Evolution and Systematics of the African achyranthoid clade of the Amaranthaceae

Dissertation

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Declaration of authorship

I hereby declare that I alone am responsible for the content of my doctoral dissertation and that I have only used the sources or references cited in the dissertation. I also declare that I have not already submitted this dissertation as part of another doctoral procedure.

"We do not inherit the Earth from our ancestors; we borrow it from our children"

American Indian proverb
(Sitting Bull, Lakota Indian)

To my beloved daughter

Preface and acknowledgements

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My studies took mainly place at the Botanischer Garten und Botanisches Museum Berlin-Dahlem and Institut für Biologie of the Freie Universität Berlin, and partly at the Addis Ababa University of Ethiopia. I thank all the institutions and organizations that made this study possible and successful.

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Summary

The Amaranthaceae are a nearly cosmopolitan flowering plant family that encompasses species of high economic value as food, forage, and ornamentals. Other species are of cultural value for traditional medicine or in traditional rituals of African tribes. Amaranthaceae are notable in terms of their diverse ecology, physiology and morphology, and represent a perfect study group for diverse scientific questions.

In recent molecular studies there was evidence for the existence of the so-called achyranthoid clade, within Amaranthaceae, which includes most of the genera with African distribution plus a few genera outside the African continent.

Many African genera of the Amaranthaceae exhibit unique inflorescences that include sterile flowers modified to form hooks or spines. Considering that the abundance of large terrestrial herbivores increased on the African continent with the expansion of grassland and savannah ecosystems, modified sterile flowers facilitating dispersal by large animals through epizoochory could have been an innovation that boosted the diversification of an African achyranthoid clade within the Amaranthaceae.

To test this hypothesis, for **Chapter 2** an extensively sampled phylogeny comprising 26 of the 31 achyranthoid genera as well as representatives of all other lineages of Amaranthaceae was generated, which also served as a base for downstream analyses presented in the other chapters. Phylogenetic tree inference employed the nuclear genomic region ITS as well as the plastid genomic regions *trnK*/*matK*, *rpl16*, and *trnL-F*, using parsimony, likelihood and Bayesian inference methods. Moreover, in the second chapter divergence times were estimated for this clade, trait-dependant changes of species diversification rates were evaluated using state-dependent speciation and extinction models, and ancestral character states for modified sterile flowers were reconstructed.

The Achyranthoids were found to be a major clade of the Amaranthaceae, comprising mostly African members. Phylogenetic relationships within this clade were well resolved and supported two main subclades. Several genera were found to be polyphyletic. According to the results, the Achyranthoids started to diversify ~28 million years ago, and modified sterile flowers evolved multiple times within this clade. An asymmetry in transition rates towards the gain of sterile flowers was observed, whereas no trait-dependent increase in species diversification rates was detected. Bayesian rate heterogeneity analyses indicated that the Achyranthoids diversified without significant rate shifts.

The accumulation of modified sterile flowers within Achyranthoids appears to result from the higher transition rates in favour of modified sterile flowers. Multiple gains suggest an adaptive value for this trait. However, epizoochory does not appear to have fuelled species diversification in Achyranthoids, possibly due to extensive gene flow through regularly migrating mammals, which limits the possibility of speciation by isolation.

In **Chapter 3** the pollen of the achyranthoid taxa was investigated, based on the phylogeny obtained in the previous chapter. The aim was to detect synapomorphies for monophyletic groups derived from analyses of molecular data. Morphological characters should thereby substantiate molecular results and facilitate the description of taxonomic entities. The complete character syndrome, to which was referred to as stellate pore ornamentation in earlier studies was shown to be limited to genera of subclade I within Achyranthoids. Several pollen characters were observed as highly discriminant between subclade I and II of Achyranthoids as well as between genera and species within these clades, such as the number of apertures in species of *Psilotrichum* Blume*,* the strongly vaulted mesoporia in *Kyphocarpa* (Fenzl) Schinz, and the dodecahedral pollen shape in *Sericocoma heterochiton* Lopr. and *Kyphocarpa,* as well as the unique pollen shape observed in *Centemopsis* Schinz, where pollen grains resemble the geometry of a football. The shape depicts a truncated icosahedral, constituted of 12 pentagons, corresponding to the placement of apertures, and 20 hexagons, corresponding to the areas of mesoporia. Also, the mesoporia was observed to be uniquely vaulted inwards while apertures appear uplifted and microspines are specially arranged throughout all species of *Centemopsis*.

Chapter 4 focused on the achyranthoid subclade II, in which a high degree of non-monophyly was observed at genus- and species level in the molecular phylogeny derived in the second chapter. In order to facilitate the description of taxonomic entities, generate a new genus concept which renders genera and species monophyletic, and complement the pollen morphological data, a set of 24 characters covering habit and vegetative morphology as well as inflorescence and floral morphology, was established to be used in ancestral character state reconstructions. In order to further improve tree resolution and node support compared to the second chapter, the sequence data were complemented with sequences of the plastid intergenic spacer between *rpl32-trnL*$_{UAG}$ and the two spacers in the *rps4-trnT-trnL*$_{UAA}$ region. Phylogenetic trees of the nuclear and the combined plastid regions were largely congruent and almost fully resolved with maximum support for most lineages and showed a further subdivision of Achyranthoids II into subclade II A (*Achyranthes* L. plus allies) and subclade II B (*Cyathula* Blume plus allies). Based on molecular and morphological results, a new genus concept for the genera and species of subclade II was established, which renders the genera of subclade II monophyletic, and a commented checklist was provided, including complete homotypic and heterotypic synonymy of the genera and species, two newly established genera, resurrection of one species name, and several new combinations.

Zusammenfassung

Die Amaranthaceae sind eine nahezu kosmopolitische Familie innerhalb der Blütenpflanzen, die Arten von hohem wirtschaftlichem Wert als Nahrungs- und Futtermittel sowie als Zierpflanzen umfasst. Einige Arten sind von großem kulturellem Wert für die traditionelle Medizin oder für traditionelle Rituale afrikanischer Stämme. Amaranthaceae sind hinsichtlich ihrer vielfältigen Ökologie, Physiologie und Morphologie bemerkenswert und stellen insgesamt eine perfekte Studiengruppe für verschiedene wissenschaftliche Fragestellungen dar.

Frühere molekulare Studien gaben Hinweise auf die Existenz des sogenannten „achyranthoid clade", der die meisten afrikanischen Taxa umfasst sowie einige Gattungen und Arten, die auf anderen Kontinenten verbreitet sind.

Viele afrikanische Gattungen der Amaranthaceae weisen komplexe Blütenstände auf, in denen sterile und fertile Blüten gemeinsam in Teilblütenständen angeordnet und die sterilen Blüten zu Haken oder Stacheln modifiziert sind. In Anbetracht der Tatsache, dass große terrestrische Pflanzenfresser auf dem afrikanischen Kontinent mit der Ausweitung von Grasland- und Savannenökosystemen quantitativ zunahmen und dabei der Samenausbreitung durch Epizoochorie dienten, könnten modifizierte sterile Blüten eine wesentliche Innovation dargestellt haben, welche die Diversifizierung dieses afrikanischen Clades beschleunigte.

Um diese Hypothese zu testen war in **Kapitel 2** zunächst die Erstellung einer umfangreichen Phylogenie notwendig, die 26 der 31 Gattungen des „achyranthoid clade" sowie Repräsentanten sämtlicher Linien der Amaranthaceae beinhaltete und als Basis für weitere Untersuchungen diente. DNA-Sequenzen der Kerngenomregion ITS sowie der Plastidengenomregionen *trnK/matK*, *rpl16* und *trnL-F* wurden dafür unter Verwendung von Parsimony-, Likelihood- und Bayes'schen Inferenzmethoden analysiert. Darüber hinaus wurde das evolutive Alter verschiedener Abstammungsgemeinschaften abgeschätzt und es wurden merkmalsabhängige Änderungen der Diversifizierungsraten unter Anwendung verschiedenen Artbildungs- und Extinktionsmodellen bewertet und Merkmalszustände bezogen auf modifizierte sterile Blüten für die gemeinsamen Vorfahren von Abstammungsgemeinschaften rekonstruiert.

Die Achyranthoiden wurden als Hauptgruppe innerhalb der Amaranthaceae aufgezeigt und bestehen hauptsächlich aus afrikanischen Taxa. Die Verwandtschaftsverhältnisse innerhalb der Gruppe konnten mit hoher statistischer Unterstützung aufgeklärt werden und es wurden zwei Untergruppen innerhalb der Achyranthoiden gefunden. Viele Gattungen erwiesen sich als polyphyletisch. Die Ergebnisse lassen vermuten, dass die Diversifizierung der Achyranthoiden vor etwa 28 Millionen Jahren begann und dass modifizierte sterile Blüten mehrfach unabhängig entstanden sind. Übergangsraten in die unterschiedlichen Merkmalszustände waren unsymmetrisch und der Wechsel zugunsten steriler modifizierter Blüten wurde häufiger beobachtet als ihr Verlust, wohingegen keine merkmalsabhängige

Erhöhung der Arten-Diversifizierungsraten festgestellt wurde. Bayes'sche Ratenheterogenitäts-Analysen zeigten, dass sich die Achyranthoiden ohne signifikante Ratenerhöhungen diversifizierten.

Die Anhäufung modifizierter steriler Blüten innerhalb der Gruppe resultierte vermutlich aus den erhöhten Übergangsraten zugunsten steriler modifizierter Blüten. Mehrere Gewinne deuten auf einen adaptiven Wert für dieses Merkmal hin. Die Epizoochorie scheint jedoch die Artendiversifizierung der Achyranthoiden nicht vorangetrieben zu haben, was möglicherweise an den regelmäßigen Wanderungen der Säugetiere liegt, aufgrund derer eine Unterbrechung des Genflusses verhindert und somit die Möglichkeit der Artbildung durch Isolierung eingeschränkt wird.

In **Kapitel 3** wurde der Pollen von Taxa der Achyranthoiden auf Grundlage der im vorherigen Kapitel erstellten Phylogenie untersucht. Ziel war es, Synapomorphien für monophyletische Gruppen zu finden, die aus Analysen molekularer Daten abgeleitet wurden. Morphologische Merkmale sollten dabei die molekularen Ergebnisse untermauern und die Beschreibung taxonomischer Einheiten erleichtern. Es wurde gezeigt, dass die sternförmige Porenverzierung innerhalb der Achyranthoiden nur in Gattungen der Untergruppe I zu finden ist. Mehrere Pollenmerkmale wurden als sehr charakteristisch zwischen den Untergruppen I und II der Achyranthoiden sowie zwischen Gattungen und Arten innerhalb dieser Gruppen beobachtet, wie z.B. die Anzahl der Aperturen innerhalb Arten der Gattung *Psilotrichum* Blume, die stark aufgewölbten Mesoporien der Gattung *Kyphocarpa* (Fenzl) Schinz, die dodekaedrische Pollenform in S*ericocoma heterochiton* Lopr. und *Kyphocarpa* und die einzigartige Pollenform, die bei *Centemopsis* Schinz beobachtet wurde. Hier repräsentieren die Pollenkörner perfekt die Geometrie eines Fußballs. Die Form stellt ein abgestumpftes Ikosaeder dar, das aus 12 Fünfecken besteht, die den Aperturen der Pollenkörner entsprechen, und aus 20 Sechsecken, die den Bereichen der Mesoporien entsprechen. Außerdem wurde beobachtet, dass die Mesoporien einzigartig nach innen gewölbt sind, während Öffnungen angehoben erscheinen und Mikrostachel innerhalb der gesamten Gattung *Centemopsis* speziell angeordnet sind.

Kapitel 4 konzentrierte sich auf die Untergruppe II innerhalb der Achyranthoiden, in welcher mit den molekularen Analysen des zweiten Kapitels ein hohes Maß an nicht-monophyletischen Taxa auf Gattungs- und Art-Ebene gefunden wurde. Um die Beschreibung taxonomischer Einheiten zu erleichtern, ein neues Gattungskonzept zu generieren, welches Gattungen und Arten monophyletisch beschreibt, und die morphologischen Daten aus dem dritten Kapitel zu ergänzen, wurde eine Matrix von 24 Merkmalen erstellt, der Habitus und vegetative Morphologie sowie Infloreszenz- und Blütenmorphologie abdeckt. Die Merkmalszustände diese Merkmale wurden dann für die gemeinsamen Vorfahren von Abstammungsgemeinschaften rekonstruiert. Um die Auflösung der Phylogenien sowie die statistische Unterstützung der Knoten im Vergleich zum 2. Kapitel weiter zu verbessern, wurden die Datensätze vor den Analysen um Sequenzen weiterer Plastiden-Regionen ergänzt, und zwar *rpl32-trnL*$_{UAG}$ und *rps4-trnT-trnL*$_{UAA}$. Die Phylogenien, welche aus nuklearen sowie plastidialen Regionen hergeleitet wurden, waren größtenteils bezüglich der Topologie kongruent, gut aufgelöst und maximal

statistisch unterstützt. Darüber hinaus zeigten sie eine weitere Unterteilung der Achyranthoiden II in die Untergruppe II A (*Achyranthes* L. plus nahe Verwandte) und die Untergruppe II B (*Cyathula* Blume plus nahe Verwandte). Basierend auf den molekularen und morphologischen Ergebnissen konnte ein neues Gattungskonzept für die Gattungen und Arten der Achyranthoiden II erarbeitet werden. Eine kommentierte Checkliste wurde erstellt, welche die komplette homo- und heterotypische Synonymie der Gattungen und Arten sowie verschiedene Umkombinationen enthält, zwei neue Gattungen etabliert und einen Artnamen wieder einführt.

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1.1 The Amaranthaceae – a cosmopolitan flowering plant family

The Amaranthaceae constitute a nearly cosmopolitan flowering plant family within the order Caryophyllales and form a well-supported lineage together with the Chenopodiaceae (Cuénoud & al., 2002; Müller & Borsch, 2005a, b, Schäferhoff & al., 2009). Due to several shared morphological, anatomical, and phytochemical synapomorphies (compare e.g., Kühn, 1993; Townsend, 1993; Kadereit & al., 2003; Acosta & al., 2009) and the non-monophyly of Chenopodiaceae (e.g., Kadereit & al. 2003), the fusion of Amaranthaceae and Chenopodiaceae into the Amaranthaceae s.l. has been suggested (The Angiosperm Phylogeny Group 2003-2016). However, most of the floras and current studies treat them as separated families. Nonetheless, the close relationship of the Amaranthaceae s.str. (excluding Chenopodiaceae; the family name will be used in this sense in the following text) and the Chenopodiaceae is accepted unanimously throughout the literature and supported by several molecular phylogenetic studies based on plastid and nuclear markers (e.g., Kadereit et al., 2003; Müller & Borsch, 2005a, b; Hohmann & al., 2006; Kadereit & al., 2017; Di Vincenzo & al., 2018) as well as in recent phylogenomic studies (Walker & al., 2018; Morales-Briones & al., 2021). In contrast, the position of Polycnemoideae and Betoideae remain controverse within the Amaranthaceae-Chenopodiaceae alliance. Müller & Borsch (2005a) proposed Polycnemoideae as sister of Amaranthaceae plus Chenopodiaceae, whereas in other studies they were mostly resolved as sister to Amaranthaceae or treated as their subfamily (e.g., Kadereit & al., 2003; Pratt, 2003; Kadereit & al., 2012; Masson & Kadereit, 2013; Hernández-Ledesma & al., 2015). In the most recent phylogenomic studies of Amaranthaceae s.l., Polycnemoideae were resolved as sister to Chenopodiaceae (Walker & al., 2018; Kadereit & al.,2021).

While the main lineages of the Chenopodiaceae were found non-monophyletic in several phylogenetic studies, the Amaranthaceae s.str. were consistently resolved as monophyletic in phylogenetic and phylogenomic studies (compare Morales-Briones & al. 2021, Fig. 1). The Amaranthaceae s.str. consist of c. 800 species in 79 genera: Hernández-Ledesma & al. (2015) recognized 79 genera, still accepting the genera *Gossypianthus* Hook., *Lithophila* Sw. and *Philoxerus* R.Br. which were later included into *Gomphrena* L. (Ortuño & Borsch, 2020). Subsequently, *Wadithamnus* T.Hammer & R.W.Davis and *Paraerva* T.Hammer were described as new genera by Hammer & al. who also reinstated the genus *Ouret* Adans. (Hammer & al., 2017, 2019). Consequently, Amaranthaceae continue to comprise 79 genera, with diversification hotspots in the Neotropics, East- and South Africa, and Australia (Townsend, 1993).

Amaranthaceae exhibit a variety of life forms (annual and perennial herbs, subshrubs, shrubs, lianas and treelets). In respect to their morphological and physiological diversity, their species are well adapted to ecologically different habitats ranging from deserts to evergreen rainforests. Adaptations to ecological niches such as dry habitats are, for example succulence, which is found in the monotypic genus

Arthraerua (Kuntze) Schinz, consisting of the single species *Arthraerua leubnitziae* Schinz, which depicts an exception within the Amaranthaceae.

Another adaptation to dry habitats are the two anatomically different C4 leaf types which were shown in Amaranthaceae to mark an independent origin of C4-photosynthesis of at least three times within the family (Kadereit & al., 2003). Sage & al. (2007) found C4-photosynthesis in c. 28% of Amaranthaceae species with five origins throughout the family. The genus *Gomphrena* contains both, C3- and C4-species (Sage & al., 2007), and in *Alternanthera* Forssk. anatomical and physiological C3-C4-intermediates have been documented (Rajendrudu & al., 1986; Devi & Raghavendra, 1993). The highest amount of C4-species is found in Gomphrenoideae, in which C4-photosynthesis evolved three times due to analysis based on plastid data (Ortuño & Borsch, 2020).

Several Amaranthaceae species have economic value as food or animal fodder (e.g., *Amaranthus* L.), or as ornamental plants (e.g., *Amaranthus*, *Celosia* L.). Others are used in traditional medicine (e.g., *Achyranthes aspera* L., *Pupalia lappacea* (L.) Juss., *Cyathula prostrata* Blume, *C. officinalis* K.C.Kuan). Additionally, *Pupalia lappacea* is used in a traditional ritual called “arsi” which is part of the initiation ceremonies performed by tribal members in Bashada (Ethiopia), and which symbolize a variation of the ‘leap across the cattle’ – a common initiation ritual within different Ethiopian tribes (Epple & Gabbert, 1999).

1.1.1 Inflorescence structure and flower morphology of the Amaranthaceae

In Amaranthaceae, tiny sessile flowers are mostly arranged in complex cymose inflorescences or cymes are reduced to a single flower with one bract and two bracteoles (Schinz, 1934; Townsend, 1993; Acosta & al., 2009). In several species, besides the fertile flowers also sterile flowers are found which are sometimes modified to form wings or long hairs, probably serving wind dispersal, or hooks and spine-like structures, presumably facilitating animal dispersal. Flowers are mostly five-parted uniseriate perigones. They normally exhibit five epitepalous stamens which insert from a hypogynous disc and in several Amaranthaceae species alternate with androecial tube appendages, formerly called “interstaminodial appendages” or “pseudostaminodes” (e.g., Eliasson, 1988; Townsend, 1993). Androecial tube appendages have shown to originate from primordia on the androecial tube rim, and thus are considered as non-homologous with the appendages on the filaments which originate from the bases of the filaments (Vrijdaghs & al., 2014; Sánchez-Del Pino & al., 2019; Ortuño & Borsch, 2020). In Amaranthaceae they are found in a wide range of shape and size. The anthers in Amaranthaceae are uni- or bilocular; carpels are fused to a superior ovary.

1.1.2 Pollen morphology of the Amaranthaceae

Pollen grains in Amaranthaceae are spheroidal (with only a few exceptions, e.g., *Pseudoplantago* Suess.) and pantoporate (except pollen of *Pseudoplantago* and *Nothosaerva* Wight) with highly variable pore numbers, e.g., six pores in *Psilotrichum boivinianum* (Baill.) Cavaco and more than 250 in *Froelichia floridana* (Nutt.) Moq. (Borsch, 1998; Borsch & Barthlott, 1998; Müller & Borsch, 2005b). Several genera possess unique pollen characters which among angiosperms occur only in Amaranthaceae. An example is the "stellate pore ornamentation" (Müller & Borsch, 2005b) which is restricted to only few subclades and genera within the family. Differences in pollen morphology are also found in shape, size, and quantity of ektexinous bodies which surrounds the pores of pollen grains. Pollen in Amaranthaceae was found to be very diverse within genera, as recently shown for the neotropical genus *Iresine* P.Browne (Borsch & al., 2018).

1.1.3 Classification history: pre-cladistic classification vs. molecular-based phylogenies

The currently used suprageneric classification of the Amaranthaceae Townsend (1993) is pre-cladistic and based on morphology only. Townsend derived his classification mainly from Schinz (1893, 1934) who had based his concept on Bentham & Hooker (1880). Townsend (1993) recognizes two subfamilies: 1. the Amaranthoideae, characterized by bilocular anthers, and 2. the Gomphrenoideae, with unilocular anthers, which were already introduced by Schinz (1893, 1934). Townsend (1993) subdivided the Amaranthoideae into the tribe Amarantheae (subtribes Aervinae and Amaranthinae) and the tribe Celosieae. He assigned 38 genera (c. half of all Amaranthaceae genera) to subtribe Aervinae (Table 1.A; for generic concepts in the achyranthoid clade see also Chapter 4).

Nowadays, phylogenetic analyses allow to test morphology-based hypothesizes. Müller & Borsch (2005a) provided the first robust phylogeny of Amaranthaceae which showed their position within Caryophyllales: Together with Chenopodiaceae they were found to form a monophyletic group with Achatocarpaceae and Caryophyllaceae appearing as successive sisters. Whereas they proposed Polycnemoideae to be sister of Amaranthaceae plus Chenopodiaceae, other studies showed them as sister to Amaranthaceae (Kadereit & al., 2003; Pratt, 2003; Kadereit & al., 2012; Masson & Kadereit, 2013) and suggested Polycnemoideae to be best classified as subfamily of the Amaranthaceae.

According to Müller & Borsch (2005a), *Bosea* L. and *Charpentiera* Gaudich. form the first branches within the monophyletic Amaranthaceae. Only the subfamily Gomphrenoideae sensu Townsend (1993) represents a natural group (Müller & Borsch, 2005a; Sánchez del Pino & al., 2009) whereas the subfamily Amaranthoideae sensu Townsend is paraphyletic relative to the Gomphrenoideae. Phylogenetic analyses by Müller & Borsch (2005a) yielded two monophyletic groups within the non-monophyletic subtribe Aervinae: Achyranthoids and Aervoids. The achyranthoid clade (mainly composed of African taxa) and a neotropical gomphrenoid clade (within subfamily Gomphrenoideae)

were hypothesized to depict two main radiations within Amaranthaceae. The gomphrenoid clade was later confirmed to constitute a neotropical major radiation comprising about 380 species (Sánchez del Pino & al., 2009). The subtribe Aervinae was shown to be non-monophyletic (Müller & Borsch, 2005a, 2005b, and this study) because *Aerva* Forssk., *Nothosaerva*, *Ptilotus* R.Br. and allies depict an independent clade (Hammer & al., 2017, 2019), which is diverging earlier than the Achyranthoids plus Gomphrenoideae.

The achyranthoid clade was not further examined in detail in any study after its detection in 2005 and therefore is subject of this doctoral thesis.

Table 1.A: History of classification systems of Amaranthaceae with a focus on the achyranthoid clade.
For Achyranthoids (highlighted in grey), subdivisions are listed beyond the generic level. For all other genera, these subdivisions - if available - are not displayed here.

	Family	Subfamily	Tribe	Subtribe	Genera	Section	Subgenus	Section
Endlicher 1837	Amarantaceae		Achyrantheae	Aerveae	*Achyranthes, Centrostachys, Psilotrichum*			
					Aerva, Nyssanthes, Ptilotus, Trichinium			
				Amaranteae	*Amarantus, Chamissoa*			
				Desmochaeteae	*Pupalia*			
					Desmochaeta, Digera			
				Polycnemeae	*Hemichroa, Polycnemum*			
			Celosieae		*Celosia, Cladostachys, Deeringia, Lanzia, Lestibudesia*			
			Gomphreneae		*Alternanthera, Frölichia, Gomphrena, Iresine, Teleianthera, Trommsdorfia*			
Moquin-Tandon 1849	Amarantaceae		Achyrantheae	Aerveae	*Achyranthes*	*Achyropsis*		
						Cadelari		
						Pandiaka		
					Centrostachys, Psilotrichum			
					Sericocoma	*Euchnoa*		
						Kyphocarpa		
					Acnida, Aerva, Amblogyna, Banalia, Charpentiera, Euxolus, Lachnostachys, Mengea, Nyssanthes, Ptilotus, Rodetia, Scleropus, Trichinium			
				Amaranteae	*Acroglochin, Amarantus, Chamissoa, Hablitzia, Lagrezia, Sarratia*			
				Desmochaeteae	*Cyathula*	*Polyscalis*		
						Telostachys		
					Pupalia	*Desmochaeta*		
						Orthochaeta		
					Digera, Saltia			
				Polycnemeae	*Hemichroa, Polyycnemum*			
			Celosieae		*Celosia, Cladostachys, Deeringia, Henonia, Hermbstaedtia*			
			Gomphreneae		*Alternanthera, Cruzeta, Froelichia, Gomphrena, Gossypianthus, Guilleminea, Ireneis, Iresine, Phyllepidum, Telanthera, Tryphera*			

Table 1.A – continued

	Family	Subfamily	Tribe	Subtribe	Genera	Section	Subgenus	Section
Bentham & Hooker 1880	Amarantaceae		Amaranteae	Achyrantheae	*Achyranthes, Achyropsis, Calicorema, Centema, Chionothrix, Cyathula, Pandiaka, Psilotrichum, Pupalia*			
					Sericocoma	*Euchroa*		
						Kyphocarpa		
						Sericorema		
					Aerva, Nothaerva, Psilostachys, Ptilotus, Saltia, Stilbanthus, Trichinium			
				Euamaranteae	*Acanthochiton, Achatocarpus, Acnida, Allmania, Amarantus, Banalia, Bosia, Chamissoa, Charpentiera, Digera, Nyssanthes, Rodetia*			
			Celosieae		*Celosia, Deeringia, Henonia, Hermbstaedtia, Melanocarpum*			
			Gomphreneae		*Alternanthera, Cladothrix, Dicraurus, Froelichia, Gomphrena, Gossypianthus, Guilleminea, Hebanthe, Iresine, Mogiphanes, Pfaffia, Philoxerus, Telanthera, Woehleria*			
Baillon 1887	Chénopodiacées		Amaranteae		*Achyranthes, Calicorema, Centema, Chionothrix, Cyathula, Pandiaka, Psilotrichum, Pupalia, Sericocoma*			
					Acanthochiton, Aerva, Allmania, Amarantus, Banalia, Chamissoa, Charpentiera, Digera, Marcellia, Nothosaerva, Nyssanthes, Pleuropterantha, Psilostachys, Rodetia, Saltia, Stilbanthus, Trichinium			
			Celosieae		*Celosia, Deeringia, Henonia, Hermbstaedtia, Pleuropetalum*			
			Gomphreneae		*Alternanthera, Cladothrix, Cruzeta, Dicraurus, Froelichia, Gomphrena, Gossypianthus, Guilleminea, Hebanthe, Pfaffia, Philoxerus, Telanthera, Woehleria*			
Schinz 1893	Amaranthaceae	Amaranthoideae	Amaranteae	Achyranthinae	*Achyranthes*		*Achyropsis*	
							Euachyranthes	*Aspera*
								Centrostachys
							Pandiaka	
					Arthraerua, Centema, Chionothrix			
					Cyathula	*Achyranthoidae*		
						Eucyathula		
					Mechowia, Psilotrichum, Pupalia			
					Sericocoma		*Eurotia*	
							Eusericocoma	
					Aerva, Nothosaerva, Nyssanthes, Ptilotus, Saltia, Stilbanthus			
				Amarantinae	*Acanthochiton, Acnida, Allmania, Amarantus, Banalia, Bosia, Chamissoa, Charpentiera, Digera, Pleuropterantha*			
			Celosieae		*Celosia, Deeringia, Henonia, Hermbstaedtia, Pleuropetalum*			
		Gomphrenoideae	Gomphreneae	Froelichinae	*Alternanthera, Froelichia, Gossypianthus, Pfaffia*			
				Gomphreninae	*Dicraurus, Gomphrena, Iresine, Woehleria*			
			Guillemineae		*Cladothrix, Guilleminea*			

Table 1.A – continued

	Family	Subfamily	Tribe	Subtribe	Genera	Section	Subgenus	Section
Standley 1917	Amaranthaceae		Amarantheae		*Acanthochiton, Acnida, Amaranthus, Chamissoa, Lagrezia*			
			Brayulineae		*Brayulinea*			
			Celosieae		*Celosia, Pleuropetalum*			
			Centrostachydeae		*Cyathula, Centrostachys*			
			Froelichieae		*Froelichia*			
			Gomphreneae		*Achyranthes*			
					Dicraurus, Gomphrena, Gossypianthus, Iresine, Lithophila, Pfaffia, Philoxerus, Tidestromia, Woehleria			
Schinz 1934	Amaranthaceae	Amaranthoideae	Amarantheae	Achyranthinae	*Achyranthes, Achyropsis, Arthraerua, Calicorema, Centema, Centemopsis, Centrostachys, Chionothrix*			
					Cyathula	*Achyranthoideae*		
						Eucyathula		
					Dasysphaera, Kyphocarpa, Leusosphaera, Lopriorea, Marcelliopsis, Mechowia, Nelsia, Nototrichium, Pandiaka, Psilotrichum, Pupalia, Sericocoma, Sericocomopsis, Sericorema, Sericostachys, Volkensinia			
					Aerva, Diptheranthemum, Neocentema, Nothosaerva, Nyssanthes, Ptilotus, Saltia, Stilbanthus			
				Amaranthinae	*Acanthochiton, Acnida, Allmania, Amaranthus, Apterantha, Banalia, Bosea, Chamissoa, Charpentiera, Digera, Lagrezia, Pleuropterantha*			
			Celosieae		*Celosia, Deeringia, Henonia, Hermbstaedtia, Pleuropetalum*			
		Gomphrenoideae	Brayulineae	Brayulineinae	*Brayulinea*			
				Tidestromiinae	*Tidestromia*			
			Gomphreneae	Froelichiinae	*Alternanthera, Froelichia, Froelichiella, Gossypianthus, Pfaffia*			
				Gomphreninae	*Dicraurus, Gomphrena, Iresine, Lithophila, Philoxerus, Pseudogomphrena, Woehleria*			

Table 1.A – continued

	Family	Subfamily	Tribe	Subtribe	Genera	Section	Subgenus	Section
Cavaco1962	Amaranthaceae	Amaranthoideae	Achyrantheae		*Achyranthes*			
					Achyropsis	*Achyropsis*		
						Aristata		
					Arthraerua, Calicorema, Centemopsis, Chionothrix, Lopriorea, Mechowia, Nototrichium			
					Pandiaka		*Achyranthopsis*	
							Pandiaka	
					Psilotrichum, Sericocoma, Sericocomopsis			
					Aerva, Allmania, Apterantha, Banalia, Bosea, Chamissoa, Charpentiera, Dipteranthemum, Lagrezia, Nothosaerva, Nyssanthes, Pseudodigera, Ptilotus, Robynsiella			
			Amarantheae		*Acanthochiton, Acnida, Amaranthus*			
			Digereae		*Centema*			
					Cyathula	*Achyranthoideae*		
						Eucyathula		
						Nelsioideae		
					Dasysphaera, Kyphocarpa, Leucosphaera, Marcelliopsis, Nelsia, Pupalia, Sericorema, Sericostachys			
					Allmaniopsis, Digera, Neocentema, Pleuropterantha, Pseudosericocoma, Saltia			
		Brayulineoideae			*Brayulinea*			
		Celosioideae			*Celosia, Deeringia, Henonia, Hermbstaedtia, Pleuropetalum*			
		Gomphrenoideae	Gomphreneae	Froelichiinae	*Alternanthera, Froelichia, Froelichiella, Gossypianthus, Pfaffia*			
				Gomphreninae	*Dicraurus, Gomphrena, Iresine, Lithophila, Philoxerus, Pseudogomphrena, Woehleria*			
			Tidestromeae		*Tidestromia*			
Townsend 1993	Amaranthaceae	Amaranthoideae	Amarantheae	Aervinae	*Achyranthes, Achyropsis, Arthraerua, Calicorema, Centema, Centemopsis, Centrostachys, Chionothrix, Cyathula, Dasysphaera, Kyphocarpa, Leucosphaera, Lopriorea, Marcelliopsis, Mechowia, Nelsia, Nototrichium, Pandiaka, Polyrhabda, Psilotrichum, Pupalia, Rosifax, Sericocoma, Sericocomopsis, Sericorema, Sericostachys, Volkensinia*			
					Aerva, Allmaniopsis, Eriostylos, Pseudosericocoma, Psilotrichopsis, Saltia, Trichuriella, Nothosaerva, Stilbanthus, Nyssanthes, Ptilotus			
				Amaranthinae	*Allmania, Amaranthus, Bosea, Chamissoa, Charpentiera, Digera, Herbstia, Indobanalia, Lagrezia, Neocentema, Pleuropterantha, Siamosia*			
			Celosieae		*Celosia, Deeringia, Henonia, Hermbstaedtia, Pleuropetalum*			
		Gomphrenoideae	Gomphreneae	Froelichiinae	*Alternanthera, Froelichia, Froelichiella, Guilleminea, Pfaffia, Tidestromia*			
				Gomphreninae	*Blutaparon, Gomphrena, Irenella, Iresine, Lithophila, Pseudogomphrena, Woehleria*			
			Pseudoplantageae		*Pseudoplantago*			

1.2 The achyranthoid clade

The achyranthoid clade was estimated to include 120-145 species, based on *matK/trnK* sequences and dependent on the placement of further so far unsampled genera (Müller & Borsch, 2005a). Unlike the situation in Gomphrenoids, which are characterized by unilocular anthers (Sánchez del Pino & al., 2009), no phenotypic synapomorphy is so far known for the Achyranthoids. Nevertheless, members of the clade have evolved many species possessing complex cymose structures with fertile and sterile flowers arranged together within the inflorescence, a character state that within Amaranthaceae only occurs in *Pseudoplantago* in the subfamily Gomphrenoideae (Covas 1939). This is a case of convergent evolution concerning Achyranthoids and *Pseudoplantago* but is also a character state that could possibly be synapomorphic for the achyranthoid clade (in a sampling that includes lineages of the Amaranthoideae only) and thus needs to be investigated through a broader taxon sampling. Furthermore, the tepals and bracteoles of sterile flowers forming terminal hooks or spines, completely falling off at maturity in several species. These modified sterile flowers are supposed to support animal dispersal. Diversification of an African achyranthoid radiation could therefore have been fueled by adaptation to open habitats with large animals serving dispersal.

According to Müller & Borsch (2005a, 2005b) and Sage & al. (2007), the achyranthoid clade encompasses the following genera, of which the latter four were shown either para- or highly polyphyletic: *Achyranthes* L., *Achyropsis* Benth. & Hook.f., *Arthraerua* (Kuntze) Schinz, *Centemopsis* Schinz, *Kyphocarpa* (Fenzl) Schinz, *Marcelliopsis* Schinz, *Mechowia* Schinz, *Nototrichium* Hillebr., *Pupalia* Juss., *Sericocoma* Fenzl ex Endl., *Sericorema* Lopr., *Sericostachys* Gilg & Lopr. ex Lopr., *Cyathula* Blume, *Calicorema* Hook.f., *Pandiaka* (Moq.) Benth. & Hook.f., and *Psilotrichum* Blume. Prior to molecular studies, all these taxa were assigned to the subtribe Aervinae sensu Townsend (1993) [subfamily Amaranthoideae, tribe Amarantheae; compare Table 1.A], which had later shown to be non-monophyletic (Müller & Borsch, 2005a). *Nototrichium*, *Achyranthes*, *Achyropsis*, *Pandiaka*, *Sericostachys* and *Cyathula* were hypothesized to constitute a subclade within Achyranthoids (Müller & Borsch, 2005b). This group seems to be the only one within the achyranthoid clade in which stellate pore ornamentation is completely missing (Müller & Borsch, 2005b).

Most of the achyranthoid genera are endemic to Africa. The only genera with few species outside the African continent are *Achyranthes* (distributed in Africa, Asia, and the Pacific Isles), *Nototrichium* (endemic to the Hawaiian Islands) *Cyathula* (species richness chiefly in Africa but additionally two species in Asia) and *Psilotrichum* (main distribution in tropical Africa but five species occurring in tropical Asia) which probably all derived from African ancestors.

The common vegetation types in which most African Achyranthoids occur are *Acacia-Commiphora* woodland and *Acacia* wooded grasslands, open bushland or grassland vegetation complexes, dry evergreen Afromontane forests, savannas, or semi-desert scrublands; for classification of vegetation types see White (1983) and Friis & al. (2011).

1.3 Epizoochory – directed dispersal in wide open habitats

Epizoochory occurs in <5 % of seed plants (Sorensen, 1986) but is frequent in Achyranthoids (>80 % of the genera). Ridley (1930) suggested that the evolution of plants exhibiting burrs as dispersal units in Africa was primarily promoted through epizoochory by herds of ungulates covering large areas of the African continent between the Eocene and the Pleistocene. He reported species of *Cyathula*, *Achyranthes* and *Pupalia* to be animal-dispersed by adhesion and described the diaspores of *Pupalia* as 'the most persistently adhesive burrs' he knows.

There are many studies showing epizoochory as a perfect (long-distance-) dispersal mechanism in vegetation types with open spaces, such as those habitats in which the majority of achyranthoid species is distributed. Animals are known to disperse diaspores by adhesion in their fur successfully over short and up to very long distances (Agnew & Flux, 1970; Stebbins, 1971; Sorensen, 1986; Mori & Brown, 1998; Lobova & Mori, 2004). Additionally, there are several studies which developed and tested models of dispersal mechanisms, measured retention times and separation forces of diaspores in different fur types (e.g. cattle, donkey, sheep, goats) and evaluated adaptations to epizoochory (e.g. hooks, spines, barbs), in order to predict attachment potentials of different diaspores (e.g. Gorb & Gorb, 2002; Römermann & al., 2005; Tackenberg & al., 2006; Will & al., 2007; Will & Tackenberg, 2008; Bullock & al., 2011). Those studies showed that retention times of diaspores in fur as well as dispersal distances depend on different factors, such as fur type, correlation between weight and size of diaspores, diaspore surface structure (availability of hooks, barbs, spines) and their noticeability for spreaders, which is negatively correlated to the retention time.

Several studies also included species of Achyranthoids in their sampling – such as *Achyrantes aspera*, *Cyathula prostrata*, *C. orthacantha* Schinz, and *Pupalia lappacea* – and showed them to be very well dispersed over long distances through epizoochory and therefore underline the idea that Achyranthoids evolved in relation to evolution of African savanna and grassland ecosystems. Moreover, Agnew & Flux (1970) observed diaspores of different plant species attached to the fur of hares in Kenya, all of which mainly possessed hooks and barbs, such as *Achyranthes aspera*, *Pupalia lappacea* and *Cyathula orthacantha*. They discussed the dry thornbush flora of East Africa to be highly adapted for zoochory but evaluated that the diaspores of *Pupalia* and *Cyathula* stick too well in the fur of hares or even hurt their skin and therefore might not fall and germinate at a favorable time. They concluded that species of *Pupalia* and *Cyathula* might be better adapted to dispersal by larger animals such as antelopes with shorter and more robust fur.

The advantage of epizoochory over anemochory is that it is more precise regarding habitat conditions: Animals migrate between similar habitats which serves for a directed dispersal of seeds and guarantees an optimal habitat for the offspring of plants. In addition, wind is unpredictable in strength, direction and timing so it is not guaranteed to have strong enough winds in the time of fruit/ seed maturity to serve for adequate dispersal (Stebbins, 1971).

The advantage of epizoochory over endozoochory is that this mechanism is more economical for plants because they don't have to invest in attracting mechanisms (fruit color, smell) and in nutritious fruits serving as a reward for their distributors. In contrast to endozoochory, epizoochory depicts a passive dispersal and thus, epizoochorous dispersed plants which grow in the neighborhood of endozoochorous dispersed plants might profit from the attracting mechanisms of their neighbors and thus statistically have a higher chance to be dispersed (Sorensen, 1986). The study of Lobova & Mori (2004) reports that diaspores of *Cyathula prostrata* have been found in faecal samples of two different bat species (*Sturnira tildae* de la Torre and *S. lilium* Geoffroy) in French Guiana. Besides those intact diaspores of *Cyathula* (which therefore are supposed to not have passed the digestive tracts of the bats but might had stuck to the bats fur) seeds of *Solanum torvum* Sw. and *Piper bartlingianum* (Miq.) C.DC., which are considered as food for species of *Sturnira* Gray and grow in the same habitat as *Cyathula prostrata*, were found in the faecal samples. Several achyranthoid species grow in the same habitats as e.g., species of *Solanum* L. or plants which are regularly browsed by large animals. These Achyranthoids might profit from their neighbors which depict a nutritious source for animals.

1.4 Molecular markers with high levels of hierarchic phylogenetic signal

The *matK/trnK* region is one of the best-established phylogenetic markers and yields large quantities of informative characters, even at low taxonomic levels (Korotkova & al., 2011). It has first been used in intrafamiliar phylogenies (e.g., Johnson & Soltis, 1994; Steele & Vigalys, 1994) and afterwards widely been employed in c. 77% of all molecular studies from 1995 to 2002 together with the *trnL-F* region (Shaw & al., 2005). *MatK* is a coding region localized in the *trnK*-Intron and encoding the maturase K. Coding regions are the more conservative parts of the chloroplast DNA, i.e., that these regions normally evolve very slow and thus are not sufficiently informative to resolve relationships of closely related taxa. *MatK* is the fastest evolving gene with the highest variability among plastid genes and therefore is more informative. *TrnK* is a group II intron (noncoding region). Noncoding regions are known to be highly variable and informative at low taxonomic levels (e.g., Borsch & al., 2009; Korotkova & al., 2011; Barniske & al., 2012). Sequencing of the whole *matK/trnK* region is promising for phylogenetic analyses of the achyranthoid clade, as it has already been successfully employed in analyses of the Amaranthaceae-Chenopodiaceae alliance (Kadereit & al., 2003; Müller & Borsch, 2005a, b; Fuentes-Bazan & al., 2012; Borsch & al., 2018; Ortuño & Borsch, 2020) to resolve relationships at low taxonomic levels (genera and species). For an efficient lab protocol with additional sequence characters, the sequencing of the *matK/trnK* region was later extended to include the *trnK-psbA* spacer.

TrnL-F and *rpl16* are plastid markers, located in the large single-copy region of the chloroplast genome. As mentioned above, *trnL-F* is widely employed in phylogenetic analyses at intraspecific and infrageneric levels (e.g., Borsch & al., 2003; Worberg & al., 2007; Sánchez del Pino & al., 2009) In phylogenetic analyses which were based on the combination of *trnL-F* and *rpl16*, better phylogenetic signals were gained than in analyses of the *matK/trnK* region, although numbers of sequenced nucleotides were comparable (Müller & al., 2006; Borsch & Quandt, 2009). *Rpl16* is known as the most variable plastid intron (Kelchner, 2002) with high interspecific variability and therefore a good phylogenetic signal between closely related taxa (Löhne & al., 2007; Sánchez del Pino & al., 2009).

The combination of different noncoding plastid regions (introns and spacers) showed well resolved trees with high phylogenetic signal in several studies (e.g., Löhne & al., 2007; Sánchez del Pino & al., 2009; Korotkova & al., 2011) . Hence, a combination of the plastid markers *trnK/matK*, *trnL-F* and *rpl16* was chosen for phylogenetic analyses of the whole achyranthoid clade in the present study, in line with other investigations in the Amaranthaceae (Borsch et al. 2018, Ortuño & Borsch, 2020). This will also allow the integration of the results from this investigation into future family-wide multiple sequence alignments and phylogenetic analyses. For analyses of subclades, sequences of the plastid intergenic spacer between *rpl32-trnL*$_{UAG}$ and the two spacers in the *rps4-trnT-trnL*$_{UAA}$ region were additionally used to further improve tree resolution and untangle relationships of closely related taxa. They rank among the most variable plastid regions (Shaw & al., 2007; Dong & al., 2012; Korotkova & al., 2014)

and yielded good hierarchical phylogenetic signal in other analyses of Caryophyllales lineages (e.g Fassou & al., 2022).

In addition, analysis of nuclear ITS was done to obtain a more reliable understanding of species level relationships. ITS was chosen because this marker works well in Amaranthaceae. Possible incongruence between ITS and plastid trees can be used to detect reticulate evolution (following Fuertes Aguilar & Nieto Feliner, 2003; Koontz & al., 2004; Löhne & al., 2008; Fuentes-Bazan & al., 2012). Reticulation points to hybridization between different species and thus is important to interpret relationships and possible occurring incongruence between molecular trees.

1.5 Study design and structure of the dissertation

This doctoral thesis had the major aims to understand the composition of the African achyranthoid clade, to evaluate its monophyly and to resolve relationships within the clade on genus- and species level. Furthermore, the diversification of the achyranthoid clade in time and space should be clarified in the curse of this study. To gain a comprehensive understanding of the clade and its taxa, a combined molecular, morphological and palynological approach was employed.

The study was embedded in the overall DFG-project "Systematics of the Amaranthaceae and evolution of pollen characters" which equally focused at the Neotropics, and Africa. In the first phase of the overall project an overall phylogeny of Amaranthaceae was generated, that indicated the existence of an achyranthoid clade (Müller & Borsch, 2005a, b). Building upon this result, the dissertation project focused on this African achyranthoid clade, and thus contributed to the aims of the overall DFG-project.

Major aims of the overall project were to generate a phylogenetic hypothesis based on molecular data, which resolves the relationships of Amaranthaceae to Chenopodiaceae, but also the intrafamilial relationships of the Amaranthaceae. The combination of phylogenetic studies on the one hand, and molecular and palynological studies on the other hand built the basis for the revision of pre-cladistic classification systems and the development of a new and modern one. The integration of morphological data allowed to reconstruct the evolution of phenotypical diversity, and to develop a scenario for the genesis of species diversity within the Amaranthaceae. Considering that pollen morphology is highly variable within Amaranthaceae, a specific aim was to reconstruct the evolution of pollen characters, in order to answer the questions of which characters are homoplastic and which are synapomorphic. The combination of (pollen-) morphological, molecular and ecological approaches moreover provided scientific data of fundamental importance for conservation.

The present dissertation is divided into 5 chapters, out of which Chapter 2 was already published in the journal "Annals of Botany" (the original publication is attached at the end of the work), Chapter 3 and 4 are to be published as manuscripts and are in preparation to be submitted. Each of these chapters contains a table of contents and is structured into the sections "introduction", "material and methods", "results", and "discussion", and is followed by its Appendices. Full references are listed after Chapter 5.

Chapter 1 is the "General introduction".

In **Chapter 2** a robust, molecular based phylogenetic hypothesis for the Achyranthoids is generated, in order to evaluate the composition of the clade. This phylogeny is the base for the application of a molecular clock, the estimation of divergence times of the clade and its relatives and for the reconstruction of the evolution of modified sterile flowers. By the application of a trait-dependent approach, the influence of modified sterile flowers on speciation, extinction, and character transition rates in the Achyranthoids is evaluated. The implementation of ancestral character reconstruction of modified sterile flowers allows to test the hypothesis that an African achyranthoid radiation could have

been reinforced by a key innovation as adaptation to open habitats with large animals serving dispersal (Chapter 2).

In addition to groups that were already established members of the achyranthoid clade, this thesis includes genera such as *Centrostachys* Wall., *Chionothrix* Hook.f., *Dasysphaera* Volkens ex Gilg, *Lopriorea* Schinz, *Nelsia* Schinz, *Polyrhabda* C.C.Towns., *Digera* Forssk., *Lagrezia* Moq., *Henonia* Moq. and *Volkensinia* Schinz, all of which had not been included into any phylogenetic analysis before. Hence, this study offers a broader sampling regarding the number of taxa, molecular markers, and characters than all previous analyses which included taxa of the achyranthoid clade. Thus, a better resolution on species level and higher support for phylogenetic hypotheses is achieved.

In **Chapter 3** the molecular data is complemented by pollen morphological studies for the whole clade. Pollen characters are reconstructed with a Bayesian approach. This helps clarifying species and genus limits and supports the molecular results.

Chapter 4 taxonomically revises subclade II of the Achyranthoids (compare the results of Chapter 2 which are published in Di Vincenzo & al. (2018)) and offers a new genus concept for the subclade which makes its genera monophyletic. Additionally, species limits in *Sericocomopsis hildebrandtii* Schinz — for which phylogenetic results (Chapter 2) provided evidence for polyphyly — are addressed. In Chapter 4 additional markers are used to fully untangle the relationships of all members of the achyranthoid subclade II. Furthermore, several morphological characters are reconstructed with a Bayesian approach and mapped on the phylogeny. Thus, the evaluation of genus limits is facilitated, and the molecular hypothesis is supported through morphology.

Chapter 5 is the “General discussion”.

– Chapter 2 –
Evolutionary diversification of the African achyranthoid clade (Amaranthaceae) in the context of sterile flower evolution and epizoochory

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2.1 Introduction

The Amaranthaceae constitute a nearly cosmopolitan family of flowering plants within the order Caryophyllales, forming a well-supported lineage with the Chenopodiaceae (Cuénoud *et al.* 2002; Schäferhoff *et al.* 2009). In the strict sense (excluding Chenopodiaceae), the Amaranthaceae contain ~800 species in 79 genera (Hernández-Ledesma *et al.* 2015), comprising a variety of life forms (herbs, shrubs, lianas and trees) that inhabit a wide range of habitats (from deserts to rainforests). Most of the diversity of the Amaranthaceae is found in the neotropics as well as in eastern and southern Africa (Townsend 1993).

Müller and Borsch (2005) found the subfamily Amaranthoideae and most of the tribes and subtribes in the classification of Schinz (1934) and Townsend (1993) to be para- or polyphyletic. A newly recovered achyranthoid clade included several genera of the subtribe Aervinae, while the genera *Aerva*, *Nothosaerva* and *Ptilotus* were identified as part of a separate lineage (the 'aervoid clade'; Müller and Borsch 2005b). The achyranthoid clade was estimated to comprise ~134–150 species in 31 genera, if currently accepted genera were upheld. However, adequate support for the clade was lacking due to limited character and taxon sampling. We assume that, next to the mostly neotropical Gomphrenoids (Sánchez del Pino *et al.* 2009), which comprise about 380 species, the achyranthoid clade constitutes the second main radiation within the Amaranthaceae. The majority of achyranthoid taxa occur in Africa, except the Hawaiian genus *Nototrichium*, species of *Achyranthes* from Asia and the Pacific, and certain species of *Cyathula* distributed in China and the Himalaya.

Members of the achyranthoid clade (Müller and Borsch 2005b) and of many Aervinae in the pre-phylogenetic circumscription of the subtribe possess cymose inflorescence structures. In several taxa, sterile modified flowers appear within partial florescences, which fall off completely at maturity as burr-like units (Acosta *et al.* 2009). The sterile flowers are modified to hooks, spines or barbs, which provide adhesive structures serving epizoochory. In some genera (e.g., *Achyranthes*), which do not exhibit sterile flowers, the midribs of bracteoles are thickened to spines, which also form adhesive appendages.

Epizoochory occurs in <5 % of seed plants (Sorensen 1986) but is frequent in Achyranthoids (>80 % of the genera). Ridley (1930) suggested that the evolution of plants exhibiting burrs as dispersal units in Africa was primarily promoted through epizoochory by herds of ungulates covering large areas of the African continent between the Eocene and the Pleistocene. He reported species of *Cyathula*, *Achyranthes* and *Pupalia* to be animal-dispersed by adhesion and described the diaspores of *Pupalia* as 'the most persistently adhesive burrs' he knows. Agnew and Flux (1970) as well as Mori and Brown (1998) showed that several achyranthoid species had been dispersed over long distances in the fur of animals. Most African Achyranthoids occur in *Acacia–Commiphora* wood- and bushlands, dry evergreen Afromontane forests, grassland vegetation complexes or semi-desert scrublands, all of which are vegetation types with open spaces (White 1983; Friis *et al.* 2011) and are thus suitable for long-distance dispersal via epizoochory.

In the present investigation, we hypothesize that the modified sterile flowers present in many African Amaranthaceae evolved during time periods when African habitats changed to more open conditions and the local diversity of terrestrial mammals increased. We propose that the diversification of the African achyranthoid clade may have been fueled by these adaptations to epizoochory. To evaluate this hypothesis, we conducted a series of phylogenetic analyses of the Amaranthaceae by following three primary aims. First, we aimed to generate a robust molecular phylogenetic hypothesis for the Achyranthoids to evaluate the composition of the achyranthoid clade initially found by Müller and Borsch (2005b). Second, we aimed to estimate divergence times and to reconstruct the evolution of modified sterile flowers based on our new phylogenetic trees. This second aim would allow us to identify the number of origins of modified sterile flowers within the Achyranthoids. Third, we aimed to evaluate the influence of morphological traits that may have an adaptive value for epizoochory in speciation and extinction in the Achyranthoids. In this third goal, we specifically aimed to understand (1) whether speciation and extinction rates are significantly different in the presence of the character 'modified sterile flowers' (and the more inclusive functional trait of 'adhesive appendages', which can be considered as any part of a dispersal unit with an adhesive property and an adaptive value for epizoochory, regardless of whether a taxon possess sterile flowers) than in their absence, (2) whether transition rates between character states are strongly asymmetrical, and (3) whether net diversification is higher in the presence of these features than in their absence. The specific research questions of the third aim were evaluated through a trait-dependent as well as a trait-independent approach. In the trait-dependent approach, we estimated and compared speciation, extinction and character transition rates in relation to morphological traits with a potential adaptive value for epizoochory. In the trait-independent approach, we explored possible diversification rate shifts across the entire family Amaranthaceae. Taken together, these analyses will allow us to evaluate whether the diversification of the African achyranthoid clade may have been fueled by the emergence of modified sterile flowers in this group.

2.2 Methods

2.2.1 Collection of plant material and taxon sampling

Plant material was primarily collected during field trips to Ethiopia and Kenya in 2012 and 2013. Taxon sampling focused on the whole subtribe Aervinae *sensu* Schinz (1934). We included almost half of all known species belonging to the achyranthoid clade in our analyses, representing 26 of 31 genera. Our sampling was thus guided to best represent the morphological diversity in the group, independently of the current classification into genera. Also, the very few species occurring outside Africa were represented in our sampling; sampling statistics are provided in Table 2.A. Taxa that were recovered with minimal support or in unexpected phylogenetic positions were supplemented by additional samples of the same species from different localities to verify the inferred positions. Species names, geographical origin, and herbarium voucher information for all taxa under study are given in Appendix 2.A and Appendix 2.B.

Table 2.A: Number and proportions of genera and species sampled for the present investigation.

	Genera (approximate sampling proportions)	Species (approximate sampling proportions)
Estimated taxon numbers of Achyranthoids	31	c. 134-150
Taxa in dataset A	26 (84%)	69 (51%)
Taxa in dataset B	26 (84%)	65 (49%)
Taxa in dataset C	23 (74%)	63 (47%)

2.2.2 Construction of datasets

For the present investigation, we generated a total of 396 new DNA sequences. In addition, we included 112 sequences from (Müller and Borsch 2005a; b) in our datasets. Three different DNA matrices were assembled: (1) a combined plastid dataset (hereafter dataset A), which consisted of the chloroplast regions *trnK*/*matK*, *rpl16* and *trnL-F* and comprised 130 samples with a focus on the achyranthoid clade but also a representative coverage of subfamily Amaranthoideae; (2) a broader plastid dataset (dataset B), which consisted of the chloroplast region *trnK*/*matK* only and comprised a total of 189 samples from across the Caryophyllales (134 samples of Amaranthaceae, 40 of Chenopodiaceae and 15 representing clades within the Caryophyllales); and (3) a nuclear ribosomal dataset (dataset C), which consisted of internal transcribed spacer (nrITS) sequences of 105 taxa with a focus on Achyranthoids plus representatives of other main Amaranthaceae clades.

