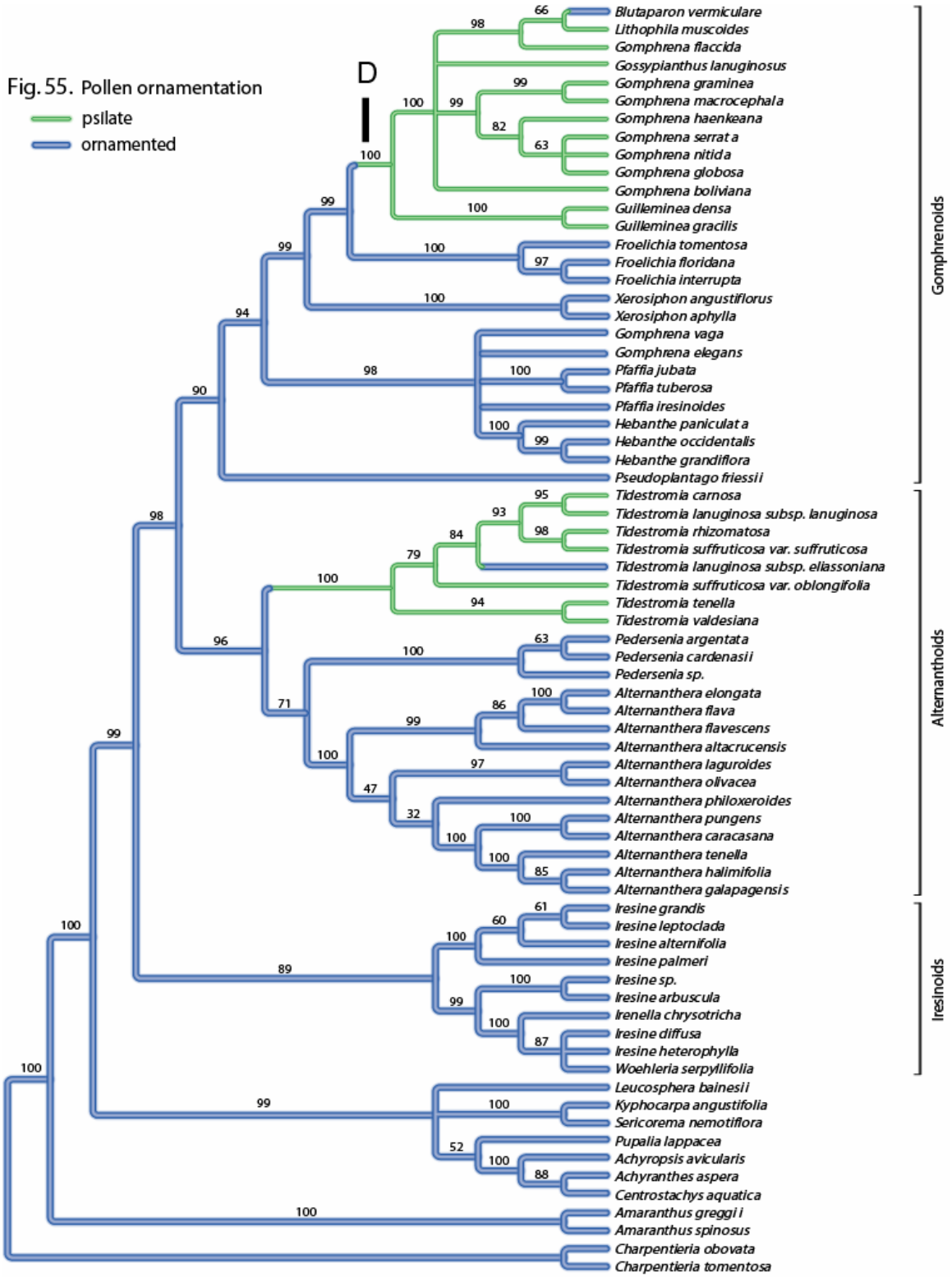

Fig. 55. Binary reconstruction of pollen ornamentation

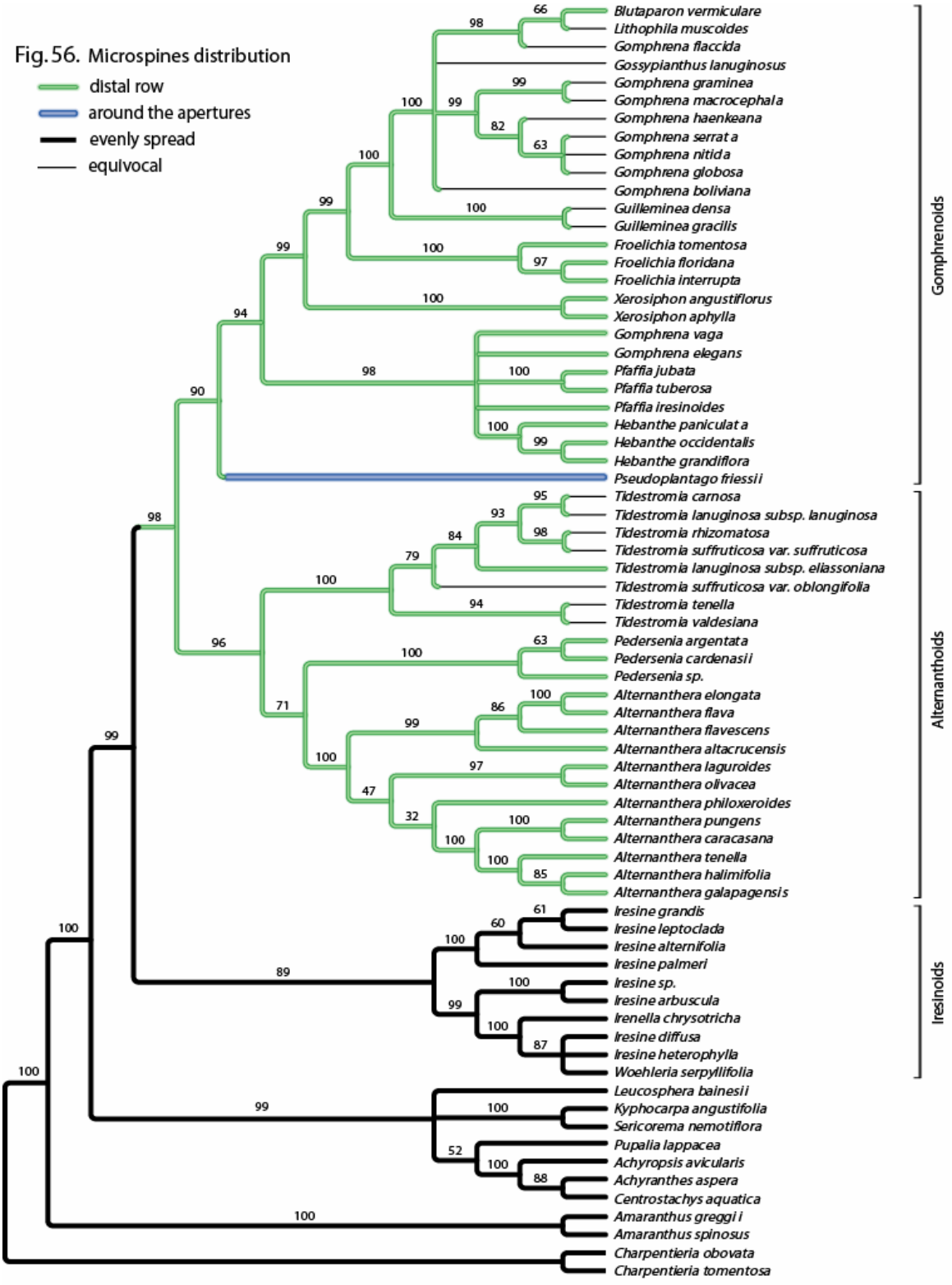

Fig. 56. Binary reconstruction of microspines distribution

Fig. 57. Multistate reconstruction of mesoporia vaultation

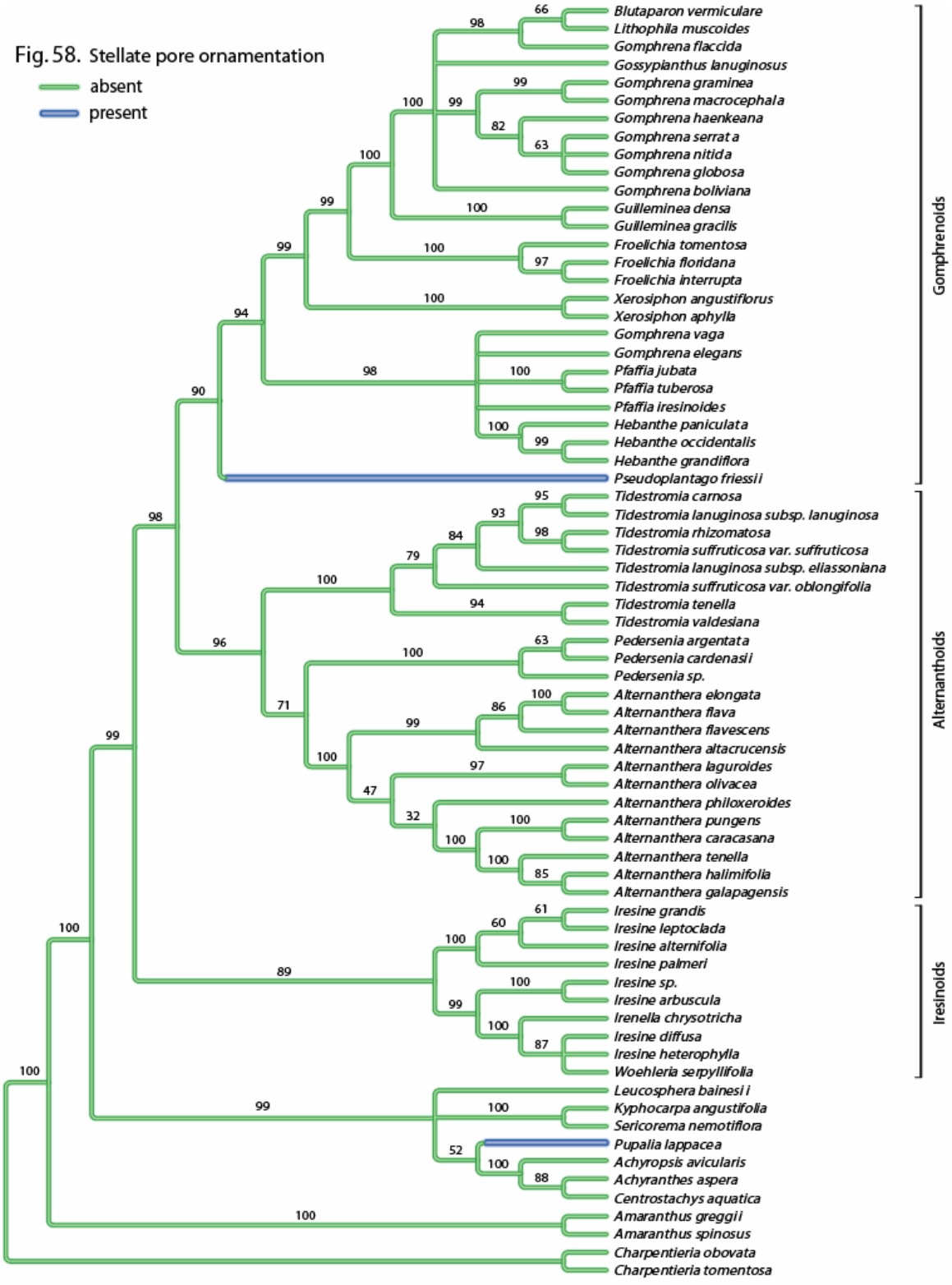

Fig. 58. Binary reconstruction of star pore ornamentation

Mesoporia vaultation (Fig. 57)

Mesoporia have four variable degrees of vaultation recognized in this study (Borsch 1998; flat, broadly vaulted, acute vaulted, and extremely narrow). The lack of resolution in the combined tree prevents the determination of the plesiomorphic condition. However, the condition extremely narrow vaultation evolved at least two times, once in *Tidestromia* and a second time within the Gomphrenoids. The acute vaulted condition occurred at least seven times within the Gomphrenoideae, once in the Alternanthoids, and six times in the Gomphrenoids.

Stellate pore ornamentation (Fig. 58)

Optimization of stellate pore ornamentation (absent and present) condition suggested that presence of stellate ornamentation occurs in parallel in the subfamily Amaranthoideae (outgroup taxa) and *Pseudoplantago* within the subfamily Gomphrenoideae.

5.4. DISCUSSION

States of floral evolution in the Amaranthaceae have focused on androecium characters rather than gynoecium structures (e. g. Eliasson 1988; Borsch 1998). These are because of the greater morphological diversity within the androecium, such as anthers dehiscence, pseudostaminodia variation, and pollen, whereas the gynoecium appears to be a less variable structure. The androecium and gynoecium characters explored in this study are documented and discussed with emphasis on the main objectives of this study.

Evolution of anthers

The first objective of this study is to test trends in evolution of anthers. The oldest hypothesis on androecium evolution in the family perhaps corresponds to Schinz (1934) who defined two subfamilies based on anther locules, but he did not clearly specify an evolutionary trend. The character states among anthers are either bisporangiate or tetrasporangiate (Appendix 3, character 1). The Schinz classification is still thought to be the most natural one, and many authors (e.g. Eliasson 1988; Townsend 1993; Borsch 1998) and molecular studies (Kadereit *et al.