We used dataset A to reconstruct detailed relationships of the achyranthoid clade; dataset B was employed for divergence dating, as it allowed (1) the placement of three fossil specimens assigned to the Chenopodiaceae as primary calibration points, and (2) the definition of three secondary calibration points by using age estimates of Bell *et al.* (2010). Dataset C allowed a comparison of tree topologies inferred from nuclear ribosomal data with those inferred from plastid DNA regions.

2.2.3 DNA isolation, amplification, and sequencing

Total genomic DNA was isolated as described in Müller and Borsch (2005a). Primer sequences and details of the PCR protocols are listed in Appendix 2.C and Appendix 2.D. Upon amplification, all PCR products were sequenced by standard Sanger sequencing at Macrogen Europe (Amsterdam, The Netherlands). Chromatograms were inspected by eye, corrected for erroneous nucleotide calls and assembled using PhyDE 0.9971 (Müller *et al.* 2010). Final DNA sequences were submitted to ENA (http://www.ebi.ac.uk/ena/) upon conversion to checklist files with custom Python scripts (https://github.com/michaelgruenstaeudl/annonex2embl). ENA/GenBank accession numbers for all taxa under study are given in Appendix 2.A and Appendix 2.B.

2.2.4 Sequence alignment, indel coding and model selection

Initial alignment files generated with Muscle 3.6 (Edgar 2004) were further refined under a motif-based approach (Löhne and Borsch 2005) using PhyDe. Areas of uncertain homology were subsequently excluded from matrices. Insertions and deletions in datasets A and C were coded according to the 'simple indel coding' scheme (Simmons and Ochoterena 2000) as implemented in SeqState 1.4.0 (Müller 2005). Inversions were reverse-complemented and coded manually. Best-fitting models of nucleotide substitution were selected via the Akaike information criterion (AIC; Akaike 1974) using the software jModeltest 2.1.7 (Darriba *et al.* 2012). The substitution model GTR+G was found to display the best fit for DNA sequences of *matK*, the *trnL* intron and the *trnL-F* intergenic spacer. The model GTR+G+I was found to display the best fit for DNA sequences of ITS1, ITS2, *rpl16* and the *trnK* intron, and the model HKY+I for the 5.8S ribosomal DNA.

2.2.5 Phylogenetic analysis

Phylogenetic inference was performed via maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). Parsimony analyses were conducted in PAUP* 4.0b10 (Swofford 2002), with 10,000 jackknife (JK) replicates to calculate node support. Likelihood analyses were conducted in RAxML 7.4.2 (Stamatakis 2006), with node support calculated via 1,000 rapid bootstrap (BS) replicates, using the RAxMLGui 1.3 as input interface (Silvestro and Michalak 2012). BI was performed with MrBayes 3.2.2 (Ronquist *et al.* 2012), with node support calculated as posterior probabilities. Four independent Markov chain Monte Carlo (MCMC) runs with four chains and 10,000,000 generations each were conducted, with trees sampled every 1,000th generation. The burn-in was set to 50 %, resulting in a combined posterior tree distribution of 20,000 trees. Majority-rule consensus trees were calculated from the combined posterior tree distribution subsampled to 1,000 trees. We rated BS/JK values of 70–84 % and posterior probability of 0.9–0.94 as moderate support, and BS/JK values of 85–100 % and posterior probability of 0.95–1 as strong support. For BI and ML analyses, datasets were partitioned to allow independent parameter estimation for each partition. For analyses in RAxML, the

model MULTIGAMMAI was employed as the nearest approximation to the model GTR+I+G. The binary character model (Lewis 2001) was applied to indel partitions. DNA sequence alignments and the main phylogenetic trees inferred were submitted to TreeBASE (number 21912).

For all trait-dependent and -independent analyses of speciation and extinction rates as well as our ancestral character state reconstructions, we reduced multiple samples of the same species in our phylogenetic trees to a single representative by randomly selecting one sequence. In cases of non-monophyly of multiple samples per taxon, one representative per phylogenetic position was kept. This strategy ensured that taxa were properly represented and simultaneously prevented species duplicates biasing the final results.

2.2.6 Molecular clock calibration

To calibrate the molecular clock, three fossils were assigned to different clades of the Chenopodiaceae. These fossils were: *Chenopodipollis multiplex*, *Salicornites massalongoi* and *Parvangula randeckensis*. *Chenopodipollis multiplex* had been found in a marine influence assemblage alongside other marine organisms (Nichols and Traverse 1971) and was assigned to Chenopodiaceae by (Kadereit *et al.* 2003). Although habitats close to coastal environments are typical of Chenopodiaceae, this is rarely the case for Amaranthaceae or Caryophyllaceae with similar pollen. Thus, we implemented a conservative calibration point for this fossil and used it as a minimum constraint on the stem node of Chenopodiaceae (excluding subfamily Polycnemoideae). The fossil *S. massalongoi* had been described as a fragment of a plant stem (Principi 1926; Collinson *et al.* 1993) and was since assigned to the Salicornioideae (Hohmann *et al.* 2006; Kadereit *et al.* 2012). Here, we used it as a minimum constraint for the stem node of Salicornioideae, assuming that the succulent stem features appeared with the split of Salicornioideae and have fostered its diversification into dry and saline habitats. The fossil *P. randeckensis* had been described as *Chenopodium*-like seeds (Gregor 1982) but, considering seed evolution in the Chenopodioideae (Sukhorukov and Zhang 2013), these seeds may belong to a lineage after divergence of the *Axyris–Krascheninnikovia* clade. The fossil was placed in different positions by different authors (Kadereit *et al.* 2003, 2012; Hohmann *et al.* 2006). We designated this fossil as a minimum age constraint for the stem of the *Axyris–Krascheninnikovia* clade. Maximum ages for all three fossils were set to 125 million years (Ma), which represents the mean age of the core eudicots [corresponding to node 29 of Appendix S5 in Bell *et al.* (2010)]. The age distribution of each of these primary calibration points was modelled as an exponential prior distribution (Ho and Phillips 2009), with an offset equal to the minimum age of the respective fossil and a mean such that the median age of the exponential distribution equalled the maximum age of the fossil. A fossil calibration so constrained renders the age estimate of the fossil the likely divergence time of the calibrated node, but also allows the inference of older ages. A summary of the primary calibration points employed in this investigation as well as their respective age ranges is given in Table 2.B.

Table 2.B: Fossils and their age ranges used for primary calibration of the molecular clock.

No.	Fossil name	Description	Origin	Age (Ma)	Epoch	Publication
1	*Chenopodipollis multiplex*	Pollen record	United States	65-56	Paleocene	Nichols and Traverse (1971)
2	*Salicornites massalongoi*	Part of an axis	Italy	35-23	Oligocene	Principi (1926)
3	*Parvangula randeckensis*	*Chenopodium*-like seeds	Germany	23-16	Early Miocene	Gregor (1982)

Additionally, we defined three secondary calibration points by using age estimates of Bell *et al.* (2010): the age for the crown group of the Caryophyllales (their node 39); the age for the crown group of the polygonoid clade (their node 41); and the maximum estimated age of the entire tree, corresponding to Gunneridae (their node 31). The age distribution of each of these secondary calibration points was modelled as a normal prior distribution and was equal to the 95 % highest posterior density (HPD) interval of the ages inferred by Bell *et al.* (2010), which were based on lognormal prior age distributions.

2.2.7 Estimation of divergence times

Divergence times were estimated using a Bayesian relaxed molecular clock model in BEAST 1.8.0 (Drummond *et al.* 2012). A preliminary evaluation of the nucleotide substitution rates across dataset B indicated that the rates did not conform to a strict molecular clock (Peterson 2006). Hence, a relaxed molecular clock with uncorrelated substitution rates sampled from an exponential distribution for the estimation of divergence times was applied (Drummond *et al.* 2006). Lineage diversification was modelled as a birth–death process, and a random starting tree was employed. Two MCMC runs with 50,000,000 generations each were performed for the estimation of divergence times, with trees sampled every 1,000 generations. We confirmed adequate parameter sampling (effective sample size >200) and the mixing of Markov chains in both runs through visual inspection of the MCMC samples using Tracer 1.4 (Rambaut and Drummond 2007). Given the results of our sampling and convergence diagnostics, the first 50 % of the MCMC generations were removed as burn-in. The remaining trees were subsampled to every fifth generation, generating a post-burn-in posterior tree distribution of 500 trees per run. The post burn-in MCMC samples of both runs were combined via LogCombiner 1.8.0 (Drummond *et al.* 2012), and the combined tree distribution was summarized as a maximum clade credibility (MCC) tree via TreeAnnotator 1.8.0 (Drummond *et al.* 2012).

2.2.8 Analyses of state-dependent speciation and extinction

To evaluate the influence of morphological traits with a potential adaptive value for epizoochory in speciation and extinction rates in the Achyranthoids, we employed a trait-dependent analysis approach using two morphological characters: modified sterile flowers and the more inclusive trait of adhesive

appendages (Figure 2.A). Presence (noted as 1) and absence (0) of both characters was coded for the achyranthoid taxa included in dataset B, using the same specimens as in the molecular phylogenetic analyses. Additional assessment and confirmation of the traits in the study taxa were conducted via extraction of morphological descriptions from the literature (e.g. protologues of taxa, genus and species descriptions of floras, morphological treatments) or via the examination of herbarium material. The complete character matrix is provided in Appendix 2.E.

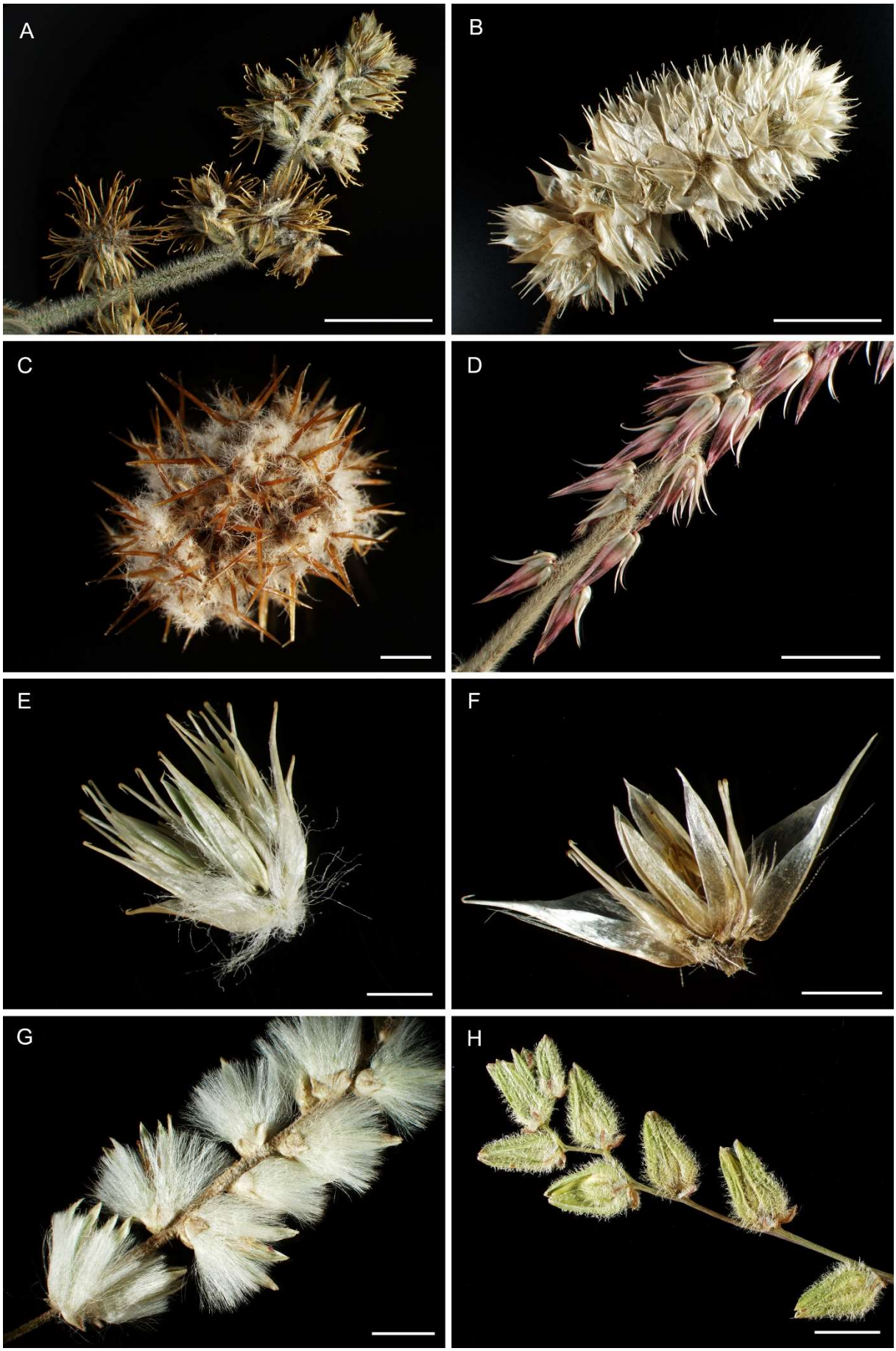

Figure 2.A: Flower morphology of Achyranthoids.

A, B: Inflorescences of *Pupalia lappacea* (Di Vincenzo *et al*., 301, B) and *Cyathula cylindrica* (Wondafrash *et al*., 3332, B) exhibiting modified sterile flowers. **C:** Adhesive dispersal unit of *Cyathula orthacantha* (Di Vincenzo *et al*., 21, B) with sterile flowers modified to spines. **D:** Inflorescence of *Achyranthes aspera* (Di Vincenzo *et al*., 9, B), with bracteoles having a thickened spine-like midrib. **E:** Partial inflorescence of *Cyathula uncinulata* (Wondafrash *et al*., 3199, B) with terminal parts of sterile and fertile flowers modified to hooks. **F:** *Cyathula cylindrica* (Wondafrash *et al*., 3332, B); cyme of one fertile flower and two lateral modified sterile flowers. **G:** Inflorescence of *Chionothrix latifolia* (Di Vincenzo *et al*., 236, B) with hairs growing at maturity and serving wind dispersal. **H:** Inflorescence of *Psilotrichum gnaphalobryum* (Di Vincenzo *et al*., 239B, B) with solitary fertile flowers. Scales bars: (A, B, D, G, H) = 1 cm; (C, E, F) = 2 mm.

To estimate speciation and extinction rates in the Achyranthoids in relation to the morphological traits, two types of statistical analysis were conducted via the application and comparison of two sets of state-dependent speciation and extinction (SSE) models: binary-state speciation and extinction (BiSSE) models (Maddison *et al.* 2007) as implemented in the R package 'diversitree', and hidden-state speciation and extinction (HiSSE) models (Beaulieu and O'Meara 2016) as implemented via the R package 'hisse'. Specifically, we conducted analyses of state-dependent speciation and extinction rates under (1) a detailed group of hierarchical BiSSE models and (2) a combined group of SSE models selected from the BiSSE and HiSSE concepts. The aim of applying model group (1) was to evaluate which of the modelled combinations of speciation, extinction and character transition rates could best explain character distribution and phylogenetic diversification of the Achyranthoids. The aim of model group (2) was to evaluate whether the inclusion of an additional, unaccounted (i.e., hidden) character state may better explain the fit of the speciation, extinction and character transition rates than the BiSSE models alone.

For model group (1), we estimated trait-dependent rates of speciation (λ), extinction (μ) and character transition (q) under 20 different BiSSE models and compared the fit of each model, given the inferred phylogenetic trees. Each of these models had six different parameters (i.e., the rates of speciation, extinction and character transition under character state 0 and character state 1) and differed in the number of constraints applied to the parameters. Two sets of models were hereby defined: models in which one or more parameters were constrained to be equal (e.g., $\lambda_0 = \lambda_1$; hereafter 'BiSSE model set 1'), and models in which one or more parameters were constrained to be zero (e.g., $\lambda_0 = 0$; hereafter 'BiSSE model set 2'). The trait-dependent rates were estimated for both states of modified sterile flowers and adhesive appendages. In addition, we calculated the net effect for each model (de Vos *et al.* 2014), which represents the difference between speciation and extinction rate under character state 1 (i.e. the net diversification under state 1) minus the difference between speciation and extinction rate under character state 0 (i.e. the net diversification under state 0). Relative model fit was evaluated within each BiSSE model set but not among the two sets. All parameter estimations and comparisons of model fit also included a model with unconstrained parameters (i.e., λ, μ and q were allowed to vary; hereafter 'unconstrained model'), which was used as a null hypothesis. A summary of all BiSSE models employed for the analysis of the character 'modified sterile flowers' is given in Table 2.C, a summary of all BiSSE models employed for the more inclusive trait 'adhesive appendages' is provided in Appendix 2.F.

Table 2.C: Model fit of different binary state-dependent speciation and extinction models regarding the presence of modified sterile flowers.
(0 = absent; 1 = present). In the models evaluated, parameters were set as equal (BiSSE model set 1) and to zero (BiSSE model set 2) across character states. Relative model fit was evaluated via the AIC and AICc; a comparison of model fit between the unconstrained and each constrained model was performed via the LRT. Parameter estimates and test statistics were calculated on the MCC tree that summarizes 1000 post-burn-in MCMC trees of the posterior tree distribution inferred during the estimation of divergence times. Asterisks indicate LRT results at $p<0.05$. Abbreviations used: DF = degrees of freedom; Trans. rate = character transition rate; lnLik = natural logarithm of the likelihood.

	Model Specification			Model fit					
	Speciation (λ)	**Extinction (μ)**	**Trans. rate (q)**	**DF**	**lnLik**	**AIC**	**AICc**	**LRT χ^2**	**LRT *p*-value**
	Unconstrained – all rates free			6	-251.656	515.311	516.494	NA	NA
BiSSE Model set 1	Free	μ0=μ1	Free	5	-252.745	515.491	516.324	2.179	0.140
	Free	Free	q01=q10	5	-251.877	513.753	514.587	0.442	0.506
	λ0=λ1	Free	Free	5	-253.045	516.091	516.924	2.779	0.096
	Free	μ0=μ1	q01=q10	4	-253.325	514.651	515.199	3.340	0.188
	λ0=λ1	Free	q01=q10	4	-253.373	514.745	515.293	3.434	0.180
	λ0=λ1	μ0=μ1	Free	4	-253.340	514.679	515.227	3.368	0.186
	λ0=λ1	μ0=μ1	q01=q10	3	-253.380	512.760	513.084 (best)	3.448	0.328
BiSSE Model set 2	λ0=0	Free	Free	5	-276.098	562.196	563.029	48.884	< 0.01*
	Free	μ0=0	Free	5	-251.847	513.693	514.527	0.382	0.536
	Free	μ1=0	Free	5	-254.647	519.295	520.128	5.983	0.014*
	Free	Free	q01=0	5	-252.394	514.789	515.622	1.477	0.224
	Free	Free	q10=0	5	-252.754	515.508	516.341	2.196	0.138
	λ0=0	μ0=0	Free	4	-273.762	555.525	556.073	44.214	< 0.01*
	λ0=0	μ1=0	Free	4	-276.098	560.196	560.743	48.884	< 0.01*
	λ0=0	Free	q01=0	4	-286.815	581.629	582.177	70.318	< 0.01*
	Free	μ0=0	q01=0	4	-253.201	514.402	514.950	3.091	0.213
	Free	μ0=0	q10=0	4	-252.755	513.509	514.057 (best)	2.198	0.333
	Free	μ1=0	q01=0	4	-254.647	517.295	517.843	5.983	0.050
	Free	μ1=0	q10=0	4	-257.465	522.930	523.478	11.619	< 0.01*

For model group (2), we estimated speciation, extinction and character transition rates under selected SSE models and again compared the fit of each model given our inferred phylogenetic trees. Parameters of the HiSSE models were set up according to the recommendations of Beaulieu and O'Meara (2016) for defining character-independent models (CIDs). Models with four diversification process parameters were set up, with the most parameterized CID model containing 13 free parameters (CID2 with unconstrained net turnover, extinction fraction and character transition rates among and between the two observed and the two hidden states) and the least parameterized CID model containing six free parameters (CID2 with unconstrained net turnover and extinction fraction parameters among and between the two observed and the two hidden states, but character transitions constrained to be equal). Relative model fit was evaluated among the selected SSE models, with the most parameterized CID and the most parameterized BiSSE model acting as null hypothesis. A summary of all CID and BiSSE models of model group (2) employed for the analysis of the character 'modified sterile flowers' is provided in Table 2.D, and a summary of all CID and BiSSE models of model group (2) employed for the more inclusive trait 'adhesive appendages' is provided in the Appendix 2.G.

Model fit was evaluated by two different procedures. First, we applied likelihood ratio tests (LRTs) between the unconstrained model and each constrained model to determine whether any of the constrained models was significantly different from the unconstrained model; this comparison was enabled by a hierarchical setup of the parameter constraints. Second, we evaluated the relative fit of each model by ML optimization, using the AIC as well as an AIC corrected for small sample size (AICc; Hurvich and Tsai 1989) to identify best-fitting models. Parameter estimates for each model and test statistics were calculated and then averaged over 100 randomly selected trees of the posterior tree distribution generated during the estimation of divergence times in order to accommodate the phylogenetic uncertainty of the tree distribution. In addition to the comparison of model fit via ML optimization, we also applied MCMC sampling to estimate marginal posterior probability densities for the diversification rate parameters of the unconstrained model. We thus conducted MCMC sampling for 10,000 generations, with 95 % HPD intervals calculated for each parameter.

To allow the application of a global sampling fraction for SSE models to correct for incomplete taxon sampling (Table 2.A), our analyses were conducted exclusively on the achyranthoid clade of our ultrametric trees inferred from dataset B. This clade comprised a total of 65 taxa. We bioinformatically extracted the achyranthoid clade from each of the 100 randomly selected trees of the posterior tree distribution using the R package 'phyloch' 1.5–5 (Heibl 2008). For modified sterile flowers, we assumed a sampling proportion of 0.44/0.41 (absence/presence); for adhesive appendages we assumed a ratio of 0.63/0.34 (Appendix 2.H).

Table 2.D: Comparison of model fit of different CID models of the HiSSE model concept and of BiSSE models regarding the presence of modified sterile flowers.

Two two-state and two four-state CID models as well as four BiSSE models were evaluated. Dual transitions between character states (e.g. q_0A ↔ q_1B; q_0B ↔ q_1A) were not included in any CID model. Relative model fit was evaluated via the AIC and AICc; the difference in model fit between the most parameterized CID or BiSSE model and all others was evaluated via the LRT. Abbreviations used: DF = degrees of freedom; Trans. rate = character transition rate; lnLik = natural logarithm of the likelihood; NA = not applicable.

		Model specification			Model fit				Comparison with *CID-2 unconstrained*		Comparison with *BiSSE unconstrained*	
		Net turnover (τ)	**Extinction fraction (ε)**	**Trans. rate (q)**	**DF**	**lnLik**	**AIC**	**AICc**	**LRT χ^2**	**LRT *p*-value**	**LRT χ^2**	**LRT *p*-value**
HiSSE models	***CID-2***	Free	Free	Free	13	-212.593	449.185	455.185	NA	NA	0.183	1.000
		Free	Free	Equal	6	-218.105	446.209	447.226	0.0705	1.000	0.026	1.000
	CID-4	Free	Free	Three rates	11	-215.528	453.057	458.038	0.0201	1.000	0.082	1.000
		Free	Free	Equal	9	-215.826	449.651	452.924	0.0244	1.000	0.074	1.000
BiSSE models		Free	Free	Free	7	-221.510	455.019	456.467	0.1832	1.000	NA	NA
		Equal	Free	Free	6	-221.545	454.108	453.091	0.1846	1.000	<0.001	1.000
		Free	Equal	Free	6	-221.714	454.445	453.428	0.1916	1.000	<0.001	1.000
		Equal	Equal	Free	5	-221.504	451.675	451.008	0.1829	0.999	<0.001	1.000

2.2.9 Ancestral character state reconstruction

We conducted ancestral character state reconstruction (ACSR) for modified sterile flowers under three different BiSSE models. The second trait, adhesive appendages, was not reconstructed, as their functionality is achieved by different, non-homologous organs (sterile flowers and bracteoles). The same matrix for presence and absence of sterile flowers was applied for ACSR as for model testing (Appendix 2.E).

We performed ACSR with the function 'asr-bisse' of the R package diversitree 0.9–7 (FitzJohn *et al.* 2009). Reconstruction was performed under the unconstrained binary-state speciation and extinction model and those BiSSE models with the smallest AICc value out of BiSSE model sets 1 and 2 (Table 2.C). To average out phylogenetic uncertainty, final ACSR was conducted over the post-burn-in posterior tree distribution as inferred during divergence dating. Inferred ancestral character states were visualized on the MCC tree. To evaluate the significance of the reconstruction results, we followed Schluter *et al.* (1997) and used likelihood ratios as measures of relative weight of evidence and the ratio 1:7.4 (i.e. 0.86) as indication for significance of a particular character state.

2.2.10 Analyses of shifts in diversification rates

In the trait-independent approach, we evaluated rate shifts across the entire family Amaranthaceae. The software BAMM 2.5.0 (Rabosky 2014) was employed to calculate the rate heterogeneity of speciation and extinction on the MCC tree inferred during the estimation of divergence times. To apply a more confident sampling fraction in BAMM, our analyses were conducted exclusively on the clade of Amaranthaceae. Sampling fractions for the main clades of the Amaranthaceae are listed in Appendix 2.I.

Analyses were conducted in four independent runs with 10,000,000 MCMC generations each, and results were sampled every 1000 generations. Post-run analyses and visualizations were performed using the R package BAMMtools 2.1.5 (Rabosky 2014) and run convergence was tested with the R package CODA 0.19–1 (Plummer *et al.* 2006). Upon removal of the first 10 % of all sampled MCMC generations as burn-in, we inferred the posterior probabilities of rate-shift configurations under different numbers of rate shifts and calculated Bayes factors (BFs) to compare all rate-shift configurations with prior and posterior probabilities greater than zero with a model without shifts. We considered BF > 20 to be substantial evidence for one model over another, and BF > 50 was considered very strong evidence in support of the model under analysis. The 95 % credible set of distinct rate-shift configurations and the single best-shift configuration with the highest posterior probabilities were generated as final results.

2.3 Results

2.3.1 Molecular datasets

The aligned matrix of dataset A comprised 4747 bp (*trnK/matK*, 2474 bp; *rpl16*, 1069 bp; *trnL-F*, 1204 bp), of which 373 bp were excluded as mutational hotspots (*trnK/matK*, five hotspots with a total of 67 bp; *rpl16*, eight hotspots with a total of 158 bp; *trnL-F*, ten hotspots with a total of 148 bp). Simple indel coding of dataset A resulted in an additional matrix of 573 characters. Six inversions (in total 39 bp) were found in *trnK/matK*, two inversions (in total 58 bp) in *rpl16* and three inversions (in total 34 bp) in *trnL-F* of dataset A. Dataset B included a total of 2474 aligned positions of the *trnK/matK* region, and five mutational hotspots with a total of 145 bp were excluded from this dataset. Eight inversions with a total of 61 bp were found in dataset B. Dataset C included a total of 706 aligned positions for nrITS, from which four mutational hotspots with a total of 83 bp were excluded. Simple indel coding of dataset C resulted in an additional matrix of 122 characters.

2.3.2 Phylogenetic analyses of dataset A

Reconstructions under BI, ML and MP yielded identical tree topologies, only differing in node support (Figure 2.B). Monophyly of the achyranthoid clade was confirmed with high support. Five of the 26 genera were identified as polyphyletic: *Psilotrichum* was inferred as four separate lineages (in addition to *P. ferrugineum* outside the clade), *Cyathula* as six separate lineages, *Sericocomopsis* as three separate lineages, *Achyranthes* as three separate lineages, and *Calicorema* as two separate lineages. The genera *Centrostachys*, *Chionothrix*, *Dasysphaera*, *Lopriorea*, *Mechowia*, *Nelsia*, *Polyrhabda* and *Volkensinia* have been included in a molecular phylogenetic analysis for the first time and were identified as part of the achyranthoid clade. The two endemic Madagascan genera *Henonia* and *Lagrezia* were resolved within the celosioid clade, and *Digera* as well as *Pleuropterantha* within the amaranthoid clade.

Furthermore, we found two strongly supported major subclades that diverged after a grade of three early diverging achyranthoid lineages (Figure 2.B). These three lineages are: (1) *Arthraerua* plus *Calicorema capitata*; (2) *Cyathula lanceolata* plus the genus *Volkensinia*; and (3) the genus *Chionothrix*. Subclade I comprises the genera *Polyrhabda*, *Pupalia*, *Dasysphaera*, *Sericocoma*, *Sericorema*, *Marcelliopsis*, *Kyphocarpa*, *Centema* and *Lopriorea*, *Centemopsis*, the species *Calicorema squarrosa* and several lineages of *Psilotrichum*. Subclade II comprises the genera *Nototrichium*, *Achyranthes*, *Achyropsis*, *Centrostachys*, *Sericocomopsis*, *Sericostachys*, *Nelsia*, *Pandiaka* and *Leucosphaera*, and several lineages of *Cyathula* (except *C. lanceolata*).

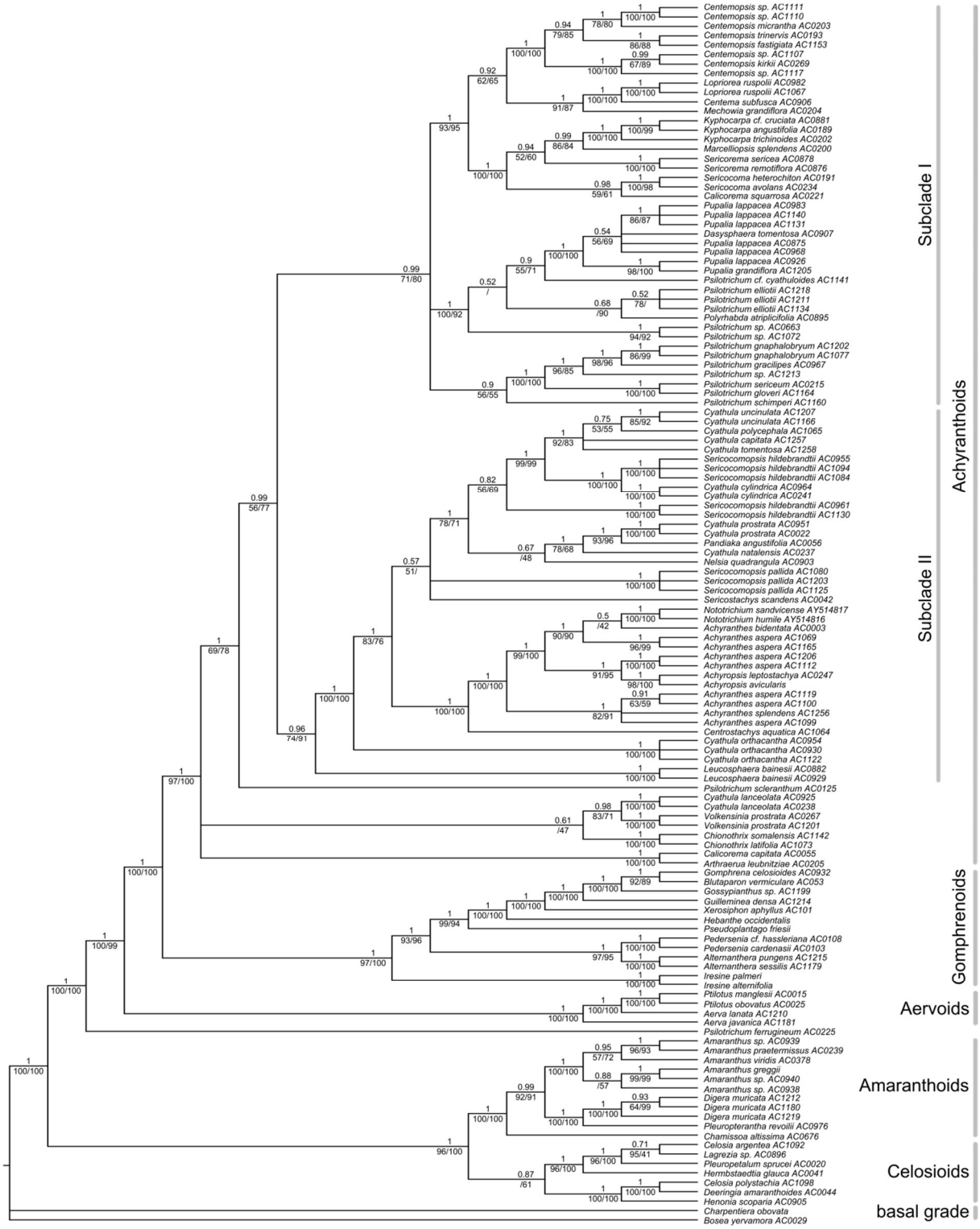

Figure 2.B: Phylogeny of the Amaranthaceae with a focus on the achyranthoid clade.
The tree topology was obtained with Bayesian inference from dataset A. Posterior probabilities of the Bayesian inference are given above branches, jackknife percentages of the maximum parsimony (left) and bootstrap percentages of the maximum likelihood analyses (right) are given below branches.

2.3.3 Phylogenetic analyses of dataset C

Reconstructions under BI, ML and MP recovered the achyranthoid clade as well as most internal nodes also found in the plastid tree (Appendix 2.J). The core of subclade II of Achyranthoids was inferred with maximum support, whereas the genus *Leucosphaera* was inconsistently recovered as sister to *Cyathula lanceolata* rather than as sister to all other taxa in subclade II. The species that were recovered as subclade I under the plastid data were inferred as a grade of three independent lineages under nrITS data.

2.3.4 Divergence dating

The age of the crown group of the Amaranthaceae was estimated to be 51.25 Ma (95 % HPD 42.97–57.55 Ma) (node number 56 in Appendix 2.K). The achyranthoid clade split from the gomphrenoid clade ~27.7 million years ago (Mya) (95 % HPD 21.42–33.86 Mya) and started to diversify 22.2 Mya (95 % HPD 15.79–27.89 Mya) (Figure 2.C; node numbers 88 and 112 in Appendix 2.K). The diversification of most subclades within the Achyranthoids was inferred to have occurred within the past 10 Ma (Figure 2.C). A list of all inferred ages including their 95 % HPD intervals is provided in Appendix 2.K.

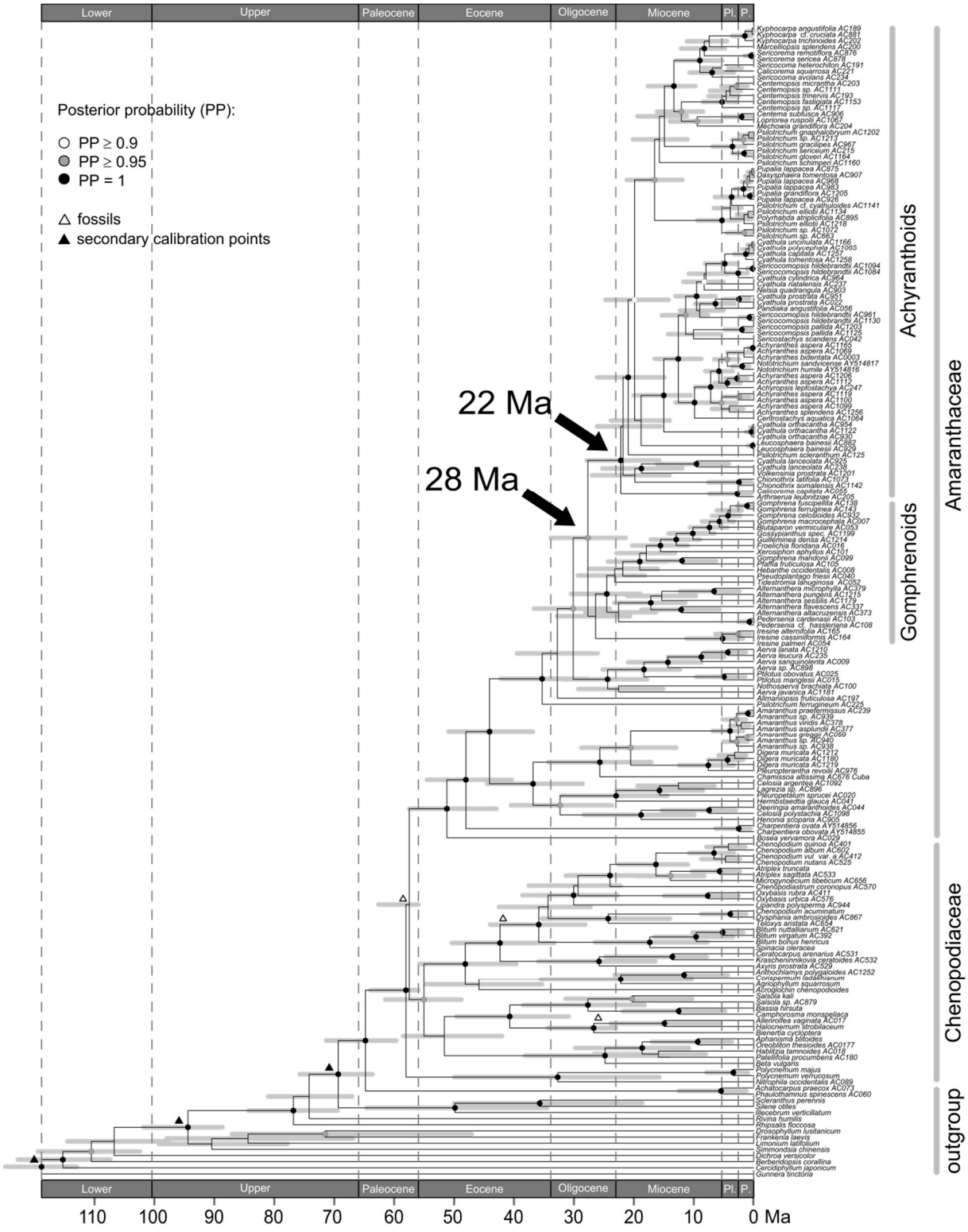

Figure 2.C: Chronogram of the Amaranthaceae–Chenopodiaceae alliance.
Fossil (white triangles) and secondary (black triangles) calibration points as well as 95 % HPD intervals (grey bars) are visualized on the MCC tree inferred from dataset B. Epoch abbreviations: Lower = Lower Cretaceous; Upper = Upper Cretaceous; Pl. = Pliocene; P. = Pleistocene. Epoch abbreviations: Lower = Lower Cretaceous; Upper = Upper Cretaceous; Pl. = Pliocene; P. = Pleistocene.

2.3.5 Analyses of state-dependent speciation and extinction

In our analyses of different state-dependent SSE models using model group (1), none of the models of BiSSE model set 1 was found to be significantly different from the unconstrained model regarding the character 'modified sterile flowers' (Table 2.C). Among the models of BiSSE model set 2, the LRTs indicated six models to be significantly different from the unconstrained model, but the fit of each of these models was worse than that of the unconstrained model. Comparisons of relative model fit via the AICc indicated that the model with all parameters constrained to be equal between state 0 and state 1 ($\lambda_0 = \lambda_1$, $\mu_0 = \mu_1$, $q_{01} = q_{10}$) had the best fit among models of BiSSE model set 1. By contrast, the model that constrained the extinction rate under state 0 as well as the character transition from state 1 to state 0 to zero ($\mu_0 = 0$, $q_{10} = 0$) was found to have the lowest AICc value within BiSSE model set 2.

Since no model was significantly better compared with the null model and AICc values were similar across most models, we calculated the parameter values for speciation, extinction and character state transitions as well as the net effect under the unconstrained model (Table 2.E). For all other models, parameter values are provided in Appendix 2.L. In the unconstrained model, both speciation and extinction rates for taxa with modified sterile flowers were found to be higher than for taxa lacking sterile flowers. However, the net effect under the unconstrained model was slightly negative, pointing to lower net diversification rates for taxa with than without modified sterile flowers. Rates for gains of modified sterile flowers were higher than for their loss (Table 2.E, Figure 2.D). The accumulation of modified sterile flowers within achyranthoid taxa therefore results from the higher transition rates in favour of modified sterile flowers.

Table 2.E: Median rates of speciation (*λ*), extinction (*μ*) and character transition (*q*) and the net effect of diversification for modified sterile flowers (0 = absent; 1 = present) under the unconstrained model.

Median rate values were calculated across 100 post-burn-in MCMC trees of the posterior tree distribution as inferred during the estimation of divergence times. Each median value is followed by the interquartile range in parentheses. A negative net effect indicates a lower diversification rate for taxa with modified sterile flowers. Abbreviations used: Trans. rate = character transition rate.

Model Specification			Parameterization						Net effect
Speciation (λ)	**Extinction (μ)**	**Trans. rate (q)**	**λ0**	**λ1**	**μ0**	**μ1**	**q01**	**q10**	**(λ1-μ1)-(λ0-μ0)**
Unconstrained – all rates free			0.28 [0.26,0.32]	0.94 [0.76,1.14]	0.05 [0,0.13]	0.87 [0.64,1.09]	0.08 [0.02,0.11]	0.03 [0,0.05]	-0.12 [-0.28,-0.06]

Concerning adhesive flower appendages as a more inclusive functional trait, our LRT evaluations indicated that none of the models of BiSSE model set 1 was found to be significantly different from the unconstrained model (Appendix 2.F). Comparisons of relative model fit via the AICc indicated that the model that constrained extinction and character transition rates to be equal between state 0 and state 1 ($\mu_0 = \mu_1$, $q_{01} = q_{10}$) had the best fit among models of BiSSE model set 1. Among the models of BiSSE model set 2, the LRTs indicated a total of seven models to be significantly different from the unconstrained model. However, none of these seven models had a better fit to our data than the null model according to the AICc values. Comparisons further indicated that the model that constrained the extinction rate under state 1 as well as the character transition from state 0 to state 1 ($\mu_1 = 0$, $q_{01} = 0$) was found to have the best relative model fit.

Similar to the results for modified sterile flowers, our analyses regarding the presence of adhesive appendages indicated that no model was significantly better than the null model, and AICc values were quite similar across most BiSSE models. We calculated the parameter values for speciation, extinction and character transition as well as the net effect under all models (Appendix 2.M). In the unconstrained model, we found higher speciation and extinction rates for taxa with adhesive appendages than for taxa without them. Here, the net effect was found to be positive, indicating that diversification rates for taxa with adhesive appendages were higher than for taxa without them. Character transition rates were found to be equal for both gains and losses (Appendix 2.M, Figure 2.D).

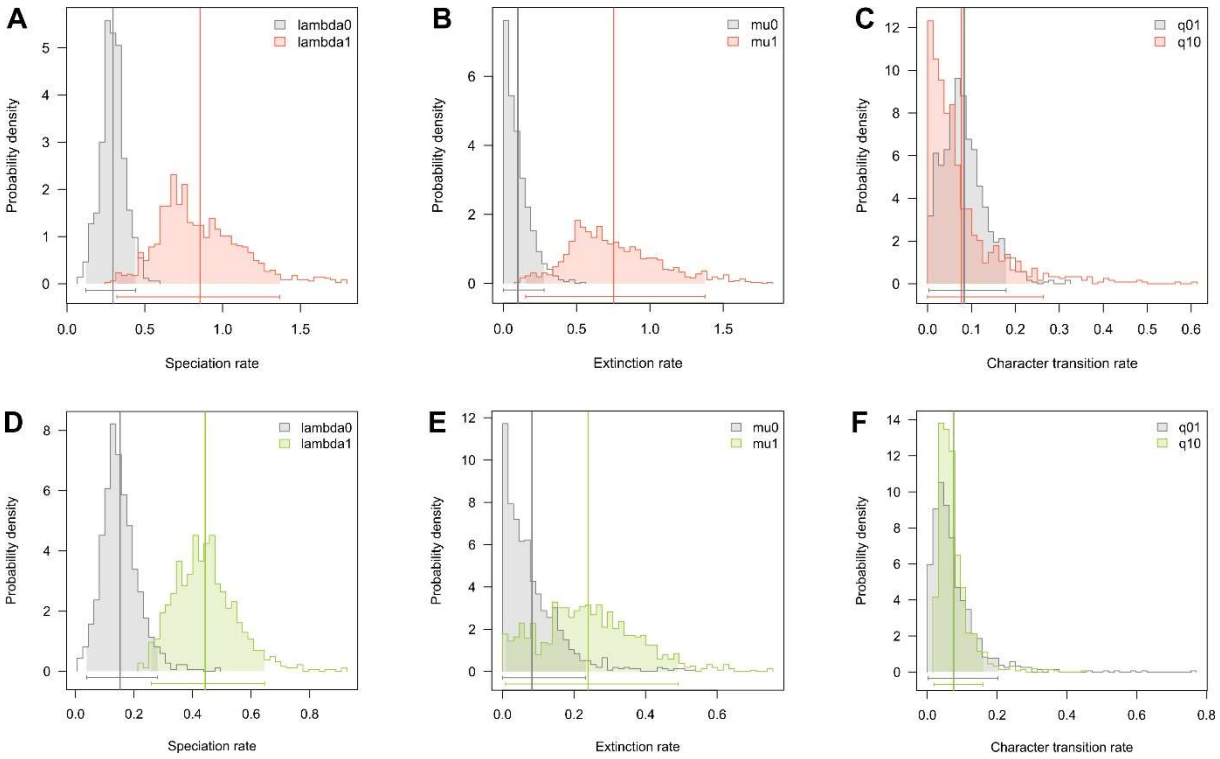

Figure 2.D: Posterior probability distributions of speciation (λ), extinction (μ) and character transition rates (q) under the unconstrained BiSSE model.
A–C: Rates are concerning the presence (1) and absence (0) of modified sterile flowers**; D–F:** Rates are concerning the adhesive flower appendages. Vertical lines represent mean values; horizontal bars and shaded areas represent the 95 % credibility intervals of posterior probability distributions.

In our analyses of different state-dependent SSE models using model group (2), none of the evaluated models was found to be significantly different from the unconstrained model (Table 2.D, Appendix 2.F). This finding was true for both morphological traits evaluated. For both traits, neither the BiSSE models nor the constrained CID models of the HiSSE concept displayed a significantly better model fit to the data than the null CID model when evaluated using an LRT. Comparisons of relative model fit via the AICc indicated that the least parameterized CID model had the best relative fit among models of model group (2). Again, this result was observed for both morphological traits evaluated. In summary, the results of our analyses of different state-dependent SSE models using model group (2) indicated that the inclusion of an additional, hidden character state did not explain the fit of the speciation, extinction and character transition rates significantly better than the BiSSE models alone.

2.3.6 Ancestral character state reconstruction

Since model testing did not infer a single best BiSSE model, we reconstructed ancestral character states for modified sterile flowers under three different BiSSE models: the unconstrained model and the two best-fitting models from BiSSE model sets 1 and 2 as inferred through the AICc (Figure 2.E). The best-fitting BiSSE models yielded very similar reconstructions for the majority of the early divergences in the achyranthoid clade but were distinctly different from the reconstructions under the unconstrained model. The best-fitting BiSSE models inferred the absence of modified sterile flowers in the most recent common ancestor (MRCA) of the Achyranthoids as more likely than the presence (BiSSE model set 1, 16 % presence, 84 % absence; BiSSE model set 2, 0 % presence, 100 % absence) and, indicated multiple origins of this trait within the clade: 14 independent origins for modified sterile flowers are inferred within the Achyranthoids under the best-fit model of BiSSE model set 1, and 15 independent origins under the best-fit model of BiSSE model set 2 (according to the significance level of 86 % for a particular character state). The unconstrained model, by contrast, inferred the presence of modified sterile flowers in the MRCA of the Achyranthoids as more likely than the absence (59 % presence, 41 % absence). Similarly, the reconstruction results for the MRCA of subclades I and II of the Achyranthoids under the best-fit BiSSE models indicated the absence of modified sterile flowers as more likely than their presence (subclade I, BiSSE model set 1, 6 % presence, 94 % absence; BiSSE model set 2, 0 % presence, 100 % absence; subclade II, BiSSE model set 1, 14 % presence, 86 % absence; BiSSE model set 2, 0 % presence, 100 % absence), whereas the reconstruction results under the unconstrained model were uncertain, indicating equal probability for both character states (subclade I, 51 % presence, 49 % absence; subclade II, 54 % presence, 46 % absence).

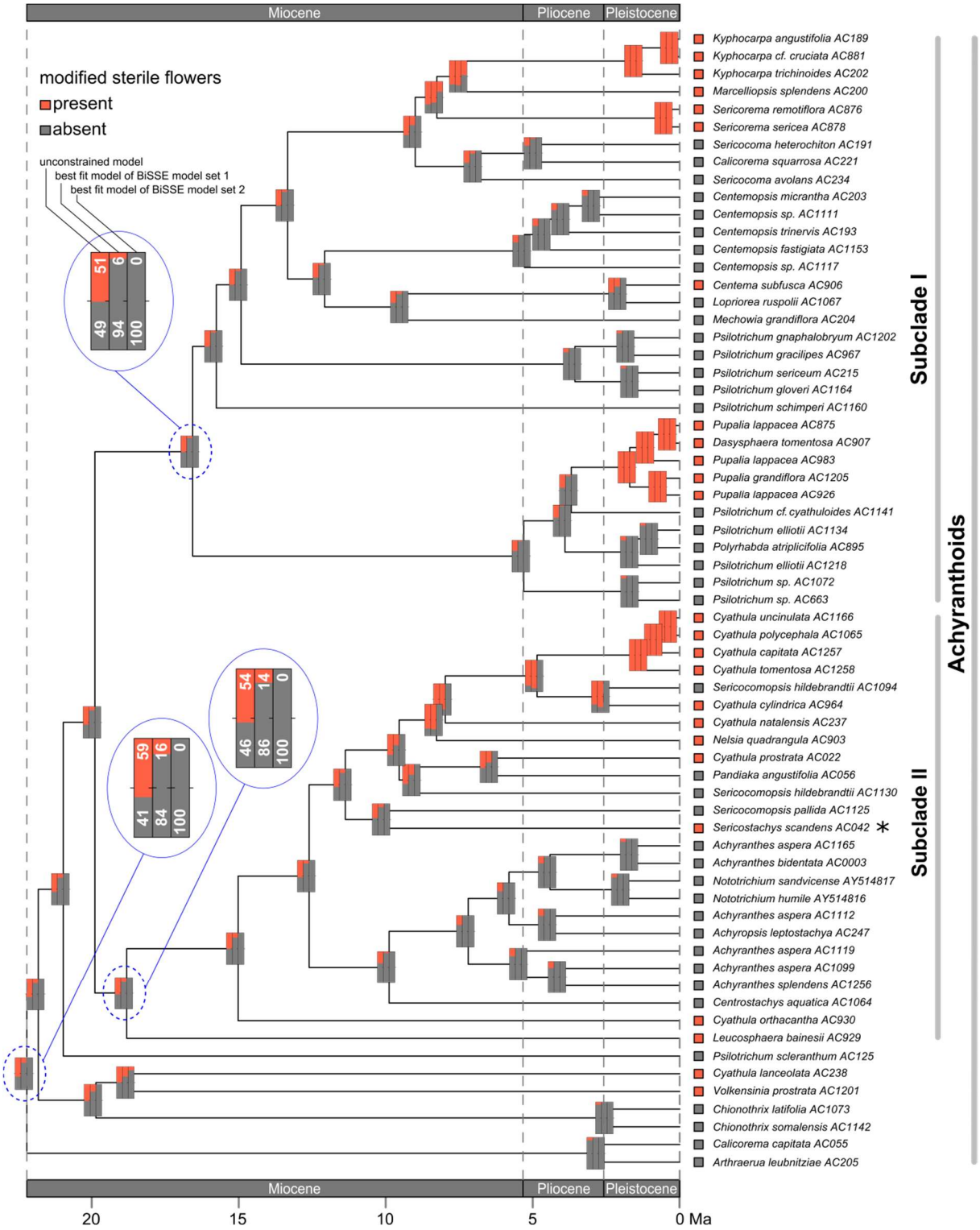

Figure 2.E:Reconstruction of ancestral character states for modified sterile flowers under three different BiSSE models.

Bars from left to right: the unconstrained model; the best fit model of BiSSE model set 1 with parameters set as equal across the character states; and the best fit model of BiSSE model set 2 with parameters set to zero across states. The asterisk marks a species that bears sterile flowers that are not modified to adhesive appendages, but instead are modified to long hairs that serve wind dispersal. Percentages in the magnified nodes represent character state probabilities.

2.3.7 Analyses of shifts in diversification rates

Convergence of BAMM analyses was assessed in four independent runs via effective sample size (ESS) values (ESS log-likelihood, 481.59–699.76; ESS number of shifts, 824.84–1151.58). Our analyses via BAMM indicated that the zero-shift configuration (null model) had the highest prior and posterior probability (posterior probability = 0.31; Figure 2.F). Model comparison via BFs did not show evidence in favour of a model with one or more shifts relative to the null model, as BFs for all models with one or more shifts were <20, ranging from 0.37 to 2.73. Computing the 95 % credible set of macroevolutionary rate shifts yielded a set of 11 distinct shift configurations, out of which three accounted for 72 % of the posterior probability (Appendix 2.N). The first configuration accounted for 40 % of the probability of the data, the second for 26 % and the third for ~6 %. The null model with zero shifts was the one with the highest posterior probability (0.40). None of the other configurations had sampling frequencies >0.06 and are therefore not shown. A phylorate plot that displays the most frequently sampled configuration out of the credible shift set is given in Appendix 2.N.

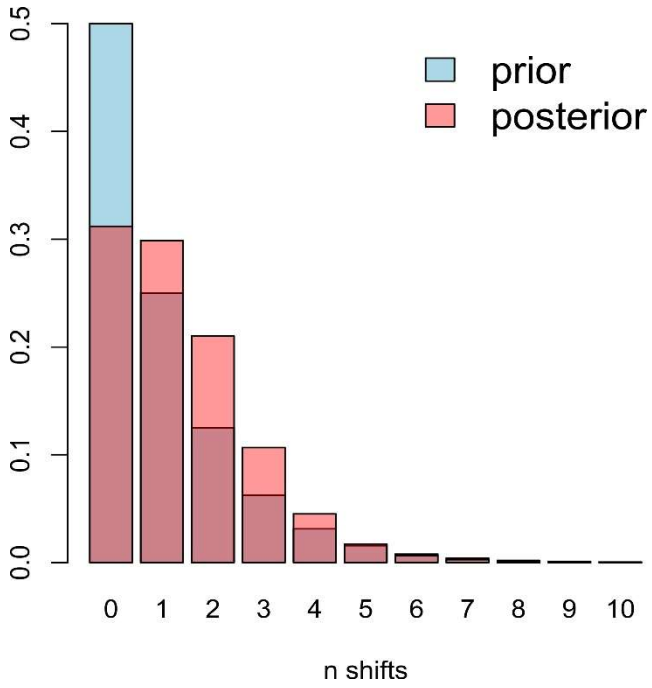

Figure 2.F: Prior and posterior probability distributions of the number of rate shifts.
Distributions of shifts are inferred via BAMM on the MCC tree inferred during the estimation of divergence times.

2.4 Discussion

2.4.1 Monophyly and overall phylogenetic structure of the achyranthoid clade

Under our increased sampling of taxa and molecular characters, the achyranthoid clade originally evidenced by Müller and Borsch (2005a; b) has been found strongly supported. In addition, the genera *Centrostachys*, *Chionothrix*, *Dasysphaera*, *Lopriorea*, *Mechowia*, *Nelsia*, *Polyrhabda*, *Sericocomopsis* and *Volkensinia* of the subtribe Aervinae sensu Townsend (1993), which had not been included in any phylogenetic study so far, have been identified as part of the achyranthoid clade.