* 2003; Müller and Borsch 2005) support it. These molecular studies suggested that the origin of bisporangiate anthers is a parallel event in the core Gomphrenoideae and the unresolved *Iresine*. The genus *Iresine* was classified by Schinz (1934) as part of the Gomphrenoideae because of their bisporangiate anthers. However, previous molecular studies (Kadereit *et al.* 2003; Müller and Borsch 2005) resolved *Iresine* as more closely related to the subfamily Amaranthoideae or as unresolved. This study suggests that character bisporangiate anthers supports the monophyly of subfamily Gomphrenoideae including *Iresine*, whereas the rest of the outgroup taxa (subfamily Amaranthoideae) have tetraesporangiate anthers. Therefore, the character bisporangiate anthers is derived from the plesiomorphic condition of tetraesporangiate anthers. In addition, the controversial *Pseudoplantago* has morphological characters shared with the Gomphrenoideae and the Amaranthoideae (Eliasson 1998; Kadereit *et al.* 2003; Müller and Borsch 2005). The position of *Pseudoplantago* in the Gomphrenodieae is supported in this study by the presence of bisporangiate anthers and several shared molecular characters (Fig. 29). The position of *Pseudoplantago* within their “core Gomphrenoideae” of Müller and Borsch (2005) is also

confirmed based on this data. Therefore, Schinz's confirmation and supports ideas about two natural groups in the Amaranthaceae based on anther locules.

Evolution of pseudostaminodia

The second objective of this study is related to the hypotheses of Eliasson (1988) about evolutionary trends in the pseudostaminodia development. Two aspects can be divided from Eliasson hypotheses: the phylogenetic and the morphological aspects. The phylogenetic aspect is explained in this section and morphological aspects are explained in the section entitled pseudostaminodia homology.

Eliasson's (1988) first hypothesis suggested division and fusion of pseudostaminodia and the extent and type of fusion of these parts (Fig. 59). He considered that perhaps two apical filament appendages in *Gomphrena* (Fig. 59 [1a]) were fused along most of their length and would result in a structure reminiscent of the pseudostaminodium that occurs in *Pseudogomphrena* (Fig. 59 [1b]). An additional fusion could give rise to the pseudostaminodium present in *Froelichia* (Fig. 59 [1c]).

The second hypothesis he suggested was a progression in the reduction of filament size, increase in pseudostaminodia size and fusion of pseudostaminodia with filaments forming staminal tubes. Eliasson (1988) considered that from pseudostaminodia of *Alternanthera* (Fig. 59 [2a]), the least advanced types in the family, derived the pseudostaminodia of *Froelichiella* (Fig. 59 [2b]). Reduction of filament length and fusion of pseudostaminodia with filaments would result in a staminal tube similar to that in *Froelichia* (Fig. 59 [2c]) and/or *Pseudogomphrena* (Fig. 59 [2d]). Pseudostaminodia of *Pseudogomphrena* are forked at the apex. Deeper division and decrease in a connation

distance between the pseudostaminodia would result in a staminal tube similar to that in *Gomphrena* (Fig. 59 [2e]).

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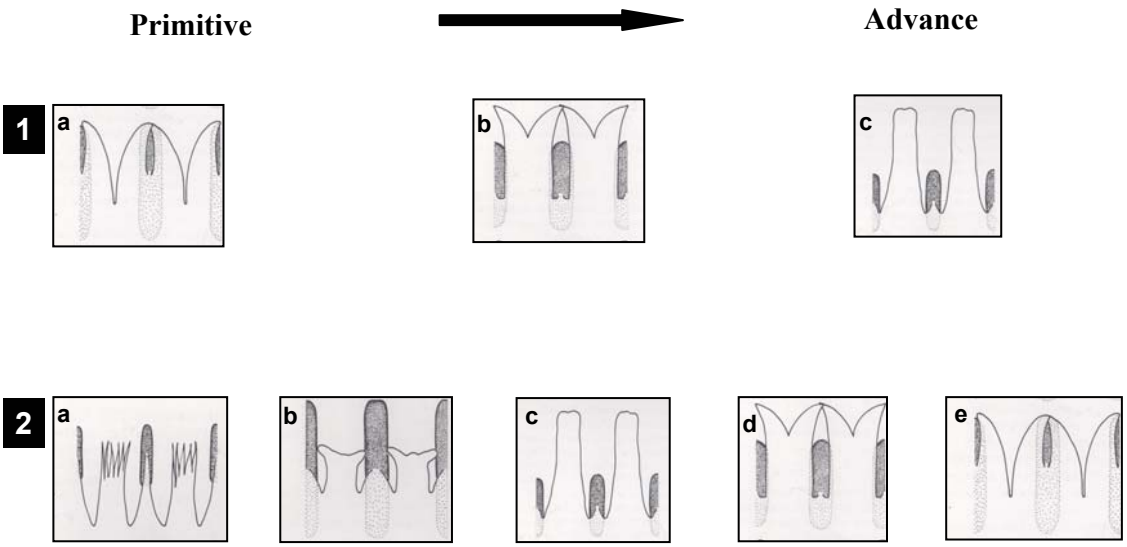

Fig. 59. Evolution of pseudostaminodia as hypothesized by Eliasson (1988) 1. The division and fusion of pseudostaminodia hypothesis. *Gomphrena* (1a), *Pseudogomphrena* (1b), and *Froelichia* (1c). **2.** The reduction of filament size and increase in pseudostaminodia size and fusion with filaments hypothesis. *Alternanthera* (2a), *Froelichiella* (2b), *Froelichia* (2c), *Pseudogomphrena* (2d), and *Gomphrena* (2e).

The characteristics pseudostaminodia length (character 4), filaments enclosed by pseudostaminodia (character 7) and androecium in staminal cup, staminal tube or free to base (character 8; Appendix 3), codified and optimized in this study are related to Eliasson's (1988) two hypotheses (Fig. 59). The results of the present analysis suggest that the development of long pseudostaminodia is derived in the Gomphrenoids clade from short pseudostaminodia (Fig. 45), but it is not in the subfamily Gomphrenoideae.

Filaments enclosed by pseudostaminodia are derived in the Gomphrenoideae (Fig. 47); and filaments fused in a staminal tube are derived (subclade C) from the plesiomorphic condition, staminal cups (although a few reversal to the plesiomorphic condition occur in subclade C; Fig. 48). It is interesting to note that *Alternanthera* and subclade C within the Gomphrenoids share the trait pseudostaminodia longer than filaments (Fig. 45). However, the difference is that *Alternanthera* filaments and pseudostaminodia are fused in a staminal cup, whereas the taxa in subclade C within Gomphrenoids have pseudostaminodia fused to the filaments to form staminal tubes (Fig. 48). These clades may share some morphological similarity, but are derived from a different development pathway. Therefore, considering the major changes we suggest that there is not evidence to support Eliasson's theories development of pseudotaminodia in the subfamily Gomphrenoideae.

Pseudostaminodia homology. The evaluation of the morphological component of Eliasson hypotheses deals with three main difficulties to define hypotheses of homology in the pseudostaminodia characters. The first problem is the definition of Pseudostaminodia. Staminodia or interstaminal appendages are other names for pseudostaminodia (Eliasson 1988). Eliasson (1988) suggested that staminodia are dentate structures present in *Lithophila* and *Woehleria*. These genera have one or two functional stamens and he suggested that the two or three dentate structures that come from the filament cups of these genera may be rudiments of stamen. The comparison using SEM of *Lithophila* and *Woehleria* (Fig. 33c) suggested that only *Woehleria* has staminodia based on form and size (*sensu* Eliasson 1988). Perhaps Eliasson (1988) suggested presence of pseudostaminodia in genera that have five functional stamens. However,

following the criteria of similarity of position, and morphology (Kitching *et al.* 1998), in this study it was considered that *Woehleria* structures are homologous to the structures called pseudostaminodia. It appears that there is not a real difference between staminodia or pseudostaminodia in the family Amaranthaceae, because staminodia definition and function (based on Stearn 1995; Walker-Larsen and Harder 2000) can be applied to structures called pseudostaminodia in the Amaranthaceae. However, Townsend (1993) suggested that the pseudostaminodia term, although unwieldy, is the preferred term because staminode is normally understood to imply a modified stamen which has become sterile and that the structures in Amaranthaceae are entirely. In this study, it was preferred to use the most conservative name; therefore these structures were called pseudostaminodia.

The second problem refers to the controversial hypotheses of homology suggested for the staminal tube in *Gomphrena*. *Gomphrena* has long staminal tubes with appendages at the end. These appendages have interpreted as apical parts of distally forked filaments, whereas other opinions suggested that these appendages are pseudostaminodia. Eliasson (1988) suggested that staminal tubes in *Gomphrena* are not different from those in *Pseudogomphrena*, *Froelichia*, and *Froelichiella*, whose interstaminal appendages he called pseudostaminodia. In addition, he hypothesized that perhaps these apical filament lobes in *Gomphrena* would be homologous with half a pseudostaminodium in other genera such as *Alternanthera* and *Froelichia*. Here we expose the difficulties to define the origin of appendages in *Gomphrena* and they can be extended to the genera *Froelichia*, *Froelichiella* and *Pseudogomphrena*. In this study, the SEM observation suggested that staminal appendages in *Gomphrena*, although

considered pseudostaminodia, are not similar to the rest of pseudostaminodia of other genera included in this study. *Gomphrena* filaments are enclosed by pseudostaminoida in an exclusive form that it was considered not homologous with any other type (character 7; Appendix 3).