Compared with the sole analyses of *trnK/matK* (Müller and Borsch 2005a; b), the internal relationships of Achyranthoids are much better resolved in this study, except for the first diverging lineages. While *Cyathula lanceolata* and *Volkensinia* appear in a medium-supported subclade, at least in the plastid tree (Figure 2.B), their relationships to *Chionothrix* and the *Calicorema capitata* plus *Arthraerua* subclade remain unclear. However, the relationship of *C. capitata* with *Arthraerua leubnitziae* is confirmed with maximum support in plastid and nuclear trees (Figure 2.B, Appendix 2.J). The affinities of both South African taxa are further supported by similar pollen morphology (Müller and Borsch 2005b).

Our analysis also confirms the position of the achyranthoid clade as sister to the Gomphrenoideae, both of which are the sister to an aervoid clade (Müller and Borsch 2005a; b). Four other genera of the paraphyletic Amaranthoideae sensu Townsend (1993) that were newly included in our phylogenetic analysis appear outside of the Achyranthoids: *Digera* and *Pleuropterantha* are close relatives of *Amaranthus* (extending the amaranthoid clade of Müller and Borsch 2005a). *Henonia* and *Lagrezia* belong to the Celosioids (Figure 2.B), indicating that tribe Celosieae in the circumscription of Schinz (1934) is not monophyletic unless *Lagrezia* [classified to Amaranthinae (Schinz 1934; Townsend 1993)] is included.

The two lineages of Achyranthoids initially found by Müller and Borsch (2005b) appear as two major subclades with our more representative taxon sampling (subclades I and II; Figure 2.B). Plastid and nrITS trees are largely congruent. Topological differences only occur among samples of *Psilotrichum* in subclade I and concerning the placement of *Cyathula orthacantha*.

Species of *Psilotrichum* are found in several different lineages. The Asian *P. ferrugineum* is confirmed in a position outside the achyranthoid clade (Müller and Borsch 2005b) and *P. scleranthum* (= *P. africanum*) is now resolved as isolated sister to subclades I plus II in the combined plastid dataset (Figure 2.B). *Psilotrichum gnaphalobryum*, *P. gracilipes*, *P. gloveri* and *P. sericeum* form a well-supported subclade in all trees (Figure 2.B, Appendix 2.J). *Psilotrichum schimperi* is also included in this subclade based on plastid data (Figure 2.B), but its position is unresolved under nrITS (Appendix 2.J). Another lineage with the remaining species of *Psilotrichum* (*P. elliotii*, *P. cyathuloides*) appears close to a *Dasysphaera–Pupalia* subclade. Interestingly, the Somalian monotypic genus *Polyrhabda* is suggested

as a close relative of *P. elliotii.* The *Dasysphaera–Pupalia* subclade is highly supported in all analyses and is one of the youngest groups that have evolved modified sterile flowers with hooks forming adhesive diaspores (Figure 2.A and Figure 2.E). *Calicorema squarrosa* is now well supported as sister to *Sericocoma* (Figure 2.B, Appendix 2.J), and not to *Sericorema* as depicted in the Bayesian *trnK/matK* tree (posterior probability = 0.81; Müller and Borsch 2005b).

In subclade II of the Achyranthoids, the genus *Achyranthes* appears paraphyletic to *Achyropsis* and *Nototrichium* (Figure 2.B, Appendix 2.J). All species of *Nototrichium* are small trees, indicating insular gigantism in these Hawaiian endemics. *Centrostachys aquatica* is sister to all other species in this subclade. It morphologically resembles the herbaceous species of *Achyranthes* but has bigger flowers and occurs in swampy habitats. All four genera are characterized by spiciform inflorescences with solitary fertile flowers. In *Centrostachys* and *Achyranthes* (Figure 2.A[D]) the flowers with their bracts and bracteoles are deflexed at maturity and bracteoles possess strongly thickened, spine-like midribs that serve for adhesion in diaspores. This feature was obviously lost in *Achyropsis* and *Nototrichium*. Furthermore, elaborate stamen tube appendages characteristic of *Achyranthes* and relatives are absent in *Nototrichium*. Different samples of *Achyranthes aspera* are found in at least three lineages, paralleling a considerable morphological variation (e.g. plant size from 10 to 150 cm, presence or absence of woodiness, dense or very sparse indumentum). The Asian *Achyranthes bidentata* is depicted close to Ethiopian and Kenyan plants of *Achyranthes aspera*, and the Hawaiian endemic *Achyranthes splendens* is found distantly from *Nototrichium*. Further taxon and character sampling is necessary to understand the complex evolutionary history in *Achyranthes*.

The species of *Cyathula* appear scattered across subclade II (Figure 2.B, Appendix 2.J), except for *C. lanceolata*, which belongs to the early diverging Achyranthoids. The deviating position of *C. lanceolata* in Müller and Borsch (2005b) is an artefact because their sequence (AC022) was mislabeled and in fact is *C. prostrata*. *Cyathula orthacantha* is isolated and diverges after *Leucosphaera* within subclade II (Figure 2.B). *Cyathula uncinulata* (Figure 2.A[E]), *C. polycephala*, *C. tomentosa* and *C. capitata* constitute a subclade of taxa with very dense and partly subglobose synflorescences that is sister to *C. cylindrica* (Figure 2.A[B]) plus three samples of the polyphyletic *Sericocomopsis hildebrandtii* (Figure 2.B, Appendix 2.J). *Cyathula natalensis*, *C. prostrata* and *Pandiaka* form a lineage with elongate, lax inflorescences. Since the genus concept of *Cyathula* is based on the presence of sterile flowers (Townsend 1993; Hernández-Ledesma *et al.* 2015), now shown as homoplastic, it is not surprising that the genus does not appear as a natural group. *Sericocomopsis* currently has two accepted species (Townsend 1993), distinguished by simple versus stellate hairs. They are resolved in three different lineages (Figure 2.A, Appendix 2.J), indicating that the simple-haired individuals currently circumscribed as *S. hildebrandtii* constitute more than one species.

2.4.2 Diversification of the achyranthoid clade through time

Compared with the Gomphrenoids, which are a Neotropical radiation (Sánchez del Pino *et al.* 2009) that started 26.4 Mya (95 % HPD 20.68–32.85 Mya) and resulted in the largest clade within the Amaranthaceae, we estimate the crown group age of the Achyranthoids as 22.2 Ma (95 % HPD 15.79–27.89 Ma) (node numbers 89 and 112 in Appendix 2.K). Most Achyranthoids occur in open habitats, mainly semi-desert scrubland, *Acacia–Commiphora* wood- and bushland as well as dry evergreen Afromontane Forest and grassland complexes. Characteristic plant groups of these habitats are *Acacia* (Fabaceae, subfamily Mimosoideae), *Commiphora* (Burseraceae) and different C4 grasses, such as *Sporobolus* (subfamily Chloridoideae), *Hyparrhenia*, *Heteropogon* and *Panicum* (subfamily Panicoideae). As shown by Bouchenak-Khelladi *et al.* (2014), these C4 grass lineages originated around 35 Mya. The crown age of the genus *Commiphora* was estimated to be ~30 Ma by Becerra *et al.* (2012). The earliest split of an African *Acacia* clade with species occurring in open environments from its American sister group is dated to early Miocene times by Bouchenak-Khelladi *et al.* (2010) but was estimated to even have occurred from around 33 Mya onward by Miller *et al.* (2013).

Since the above-mentioned genera are dominant in the vegetation in which Achyranthoids occur, we may assume that those habitats evolved from ~30 Mya onward. These findings are in line with our age estimation for the Achyranthoids. The inferred origins of all these plants, being indicators of open arid and semi-arid habitats, furthermore coincide with the assumed stepwise aridification of East Africa since Eocene–Oligocene times in conjunction with the uplift of the East African Rift system (e.g. Sepulchre *et al.* 2006).

As shown in our chronogram (Figure 2.C) the two major subclades of Achyranthoids started to diversify in the early Miocene 16.6 Mya (95 % HPD 11.94–21.22 Mya) and 18.8 Mya (95 % HPD 14.05–23.87 Ma), respectively (node numbers 155 and 121 in Appendix 2.K). Achyranthoid species are mostly herbs and small shrubs which are not competitive in broad-leaved forests. Thus, the opening of forests is likely to have offered new niches for achyranthoid species to diversify. Many achyranthoid taxa, such as the *Dasysphaera–Pupalia* lineage or the *Cyathula* species with dense inflorescences (node number 152 in Appendix 2.K), evolved in Pliocene–Pleistocene times, shortly after C4 grasslands became ecologically dominant in large parts of East Africa (8–6 Mya; Bouchenak-Khelladi *et al.* 2014).

2.4.3 Evolution of modified sterile flowers

At the selected significance level, the reconstruction of ancestral character states suggests that modified sterile flowers originated at least 14 times independently in the Achyranthoids (Figure 2.E). Reconstruction results for deeper nodes under the unconstrained model are uncertain, indicating almost equal probabilities for both character states. The transition rates under the unconstrained model were found to favour gains of modified sterile flowers during the evolution of Achyranthoids (Table 2.E). This is in line with our ACSR (Figure 2.E), which suggests more gains than losses of this character over

time. For the more inclusive functional trait of adhesive appendages, by contrast, transition rates are equal in both directions (Appendix 2.M). This also suggests that a reversal of the bracteole midrib morphology [from thickened and thorn-like, as in *Achyranthes* (Figure 2.A[D]), to the unmodified state] is more probable than the loss of sterile flowers, which would require a change in inflorescence architecture.

The first occurrence of modified sterile flowers above the significance level was inferred to the early Pliocene under the constrained model of BiSSE model set 1. However, it seems more parsimonious regarding the number of independent origins that the first achyranthoid lineages with modified sterile flowers originated in the second half of the Miocene (once in the stem node incorporating *Nelsia quadrangula* and several species of *Cyathula*, and a second time in the ancestor of *Kyphocarpa*, *Marcelliopsis* and *Sericorema*). Several independent origins of this trait occurred during the Pleistocene in subclades I and II. Our reconstructions also indicated that sterile flowers in several early-diverging taxa (e.g. *Volkensinia*, *Leucosphaera*, *Cyathula lanceolata* and *Cyathula orthacantha*) could have evolved on terminal branches, and therefore dating the origin of the trait is not possible. We assume that the asymmetries in transition rates contributed highly to the prevalence of modified sterile flowers in many achyranthoid species.

2.4.4 Species diversification and epizoochory in achyranthoid evolution

To illustrate the effect of modified sterile flowers (and the more inclusive functional trait of adhesive appendages) on achyranthoid diversification, we evaluated the rates of speciation, extinction and net diversification by comparing lineages with and without these traits under several BiSSE models (Table 2.E, Appendix 2.L, Appendix 2.M). Speciation as well as extinction rates were found to be higher in the presence than in the absence of both traits (Figure 2.D, Table 2.E, Appendix 2.L, Appendix 2.M). Thus, net diversification was only slightly different in the presence than in the absence of these traits.

The reliability of analyses with SSE models on datasets with <300 terminals has been evaluated in several investigations (e.g. Davis *et al.* 2013; Rabosky and Goldberg 2015). Even though the sample size in this investigation was <300, it is well in line with recently recommended sample sizes for SSE models (Gamisch 2016). Beaulieu and O'Meara (2016) developed proper null models (CID-2 and CID-4), which we used to validate our data, and which confirmed the lack of diversification signal in the observed traits. Additionally, we employed BAMM to test for trait-independent rate heterogeneity among the Amaranthaceae, specifically within certain subclades of the Achyranthoids or the clade as a whole. The idea was to look for diversification rate shifts that, if present, could be correlated to the dated origins of modified sterile flowers. However, our results indicate the absence of diversification rate shifts within the diversification of the Achyranthoids (Figure 2.F, Appendix 2.N).

Beaulieu and Donoghue (2013) argued that "the origin of a trait may not, in itself, be sufficient to increase diversification rate, but rather, requires the right combination of traits". This view is shared by

different authors (e.g. de Queiroz 2002; Maddison *et al.* 2007; Beaulieu and O'Meara 2016). In Achyranthoids there is no significant boost of diversification after the acquisition of traits that can serve epizoochory. Nevertheless, the high number of independent origins of sterile flowers, connected with biased transition rates, suggests an adaptive value. The evolution of open habitats in East Africa has probably been an ecological opportunity for the Achyranthoids. Nearly all members of this clade occur in habitats with large animals (with a few exceptions, such as *Sericostachys*, which evolved a liana habit secondarily). Our inferred divergence times indicate that the evolution of achyranthoid diversity coincides with times in which habitats changed and appropriate dispersal agents became abundant (Figure 2.C). Large terrestrial mammals started to diversify in the Oligocene. Fossil evidence pointed to a further increase in diversity of large herbivores (including bovids, which represent the largest group of herbivores in Africa today) during the Late Miocene (8–6 Mya) due to a well-established Eurasian connection that allowed massive migration to Africa. A peak of diversity was reached in the Pliocene due to the global transition from C3 to C4 vegetation (Bobe 2006; Charles-Dominique *et al.* 2016).

Several investigations developed models to measure and predict the adhesive ability of different diaspore types. Factors such as retention times and separation forces strongly influence the persistence of diaspores in fur and, by extension, dispersal distances (Gorb and Gorb 2002; Will *et al.* 2007; Couvreur *et al.* 2008; Will and Tackenberg 2008; Bullock *et al.* 2011). Animals migrate between similar habitats, leading to directed dispersal of plants to habitats with optimal conditions for their offspring (Stebbins 1971). Since most animals use the same route during migration and have been shown to be mobile links between fragmented and therefore isolated ecosystems (Couvreur *et al.* 2004), there is only a limited possibility for populations to become isolated due to an interruption in gene flow. In Achyranthoids, epizoochory may therefore be a successful dispersal mechanism, but rapid diversification may be counteracted by regular interchange of gene pools. More work is needed to evaluate this possibility, particularly by analyzing the phylogeography and population structure of widespread achyranthoid species.

2.5 Appendix 2.A-N Supplementary material for Chapter 2

Appendices

Appendix 2.A: List of analysed taxa of the Amaranthaceae, including geographic origin, voucher information and ENA/GenBank accession numbers. *48*

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Appendix 2.A: List of analysed taxa of the Amaranthaceae, including geographic origin, voucher information and ENA/GenBank accession numbers.

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	ITS
Achyranthes aspera L.	M. Wondafrash 4076 (ETH, B)	Ethiopia	AC1069	LT992592 this study	LT993043 this study	LT992953 this study	LT992992 this study
Achyranthes aspera L.	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 281 (EA, B, ETH)	Kenya	AC1099	LT992595 this study	LT993046 this study	LT995259 this study	LT992995 this study
Achyranthes aspera L.	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 287 (EA, B, ETH)	Kenya	AC1100	LT992596 this study	LT993047 this study	LT995260 this study	LT992996 this study
Achyranthes aspera L.	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 308 (EA, B, ETH)	Kenya	AC1112	LT992597 this study	LT993048 this study	LT995261 this study	LT992997 this study
Achyranthes aspera L.	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 325 (EA, B, ETH)	Kenya	AC1119	LT992598 this study	LT993049 this study	LT992955 this study	LT992998 this study
Achyranthes aspera L.	V. Di Vincenzo, N. Korotkova 360 (B, ETH)	Ethiopia	AC1165	LT992602 this study	LT993053 this study		LT993002 this study
Achyranthes aspera L.	V. Di Vincenzo, A. Hailu 85 (B, ETH)	Ethiopia	AC1206	LT992607 this study	LT993057 this study	LT995255 this study	LT993006 this study
Achyranthes bidentata Blume	T. Borsch s. n.	China, Kunming	AC0003	LT992574 this study	LT993014 this study	LT992944 this study	LT992958 this study
Achyranthes splendens Mart. Ex Moq.	S. Perlman 5803	Hawaii	AC1256	LT999872 this study			LT995187 this study
Achyropsis avicularis (E.Mey. ex Moq.) Benth. & Hook.f. ex B.D.Jacks.	Venter 9671 (NY)	South Africa			EF688731 Sanchez del-Pino et al. (2009)	EF688660 Sanchez del-Pino et al. (2009)	
Achyropsis leptostachya (E. Mey. ex Meisn.) Baker & C.B. Clarke	K. Müller 876 (B, PRE)	South Africa	AC0247	AY998117 Müller & Borsch (2005b)	LT993028 this study	LT995234 this study	LT992975 this study
Aerva javanica (Burm. f.) Juss. ex Schult.	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 314 (EA, B, ETH)	Kenya	AC1181	LT992605 this study	LT993055 this study	LT995253 this study	LT993004 this study
Aerva lanata (L.) Juss.	V. Di Vincenzo, A. Hailu 142 (B, ETH)	Ethiopia	AC1210	LT992608 this study	LT993058 this study	LT995256 this study	LT993007 this study

Appendix 2.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	ITS
***Aerva leucura* Moq.**	K. Müller 843 (B, PRE)	South Africa	AC0235	DQ317308 Müller & Borsch (2005b)			
***Aerva* sp.**	I. Kiefer 9497 (B)	Madagascar	AC0898	LT999831 this study			
***Aerva sanguinolenta* (L.) Blume**	T. Borsch 3898 (B)	BG Bonn 11115-90	AC0009	AM887482 Sage et al. (2007)			
***Allmaniopsis fruticulosa* Suess.**	R. B. & A. J. Faden 74/766 (PRE, MO)		AC0197	AY998116 Müller & Borsch (2005b)			
***Alternanthera altacruzensis* Suess.**	Borsch & Ortuno 3628	Bolivia	AC0373	AM887483 (Sage et al. 2007)			
***Alternanthera flavescens* Kunth**	Borsch & Ortuno 3509		AC0337	AM887484 (Sage et al. 2007)			
***Alternanthera microphylla* R.E.Fr.**	Borsch & Ortuno 3670	Bolivia	AC0379	AM887485 (Sage et al. 2007)			
***Alternanthera pungens* Kunth**	V. Di Vincenzo, A. Hailu 181 (B, ETH)	Ethiopia	AC1215	LT992612 this study	LT993062 this study	LT995245 this study	LT993011 this study
***Alternanthera sessilis* (L.) R.Br. ex DC.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 309 (EA, B, ETH)	Kenya	AC1179	LT992603 this study		LT995251 this study	
***Amaranthus asplundii* Thell.**	T. Borsch & T. Ortuño 3757 (B, LPB)	Bolivia	AC0377	AM887486 Sage et al. (2007)			
***Amaranthus greggii* S.Watson**	D. Pratt, K. Müller, T. Borsch 207 (BO, ISC)	USA, Texas	AC0059	AY514808 Müller & Borsch (2005a)			
***Amaranthus greggii* S.Watson**	Sánchez-del Pino 106 & Flores (MEXU)	Mexico			EF688742 Sanchez del-Pino et al. (2009)	EF688670 Sanchez del-Pino et al. (2009)	
***Amaranthus praetermissus* Brenan**	K. Müller 873 (B, PRE)	South Africa	AC0239	AM887487 (Sage et al. 2007)			

Appendix 2.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	ITS
***Amaranthus* sp.**	M. Wondafrash 2254 (ETH, B)	Ethiopia	AC0938	LT999837 this study		LT999824 this study	LT999799 this study
***Amaranthus* sp.**	M. Wondafrash 2263 (ETH, B)	Ethiopia	AC0939	LT999838 this study		LT999815 this study	LT999800 this study
***Amaranthus* sp.**	M. Wondafrash 2265 (ETH, B)	Ethiopia	AC0940	LT999839 this study	LT996928 this study	LT999816 this study	LT999801 this study
***Amaranthus viridis* L.**	T. Borsch & J. Coimbra 3451 (B, LPB)	Bolivia	AC0378	AM887488 Sage et al. (2007)			
***Arthraerua leubnitziae* (Kuntze) Schinz**	B. Leuenberger, T. Raus, C. Schiers 3330 (PRE)	Namibia	AC0205	AY998115 Müller & Borsch (2005b)	LT993023 this study	LT992946 this study	LT992970 this study
Blutaparon vermiculare (L.) Mears	T. Borsch, K. Müller & D. Pratt 3444 (B, ISC)	USA, Texas	AC0053	AY514798 Müller & Borsch (2005a)			
***Blutaparon vermiculare* (L.) Mears**	Liogier 34352 (NY)				EF688748 Sanchez del-Pino et al. (2009)	EF688676 Sanchez del-Pino et al. (2009)	
***Bosea yervamora* L.**	K. Müller 751 (B)	Canary Islands, Tenerife	AC0029	AY514810 Müller & Borsch (2005a)			
***Calicorema capitata* (Moq.) Hook. f.**	C. Neinhuis s.n. (B)	Namibia	AC0055	AY514807 Müller & Borsch (2005a)	LT993015 this study	LT995223 this study	LT992960 this study
***Calicorema squarrosa* (Schinz) Schinz**	H. O. Volk 12630 (M)		AC0221	AY998114 Müller & Borsch (2005b)	LT993025 this study	LT995231 this study	LT992972 this study
***Celosia argentea* L.**	M. Wondafrash 2211 (ETH, B)	Ethiopia	AC1092	LT992594 this study	LT993045 this study	LT995258 this study	LT992994 this study
***Celosia polystachia* (Forssk.) C. C. Towns.**	M. Wondafrash 4012 (ETH, B)	Ethiopia	AC1098	LT999852 this study		LT995207 this study	LT995175 this study
***Centema subfusca* (Moq.) T. Cooke**	P. Bamps (Moq.) Lopr. (BR)	Süd-Afrika	AC0906	LT999833 this study	LT995131 this study	LT995193 this study	LT995161 this study

Appendix 2.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	ITS
***Centemopsis fastigiata* (Suess.) C. C. Towns.**	S. Bidgood, D. Sitoni, K. Vollesen, C. Whitehouse 3528, 28.04.1997 (EA)	Tanzania	AC1153	LT999862 this study	LT995148 this study	LT995211 this study	LT995179 this study
Centemopsis kirkii	M. Wondafrash 2147 (ETH, B)	Ethiopia	AC0269	LT992576 this study	LT993029 this study	LT995235 this study	LT992976 this study
***Centemopsis micrantha* Chiov.**	J. B. Gillet & C. F. Hemming 24874 (PRE)	Namibia	AC0203	AY998105 Müller & Borsch (2005b)	LT993022 this study	LT995230 this study	LT992968 this study
***Centemopsis* sp.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 322 (EA, B, ETH)	Kenya	AC1107	LT999853 this study	LT996930 this study	LT999818 this study	LT999803 this study
***Centemopsis* sp.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 304 (EA, B, ETH)	Kenya	AC1110	LT999854 this study	LT996931 this study	LT999819 this study	LT999804 this study
***Centemopsis* sp.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 307 (EA, B, ETH)	Kenya	AC1111	LT999855 this study	LT996932 this study	LT999820 this study	LT999805 this study
***Centemopsis* sp.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 318 (EA, B, ETH)	Kenya	AC1117	LT999856 this study	LT996933 this study	LT999821 this study	LT999806 this study
***Centemopsis trinervis* Hauman**	M. Reekmans 31981 (PRE)	Namibia	AC0193	AY998107 Müller & Borsch (2005b)	LT993019 this study	LT995227 this study	LT992965 this study
***Centrostachys aquatica* (R. Br.) Wall.**	K. Balkwill & M.-J. Balkwill 4356 (B)	Nigeria	AC1064	LT992591 this study		LT992952 this study	LT992991 this study
***Chamissoa altissima* (Jacq.) Kunth**	W. Greuter et al. 26968	Cuba	AC0676	LT992577 this study			
***Charpentiera obovata* Gaudich.**	B. A. Prigge 15251 (LA)	USA, Hawaii		AY514855 Müller & Borsch (2005a)			
***Charpentiera obovata* Gaudich.**	HLA voucher 10254 (HLA)	USA, Hawaii			EF688749 Sanchez del-Pino et al. (2009)	EF688678 Sanchez del-Pino et al. (2009)	
***Charpentiera ovata* Gaudich.**	B. A. Prigge 15252 (LA)	USA, Hawaii		AY514856 Müller & Borsch (2005a)			

Appendix 2.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	ITS
***Chionothrix latifolia* Rendle**	M. Wondafrash 2142 (ETH, B)	Ethiopia	AC1073	LT999848 this study	LT995141 this study	LT995203 this study	LT995171 this study
***Chionothrix somalensis* (S. Moore) Hook. f.**	J.B. Gillet, W.M. Watson 23583, 22.06.1981 (EA)	Somalia	AC1142	LT999861 this study			
***Cyathula capitata* Moq.**	(KUN)	China	AC1257	LT999873 this study	LT995155 this study	LT995219 this study	LT995188 this study
***Cyathula cylindrica* Moq.**	K. Müller 877 (B, PRE)	South Africa	AC0241	LT992575 this study	LT993027 this study	LT995233 this study	LT992974 this study
***Cyathula cylindrica* Moq.**	M. Wondafrash 3605A (ETH, B)	Ethiopia	AC0964	LT992587 this study	LT993039 this study	LT992949 this study	LT992987 this study
***Cyathula lanceolata* Schinz**	K. Müller 867 (B, PRE)	Süd-Afrika (RSA), Northern Province	AC0925	LT999835 this study	LT993035 this study	LT995240 this study	LT992982 this study
***Cyathula lanceolata* Schinz**	K. Müller 865 (B, PRE)	South Africa	AC0238	AY514862 Müller & Borsch (2005b)	LT995128 this study	LT995190 this study	LT995157 this study
***Cyathula natalensis* Sond.**	K. Müller 879 (B, PRE)	South Africa	AC0237	LT999825 this study	LT995127 this study	LT995189 this study	LT995156 this study
***Cyathula orthacantha* (Hochst. ex Asch.) Schinz**	K. Müller 872 (B, PRE)	Süd-Afrika (RSA), Northern Province	AC0930	LT999836 this study	LT995133 this study	LT995195 this study	LT995163 this study
***Cyathula orthacantha* (Hochst. ex Asch.) Schinz**	M. Wondafrash 3461 (ETH, B)	Ethiopia	AC0954	LT999840 this study	LT995134 this study	LT995196 this study	LT995164 this study
***Cyathula orthacantha* (Hochst. ex Asch.) Schinz**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 330 (EA, B, ETH)	Kenya	AC1122	LT999857 this study	LT995145 this study	LT995208 this study	LT995176 this study
***Cyathula polycephala* Baker**	M. Wondafrash 3394 (ETH, B)	Ethiopia	AC1065	LT999845 this study	LT995139 this study	LT995201 this study	LT995169 this study
***Cyathula prostrata* (L.) Blume**	M. Wondafrash 3434 (ETH, B)	Ethiopia	AC0951	LT992586 this study		LT995244 this study	LT992986 this study

Appendix 2.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	ITS
Cyathula prostrata (L.) Blume	E. Russo 114 (B, Herb. Russo)	Peru	AC0022	AY514862 Müller & Borsch (2005a)		LT995221 this study	LT992959 this study
***Cyathula tomentosa* (Roth.) Moq.**	(KUN)	Tibet	AC1258	LT999875 this study			
***Cyathula uncinulata* (Schrad.) Schinz**	V. Di Vincenzo, N. Korotkova 361 (B, ETH)	Ethiopia	AC1166	LT999865 this study	LT995150 this study	LT995214 this study	LT995182 this study
***Cyathula uncinulata* (Schrad.) Schinz**	V. Di Vincenzo, A. Hailu 86 (B, ETH)	Ethiopia	AC1207	LT999869 this study	LT995154 this study	LT995218 this study	LT995186 this study
***Dasysphaera tomentosa* Volkens ex Lopr.**	K. Vollesen 96-33 (BR)	Tansania	AC0907	LT999834 this study	LT995132 this study	LT995194 this study	LT995162 this study
***Deeringia amaranthoides* (Lam.) Merr.**	Moore 746 (BONN)	Guam, Philipp. Sea	AC0044	AY514814 Müller & Borsch (2005a)			
***Digera muricata* subsp. *trinervis* var. *trinervis* C. C. Towns.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 311 (EA, B, ETH)	Kenya	AC1180	LT992604 this study	LT993054 this study	LT995252 this study	LT993003 this study
***Digera muricata* subsp. *trinervis* var. *patentipilosa* C. C. Towns.**	V. Di Vincenzo, A. Hailu 152 (B, ETH)	Ethiopia	AC1212	LT992610 this study	LT993060 this study	LT992956 this study	LT993009 this study
***Digera muricata* subsp. *trinervis* var. *macroptera* C. C. Towns.**	V. Di Vincenzo, A. Hailu 251 (B, ETH)	Ethiopia	AC1219	LT992614 this study	LT993064 this study	LT995247 this study	LT993013 this study
***Froelichia floridana* (Nutt.) Moq.**	Th. Thiel s.n. (B)	BG Bonn 03615-90	AC0016	AY514799 Müller & Borsch (2005a)			
***Gomphrena celosioides* Mart.**	K. Müller 882 (B, PRE)	Süd-Afrika (RSA), Gauteng	AC0932	LT992585 this study	LT993038 this study	LT995243 this study	LT992985 this study
***Gomphrena ferruginea* Pedersen**	T. Borsch &T. Ortuño 3650 (B, LPB)	Bolivia	AC0143	AM887524 Sage et al. (2007)			
***Gomphrena fuscipellita* T.Ortuño & Borsch**	T. Borsch & T. Ortuño 3594 (B, LPB)	Bolivia	AC0138	AM887525 Sage et al. (2007)			

Appendix 2.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	ITS
***Gomphrena macrocephala* A.St.-Hil.**	E. Zardini 45564 (B, MO)	Paraguay	AC0007	AY514802 Müller & Borsch (2005a)			
***Gomphrena mandonii* R.E.Fr.**	S. Beck 18720 (B, LPB)	Bolivia	AC0099	AY514801 Müller & Borsch (2005a)			
***Gossypianthus* sp.**	I. Castaneda N-UCLV 10185 (UCLV)	Cuba, Villa Clara, 9-9-2012	AC1199	LT999994 Ortuño & Borsch (unpublished)	LT999882 Ortuño & Borsch (unpublished)	LT999993 Ortuño & Borsch (unpublished)	LT999992 Ortuño & Borsch (unpublished)
***Guilleminea densa* (Willd.) Moq.**	V. Di Vincenzo, A. Hailu 180 (B, ETH)	Ethiopia	AC1214	LT992611 this study	LT993061 this study	LT992957 this study	LT993010 this study
***Hebanthe occidentalis* (R.E.Fr.) Borsch & Pedersen var. occidentalis**	E. Zardini 45377 (BONN, MO)	Paraguay	AC0008	AY514821 Müller & Borsch (2005a)			
***Hebanthe occidentalis* (R.E.Fr.) Borsch & Pedersen var. occidentalis**	Nee & Saldias 47054 (NY)	Bolivia			EF688767 Sanchez del-Pino et al. (2009)	EF688697 Sanchez del-Pino et al. (2009)	
***Henonia scoparia* Moq.**	P.B. Phillipson 2419 (BR, MO)	Madagascar	AC0905	LT999832 this study	LT995130 this study	LT995192 this study	LT995160 this study
***Hermbstaedtia glauca* Reichb. ex Steud.**	C. Neinhuis s.n. (B)	Namibia	AC0041	AY514812 Müller & Borsch (2005a)			
***Iresine alternifolia* S. Watson**	R. S. Felger, M. F. Wilson 95-270 (MEXU, ARIZ)	Mexico, Sonora	AC0165	AM887490 Borsch et al. Iresine phylogeny unpubl.			
***Iresine alternifolia* S. Watson**	Daniel 2340, (NY)	Mexico			EF688770 Sanchez del-Pino et al. (2009)	EF688700 Sanchez del-Pino et al. (2009)	
***Iresine cassiniiformis* Schauer**	T. Borsch, H. Flores, H. Ochoterena 3679 (B, MEXU)	Mexico, Puebla	AC0164	AM887489 Borsch et al. Iresine phylogeny unpubl.			
***Iresine palmeri* (S. Watson) Standl.**	T. Borsch, K. Müller & D. Pratt 3445 (B, ISC)	USA, Texas	AC0054	AY514804 Müller & Borsch (2005a)			
***Iresine palmeri* (S. Watson) Standl.**	Nee & Taylor 29041 (NY)	Mexico			EF688775 Sanchez del-Pino et al. (2009)	EF688705 Sanchez del-Pino et al. (2009)	

Appendix 2.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	ITS
Kyphocarpa angustifolia (Moq.) Lopr.	K. Müller 860 (BONN, PRE)	South Africa	AC0189	AY998111 Müller & Borsch (2005b)	LT993017 this study	LT995225 this study	LT992963 this study
Kyphocarpa cf. *cruciata* (Schinz) Schinz	K. Müller 862 (B, PRE)	Süd-Afrika (RSA), Northern Province	AC0881	LT999874 this study	LT996926 this study	LT999813 this study	LT999797 this study
Kyphocarpa trichinoides (Fenzl) Lopr.	A. E. van Wyk 12403 (PRE)	South Africa	AC0202	AY998106 Müller & Borsch (2005b)	LT993021 this study	LT995229 this study	LT992967 this study
Lagrezia sp.	I. Kiefer 9498 (B)	Madagascar	AC0896	LT999830 this study	LT996927 this study	LT999814 this study	LT999798 this study
Leucosphaera bainesii (Hook.f.) Gilg	K. Müller 866 (B, PRE)	Süd-Afrika (RSA), Northern Province	AC0882	LT992581 this study	LT993033 this study	LT995238 this study	LT992980 this study
Leucosphaera bainesii (Hook.f.) Gilg	K. Müller 871 (B, PRE)	Süd-Afrika (RSA), Northern Province	AC0929	LT992584 this study	LT993037 this study	LT995242 this study	LT992984 this study
Lopriorea ruspolii (Lopr.) Schinz	M. Wondafrash 3921 (ETH, B)	Ethiopia	AC0982	LT999844 this study	LT995138 this study	LT995200 this study	LT995168 this study
Lopriorea ruspolii (Lopr.) Schinz	M. Wondafrash 3929 (ETH, B)	Ethiopia	AC1067	LT999846 this study	LT995140 this study	LT995202 this study	LT995170 this study
Marcelliopsis splendens (Schinz) Schinz	M. Müller & W. Giess 370 (PRE)	Namibia	AC0200	AY998112 Müller & Borsch (2005b)	LT993020 this study	LT995228 this study	LT992966 this study
Mechowia grandiflora Schinz	R. Mendes dos Santos 1780 (PRE)	Namibia	AC0204	AY998113 Müller & Borsch (2005b)		LT992945 this study	LT992969 this study
Nelsia quadrangula (Engl.) Schinz	E. Coppejam (BR)		AC0903	LT992582 this study	LT993034 this study	LT995239 this study	LT992981 this study
Nothosaerva brachiata (L.) Wight	Anonymous s.n. (FR)	Burkina Faso	AC0100	AY514806 Müller & Borsch (2005a)			
Nototrichium humile Hillebr.	B. A. Prigge 15249 (LA)	USA, Hawaii		AY514816 Müller & Borsch (2005a)			

Appendix 2.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	ITS
***Nototrichium sandwicense* Hillebr.**	B. A. Prigge 15250 (LA)	USA, Hawaii		AY514817 Müller & Borsch (2005a)			
***Pandiaka angustifolia* (Vahl) Hepper**	J. Müller 324 (FR)	Burkina Faso	AC0056	AY514818 Müller & Borsch (2005a)	LT993016 this study	LT995224 this study	LT992961 this study
***Pedersenia cardenasii* (Standl.) Holub**	T. Borsch & T. Ortuno 3504 (LPB, B, MEXU)		AC0103	AM887491 Sage et al. (2007)	EF688782 Sanchez del-Pino et al. (2009)	EF688712 Sanchez del-Pino et al. (2009)	
***Pedersenia* cf. *hassleriana* (Mart.) Holub**	T. Borsch & P. Ibisch 3532 (LPB, B)		AC0108	AM887522 Sage et al. (2007)	EF688783 Sanchez del-Pino et al. (2009)	EF688713 Sanchez del-Pino et al. (2009)	LT999795 this study
Pedersenia volubilis			AC0154				LT992962 this study
***Pfaffia fruticulosa* Suess.**	Borsch, Th. 3522 (LPB, B)	Bolivia	AC0105	AM887492 Borsch, Ortuno & Nee (2011)			
***Pleuropetalum sprucei* (Hook.f.) Standl.**		BG Bonn 16484	AC0020	AF542596 Hilu et al. (2003)			
***Pleuropterantha revoilii* Franch.**	M. Wondafrash 3846 (ETH, B)	Ethiopia	AC0976	LT992589 this study	LT993041 this study	LT992951 this study	LT992989 this study
***Polyrhabda atriplicifolia* C. C. Towns.**	P. Kucher et al. 17890 (ETH)	Ethiopia	AC0895	LT999829 this study			LT995159 this study
***Pseudoplantago friesii* Suess.**	T. M. Pedersen 15792 (CTES, C)	Argentina	AC0040	AY514820 Müller & Borsch (2005a)	EF688784 Sanchez del-Pino et al. (2009)	EF688714 Sanchez del-Pino et al. (2009)	
***Psilotrichum* cf. *cyathuloides* Suess. & Launert**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 358 (EA, B, ETH)	Kenya	AC1141	LT999860 this study	LT996934 this study	LT999822 this study	LT999807 this study
***Psilotrichum elliotii* Baker & C.B. Clarke**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 346A (EA, B, ETH)	Kenya	AC1134	LT992600 this study	LT993051 this study	LT995249 this study	LT993000 this study
***Psilotrichum elliotii* Baker & C.B. Clarke**	V. Di Vincenzo, A. Hailu 145 (B, ETH)	Ethiopia	AC1211	LT992609 this study	LT993059 this study	LT995257 this study	LT993008 this study

Appendix 2.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	ITS
***Psilotrichum elliotii* Baker & C.B. Clarke**	V. Di Vincenzo, A. Hailu 245 (B, ETH)	Ethiopia	AC1218	LT992613 this study	LT993063 this study	LT995246 this study	LT993012 this study
Psilotrichum ferrugineum	K. Larsen & S. Larsen 34281		AC0225	AY998108 Müller & Borsch (2005b)			
***Psilotrichum gloveri* Suess.**	P. Kuchar, A. Abdihakim 19609, 20.07.1993 (ETH)	Ethiopia	AC1164	LT999864 this study	LT995149 this study	LT995213 this study	LT995181 this study
***Psilotrichum gnaphalobryum* (Hochst.) Schinz**	M. Wondafrash 2200 (ETH, B)	Ethiopia	AC1077	LT992593 this study	LT993044 this study	LT992954 this study	LT992993 this study
***Psilotrichum gnaphalobryum* (Hochst.) Schinz**	M. Wondafrash 4253 (ETH, B)	Ethiopia	AC1202	LT992606 this study	LT993056 this study	LT995254 this study	LT993005 this study
***Psilotrichum gracilipes* Hutch. & E. A. Bruce**	M. Wondafrash 3683 (ETH, B)	Ethiopia	AC0967	LT999843 this study	LT995137 this study	LT995199 this study	LT995167 this study
***Psilotrichum schimperi* Engl.**	T. Mesfin & G.Y. Kagnew 1791, 01.11.1981 (ETH)	Ethiopia	AC1160	LT999863 this study		LT995212 this study	LT995180 this study
***Psilotrichum scleranthum* Thwaites**	K. Müller 878 (B, PRE)	South Africa	AC0125	AY514822 Müller & Borsch (2005a)	LT996924 this study	LT999811 this study	LT999809 this study
***Psilotrichum sericeum* (J. König ex Roxb.) Dalzell**	W. R. G. Luke & P. A. Luke 3832 (PRE)		AC0215	AY998109 Müller & Borsch (2005b)	LT993024 this study	LT992947 this study	LT992971 this study
***Psilotrichum* sp.**	M. Wondafrash 3460	Ethiopia	AC0663	LT999827 this study	LT996925 this study	LT999812 this study	LT999796 this study
***Psilotrichum* sp.**	M. Wondafrash 4114 (ETH, B)	Ethiopia	AC1072	LT999847 this study	LT996929 this study	LT999817 this study	LT999802 this study
***Psilotrichum* sp.**	V. Di Vincenzo, A. Hailu 153 (B, ETH)	Ethiopia	AC1213	LT999870 this study	LT996935 this study	LT999823 this study	LT999808 this study
***Ptilotus manglesii* F.Muell.**	T. Borsch 3543 (BONN)	BG Bonn 12999 (Australia)	AC0015	AY514824 Müller & Borsch (2005a)		LT995220 this study	

Appendix 2.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	ITS
***Ptilotus obovatus* (Gaudich.) F. Muell.**		BG Bonn s.n. [Australia]	AC0025	AY514823 Müller & Borsch (2005a)		LT995222 this study	
***Pupalia grandiflora* Peter**	V. Di Vincenzo, A. Hailu 84 (B, ETH)	Ethiopia	AC1205	LT999868 this study	LT995153 this study	LT995217 this study	LT995185 this study
***Pupalia lappacea* (L.) A. Juss.**	K. Müller 861 (B, PRE)	Süd-Afrika, Northern Province	AC0875	LT992578 this study	LT993030 this study	LT992948 this study	LT992977 this study
***Pupalia lappacea* (L.) A. Juss.**	K. Müller 868 (B, PRE)	Süd-Afrika (RSA), Northern Province	AC0926	LT992583 this study	LT993036 this study	LT995241 this study	LT992983 this study
***Pupalia lappacea* (L.) A. Juss.**	M. Wondafrash 3650 (ETH, B)	Ethiopia	AC0968	LT992588 this study	LT993040 this study	LT992950 this study	LT992988 this study
***Pupalia lappacea* (L.) A. Juss.**	M. Wondafrash 3922 (ETH, B)	Ethiopia	AC0983	LT992590 this study	LT993042 this study	LT995262 this study	LT992990 this study
***Pupalia lappacea* (L.) A. Juss.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 342 (EA, B, ETH)	Kenya	AC1131	LT992599 this study	LT993050 this study	LT995248 this study	LT992999 this study
***Pupalia lappacea* (L.) A. Juss.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 354 (EA, B, ETH)	Kenya	AC1140	LT992601 this study	LT993052 this study	LT995250 this study	LT993001 this study
***Sericocoma avolans* Fenzl**	K. Müller 859 (B, PRE)	South Africa	AC0234	AY998103 Müller & Borsch (2005b)	LT993026 this study	LT995232 this study	LT992973 this study
***Sericocoma heterochiton* Lopr.**	K. Müller 851 (B, PRE)	South Africa	AC0191	AY998104 Müller & Borsch (2005b)	LT993018 this study	LT995226 this study	LT992964 this study
***Sericocomopsis hildebrandtii* Schinz**	M. Wondafrash 3500 (ETH, B)	Ethiopia	AC0961	LT999842 this study	LT995136 this study	LT995198 this study	LT995166 this study
***Sericocomopsis hildebrandtii* Schinz**	M. Wondafrash 3462 (ETH, B)	Ethiopia	AC0955	LT999841 this study	LT995135 this study	LT995197 this study	LT995165 this study
***Sericocomopsis hildebrandtii* Schinz**	M. Wondafrash 2065 (ETH, B)	Ethiopia	AC1084	LT999850 this study	LT995143 this study	LT995205 this study	LT995173 this study

Appendix 2.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	ITS
***Sericocomopsis hildebrandtii* Schinz**	M. Wondafrash 2213 (ETH, B)	Ethiopia	AC1094	LT999851 this study	LT995144 this study	LT995206 this study	LT995174 this study
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 339 (EA, B, ETH)	Kenya	AC1130	LT999859 this study	LT995147 this study	LT995210 this study	LT995178 this study
***Sericocomopsis pallida* (S. Moore) Schinz**	M. Wondafrash 3928 (ETH, B)	Ethiopia	AC1080	LT999849 this study	LT995142 this study	LT995204 this study	LT995172 this study
***Sericocomopsis pallida* (S. Moore) Schinz**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 334A (EA, B, ETH)	Kenya	AC1125	LT999858 this study	LT995146 this study	LT995209 this study	LT995177 this study
***Sericocomopsis pallida* (S. Moore) Schinz**	V. Di Vincenzo, M. Wondafrash 42 (B, ETH)	Ethiopia	AC1203	LT999867 this study	LT995152 this study	LT995216 this study	LT995184 this study
***Sericorema remotiflora* (Hook.) Lopr.**	K. Müller 845 (B, PRE)	Süd-Afrika (RSA), Northern Cape	AC0876	LT992579 this study	LT993031 this study	LT995236 this study	LT992978 this study
***Sericorema sericea* (Schinz) Lopr.**	K. Müller 849 (B, PRE)	Süd-Afrika (RSA), Northern Cape	AC0878	LT992580 this study	LT993032 this study	LT995237 this study	LT992979 this study
***Sericostachys scandens* Gilg & Lopr.**	E. Fischer s.n. (B)	Rwanda	AC0042	AY514819 Müller & Borsch (2005a)			
***Tidestromia lanuginosa* (Nutt.) Standl.**	T. Borsch, K. Müller & D. Pratt 3439 (B, ISC)	USA, Texas	AC0052	AY514797 Müller & Borsch (2005a)			
***Volkensinia prostrata* (Volkens ex Gilg) Schinz**	M. Wondafrash 2121 (ETH, B)	Ethiopia	AC0267	LT999826 this study	LT995129 this study	LT995191 this study	LT995158 this study
***Volkensinia prostrata* (Volkens ex Gilg) Schinz**	M. Wondafrash 4200 (ETH, B)	Ethiopia	AC1201	LT999866 this study	LT995151 this study	LT995215 this study	LT995183 this study
***Xerosiphon aphyllus* (Pohl ex Moq.) Pedersen**	S. Splett 701 (B)	Brasil	AC0101	AM887523 Sage et al. (2007)			
***Xerosiphon aphyllus* (Pohl ex Moq.) Pedersen**	Fonseca 1303 et al (NY)	Brasil			EF676100 Sanchez del-Pino et al. (2009)	EF688729 Sanchez del-Pino et al. (2009)	

Appendix 2.B: List of analyzed taxa of the Caryophyllales, including geographic origin, voucher information and ENA/GenBank accession numbers.

Taxa of the Amaranthaceae are excluded here and are listed in Appendix 2.A.

Family	Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	matK
Achatocarpaceae	Achatocarpus praecox Griseb.	B. Leuenberger, Arroya-Leuenberger & U. Eggli 4345 (B)	Bot. Gard. Berlin 142-78-94-10 [Argentina]	AC0073	AY514845 Müller & Borsch (2005a)
Achatocarpaceae	Phaulothamnus spinescens A. Gray	T. Borsch, K. Müller & D. Pratt 3446		AC0060	AY514846 Müller & Borsch (2005a)
Berberidopsidaceae	Berberidopsis corallina Hook. f.	M. J. Moore 326 (FLAS)			EU002171 Wang et al. (2009)
Cactaceae	Rhipsalis floccosa Salm-Dyck ex Pfeiff.	Leuenberger & Eggli 4643 (ZSS 18976)	Argentina		AY015342 Nyffeler (2002)
Caryophyllaceae	Illecebrum verticillatum L.	T. Borsch & K. Müller 3541 (B)	Germany		AY514849 Müller & Borsch (2005a)
Caryophyllaceae	Scleranthus perennis L.	T. Borsch 3389 (B)	Germany		AY514847 Müller & Borsch (2005a)
Caryophyllaceae	Silene otites (L.) Wibel	T. Borsch 3495 (B)	Netherlands		AY514848 Müller & Borsch (2005a)
Cercidiphyllaceae	Cercidiphyllum japonicum Siebold & Zucc. ex J. J. Hoffm. & J. H. Schult. bis	T. Borsch s.N. (B)	BG Bonn		AM396508 Worberg et al. (2007)
Chenopodiaceae	Acroglochin chenopodioides Schrad. ex Schult.; syn. of	Anonymous s.n. (KAS)	BG Kassel ex BG Lyon		AY514826 Müller & Borsch (2005a)
Chenopodiaceae	Agriophyllum squarrosum (L.) Moq.	H. Freitag 28.196a (KAS)	SE Russia, Prov. Astrakhan		AY514827 Müller & Borsch (2005a)
Chenopodiaceae	Allenrolfea vaginata (Griseb.) Kuntze		BG Bonn 2488	AC0017	AY514828 Müller & Borsch (2005a)
Chenopodiaceae	Anthochlamys polygaloides (Fisch. & C. A .Mey.) Moq.	Sh. Zarre et al 35225 (B)	Iran	AC1252	LT999871 this study
Chenopodiaceae	Aphanisma blitoides Nutt. ex Moq.	S. Junak SR-987 (SBBG)	USA, California		AY514844 Müller & Borsch (2005a)
Chenopodiaceae	Atriplex sagittata Borkh.	S. Fuentes 021 (B)	Berlin Bot. Gard. No: 063119110 [Germany]	AC0533	HE855637 Fuentes et al. (2012)
Chenopodiaceae	Atriplex truncata (Torr.) A. Gray	T. Borsch, D. Pratt & K. Müller 3425 (ISC, B, KAS)	USA, Texas		AY514830 Müller & Borsch (2005a)
Chenopodiaceae	Axyris prostrata L.	E. v. Raab-Straube 020232a (B)	Russia	AC0529	HE855600 Fuentes et al. (2012)
Chenopodiaceae	Bassia hirsuta (L.) Asch.; syn. of Bassia crassifolia (Pall.) Soldano	T. Borsch, D. Pratt & K. Müller 3430 (ISC, B)	USA, Texas		AY514831 Müller & Borsch (2005a)
Chenopodiaceae	Beta vulgaris L.	G. Kadereit 99/255 (MJG)	Germany, Baltic Sea		AY514832 Müller & Borsch (2005a)
Chenopodiaceae	Bienertia cycloptera Bunge	H. Akhani s.n., 16.11.2000 (Hb. Akhani, KAS)	Iran, Kavir protected area near Mobarakiyeh		AY514833 Müller & Borsch (2005a)
Chenopodiaceae	Blitum bonus henricus (L.) Rchb.		Bot. Gard. Mainz, without voucher		AY514834 Müller & Borsch (2005a)
Chenopodiaceae	Blitum nuttallianum Schult.	R. C. Holmgren 317 (B)	USA (Utah)	AC0621	HE855621 Fuentes et al. (2012)
Chenopodiaceae	Blitum virgatum L.	S. Fuentes 003 (B)	Bonn Bot. Gard. No: 19117 [Germany]	AC0392	HE855617 Fuentes et al. (2012)

Appendix 2.B – continued

Family	Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*
Chenopodiaceae	*Camphorosma monspeliaca* L.	H. Freitag 28.133 (KAS)	Kasakhstan, Prov. Gur'yev (Atyrau), Makhambat		AY514829 Müller & Borsch (2005a)
Chenopodiaceae	*Ceratocarpus arenarius* L.	A. Romanovsch (B)	Romania, Navodari	AC0531	HE855601 Fuentes et al. (2012)
Chenopodiaceae	*Chenopodiastrum coronopus* (Moq.) S. Fuentes, Uotila, Borsch	Royl 6823 (B)	Spain, La Palma	AC0570	HE855636 Fuentes et al. (2012)
Chenopodiaceae	*Chenopodium acuminatum* Willd.	M. Azuma s.n. 28.11.1984 (FR)	Mongolia		AY514836 Müller & Borsch (2005a)
Chenopodiaceae	*Chenopodium album* L.	Weber (B)	Germany, Usedom	AC0602	HE855665 Fuentes et al. (2012)
Chenopodiaceae	*Chenopodium nutans* (R.Br) S.Fuentes & Borsch	S. Fuentes 019 (B)	Berlin Bot. Gard. No: 187199 [Australia]	AC0525	HE855662 Fuentes et al. (2012)
Chenopodiaceae	*Chenopodium quinoa* Willd.	S. Fuentes 013 (B)	USA ARS GRIN Ames 13214 [Bolivia]	AC0401	HE855649 Fuentes et al. (2012)
Chenopodiaceae	*Chenopodium vulvaria* L.	T. Borsch 3918 (B)	Spain	AC0412	HE855640 Fuentes et al. (2012)
Chenopodiaceae	*Corispermum ladakhianum* Grey-Wilson & Wadhwa; syn. of *Corispermum*	D. Pratt 204 (ISC, B)	USA, Utah		AY514837 Müller & Borsch (2005a)
Chenopodiaceae	*Dysphania ambrosioides* (L.) Mosyakin & Clemants	T. Borsch & al. 4397 (B)	Cuba	AC0867	HE855611 Fuentes et al. (2012)
Chenopodiaceae	*Hablitzia tamnoides* M.Bieb.	T. Borsch 3546 (B)	BG Bonn 03609-90	AC0018	AY514825 Müller & Borsch (2005a)
Chenopodiaceae	*Halocnemum strobilaceum* (Pall.) M. Bieb.	H. Freitag & Adigüzel 28.783 (KAS, GAZI)	Turkey		AY514842 Müller & Borsch (2005a)
Chenopodiaceae	*Kraschenninnikovia ceratoides* (L.) Gueldenst.	R. Hand 1536 (B)	Russia	AC0532	HE855602 Fuentes et al. (2012)
Chenopodiaceae	*Lipandra polysperma* (L.) S. Fuentes, Uotila, Borsch	T. Borsch s/n (B)	Germany	AC0944	HE855631 Fuentes et al. (2012)
Chenopodiaceae	*Microgynoecium tibeticum* Hook. f.	B. Dickoré 4284 (B)	China	AC0656	HE855639 Fuentes et al. (2012)
Chenopodiaceae	*Nitrophila occidentalis* (Moq.) S. Watson	D. Pratt 204 (ISC, B)	USA, Utah	AC0089	AY514840 Müller & Borsch (2005a)
Chenopodiaceae	*Oreobliton thesioides* Durieu & Moq.	P. Stipacek & C. Scheuer s.n. (M)	Tunisia	AC0177	AY875638 Müller & Borsch (2005b)
Chenopodiaceae	*Oxybasis rubra* (L.) S. Fuentes, Uotila, Borsch	T. Borsch [08.07] (B)	Germany, North See	AC0411	HE855624 Fuentes et al. (2012)
Chenopodiaceae	*Oxybasis urbica* (L.) S. Fuentes, Uotila, Borsch	R. & E. Willing 146.1979 (B)	Greece, Fthiotis	AC0576	HE855630 Fuentes et al. (2012)
Chenopodiaceae	*Patellifolia procumbens* (C. Sm.) A. J. Scott, Ford-Lloyd & J. T. Williams	K. Müller 752 (B)	Tenerife, Canary Islands	AC0180	AY875637 Müller & Borsch (2005b)
Chenopodiaceae	*Polycnemum majus* A. Braun		Bot. Gard. Mainz s.n.		AY514839 Müller & Borsch (2005a)
Chenopodiaceae	*Polycnemum verrucosum* Láng	KP Buttler 15976 (FR)	Turkey		AY514838 Müller & Borsch (2005a)

Appendix 2.B – continued

Family	Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*
Chenopodiaceae	*Salsola kali* L.	G. Kadereit 1999/211 (MJG)	Germany, Graswarder, Baltic Sea Coast		AY514843 Müller & Borsch (2005a)
Chenopodiaceae	*Salsola* sp.	K. Müller 852 (B, PRE)	South Africa (RSA), Northern Cape	AC0879	LT999828 this study
Chenopodiaceae	*Spinacia oleracea* L.				AJ400848.1
Chenopodiaceae	*Teloxys aristata* (L.) Moq.	S. Fuentes 183 (B)	USA ARS GRIN Ames 25314 [Mongolia]	AC0654	HE855612 Fuentes et al. (2012)
Drosophyllaceae	*Drosophyllum lusitanicum* (L.) Link		BG Bonn 09015		AY514860 Müller & Borsch (2005a)
Frankeniaceae	*Frankenia laevis* L.	K. Müller 884 (BONN)	BG Bonn s.n.		AY514853 Müller & Borsch (2005a)
Gunneraceae	*Gunnera tinctoria* (Molina) Mirb.	N. Korotkova 50 (BONN)	BG Bonn		AM396506 Worberg et al. (2007)
Hydrangeaceae	*Dichroa versicolor* (Fortune) D. R. Hunt	PG 11339 (GENT)	BG Ghent University 2007-1346 [Thailand]		GU217319 Samain et al. (2010)
Phytolaccaceae	*Rivina humilis* L.	T. Borsch 3542 (BONN)	BG Bonn 9865		AY514850 Müller & Borsch (2005a)
Plumbaginaceae	*Limonium latifolium* (Sm.) Kuntze	K. Müller 883 (BONN)	BG Bonn 03678		AY514861 Müller & Borsch (2005a)
Simmondsiaceae	*Simmondsia chinensis* C. K. Schneid.		Bot. Gard. Bonn 310700001		AY514854 Müller & Borsch (2005a)

Appendix 2.C: Primers used for amplification and sequencing of the plastid *trnK/matK*, *rpl16*, *trnL-F* and the nrITS genomic regions.

primer name	sequence 5' → 3'	dir	source	region
trnKF	GGGTTGCTAACTCAATGGTAGAG	F	Wicke & Quandt 2009	*trnK*
trnK2R	AACTAGTCGGATGGAGTAG	R	Johnson & Soltis 1995	*trnK*
psbA5'R	AACCATCCAATGTAAAGACGGTTT	R	Shaw et al. 2005	*trnK-psbA*
ACmatK490F	CTGGTTGAAAGATGCTTCKTC	F	Müller & Borsch 2005a	*matK*
ACmatK500F	TTCTTCTTTGCATTTATTACG	F	Müller & Borsch 2005a	*matK*
ACmatK1400R	GGATTCGTATTCACATACA	R	Müller & Borsch 2005a	*matK*
ACmatK1401R	ATGGATTCGTATTCACATAC	R		*matK*
ACmatK100F	CTCGACTGTATCAACAGAATC	F	Müller & Borsch 2005a	*matK*
ACmatK200R	GAATTTCCACAATGACTGC	R	Müller & Borsch 2005a	*matK*
ACmatK1300R	TRCGAGACAAAGTTCTAGCAC	R	Müller & Borsch 2005a	*matK*
ACmatK1250F	CTCATTATTATAGTGGCTCYTC	F	Müller & Borsch 2005a	*matK*
rpl16-1516R	CCCTTCATTCTTCCTCTATGTTG	R	Kelchner & Clarck 1997	*rpl16*
rpl16F	CTATGCTTAGTGTGTGACTC	F	Löhne et al. 2007, but in that study, it was cited as Campagna & Downie 1998 but the sequence of the primer is different	*rpl16*
trnT-AC1	AARCATTTTTCGGTATAG	F	Borsch 2006	*trnT-F*
trnTf	ATTTGAACTGGTGACACGAG	R	Taberlet et al. 1991	*trnT-F*
AC-ITS5	GGAAGGAGAAGTCGWAACARGG	F	Kai Mueller 2002	ITS
ITS4	TCCTCCGCTTATTGATATGC	R	White et al. 1990	ITS

Appendix 2.D: PCR protocol used for amplification of the plastid *trnK/matK*, *rpl16*, *trnL-F* and the nrITS genomic regions.