The third problem refers to staminal structures in *Pfaffia*. This genus has strap-shaped filaments (Fig. 34f, g). Eliasson (1988) mentioned that wide filaments are partly of pseudostaminodial origin. Similar wide filaments are observed in *Gomphrena flaccida*. In this study, it was impossible to determine the origin of these structures, because they could be filaments or a fusion of pseudostaminodia and filaments. Therefore, these characters were coded as inapplicable.

In order to asses the morphological component of Eliasson hypotheses, it is critical to make more investigations exploring more specific tools such as ontogenetic studies. It is necessary to ascertain the ontogenetic origin of the morphological characters of pseudostaminodia to define the correct homologies which are basic to test the phylogenetic species componets of Eliasson hypotheses critically. Based on our analyses, there is little or no support for either of his androecial development hypotheses.

Evolution of stigmas

The second objective of this study was to explore trends in evolution of stigmatic characters. The characters in stigmas have been poorly studied. Among the three informative stigmatic characters none of them was relevant to propose a trend in the evolution in the Gomphrenoids.

Evolution of pollen

The third objective was to evaluate trends in pollen character evolution within the Gomphrenoideae with more comprehensive sampling among genera. Borsch (1998) mentioned that parallel evolution occurs in two pollen characters: metareticulate structure and stellate pore ornamentation. The metareticulate structure has occurred in the genus *Psilotrichopsis* which is part of the tribe Amarantheae within the subfamily Amaranthoideae and also characterizes the subfamily Gomphrenoideae. Metareticulate pollen is a synapomorphy of the core Gomphrenoideae according to Müller and Borsch (2005b) and parallel evolution of stellate pore ornamentation was again confirmed by Müller (2005b).

Among the seven characters tested in this study the character related to pollen structure as metareticulate has already expressed its phylogenetic value and is corroborated by this study with a larger sampling. The metareticulate character based on these results is a derived character in the subfamily Gomphrenoideae. This study also corroborates stellate pore ornamentation as a phylogenetic synapomorphy for the lineage (character 18, Appendix 3). Previous authors (Borsch 1998; Eliasson 1988) suggested a close relationship of the monotypic tribe Pseudoplantageae (within the subfamily Gomphrenoideae) to the subfamily Amaranthoideae based on several floral characters. In addition, recent pollen studies indicated that stellate pore ornamentation in Pseudoplantageae occurs in members of the subfamily Amaranthoideae. Borsch (1998) described three types of pollen based on stellate pore ornamentation: *Mechowia*-type (in the genus *Mechowia*), *Pseudoplantago*-type (the genus *Pseudoplantago*), and *Psilotrichum*-type (the genera *Psilotrichum*, *Pupalia*, *Sericocoma*, and *Stilbanthus*) and

he mentioned that stellate ornamentation is a derived character and that among all the Angiosperms it is only known in the family Amaranthaceae. Stellate pore ornamentation also occurrs in *Pupalia*. A recent study concluded that stellate pore ornamentation evolved in parallel in the Amaranthaceae (Müller and Borsch 2005). The results in this study suggested that *Pseudoplantago* is part of the Gomphrenoids and stellate pore ornamentation is a derived state in the group lending support to the conclusion that this character has evolved in parallel in the Amaranthaceae.

The characters pollen shape, completeness of tectum in pollen grains, tectum ornamentation, and distribution of pollen ornamentation have not been tested before in the subfamily. The completeness of tectum is plesiomorphic and the derived conditions tectum present on distal bands (although *B. vermiculare* has the plesiomorphic condition). This confirms Borsch (1998) observations when he mentioned that the semitectate *Gomphrena*-type (Fig. 40) pollen is even more specialized that the tectate *Pfaffia*-type (Fig. 41 a-c). The *Gomphrena*-type and *Pfaffia*-type are two of the 17 types of pollen proposed by Borsch (1998). The plesiomorphic condition is microspines evenly distributed and the derived condition is the distribution of microspines in rows at the distal part of the mesoporia present in the Alternanthoid and Gomphrenoid clades, whereas microspines around the apertures only occurs in *Pseudoplantago friessii*.

Pollen form and tectum ornamentation show a parallel signal. Three different pollen forms have been described within Amaranthaceae. The dodecahedric and cubic are derived, whereas spheroidal is the most common form within the Gomphrenoideae, which occurrs in the Iresinoid and Gomphrenoid clades. Dodecahedric pollen is informative in the Alternanthoid clade. The psilate pollen evolved in two clades: the

Tidestromia clade and the subclade D within the Gomphrenoideae. However, within the *Tidestromia* clade the reversal to plesiomorphic condition occurred in *T. lanuginosa* subsp. *eliassoniana* (with ornamented pollen) and in *Blutaparon vermiculare* within the subclade D. In summary, only pollen structure, completeness of tectum, and distribution of microspines on the layer tectum are phyllogenetically informative in the evolution of pollen in the subfamily Gomphrenoideae.

5.5. CONCLUSIONS

Anther dehiscence is a useful character to recognize subfamilies as was suggested by Schinz (1934). The subfamily Gomphrenoideae has the derived condition with anthers bisporangiate. The bisporangiate anther is a morphological synapomorphy for the subfamily Gomphrenoidea.

The characters coded in this study to evaluate the phylogenetic aspect of Eliasson (1988) hypotheses suggest that there is not a continuous developmental trend in evolution of the character pseudostaminodia within the Gomphrenoideae. However, considering that the evolution of these traits occurs many times in the lineage, there is not a continuous developmental trend in character evolution in pseudostaminodia within Gomphrenoideae. In addition, the evaluation of the morphological aspect of Eliasson's hypotheses indicated that ontogenetic studies for floral evolution are needed in the Amaranthacea to better understand and define homology assessments with special emphasis in *Gomphrena*, *Froelichia*, *Froelichiella*, *Pseudogomphrena*, and *Pfaffia*. This study reveled that pseudostaminodia synapomorphies help define the genera *Pedersenia*, *Tidestromia*, and *Alternathera*. A better knowledge of these structures can help to

understand pseudostaminodia function and frequency in the family, and about the possible mechanisms involved in pollination.

Although Eliasson (1988) already mentioned that evolutionary trends in the structure of the stigma are difficult to trace, they habe been important in taxonomic classifications. Therefore, we wished to re-examine them in a phylogenetic context (e.g. Moquin-Tandom 1849; Schinz 1934; Townsend 1993). Gynoecium characters were not phylogenetically informative within the subfamily Gomphrenoideae. The results obtained from this study of pistils by SEM correlated Eliasson's (1988) opinions that stigma characteristics can define individual genera, but there is no correlation to the higher taxonomic levels. In this study, we found no correlation among characters of stigma with pollen or pseudostaminodial features. This idea is clearly observed with *Iresine* and *Tidestromia* with constant stigmatic characters and variable androecium characters.

Metareticulate pollen, tectum on distal bands, and microspines distributed either around the apertures or in distal row are derived characters in the subfamily Gomphrenoideae. These characters are diagnostic for recognizing the pollen types defined by Borsch (1998), but also are useful in a phylogenetic context. The understanding of pollen characteristics can be correlated to pollination biology. Perhaps, mesoporia (meshes) and pore aperture location offer a more efficient surface area for pollen development and an elaborate pollen design can aid in pollination.