Chemicals for PCR reaction (50µl)
2 µl solution of DNA template
15.2 µl H2O ultrarein
5 µl 10x peqLab Taq.Buffer S incl. $MgCl_2$
10 µl Betaine 5 M
1 µl BSA
2.5 µl 25mM $MgCl_2$
2 µl Forward Primer (20pm/µl)
2 µl Reverse Primer (20pm/µl)
10 µl dNTP (each 1.25mM)
0.3 µl peqLab Taq. Polymerase @ 5units/µl

Appendix 2.E: Character matrix coding the presence (1) and absence (0) of traits within the Achyranthoids.

Taxon	Modified sterile flowers	Adhesive appendages
Achyranthes aspera	0	1
Achyranthes bidentata	0	1
Achyranthes splendens	0	1
Achyropsis leptostachya	0	0
Arthraerua leubnitziae	0	0
Calicorema capitata	0	0
Calicorema squarrosa	0	0
Centema subfusca	1	1
Centemopsis fastigiata	0	1
Centemopsis sp.	0	1
Centemopsis micrantha	0	1
Centemopsis trinervis	0	1
Centrostachys aquatica	0	1
Chionothrix latifolia	0	0
Chionothrix somalensis	0	0
Cyathula capitata	1	1
Cyathula cylindrica	1	1
Cyathula lanceolata	1	1
Cyathula natalensis	1	1
Cyathula orthacantha	1	1
Cyathula polycephala	1	1
Cyathula prostrata	1	1
Cyathula tomentosa	1	1
Cyathula uncinulata	1	1
Dasysphaera tomentosa	1	1
Kyphocarpa angustifolia	1	1
Kyphocarpa cf. *cruciata*	1	1
Kyphocarpa trichinoides	1	1
Leucosphaera bainesii	1	1
Lopriorea ruspolii	0	0
Marcelliopsis splendens	1	1
Mechowia grandiflora	0	0
Nelsia quadrangula	1	1
Nototrichium humile	0	0
Nototrichium sandvicense	0	0
Pandiaka angustifolia	0	1
Polyrhabda atriplicifolia	0	0
Psilotrichum cf. *cyathuloides*	0	0
Psilotrichum elliotii	0	0
Psilotrichum gloveri	0	0
Psilotrichum gnaphalobryum	0	0
Psilotrichum gracilipes	0	0
Psilotrichum schimperi	0	0
Psilotrichum scleranthum	0	0
Psilotrichum sericeum	0	0
Psilotrichum sp.	0	0
Pupalia grandiflora	1	1
Pupalia lappacea	1	1
Sericocoma avolans	0	0
Sericocoma heterochiton	0	0
Sericocomopsis hildebrandtii	0	0
Sericocomopsis pallida	0	0
Sericorema remotiflora	1	1
Sericorema sericea	1	1
Sericostachys scandens	1	0
Volkensinia prostrata	1	1

Appendix 2.F: Model fit of different binary state-dependent speciation and extinction models regarding the presence of adhesive flower appendages. (0 = absent; 1 = present). In the models evaluated, parameters were set as equal (BiSSE model set 1) and to zero (BiSSE model set 2) across character states. Relative model fit was evaluated via the AIC and AICc; a comparison of model fit between the unconstrained and each constrained model was performed via the LRT. Parameter estimates and test statistics were calculated on the MCC tree that summarizes 1000 post-burn-in MCMC trees of the posterior tree distribution inferred during the estimation of divergence times. Asterisks indicate LRT results at $p<0.05$. Abbreviations used: DF = degrees of freedom; Trans. rate = character transition rate; lnLik = natural logarithm of the likelihood.

Model Specification					Model fit				
	Speciation (λ)	**Extinction (μ)**	**Trans. rate (q)**	**DF**	**lnLik**	**AIC**	**AICc**	**LRT χ^2**	**LRT *p*-value**
	unconstrained – all rates free			6	-223.648	459.297	460.745	NA	NA
BiSSE Model set 1	free	μ0=μ1	free	5	-223.698	457.397	458.414	0.100	0.752
	free	free	q01=q10	5	-223.780	457.560	458.577	0.263	0.608
	λ0=λ1	free	free	5	-224.519	459.038	460.055	1.741	0.187
	free	μ0=μ1	q01=q10	4	-224.412	456.824	457.491 (best)	1.528	0.466
	λ0=λ1	free	q01=q10	4	-225.789	459.578	460.245	4.282	0.118
	λ0=λ1	μ0=μ1	free	4	-226.285	460.569	461.236	5.273	0.072
	λ0=λ1	μ0=μ1	q01=q10	3	-226.433	458.867	459.260	5.570	0.135
BiSSE Model set 2	λ0=0	free	free	5	-234.189	478.379	479.396	21.082	< 0.01*
	free	μ0=0	free	5	-223.720	457.441	458.458	0.144	0.704
	free	μ1=0	free	5	-223.980	457.961	458.978	0.664	0.415
	free	free	q01=0	5	-223.725	457.449	458.466	0.153	0.696
	free	free	q10=0	5	-229.475	468.950	469.967	11.654	< 0.01*
	λ0=0	μ0=0	free	4	-234.189	476.379	477.045	21.082	< 0.01*
	λ0=0	μ1=0	free	4	-234.277	476.555	477.222	21.258	< 0.01*
	λ0=0	free	q01=0	4	-237.562	483.125	483.791	27.828	< 0.01*
	free	μ0=0	q01=0	4	-224.017	456.033	456.700	0.737	0.692
	free	μ0=0	q10=0	4	-229.475	466.950	467.617	11.654	< 0.01*
	free	μ1=0	q01=0	4	-223.982	455.964	456.631 (best)	0.668	0.716
	free	μ1=0	q10=0	4	-231.275	470.550	471.217	15.254	< 0.01*

Appendix 2.G: Comparison of model fit of different CID models of the HiSSE model concept and of BiSSE models regarding the presence of adhesive flower appendages.

Two two-state and two four-state CID models as well as four BiSSE models were evaluated. Dual transitions between character states (e.g. q_0A ↔ q_1B; q_0B ↔ q_1A) were not included in any CID model. Relative model fit was evaluated via the AIC and AICc; the difference in model fit between the most parameterized CID or BiSSE model and all others was evaluated via the LRT. Abbreviations used: DF = degrees of freedom; Trans. rate = character transition rate; lnLik = natural logarithm of the likelihood; NA = not applicable.

Model specification						Model fit			Comparison to *CID-2 unconstrained*		Comparison to *BiSSE unconstrained*	
		Net turnover (τ)	**Extinction fraction (ε)**	**Trans. rate (q)**	**DF**	**lnLik**	**AIC**	**AICc**	**LRT χ^2**	**LRT *p*-value**	**LRT χ^2**	**LRT *p*-value**
HiSSE models	***CID-2***	free	free	free	13	-223.544	471.089	477.089	NA	NA	<0.001	1.000
		free	free	equal	6	-222.848	455.696	456.713	0.001086	1.000	0.002	1.000
	CID-4	free	free	three rates	11	-220.243	462.486	467.467	0.024558	1.000	0.03	1.000
		free	free	equal	9	-220.960	459.920	463.193	0.015023	1.000	0.019	1.000
BiSSE models		free	free	free	7	-223.899	459.797	461.245	0.000280	1.000	NA	NA
		equal	free	free	6	-224.643	460.303	459.286	0.002694	1.000	0.001	1.000
		free	equal	free	6	-223.849	458.716	457.699	0.000208	1.000	<0.001	1.000
		equal	equal	free	5	-226.859	462.384	461.717	0.024389	1.000	0.019	1.000

Appendix 2.H: Global sampling fractions specified for BiSSE analyses.

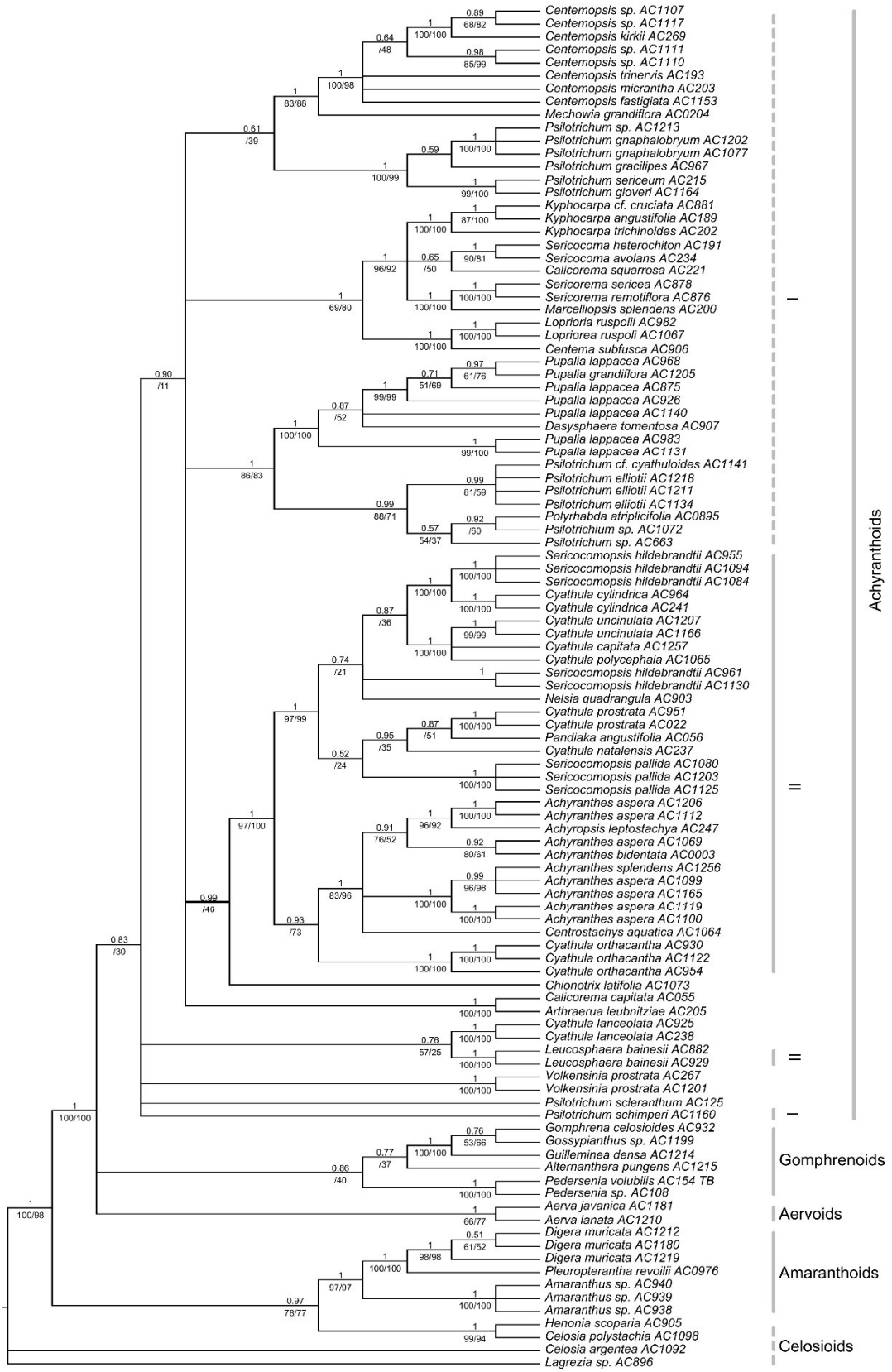
Trait	Sampling fraction	
	trait absent	trait present
(1) modified sterile flowers serving epizoochory	0.44	0.41
(2) adhesive flower appendages within dispersal units serving epizoochory	0.63	0.34

Appendix 2.I: Clade-specific sampling fractions specified for BAMM analyses and species numbers assumed for Amaranthaceae.

Subclade of Amaranthaceae	Estimated species-no. per clade	Sampling fraction
Achyranthoids	150	0.44
Aervoids	112	0.07
Gomphrenoids	377	0.06
*Allmaniopsis**	1	1.00
*Psilotrichum ferrugineum***	1	1.00
Amaranthoids	67	0.15
Celosioids	88	0.08
*Charpentiera**	6	0.33
*Bosea**	3	0.33

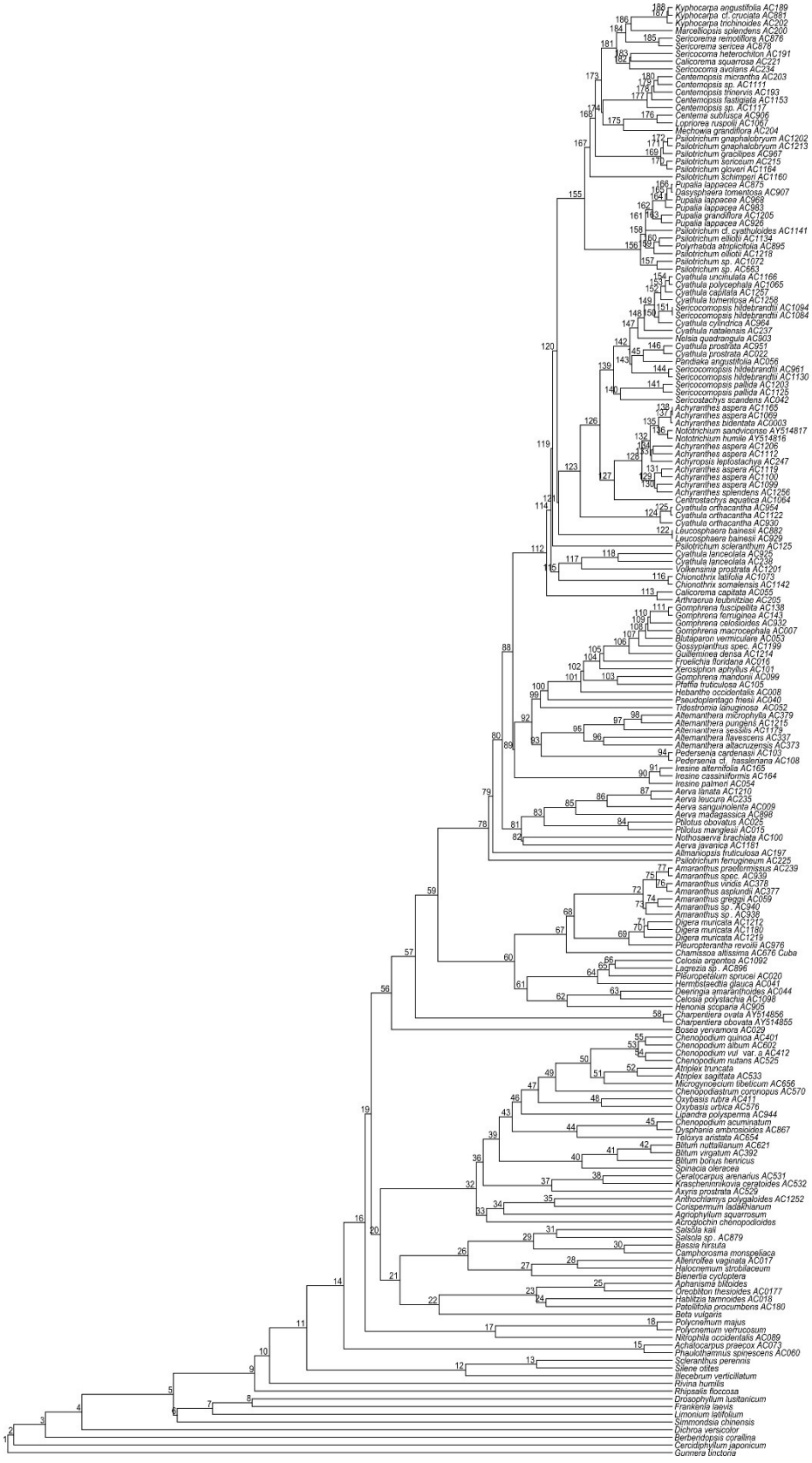
Names correspond to main subclades of Amaranthaceae s.str. with the following exceptions: *name corresponds to a single genus, **name corresponds to a single species.

Appendix 2.J: Phylogeny of the achyranthoid clade based on nrITS sequences inferred via BI.
Posterior probabilities are given above branches, JK percentages from MP (left) and BS percentages from ML analyses (right) are given below branches.



Appendix 2.K: Chronogram of the Amaranthaceae-Chenopodiaceae alliance and associated table with ages and confidence intervals inferred from dataset C via divergence dating.

Inferred ages and confidence intervals (given in the format "median age [95% HPD age interval]") from analyses under different speciation models and prior distributions are listed in the table below the chronogram; numbers in the table refer to the node numbers in the chronogram.



node	Tree prior: Yule			Tree prior: Birth-Death		
	Prior distr.: uniform	Prior distr.: exponential	Prior distr.: lognormal	Prior distr.: uniform	Prior distr.: exponential	Prior distr.: lognormal
1	118,19 [112,32-123,9]	118.54 [112.51-124.21]	118.22 [112.38-124.15]	118.91 [113.18-125.15]	118.93 [113.08-125.04]	118.7 [112.88-125.11]
2	113,74 [104,99-121,09]	114.53 [106.09-122.34]	114.2 [105.66-121.53]	115.12 [106.64-121.98]	115.38 [107.46-122.89]	114.51 [104.64-122.63]
3	108 [99,69-116,63]	109.27 [100.64-117.03]	108.67 [100.01-116.46]	109.64 [100.98-117.58]	110.61 [102.52-119.54]	108.99 [99.89-117.44]
4	103,66 [95,35-111,46]	104.96 [97.38-113.54]	104.26 [96.07-112.1]	105.63 [96.78-112.59]	106.8 [97.73-114.64]	104.33 [95.94-112.84]
5	94,52 [87,6-101,75]	93.44 [85.85-100.05]	92.88 [86.81-99.64]	94.42 [87.26-101.05]	94.49 [88.74-101.92]	94.35 [88.12-101.97]
6	88,19 [69,03-99,34]	79.68 [59.55-95.23]	87.17 [66.66-98.01]	82.3 [61.01-96.18]	90.51 [77.85-99.33]	83.26 [66.72-97.86]
7	78,55 [54,88-94,71]	58.85 [37.34-82.38]	77.75 [52.78-93.08]	60.89 [27.03-83.71]	84.47 [66.91-98.24]	65.71 [37.94-87.57]
8	60,12 [37,24-82,97]	91.01 [83.77-99.79]	56.75 [32.33-78.51]	92.24 [83.08-98.79]	71.6 [47.1-87.14]	91.39 [82.35-97.93]
9	77,13 [68,7-85,79]	76.42 [68.82-84.54]	75.87 [68.35-83.18]	76.68 [68.99-85.16]	76.85 [69.52-84.39]	75.5 [68.25-84.03]
10	75 [68,34-83,46]	73.95 [66.99-81.55]	73.34 [66.77-81.1]	74.05 [67.24-81.63]	74.24 [67.07-81.1]	73.71 [66.49-80.69]
11	70,54 [64,47-77,95]	69.3 [63.55-75.7]	69.05 [63.41-74.89]	69.81 [63.52-75.71]	69.39 [63.67-75.82]	68.52 [62.15-74.62]
12	51,43 [38,73-63,91]	48.45 [34.32-64.57]	48.71 [34.71-60.51]	48.06 [32.47-60.23]	49.88 [34.48-64.64]	49.14 [34.65-61.11]
13	36,91 [24,82-50,98]	32.6 [18.28-47.7]	33.74 [16.02-48.85]	29.8 [18.07-43.82]	35.74 [18.64-50.03]	34.4 [20.79-47.32]
14	65,99 [60,01-72,49]	64.93 [60.04-70.71]	64.34 [60.01-70.35]	65.17 [59.62-71.5]	64.84 [59.71-71.39]	63.76 [59.18-70.91]
15	6,77 [1,59-16,06]	6.94 [1.64-16.04]	6.7 [1.5-15.84]	5.7 [1.59-14]	5.46 [1.2-12.53]	5.65 [1.49-13.87]
16	59,92 [56,02-66,28]	58.57 [56.01-63.39]	57.51 [56.02-61.88]	58.68 [56.07-63.95]	58.08 [56.02-64.01]	57.15 [56.02-63.06]
17	33,38 [16,89-48,83]	55.74 [50.49-61.29]	54.99 [49.91-60.55]	55.91 [50.33-61.75]	32.74 [15.87-49.99]	28.93 [13.98-44.45]
18	3,98 [0,77-9,59]	52.63 [44.57-59.01]	52.03 [43.87-57.27]	53.09 [45.37-58.92]	3.41 [0.99-7.86]	3.4 [0.95-7.63]
19	59,36 [56,05-65,85]	28.5 [13.88-40.97]	28.26 [15.41-42.81]	25.66 [14.68-39.74]	57.52 [56-62.69]	56.65 [56.01-60.24]
20	57,16 [51,76-64,15]	21.58 [11.05-33.64]	21.4 [10.8-34.32]	19.26 [9.87-28.92]	55.07 [48.83-61.54]	54.56 [47.93-59.21]
21	53,76 [45,07-61,14]	17.93 [7.19-29.8]	18.12 [8.15-31.14]	9.36 [3.7-16.2]	51.68 [42.04-58.57]	51.18 [42.68-57.42]
22	30,17 [15,94-43,97]	10.51 [4.61-21.65]	10.58 [4.33-19.89]	16.19 [7.7-26.68]	24.85 [14.35-38.18]	25.41 [16.09-39.22]
23	22,87 [12,44-36,03]	42.45 [32.42-50.87]	40.76 [31.77-50.72]	42.63 [33.56-51.52]	18.65 [10.79-29.8]	19.02 [10.52-29.56]
24	19,05 [9,16-31,96]	26.93 [23.02-35.83]	24.34 [23-30.94]	27.46 [23.02-36.26]	15.93 [8-26.7]	9.13 [4.02-17.47]
25	11,43 [4,51-22,01]	15.54 [5.57-23.81]	14.25 [6.11-21.91]	15.1 [4.95-25.31]	9.36 [3.67-17]	16.19 [7.01-27.16]
26	42,82 [33,93-52,47]	30.23 [20.57-39.79]	29.56 [18.73-40.67]	29.13 [17.9-40.67]	40.76 [30.92-49.63]	39.72 [31.33-48.57]
27	28,33 [23-38,17]	23.33 [12.32-33.84]	14.57 [6.37-25.53]	21.9 [10.67-33.21]	26.74 [23.01-34.37]	24.09 [23-29.95]
28	16,47 [6,33-26,49]	14.71 [6.4-24.34]	22.56 [9.99-32.71]	13.34 [5.42-22.93]	14.92 [5.46-23.86]	14.09 [5.57-22.43]
29	30,52 [19,38-40,64]	49.99 [42.85-57.08]	49.57 [42.78-55.71]	49.9 [41.4-56.66]	27.69 [18.23-38.52]	26.94 [17.18-37.89]
30	14,52 [6,49-23,77]	47.73 [39.79-56.37]	47.25 [38.33-54.37]	47.68 [37.81-56.7]	12.53 [4.68-21.71]	12.64 [5.79-22.61]
31	22,72 [12,87-35,65]	24.71 [12.06-38.1]	25.26 [13.53-40.56]	23.2 [11.11-38.34]	20.32 [10.27-31.42]	20.07 [9.61-30.43]
32	51,65 [43,12-58,41]	13.94 [6.07-26.99]	14.18 [6.65-26.4]	13.2 [4.23-25.02]	48.17 [39.66-55.84]	47.94 [38.13-54.35]
33	49,45 [39,94-58,46]	44.59 [37.74-52.53]	44.85 [37.73-51.52]	44.49 [36.56-53.26]	45.89 [35.34-54.73]	45.62 [35.74-53.75]
34	25,51 [12,77-39,29]	29.7 [21.14-40.67]	29.91 [19.74-39.91]	29.68 [20.36-39.34]	22.19 [10.36-35.59]	21.74 [11.02-35.42]
35	14,52 [5,8-26,84]	17.36 [9.21-27.49]	17.24 [8.85-26.85]	16.72 [8.95-26.01]	11.64 [4.33-23.23]	12.06 [4.57-24.97]
36	46,9 [38,72-53,89]	38.29 [31.27-46.55]	38.79 [30.96-45.45]	36.75 [28.47-45.4]	42.38 [33.33-50.42]	42.45 [33.27-50.46]
37	31,61 [19,53-43,43]	35.77 [29.17-45.51]	20.53 [10.65-31.52]	19.25 [9.57-29.72]	25.84 [16.44-35.97]	25.7 [14.49-35.97]
38	18,47 [10,81-27,85]	26.33 [16.53-35.82]	11.46 [5.33-19.75]	10.16 [4.46-17.82]	13.58 [7.86-24.72]	12.77 [5.3-23.06]
39	40,31 [32,23-48,57]	4.46 [1.04-10.28]	6.05 [2.08-11.98]	5.28 [1.51-10.97]	35.89 [28.15-44]	35.99 [27.99-43.23]
40	21,37 [11,67-33,22]	20.71 [12.19-31.53]	36.88 [30.14-44.7]	35.16 [27.72-43.69]	17.35 [7.73-26.5]	33.34 [25.56-42.37]
41	11,8 [5,54-21,12]	11.59 [5.77-20.09]	26.2 [15.97-37.23]	24.43 [13.05-34.2]	9.62 [3.46-16.59]	23.02 [11.99-33.79]
42	6,51 [2,58-12,76]	6.25 [2.02-12.31]	4.48 [1.43-10.29]	4.09 [1.31-8.71]	5.18 [1.74-10.2]	4.02 [1.3-8.68]
43	38,91 [31,08-46,66]	32.25 [24.51-38.88]	32.81 [25.66-41.07]	30.12 [22.51-38.63]	34.35 [27.27-42.54]	17.93 [9.3-28.5]
44	27,03 [14,76-37,61]	31.04 [25.32-39.52]	31.79 [24.64-40.07]	27.46 [18.62-36.57]	24.3 [13.99-35.42]	9.78 [4.49-17.32]
45	4,72 [1,47-10,45]	9.02 [3.1-17.92]	9.12 [2.83-16.94]	8.28 [3.23-16.19]	3.92 [1.26-8.8]	5.09 [1.85-10.09]
46	33,98 [25,56-41,47]	25.58 [18.5-32.47]	26.41 [19.15-33.83]	23.49 [17.32-32.12]	30.08 [22.72-38.62]	30.02 [23.29-38.76]
47	31,96 [23,68-40,37]	18.16 [13.06-24.78]	18.45 [13.1-24.97]	17.15 [11.49-23.28]	29.35 [22.24-37.57]	28.98 [22.09-36.46]

node	Tree prior: Yule			Tree prior: Birth-Death		
	Prior distr.: uniform	Prior distr.: exponential	Prior distr.: lognormal	Prior distr.: uniform	Prior distr.: exponential	Prior distr.: lognormal
48	9,22 [2,66-17,11]	15.67 [10.29-21.01]	15.77 [10.41-21.84]	14.79 [9.68-21.07]	7.68 [2.53-14.99]	7.26 [2.34-14.82]
49	26,9 [19,66-35,08]	6.69 [2.92-11.13]	6.63 [2.85-10.77]	6.02 [2.77-10.25]	24 [17.5-31.38]	23.61 [17.15-31.61]
50	18,87 [13,3-26,53]	8.07 [4.19-13.53]	8.13 [4.04-13.29]	7.01 [3.58-11.65]	16.33 [10.99-22.57]	16.39 [11.08-22.69]
51	16,18 [10,42-22,95]	5.29 [1.76-10.41]	6.97 [3.26-11.03]	5.63 [2.87-9.48]	13.92 [8.28-19.39]	13.99 [7.94-19.34]
52	6,99 [2,44-12]	6.1 [2.75-11.09]	5.41 [2.23-9.94]	4.51 [1.72-8.23]	5.71 [2.31-10.3]	5.53 [2.59-10.83]
53	8,37 [4,32-13,75]	57.37 [53.53-63.1]	56.32 [53.95-61.56]	57.61 [54.34-63.85]	6.64 [3.29-10.74]	6.88 [3.28-11.22]
54	7,14 [3,98-12,03]	31.33 [15.1-47.3]	31.18 [16.14-46.75]	32.31 [16.98-47.86]	4.7 [2.3-8.75]	5.86 [3.17-9.93]
55	5,46 [2,4-10,13]	3.84 [0.97-8.68]	3.74 [0.79-9.13]	3.56 [0.62-8]	4.24 [1.45-7.89]	4.49 [2-8.55]
56	54,02 [47,14-61,57]	52.56 [45.29-58.55]	51.98 [46.4-57.71]	52.14 [44.36-57.97]	51.25 [42.97-57.55]	50.33 [43.41-56.13]
57	51,11 [43,31-58,26]	49.79 [43.3-56.28]	49.31 [43-54.8]	48.85 [41.19-55.66]	48.08 [40.43-54.58]	47.21 [40.71-53.27]
58	3,08 [0,57-7,84]	2.92 [0.68-7.97]	3.01 [0.61-7.53]	2.59 [0.6-6.25]	2.5 [0.54-6.38]	2.48 [0.58-6.04]
59	47,69 [39,75-54,68]	46.32 [39.71-52.84]	45.88 [39.86-51.61]	44.92 [37.63-51.49]	44.13 [36.87-50.97]	43.45 [36.81-49.1]
60	41,03 [32,09-49,03]	39.28 [30.18-46.54]	39.5 [31.54-46.37]	37.87 [29.12-45.49]	36.86 [28.6-44.52]	36.51 [27.79-44.64]
61	36,5 [27,79-45,92]	35.17 [26.34-43.24]	35.17 [27.73-43.45]	33.56 [25.58-42.73]	32.33 [23.68-40.55]	32.06 [23.81-40.87]
62	21,16 [11,16-32,86]	20.71 [10.48-31.36]	20.55 [10.91-30.6]	19.02 [9.32-29.53]	18.82 [9.94-28.37]	18.23 [9.69-29.18]
63	9,19 [3,28-16,58]	8.86 [2.55-17.1]	8.57 [3.02-16.66]	7.46 [2.98-14.99]	7.46 [2.98-15.33]	7.54 [2.7-14.56]
64	26,76 [15,71-35,84]	25.53 [17.03-35.63]	25.57 [17.2-34.53]	24.06 [15.46-33.66]	22.98 [14.42-32.11]	23.13 [14.61-33.39]
65	18,53 [10,17-28,18]	17.88 [10.39-25.85]	17.93 [10.33-26.66]	16.64 [8.49-24.29]	15.73 [8.45-23.01]	15.46 [9.16-24.12]
66	14,71 [6,97-23,56]	14.25 [7.3-21.93]	14.52 [7.85-23.08]	13.16 [6.33-20.37]	12.58 [6.55-19.38]	12.1 [6.69-20.49]
67	28,84 [20,18-39,89]	27.34 [19.15-37.62]	27.66 [18.92-37.05]	25.82 [16.3-35.18]	25.7 [17.12-34.3]	25.24 [16.73-34]
68	23,21 [15,07-33,26]	21.76 [12.88-30.19]	22.36 [14.25-32.26]	20.5 [11.78-29.69]	20.56 [12.95-28.79]	19.99 [12.55-28.04]
69	9,57 [4,98-15,18]	8.75 [4.76-14.53]	9.08 [4.67-14.96]	8.41 [4.52-13.85]	7.6 [3.34-12.43]	7.45 [3.82-12.06]
70	5,88 [2,84-10,16]	5.21 [2.04-9.25]	5.42 [2.61-10.02]	5.1 [2.76-9.08]	4.37 [1.77-7.58]	4.34 [2.33-7.66]
71	4,54 [1,88-8,11]	3.86 [1.41-7.14]	4.08 [1.78-8.1]	4.02 [1.94-7.27]	3.19 [1.27-5.87]	3.23 [1.44-5.77]
72	5,15 [2,72-8,75]	5.02 [2.26-8.36]	4.99 [2.48-8.82]	4.27 [2.16-6.96]	3.96 [1.81-6.86]	4.12 [2.1-7.13]
73	3,49 [1,42-6,87]	3.47 [1.15-6.5]	3.42 [1.19-6.54]	2.96 [1.01-5.33]	2.85 [1-5.04]	2.92 [1.27-5.28]
74	1,51 [0,27-3,63]	1.47 [0.26-3.44]	1.45 [0.14-3.29]	1.26 [0.2-2.93]	1.23 [0.17-2.74]	1.25 [0.16-2.88]
75	3,7 [1,66-6,28]	3.59 [1.65-6.43]	3.49 [1.57-6.62]	3.08 [1.39-5.28]	2.89 [1.3-5.18]	2.98 [1.41-5.44]
76	2,61 [0,84-5,23]	2.87 [1.39-5.36]	1.13 [0.18-2.82]	2.28 [0.69-4.39]	2.1 [0.74-3.98]	2.73 [1.37-4.91]
77	1,17 [0,25-3,14]	1.18 [0.15-2.97]	2.57 [0.88-5.35]	0.99 [0.16-2.32]	0.99 [0.18-2.39]	0.94 [0.19-2.24]
78	39,87 [33,05-47,59]	38.61 [32.44-45.03]	38.39 [32.42-45.01]	37.24 [30.01-43.43]	35.33 [27.69-42.27]	35.72 [29.76-42.13]
79	37,24 [29,98-44,2]	36.24 [30.28-43.11]	36.04 [29.9-41.89]	34.65 [28.26-41.33]	32.8 [26.08-39.55]	33.26 [28.05-39.9]
80	34,74 [28,29-41,03]	33.69 [28.19-39.89]	33.5 [28.06-39.99]	32.09 [26.15-38.16]	30.15 [24.06-36.71]	30.68 [25.66-36.49]
81	28,66 [20,37-35,35]	27.58 [21.18-34.92]	27.52 [20.58-34.62]	26.08 [19.18-32.24]	24.41 [17.82-31.71]	24.64 [18.94-31.13]
82	26,55 [17,21-34,38]	25.53 [17.15-32.9]	25.83 [18.43-32.83]	24.19 [17.64-31.75]	22.57 [15.13-29.3]	23.14 [16.85-30.18]
83	21,68 [15,19-29,75]	21.02 [14.38-28.29]	20.55 [13.52-27.3]	19.26 [13.75-27.2]	18.31 [12.34-25.22]	18.52 [13.17-24.73]
84	6,45 [2,55-12,53]	5.93 [1.43-11.76]	6.11 [2.29-11.83]	5.61 [2.1-10.97]	4.97 [1.43-9.59]	5.14 [1.95-9.78]
85	17,32 [10,08-24,3]	16.69 [11.06-24.25]	16.03 [10.5-23.06]	15.14 [9.6-22.14]	14.34 [8.75-20.97]	14.37 [9.01-21.03]
86	10,21 [5,71-17,29]	9.86 [5.29-16.34]	9.66 [5.08-16.07]	8.66 [4.15-13.97]	8.76 [4.88-14.32]	8.46 [4.1-13.21]
87	5,1 [1,76-9,6]	4.75 [1.89-9.15]	4.69 [1.32-8.94]	4 [1.3-7.86]	4.34 [1.34-8.31]	4.09 [1.59-7.72]
88	32,36 [26,11-38,42]	31.49 [26.39-37.14]	31.19 [25.64-37.06]	29.55 [23.1-34.84]	27.7 [21.42-33.86]	28.32 [23.06-33.59]
89	6,66 [2,51-14,92]	30.1 [24.7-35.82]	29.91 [24.6-36.27]	28.34 [21.26-33.29]	26.4 [20.68-32.85]	27.08 [21.89-31.89]
90	3,25 [0,72-8,06]	6.44 [1.91-13.96]	6.42 [2.42-13.96]	5.41 [1.87-11.5]	5.19 [1.82-11.21]	5.35 [1.59-11.42]
91	31,95 [26,09-36,75]	3.17 [0.76-7.45]	3.15 [0.8-7.28]	2.72 [0.8-6.34]	2.61 [0.61-6.38]	2.72 [0.62-6.47]
92	28,8 [23,23-35,41]	28.13 [23.11-33.86]	27.99 [22.46-33.78]	26.51 [20.46-31.87]	24.53 [18.94-30.5]	25.36 [20.16-30.05]
93	25,71 [15,78-33,68]	25.75 [20.18-31.21]	25.29 [16.51-32.46]	23.83 [15.24-31.36]	22.58 [16.29-28.15]	22.77 [13.8-28.69]
94	27,25 [22,13-33,68]	0.86 [0.05-2.9]	26.67 [22.04-32.8]	24.76 [19.46-30.39]	0.73 [0.07-2.53]	24.01 [19.51-28.72]

node	Tree prior: Yule			Tree prior: Birth-Death		
	Prior distr.: uniform	Prior distr.: exponential	Prior distr.: lognormal	Prior distr.: uniform	Prior distr.: exponential	Prior distr.: lognormal
95	20,48 [14,18-27,03]	20.19 [13.82-26.27]	20.14 [14.08-26.14]	18.42 [12.58-24.1]	17.18 [11.45-23.25]	17.83 [12.13-23.81]
96	13,85 [6,98-21,39]	14.15 [7.94-21.62]	13.82 [7.2-21.27]	12.6 [6-17.97]	12.12 [5.67-18.66]	12.52 [6.34-18.18]
97	18,36 [11,77-25,23]	17.82 [11.57-25.14]	18.11 [10.79-24.11]	16.51 [10.99-22.4]	15.36 [9.67-21.1]	15.78 [9.37-21.45]
98	8,14 [3,02-15,23]	7.84 [2.72-14.07]	8.02 [3.09-14.59]	7.12 [2.37-12.99]	6.64 [2.4-12.32]	6.77 [2.04-12.25]
99	26,27 [21,2-32,95]	26.14 [22.39-32.15]	25.58 [20.76-31.62]	23.7 [18.41-28.92]	23.21 [18.21-29.38]	22.84 [18.22-27.33]
100	0,86 [0,07-2,96]	25.27 [20.38-30.49]	0.83 [0.07-2.62]	0.79 [0.05-2.45]	21.87 [16.02-27.48]	0.7 [0.03-2.38]
101	22,43 [16,8-28,21]	22.09 [17.4-27.32]	21.97 [17.22-27.63]	20.64 [15.75-26.26]	19.04 [14.53-24.32]	19.44 [15.43-24.12]
102	21,2 [16,29-26,66]	20.71 [16.14-25.36]	20.7 [16.18-25.86]	19.49 [14.3-23.82]	17.93 [13.16-22.91]	18.38 [14.44-22.86]
103	14,58 [8,33-21,15]	14.37 [8.33-20.54]	14.28 [8.12-21.1]	13.07 [7.8-19.36]	11.97 [6.28-17.6]	12.52 [6.94-18.06]
104	18,28 [13,67-24,02]	18.06 [13.75-23.17]	17.91 [13.97-23.84]	16.89 [12.17-21.99]	15.58 [11.01-20.29]	15.53 [11.49-20.24]
105	15,32 [10,42-20,36]	15.18 [10.52-19.95]	15.16 [10.91-19.95]	14.18 [9.89-18.87]	12.94 [9.01-17.18]	13.01 [9.37-17.36]
106	11,95 [8,03-16,77]	11.98 [8.27-16.34]	11.92 [8-16.21]	11.19 [7.24-15.34]	10.16 [6.57-14.24]	10.27 [6.9-13.85]
107	8,9 [5,68-13,02]	8.91 [5.27-12.23]	8.85 [5.36-12.39]	8.05 [4.84-11.71]	7.4 [4.39-10.68]	7.43 [4.93-10.6]
108	7,01 [4,41-10,69]	6.94 [4.3-10.02]	6.95 [4.15-10.18]	6.3 [3.5-9.4]	5.73 [3.33-8.64]	5.72 [3.62-8.38]
109	5,35 [3,04-8,26]	5.28 [2.75-8.09]	5.32 [2.99-8.38]	4.81 [2.43-7.51]	4.31 [2.3-6.97]	4.4 [2.5-6.7]
110	4,72 [2,02-7,54]	1.27 [0.23-3.04]	4.83 [2.49-7.67]	1.24 [0.28-2.74]	3.93 [2.02-6.17]	4.03 [2.33-6.27]
111	1,37 [0,32-3,12]	4.77 [2.42-7.76]	1.27 [0.33-3.03]	4.23 [2.18-6.94]	1.12 [0.29-2.7]	1.09 [0.21-2.47]
112	27 [21,25-32,59]	25.69 [21.35-31.27]	25.55 [20.37-31.59]	23.96 [18.48-29.51]	22.21 [15.79-27.89]	22.65 [18.45-27.6]
113	3,5 [0,53-9,36]	24.65 [19.66-29.79]	24.44 [18.64-30.46]	21.69 [16.28-27.65]	2.77 [0.47-7.41]	2.71 [0.41-6.46]
114	25,87 [21,28-31,88]	3.29 [0.74-8.73]	3.27 [0.61-8.36]	2.75 [0.43-7.74]	21.83 [15.49-26.19]	22.01 [17.53-27.19]
115	24,62 [17,93-31,36]	23.39 [17.59-29.52]	23.42 [17.3-28.97]	20.37 [14.38-26.76]	19.86 [14.1-27.01]	20.62 [15.61-26]
116	3,26 [0,51-9,71]	3.05 [0.43-8.93]	3.07 [0.4-9.11]	10.47 [4.4-16.99]	2.46 [0.27-7.56]	2.63 [0.37-7.41]
117	23,08 [15,51-29,47]	22.09 [16.06-27.97]	21.98 [15.65-28.19]	22.95 [17.4-28.37]	18.76 [11.93-25.26]	19.37 [14.68-25.72]
118	11,43 [4,97-18,38]	11.1 [5.19-17.72]	11.43 [5.09-18.2]	2.79 [0.53-6.96]	9.57 [4.18-16.08]	9.84 [4.75-15.65]
119	25,6 [20,73-31,89]	24.35 [19.45-29.38]	24.11 [19.59-30.58]	22.51 [16.59-27.7]	20.97 [14.92-26.17]	21.18 [17.23-26.48]
120	24,3 [19,05-30,16]	23.35 [18.37-27.91]	22.99 [18.29-28.92]	21.33 [15.64-26.33]	19.88 [14.22-24.77]	20.26 [15.94-25.15]
121	20,37 [14,8-26,36]	19.56 [14.35-24.44]	22.04 [17.13-27.64]	17.53 [13.01-22.6]	18.81 [14.05-23.87]	16.87 [11.87-20.91]
122	14,42 [7,25-20,87]	15.9 [11.44-20.44]	0.2 [0-1.27]	5.43 [2.59-9.4]	0.19 [0-0.95]	13.5 [9.53-17.55]
123	4,29 [1,65-8,25]	14.52 [10.06-19.09]	17.97 [13.56-22.34]	3.39 [1.17-6.09]	15.02 [10.51-20.03]	12.37 [8.48-16.59]
124	1,91 [0,44-4,31]	11.28 [6.51-16.42]	0.42 [0.02-1.7]	1.67 [0.25-4.7]	0.38 [0.01-1.28]	9.52 [5.49-13.98]
125	2,03 [0,56-4,55]	2.35 [0.47-5.73]	0.13 [0-0.77]	1.72 [0.41-3.81]	0.11 [0-0.58]	1.9 [0.42-4.72]
126	0,99 [0,05-2,81]	6.2 [3.19-9.59]	15.11 [11.7-19.19]	1.16 [0.13-3.06]	12.59 [8.78-16.52]	5.28 [2.62-8.05]
127	19,08 [14,16-24,28]	5.44 [2.62-8.21]	12.02 [8.37-15.68]	3.71 [1.8-6.49]	9.89 [6.54-13.05]	4.52 [2.38-6.89]
128	6,51 [3,06-11,93]	4.83 [2-7.52]	8.9 [6.24-11.88]	1.78 [0.84-3.22]	7.2 [4.81-9.74]	4.19 [2.12-6.84]
129	1,97 [0,2-5,39]	3.5 [1.16-6.4]	6.8 [3.45-10.32]	0.66 [0.06-1.58]	5.39 [2.92-8.13]	2.96 [1.18-5.62]
130	4,67 [2,3-7,51]	11.05 [6.69-15.49]	5.43 [2.46-8.73]	1.12 [0.39-2.12]	4.08 [1.72-6.95]	9.01 [5.65-12.47]
131	2,06 [0,48-4,62]	8.47 [4.12-12.42]	2.87 [0.71-6]	0.69 [0.16-1.49]	2.44 [0.71-5.01]	6.96 [3.88-10.82]
132	1,26 [0,16-3,41]	6 [2.5-10.15]	7.16 [4.86-10.25]	0.44 [0.04-1.17]	5.81 [3.56-8.12]	4.93 [2.21-8.87]
133	4,27 [2,26-7,89]	10.02 [5.91-14.44]	5.4 [2.9-7.97]	16.93 [12.56-21.8]	4.42 [2.22-6.53]	8.32 [5.11-11.95]
134	2,12 [0,88-3,75]	1.81 [0.36-4.59]	3.34 [1.54-6.25]	12.14 [6.37-18.74]	2.82 [1.04-4.76]	7.5 [4.26-10.85]
135	0,77 [0,07-1,88]	0.29 [0-1.64]	5.44 [3.18-7.92]	3.61 [1.38-7.04]	4.41 [2.51-6.61]	0.42 [0.01-1.54]
136	1,3 [0,46-2,57]	9.09 [4.83-13.07]	2.39 [0.9-4.28]	1.63 [0.42-3.52]	1.92 [0.79-3.54]	1.42 [0.3-3.59]
137	0,81 [0,12-1,59]	0.5 [0.01-1.86]	1.91 [0.19-5.32]	1.76 [0.42-3.71]	1.63 [0.22-4.37]	0.28 [0-1.32]
138	0,47 [0,05-1,24]	17.66 [12.17-23.88]	0.2 [0-1.15]	1.1 [0.21-2.6]	0.16 [0-0.92]	15.17 [10.43-20.18]
139	16,74 [12,04-22,11]	13.73 [7.48-20.69]	13.7 [10.16-17.69]	14.37 [9.89-18.55]	11.37 [7.65-15.32]	11.96 [6.38-17.6]
140	15,11 [10,53-20,46]	4.28 [1.85-7.96]	12.45 [8.23-16.99]	13.23 [8.86-17.76]	10.06 [5.46-14.31]	3.57 [1.43-6.36]
141	11,84 [6,57-19,28]	1.94 [0.37-3.98]	2.26 [0.33-6.26]	10.15 [5.42-15.1]	1.97 [0.32-4.87]	1.58 [0.33-3.28]

node	Tree prior: Yule			Tree prior: Birth-Death		
	Prior distr.: uniform	Prior distr.: exponential	Prior distr.: lognormal	Prior distr.: uniform	Prior distr.: exponential	Prior distr.: lognormal
142	2,36 [0,43-6,1]	1.92 [0.45-4.2]	11.5 [8.74-15.32]	2.05 [0.35-4.94]	9.54 [6.46-13.12]	1.66 [0.43-3.63]
143	6,33 [3,59-10,37]	1 [0.12-2.71]	10.64 [7.99-14.39]	5.38 [2.82-8.04]	9.03 [5.35-12.47]	0.76 [0.1-2.21]
144	5,51 [2,77-8,76]	6.28 [3.08-11.24]	0.84 [0.02-2.63]	4.63 [2.41-7.25]	0.73 [0.05-2.25]	5.06 [2.61-9.12]
145	4,9 [2,43-8,03]	1.89 [0.15-5.52]	7.81 [4.24-11.43]	4.11 [1.96-6.4]	6.39 [3.26-9.8]	1.68 [0.21-4.34]
146	3,55 [1,41-6,95]	4.55 [2.49-7.62]	2.99 [0.62-5.75]	2.99 [1.04-5.41]	2.44 [0.73-4.97]	3.77 [2.12-6.18]
147	11,7 [7,02-15,89]	2.06 [0.47-4.5]	9.96 [6.71-13.28]	9.81 [6.28-13.68]	8.27 [5.07-11.75]	1.73 [0.39-3.8]
148	8,85 [4,59-13,51]	1.19 [0.12-3.16]	9.15 [5.64-12.28]	7.51 [3.6-11.4]	7.98 [4.31-10.85]	1.01 [0.13-3.11]
149	6,43 [2,11-10,3]	4.28 [2.13-7.54]	6.02 [2.99-9.15]	5.37 [1.98-9.28]	4.86 [2.68-8.14]	3.53 [1.61-6.01]
150	10,7 [6,67-15,6]	2.02 [0.82-3.86]	3.22 [0.94-5.86]	9.07 [5.74-13.33]	2.6 [1.05-5.37]	1.69 [0.69-2.99]
151	9,74 [5,19-14,49]	0.75 [0.15-1.86]	0.24 [0-1.3]	1.62 [0.41-4.07]	0.2 [0-1.08]	0.63 [0.14-1.57]
152	0,5 [0,01-1,93]	1.3 [0.43-2.43]	1.56 [0.27-3.84]	0.28 [0-1.41]	1.32 [0.21-3.19]	1.1 [0.34-2.08]
153	1,88 [0,47-4,61]	0.78 [0.17-1.76]	0.77 [0.11-1.93]	8.15 [5.02-12.77]	0.8 [0.1-1.9]	0.67 [0.16-1.45]
154	0,33 [0-1,83]	0.44 [0.03-1.24]	0.38 [0.02-1.18]	0.5 [0.02-1.87]	0.31 [0-1.04]	0.39 [0.02-1.16]
155	23,24 [18,33-29,51]	22.46 [17.35-27.54]	19.29 [13.92-24.15]	20.36 [15.21-25.1]	16.57 [11.94-21.22]	19.28 [15.26-23.86]
156	0,21 [0-1,27]	0.22 [0-1.36]	13.57 [6.67-20.12]	0.21 [0-1.14]	5.31 [2.43-9.42]	0.18 [0-1]
157	18,48 [13,8-23,86]	18 [13.83-22.21]	4.19 [1.64-7.85]	15.75 [11.59-20.43]	1.61 [0.22-4.49]	15.19 [11.79-18.88]
158	0,47 [0,01-1,76]	0.48 [0.01-1.59]	1.85 [0.52-4.15]	0.39 [0.01-1.36]	3.91 [2.11-6.65]	0.37 [0.01-1.3]
159	0,15 [0-0,72]	0.14 [0-0.74]	2 [0.43-4.44]	0.13 [0-0.62]	1.62 [0.34-3.76]	0.12 [0-0.56]
160	15,69 [11,61-20,09]	15.31 [11.78-19.28]	0.96 [0.06-2.84]	13.06 [9.64-17.13]	0.95 [0.19-2.78]	12.63 [9.83-16.11]
161	12,47 [8,29-16,57]	12 [8.73-15.71]	18.05 [14.48-23.62]	10.36 [7.42-14.08]	3.69 [1.59-6.36]	10 [7.28-12.93]
162	9,1 [5,83-12,33]	8.76 [6.3-12.01]	6.11 [3.15-11.05]	7.59 [5.32-10.7]	1.7 [0.76-3.14]	7.21 [4.98-9.81]
163	6,92 [3,65-10,58]	6.71 [3.75-10.37]	4.36 [2.11-8.07]	5.82 [3.12-8.9]	0.66 [0.07-1.55]	5.53 [3-8.13]
164	5,49 [2,31-9,29]	5.41 [2.53-8.63]	1.95 [0.28-5.36]	2.67 [0.72-5.11]	1.08 [0.36-1.98]	4.61 [2.21-7.6]
165	3,02 [0,75-6,36]	2.99 [0.93-5.93]	2.11 [0.43-4.4]	4.57 [1.72-7.52]	0.68 [0.09-1.42]	2.31 [0.57-4.65]
166	7,38 [4,55-10,32]	6.92 [4.44-9.68]	1.22 [0.19-3.27]	6.08 [4.16-8.53]	0.34 [0.02-0.94]	5.75 [3.78-7.88]
167	5,5 [3,08-8,7]	5.28 [2.78-8.3]	4.2 [1.8-7.34]	4.57 [2.47-6.91]	15.76 [11.07-20.15]	4.38 [2.43-6.7]
168	3,4 [1,55-6,16]	3.39 [1.37-5.83]	2.05 [0.82-3.59]	2.89 [0.87-4.86]	14.92 [11.15-19.42]	2.78 [0.93-4.72]
169	5,58 [2,88-8,31]	5.32 [3.06-7.93]	0.79 [0.11-1.82]	4.71 [2.81-6.78]	3.57 [1.48-6.88]	4.44 [2.71-6.55]
170	2,5 [0,92-4,36]	2.26 [0.96-4.28]	1.26 [0.44-2.61]	2.06 [0.83-3.6]	1.61 [0.49-3.57]	1.93 [0.84-3.43]
171	1,79 [0,19-5,29]	1.83 [0.18-5.16]	0.82 [0.21-1.86]	1.65 [0.21-4.68]	1.75 [0.47-3.78]	1.6 [0.17-4.22]
172	0,19 [0-1,07]	0.17 [0-1.1]	0.46 [0.03-1.31]	0.17 [0-0.93]	0.84 [0.11-2.14]	0.17 [0-1.08]
173	14,33 [10,47-18,49]	13.75 [10.2-17.49]	15.58 [11.56-20.82]	11.79 [8.45-15.18]	13.35 [9.75-17.69]	11.48 [8.39-14.79]
174	13,8 [10,73-18,83]	13.2 [9.82-16.94]	14.27 [10.14-19.51]	10.53 [5.32-14.02]	12.09 [8.45-16.15]	10.84 [7.91-14.17]
175	2,29 [0,23-6,11]	2.22 [0.33-5.97]	11.02 [5.88-16.44]	1.94 [0.27-5.26]	9.44 [5.39-13.98]	1.94 [0.23-4.84]
176	12,05 [8,38-15,98]	11.65 [8.38-15.17]	2.32 [0.54-5.62]	9.92 [6.93-12.61]	2.03 [0.4-5.1]	9.49 [6.62-12.96]
177	8,17 [4,27-12,06]	8.01 [4.45-11.59]	6.14 [3.62-9.95]	9.41 [6.57-12.38]	5.28 [2.77-8.47]	8.85 [5.67-11.97]
178	3,19 [0,92-6,47]	3.1 [0.85-6.14]	5 [2.2-7.93]	0.75 [0.03-2.4]	4.62 [2.53-7.57]	0.72 [0.04-2.13]
179	11,33 [7,94-15,45]	11.05 [7.78-14.49]	4.57 [2.53-7.57]	6.74 [3.92-9.67]	3.97 [2.19-7.3]	6.52 [3.62-9.87]
180	0,89 [0,03-3,01]	0.86 [0.1-3.01]	3.33 [1.38-6.23]	2.51 [0.94-5.13]	2.92 [1.05-5.41]	2.67 [0.76-5.08]
181	10,45 [6,69-13,86]	10.06 [6.89-13.98]	10.67 [6.54-15.02]	8.61 [5.96-11.67]	9 [5.27-12.96]	8.12 [5.17-11.65]
182	9,85 [6,46-13,82]	9.23 [5.9-12.52]	8.19 [4.79-13.12]	8.1 [5.36-11.02]	6.95 [3.33-10.7]	7.56 [4.71-10.8]
183	6,18 [2,63-10,11]	5.95 [2.92-9.57]	5.88 [2.21-10.22]	5.13 [2.75-8.48]	4.89 [2-8.59]	4.89 [2.17-7.64]
184	3,39 [1,35-6,52]	3.15 [0.85-6.07]	9.76 [5.63-14.08]	2.76 [0.98-5.42]	8.26 [4.85-12.16]	2.59 [0.96-5.09]
185	0,25 [0-1,32]	0.24 [0-1.36]	9.08 [4.93-13.09]	0.22 [0-1.3]	0.46 [0.02-1.53]	0.2 [0-1.11]
186	1,6 [0,26-3,83]	1.57 [0.42-3.9]	0.5 [0.02-1.66]	1.33 [0.34-3.2]	7.42 [4.45-11.15]	1.3 [0.21-3.02]
187	0,89 [0,15-2,61]	0.89 [0.17-2.14]	1.66 [0.3-4.11]	0.55 [0.02-1.72]	1.48 [0.39-3.57]	0.66 [0.16-1.58]
188	0,34 [0,01-1,25]	0.35 [0-1.25]	0.33 [0-1.73]	0.29 [0.02-1]	0.26 [0-1.2]	0.29 [0-1]

Appendix 2.L: Median rates of speciation (λ), extinction (μ) and character transition (q) as well as the net effect of diversification for modified sterile flowers under all models.

Median rate values were calculated across 100 post burn-in MCMC trees of the posterior tree distribution as inferred during the estimation of divergence times. Each median value is followed by the interquartile range in square brackets. Abbreviations used: Trans. rate = character transition rate.

Model Specification				Parameterization						Net effect
	Speciation (λ)	**Extinction (μ)**	**Trans. rate (q)**	**λ0**	**λ1**	**μ0**	**μ1**	**q01**	**q10**	**(λ1-μ1)-(λ0-μ0)**
BiSSE Model set 1	free	μ0=μ1	free	NaN	NaN	NaN	NaN	NaN	NaN	NaN
	free	free	q01=q10	0.27 [0.24,0.33]	0.98 [0.82,1.16]	0.09 [0.01,0.14]	0.88 [0.71,1.09]	0.05 [0.03,0.06]	0.05 [0.03,0.06]	-0.08 [-0.13,-0.06]
	λ0=λ1	free	free	NaN	NaN	NaN	NaN	NaN	NaN	NaN
	free	μ0=μ1	q01=q10	0.41 [0.37,0.45]	0.44 [0.41,0.47]	0.25 [0.2,0.31]	0.25 [0.2,0.31]	0.03 [0.03,0.04]	0.03 [0.03,0.04]	0.02 [0.01,0.05]
	λ0=λ1	free	q01=q10	0.43 [0.4,0.45]	0.43 [0.4,0.45]	0.26 [0.23,0.31]	0.29 [0.23,0.35]	0.04 [0.03,0.04]	0.04 [0.03,0.04]	-0.03 [-0.04,-0.01]
	λ0=λ1	μ0=μ1	free	0.43 [0.4,0.45]	0.43 [0.4,0.45]	0.27 [0.24,0.32]	0.27 [0.24,0.32]	0.02 [0.01,0.03]	0.07 [0.03,0.08]	0 [0,0]
	λ0=λ1	μ0=μ1	q01=q10	0.43 [0.4,0.45]	0.43 [0.4,0.45]	0.27 [0.24,0.32]	0.27 [0.24,0.32]	0.03 [0.03,0.04]	0.03 [0.03,0.04]	0 [0,0]
BiSSE Model set 2	λ0=0	free	free	NaN	NaN	NaN	NaN	NaN	NaN	NaN
	free	μ0=0	free	NaN	NaN	NaN	NaN	NaN	NaN	NaN
	free	μ1=0	free	0.3 [0.27,0.33]	0.31 [0.28,0.33]	0.12 [0.08,0.17]	0 [0,0]	0.02 [0.01,0.02]	0.05 [0.04,0.1]	0.14 [0.11,0.16]
	free	free	q01=0	0.34 [0.31,0.38]	0.72 [0.65,0.8]	0.23 [0.19,0.3]	0.59 [0.5,0.68]	0 [0,0]	0.06 [0.05,0.07]	0.03 [0.01,0.05]
	free	free	q10=0	0.29 [0.28,0.31]	1.01 [0.84,1.2]	0 [0,0.01]	1.01 [0.83,1.23]	0.11 [0.11,0.13]	0 [0,0]	-0.28 [-0.33,-0.26]
	λ0=0	μ0=0	free	0 [0,0]	1.1 [0.99,1.22]	0 [0,0]	0.7 [0.6,0.84]	0.52 [0.45,0.78]	0.97 [0.87,1.42]	0.39 [0.34,0.44]
	λ0=0	μ1=0	free	0 [0,0]	0.77 [0.73,0.85]	0.22 [0.16,0.29]	0 [0,0]	0.36 [0.32,0.64]	1.28 [1.1,1.58]	0.97 [0.91,1.1]
	λ0=0	free	q01=0	0 [0,0]	0.71 [0.69,0.75]	0.67 [0.6,0.72]	0 [0,0]	0 [0,0]	0.66 [0.64,0.7]	1.38 [1.31,1.49]
	free	μ0=0	q01=0	0.22 [0.21,0.24]	0.67 [0.61,0.76]	0 [0,0]	0.56 [0.47,0.64]	0 [0,0]	0.03 [0.03,0.04]	-0.1 [-0.12,-0.09]
	free	μ0=0	q10=0	0.29 [0.27,0.3]	1.02 [0.86,1.2]	0 [0,0]	1.03 [0.85,1.24]	0.12 [0.11,0.14]	0 [0,0]	-0.29 [-0.33,-0.27]
	free	μ1=0	q01=0	0.37 [0.35,0.43]	0.3 [0.28,0.33]	0.28 [0.23,0.37]	0 [0,0]	0 [0,0]	0.15 [0.14,0.17]	0.22 [0.18,0.26]
	free	μ1=0	q10=0	0.26 [0.25,0.29]	0.27 [0.24,0.31]	0.04 [0,0.09]	0 [0,0]	0.03 [0.03,0.04]	0 [0,0]	0.06 [0.04,0.08]

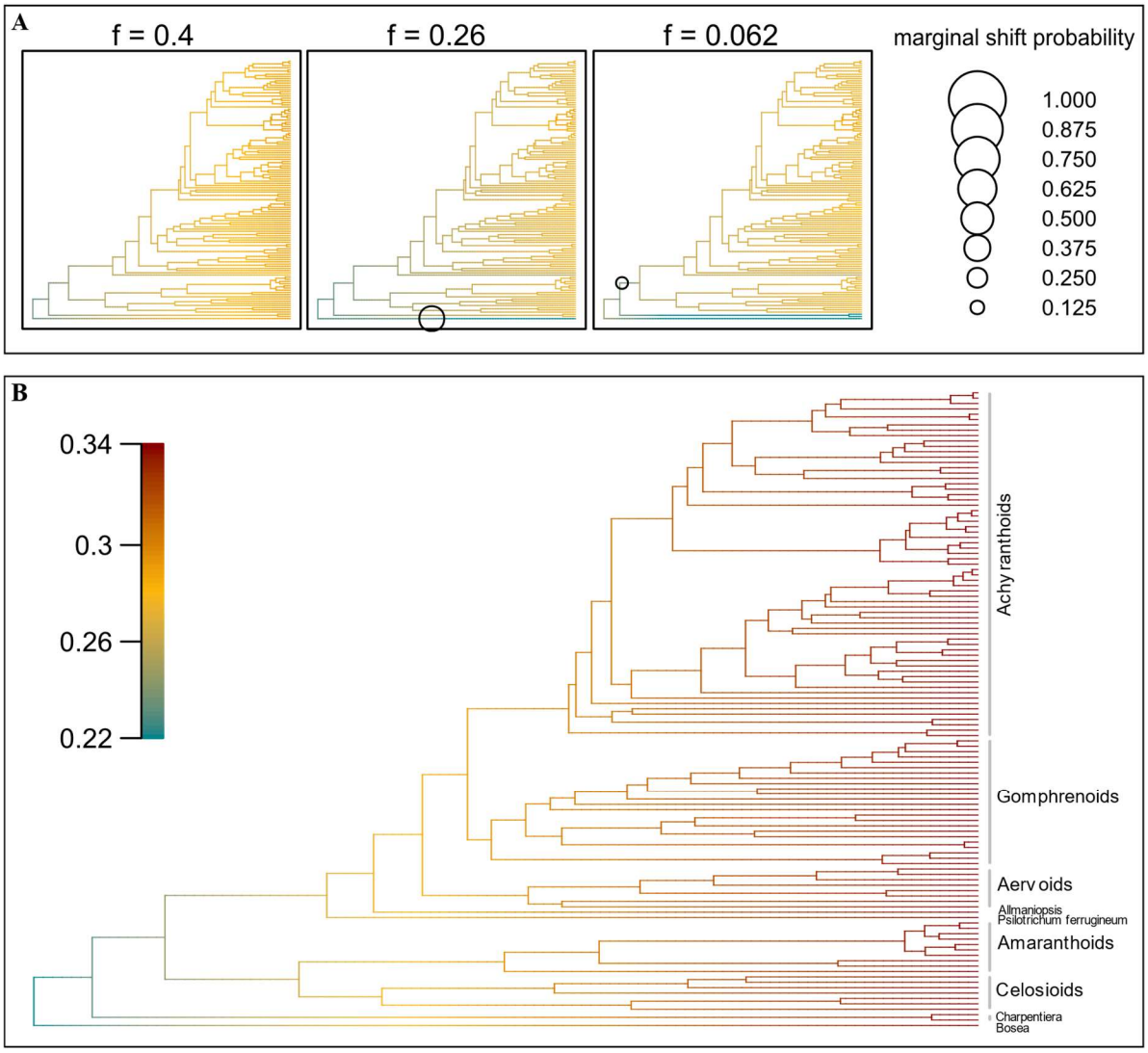
Appendix 2.M: Median rates of speciation (λ), extinction (μ) and character transition (q) as well as the net effect of diversification for adhesive flower appendages under all models.

Median rate values were calculated across 100 post burn-in MCMC trees of the posterior tree distribution as inferred during the estimation of divergence times. Each median value is followed by the interquartile range in square brackets. Abbreviations used: Trans. rate = character transition rate.

Model Specification				Parameterization						Net effect
	Speciation (λ)	**Extinction (μ)**	**Trans. rate (q)**	**λ0**	**λ1**	**μ0**	**μ1**	**q01**	**q10**	**(λ1-μ1)-(λ0-μ0)**
	Unconstrained – all rates free			0.14 [0.12,0.16]	0.44 [0.42,0.5]	0 [0,0.06]	0.27 [0.22,0.33]	0.04 [0.03,0.05]	0.04 [0.04,0.05]	0.07 [0.04,0.08]
BiSSE Model set 1	Free	μ0=μ1	Free	0.08 [0.02,0.14]	0.21 [0.13,0.26]	0.09 [0.05,0.11]	0.15 [0.11,0.21]	0.05 [0.04,0.06]	0.04 [0.03,0.05]	0.07 [0.03,0.08]
	Free	Free	q01=q10	0.04 [0.01,0.07]	0.26 [0.25,0.26]	0.12 [0.01,0.22]	0.09 [0.02,0.19]	0.06 [0.02,0.1]	0.07 [0.02,0.13]	0.25 [0.24,0.31]
	λ0=λ1	Free	Free	0.13 [0.03,22]	0.18 [0.12,0.24]	0 [0,0.10]	0.03 [0.01,0.04]	0.06 [0.04,0.08]	0.06 [0.05,0.06]	0.02 [0,0.04]
	Free	μ0=μ1	q01=q10	0.19 [0.16,0.2]	0.32 [0.3,0.34]	0.09 [0.04,0.13]	0.09 [0.04,0.13]	0.06 [0.05,0.06]	0.06 [0.05,0.06]	0.13 [0.12,0.14]
	λ0=λ1	Free	q01=q10	0.27 [0.26,0.29]	0.27 [0.26,0.29]	0.15 [0.11,0.2]	0.06 [0.02,0.1]	0.06 [0.05,0.06]	0.06 [0.05,0.06]	0.09 [0.08,0.1]
	λ0=λ1	μ0=μ1	Free	0.27 [0.26,0.29]	0.27 [0.26,0.29]	0.09 [0.05,0.13]	0.09 [0.05,0.13]	0.03 [0.03,0.04]	0.05 [0.04,0.05]	0 [0,0]
	λ0=λ1	μ0=μ1	q01=q10	0.27 [0.26,0.29]	0.27 [0.26,0.29]	0.09 [0.05,0.13]	0.09 [0.05,0.13]	0.05 [0.04,0.05]	0.05 [0.04,0.05]	0 [0,0]
BiSSE Model set 2	λ0=0	Free	Free	0.08 [0.01,0.15]	0.45 [0.42,0.50]	0 [0,0.08]	0.21 [0.13,0.26]	0.52 [0.40,0.64]	0.35 [0.29,0.40]	0.16 [0.14,0.18]
	Free	μ0=0	Free	0.04 [0.02,0.06]	0.39 [0.28,0.44]	0.02 [0.01,0.03]	0.22 [0.14,0.24]	0.36 [0.33,0.39]	0.37 [0.30,0.42]	0.15 [0.11,0.19]
	Free	μ1=0	Free	0.13 [0.03,22]	0.34 [0.31,0.37]	0 [0,0.15]	0.03 [0.03,0.04]	0.48 [0.35,0.66]	0.29 [0.28,0.31]	0.18 [0.12,0.24]
	Free	Free	q01=0	0.15 [0.13,0.18]	0.4 [0.37,0.45]	0.12 [0.08,0.16]	0.21 [0.15,0.25]	0 [0,0]	0.05 [0.05,0.06]	0.16 [0.14,0.18]
	Free	Free	q10=0	0.26 [0.25,0.28]	0.49 [0.45,0.54]	0 [0,0]	0.43 [0.38,0.51]	0.17 [0.15,0.18]	0 [0,0]	-0.22 [-0.24,-0.18]
	λ0=0	μ0=0	Free	0 [0,0]	0.46 [0.41,0.51]	0 [0,0]	0.21 [0.12,0.26]	0.52 [0.39,0.66]	0.35 [0.29,0.41]	0.25 [0.24,0.31]
	λ0=0	μ1=0	Free	0 [0,0]	0.35 [0.33,0.37]	0 [0,0.11]	0 [0,0]	0.47 [0.36,0.65]	0.38 [0.31,0.44]	0.36 [0.33,0.47]
	λ0=0	Free	q01=0	0 [0,0]	0.39 [0.37,0.41]	0.65 [0.53,0.72]	0 [0,0]	0 [0,0]	0.27 [0.24,0.29]	1.01 [0.92,1.14]
	Free	μ0=0	q01=0	0.11 [0.1,0.12]	0.39 [0.37,0.42]	0 [0,0]	0.2 [0.15,0.24]	0 [0,0]	0.04 [0.03,0.04]	0.08 [0.07,0.1]
	Free	μ0=0	q10=0	0.26 [0.25,0.28]	0.49 [0.45,0.54]	0 [0,0]	0.43 [0.38,0.51]	0.17 [0.15,0.18]	0 [0,0]	-0.22 [-0.24,-0.19]
	Free	μ1=0	q01=0	0.16 [0.14,0.19]	0.27 [0.25,0.28]	0.12 [0.08,0.17]	0 [0,0]	0 [0,0]	0.07 [0.06,0.08]	0.23 [0.21,0.26]
	Free	μ1=0	q10=0	0.2 [0.19,0.22]	0.24 [0.22,0.26]	0 [0,0]	0 [0,0]	0.08 [0.08,0.09]	0 [0,0]	0.04 [0.02,0.05]

Appendix 2.N: Shift configurations of the BAMM analyses.

A: The shift configurations with the highest posterior probabilities out of the 95% credible set of distinct shift configurations. The plots display model-averaged mean rate parameters across all samples of a given configuration. **B:** Phylorate plot of the single best overall shift configuration taken from the 95% credible set of distinct shift configurations. In both plots, only speciation rates are illustrated.



– Chapter 3 – Pollen morphology of the achyranthoid clade (Amaranthaceae)

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3.1 Introduction

Pollen in Amaranthaceae is known to be diverse and informative at different taxonomic levels from several studies which mainly focused on the description and classification of pollen types (e.g., Erdtman 1952; Vishnu-Mittre, 1963; Eliasson 1988; Townsend 1993; Nowicke, 1975, 1994; Borsch 1998; Borsch & Barthlott 1998; Müller & Borsch 2005b). Erdmann (1952) described two major pollen types: the *Amaranthus* type as characteristic for the Amaranthoideae and the the *Gomphrena* type as characteristic for Gomphrenoideae, whereas Vishnu-Mittre (1963) distinguished nine pollen types within Indian Amaranthaceae based on size and tectum characters. Nowicke (1975) described five pollen types within Centrospermae, out of which one type respectively covered the *Amaranthus* and the *Gomphrena* type earlier described by Erdtman. Borsch (1998) delimited 17 pollen types within the whole family which were either distinguished by a syndrome of characters or a single very distinctive character. According to Borsch (1998), the pollen grains of the genera which were shown to constitute the achyranthoid clade in Chapter 2, belong to the following nine pollen types: the *Arthraerua* type (11 genera), the *Centemopsis* type (2 genera), the *Chionothrix* type (1 genus), the *Deeringia* type (3 genera), the *Kyphocarpa* type (1 genus), the *Marcelliopsis* type (2 genera), the *Mechowia* type (1 genus), the *Psilotrichum* type (4 genera), and the *Sericorema* type (1 genus). The *Arthraerua* type was shown to be the most frequent among Achyranthoids.

Livingstone et al. (1973) detected stellate operculate extrusions from the pores in several Old World genera (e.g., *Psilotrichum, Pupalia* and *Centemopsis*), and referred to this characteristic as "stellate pore ornamentation". Within New World genera this characteristic was only observed in *Pseudoplantago*, which differs from the above-mentioned genera in its pyramidiform, prismatiform or cubiform pollen shape, which within Amaranthaceae is considered as unique (Townsend 1988). Müller and Borsch (2005b) focused on aperture characters in their Amaranthaceae pollen study and described stellate pore ornamentation as a complex character syndrome which is rare in angiosperm pollen and within Gomphrenoids they confirmed it to occur only in the genus *Pseudoplantago*, while it was observed in nine genera of the achyranthoid clade. Müller and Borsch (2005b) investigated six characters to reconstruct the evolution of stellate pore ornamentation and found clear evidence for several independent origins and reversals of this character complex within Achyranthoids. However, the underlying phylogeny was based on analyses of *trnK/matK* sequences only and therefore not fully resolved and lacking important achyranthoid species and genera. Furthermore, analyses focused on a few aperture characters only and do not depict a complete pollen morphological study.

Borsch et al. (2018) conducted a comprehensive study of the genus *Iresine* and allied genera, in which they also included three achyranthoid species as representatives of the achyranthoid clade (*Cyathula lanceolata*, *Pandiaka angustifolia*, *Psilotrichum scleranthum*). They reconstructed 15 pollen characters in order to characterize the *Iresine* clade.

With this chapter, I am aiming in complementing recent studies on Amaranthaceae pollen with a focus on the achyranthoid clade and therefore mainly the character definitions from Borsch et al. (2018) and partially also from Müller and Borsch (2005b) will be involved. Compared to Müller and Borsch (2005b) a broader sampling regarding both number of analyzed pollen characters and number of sampled achyranthoid taxa is applied here, and characters are reconstructed based on the better resolved phylogeny published in Chapter 2 (Di Vincenzo et al., 2018), which was gained from combined analyses of the chloroplast markers *trnK*/*matK*, *rpl16*, and *trnL-F*.

Since stellate pore ornamentation is characteristic for Achyranthoids and has already been analyzed in detail (Müller and Borsch, 2005b), within this chapter it will be possible due to the denser taxon sampling of achyranthoid genera and species to further locate the position of this character syndrome within the achyranthoid clade. However, the major aim is to find synapomorphies for the whole of Achyranthoids and internal lineages or discover descriptive pollen characters, in order to substantiate the molecular based phylogenies from Chapter 2 (Di Vincenzo et al., 2018) with pollen morphological characters.

3.2 Methods

3.2.1 Field work and taxon sampling

Analyses of the present chapter are based on the sample set presented in Chapter 2 (DiVincenzo et al., 2018) that was largely achieved by field work in Ethiopia and Kenya in 2012 and 2013. The data set was slightly modified in order to complement missing data and to remove multiple accessions of the same species. Only in cases of unclear species limits when individuals of a single taxon occur in different positions within the phylogeny, multiple accessions were kept in the data set. Sequences were chosen according to the availability of pollen material so that whenever possible the DNA samples and the pollen material was taken from the same individual. This is particularly important in cases of uncertain species limits, in which multiple accessions remained in the dataset.

Detailed information on the specimens examined for pollen morphology and included in the molecular trees is provided in Appendix 3.A in the end of this chapter.

3.2.2 Composition of molecular datasets

A combined plastid dataset (*trnK*/*matK*, *rpl16*, and *trnL-F*) was generated with a dense taxon sampling of the Achyranthoids of Amaranthaceae but including a representation of genera from their sister group Gomphrenoids and one outgroup from the Aervoids. Sequences from *trnK*/*matK*, *rpl16* and *trnL-F* were largely available from the previous chapter and only 15 sequences were newly sequenced for this chapter. Further species of *Pandiaka* were added, which were not analyzed in phylogenetic studies before, including the type species of the genus *P. involucrata*. Additionally, Cyathula *achyranthoides* was included which remained unsampled so far. For *Sericocomopsis hildebrandtii*, *Achyranthes aspera* and *Pupalia lappacea* several accessions were included in the dataset, as each of them represent a separated lineage.

3.2.3 Processing of DNA samples and generation of molecular datasets

DNA isolation and sequencing followed the same procedures as described in Chapter 1 (Di Vincenzo & al., 2018). Sanger sequencing was carried out at Macrogen Europe (Amsterdam, The Netherlands). Chromatograms were inspected by eye and corrected for erroneous nucleotide calls. Assembly and a motif-based alignment (as outlined in Löhne & Borsch, 2005) was conducted manually using PhyDE 0.9971 (Müller & al., 2010), building upon the multiple sequence alignments of Chapter 1 (Di Vincenzo & al., 2018). Areas of uncertain homology were excluded from matrices and inversions were reverse-complemented and coded as presence-absence data. For coding insertions and deletions, I applied 'simple indel coding' (Simmons & Ochoterena, 2000) as implemented in SeqState 1.4.1 (Müller, 2005) New DNA sequences from this chapter will be submitted to ENA (http://www.ebi.ac.uk/ena/) upon

conversion to checklist files with custom Python scripts (https://github.com/michaelgruenstaeudl/annonex2embl). ENA/GenBank accession numbers from already published sequences are given in Appendix 3.A along with the voucher data.

3.2.4 Model selection and phylogenetic analysis

The phylogeny was obtained with Bayesian Inference (BI) as a basis for the analyses of morphological characters. Since tree topologies inferred from BI, Maximum parsimony (MP) and Maximum likelihood (ML) analyses were already compared in the second chapter and were largely congruent within Achyranthoids, this comparison is not part of the present chapter. Preceding the analyses in MrBayes 3.2.7a (Ronquist et al., 2012) available from the CIPRES Science Gateway (Miller & al., 2010), the dataset was annotated and partitioned to allow for independent parameter estimation. Best-fitting models of nucleotide substitution were selected via the Akaike information criterion corrected for small sample sizes (AICc; Akaike, 1974) for each partition with ModelTest-NG v0.1.7 (Darriba & al., 2020), which is implemented in the software raxmlGUI 2.0.5 (Edler & al., 2021),. Results of model testing are summarized in Appendix 3.B.

Node support in MrBayes was calculated as posterior probabilities from four independent Markov chain Monte Carlo (MCMC) runs with four chains and 5,000,000 generations each. Trees were sampled every 1,000th generation resulting in 20,004 trees. Upon removal of a burn-in of 10 percent, resulting in a combined posterior tree distribution of 18,004 trees, the maximum clade credibility (MCC) tree was calculated. Convergence of each independent run was assessed based on the standard deviation of split frequencies and the effective sample size (ESS) using Tracer v. 1.7.2 (Rambaut & al., 2018).

3.2.5 Scanning electron microscopy

Air dried pollen from herbarium specimens was taken from different anthers of the same individual. Anthers were carefully removed without destruction of herbarium specimens and bagged in filter paper, soaked in dimethoxypropane for at least five days, then in acetone for three days and critical point dried (Halbritter, 1998). Afterwards, the dry pollen grains were placed on silicium platelets mounted on aluminum stubs. Pollen material was examined at the Botanical Garden and Botanical Museum Berlin with a Hitachi SU8070 microscope using cold field emission at 1 kV. Pollen grains were sputtered for 0.75 minutes only, to minimize artificial layer which might obscure pollen surfaces. Whenever sufficient material was available, identical individuals were used for both – DNA extraction and pollen analysis.

In the case of *Sericocomopsis hildebrandtii*, *Achyranthes aspera* and *Pupalia lappacea* pollen grains of multiple individuals were examined as each of them represent a different lineage in the tree. For the taxa of Gomphrenoids (representatives of the sister lineage of Achyranthoids) and Aervoids (outgroup), pollen morphological data was taken from Borsch et al. (2018). For some achyranthoid taxa pollen

morphological data was available from Müller & Borsch (2005b). Detailed voucher information is given in Appendix 3.A.

3.2.6 Assessment of pollen morphological characters and ancestral character state reconstruction (ACSR)

In order to reconstruct the evolution of pollen characters and find synapomorphies for clades within the Achyranthoids, I defined a set of 15 characters. These were mainly chosen according to Borsch et al. (2018). Their character 7 "tectum completeness" is not reconstructed here, due to the missing variation within Achyranthoids. The tectum of pollen grains in achyranthoid taxa is always complete punctate and punctae are evenly spread on mesoporia. This corresponds to state 0 in Borsch et al. (2018). Instead, here the homogeneity of tectum perforations is coded (char 7). Additionally, I included the character "Size of ektexinous bodies on aperture membrane" (char 15) from Müller & Borsch (2005b, their character 2). Where necessary, state categories were adjusted and/or added according to own character state observations. Definitions of pollen characters and their states are given in Appendix 3.C, the full character matrix is depicted in Appendix 3.D.

BayesTraits v. 2 (Pagel & al., 2004) was used to infer ancestral character states. ACSR was calculated over 500 random samples which were extracted from the post-burn in tree distribution of the Bayesian analyses using Mesquite v. 3.70 (Maddison & Maddison, 2021). BayesTraits input was generated with TreeGraph2 v. 2.14.0-771 (Stöver & Müller, 2010). I selected a multistate analysis under MCMC searches using the Reverse Jump Hyper-prior to analyze each character separately. The BayesTraits output log files were imported to TreeGraph2 where the relative probabilities of ancestral states were visualized on the MCC tree.

3.3 Results

3.3.1 Sequence datasets

The dataset included 55 samples and resulted in a multiple sequence alignment of 6.419 aligned positions including hotspots. The final matrix (excluding hotspots) comprised 5.328 aligned positions and simple indel coding yielded an additional matrix of 387 characters. For detailed alignment statistics compare Appendix 3.E.

3.3.2 Position of newly sequenced species in the phylogenetic tree

The topology of the phylogenetic tree inferred here from analyses in MrBayes was identical with the tree topology inferred from Bayesian analyses of the larger dataset A in the second chapter (Figure 2.B). Major lineages, as well as the majority of internal relationships were resolved with maximum support (compare Figure 3.C - Figure 3.E). For a detailed description and discussion of results concerning overall phylogenetic relationships of the achyranthoid clade and sister lineages compare the respective paragraphs of Chapter 2. The species *Pandiaka involucrata, P. lanuginosa* and *Cyathula achyranthoides* had not been included in molecular analyses before and all of them were found within subclade II of Achyranthoids. *Cyathula achyranthoides* was resolved as sister to *C. prostrata* with maximum support. Both together were resolved in a clade also containing *Pandiaka involucrata*, which is the type of *Pandiaka*, and *P. angustifolia*. The only species of *Pandiaka* which is found in a distant position and nested within species of *Achyranthes*, *Nototrichium*, *Achyropsis*, and *Centrostachys*, is *P. lanuginosa* (compare Figure 3.C - Figure 3.E). However, phylogenetic results are not further explained here, since this is not the scope of the present chapter and has already been done for a broader dataset in Chapter 2. For the here newly sequenced species, a detailed phylogenetic analyses and discussion will be carried out in the subsequent Chapter 4 which focuses on internal relationships of subclade II of Achyranthoids, their morphology, and taxonomy. In Chapter 4 I will then, among other questions closely examine the present phylogenetic implications by application of an extended marker set compared to Chapter 2 and the present chapter.

3.3.3 Distribution and ancestral states of pollen morphological characters

The taxa included in the achyranthoid clade of Amaranthaceae exhibit a great pollen grain diversity, which is depicted in Figure 3.A for chosen representatives of the clade.

Ancestral states of all 15 characters analyzed are compiled in Figure 3.C - Figure 3.E and separated figures are additionally given for each single character in the Appendix 3.F. The definition of characters and their states as well as the full matrix used for the reconstruction is given in Appendix 3.C and Appendix 3.D.

3.3.3.1 Pollen shape, size, and apertures

Ancestral state probabilities for characters concerning pollen shape, size, and apertures (chars 1-6) are depicted as pie charts, illustrated in Figure 3.C.

The spheroidal pollen shape (char 1, state 0) is common in Amaranthaceae and depicts the ancestral state for the whole achyranthoid clade, whereas dodecahedral pollen (char 1, state 1) only derived two times in the subclade of Achyranthoids I, comprising the genera *Sericocoma*, *Kyphocarpa*, *Sericorema*, and *Marcelliopsis*. The truncated icosahedral pollen shape (char 1, state 3; compare Figure 3.A, photo I and Figure 3.B) only derived once within Achyranthoids and depicts an autapomorphy for the genus *Centemopsis*. This pollen shape is not known for any other genus within Amaranthaceae.

Pollen size is variable even within single genera (e.g., *Pupalia*, *Sericocoma, Achyranthes*). However, most frequent within Achyranthoids is a pollen size ranging between 15 and 24 µm (char 2, state 1), as this was also inferred as the ancestral state for the whole clade as well as for the subclades I and II.

Aperture numbers within Achyranthoids are ranging from 6 in subclade I (char 3, state 0 in *Psilotrichum sericeum*) to numbers between 36 and 80 in the genera of subclade II (char 3, state 4, e.g., *Achyranthes*, *Cyathula*). As ancestral state an aperture number of 20-35 was inferred. The aperture diameter (char 4) is ranging from 1.3 up to 5.7 µm among Achyranthoids. The largest apertures with diameters of 4 - 5.7 µm (char 4, state 4) were found in Achyranthoids I, where they evolved several times.

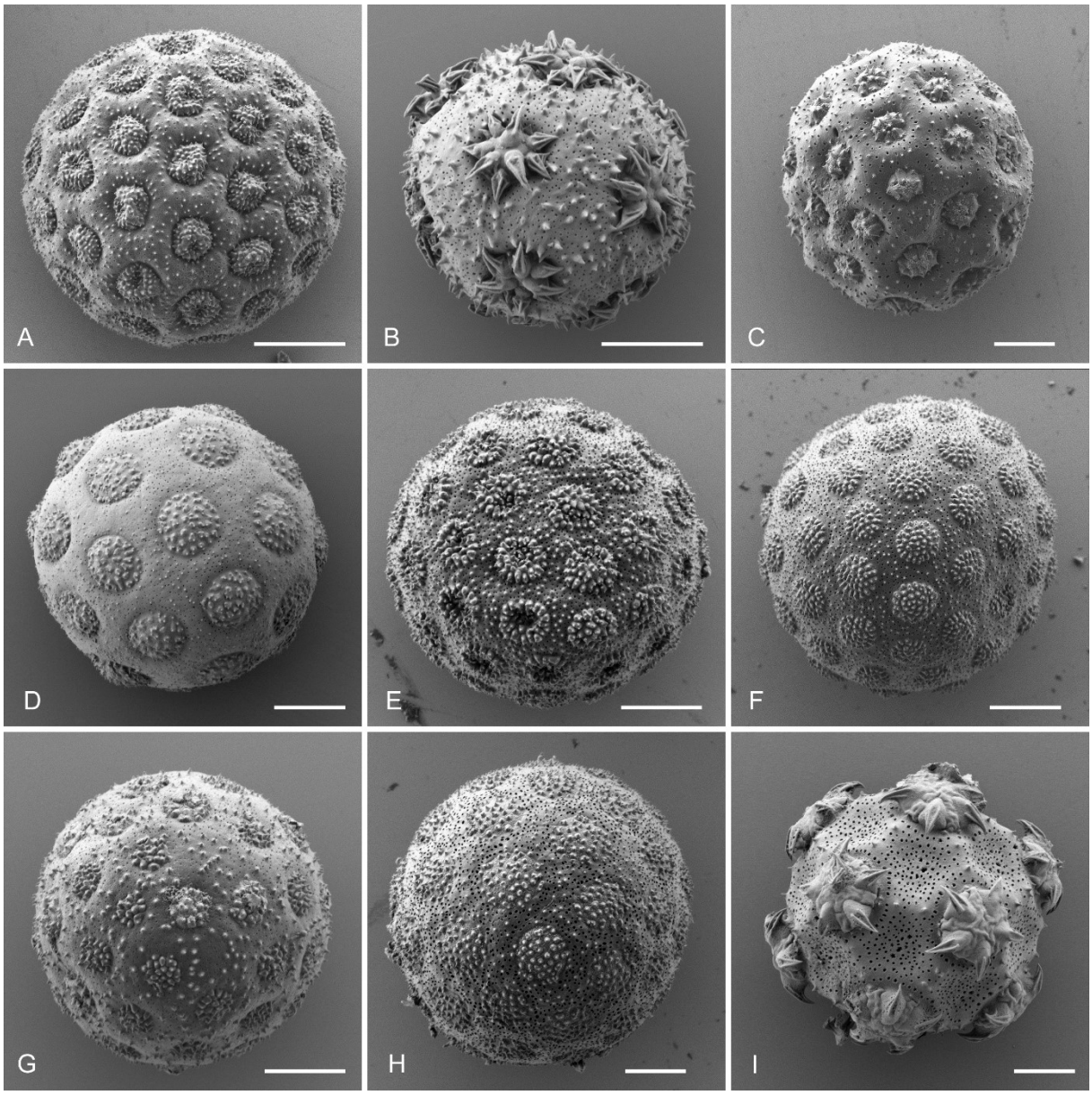

Figure 3.A: Pollen grain diversity within the achyranthoid clade of Amaranthaceae.
A: *Centrostachys aquatica* (Wondafrash *et al*. 4049, B); **B:** *Psilotrichum gnaphalobryum* (Wondafrash *et al*. 2195, B); **C:** *Lopriorea ruspolii* (Wondafrash *et al*. 3921, B); **D:** *Leucosphaera bainesii* (Walter 1044, B); **E:** *Cyathula orthacantha* (Wondafrash *et al*. 3461, B); **F:** *Cyathula cylindrica* (Di Vincenzo *et al*. 280, B); **G:** *Sericocomopsis pallida* (Wondafrash *et al*. 3926, B); **H:** *Sericocomopsis hildebrandtii* (Di Vincenzo *et al*. 339, B); **I:** *Centemopsis sp.* (Di Vincenzo *et al*. 318, B). White scale bars correspond to a length of 5 µm.

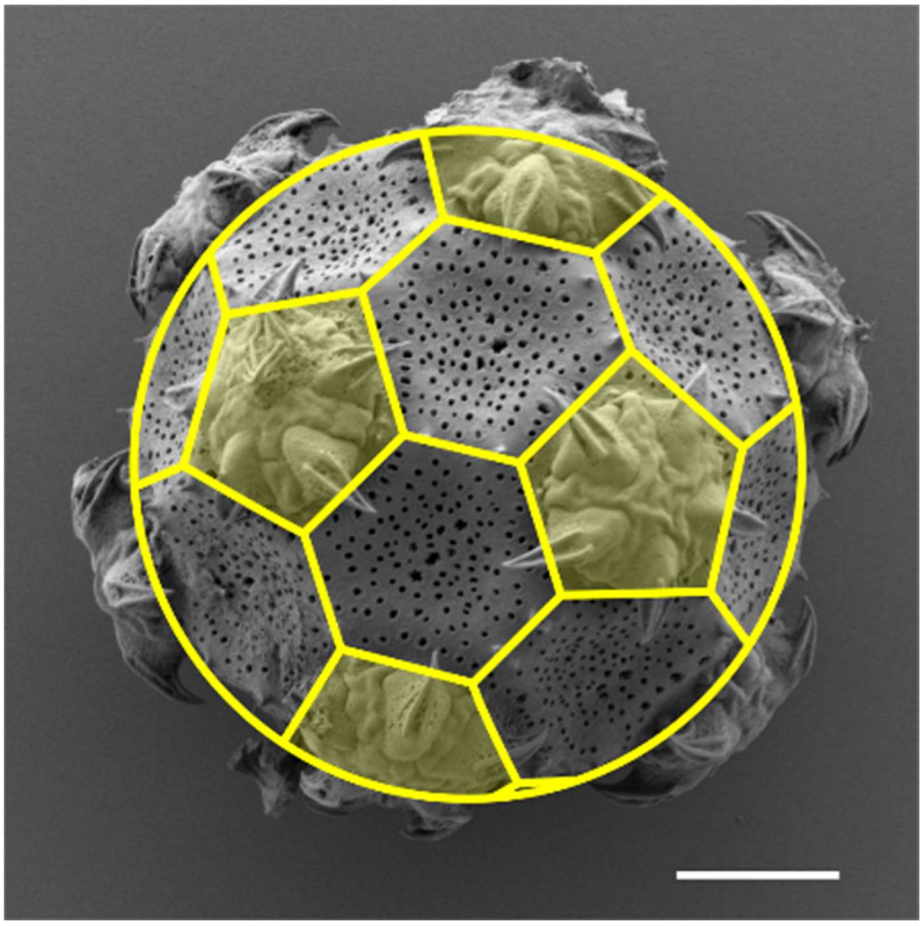

Figure 3.B: Unique pollen shape in the genus *Centemopsis*.
Pollen grain resembles the geometry of a football (represented by the yellow outlines). The shape is a truncated icosahedral, constituted of 12 pentagons (corresponding to the placement of apertures) and 20 hexagons (corresponding to the areas of mesoporia). *Pollen grain of Centemopsis sp.* (Di Vincenzo *et al*. 318, B). White scale bar corresponds to a length of 5 µm.

Whereas mesoporia is strongly vaulted in several genera of Gomphrenoids (compare char 5, state 1), within Achyranthoids this character state is limited to the genus *Kyphocarpa* of subclade I. The majority of Achyranthoids bear flat or slightly vaulted mesoporia (char 5, state 0), and this state is also inferred as ancestral for the whole clade. For *Centemopsis* a unique shape of mesoporia was observed as autapomorphic: Mesoporia are pentagonal protruding around pores, which thus appear uplifted, and between the pores mesoporia is vaulted inwards so that pores appear surrounded by 5 irregular concave hexagons each (compare Figure 3.A, photo I and Figure 3.B). This character state is not known from any other taxon within Amaranthaceae. The diameter of mesoporia was inferred to range between 2 and 3 µm in the ancestor of Achyranthoids and can reach diameters of up to 5.5 µm within several genera of subclade I. In subclade II mesoporia diameter was reduced to 1.1 - 2 µm in the most recent common ancestor (MRCA) and all other taxa, followed by a further reduction to 0.3-1.09 µm in the lineage including *Sericocomopsis hildebrandtii*, *Cyathula cylindrica* and their stem node.

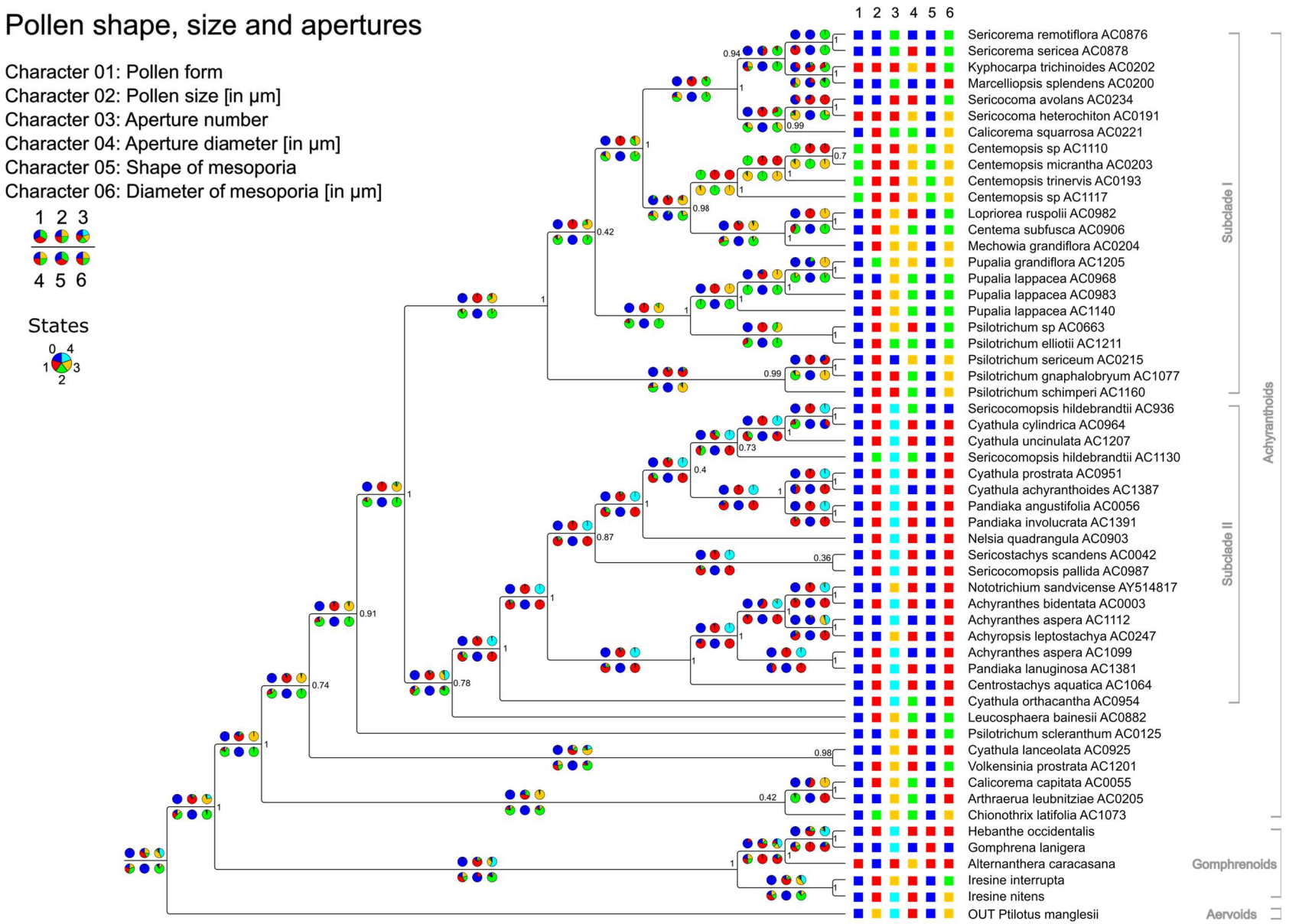

Figure 3.C: Evolution of pollen grains and apertures (character 1-6).
Pie charts depict the ancestral states reconstructed on the maximum clade credibility (MCC) tree of the plastid analysis. For character and state definitions see Appendix 3.C.

3.3.3.2 Pollen ornamentation and tectum perforations

Ancestral state probabilities for characters concerning pollen ornamentation and tectum perforations (chars 7-12) are depicted as pie charts, illustrated inFigure 3.D.

Tectum perforations are variable in size (char 8) and number (char 9) throughout the genera of Amaranthaceae, but the size of perforations is widely homogeneous within single pollen grains (char 7) in most of the taxa, except in *Chionothrix*, in a few taxa of subclade I and in *Achyropsis leptostachya* (compare Figure 3.D).

Microspines are evenly spread in most Achyranthoids (char 10, state 0) as well as in their MRCA. In Gomphrenoids they can also be arranged in a single row distally on mesoporia (char 10, state 1), which has otherwise only been observed in the genus *Kyphocarpa* and thus depicts an autapomorphy for the genus within Achyranthoids. In *Mechowia grandiflora* only a few microspines were found irregularly surrounding the pores (char 10, state 3) which was shown as a unique feature for this taxon (compare Fig 3D in Müller & Borsch, 2005b). Another exclusive state was observed in *Centemopsis*, where microspines occurred only on the uplifted hexagon sides (char 10, state 2; shown in Figure 3.A, photo I and Figure 3.B). The height of microspines was inferred as small or medium sized (char 11, state 0 & 1) in the MRCA of Achyranthoids as well as in most achyranthoid taxa. Only in subclade I and in *Ptilotus manglesii* (outgroup), large microspines of more than 500 nm height evolved several times. The number of microspines is also variable and ranging from less than 10 up to more than 41 per 10 µm. In total higher numbers were inferred for taxa of subclade II, where a number of 21-40 microspines was estimated for the MRCA, in contrast to subclade I for which a number of 11-20 microspines was inferred as the ancestral state.

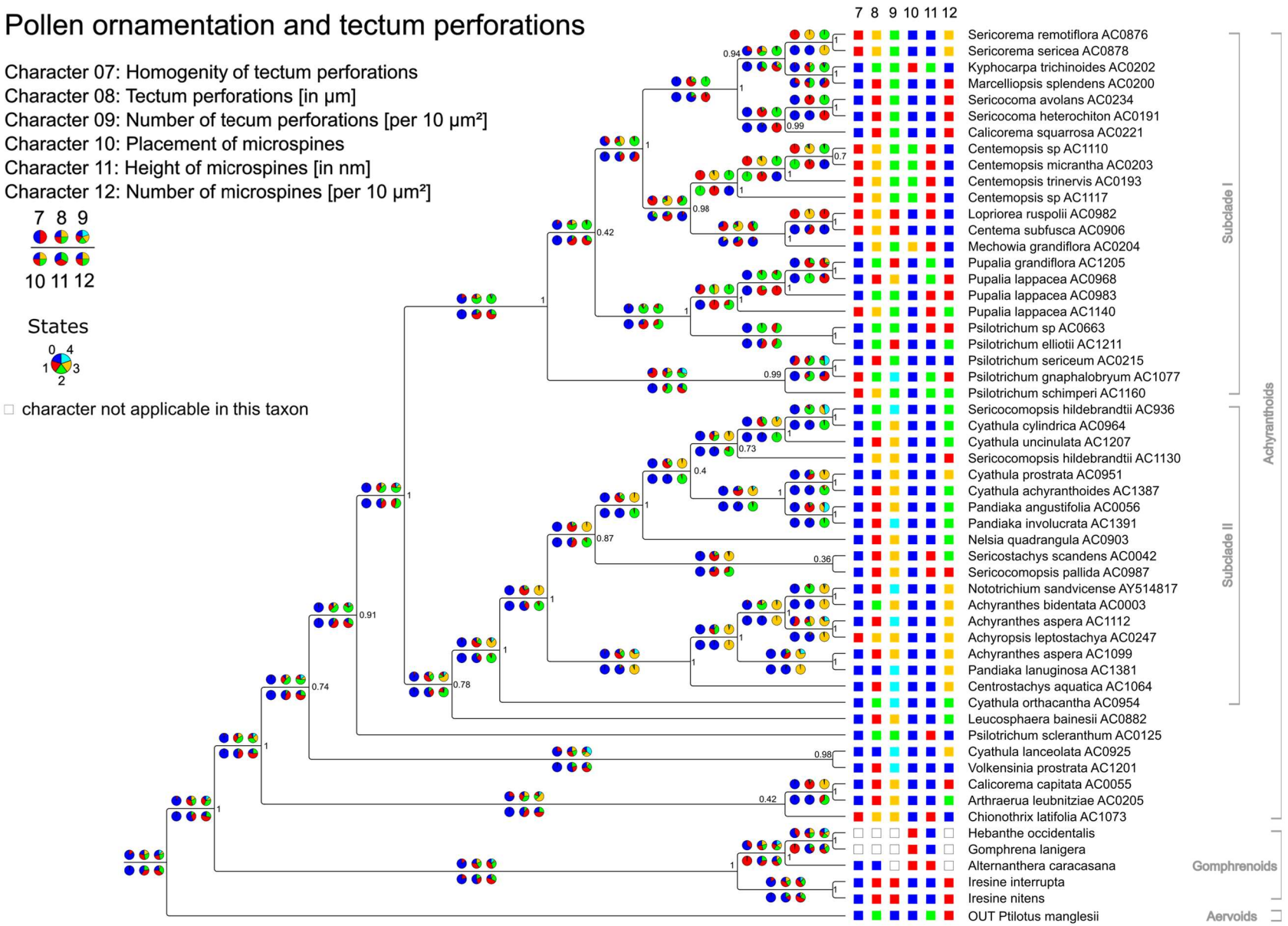

Figure 3.D: Evolution of pollen ornamentation and tectum perforations (character 7-12).
Pie charts depict the ancestral states reconstructed on the maximum clade credibility (MCC) tree of the plastid analysis. For character and state definitions see Appendix 3.C.

3.3.3.3 Pore ornamentation

Ancestral state probabilities for characters concerning pore ornamentation (chars 13-15) are depicted as pie charts, illustrated in Figure 3.E.

The number of ektexinous bodies on the aperture membrane was inferred to 7-10 (char 13, state 1) in the ancestor of Achyranthoids as well as in the MRCA of subclade I. In most taxa of subclade II, the number is elevated to 20-40 (char 13, state 3), which is also the ancestral state of this clade. Higher numbers of above 40 were only observed in *Alternanthera caracasana* of the Gomphrenoids. The smallest number of ektexinous bodies (≤6; char 13, state 0) was found in species of *Centemopsis*, *Psilotrichum sericeum*, and *Calicorema squarrosa*, all of which are nested in subclade I of Achyranthoids, and besides was only observed in *Ptilotus manglesii* (outgroup). Ektexinous bodies are closely adjoining in all taxa of Achyranthoids II, as well as in the majority of taxa within the whole achyranthoid clade and their MRCA (char 14, state 0). Ekexinous bodies which are well separated from each other (char 14, state 1) evolved twice in subclade I (in *Marceliopsis splendens* and in *Centema subfusca*), twice in basal lineages of Achyranthoids (in *Chionothrix latifolia* and in *Volkensinia prostrata* or maybe already in the MRCA of *Volkensinia* plus *Cyathula lanceolata* [probabilities are almost equal for state 0 and 1 in the MRCA and thus the origin cannot clearly be reconstructed]) and once in *Leucosphaera bainesii*. Ektexinous bodies most likely were medium sized (char 15, state 1) in the ancestor of Achyranthoids, as well as in several lineages of subclade I, where most likely, they were secondarily reduced after a primary enlargement (char 15, state 2) in the MRCA of Achyranthoids I. In Achyranthoids I, all three character states are present, which is – according to the state probabilities – interpreted as a stepwise reduction within this clade. In all taxa of subclade II including the stem node, as well as in all taxa of the basal lineages of Achyranthoids, ektexinous bodies were inferred to be smaller than the pore radius (char 15, state 0), as a consequence of secondary reduction.

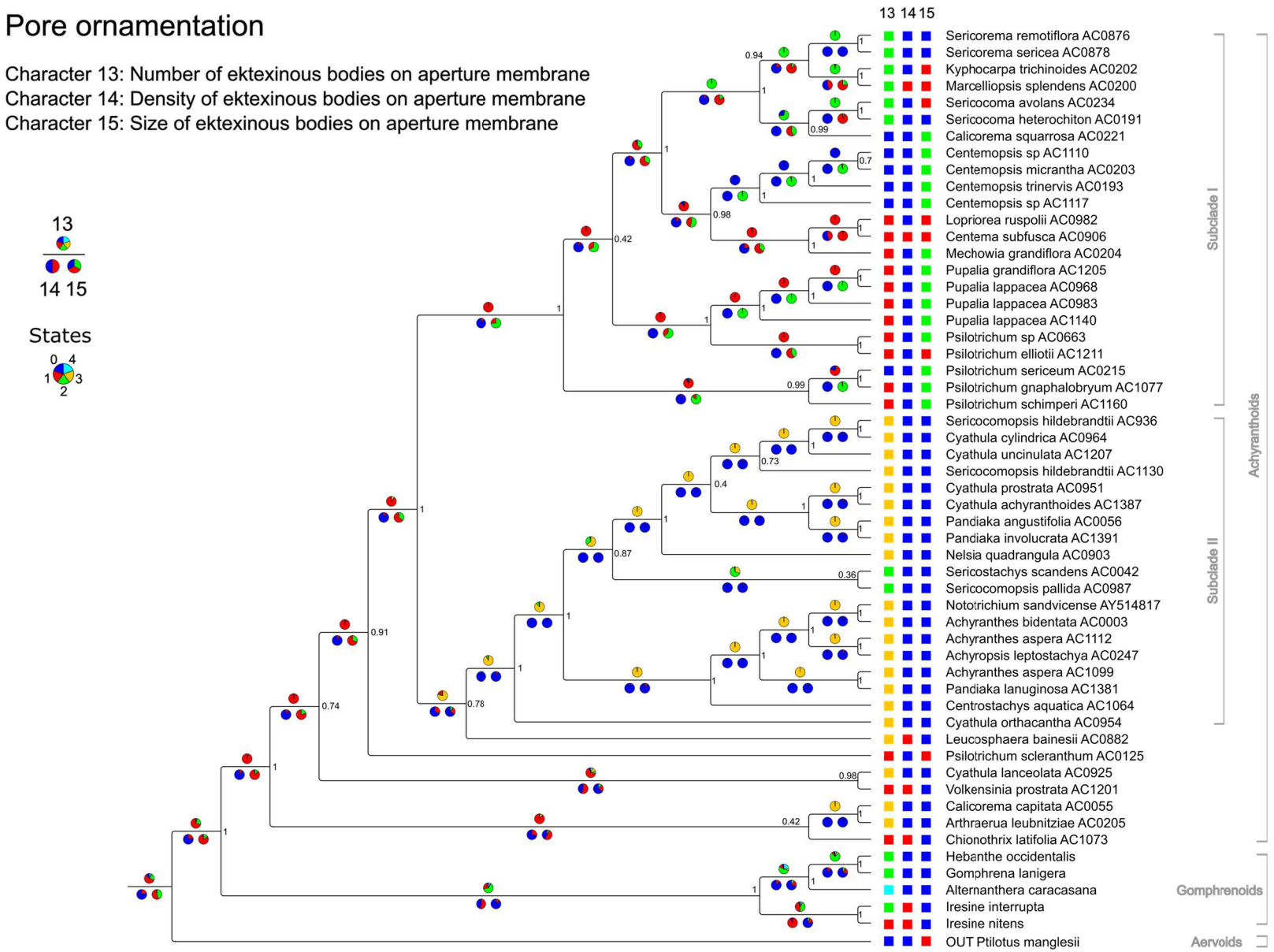

Figure 3.E: Evolution of pore ornamentation (character 13-15).
Pie charts depict the ancestral states reconstructed on the maximum clade credibility (MCC) tree of the plastid analysis. For character and state definitions see Appendix 3.C.