The floral characters tested in this study were for useful defining groups of clades both the genera (*Alternanthera*, *Pedersenia*, *Tidestromia*, *Pseudoplantago*, *Froelichia*, *Guilleminea*, and *Gossypianthus*) and subfamily level. The use of molecular data (Chapter 2) resolved in three major clades within the Gomphrenoideae. However, just

two clades were supported by morphological synapomorphies in the combined tree (Fig. 29). These synapomorphies are basically homoplasious characters. The Alternanthoid clade is supported by presence of dodecahedric pollen. This character is shared with the subfamily Amaranthoideae, dodecahedric pollen occurs only once in the subfamily Gomphrenoideae. Therefore, dodecahedric pollen can be useful in the taxonomy of the subfamily Gomphrenoideae. On the other hand, the Gomphrenoid clade supported by the presence of pseudostaminodia 1/3 longer than filaments includes subclades that can be well defined based on morphological characters and can also help in the taxonomy of this group. Subclade B with sessile stigmas, subclade C with pseudostaminodia longer than filaments, filaments fused in a staminal tube and the homology tectum complete, and subclade D with psilate pollen.

APPENDIX

Appendix 1. Characters and character states.

1. Habit. 0 = annual, 1 = perennial. The codification of this character was based on field observations and the study of herbarium material. Annual herbs have non-ligneous roots and herbaceous stems. Perennials have structures such as rhizomes, woody roots, or woody stems at the base (suffrutescent). *Tidestromia* includes both annual and perennial herbs, whereas *Alternanthera* and *Pedersenia* are perennials.

2. Type of trichomes on tepals. 0 = simple, 1 = dendritic, 2 = barbed. Three types of trichomes were characterized based on apices and direction of projections along the stalk. Barbed trichomes have the apex bent back acutely and the sides with reflexed barbs (Radford *et al.* 1974); this type is observed in *Tidestromia tenella, T. valdesiana*, and *Alternanthera tenella*. Dendritic trichomes have flexuous main axis with several divergent branches or rays apparently distributed at random, and the apices are variously oriented but never reflexed; the remaining species of *Tidestromia* have this type of trichomes. *Pedersenia* and *Alternanthera laguroides* have simple trichomes.

3. Phyllotaxy. 0 = alternate, 1 = opposite. Phyllotaxy in *Tidestromia* has been variously described either as opposite (Standley 1917; Shreve and Wiggins 1964; Robertson 1981, Eliasson 1988) or alternate to opposite or even whorled (Henrickson 1993). Observations of herbarium specimens**,** and cleared leaves revealed that each node has two involucral leaves, and one true leaf. The involucral leaves are subopposite, smaller, sessile or short petiolated, with veins oriented toward one side of the blade and associated with flowers. The true leaves are larger, long petiolated, have veins erect, and are not associated with

flowers. Therefore, phyllotaxy in *Tidestromia* is considered alternate. *Alternanthera* and *Pedersenia* have true opposite leaves.

4. Leaf texture. 0 = succulent, 1 = incrassate, 2 = chartaceous. Three types of leaf texture are distinguished in the taxa included in this study. Succulent leaves of *Tidestromia carnosa*, *T. tenella* and some specimens of *T. lanuginosa*, are fleshy in the field and membranous when dried. Specimens of *T. rhizomatosa* are distinguished by being very thick, including the veins (incrassate). Chartaceous leaves of the remaining species of *Tidestromia*, *Alternanthera*, and *Pedersenia* are thin and quite opaque. Incrassate and chartaceous leaves maintain their texture in fresh and dry material.

5. Involucre. 0 = absent, 1 = present. *Tidestromia* has been characterized by having an involucre formed by a group of leaves that subtend axillary glomerules and become hard and more or less connate with age (Standley 1916; Eliasson 1988). *Tidestromia valdesiana* has involucral leaves that do not form an involucre (Sánchez-del Pino and Flores Olvera 2002). Therefore, the involucre is not a synapomorphic character in the genus.

6. Stem adnation to fruiting involucres. 0 = absent, 1 = present. *Tidestromia* has two types of fruiting involucres on secondary branches. One type is characterized by having involucral leaf bases connate and sometimes adnate to the true leaf base, but never to the stem as in *T. carnosa* and *T. tenella*. The second type is characterized by having the stem adnate to involucral leaf petioles or the true leaf petiole. Involucres become indurate at fruiting time and usually contain one, rarely two, well developed seeds. The fruiting involucres detach from the parent plant to be dispersed. The character is inapplicable for *T. valdesiana*, which does not have involucres, as well as the remaining taxa.

7. Inflorescence. 0 = dichasium, 1 = simple spike, 2 = spikes arranged in thyrses, 3 = synflorescence panicle. The type of inflorescences in *Tidestromia* is indeterminate, formed by three flowers with the center flower maturing first followed by the lateral flowers, thus it can be characterized as a dichasium. Species of *Alternanthera* have flowers in short or long spikes, described as globose heads and subglobose or shortly to elongate capitate by some authors (Standley 1917; Mears 1977; Eliasson 1987; Borsch 2001). Sessile inflorescences are observed in *A. tenella*. *Alternanthera laguroides* has long pedunculate spikes usually arranged in thyrses. In addition, inflorescences of *Pedersenia* have been described as a synflorescence of heads (Borsch and Pedersen 1997).

8. Tepal apices. 0 = aristate, 1 = acute to obtuse, 2 = mucronate. Aristate and mucronate apices refer to a midrib extension from the blade below the apex of the tepal (Stearn 1995). Acute and obtuse apices are formed by a blade apex without a midrib extension. Aristate and mucronate apices are straight and stiff, but the former is prolonged (three times longer than wide) and the latter is smaller (less than three times longer than wide) (Radford *et al.* 1974). Acute and obtuse apices have convex margins, but the former has an angle of 45^{o}-90^{o} and the latter an angle of more than 90^{o}. Acute and obtuse apices were constantly observed together in individual species and they were coded as a single state. *Alternanthera tenella* and *Tidestromia valdesiana* have aristate tepals; *A. laguroides* and *Pedersenia cardenasii* have mucronate tepals; *P. argentata* and the remaining *Tidestromia* taxa have acute to obtuse tepals.

9. Stamen length relative to pistil length. 0 = same to slightly smaller, 1 = longer. Two states were recognized based on differences in stamen length compared to the pistil

length during anthesis. The outgroup taxa have stamens as long as or smaller than the pistil, and *Tidestromia* stamens longer than the pistil.

10. Stigma. 0 = entire, 1 = bilobed. Previous authors (Nuttall 1820; Moquin-Tandom 1849; Standley 1917; Robertson 1981; Eliasson 1988; Henrickson 1993) have considered *Tidestromia* to have capitate or 2-lobed stigmas. Detailed observations in many specimens of *Tidestromia* at anthesis, confirmed that the bilobed condition is the most common in the genus and trilobed stigmas were observed only in *T. suffruticosa* var. *oblongifolia*. Stigmas at the same stage of development in *Pedersenia* are bilobed, and in *Alternanthera* are entire.

11. Pollen exine. 0 = psilate, 1 = ornamented. Pollen ornamentation in *Tidestromia* was previously discussed by Sánchez-del Pino and Flores Olvera (2002). *Alternanthera*, *Pedersenia*, and *Tidestromia lanuginosa* subsp. *eliassoniana* have pollen ornamented with micro-spinules. The remaining *Tidestromia* taxa have psilate pollen.

12. Mesoporia vaultation. 0 = wide, 1 = narrow. Eliasson (1988) indicated that pollen of *Tidestromia* is characterized by an extraporal sexine forming ridges which are triangular in cross-section. Borsch and Barthlott (1998) proposed the term metareticulate to describe the type of pollen in the Amaranthaceae, considering that a reticulate tectum is not homologous to a metareticulate structure. Metareticulate pollen is formed by vaulted mesoporia and sunken pores. Mesoporia seems to be an informative character because genera in the family have different vaultation. According to Borsch (1998) *Tidestromia* is the only genus in the family that has mesoporia vaultation that continuously narrows distally, and appears triangular in cross section.

13. Floral bract. 0 = widely ovate, 1 = lanceolate. The species of *Tidestromia* and *Pedersenia* have widely ovate bracts and acute apices, whereas *Alternanthera* has floral bracts lanceolate and acute apices.

Appendix 2. Matrix of morphological character states used in a phylogenetic analysis of *Tidestromia*. "-" = inapplicable.

Taxon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Pedersenia cardenasii	1	0	1	2	0	-	3	2	0	1	1	0	0
P. argentata	1	0	1	2	0	-	3	1	0	1	1	0	0
Alternanthera laguroides	1	0	1	2	0	-	1, 2	2	0	0	1	0	1
A. tenella	1	1	1	2	0	-	1	0	0	0	1	0	1
Tidetromia carnosa	0	1	0	0	1	0	0	1	1	1	0	1	0
T. lanuginosa subsp. *eliassoniana*	0	1	0	0, 2	1	1	0	1	1	1	1	1	0
T. lanuginosa subsp. *lanuginosa*	0	1	0	0, 2	1	1	0	1	1	1	0	1	0
T. rhizomatosa	1	1	0	1	1	1	0	1	1	1	0	1	0
T. suffruticosa var. *oblongifolia*	1	1	0	2	1	1	0	1	1	1	0	1	0
T. suffruticosa var. *suffruticosa*	1	1	0	2	1	1	0	1	1	1	0	1	0
T. tenella	0	2	0	0	1	0	0	1	1	1	0	1	0
T. valdesiana	1	2	0	2	0	-	0	0	1	1	0	1	0

Appendix 3. Characters and character states.

1. Anthers. 0 = tetrasporangiate, 1 = bisporangiate. Bisporangiate anthers become unilocular at maturity and have one line of dehiscence whereas tetrasporangiate anthers become bilocular at maturity and have two lines of dehiscence (Eliasson 1988). The ingroup taxa have bisporangiate anthers and the outgroup have the tetrasporangiate type.

2. Pseudostaminodia. 0 = absent, 1 = present. Pseudostaminodia are characteristic structures in many genera of the family Amaranthaceae. Eliasson (1988) indicated that lack of pseudostaminodia occurs in *Amaranthus* and other genera within the subfamily Amaranthoideae from the New World and the genera *Guilleminea*, *Gossypianthus*, *Irenella*, *Blutaparon*, *Lithophila*, and *Woehleria* within the subfamily Gomphrenoideae. However, after an evaluation of androecia characters of several genera of Gomphrenoideae, it was found in this study that the genus *Woehleria* has pseudostaminodia. This genus develops one stamen and there are one or two lanceolate structures close to the single stamen, which based on form and position are considered pseudostaminodia. Eliasson (1988) indicated that pseudostaminodia are conspicuous structures. In this study, the observation of androecium in *Iresine* and *Pedersenia* by SEM was useful to determine differences between the cells that form part of the filament and thus pseudostaminodia (Fig. 5g, 6b). Therefore, filament structures in this sampling were codified as pseudostaminodia. This includes the genus *Tidestromia* which has short lobes between each filament. In this study, the presence of pseudostaminodia in *Pfaffia* is a character that was considered inapplicable, because there are different opinions in the literature about *Pfaffia* appendages; pseudostaminodia can not be distinguished from filaments based on SEM observations (Fig. 7f, g); and there are not enough elements to

evaluate until a future work focused on an ontogenetic study that can resolve the doubt about if these structures are filaments, pseudostaminodia or a fusion of filaments and pseudostaminodia. This study agrees with Eliasson (1988) that filament appendages in *Gomphrena* can be pseudostaminodia considering that there are not big differences with respect to *Froelichia*, *Froelichiella* and *Pseudogomphrena* staminal tube form. Only *Gomphrena flaccida* (Fig. 7e) has anthers located in the tip of flat structures and it is not clear if they are pseudostaminodia fused to filaments or filaments and therefore considered inapplicable in this study. The rest of the sampling shows that excluding *Charpentieria*, *Achyranthes* and *Centrostachys*, the rest of the outroup taxa do not have pseudostaminodia whereas *Pseudoplantago*, *Xerosiphon* and only *Hebanthe paniculata* have pseudostaminodia.

3. Pseudostaminodia form. 0 = triangular, 1 = oblong, 2 = lanceolate, 3 = cordate, 4 = lobed. Excluding *Alternanthera pungens*, which has triangular pseudostaminodia, all *Alternanthera* has lanceolate pseudostaminodia as well as *Froelichia*, *Xerosiphon*, *Pseudoplantago*, *Woehleria*, and three species of *Iresine*. The outgroup taxon *Centrostachys* also has lanceolate pseudostaminodia. *Pedersenia* has the exclusive cordate pseudostaminodia form. Oblong are in two species of *Iresine* as well as *Achyranthes* and *Achyranthopsis*. *Hebanthe paniculata*, two species of *Iresine*, infraspecific taxa of *Tidestromia* and *Alternanthera pungens* has triangular pseudostaminodia. The remaining taxa have lobed pseudostaminodia.

4. Pseudostaminodia size. 0 = shorter than filaments, 1 = 2/3 longer or more than filaments, 2 = equal to filaments, 3= 1/3 longer than filaments. Pseudostaminodia 2/3 longer or more that filaments are in: *Xerosiphon*, *Froelichia*, *Gomphrena* and most of the

species of *Alternanthera*. Pseudostaminodia equal to filaments characterized *Tidestromia rhizomatosa* and four species of *Alternanthera*. Two species of *Alternanthera* and two of *Gomphrena* as well as *Pseudoplantago* and the outgroup taxon *Centrostachys* have pseudostaminodia 1/3 longer than filaments. The remainding taxa have short pseudostaminodia.

5. Pseudostaminodia margin. 0 = entire, 1 = ciliate, 2 = laciniate, 3 = crenate. The genus *Iresine* has ciliate pseudostaminodia; *Achyropsis*, *Achyranthes*, *Centrostachys* and *Alternanthera* have laciniate pseudostaminodia; *Iresine arbuscula*, *Gomphrena*, *Hebanthe paniculata*, *Xerosiphon*, infraspecific categories of *Tidestromia*, *Alternanthera caracasana* and *A. pungens* have crenate pseudostaminodia; and the remaining taxa have entire pseudostaminodia.

6. Pseudostaminodia adnate to a second layer. 0 =absent, 1 = present. *Achyropsis*, *Achyranthes*, and *Centrostachys* have pseudostaminodia in two layers, whereas the rest of the sampling has only one layer.

7. Filaments enclosed by pseudostaminodia. 0 = absent, 1 = present. This study codified the pseudostaminodia of *Gomphrena* as a specific character not homologous with another type of pseudostaminodia within the Gomphrenoideae. The reason is because the pseudostaminodia enclose the filament and it does not happen in any other genera. The filament is placed in the middle of two lobes of the pseudostaminodium and kind of enclosed for them. *Gomphrena flaccida* is the only species of this sampling where these differences are not observed. The anther is on the tip of a wide structure that it is not possible to recognize the usual filament enclosed by the pseudostaminodium.

Therefore, this character was inapplicable in *Gomphrena flaccida* and absent in the remaining sampling.

8. Filaments and pseudostaminodia length adnation. 0 = in a staminal cup, 1 = in a staminal tube, 2 = free to base. Staminal tubes are present in *Gomphrena* (except for *Gomphrena vaga*), *Froelichia*, and *Xerosiphon*, whereas the rest of the taxa have staminal cups. *Amaranthus* has filaments free to base.