3.4 Discussion

3.4.1 Evolution of pollen shape, size, and apertures

Pollen in Achyranthoids is always pantoporate, as it is in the whole Amaranthaceae family, for which the pollen shape was described as tetrahedral, cubical, dodahedric or spheroidal (Nowicke 1994, Borsch 1998). For Achyranthoids spheroidal pollen was observed as the ancestral and most common state, as it was in Iresinoids (Borsch et al., 2018) and other Amaranthaceae, whereas dodecahedral pollen, which is common in Alternantheroids (Sánchez-del Pino et al., 2016), is rare within Achyranthoids and only found convergent in two lineages of subclade I. The truncated icosahedral pollen shape which was observed in all genera of *Centemopsis* is unique at least within Amaranthaceae and resembles the regular football geometry (Figure 3.A, photo I and Figure 3.B). In Borsch (1998) the *Centemopsis* type was considered as "nearly spheroidal" but pollen photos were poorly resolved compared to the high-resolution pictures of the present study, thus a more precise evaluation of the conspicuous pollen shape was possible here and was depicted as autapomorphy for the genus *Centemopsis*.

Pollen size is largely homogeneous throughout Achyranthoids and thus not distinctive for major clades (medium-sized grains with diameters of 15 - 24 µm [char 2, state 1] were inferred as the ancestral state for Achyranthoids as well as for subclades I and II), whereas the aperture number seems to be negatively correlated to the aperture size and also to the diameter of mesoporia: Achyranthoids II exhibit the highest pore numbers within Achyranthoids in combination with reduced aperture diameters and reduced mesoporia diameters. In contrast, Achyranthoids I exhibit comparatively fewer apertures with increased aperture diameters and increased mesoporia diameters. Deviating from that, for the Iresine angustifolia–I. nigra lineage in Borsch et al. (2018) a reduction in aperture number in correlation with an increase in pore diameter, and a decrease in mesoporia diameter was observed. For Achyranthoids, the flat or slightly vaulted mesoporia was shown as plesiomorphic, whereas the unique mesoporia shape in *Centemopsis* (char 5, state 2; Figure 3.C,compare Figure 3.A, photo I and Figure 3.B) depicts an autapomorphy, and the strongly vaulted mesoporia in *Kyphocarpa* evolved convergent in lineages of Gomphrenoids.

3.4.2 Evolution of pollen ornamentation and tectum perforations

The tectum of pollen grains in achyranthoid taxa is always complete punctate and punctae are evenly spread on mesoporia. This character was excluded here since it yielded no variability among Achyranthoids but the same results which were already shown in Borsch (2018) for representative of Achyranthoids, as well as for Iresinoids. Additionally, both groups were characterized by evenly spread microspines (Borsch et al., 2018), confirmed here as a plesiomorphic state for the entire achyranthoid clade (char 10, state 0), with exceptions in *Centemopsis* (state 2; compare Figure 3.B) and *Mechowia* (state 3; compare Fig 3D in Müller & Borsch, 2005b), where the placement of microspines reveals

autapomorphies for each genus, and in *Kyphocarpa* where microspines arranged in a single row distally on mesoporia (state 1) evolved in convergence to lineages of Gomphrenoids. The size of tectum perforations appears homoplastic with several transitions between character states (char 8) but with a general tendency to larger perforations among taxa of subclade I. However, tectum perforations are largely homogeneous within single pollen grains (char 7) in most taxa, with exceptions found in *Chionothrix*, within subclade I in *Sericorema*, *Centemopsis* and accessions of *Pupalia* and *Psilotrichum*, and within subclade II in *Achyropsis leptostachya* (compare Figure 3.D). In these taxa the size of perforations was strongly varying within single pollen grains from very small punctae up to large perforations. The frequency of tectum perforations (char 9) is characteristic for major lineages within Achyranthoids: in subclade II it is stepwise increased and compared to subclade I generally higher, reaching numbers of 51 up to more than 91 perforations per 10 µm², whereas clade I exhibit frequencies of 21 - 50 in the majority of taxa, and in some taxa further reduced frequencies of 11 - 20 perforations per 10 µm², a state otherwise only observed in Iresine here. The high frequency observed in subclade II is somehow surprising, since the mesoporia was shown to be reduced in diameter in taxa of Achyranthoids II, resulting in less space for perforations. Otherwise perforations are generally smaller in size in most taxa of Achyranthoids II, which might be correlated to the increase in frequencies. Less than 10 perforations per 10 µm² were only observed in *Ptilotus manglesii* (outgroup).

The largest microspines of more than 500 nm height were only observed within subclade I in *Kyphocarpa* and accessions of *Pupalia* and *Psilotrichum*, as well as in the outgroup (*Ptilotus manglesii*).

The size of microspines (char 11) is homoplastic with several shifts observed between the three character states: The MRCA of subclade I possessed medium sized microspines, which were reduced in size in the lineage comprising *Sericorema*, *Sericocoma* and allies, subsequently followed by a large increase in size in *Kyphocarpa*. Additionally, the ancestor of subclade II possessed small microspines, which were subsequently increased in size in the ancestor of a lineage comprising *Cyathula*, *Sericocomopsis*, *Nelsia* and *Pandiaka*, followed by a reversal back to smaller sized microspines in the highly supported lineage, which excludes *Sericocomopsis pallida* and *Sericostachys*. However, support for the relationship and the position of the latter two taxa is lacking and thus the ancestral state for this group cannot be accessed with security. The assumption of several transitions between character states however is not parsimonious in the evolutionary sense. Also, the frequency of microspines (char 12) was shown to be homoplastic with several transitions between character states but a general tendency to higher numbers as a derived feature of subclade II (most taxa observed in state 2 & 3), in comparison to the lower numbers inferred in subclade I (most taxa observed in state 0 & 1). The ancestral state for Achyranthoids I and probably within the entire Achyranthoids is a frequency of 11-20 microspines per 10µm².

3.4.3 Evolution of pore ornamentation

The ancestor of Achyranthoids possessed 7-10 ektexinous bodies on the aperture membrane which retained plesiomorphic in subclade I (char 13, state 1), whereas the increased number of ektexinous bodies appeared as a derived feature of subclade II (char 13, state 3). Higher numbers (>40, state 4) were only derived in *Alternanthera caracasana* of the Gomphrenoids. Within subclade I, the number of ektexinous bodies was reduced to ≤6 (char 13, state 0) in single taxa of those which exhibit the full syndrome of stellate pore ornamentation (according to Müller and Borsch, 2005b) such as *Centemopsis*, *Psilotrichum sericeum*, and *Calicorema squarrosa*, and besides such a reduction is only known from *Ptilotus manglesii* (outgroup), which does not exhibit the stellate pore ornamentation.

Closely adjoined ektexinous bodies are the plesiomorphic state for Achyranthoids and were retained in all taxa of Achyranthoids II, as well as in most taxa of subclade I and basal lineages (char 14, state 0). Ektexinous bodies which are well separated from each other (char 14, state 1) were derived several times within Achyranthoids: i) in *Marcelliopsis splendens* and *Centema subfusca*, both subclade I; ii) in *Chionothrix latifolia* and *Volkensinia prostrata* of early diverging Achyranthoids or more likely already in the MRCA of *Volkensinia* and *Cyathula lanceolata*, which would then lead to a subsequent loss in *C. lanceolata* (probabilities are almost equal and thus the origin cannot be clearly assessed); and iii) in *Leucosphaera bainesii*, for which the phylogenetic position remains not fully resolved.

Ektexinous bodies were inferred as medium-sized (char 15, state 1) in the ancestor of Achyranthoids. The enlargement of ektexinous bodies, which depicts one major characteristic of the stellate pore ornamentation and goes hand in hand with the star-shaped arrangement of ektexinous bodies (see Appendix 3.C, char 15, state 2), within this dataset was only observed once in the ancestor of subclade I, what is in line with the findings in Müller & Borsch (2005b), subsequently followed by several independent reversals, whereas a reduction to small sizes (char 15, state 0) was found several times, once in the MRCA of subclade II and *Leucosphaera*, and additionally in *Sericorema* as well as in *Sericocoma heterochiton* (both subclade I), where this feature derived from medium-sized ancestors. Thus, changes in size of ektexinous bodies appear as stepwise evolutionary processes, and within this dataset a reversal from small sized ektexinous bodies back to medium- or even large-sized ektexinous bodies could not been observed, whereas a prior enlargement followed by subsequent reduction was observed.

3.4.4 Pollen morphological characterization of Achyranthoids and sublineages

Achyranthoids are generally characterized by pantoporate, tectate pollen grains in which the tectum is complete punctate and punctae are evenly spread on mesoporia. The majority of taxa exhibit spheroidal pollen grains (char 1, state 0) with few exceptions which derived within subclade I. The shape of mesoporia is commonly flat or slightly vaulted (char 5, state 0), also with a few derived exceptions

within subclade I. In most genera the microspines are evenly spread (char 10, state 0), and ektexinous bodies are closely adjoined (char 14, state 0) but again, exceptions are found in subclade I and for the latter character also within early diverging lineages.

Subclade I is deviant from subclade II by the following characters: **i)** the inclusion of lineages with non-spheroidal pollen grains, for which the pollen shape largely coincides with vaulted (inwards or outwards) mesoporia, unevenly spread microspines, and lower frequencies of microspines; **ii)** lower aperture numbers; **iii)** enlarged mesoporia diameters; **iv)** enlarged apertures; **v)** enlarged tectum perforations in most genera which are then correlated to lower frequencies of tectum perforations; **vi)** lower frequencies of ektexinous bodies, which are then strongly enlarged. Some of the above-mentioned features contribute to the character syndrome of stellate pore ornamentation which is limited to several taxa of subclade I.

Within Amaranthaceae the pollen grains of the genus *Centemopsis* exhibit several autapomorphies: these are the unique truncated icosahedral pollen shape; the inwards vaulted mesoporia around the uplifted pores; the specific placement and significant quantitative reduction of microspines.

Thus, pollen in Achyranthoids is confirmed to be very informative on different taxonomic levels and main lineages as well as genera can be characterized by the combination of different pollen characters. Pollen morphology thus can be used in addition to further morphological characters and contribute to detailed descriptions which facilitate the delimitation of taxonomic entities within Achyranthoids.

3.5 Appendix 3.A-F Supplementary material for Chapter 3

Appendices

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Appendix 3.A: List of analysed taxa of Amaranthaceae, including geographic origin, voucher information and ENA/GenBank accession numbers.

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	Pollen morphology
***Achyranthes aspera* L.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 281 (EA, B, ETH)	Kenya	AC1099	LT992595 Di Vincenzo & al. (2018)	LT993046 Di Vincenzo & al. (2018)	LT995259 Di Vincenzo & al. (2018)	Same individual as DNA
***Achyranthes aspera* L.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 308 (EA, B, ETH)	Kenya	AC1112	LT992597 Di Vincenzo & al. (2018)	LT993048 Di Vincenzo & al. (2018)	LT995261 Di Vincenzo & al. (2018)	Same individual as DNA
***Achyranthes bidentata* Blume**	T. Borsch s. n.	China, Kunming	AC0003	LT992574 Di Vincenzo & al. (2018)	LT993014 Di Vincenzo & al. (2018)	LT992944 Di Vincenzo & al. (2018)	B.Bartholomew et al. 1500 B 10 1074034 (B)
***Achyropsis leptostachya* (E. Mey. ex Meisn.) Baker & C.B. Clarke**	K. Müller 876 (B, PRE)	South Africa	AC0247	AY998117 Müller & Borsch (2005b)	LT993028 Di Vincenzo & al. (2018)	LT995234 Di Vincenzo & al. (2018)	Drege s.n. HBG-503189 (HBG)
***Alternanthera caracasana* Kunth**	Sanchez-del Pino et al. 20 (MEXU)	Mexico		AY514794	EF688733	EF688662	Character states from Borsch et al. (2018)
***Arthraerua leubnitziae* (Kuntze) Schinz**	B. Leuenberger, T. Raus, C. Schiers 3330 (PRE)	Namibia	AC0205	AY998115 Müller & Borsch (2005b)	LT993023 Di Vincenzo & al. (2018)	LT992946 Di Vincenzo & al. (2018)	D. G. Long & D. A. H. Rae 760 (MO, PRE) Müller & Borsch (2005b)
***Calicorema capitata* (Moq.) Hook. f.**	C. Neinhuis s.n. (B)	Namibia	AC0055	AY514807 Müller & Borsch (2005a)	LT993015 Di Vincenzo & al. (2018)	LT995223 Di Vincenzo & al. (2018)	G. Kunkel 12484 (FR) Müller & Borsch (2005b)
***Calicorema squarrosa* (Schinz) Schinz**	H. O. Volk 12630 (M)		AC0221	AY998114 Müller & Borsch (2005b)	LT993025 Di Vincenzo & al. (2018)	LT995231 Di Vincenzo & al. (2018)	Same individual as DNA Müller & Borsch (2005b)
***Centema subfusca* (Moq.) T. Cooke**	P. Bamps (Moq.) Lopr. (BR)	Süd-Afrika	AC0906	LT999833 Di Vincenzo & al. (2018)	LT995131 Di Vincenzo & al. (2018)	LT995193 Di Vincenzo & al. (2018)	Strey 4658 (B)
***Centemopsis micrantha* Chiov.**	J. B. Gillet & C. F. Hemming 24874 (PRE)	Namibia	AC0203	AY998105 Müller & Borsch (2005b)	LT993022 Di Vincenzo & al. (2018)	LT995230 Di Vincenzo & al. (2018)	Same individual as DNA Müller & Borsch (2005b)
***Centemopsis* sp.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 304 (EA, B, ETH)	Kenya	AC1110	LT999854 Di Vincenzo & al. (2018)	LT996931 Di Vincenzo & al. (2018)	LT999819 Di Vincenzo & al. (2018)	Same individual as DNA
***Centemopsis* sp.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 318 (EA, B, ETH)	Kenya	AC1117	LT999856 Di Vincenzo & al. (2018)	LT996933 Di Vincenzo & al. (2018)	LT999821 Di Vincenzo & al. (2018)	Same individual as DNA
***Centemopsis trinervis* Hauman**	M. Reekmans 31981 (PRE)	Namibia	AC0193	AY998107 Müller & Borsch (2005b)	LT993019 Di Vincenzo & al. (2018)	LT995227 Di Vincenzo & al. (2018)	Same individual as DNA Müller & Borsch (2005b)
***Centrostachys aquatica* (R. Br.) Wall.**	K. Balkwill & M.-J. Balkwill 4356 (B)	Nigeria	AC1064	LT992591 Di Vincenzo & al. (2018)		LT992952 Di Vincenzo & al. (2018)	M. Wondafrash 4049 (ETH, B)

Appendix 3.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	Pollen morphology
***Chionothrix latifolia* Rendle**	M. Wondafrash 2142 (ETH, B)	Ethiopia	AC1073	LT999848 Di Vincenzo & al. (2018)	LT995141 Di Vincenzo & al. (2018)	LT995203 Di Vincenzo & al. (2018)	V. Di Vincenzo, A. Hailu 222 (B, ETH)
***Cyathula achyranthoides* (Kunth) Moq.**	I. Friis, M.G. Gilbert & K. Vollesen 3598 (B)	Ethiopia, Bale	AC1387	this study	this study	this study	Same individual as DNA
***Cyathula cylindrica* Moq.**	M. Wondafrash 3605A (ETH, B)	Ethiopia	AC0964	LT992587 Di Vincenzo & al. (2018)	LT993039 Di Vincenzo & al. (2018)	LT992949 Di Vincenzo & al. (2018)	Same individual as DNA
***Cyathula lanceolata* Schinz**	K. Müller 867 (B, PRE)	Süd-Afrika (RSA), Northern	AC0925	LT999835 Di Vincenzo & al. (2018)	LT993035 Di Vincenzo & al. (2018)	LT995240 Di Vincenzo & al. (2018)	Peter 41059 B10 0154184 (B)
***Cyathula orthacantha* (Hochst. ex Asch.) Schinz**	M. Wondafrash 3461 (ETH, B)	Ethiopia	AC0954	LT999840 Di Vincenzo & al. (2018)	LT995134 Di Vincenzo & al. (2018)	LT995196 Di Vincenzo & al. (2018)	Same individual as DNA
***Cyathula prostrata* (L.) Blume**	M. Wondafrash 3434 (ETH, B)	Ethiopia	AC0951	LT992586 Di Vincenzo & al. (2018)		LT995244 Di Vincenzo & al. (2018)	U.Scholz et al. 541 B 10 1077145 (B)
***Cyathula uncinulata* (Schrad.) Schinz**	V. Di Vincenzo, A. Hailu 86 (B, ETH)	Ethiopia	AC1207	LT999869 Di Vincenzo & al. (2018)	LT995154 Di Vincenzo & al. (2018)	LT995218 Di Vincenzo & al. (2018)	Same individual as DNA
***Gomphrena lanigera* Pohl**	Wood 19964 (B, LPB)	Bolivia, Santa Cruz	AC1029	LS451270 Borsch et al. (2018)	LS453411 Borsch et al. (2018)	LS451006 Borsch et al. (2018)	Character states from Borsch et al. (2018)
***Hebanthe occidentalis* (R.E.Fr.) Borsch & Pedersen var.**	E. Zardini 45377 (B, MO)	Paraguay	AC0008	AY514821 Müller & Borsch (2005a)			Character states from Borsch et al. (2018)
***Hebanthe occidentalis* (R.E.Fr.) Borsch & Pedersen var.**	Nee & Saldias 47054 (NY)	Bolivia			EF688767 Sanchez del-Pino et al. (2009)	EF688697 Sanchez del-Pino et al. (2009)	Character states from Borsch et al. (2018)
***Iresine interrupta* Benth.**	Zumaya, Esquivel & León 62 (B, MEXU)	Mexico, Hidalgo	AC166	LS451239 Borsch et al. (2018)	LS453378 Borsch et al. (2018)	LS450973 Borsch et al. (2018)	Character states from Borsch et al. (2018)
***Iresin. nitens* Standl.**	Borsch, Flores Olvera & Zumaya 3781 (B, MEXU)	Mexico, Puebla	AC283	LS451245 Borsch et al. (2018)	LS453384 Borsch et al. (2018)	LS450979 Borsch et al. (2018)	Character states from Borsch et al. (2018)
***Kyphocarpa trichinoides* (Fenzl) Lopr.**	A. E. van Wyk 12403 (PRE)	South Africa	AC0202	AY998106 Müller & Borsch (2005b)	LT993021 Di Vincenzo & al. (2018)	LT995229 Di Vincenzo & al. (2018)	Same individual as DNA Müller & Borsch (2005b)
***Leucosphaera bainesii* (Hook.f.) Gilg**	K. Müller 866 (B, PRE)	Süd-Afrika (RSA), Northern	AC0882	LT992581 Di Vincenzo & al. (2018)	LT993033 Di Vincenzo & al. (2018)	LT995238 Di Vincenzo & al. (2018)	Walter 1044 (B)

Appendix 3.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	Pollen morphology
***Lopriorea ruspolii* (Lopr.) Schinz**	M. Wondafrash 3921 (ETH, B)	Ethiopia	AC0982	LT999844 Di Vincenzo & al. (2018)	LT995138 Di Vincenzo & al. (2018)	LT995200 Di Vincenzo & al. (2018)	Same individual as DNA
***Marcelliopsis splendens* (Schinz) Schinz**	M. Müller & W. Giess 370 (PRE)	Namibia	AC0200	AY998112 Müller & Borsch (2005b)	LT993020 Di Vincenzo & al. (2018)	LT995228 Di Vincenzo & al. (2018)	W. Giess 3643 (MO) Müller & Borsch (2005b)
***Mechowia grandiflora* Schinz**	R. Mendes dos Santos 1780 (PRE)	Namibia	AC0204	AY998113 Müller & Borsch (2005b)		LT992945 Di Vincenzo & al. (2018)	Mechow 235 (GOET) Müller & Borsch (2005b)
***Nelsia quadrangula* (Engl.) Schinz**	E. Coppejam (BR)		AC0903	LT992582 Di Vincenzo & al. (2018)	LT993034 Di Vincenzo & al. (2018)	LT995239 Di Vincenzo & al. (2018)	BM000910354 (BM), Walter 4491 (B)
***Nototrichium sandwicense* Hillebr.**	B. A. Prigge 15250 (LA)	USA, Hawaii		AY514817 Müller & Borsch (2005a)			Remy 2071 (GH)
***Pandiaka angustifolia* (Vahl) Hepper**	J. Müller 324 (FR)	Burkina Faso	AC0056	AY514818 Müller & Borsch (2005a)	LT993016 Di Vincenzo & al. (2018)	LT995224 Di Vincenzo & al. (2018)	Thonning s.n._S-G-7065 (S), Heudelot 280 (P)
***Pandiaka involucrata* (Moq.) B.D. Jacks**	U. Scholz, P. Koumassi, H. Kühn & H. Peuker 451 (B)	Togo	AC1391	this study		this study	Same individual as DNA
***Pandiaka lanuginosa* Schinz**	WRQ Luke 15344 (EA)	Kenya	AC1381	this study	this study		E.Milne-Redhead & P.Taylor 11191 B 10 1074059 (B)
***Psilotrichum elliotii* Baker & C.B. Clarke**	V. Di Vincenzo, A. Hailu 145 (B, ETH)	Ethiopia	AC1211	LT992609 Di Vincenzo & al. (2018)	LT993059 Di Vincenzo & al. (2018)	LT995257 Di Vincenzo & al. (2018)	Same individual as DNA
***Psilotrichum gnaphalobryum* (Hochst.) Schinz**	M. Wondafrash 2200 (ETH, B)	Ethiopia	AC1077	LT992593 Di Vincenzo & al. (2018)	LT993044 Di Vincenzo & al. (2018)	LT992954 Di Vincenzo & al. (2018)	M. Wondafrash 2195 (ETH, B)
***Psilotrichum schimperi* Engl.**	T. Mesfin & G.Y. Kagnew 1791, 01.11.1981 (ETH)	Ethiopia	AC1160	LT999863 Di Vincenzo & al. (2018)		LT995212 Di Vincenzo & al. (2018)	M. Wondafrash 2685 (ETH, B)
***Psilotrichum scleranthum* Thwaites**	K. Müller 878 (B, PRE)	South Africa	AC0125	AY514822 Müller & Borsch (2005a)	LT996924 Di Vincenzo & al. (2018)	LT999811 Di Vincenzo & al. (2018)	Same individual as DNA Müller & Borsch (2005b)
***Psilotrichum sericeum* (J. König ex Roxb.) Dalzell**	W. R. G. Luke & P. A. Luke 3832 (PRE)		AC0215	AY998109 Müller & Borsch (2005b)	LT993024 Di Vincenzo & al. (2018)	LT992947 Di Vincenzo & al. (2018)	Same individual as DNA Müller & Borsch (2005b)
***Psilotrichum* sp.**	M. Wondafrash 3460 (ETH)	Ethiopia	AC0663	LT999827 Di Vincenzo & al. (2018)	LT996925 Di Vincenzo & al. (2018)	LT999812 Di Vincenzo & al. (2018)	Same individual as DNA

Appendix 3.A – continued

Taxon	Voucher/Herbarium	Field/Garden Origin	Lab No	*matK*	*trnL-F*	*rpl16*	Pollen morphology
***Ptilotus manglesii* (Lindl.) F.Muell.**	T. Borsch 3543 (BONN)	BG Bonn 12999 (Australia)	AC0015	AY514824 Müller & Borsch (2005a)	LS453368 Borsch et al. (2018)	LT995220 Di Vincenzo & al. (2018)	Character states from Borsch et al. (2018)
***Pupalia grandiflora* Peter**	V. Di Vincenzo, A. Hailu 84 (B, ETH)	Ethiopia	AC1205	LT999868 Di Vincenzo & al. (2018)	LT995153 Di Vincenzo & al. (2018)	LT995217 Di Vincenzo & al. (2018)	Same individual as DNA
***Pupalia lappacea* (L.) A. Juss.**	M. Wondafrash 3650 (ETH, B)	Ethiopia	AC0968	LT992588 Di Vincenzo & al. (2018)	LT993040 Di Vincenzo & al. (2018)	LT992950 Di Vincenzo & al. (2018)	Same individual as DNA
***Pupalia lappacea* (L.) A. Juss.**	M. Wondafrash 3922 (ETH, B)	Ethiopia	AC0983	LT992590 Di Vincenzo & al. (2018)	LT993042 Di Vincenzo & al. (2018)	LT995262 Di Vincenzo & al. (2018)	Same individual as DNA
***Pupalia lappacea* (L.) A. Juss.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 354 (EA, B, ETH)	Kenya	AC1140	LT992601 Di Vincenzo & al. (2018)	LT993052 Di Vincenzo & al. (2018)	LT995250 Di Vincenzo & al. (2018)	Same individual as DNA
***Sericocoma avolans* Fenzl**	K. Müller 859 (B, PRE)	South Africa	AC0234	AY998103 Müller & Borsch (2005b)	LT993026 Di Vincenzo & al. (2018)	LT995232 Di Vincenzo & al. (2018)	Same individual as DNA Müller & Borsch (2005b)
***Sericocoma heterochiton* Lopr.**	K. Müller 851 (B, PRE)	South Africa	AC0191	AY998104 Müller & Borsch (2005b)	LT993018 Di Vincenzo & al. (2018)	LT995226 Di Vincenzo & al. (2018)	Same individual as DNA Müller & Borsch (2005b)
***Sericocomopsis hildebrandtii* Schinz**	M. Wondafrash 2052 (ETH, B)	Ethiopia	AC0936	this study	this study	this study	Same individual as DNA
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 339 (EA, B, ETH)	Kenya	AC1130	LT999859 Di Vincenzo & al. (2018)	LT995147 Di Vincenzo & al. (2018)	LT995210 Di Vincenzo & al. (2018)	Same individual as DNA
***Sericocomopsis pallida* (S. Moore) Schinz**	M. Wondafrash 3926 (ETH, B)	Ethiopia	AC0987	this study	this study	this study	Same individual as DNA
***Sericorema remotiflora* (Hook.) Lopr.**	K. Müller 845 (B, PRE)	Süd-Afrika (RSA), Northern Cape	AC0876	LT992579 Di Vincenzo & al. (2018)	LT993031 Di Vincenzo & al. (2018)	LT995236 Di Vincenzo & al. (2018)	Müller & Borsch (2005b)
***Sericorema sericea* (Schinz) Lopr.**	K. Müller 849 (B, PRE)	Süd-Afrika (RSA), Northern Cape	AC0878	LT992580 Di Vincenzo & al. (2018)	LT993032 Di Vincenzo & al. (2018)	LT995237 Di Vincenzo & al. (2018)	South Africa; R. Seidel 390 (MO) Müller & Borsch (2005b)
***Sericostachys scandens* Gilg & Lopr.**	E. Fischer s.n. (B)	Rwanda	AC0042	AY514819 Müller & Borsch (2005a)	this study	this study	Zenker 1420 (GOET) HBG-502314 (HBG)
***Volkensinia prostrata* (Volkens ex Gilg) Schinz**	M. Wondafrash 4200 (ETH, B)	Ethiopia	AC1201	LT999866 Di Vincenzo & al. (2018)	LT995151 Di Vincenzo & al. (2018)	LT995215 Di Vincenzo & al. (2018)	J.B.Gillett 19789 B 10 1077233 (B)

Appendix 3.B: Data set partitions and nucleotide substitution models for phylogenetic analyses.
Best-fitting models were selected according to the results from model selection in ModelTest-NG v0.1.7 via AICc (Akaike Information Criterion corrected for small sample sizes).

Partition name	Model
trnk-1	GTR+I+G
matk-gen	GTR+G
trnk-2	GTR+I+G
rpl16	GTR+G
trnT-spacer incomplete	GTR+G
trnL-5-Exon	JC
trnL-Intron	GTR+G
trnL-3-Exon	JC
trnL-F-spacer	GTR+G
trnF-Exon	JC

Appendix 3.C: Pollen morphological character definitions and their states applied for ancestral character state reconstructions in Achyranthoids.
Characters were chosen according to Borsch et al. (2018). Their character 7 "tectum completeness" is not reconstructed here, due to the missing variation within Achyranthoids. The tectum of pollen grains in achyranthoid taxa is always complete punctate and punctae are evenly spread on mesoporia. This corresponds to state 0 in Borsch et al. (2018). Instead, here the homogeneity of tectum perforations is coded (char 7). Where necessary, state categories were adjusted and/or added according to own character state observations.

Character group	No.	Character definition
Pollen shape, size, and apertures	1	**Pollen shape** State 2 is only present in the genus Centemopsis and resembles the geometry of a football (compare Figure 3.B). ***States:*** *sphaeroidal* ***(0)****, dodecahedral* ***(1)****, truncated icosahedral* ***(2)***
	2	**Pollen size** [in µm] Considering the strongly deviating sizes of pollen grains in the Amaranthaceae (Borsch, 1998), categories that allow the reconstruction of significant shifts in our data set are distinguished. ***States:*** *11.0-14.99* ***(0)****, 15.0-23.99* ***(1)****, 24.0-34.99* ***(2)****, 35.0-45.0* ***(3)***
	3	**Aperture number** An aperture number of 13 does not exist due to form constraints (Borsch & Barthlott, 1998). ***States:*** *6* ***(0)****, 12* ***(1)****, 14-19* ***(2)****, 20-35* ***(3)****, 36-80* ***(4)***
	4	**Aperture diameter** [in µm] ***States:*** *1.3-1.99* ***(0)****, 2.0-2.99* ***(1)****, 3.0-3.99* ***(2)****, 4.0-5.7* ***(3)***
	5	**Shape of mesoporia** In state 1 mesoporia must be higher than wide following Borsch & Barthlott (1998). State 2 is unique for the genus *Centemopsis* (see Figure 3.B*)*. ***States:*** *flat or slightly vaulted* ***(0)****, strongly vaulted* ***(1)****, pentagonal protruding around pores, which thus appear uplifted; between the pores mesoporia vaulted inwards and thus pores appear surrounded by 5 irregular concave hexagons each* ***(2)***
	6	**Diameter of mesoporia** [in µm] ***States:*** *0.3-1.09* ***(0)****, 1.1-1.99* ***(1)****, 2.0-2.99* ***(2)****, 3.0-5.5* ***(3)***

Appendix 3.C – continued

Character group	No.	Character definition
Ornamentation and tectum perforations	7	**Homogenity of tectum perforations** ***States***: *tectum perforations almost uniform in size* ***(0)****, diameter of tectum perforations largely deviating in single pollen grains* ***(1)***
	8	**Tectum perforations** [in nm] This character is not applicable in pollen grains with reduced tectum (compare char. 7, states 3 and 4 in Borsch et al. (2018)). Some species such as *Sericorema remotiflora*, *S. sericea*, *Chionothrix latifolia*, *Achyropsis leptostachya* and species of *Centemopsis* have largely deviating diameters of perforations in single pollen grains (compare character 7, state 1 of this study). In these cases, only the large perforations were measured. ***States****: <50* ***(0)****, 50-110* ***(1)****, >110 to 200* ***(2)****, >200 to 690* ***(3)***
	9	**Number of tecum perforations** [per 10 µm^2] This character is not applicable in pollen grains with reduced tectum (compare char. 7, states 3 and 4 in Borsch et al. (2018)). ***States****: ≤ 10* ***(0)****, 11-20* ***(1)****, 21-50* ***(2)****, 51-90* ***(3)****, ≥91* ***(4)***
	10	**Placement of microspines** State 2 is unique for the genus *Centemopsis* (see Figure 3.B). State 3 is a unique feature in *Mechowia grandiflora* (compare Fig 3D in Müller & Borsch, 2005b). ***States****: evenly spread* ***(0)****, arranged in a single row distally on mesoporia* ***(1)****, microspines occurring only on the uplifted hexagon sides* ***(2)****, only a few microspines irregularly surrounding the pores* ***(3)***
	11	**Height of microspines** [in nm] *States: < 250 (0), 250-500 (1), > 500 (2)25*
	12	**Number of microspines** [per 10 µm^2] ***States****: ≤ 10* ***(0)****, 11-20* ***(1)****, 21-40* ***(2)****, ≥41****(3)***

Appendix 3.C – continued

Character group	No.	Character definition
Pore ornamentation	13	**Number of ektexinous bodies on aperture membrane** ***States**: ≤ 6 **(0)**, 7-10 **(1)**, 11-19 **(2)**, 20-40 **(3)**, ≥41 **(4)***
	14	**Density of ektexinous bodies on aperture membrane** The definition of this character follows Borsch (1998). ***States**: closely adjoining **(0)**, well separated from each other **(1)***
	15	**Size of ektexinous bodies on aperture membrane** This character is according to character 2 in Müller und Borsch (2005b). ***States**: < pore radius **(0)**, some reaching pore radius **(1)**, exceeding pore radius (=stellate) **(2)***

Appendix 3.D: Pollen morphological character matrix applied for ancestral character reconstructions

Taxon	Char 1	Char 2	Char 3	Char 4	Char 5	Char 6	Char 7	Char 8	Char 9	Char 10	Char 11	Char 12	Char 13	Char 14	Char 15
Achyranthes_aspera_AC1099	0	1	4	0	0	1	0	1	3	0	0	3	3	0	0
Achyranthes_aspera_AC1112	0	0	4	0	0	1	0	1	4	0	0	3	3	0	0
Achyranthes_bidentata_AC0003	0	1	4	1	0	1	0	2	3	0	0	3	3	0	0
Achyropsis_leptostachya_AC0247	0	0	3	1	0	1	1	3	3	0	0	3	3	0	0
Alternanthera_caracasana	1	0	1	3	1	1	0	0	n.a.	1	1	n.a.	4	0	0
Arthraerua_leubnitziae_AC0205	0	0	3	2	0	1	0	1	3	0	0	2	3	0	0
Calicorema_capitata_AC0055	0	1	3	2	0	1	0	1	3	0	0	1	3	0	0
Calicorema_squarrosa_AC0221	0	1	2	2	0	3	0	1	2	0	0	1	0	0	2
Centema_subfusca_AC0906	0	1	3	2	0	2	1	3	1	0	0	0	1	1	1
Centemopsis_micrantha_AC0203	2	1	1	3	2	3	1	3	2	2	1	0	0	0	2
Centemopsis_sp_AC1110	2	1	1	3	2	3	1	3	2	2	1	0	0	0	2
Centemopsis_sp_AC1117	2	1	1	3	2	3	1	3	2	2	1	0	0	0	2
Centemopsis_trinervis_AC0193	2	1	1	3	2	3	1	3	2	2	1	0	0	0	2
Centrostachys_aquatica_AC1064	0	1	4	1	0	1	0	1	4	0	0	3	3	0	0
Chionothrix_latifolia_AC1073	0	2	3	2	0	3	1	3	3	0	1	0	1	1	0
Cyathula_achyranthoides_AC1387	0	1	4	0	0	1	0	1	3	0	0	2	3	0	0
Cyathula_cylindrica_AC0964	0	1	4	1	0	1	0	2	3	0	0	2	3	0	0
Cyathula_lanceolata_AC0925	0	0	3	1	0	1	0	0	4	0	0	3	3	0	0
Cyathula_orthacantha_AC0954	0	1	4	2	0	1	0	2	4	0	0	2	3	0	0
Cyathula_prostrata_AC0951	0	1	4	1	0	1	0	0	3	0	0	3	3	0	0
Cyathula_uncinulata_AC1207	0	1	4	1	0	1	0	1	3	0	0	2	3	0	0
Gomphrena_lanigera	0	0	4	0	1	0	n.a.	n.a.	n.a.	1	0	n.a.	2	0	0
Hebanthe_occidentalis	0	1	4	1	1	1	n.a.	n.a.	n.a.	1	0	n.a.	2	0	0
Iresine_interrupta	0	1	3	1	0	2	0	1	1	0	0	1	2	1	0
Iresine_nitens	0	1	4	1	0	3	0	1	1	0	0	1	1	1	0
Kyphocarpa_trichinoides_AC0202	1	1	1	3	1	2	0	2	2	1	2	0	2	0	1
Leucosphaera_bainesii_AC0882	0	1	3	2	0	2	0	1	3	0	0	2	3	1	0
Lopriorea_ruspolii_AC0982	0	1	3	1	0	2	1	3	1	0	1	0	1	0	1
Marcelliopsis_splendens_AC0200	0	0	2	0	0	1	0	1	2	0	0	1	2	1	1
Mechowia_grandiflora_AC0204	0	1	3	3	0	3	0	3	2	3	1	0	1	0	2

Appendix 3.D – continued

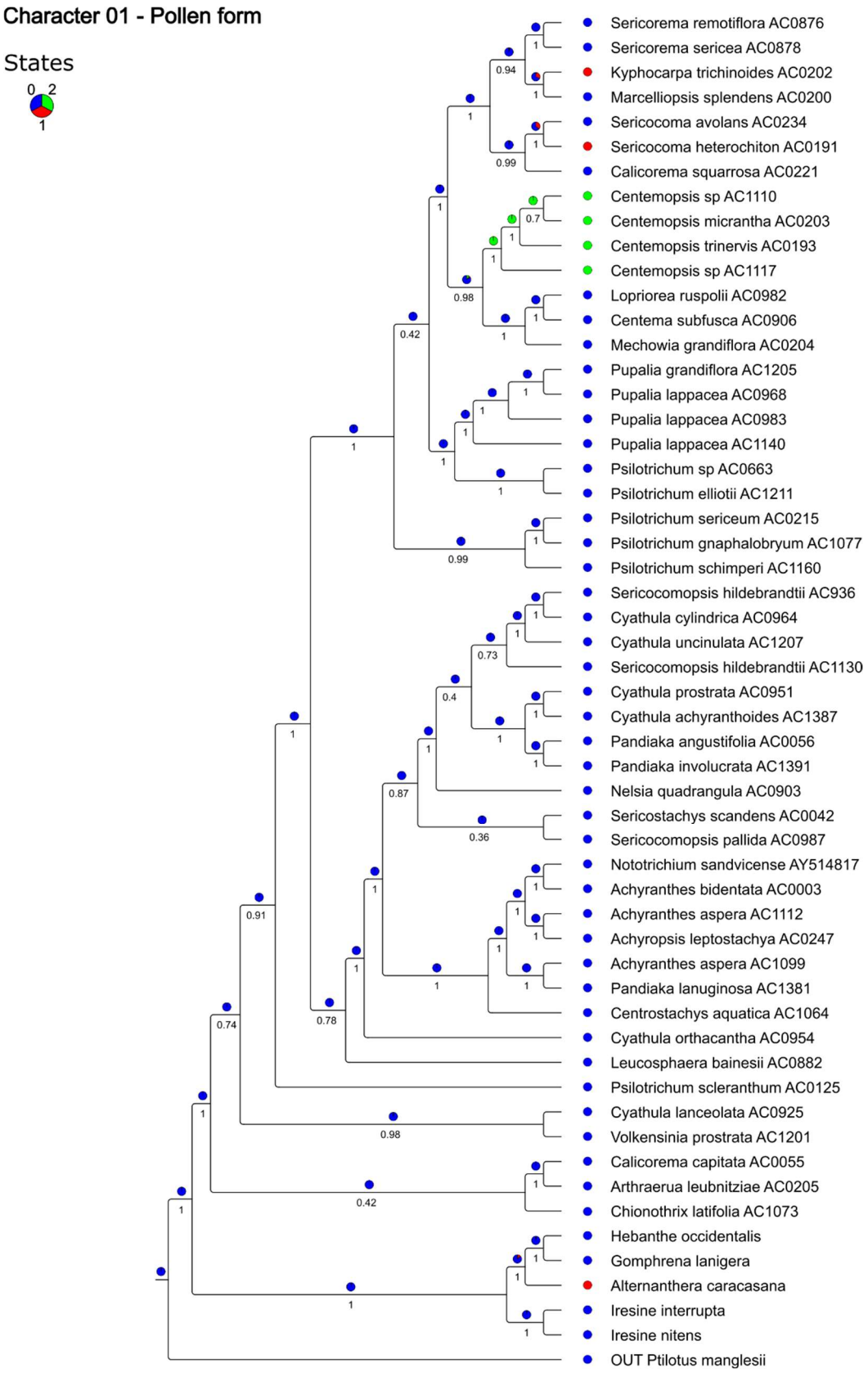
Taxon	Char 1	Char 2	Char 3	Char 4	Char 5	Char 6	Char 7	Char 8	Char 9	Char 10	Char 11	Char 12	Char 13	Char 14	Char 15
Nelsia_quadrangula_AC0903	0	1	4	1	0	1	0	1	3	0	0	2	3	0	0
Nototrichium_sandvicense_AY514817	0	0	3	1	0	1	0	1	4	0	0	3	3	0	0
OUT_Ptilotus_manglesii	0	3	4	1	0	3	0	2	0	0	2	1	0	0	1
Pandiaka_angustifolia_AC0056	0	1	4	1	0	1	0	1	3	0	0	2	3	0	0
Pandiaka_involucrata_AC1391	0	1	4	1	0	1	0	1	4	0	0	2	3	0	0
Pandiaka_lanuginosa_AC1381	0	1	4	1	0	1	0	0	4	0	0	3	3	0	0
Psilotrichum_elliotii_AC1211	0	1	2	2	0	2	0	2	1	0	0	2	1	0	1
Psilotrichum_gnaphalobryum_AC1077	0	1	1	2	0	3	1	2	4	0	2	1	1	0	2
Psilotrichum_schimperi_AC1160	0	1	1	2	0	3	1	3	2	0	2	2	1	0	2
Psilotrichum_scleranthum_AC0125	0	0	3	1	0	2	0	2	2	0	1	0	1	0	1
Psilotrichum_sericeum_AC0215	0	1	0	3	0	3	0	1	2	0	0	0	0	0	2
Psilotrichum_sp_AC0663	0	1	3	1	0	2	0	2	2	0	1	1	1	0	2
Pupalia_grandiflora_AC1205	0	2	3	3	0	3	0	2	1	0	2	0	1	0	2
Pupalia_lappacea_AC0968	0	0	3	2	0	2	0	1	3	0	2	1	1	0	2
Pupalia_lappacea_AC0983	0	1	3	2	0	2	0	2	2	0	1	1	1	0	2
Pupalia_lappacea_AC1140	0	1	3	2	0	2	1	3	2	0	1	2	1	0	2
Sericocoma_avolans_AC0234	0	0	1	1	0	2	0	1	2	0	0	1	2	0	1
Sericocoma_heterochiton_AC0191	1	1	1	3	0	3	0	1	2	0	0	1	2	0	0
Sericocomopsis_hildebrandtii_AC1130	0	2	4	2	0	1	0	3	3	0	0	1	3	0	0
Sericocomopsis_hildebrandtii_AC936	0	1	4	2	0	0	0	2	4	0	0	2	3	0	0
Sericocomopsis_pallida_AC0987	0	1	4	1	0	1	0	1	3	0	1	1	2	0	0
Sericorema_remotiflora_AC0876	0	0	2	0	0	2	1	3	2	0	0	3	2	0	0
Sericorema_sericea_AC0878	0	0	2	1	0	2	1	3	2	0	0	3	2	0	0
Sericostachys_scandens_AC0042	0	1	4	1	0	1	0	1	3	0	1	2	2	0	0
Volkensinia_prostrata_AC1201	0	1	3	1	0	2	0	1	4	0	0	0	1	1	0

Appendix 3.E: Alignment statistics of the combined plastid dataset for molecular analyses

	trnK/matK		*rpl16*	*trnL-F*					
Dataset including hotspots	*trnK* intron (I; II)	*matK*	*rpl16*	*trnT-L* spacer	*trnL-F* exon 5`	*trnL-F* intron	*trnL-F* exon 3`	*trnL-F* spacer	*trnF*
Position in the alignment	1-923; 2508-3148	924-2507	3149-4806	4807-4894	4895-4981	4982-5939	5940-5990	5991-6360	6361-6419
Length range	0-749; 0597	498-1536	0-1205	0-80	0-86	0-687	0-51	0-330	0-55
Mean length (SD)	696.5 (106.2); 288.8 (158.9)	1486.1 (146.7)	988.7 (224.3)	36.7 (23.3)	61.6 (33)	561.6 (197.6)	45.6 (15.7)	283.8 (100.3)	38.1 (16.5)
GC content (%)	29.6; 35.3	31,6	29,6	31,9	44,1	27,2	43,6	32,2	48,1
Hotspots	2; 0	0	6	0	1	5	0	2	0
Hotspot postion in the aligment	867-874, 904-921; -	-	3211-3215, 3357-3362, 3484-3490, 4023-4030, 4133-4207, 4266-4290	-	4937-4943	5122-5144, 5177-5186, 5327-5336, 5412-5419, 5629-5686	-	6023-6028, 6287-6296	-
Inversions	0	0	1	0	0	1	0	0	0
Inversion postion in the aligment	-	-	4049-4087	-	-	5412-5516	-	-	-
Dataset excluding region incomplet endings and hotspots; and reinverted inversions									
Position in the alignment	1-865; 2448-2683	866-2447	2684-3945	3946-3991	3992-4069	4070-4898	4899-4948	4949-5303	5304-5328
Length range	0-727; 0-214	497-1537	0-1002	0-45	0-78	0-646	0-50	0-323	0-25
Mean length (SD)	679.4 (102.9); 190.8 (36.8)	1486.3 (147.0)	884.0 (194.7)	27.4 (16.6)	58.4 (31.5)	532.8 (187.2)	44.7 (15.3)	277.6 (97.9)	18.1 (7.1)
Variable characters (%)	28.2; 29.7	31	30,7	28,3	15,4	20,5	10	35,5	28
Informative characters (%)	13.9; 14	14,3	14,3	13	6,4	7,6	4	13,8	12
Number of coded indels	92; 18	17	133	6	2	98	0	20	1

Appendix 3.F: Ancestral states of characters 1-24 for the achyranthoid subclade II.

The pie charts represent probabilities for ancestral states as reconstructed with BayesTraits and plotted on the maximum clade credibility tree obtained by Bayesian Inference of the combined plastid dataset. See Appendix 3.C for the definition of characters and their states as well as the full matrix in Appendix 3.D.



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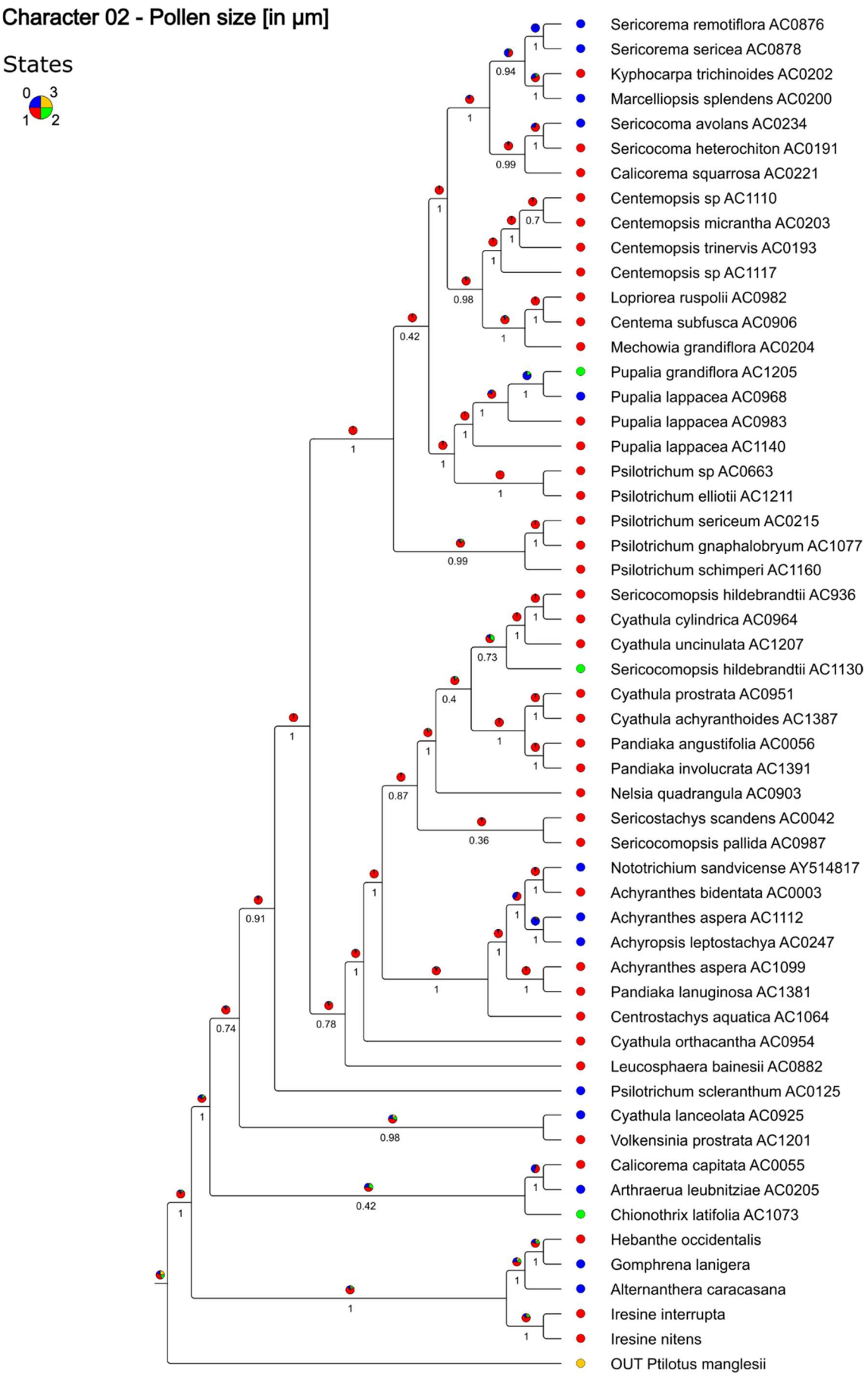

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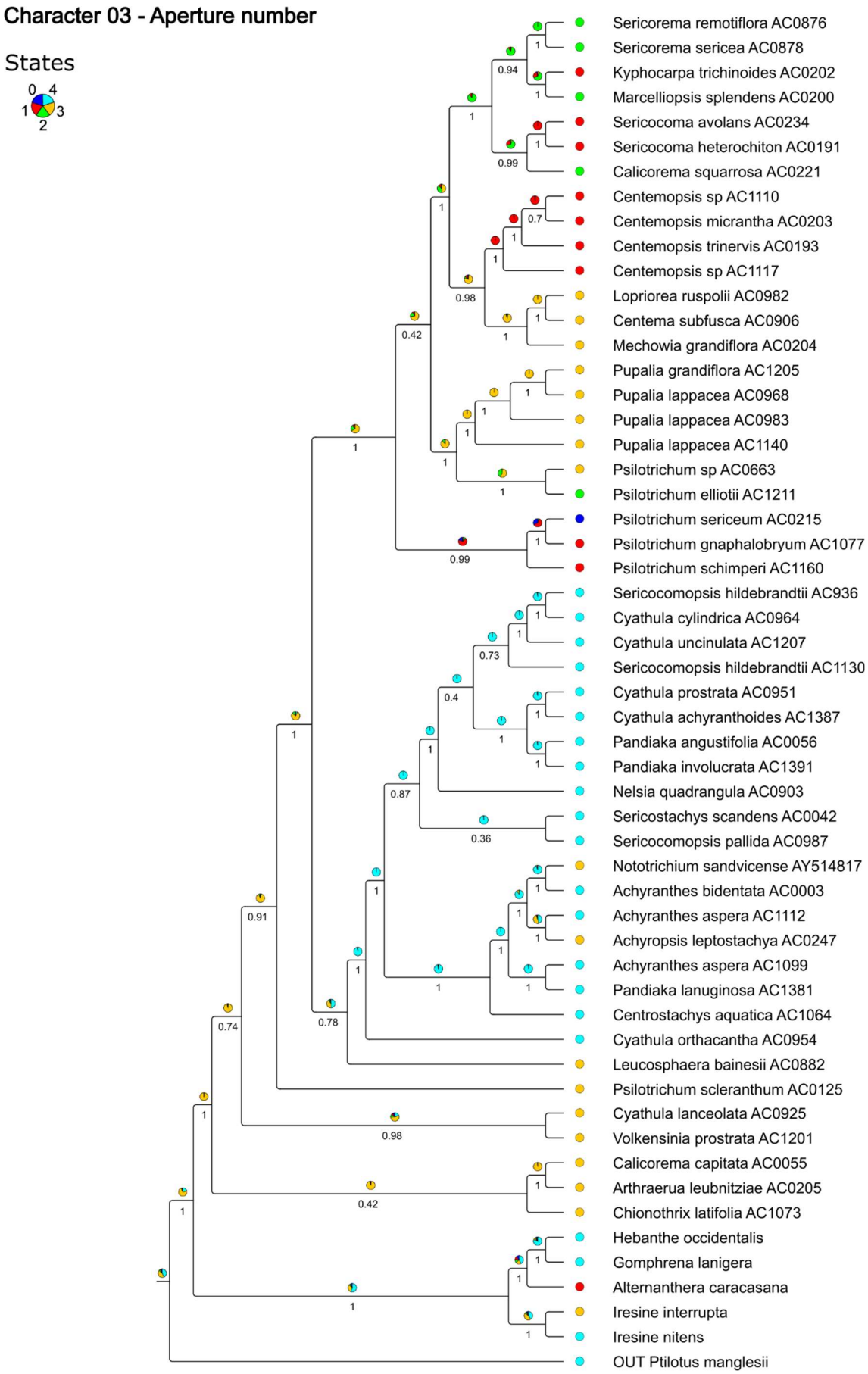