9. Stigma form. 0 = entire, 1 = 2-divided, 2 = 3-divided. Stigmas at the same stage of development in *Achyropsis*, *Achyranthes*, *Alternanthera*, *Centrostachys*, *Kyphocarpa*, *Leucosphera*, *Pseudoplantago*, *Pupalia*, and *Sericorema* are entire. The remaining sampling has bilobed stigmas. Only *Amaranthus* and *Tidestromia suffruticosa* var. *oblongifolia* can also have trilobed stigmas.

10. Papillae distribution. 0 = troughout the stigma, 1 = inner sides. Most of the taxa have papillae widely distributed along the stigma lobules, while *Amaranthus*, *Charpentiera*, *Gossypianthus*, and *Guilleminea* have papillae restricted to the inner areas of the stigmatic lobules.

11. Style size. 0 = sessile, 1 = short, 2 = long. *Achyropsis*, *Achyranthes*, *Leucosphera*, *Pupalia*, *Pseudoplantago* and *Froelichia* have long stiles; *Amaranthus*, *Pfaffia*, *Gomphrena vaga*, *Hebanthe*, *Guilleminea* and *Gossypianthus* have sessile stigmas. The rest of the sampling has short styles.

12. Pollen form: 0 = spheroidal, 1 = dodecahedric, 2 = cubic. *Alternanthera*, *Pedersenia*, *Tidestromia* and the outgroup taxon *Kyphocarpa* have dodecahedric pollen; *Pseudoplantago* has cubic pollen, and the rest of the taxa have spheroidal pollen.

13. Pollen structure: 0 = metareticulate, 1 = not metareticulate. *Pseudoplantago* was considered not metareticulate (Müller and Borsch 2005) but in this analysis the character was codified as inapplicable because of the unusual cubic pollen that for geometrical reasons need further explanation. The outgroup and *Iresine* do not have metareticulate pollen whereas the rest of the ingroup taxa have metareticulate pollen.

14. Tecta. 0 = complete, 1 = only in distal bands. The tecta for some genera of Amaranthaceae was described by Eliasson (1988), Borsch (1998), and Borsch and Barthlott (1998). *Alternanthera*, *Blutaparon vermiculare*, *Gomphrena elegans*, *G. vaga*, *Hebanthe*, *Iresine*, *Pedersenia*, *Pfaffia*, *Pseudoplantago*, *Iresine*, *Tidestromia*, *Woehleria* and the outgroup taxa have continuous tectum on the lateral sides of the mesoporia, so that no columellae can be seen from outside. Tecta in *Hebanthe* and some species of *Pfaffia* have large perforations on the vertical parts of the mesoporia that give the appearance of being columellae. However, those pollen grains were codified as tectum complete. The remaining taxa have pollen grains with the tectum only distally present, and thus exposed columellae.

15. Mesoporia vaultation. 0 = flat, 1 = broadly vaulted, 2 = acute vaulted, 3 = extremely narrow. According to Borsch (1998) pollen with flat mesoporia includes the genera *Pupalia* and *Sericorema*; flat mesoporia includes the genera *Achyranthes*, *Achyropsis*, *Amaranthus*, *Centrostachys*. Pollen with broadly vaulted mesoporia are in the genera: *Charpentiera*, *Iresine*, *Irenella*, and *Woehleria*. Pollen with acute vaulted mesoporia includes *Kyphocarpa*, *Pseudoplantago*, *Pfaffia*, *Guilleminea*, *Gossypianthus*, *Blutaparon*, *Lithophila*, *Pedersenia*, and *Alternanthera*. Pollen with extremely narrow mesoporia includes *Hebanthe*, *Xerosiphon*, *Froelichia*, *Gomphrena*, and *Tidestromia*.

16. Pollen ornamentation. 0 = psilate, 1 = ornamented. Excluding *Gomphrena vaga* and *G. elegans*, the genus *Gomphrena*, *Gossypianthus*, *Guilleminea*, *Lithophila*, and *Tidestromia* (except for *Tidestromia lanuginosa* subsp. *Eliassoniana*) have pollen non ornamented (psilate pollen). The rest of the sampling has pollen ornamented with microspines.

17. Microspines distribution: 0 = distal row, 1 = arranged around the apertures, 2 = evenly spread. *Pseudoplantago* has microspines distributed around the apertures; *Irenella*, *Iresine*, *Woehleria* and all the outgroup taxa have microspines distributed evenly over the total tectum surface, and the remaining taxa have microspines distributed in rows at the distal part of the mesoporia.

18. Stellate pore ornamentation. 0 = absent, 1 = present. Stellate pore ornamentation was defined as flecks of ektexine (Skvarla and Nowicke 1976) or ektexinous bodies that project in hooks (Borsch 1998) and cover the pore membrane. This characte is exclusive in this study sampling for the genus *Pseudoplantago* and the outgroup taxon *Pupalia*.

Appendix 4. Matrix of morphological character states used in a phylogenetic analysis of Gomphrenoideae. "-" = inapplicable.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Achyranthes aspera	0	1	1	0	2	1	0	0	0	0	2	0	1	0	0,1	1	2	0
Achyropsis Avicularia	0	0	1	0	2	1	0	0	0	0	2	0	1	0	0,1	1	2	0
Alternanthera altacrucensis	1	1	2	1	2	0	0	0	0	0	1	1	0	0	2	1	0	0
Alternanthera caracasana	1	1	2	2,3	0,3	0	0	0	0	0	1	1	0	0	2	1	0	0
Alternanthera elongata	1	1	2	3	2	0	0	0	0	0	1	1	0	0	2	1	0	0
Alternanthera flava	1	1	2	1	2	0	0	0	0	0	1	1	0	0	2	1	0	0
Alternanthera flavescens	1	1	2	1	2	0	0	0	0	0	1	1	0	0	2	1	0	0
Alternanthera galapagensis	1	1	2	2	2	0	0	0	0	0	1	1	0	0	2	1	0	0
Alternanthera halimifolia	1	1	2	1	2	0	0	0	0	0	1	1	0	0	2	1	0	0
Alternanthera laguroides	1	1	2	1,0	2	0	0	0	0	0	1	1	0	0	2	1	0	0
Alternanthera olivacea	1	1	2	2	2	0	0	0	0	0	1	1	0	0	2	1	0	0
Alternanthera philoxeroides	1	1	2	1	2	0	0	0	0	0	1	1	0	0	2	1	0	0
Alternanthera pungens	1	1	0	0	3	0	0	0	0	0	1	1	0	0	2	1	0	0
Alternanthera tenella	1	1	2	1,2	2	0	0	0	0	0	1	1	0	0	2	1	0	0
Amaranthus greggii	0	0	-	-	-	-	-	2	1,2	1	0	0	1	0	0,1	1	2	0
Amaranthus spinosus	0	0	-	-	-	-	-	2	1,2	1	0	0	1	0	0,1	1	2	0
Blutaparon vermiculare	1	0	-	-	-	-	0	0	1	0	1	0	0	0	2	1	0	0
Centrostachys aquatica	0	1	2	3	2	1	0	0	0	0	2	0	1	0	0,1	1	2	0

Appendix 4. Matrix of morphological character states used in a phylogenetic analysis of Gomphrenoideae. "-" = inapplicable.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Charpentiera obovata	0	1	4	0	0	0	0	0	1	1	1	0	1	0	1,2	1	2	0
Charpentiera tomentosa	0	1	4	0	0	0	0	0	1	1	1	0	1	0	1,2	1	2	0
Froelichia floridana	1	1	2	1	0	0	0	1	1	0	2	0	0	1	3	1	0	0
Froelichia interrupta	1	1	2	1	0	0	0	1	1	0	2	0	0	1	3	1	0	0
Froelichia tomentosa	1	1	2	1	0	0	0	1	1	0	2	0	0	1	3	1	0	0
Gomphrena boliviana	1	1	-	1	0,3	0	1	1	1	0	1	0	0	1	3	0	-	0
Gomphrena elegans	1	1	-	3	0,3	0	1	1	1	0	1	0	0	0	3	1	0	0
Gomphrena flaccida	1	-	-	-	-	-	-	1	1	0	1	0	0	1	3	0	-	0
Gomphrena globosa	1	1	-	1	0,3	0	1	1	1	0	1	0	0	1	3	0	-	0
Gomphrena graminea	1	1	-	1	0,3	0	1	1	1	0	1	0	0	1	3	0	-	0
Gomphrena haenkeana	1	1	-	1	0,3	0	1	1	1	0	1	0	0	1	3	0	-	0
Gomphrena macrocephala	1	1	-	1	0,3	0	1	1	1	0	1	0	0	1	3	0	-	0
Gomphrena nitida	1	1	-	1	0,3	0	1	1	1	0	1	0	0	1	3	0	-	0
Gomphrena serrata	1	1	-	1	0,3	0	1	1	1	0	1	0	0	1	3	0	-	0
Gomphrena vaga	1	1	-	3	0,3	0	1	0	1	0	0	0	0	0	3	1	0	0
Gossypianthus lanuginosus	1	0	-	-	-	-	0	0	1	1	0	0	0	1	2	0	-	0
Guilleminea densa	1	0	-	-	-	-	0	0	1	1	0	0	0	1	2	0	-	0
Guilleminea gracilis	1	0	-	-	-	-	0	0	1	1	0	0	0	1	2	0	-	0

Appendix 4. Matrix of morphological character states used in a phylogenetic analysis of Gomphrenoideae. "-" = inapplicable.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Hebanthe grandiflora	1	0	-	-	-	-	-	0	1	0	0	0	0	0	3	1	0	0
Hebanthe occidentalis	1	0	-	-	-	-	-	0	1	0	0	0	0	0	3	1	0	0
Hebanthe paniculada	1	1	0,4	0	0,3	0	0	0	1	0	0	0	0	0	3	1	0	0
Irenella chrysotricha	1	0	-	-	-	-	-	0	1	0	1	0	1	0	1,2	1	2	0
Iresine alternifolia	1	1	2	0	1	0	0	0	1	0	1	0	1	0	1,2	1	2	0
Iresine arbuscula	1	1	1	0	3	0	0	0	1	0	1	0	1	0	1,2	1	2	0
Iresine difusa	1	1	0	0	0	0	0	0	1	0	1	0	1	0	1,2	1	2	0
Iresine grandis	1	1	2	0	1	0	0	0	1	0	1	0	1	0	1,2	1	2	0
Iresine heterophylla	1	0,1	0,4	0	0	0	0	0	1	0	1	0	1	0	1,2	1	2	0
Iresine leptoclada	1	1	2	0	1	0	0	0	1	0	1	0	1	0	1,2	1	2	0
Iresine palmeri	1	0	-	-	-	-	-	0	1	0	1	0	1	0	1,2	1	2	0
Iresine sp.	1	1	1	0	1	0	0	0	1	0	1	0	1	0	1,2	1	2	0
Kyphocarpa angustifolia	0	0	4	0	0	0	0	0	0	0	¿	1	1	0	2	1	2	0
Leucosphera bainesii	0	0	-	-	-	-	-	0	0	0	2	0	1	0	0,1	1	2	0
Lithophila muscoides	1	0	-	-	-	-	0	0	1	0	1	0	0	1	2	0	-	0
Pedersenia argentata	1	1	3	0	0	0	0	0	1	0	1	1	0	0	2	1	0	0
Pedersenia cardenasii	1	1	3	0	0	0	0	0	1	0	1	1	0	0	2	1	0	0
Pedersenia sp.	1	1	3	0	0	0	0	0	1	0	1	1	0	0	2	1	0	0

Appendix 4. Matrix of morphological character states used in a phylogenetic analysis of Gomphrenoideae. "-" = inapplicable.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Pfaffia jubata	1	-	-	-	-	-	-	0	1	0	0	0	0	0	2	1	0	0
Pfaffia iresinoides	1	-	-	-	-	-	-	0	1	0	0	0	0	0	2	1	0	0
Pfaffia tuberosa	1	-	-	-	-	-	-	0	1	0	0	0	0	0	2	1	0	0
Pseudoplantago friessii	1	1	2	3	0	0	0	0	0	0	2	2	-	0	2	1	1	1
Pupalia lappacea	0	0	-	-	-	-	-	0	0	0	2	0	1	0	0	1	2	1
Sericorema nemotiflora	0	0	-	-	-	-	-	0	0	0	1	0	1	0	0	1	2	0
Tidestromia carnosa	1	0,1	4	0	0	0	0	0	1	0	1	1	0	0	3	0	-	0
Tidestromia lanuginosa subs. *eliassoniana*	1	0,1	0,4	0	0,3	0	0	0	1	0	1	1	0	0	3	0	-	0
Tidestromia lanuginosa subs. *lanuginosa*	1	0,1	0,4	0	0,3	0	0	0	1	0	1	1	0	0	3	0	-	0
Tidestromia suffruticosa var. *oblongifolia*	1	0,1	0,4	0	0,3	0	0	0	1,2	0	1	1	0	0	3	0	-	0
Tidestromia rhizomatosa	1	1	2	2	0	0	0	0	1	0	1	1	0	0	3	0	-	0
Tidestromia suffruticosa var. *suffruticosa*	1	0,1	0,4	0	0,3	0	0	0	1	0	1	0,1	0	0	3	0	-	0
Tidestromia tenella	1	0,1	4	0	0	0	0	0	1	0	1	1	0	0	3	0	-	0
Tidestromia valdesiana	1	0,1	4	0	0	0	0	0	1	0	1	1	0	0	3	0	-	0

Appendix 4. Matrix of morphological character states used in a phylogenetic analysis of Gomphrenoideae. "-" = inapplicable.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Woeheleria serpyllifolia	1	1	2	0	0	0	0	-	1	0	1	0	1	0	1,2	1	2	0
Xerosiphon angustiflorus	1	1	2	1	0,3	0	0	1	1	0	1	0	0	1	3	1	0	0
Xerosiphon aphylla	1	1	2	1	0,3	0	0	1	1	0	1	0	0	1	3	1	0	0

SUMMARY

This study used a representative sampling of 16 genera and showed that the subfamily Gomphrenoideae as monophyletic. However, it will be interesting in the future to include the genera *Pseudogomphrena*, *Hebanthodes*, and *Froelichiella* in order to further test the monophyly of subfamily Gomphrenoideae. The generic circumscription of those genera has been questioned. *Pfaffia* and *Gomphrena* are paraphyletic in this study and more extensive molecular or morphological researches can perhaps be used to resolve their circumscription. The study of stigma morphology using SEM suggested that the clade that includes *Gomphrena vaga*, *G. elegans*, *Pfaffia*, and *Hebanthe* that share the sessile stigma, character which can be useful for a new taxonomic classification of this clade.

The infraspecific classification of genus *Tidestromia* has changed when examined with molecular data. Future work will include nomenclatural changes elevating the infraspecific taxa to the species level. Cytological studies in the genus will be useful to study hybridization in the genus. This genus is a perfect taxa research group to study ecological aspects related to edaphic adaptations to various soils types.

The genus *Alternanthera* is a large genus that needs a taxonomic monograph. The total number of species is still unknown. The present study suggested sectional treatments need further investigation. Both biogeography and evolution of secondary xylem (wood) are interesting aspects of this genus which require further study.

Finally, this work suggests that the use of ontogenetic research will be needed to test the proposed homology assessments for pseudostaminodia development and to define interstaminal appendages in *Gomphrena*, *Froelichia*, *Pseudogomphrena*, and *Pffafia*.

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