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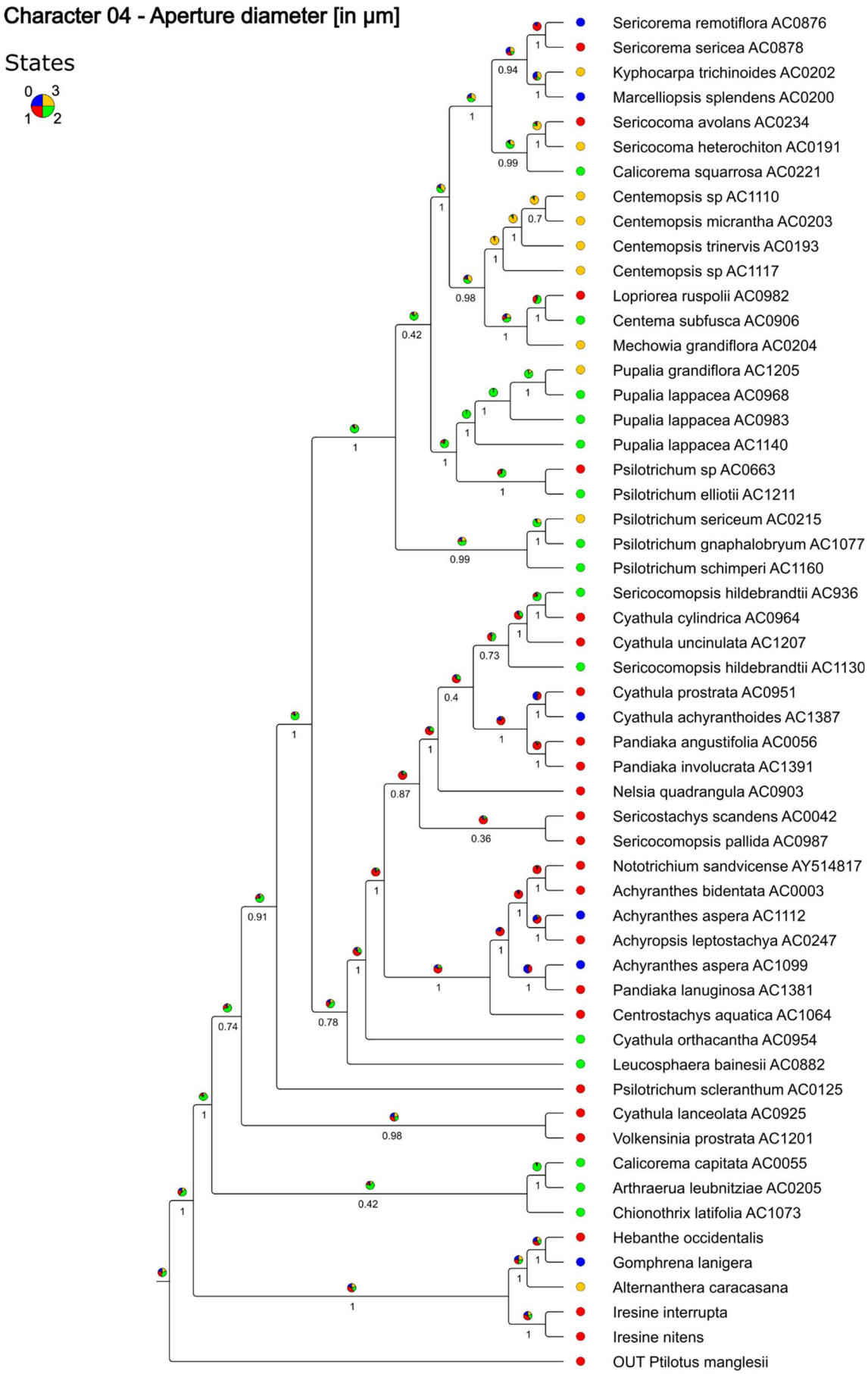

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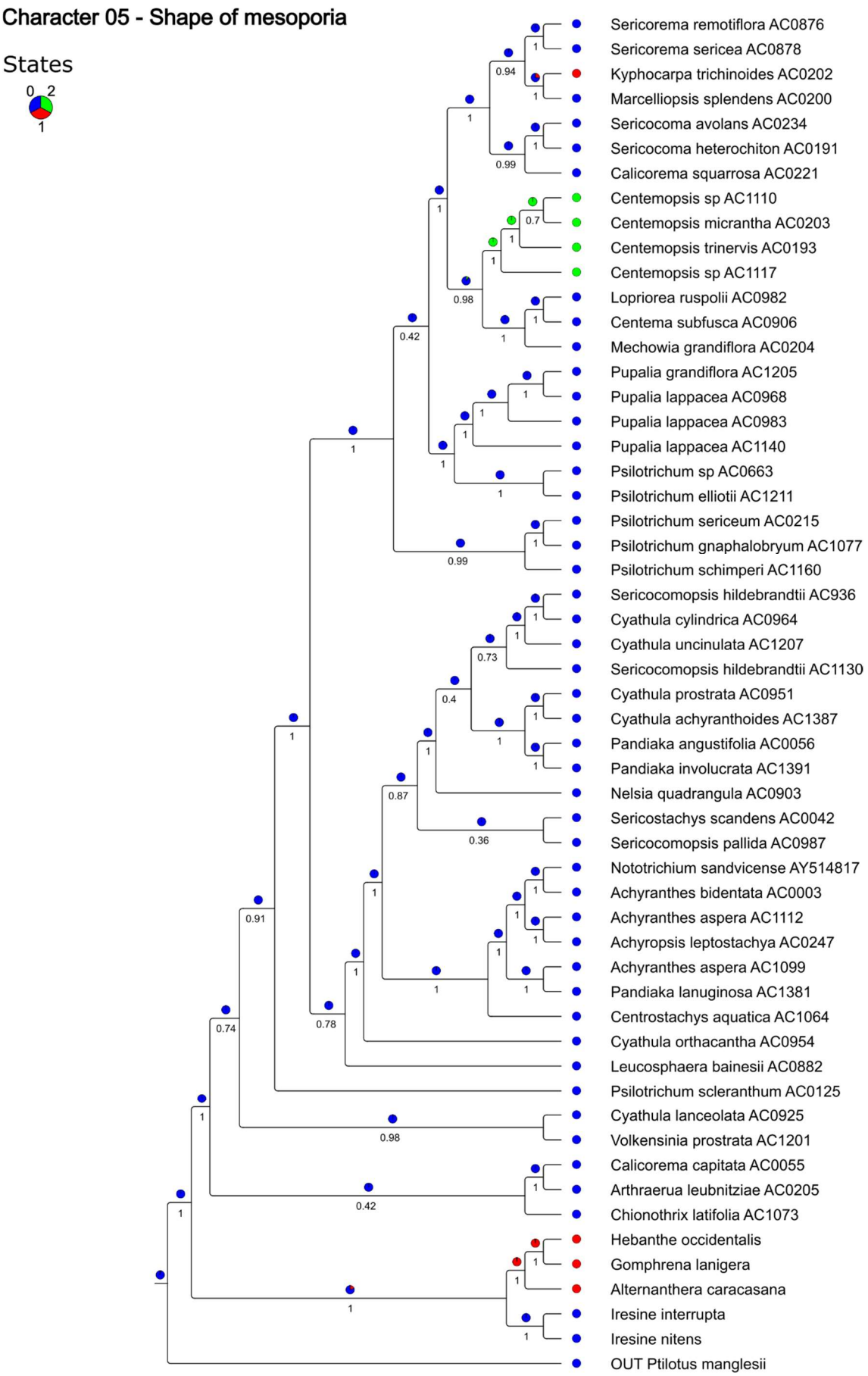

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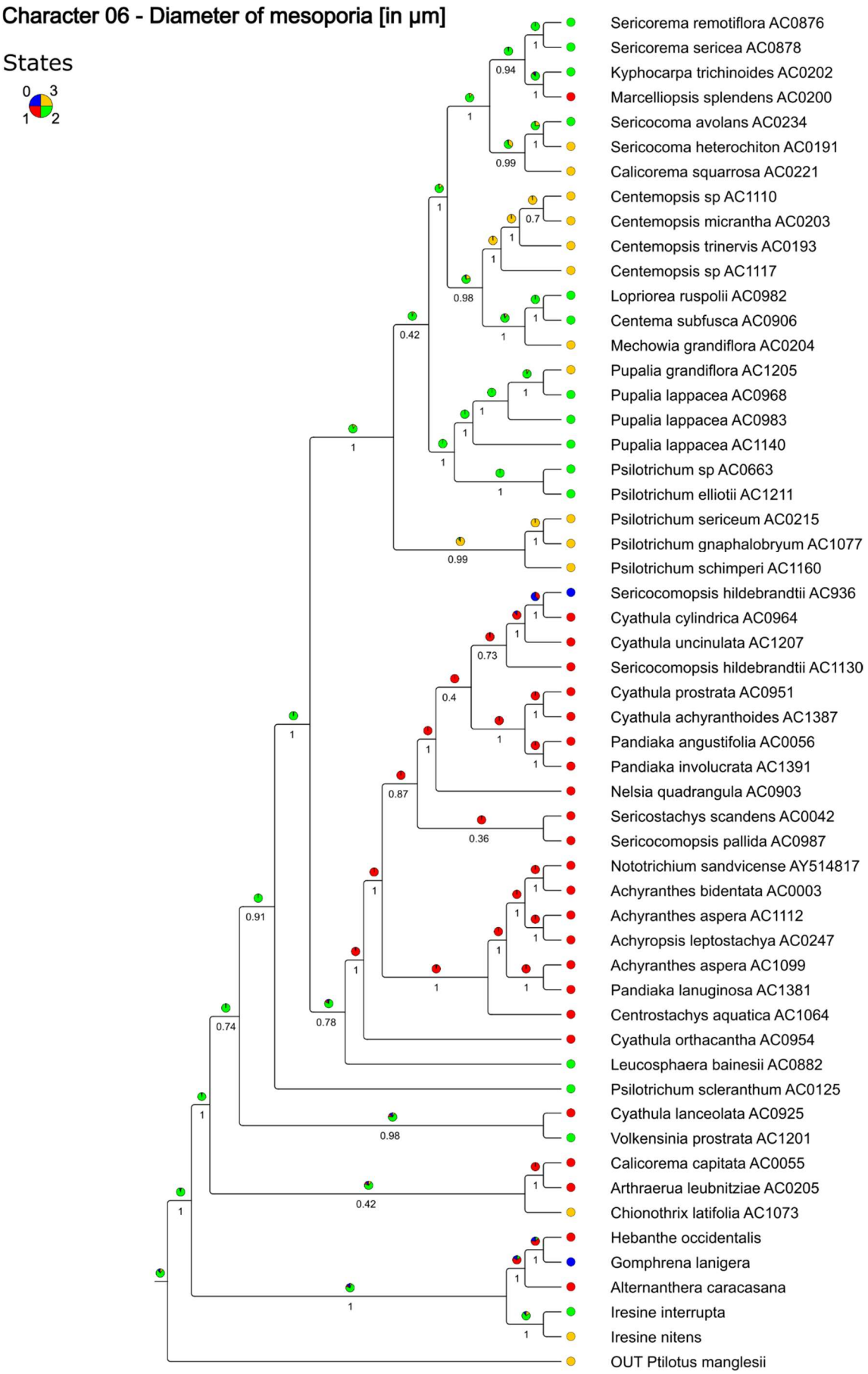

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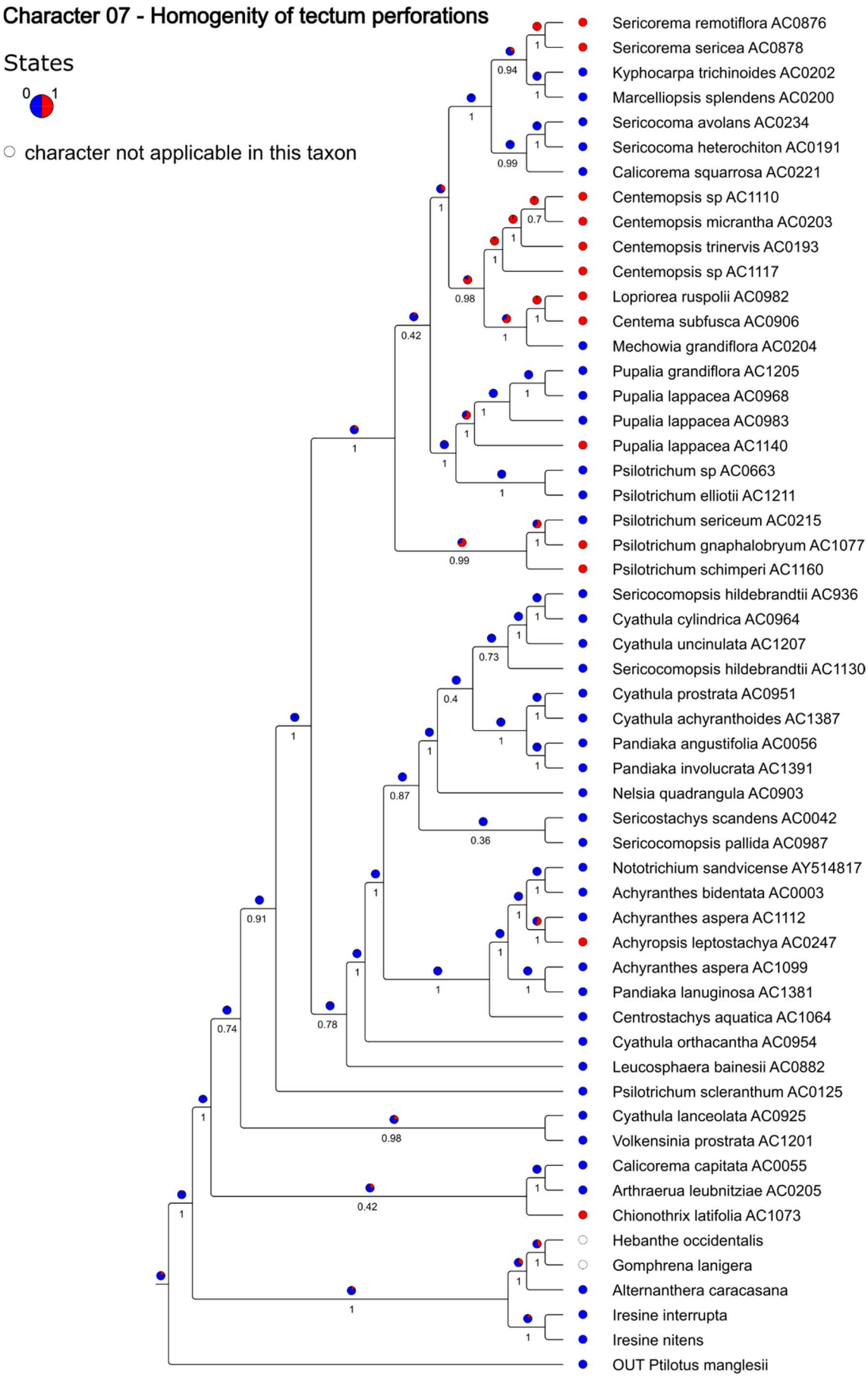

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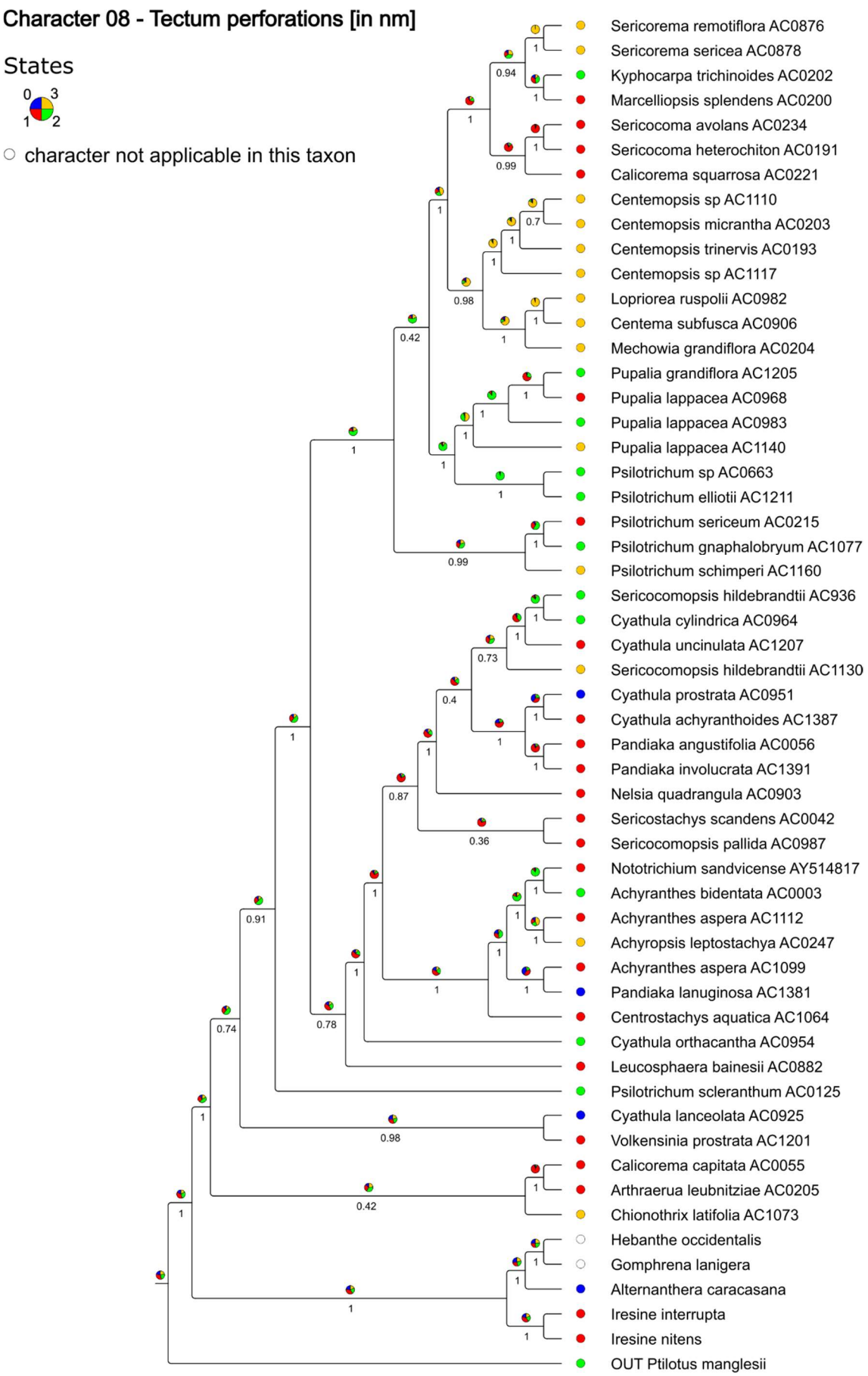

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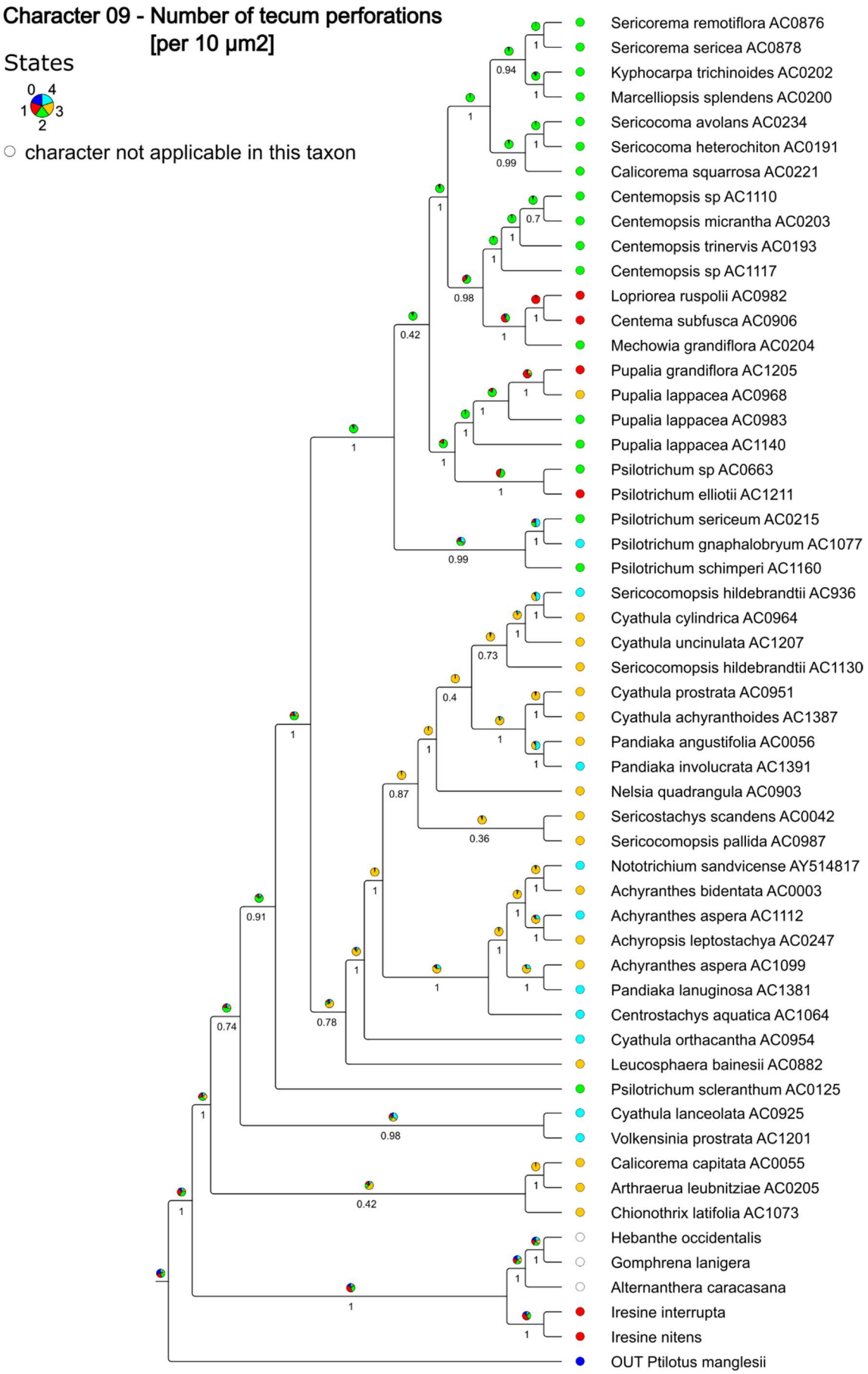

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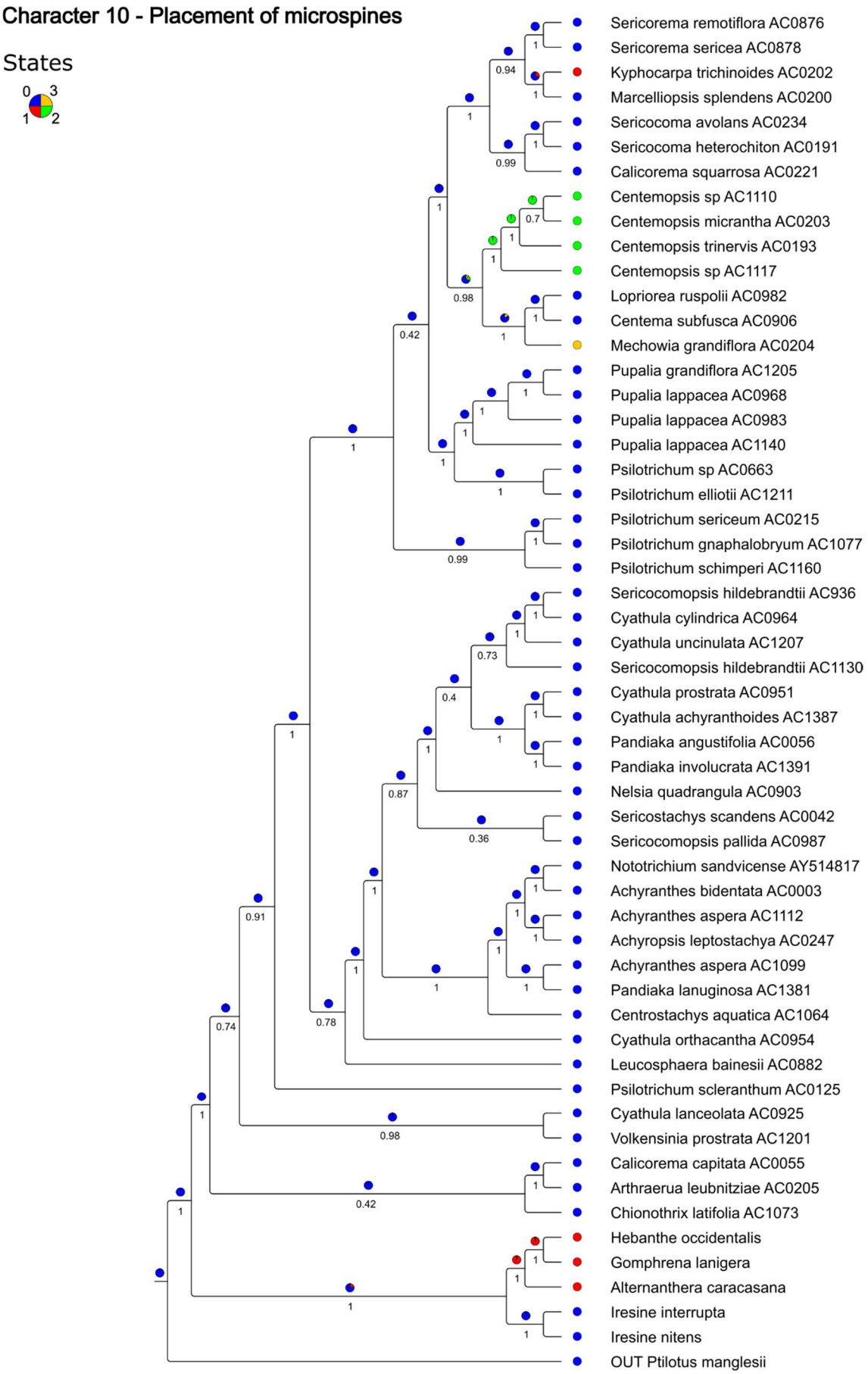

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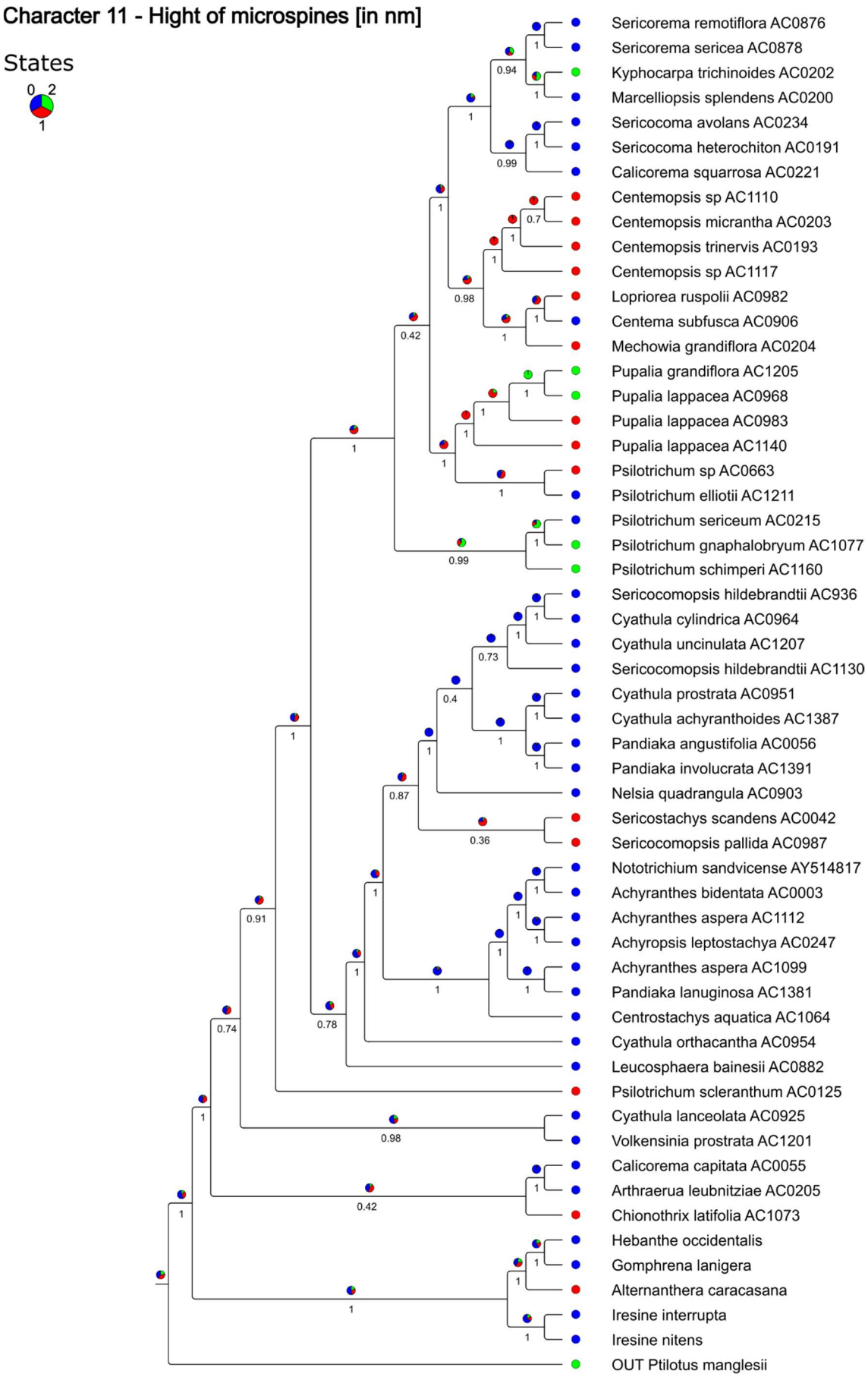

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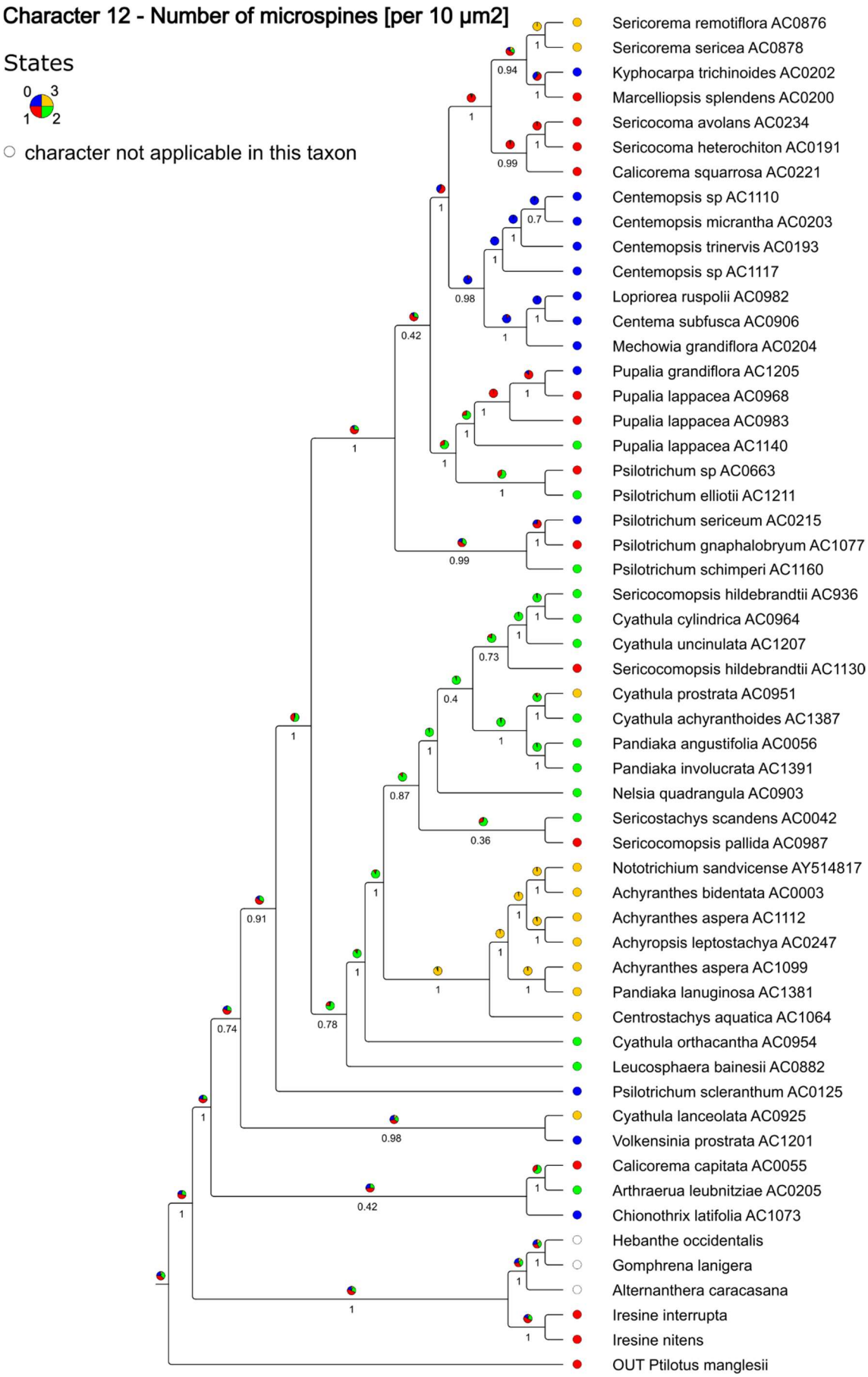

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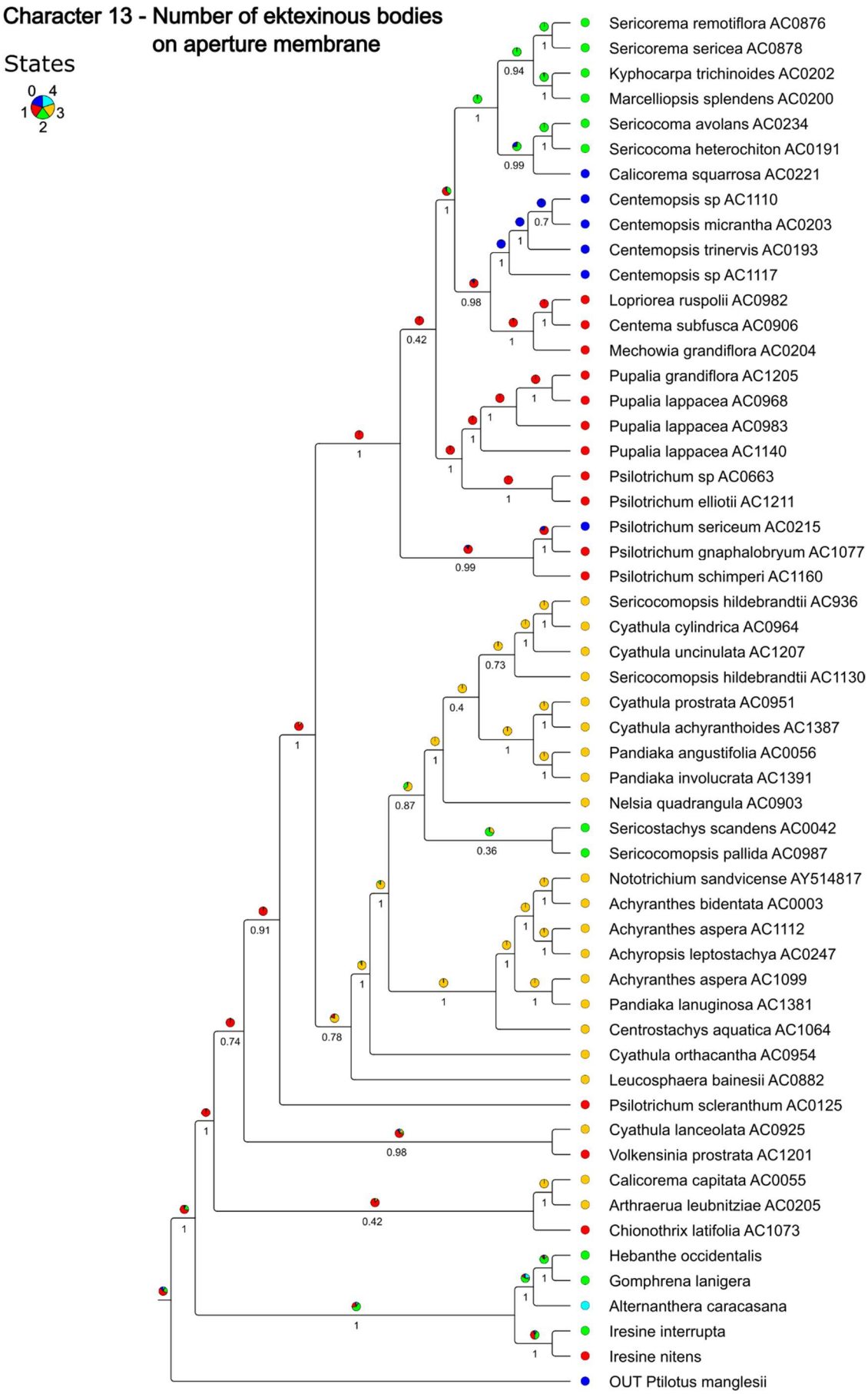

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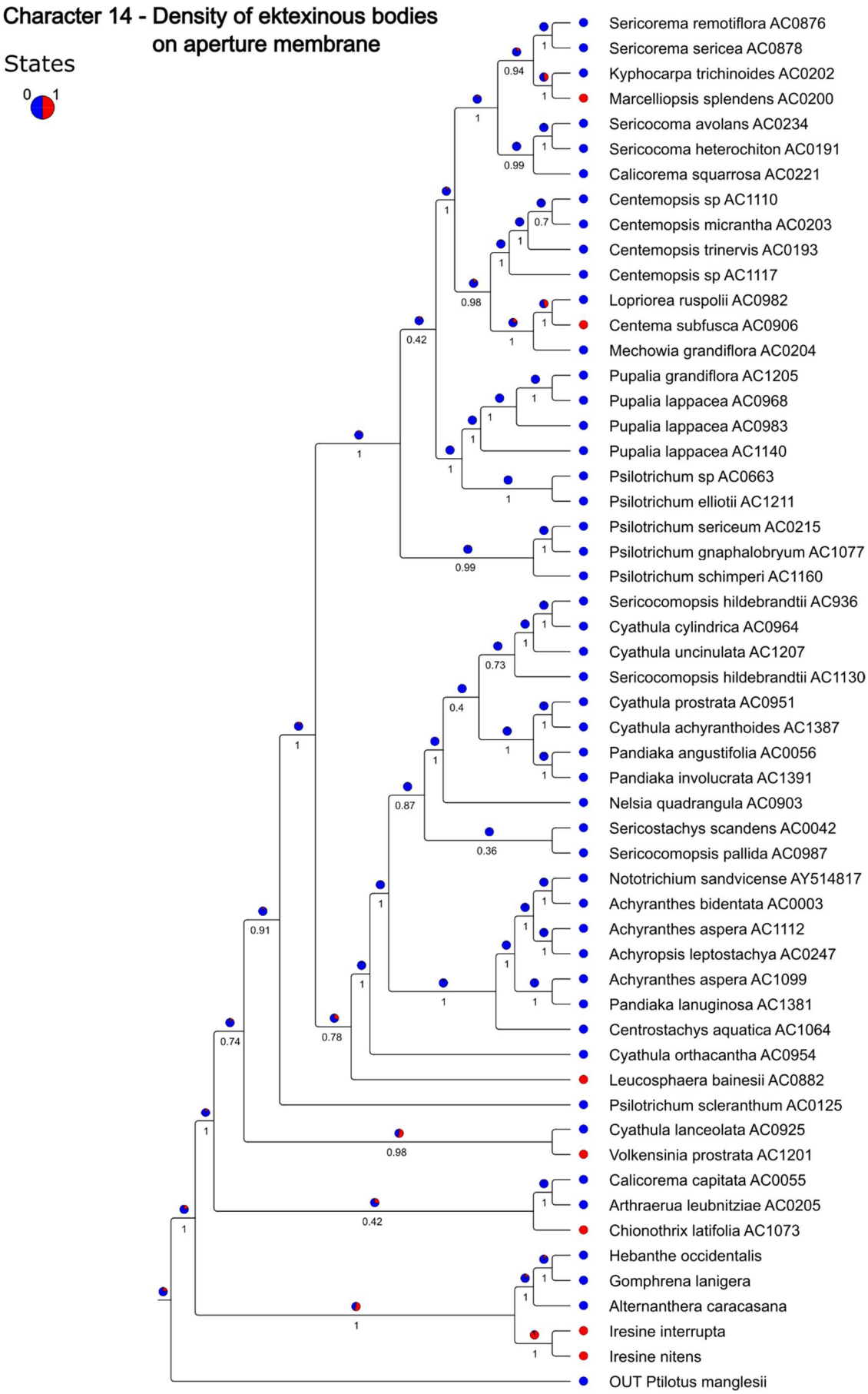

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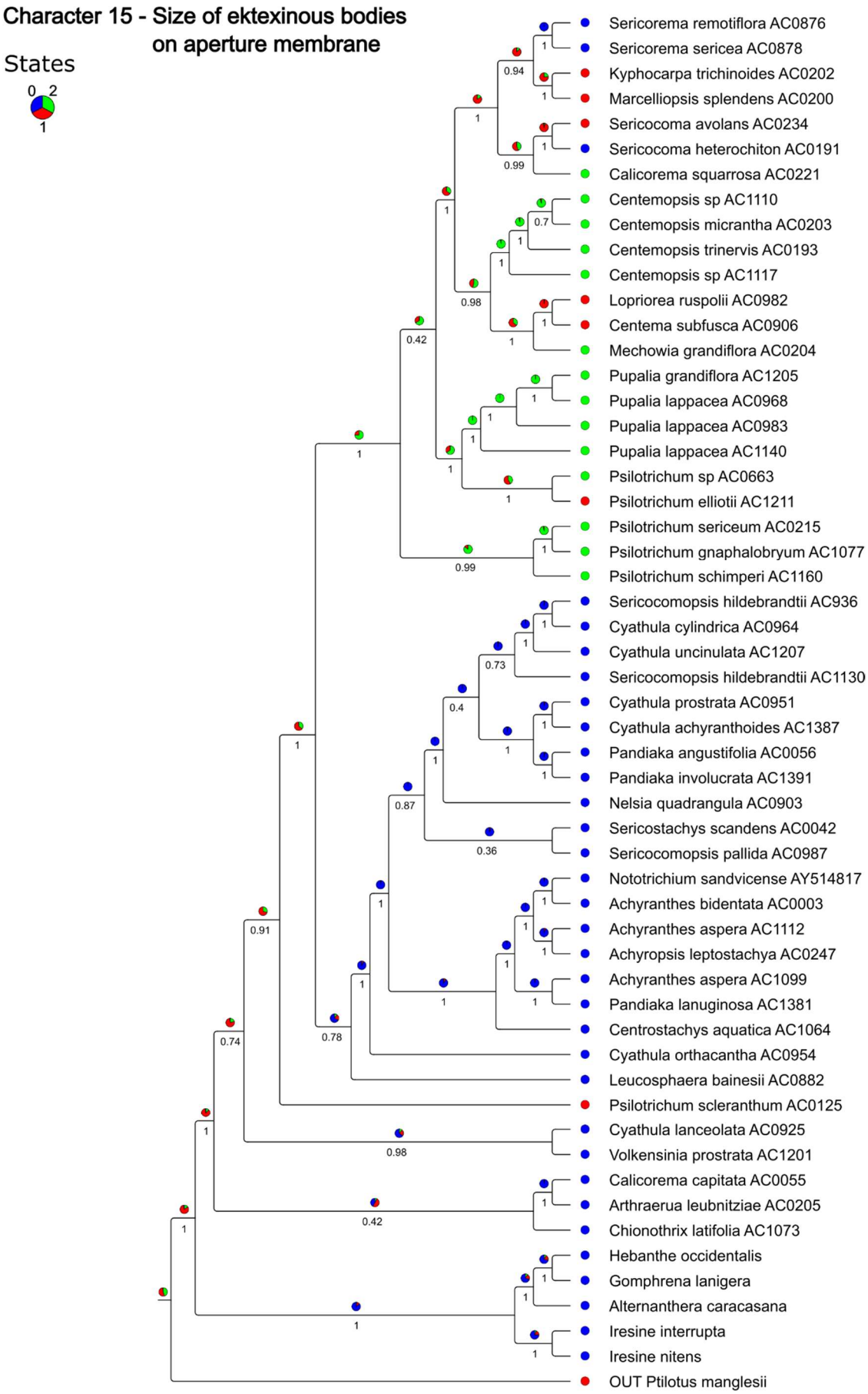

– Chapter 4 –

Phylogenetics, morphological character evolution and taxonomy of the achyranthoid clade (Amaranthaceae): Evidence to make the genus Cyathula *Blume monophyletic and to resurrect a third species of the former genus* Sericocomopsis *Schinz in East Africa*

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4.1 Introduction

The achyranthoid clade of the Amaranthaceae was newly recovered by Müller & Borsch (2005a, 2005b) and shown to be constituted mainly by African taxa. The Achyranthoids with 31 genera accepted currently were estimated to comprise c. 140 species (Di Vincenzo & al., 2018) and form the second most speciose clade in the Amaranthaceae s.str. (with 800 species estimated in total) after the neotropical Gomphrenoids (c. 380 species; Sánchez del Pino & al., 2009). In terms of genera, the achyranthoid clade represents a high proportion (nearly 40%) of a total of 79 genera: Hernández-Ledesma & al. (2015) recognized 79 genera, but included *Gossypianthus* Hook., *Lithophila* Sw.and *Philoxerus* R.Br. which no longer remain accepted (Ortuño & Borsch, 2020); however, *Wadithamnus* T.Hammer & R.W.Davis, *Paraerva* T.Hammer and *Ouret* Adans. were subsequently added (Hammer & al., 2017, 2019). According to Townsend (1993), who derived his classification mainly from Schinz (1893, 1934), all taxa of the achyranthoid clade had been assigned to the subtribe Aervinae of the tribe Amarantheae. However, this subtribe was shown to be not monophyletic (Müller & Borsch, 2005a, 2005b; Di Vincenzo & al., 2018) because *Aerva* Forssk., *Nothosaerva* Wight, *Ptilotus* R.Br. (Hammer & al., 2017, 2019) and allies constitute an independent clade from the achyranthoid clade that diverges earlier than the divergence of the Achyranthoids from the unilocular Gomphrenoideae (Müller & Borsch 2005b).

Compared to Müller & Borsch (2005a, 2005b), Di Vincenzo & al. (2018) implemented a more extensive phylogenetic analysis of the achyranthoid clade, both in terms of sequence data (*trnK/matK*, *rpl16*, *trnL-F* and nrITS) and taxon sampling (inclusion of nine additional achyranthoid genera and c. 60 additional achyranthoid species). As a result, an increased support for the achyranthoid clade (1.00 PP, 97% MP-JK, 100% ML-BS) itself was found. Di Vincenzo & al. (2018) discovered two main subclades within the achyranthoid clade (informally called achyranthoid subclade I and II), whereas genera such as *Arthraerua* (Kuntze) Schinz, *Chionothrix* Hook.f. and *Volkensinia* Schinz were placed as early diverging lineages outside the two subclades. Subclade I comprises 12 genera, including *Calicorema* Hook.f., *Centemopsis* Schinz, *Psilotrichum* Blume and *Pupalia* Juss. Achyranthoid subclade II comprises nine genera: *Cyathula* Blume, *Nelsia* Schinz, *Pandiaka* Hook.f., *Sericocomopsis* Schinz, *Sericostachys* Gilg & Lopr. ex Lopr., and then *Achyranthes* L., *Achyropsis* (Moq.) Hook.f., *Centrostachys* Wall. and *Nototrichium* Hillebr. The first five and the latter four genera form sister clades, respectively. *Achyranthes* is paraphyletic to *Achyropsis* and *Nototrichium,* and altogether they are sister to the monotypic *Centrostachys aquatica* L., whereas *Cyathula* and *Sericocomopsis* are polyphyletic (Di Vincenzo & al., 2018). Here we focus on the achyranthoid subclade II, members of which are representatively compiled within their natural habitats in Figure 4.A, which also shows their morphological diversity.

Figure 4.A: Morphological diversity of Achyranthoids II shown in their natural habitats in Ethiopia.
A: *Cyathula cylindrica*; **B:** *Sericocomopsis hildebrandtii* growing together with *Achyranthes aspera*; **C:** *Cyathula orthacantha*; **D:** *Achyranthes aspera*; **E**: *Cyathula uncinulata*; **F:** *Cyathula prostrata*; **G:** *Centrostachys aquatica*; **H:** *Sericocomopsis pallida*; **I:** *Pandiaka angustifolia*; **J:** *Cyathula polycephala*.

The plastid and nrITS trees of Di Vincenzo & al. (2018) further indicated that not all currently applied taxon concepts at species level reflect monophyletic species. The most striking case became evident for the genus *Sericocomopsis*, with three separate lineages for only two currently accepted species. One of these lineages comprises all samples of *S. pallida* (S.Moore) Schinz and was found in a polytomy with *Sericostachys scandens* L. Samples of *S. hildebrandtii* Schinz constituted the other two clades, one of which (AC0955, AC1084, AC1094, all from Ethiopia) was sister to *Cyathula cylindrica* Moq., whereas two other individuals (AC0961, from Ethiopia, Sidamo region and AC1130 from Kenya, Mwingi District) were recovered as a clade in a more isolated position as sister to the whole clade of *C. cylindrica* plus *S. hildebrandtii* (AC0955, AC1084, AC1094), *C. uncinulata* (Schrad.) Schinz, *C. polycephala* Baker, *C. capitata* Moq., and *C. tomentosa* (Roth) Moq. Individuals of the two *S. hildebrandtii*-clades showed differences in the density of hairs within inflorescences and in leaf morphology underscoring the need for a closer examination of morphological characters in comparison with other members of the achyranthoid II clade but also the sampling of further

individuals to test species limits. As a consequence of this high level of polyphyly, the whole achyranthoid subclade II had to be included into a detailed analysis of genus concepts to provide the basis for a taxonomic treatment of biologically meaningful entities. Current Flora treatments (Townsend 1985, Flora of Tropical East Africa; Townsend 2000, Flora of Ethiopia) distinguish only two species of *Sericocomopsis*: *S. hildebrandtii* with simple hairs and *S. pallida* with stellate hairs.

Most Achyranthoids are dispersed by animals. For that reason, many of the species exhibit adaptations such as hooks and spines which originated multiple times within the achyranthoid clade by modifications of sterile flowers and other inflorescence parts (Di Vincenzo & al., 2018). The reconstruction of ancestral character states for modified sterile flowers suggested at least 14 independent origins in early branching achyranthoid lineages and within subclade I and II since the Miocene (Di Vincenzo & al. 2018). Considering that the genera in Amaranthoideae were historically defined to a large extent on the basis of inflorescence characters including the presence and absence of modified sterile flowers (Schinz, 1893, 1934; Cavaco, 1962; Townsend, 1993), it is not surprising that several of these genera are now found as non-monophyletic.

The earliest genus described from the achyranthoid clade is *Achyranthes* (Linnaeus, 1753; in *Species Plantarum*). Generic circumscriptions considerably varied over time, and some of this was due to diverging interpretations of the Linnaean genus concept. Linnaeus classified *Achyranthes* into the class ‘Pentandria’ and order ‘Monogynia’ based on the presence of five stamens and one pistil and included five species in the genus (*A. aspera* L., *A. lappacea* L., *A. lanata* L., *A. repens* L., *A. corymbosa* L.). Today these are found in the genera *Achyranthes*, *Pupalia* Juss., segregated by Jussieu (1803) with *A. lappacea* as type species, *Aerva* Forssk. and *Alternanthera* Forssk., as well as *Polycarpaea* Lam. (Caryophyllaceae). Except *Achyranthes*, only *Pupalia* belongs to the Achyranthoids, but to subclade I. Endlicher (1837), who established a classification system for the Amaranthaceae with three tribes,

accepted these four genera. Moquin-Tandon (1849) further subdivided the now more narrowly defined genus *Achyranthes* into the sections *Cadelari* Adans., *Achyropsis* Moq., and *Pandiaka* Moq., of which the latter two were raised to generic rank by Bentham & Hooker (1880). Schinz (1893) divided *Achyranthes* into three subgenera (*Achyropsis* Moq., *Euachyranthes* Schinz, *Pandiaka* Moq.), of which one was further split into two sections (sect. *Aspera* Boerl. and sect. *Centrostachys* Wall. of subg. *Euachyranthes*) but in the second edition of *Die natürlichen Pfanzenfamilien* (Schinz 1934) he shifted to accepting generic rank for *Achyropsis* and *Pandiaka*, like for *Achyranthes*. These are also the concepts for these three genera as used until now (Townsend 1993, Hernandez-Ledesma et al. 2015).

In order to complete the picture, it may be noted that Standley (1915) reported the nomenclatural type species to be *Achyranthes repens* (= *Alternanthera* in tribe Gomphreneae), although the name *Achyranthes* commonly had been applied to a different group of species (tribe Achyrantheae) at that time, and implemented various new combinations according to his genus concept. Later, a proposal to conserve the genus name *Achyranthes* L. with its type species *A. aspera* L. selected by Hitchcock (1929; in Sprague, Nom. Prop. Brit. Bot.: 135) was accepted (Jarvis, 1992).

Cyathula Blume [non Lour.; *Cyathula* Lour. has been shown to be synonym of *Achyranthes* L. and thus was rejected vs. *Cyathula* Blume (nom. cons.); compare Appendix III in the *Shenzhen Code* (Turland & al., 2018)] also suffered a complex nomenclatural history since its publication in 1826. Moquin-Tandon (1849) split *Cyathula* into the two sections *Polyscalis* Moq. and *Telostachys* Moq. Schinz (1893) classified *Cyathula* into the sections *Eucyathula* Schinz with cylindrical or capitate inflorescences [to which he assigned *C. cylindrica* (Bojer) Moq., *C. schimperiana* (Hochst.) Moq., *C. globulifera* (Bojer) Moq., *C. capitata* (Wall.) Moq., *C. ceylanica* Hook.] and *Achyranthoideae* Schinz with slender inflorescences [to which he assigned *C. achyranthoides* (Kunth) Moq., *C. geminata* Moq., *C. prostrata* (L.) Blume, *C. triuncinata* Moq.]. In contrast, Standley (1917) accepted *Cyathula* in the sense of Loureiro (1790) with the type species *C. geniculata* Lour., which was considered conspecific with *Achyranthes aspera* by different authors, e.g., (Hiern, 1900; Moore, 1925; Hokche & al., 2008). Schinz (1934) did not accept the classification of Standley (1917) and maintained his classification from 1893. He later also described the monotypic genus *Nelsia* (Schinz 1911) based on *Sericocoma quadrangula* Engl. as type species. Cavaco (1962) accepted the sections of Schinz and described the additional section *Nelsioideae* Cavaco with the sole and type species *C. pobeguinii* Jac.-Fel.

The genus *Sericocomopsis* Schinz was described by Schinz (1895) as characterized by plumose or sericeous inflorescences which are either globose or spicate, with flowers arranged in 3-flowered cymes, in which sterile flowers are completely missing, pseudostaminodes are fimbriate or missing, and the ovary is glabrous or pilose. Within the new genus, he described a new species, *S. hildebrandtii* Schinz, and further transferred two species from the genus *Sericocoma* Fenzl ex Endlicher to *Sericocomopsis*, namely *S. bainesii* (Hook.) Schinz and *S. pallida* (S.Moore) Schinz. *Sericocomopsis pallida* was included in the dichotomous key and Schinz indirectly referred to its basionym by citing its author in

brackets, which was sufficient for the valid publication of new combinations before 1 January 1953 according to the International Code of Nomenclature for algae, fungi, and plants 2018 (Turland & al., 2018); Art. 41.3). In a later publication (Schinz, 1911) Schinz referred to the basionym and added a detailed species description of *S. pallida*.

Two years after Schinz transferred *Sericocoma bainesii* Hook.f. to his genus *Sericocomopsis*, Gilg (1897) transferred this species to his new genus *Leucosphaera* Gilg. He characterized the genus by sericeous, globose inflorescences, with partial florescences constituted of two fertile flowers only, in which pseudostaminodes are missing. Phylogenetic results showed *Leucosphaera bainesii* (Hook.f.) Gilg. to be distantly related to species of both *Sericocomopsis* and *Sericocoma* (Di Vincenzo & al. (2018). The transfer of *Sericocomopsis pallida* and *S. hildebrandtii* to the genus *Kyphocarpa* (Clarke 1913) was not maintained by other authors.

The specific objectives of this investigation are to further test relationships within subclade II of the Achyranthoids and to evaluate currently used genus concepts in a phylogenetic context and by studying morphological characters. We further aim at clarifying species limits of *S. hildebrandtii* which requires a closer examination of the genus *Cyathula* as a whole, considering that two groups of individuals currently identified as *S. hildebrandtii* were found nested in different parts of *Cyathula*. Due to the complex classification history of this group of Amaranthaceae, and the need to closely evaluate previously published taxon concepts at genus and also species levels, another goal was to develop a comprehensive checklist of the achyranthoid subclade II to form part of the ongoing work on an Amaranthaceae species level taxonomic backbone in the monographic synthesis of the order Caryophyllales (Borsch & al., 2015). We aimed at integrating all names for the achyranthoid subclade II to complement a new treatment at the genus level.

4.2 Methods

4.2.1 Field work and specimen information

The sample set presented in Di Vincenzo et al. (2018) that was largely achieved by field work in Ethiopia and Kenya in 2012 and 2013 was complemented by further material available at B and ETH (partially collected in the frame of the DFG-funded Amaranthaceae project from 2001 onwards by different researchers). Additional specimens for molecular analyses came from the Botanic Garden Meise (BR), and for physical examination of (type-) specimen, material was available on loan from the herbaria PRE, GOET, M, NYBG, GH, S, C, HBG, BR, P, BM, and K. Detailed information on samples used in analyses is provided in Appendix 4.A.

4.2.2 Composition of molecular datasets

We generated two datasets focusing with a dense taxon sampling on the Achyranthoids II of Amaranthaceae but also including a representation of genera from Achyranthoids I and early branching achyranthoid lineages: i) **dataset A** is a combined plastid dataset, of the regions *trnK*/*matK*, *rpl16*, *trnL-F*, and in order to improve tree resolution and node support, we complemented data from all samples with sequences of the plastid intergenic spacer between *rpl32-trnL*$_{UAG}$ and the two spacers in the *rps4-trnT-trnL*$_{UAA}$ region; and ii) **dataset B** is a nuclear ribosomal dataset which consisted of internal transcribed spacer (nrITS) sequences. Sequences for ITS, *trnK*/*matK*, *rpl16* and *trnL-F* of a large fraction of samples were used from the second chapter (Di Vincenzo & al., 2018). R*pl32-trnL*$_{UAG}$, *rps4-trnT-trnL*$_{UAA}$ rank among the most variable plastid regions: Shaw & al. (2007) and Korotkova & al. (2014) showed fragments of approx. 1,000 and 1,200 nt that promised efficient lab work and yielded good hierarchical phylogenetic signal in other analyses of Caryophyllales lineages (e.g., Fassou & al. 2022). We added further species of *Pandiaka* and *Cyathula* not analyzed in phylogenetic studies before, and to test species limits within *Sericocomopsis*, we extended the sampling of *S. pallida* and *S. hildebrandtii*, to better represent the geographic range and morphological variation of putative species.

4.2.3 Processing of DNA samples and generation of molecular datasets

For DNA isolation and sequencing we followed the same procedures as described in Di Vincenzo & al. (2018). Additional primers used for the regions *rpl32-trnL*$_{UAG}$ and *rps4-trnT-trnL*$_{UAA}$ and for difficult samples of *trnK/matK*, *rpl16* and *trnL-F* are listed in Appendix 4.B. Sanger sequencing was carried out at Macrogen Europe (Amsterdam, The Netherlands). Chromatograms were inspected by eye and corrected for erroneous nucleotide calls. Assembly and a motif-based alignment (as outlined in Löhne & Borsch, 2005) was conducted manually using PhyDE 0.9971 (Müller & al., 2010), building upon the multiple sequence alignments of Di Vincenzo & al. (2018). Areas of uncertain homology were excluded from matrices and inversions were reverse-complemented and coded as presence-absence data. For

coding insertions and deletions, we applied 'simple indel coding' (Simmons & Ochoterena, 2000) as implemented in SeqState 1.4.1 (Müller, 2005). New DNA sequences from this chapter will be submitted to ENA (http://www.ebi.ac.uk/ena/) upon conversion to checklist files with custom Python scripts (https://github.com/michaelgruenstaeudl/annonex2embl). ENA/GenBank accession numbers from already published sequences are given in Appendix 4.A along with the voucher data.

4.2.4 Model selection and phylogenetic analysis

Phylogenetic inference was obtained with maximum parsimony (MP), maximum likelihood (ML) and Bayesian Inference (BI). For the latter two analyses, datasets were annotated and partitioned to allow for independent parameter estimation. Best-fitting models of nucleotide substitution were selected via the Akaike information criterion corrected for small sample sizes (AICc; Akaike, 1974) for each partition with ModelTest-NG v0.1.7 (Darriba & al., 2020), which is implemented in raxmlGUI 2.0.5 (Edler & al., 2021). Results of model testing are summarized in Appendix 4.C and Appendix 4.D. Parsimony analyses were conducted in PAUP* 4.0b10 (Swofford, 2002), and node support was calculated via 10,000 jackknife (JK) replicates. Final ML analyses were conducted by application of the MULTIGAMMA model for dataset A and the MULTICAT model for dataset B in RAxML 8.2.10 (Stamatakis, 2014) with node support calculated via 1,000 rapid bootstrap (BS) replicates, using the RAxMLGui 2.0.4 (Silvestro & Michalak, 2012) as input interface. BI was performed in MrBayes 3.2.7a (Ronquist et al., 2012) available from the CIPRES Science Gateway (Miller & al., 2010). Node support was calculated as posterior probabilities from four independent Markov chain Monte Carlo (MCMC) runs with four chains and 5,000,000 generations each. Trees were sampled every 1,000th generation resulting in 20,004 trees. The burn-in was set to 25%, resulting in a combined posterior tree distribution of 15,004 trees out of which a majority-rule consensus tree was calculated. Convergence of each independent run was assessed based on the standard deviation of split frequencies and the effective sample size (ESS) using Tracer v. 1.7.2 (Rambaut & al., 2018). We rated BS/JK values of 70–84 % and posterior probability of 0.9–0.94 as moderate support, and BS/JK values of 85–100 % and posterior probability of 0.95–1 as strong support.

4.2.5 Dynamic editing of the taxonomic backbone in the EDIT platform

The checklist of all names and taxa included in the achyranthoid subclade II was compiled using the EDIT Platform for Cybertaxonomy, which comprises tools for editing, managing and publishing of taxonomic data (Berendsohn, 2010; Berendsohn & al., 2011; BGBM, 2011+; Ciardelli & al., 2009, EDIT 2008+). The platform is used, inter alia, by the Caryophyllales Network (Borsch & al., 2015; Hernández-Ledesma & al., 2015; Berendsohn & al., 2018, Korotkova & al. 2021), and the taxonomic treatment here included was directly generated from that database. Initially, names were imported from the World Flora Online (2018) taxonomic backbone, including their WFO IDs and taxonomic status

(thus essentially mirroring the treatment in The Plant List v. 1.1; TPL, 2013). Names and protologues were checked against available online resources such as the International Plant Name Index (IPNI, 2000+), Index Nominum Genericorum (ING, 1996+) and Tropicos (1986+). Original publications were ascertained using the Biodiversity Heritage Library (BHL, 2007+; Gwinn & Rinaldo, 2009) and where necessary physically in the BGBM library. Additionally, this dataset was matched with the dataset of the World Checklist of Vascular Plants (WCVP) received from Royal Botanic Gardens, Kew in December 2019 (WCVP, 2019), and subsequently cross-checked with relevant taxonomic treatments as cited in the introduction. Morphological descriptions from these sources were compared with data from own examination of type material in several cases and original publications. If present at the Berlin herbarium or available on loan, type specimen were examined physically (as indicated by "!") and/or examined in a digital form [image!] if available on JSTOR Global Plants (JSTOR, 2004+) or the respective virtual herbaria as indicated in the systematic treatment. For more details on data import and processing of data in the EDIT Platform see Fassou et al. (2022). All nomenclatural changes are presented in the taxonomic treatment, whereas in the phylogenetic trees hitherto used accepted names are annotated.

4.2.6 Assessment of morphological characters and ancestral character state reconstructions (ACSR) for Achyranthoids II

In order to reconstruct the evolution of morphological characters and evaluate in how far they are diagnostic for genus concepts to be applied in the achyranthoid subclade II, we defined a set of 24 characters covering habit and vegetative morphology, inflorescences and floral morphology, similar to Ortuño & Borsch (2020). Character states were mainly assessed through examination of herbarium specimens corresponding to the samples analyzed for the molecular data set A. Regarding the characters "life cycle" and "life form", the label information from specimens was sometimes incomplete, and state information needed to be assessed from morphological descriptions from original publications or included in floral treatments. Definitions of characters and their states are given in Appendix 4.E, the full character matrix is depicted in Appendix 4.F. In cases, where more than one character state is given in a single cell of the character matrix, more than one state has been observed within a single individual (e.g., the "leaf shape" is variable in single individuals, that means one individual possesses leaves with different shapes.

BayesTraits v. 2 (Pagel & al., 2004) was used to infer ancestral character states. ACSR was calculated over 1,000 random samples which were extracted from the post-burn in tree distribution of the Bayesian analyses of dataset A using Mesquite v. 3.70 (Maddison & Maddison, 2021). BayesTraits input was generated with TreeGraph2 v. 2.14.0-771 (Stöver & Müller, 2010). We selected a multistate analysis under MCMC tree optimization, using the Reverse Jump Hyper-prior. The BayesTraits output files were

imported to TreeGraph2 where probabilities of ancestral states were visualized on the maximum clade credibility (MCC) tree.

4.2.7 Testing species limits in *Sericocomopsis hildebrandtii* Schinz

For evaluation of species limits in *S. hildebrandtii*, several individuals per lineage needed to be examined, unlike as it was done for the morphological studies described above in which only a single specimen was investigated to assess character state information. With regard to this quantitatively more extensive investigation, we reduced the number of characters to the most important distinguishing features – the hairiness of the inflorescence (“less hairy” and “dense hairy"), the shape of the leaves (corresponds to char 4 in ACSR), the shape of paracladia (corresponds to char 6 in ACSR), and the curvature of bracteoles at anthesis (corresponds to char 21 in ACSR) – which made it possible to morphological divide all individuals into two groups. Individuals of both groups were then compared to the type specimen of *S. hildebrandtii*, as well as to the type specimen of presently considered synonym names which thus depict priority candidates for naming the individuals of the group which cannot remain as *S. hildebrandtii*. The examined characters were photographed for each individual for documentation and the photographs of one representative per group were compiled for the illustration of character states (Figure 4.D - Figure 4.F).

4.3 Results

4.3.1 Molecular phylogeny

We generated the following new sequences: 26 *trnK/matK*, 24 *rpl16*, 66 *rpl32-trnL*$_{UAG}$, 72 *rps4-trnT-trnL*$_{UAA}$, 20 *trnL-F* and 19 ITS. Dataset A included 75 samples and resulted in a multiple sequence alignment of 8.932 aligned positions including hotspots. The final matrix (excluding hotspots) comprised 7.390 aligned positions and simple indel coding yielded an additional matrix of 416 characters. For detailed alignment statistics compare Appendix 4.G.

Dataset B included 63 samples and sequences and resulted in a multiple sequence alignment of 834 aligned positions including hotspots. The final matrix (excluding hotspots) comprised 704 aligned positions and simple indel coding yielded an additional matrix of 116 characters. For detailed alignment statistics compare Appendix 4.H.

The phylogeny inferred from the concatenated plastid regions (dataset A, Figure 4.B) and the tree from nr ITS (dataset B, Figure 4.C) depicts two principal subclades of Achyranthoids II consisting of *Achyranthes* plus allies and *Cyathula* plus allies. In the plastid tree these appear as sisters (1 BI PP, 99 ML BS, 93 MP JK) to which a maximum supported isolated lineage comprising all individuals of *Cyathula orthacantha* appears as sister. Based on nrITS we found *C. orthacantha* as moderately supported sister to the clade comprising *Achyranthes* and allies (Figure 4.C).

All trees converge on three lineages of *Sericocomopsis,* all of which are nested within subclade II B: i) a lineage of *S. hildebrandtii* occurring as sister to *Cyathula cylindrica, ii)* a lineage, referred to as *Sericocomopsis hildebrandtii* ['meruensis'] in the depiction of phylogenies, occurring as sister to the clade which encompasses *Cyathula cylindrica*, *C. uncinulata*, *C. polycephala*, *C. tomentosa*, *C. capitata*, and *S. hildebrandtii*, and iii) a lineage including all accessions of *S. pallida*. Since individuals of all three lineages appear non-monophyletic in the phylogenetic trees, it is clear that the genus Sericocomopsis, as well as its species cannot be maintained under its current circumscription. *S. pallida* was found as sister to *Sericostachys scandens* in BI, ML and MP analysis of dataset A but this relationship remained weekly supported. Both species together were resolved as sister to all remaining taxa within subclade II B. In BI, ML and MP analysis of dataset B *S. pallida* alone is highly supported as sister to all remaining taxa within subclade II B. The following four species of *Pandiaka* and two species of *Cyathula* had never been included in any molecular analyses before: *P. involucrata* (Moq.) B.D.Jacks, which is the type of the genus, *P. lanuginosa* Schinz, *P. rubrolutea* (Lopr.) C.C.Towns., *P. welwitschii* (Schinz) Hiern, *Cyathula divulsa* Suess. (only ITS could be sequenced for the latter two species) and *C. achyranthoides.* In all analyses, *P. lanuginosa* was resolved as a member of subclade II A together with *Achyranthes*, *Nototrichium* and *Centrostachys*, while the other five species were found as members of subclade II B. *Cyathula divulsa* formed a fully supported clade together with *C. uncinulata*, *C. polycephala* and *C. capitata* in all analyses of dataset B. *Pandiaka involucrata* was

generally found as sister to *P. angustifolia*, whereas the other species, *Pandiaka rubrolutea* and *P. welwitschii,* appear in a subclade together with *C. achyranthoides* and *C. prostrata*, which is the generic type, to which again *C. natalensis* is resolved as sister with high support in the plastid tree (Figure 4.B). However, ITS does not resolve the latter node but shows maximum support for a close relationship of *C. achyranthoides*, *C. prostrata* with *P. rubrolutea* and *P. welwitschii* (Figure 4.C). In contrast, *P. lanuginosa* was resolved in a lineage together with *Achyranthes*, *Nototrichium* and *Centrostachys* by datasets A and B (Figure 4.B, Figure 4.C).

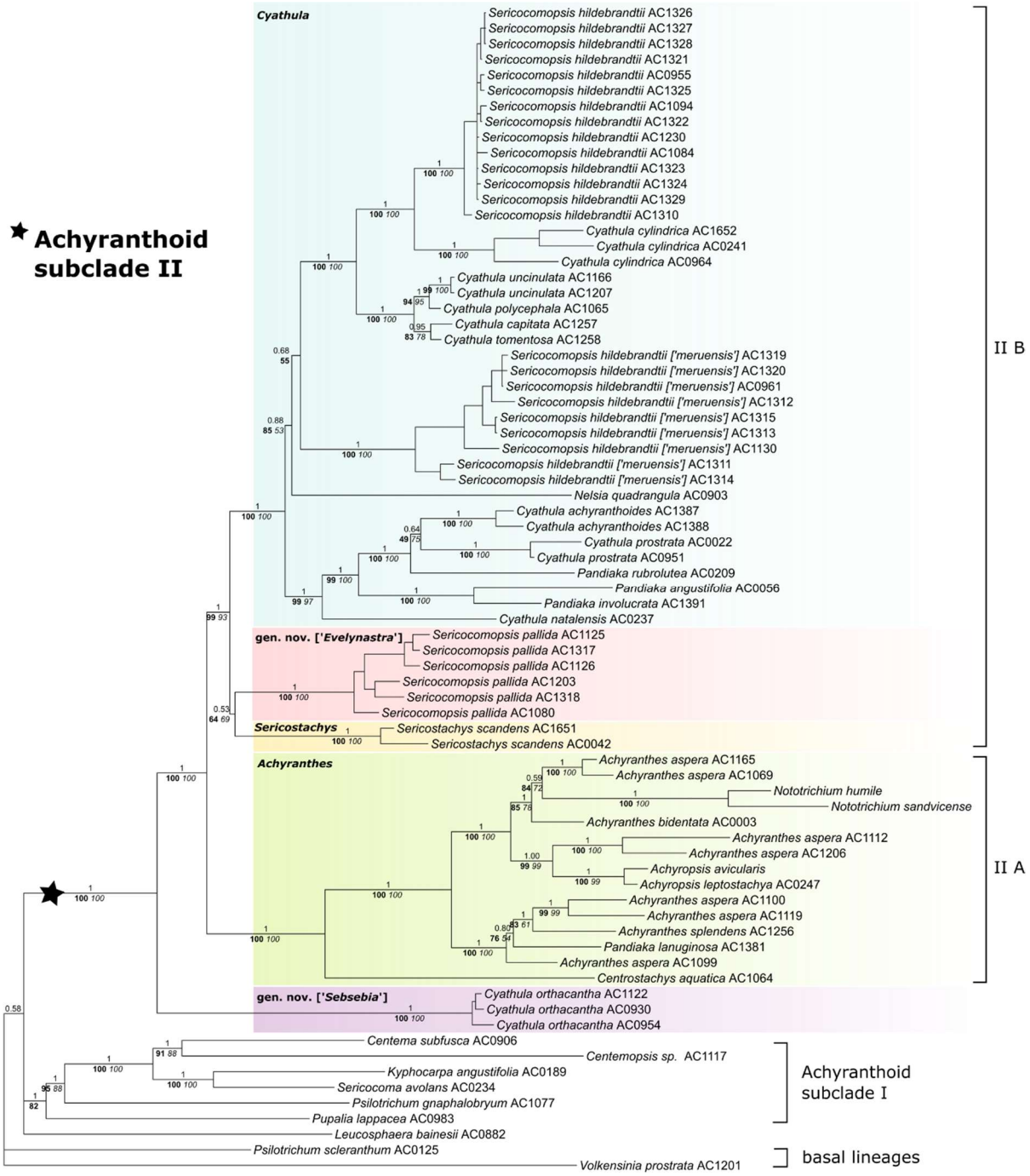

Figure 4.B: Phylogeny of the achyranthoid clade of Amaranthaceae with a focus on subclade II.
The tree topology was obtained via Bayesian inference on dataset A (combined plastid marker). Posterior probabilities of the Bayesian inference are given above branches, bootstrap percentages of the maximum likelihood analyses in bold letters (left) and jackknife percentages of the maximum parsimony analyses in italics (right) are given below branches. Genera within subclade II are shaded in different colours according to the new genus concept with the respective genus names written in bold italics in the upper left side of each clade. For gen. nov. the names in brackets are the informal genus names which are to be published. For *Sericocomopsis hildebrandtii* [‘*meruensis*’] the citation in square brackets corresponds to a present synonym of the species name which needs to be resurrected as the correct name (details are given in the discussion part and in the checklist part).

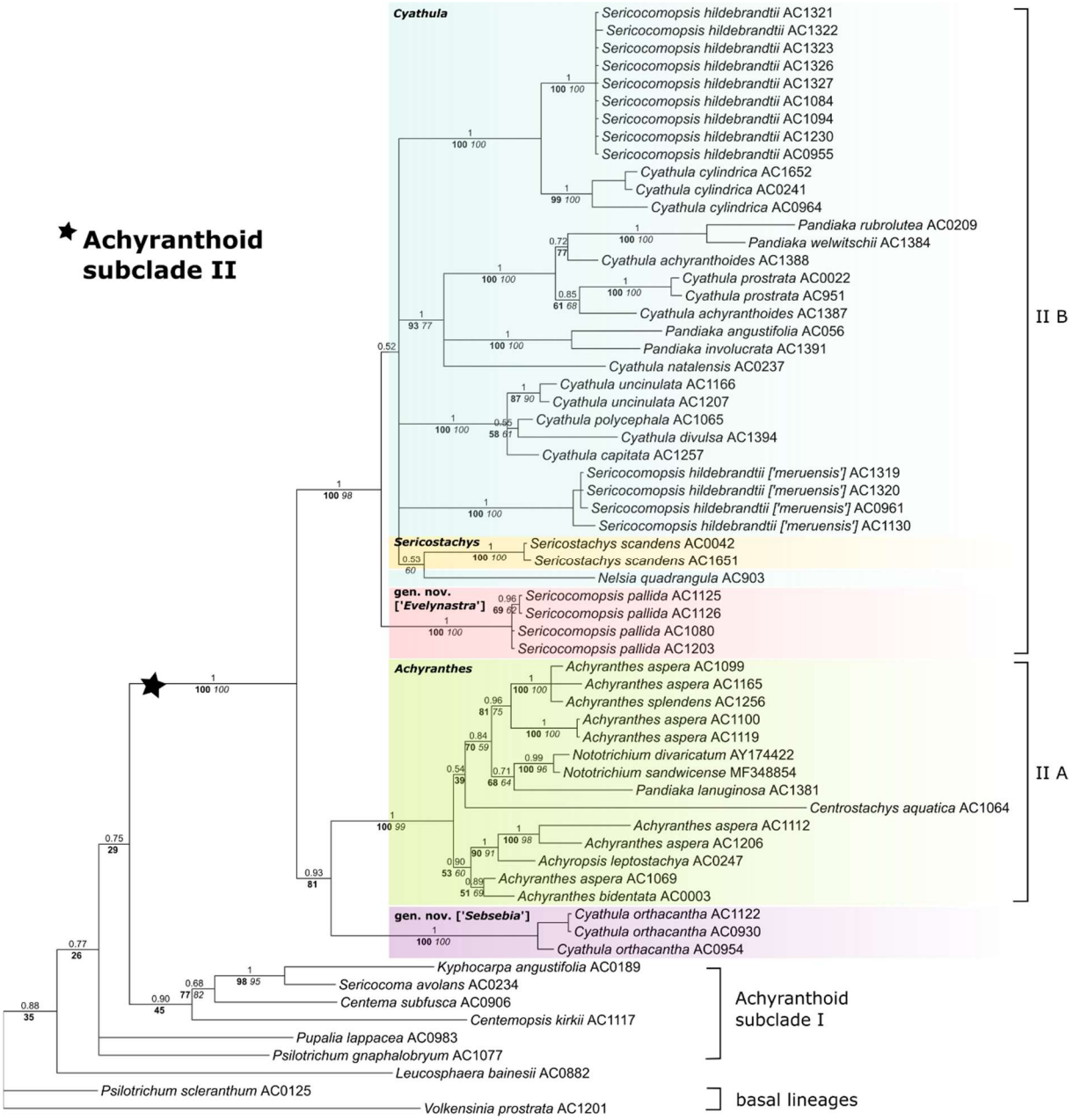

Figure 4.C: Phylogeny of the achyranthoid clade of Amaranthaceae with a focus on subclade II.
The tree topology was obtained via Bayesian inference on dataset B (nuclear ITS). Posterior probabilities of the Bayesian inference are given above branches, bootstrap percentages of the maximum likelihood analyses in bold letters (left) and jackknife percentages of the maximum parsimony analyses in italics (right) are given below branches. Genera within subclade II are shaded in different colors according to the new genus concept with the respective genus names written in bold italics in the upper left side of each clade. For gen. nov. the names in brackets are the informal genus names which are to be published. For *Sericocomopsis hildebrandtii* [‘*meruensis*’] the citation in square brackets corresponds to a present synonym of the species name which needs to be resurrected as the correct name (details are given in the discussion part and in the checklist part).

4.3.2 Polyphyly of *Sericocomopsis hildebrandtii* Schinz

The individuals identified as *Sericocomopsis hildebrandtii* according to the currently accepted classification appear in two distant lineages, in both the plastid and nuclear trees depicting well supported crown groups on a rather long branch (Figs. 2, 3), indicating that the species concept needed to be revised and also taxonomically and morphologically reassessments were necessary.

We found clear differences in the inflorescences and in the shape of cauline leaves (char 4) between the individuals of either lineage: Hairs on stems and inflorescence parts are much longer and denser in individuals assigned to *S. hildebrandtii* that are related to *C. cylindrica* than in those appearing in the other lineage, especially on bracts and bracteoles where hairs are mainly located on the midrib. Also, on the outer tepals this difference is clearly visible (compare Figure 4.D and Figure 4.E). Furthermore, we discovered differences regarding the shape of paracladia (char 6), with cymes arranged much denser on the main inflorescence axis in individuals assigned to *S. hildebrandtii* that are related to *C. cylindrica* than in those appearing in the other lineage (compare Figure 4.D, photo C & E). The texture of tepals at maturity (char 18) remained herbaceous or scarious and more hyaline in specimen assigned to *S. hildebrandtii* that are related to *C. cylindrica*, while it was found slightly indurate at the base in accessions appearing in the other lineage (compare Figure 4.E, photo C - F). The bracteoles were found strongly recurved in all fertile flowers at anthesis in the individuals assigned *to S. hildebrandtii* that are related to *C. cylindrica*, while they remained not or only slightly recurved in those appearing in the other lineage (char 21; compare Figure 4.E, photo G & H). The cauline leave shape (char 4) varied within single individuals, but in the lineage which is sister to *C. cylindrica,* the broadest portion was observed in the lower half, while in the other lineage the widest part was observed in the upper half of the leaf (compare Figure 4.F; see paragraphs on morphology and ancestral character state reconstructions). All specimens included in the phylogeny display the above-mentioned characteristics and could be sorted into two groups independent of the molecular analyses, indicating that two species from what is so far considered *S. hildebrandtii* can also be clearly differentiated morphologically.

The following names are presently considered synonyms of *Sericocomopsis hildebrandtii* Schinz and thus are priority candidates for naming the individuals of the second clade that does not resemble the type specimen of *S. hildebrandtii*: *Sericocomopsis grisea* Suess., *Sericocomopsis meruensis* Suess. and *Sericocomopsis lindaviana* Peter.

Figure 4.D: *Sericocomopsis* Schinz; inflorescences and indumentum of the upper leaf surface.
A: cyme of *S. pallida* (sample AC1203 in the phylogenetic trees depicted in Figure 4.B and Figure 4.C); **B**: stellate indumentum of *S. pallida* (sample AC1125 in the phylogenetic trees); **C, E**: inflorescences of different individuals of *S. hildebrandtii* with several partial florescences; **D, F**: simple indumentum of different individuals of *S. hildebrandtii*; **C** & **D:** *Sericocomopsis hildebrandtii* (sample AC1094 in the phylogenetic trees); **E** & **F:** *Sericocomopsis hildebrandtii* [‘*meruensis*’] (sample AC0961 in the phylogenetic trees).

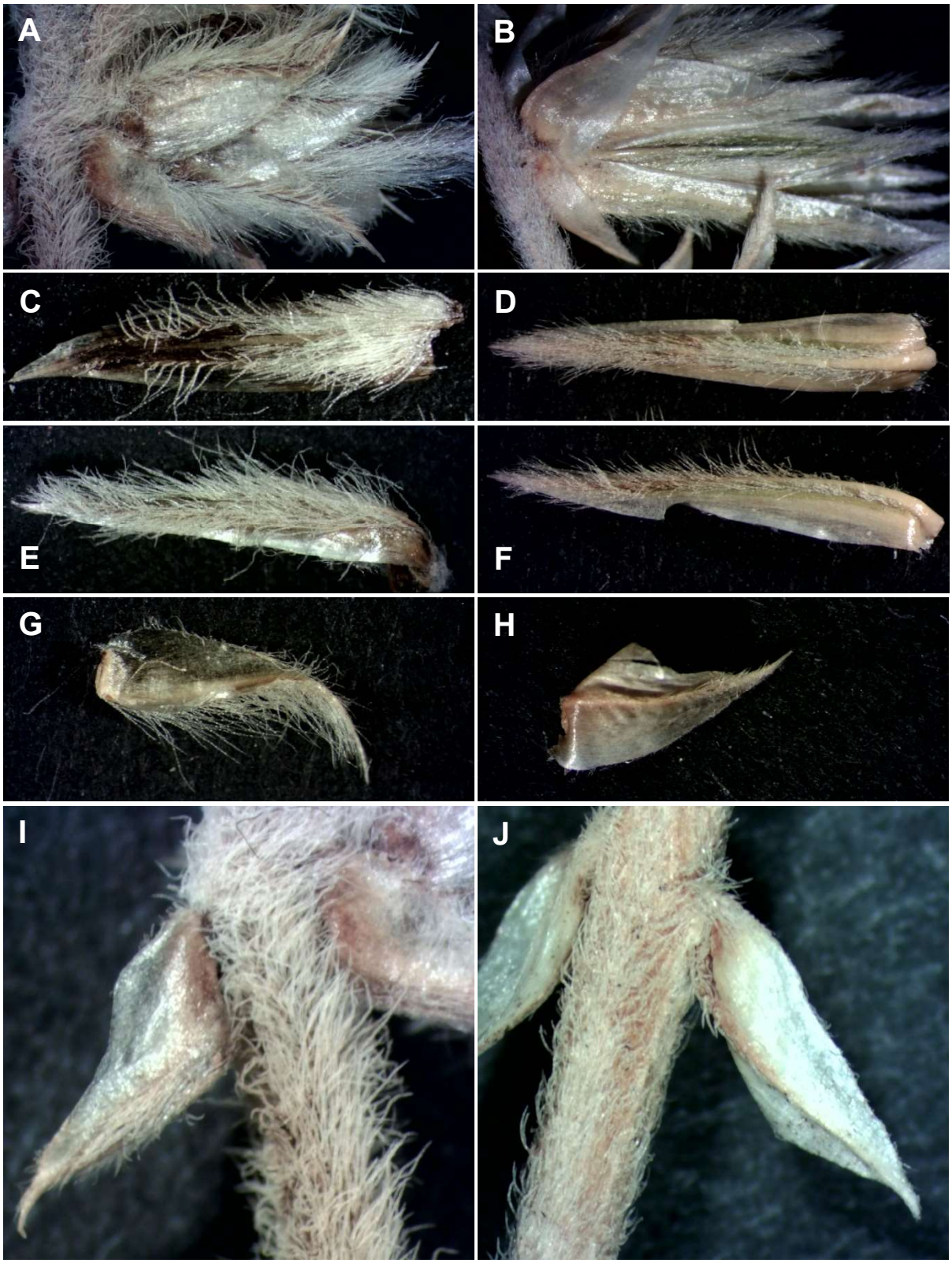

Figure 4.E: Partial florescences, stem parts with bracts, and cauline leaves of the two morphological different *Sericocomopsis hildebrandtii* lineages.
A, C, E, G, I: *Sericocomopsis hildebrandtii* (sample AC1325 in the phylogenetic trees depicted in Figure 4.B and Figure 4.C); **B, D, F, H, J**: *Sericocomopsis hildebrandtii* [‘*meruensis*’] (sample AC0961 in the phylogenetic trees).
Top down: partial florescence; tepal 1; tepal 2; bracteole; stem part with bracts..

Figure 4.F: Cauline leaves of the two morphological different *Sericocomopsis hildebrandtii* lineages.
A: *Sericocomopsis hildebrandtii* (sample AC1325 in the phylogenetic trees depicted in Figure 4.B and Figure 4.C);
B: *Sericocomopsis hildebrandtii* [‘*meruensis*’] (sample AC0961 in the phylogenetic trees).

4.3.3 Distribution and ancestral states of morphological characters

The definition of characters and their states and the matrix used for ancestral character state reconstruction is given in Appendix 4.E and Appendix 4.F, respectively. The probabilities for ancestral states of all 24 characters analysed are presented in Appendix 4.I. The ancestral state probabilities for the most relevant characters are illustrated as pie charts in Figure 4.G - Figure 4.L starting with habit and vegetative morphology (chars 1 – 4, Figure 4.G), inflorescence morphology (Figure 4.H), floral morphology (Figure 4.I - Figure 4.K), and morphology of bracteoles (Figure 4.L).

Annuals are derived several times (char 1, state 1) from plants with a perennial life cycle whereas multiple shifts between woody shrubs and subshrubs and herbaceous plants (life form, char 2) were inferred. The conspicuous woody liana in *Sericostachys scandens* (char 2, state 4) is an autapomorphy for the genus within the Achyranthoids, as are treelets of the Hawaiian island endemic *Nototrichium*, which derived from herbs (Figure 4.G). The stellate indumentum of stems and cauline leaves is a derived feature of all individuals of *Sericocomopsis pallida* (Figure 4.G).

Paracladia within Achyranthoids II are mostly spiciform with cymes or solitary flowers (reduced cymes) clearly separated during anthesis. This is also the ancestral state (char 6, state 0; Figure 4.H) whereas denser inflorescences (states 1, 2) derived several times. Paracladia are usually solitary on main axes and lateral branches, whereas the arrangement in a complex thyrsoid structure derived twice, once in *Sericostachys* and once in *Psilotrichum gnaphalobryum* outside the Achyranthoids II (char 7, Figure 4.H). Cymes commonly have more than one order of branches in Achyranthoids, which is also the plesiomorphic state. Our ancestral state reconstruction shows that they were reduced to single flowers in the whole *Achyranthes* subclade (clade “A” in Figure 4.B and Figure 4.C) but also in *Pandiaka* (char 8, Figure 4.I). Cymes with first order branches are rare and within Achyranthoids II autapomorphic for *Sericostachys* (char 8, state 1, Figure 4.I). The common ancestor of *Achyranthes* and *Centrostachys*

evolved cymes which are deflexed and appressed to the inflorescence axis at maturity (char 9, Figure 4.I). This conspicuous feature apparently has been lost in the lineages nested within *Achyranthes* that are currently classified under *Achyropsis*, *Nototrichium*, and *Pandiaka lanuginosa*.

The presence of sterile flowers is a plesiomorphic condition in Achyranthoids (char 10, state 1), and sterile flowers got lost in conjunction with the evolution of single-flowered cymes. Sterile flowers got also lost in the two lineages of *Sericocomopsis hildebrandtii*, but not in *Sericocomopsis pallida* (Figure 4.I). The latter species further deviates from the rest in that it retained unmodified sterile flowers, that became modified into hooks in those species currently classified as *Cyathula* (char 11, state 1), except *C. orthacantha* that evolved them to straight spines. In the liana *Sericostachys* filiform, feathery hairs serving dispersal develop from the midribs of the tepals of sterile flowers, being an autapomorphic feature in Amaranthaceae. Androecial tube appendages (char 12, Figure 4.J) derived multiple times, once in the common ancestor of Achyranthoids II but were lost in *Nototrichium* (Figure 4.J). The ovary in Achyranthoids II is always glabrous, whereas it can be pilose or lanate within Achyranthoids I (char 24, Figure 4.J).

Tepals with equal size and shape (char 13, state 0) are the most common feature in the Achyranthoids II A, while Achyranthoids II B commonly possess flowers with the three inner tepals smaller than the 2 outer but similar in shape (char 13, state 3) or further developed outer and inner tepals distinctly different in shape (char 13, state 2) e.g., in *C. polycephala* and close relatives (Figure 4.K). This group of species also stands out by excurrent and uncinately hooked midribs as a derived state of character 15 (Figure 4.K), contributing to the function of attaching diaspores to large animals in addition to the hooks in modified sterile flowers. The number of veins in outer and inner tepals is homoplastic (char 16, 17; Figure 4.K) with reductions in vein number appearing correlated to the very dense inflorescences in *C. uncinulata* and relatives on the one hand, but derived many-veined tepals in lineages with rather isolated flowers like *Sericostachys* and *Centrostachys*. Different degrees of induration of tepals at maturity (char 18) are also derived in particular in species with less dense inflorescences, including a noteworthy difference in the two lineages of Sericocomopsis hildebrandtii (Figure 4.K) but a trend to stronger induration in achyranthoid subclade I. The morphology of bracteoles (Chars 19-21) also appears homoplastic and orbicular bracteoles with an emarginate tip (char 19, state 4) are shown to be plesiomorphic in clade IIA and present in taxa currently classified within *Achyranthes* and *Centrostachys* (Figure 4.L).

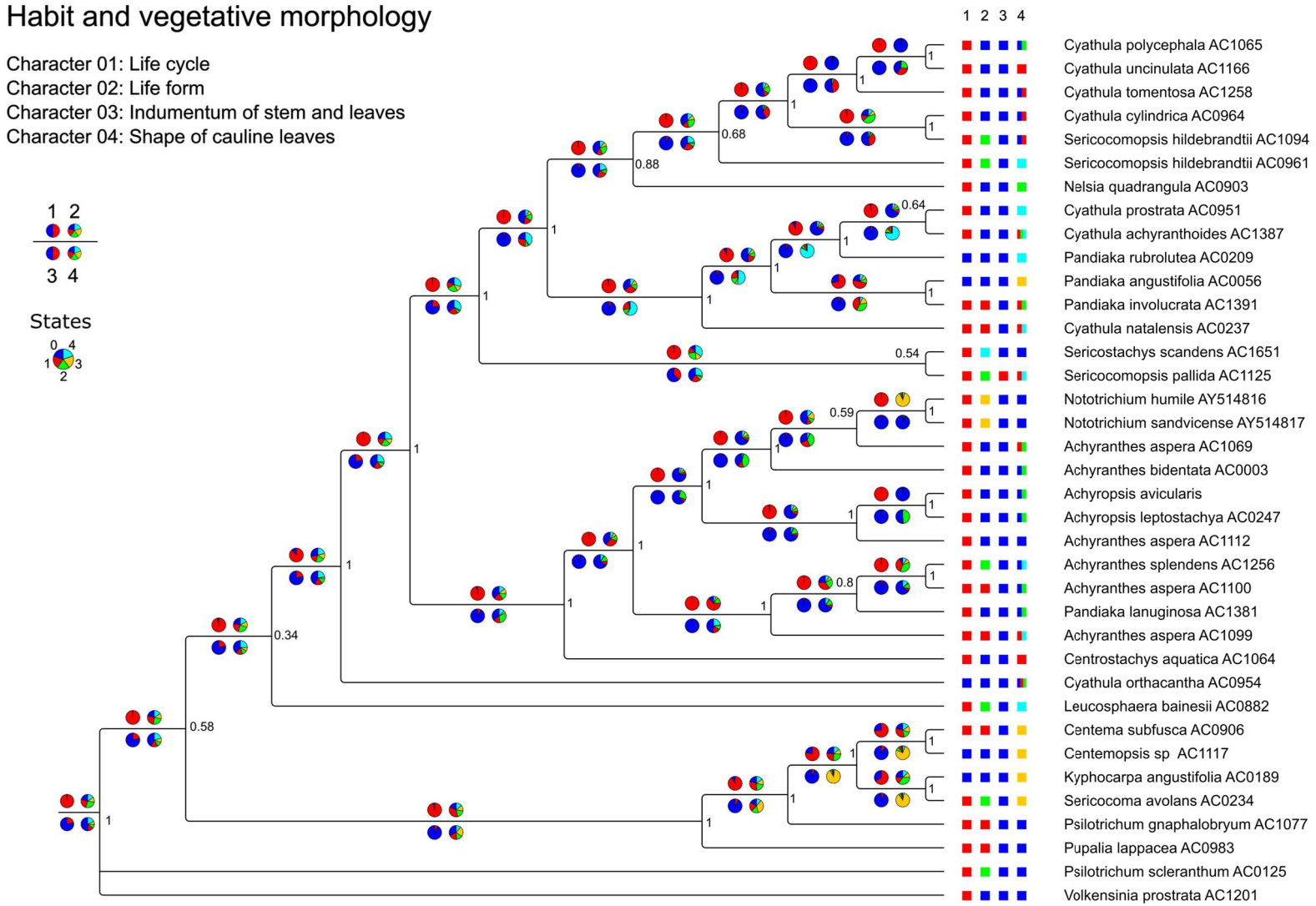

Figure 4.G: Evolution of habit and vegetative morphology in Achyranthoids with a focus on subclade II (character 1-4).
Pie charts depict the ancestral states reconstructed on the maximum clade credibility (MCC) tree of the plastid analysis. For character and state definitions see Appendix 4.E.

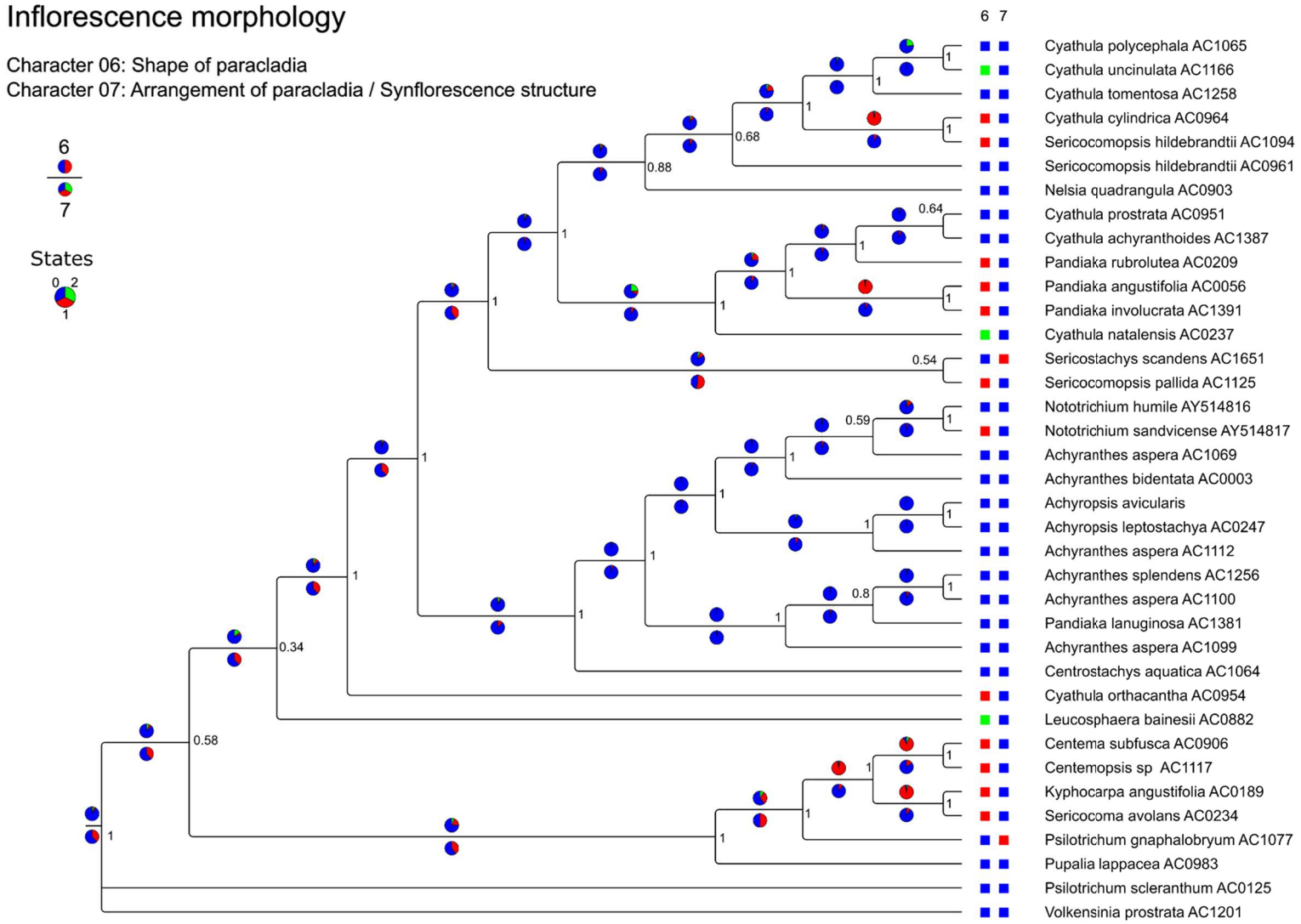

Figure 4.H: Evolution of inflorescence morphology (paracladia) in Achyranthoids with a focus on subclade II (character 6 & 7).
Pie charts depict the ancestral states reconstructed on the maximum clade credibility (MCC) tree of the plastid analysis. For character and state definitions see Appendix 4.E.

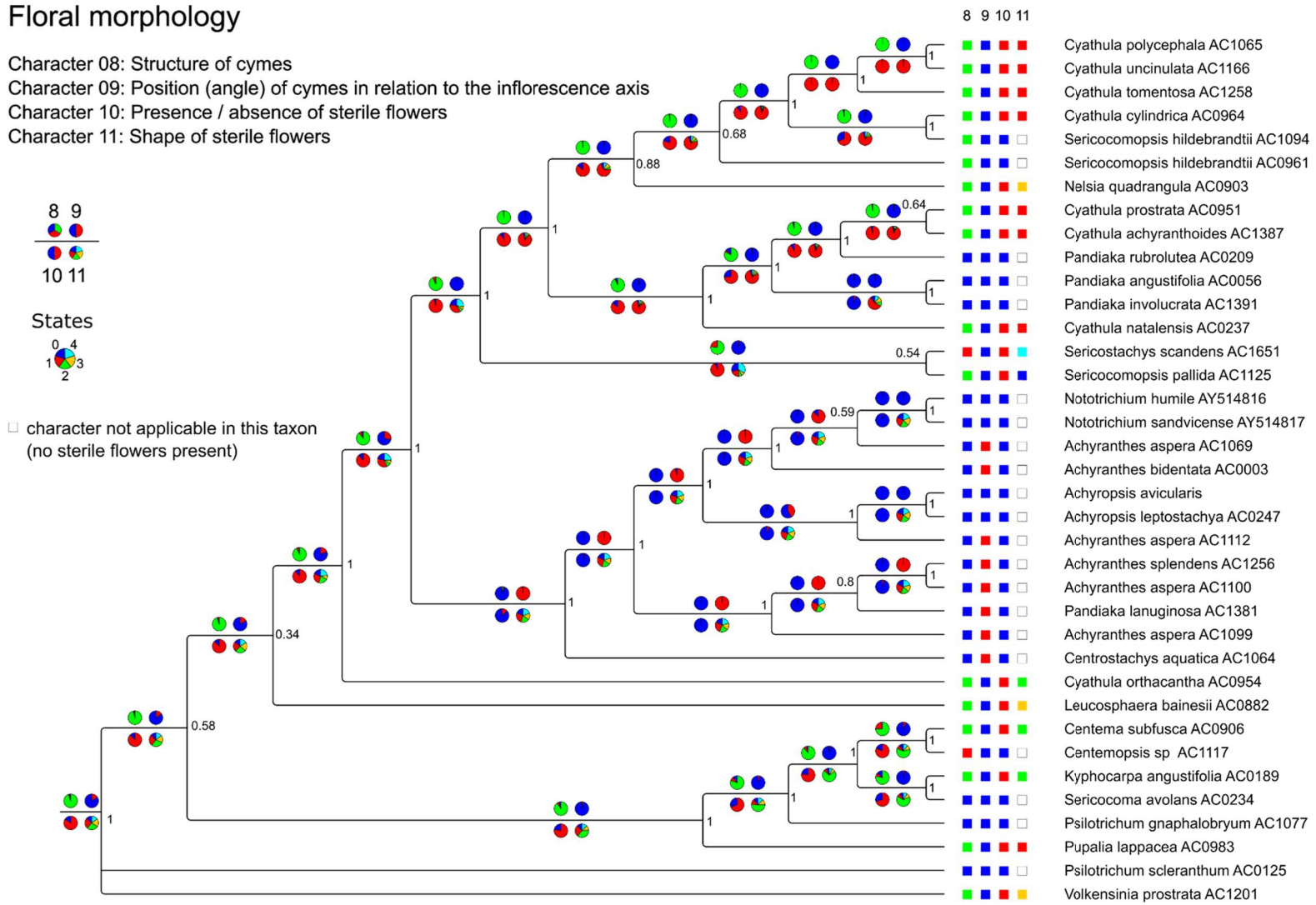

Figure 4.I: Evolution of floral morphology (cymes and sterile flowers) in Achyranthoids with a focus on subclade II (character 8-11).
Pie charts depict the ancestral states reconstructed on the maximum clade credibility (MCC) tree of the plastid analysis. For character and state definitions see Appendix 4.E.

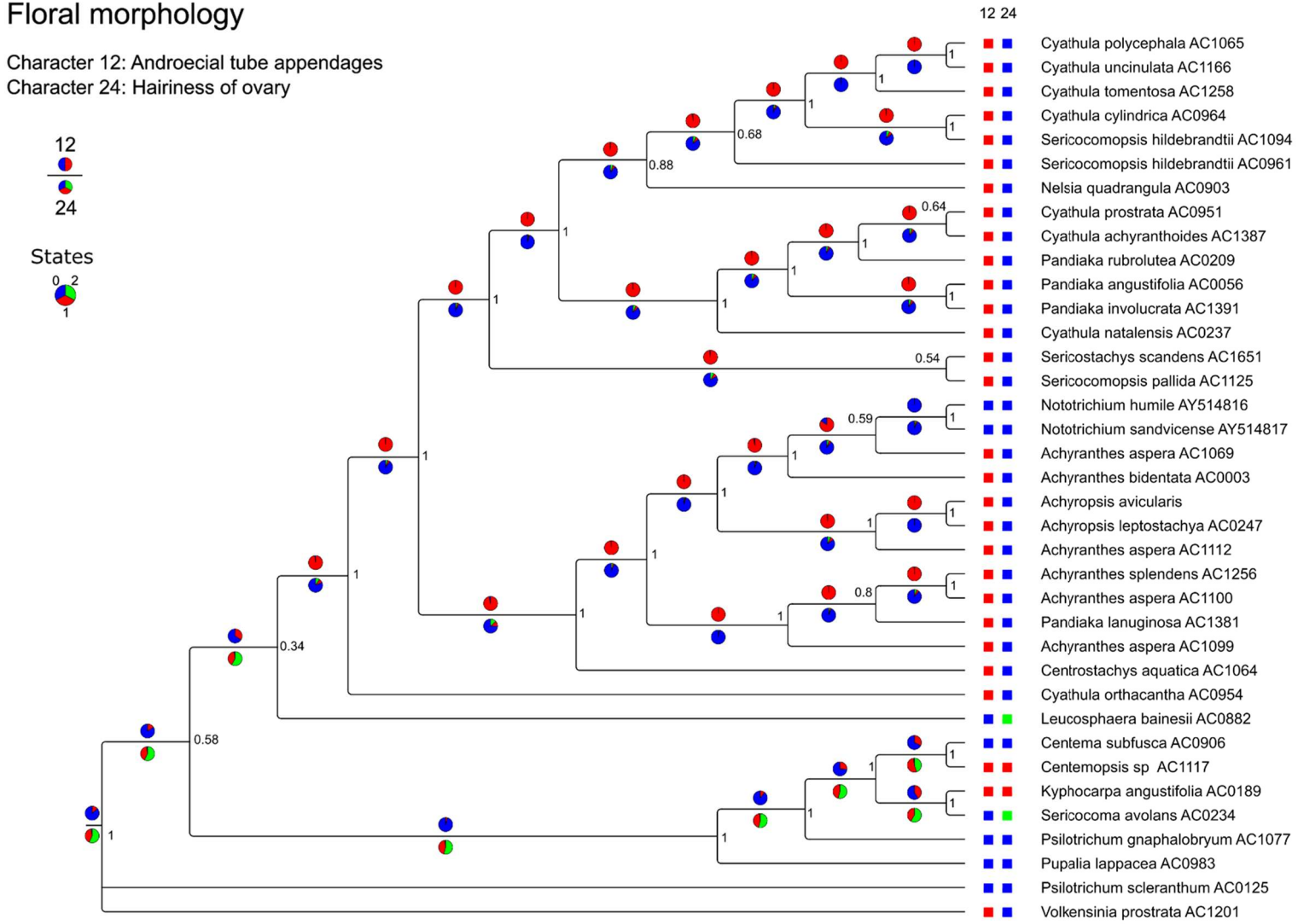

Figure 4.J: Evolution of floral morphology in Achyranthoids with a focus on subclade II (character 12 & 24).
Pie charts depict the ancestral states reconstructed on the maximum clade credibility (MCC) tree of the plastid analysis. For character and state definitions see Appendix 4.E.

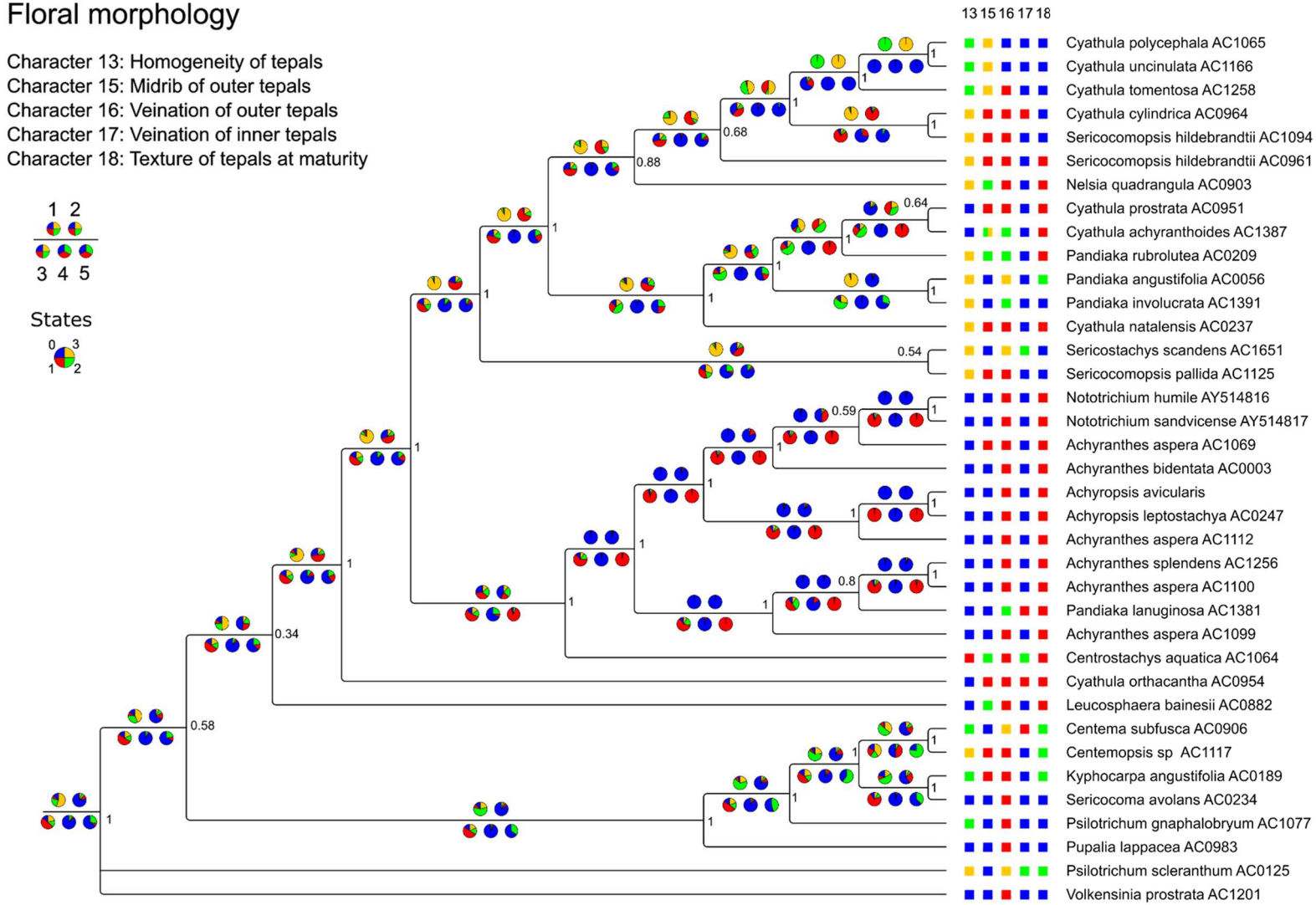

Figure 4.K: Evolution of floral morphology (tepals) in Achyranthoids with a focus on subclade II (character 13 & 15-18).
Pie charts depict the ancestral states reconstructed on the maximum clade credibility (MCC) tree of the plastid analysis. For character and state definitions see Appendix 4.E.

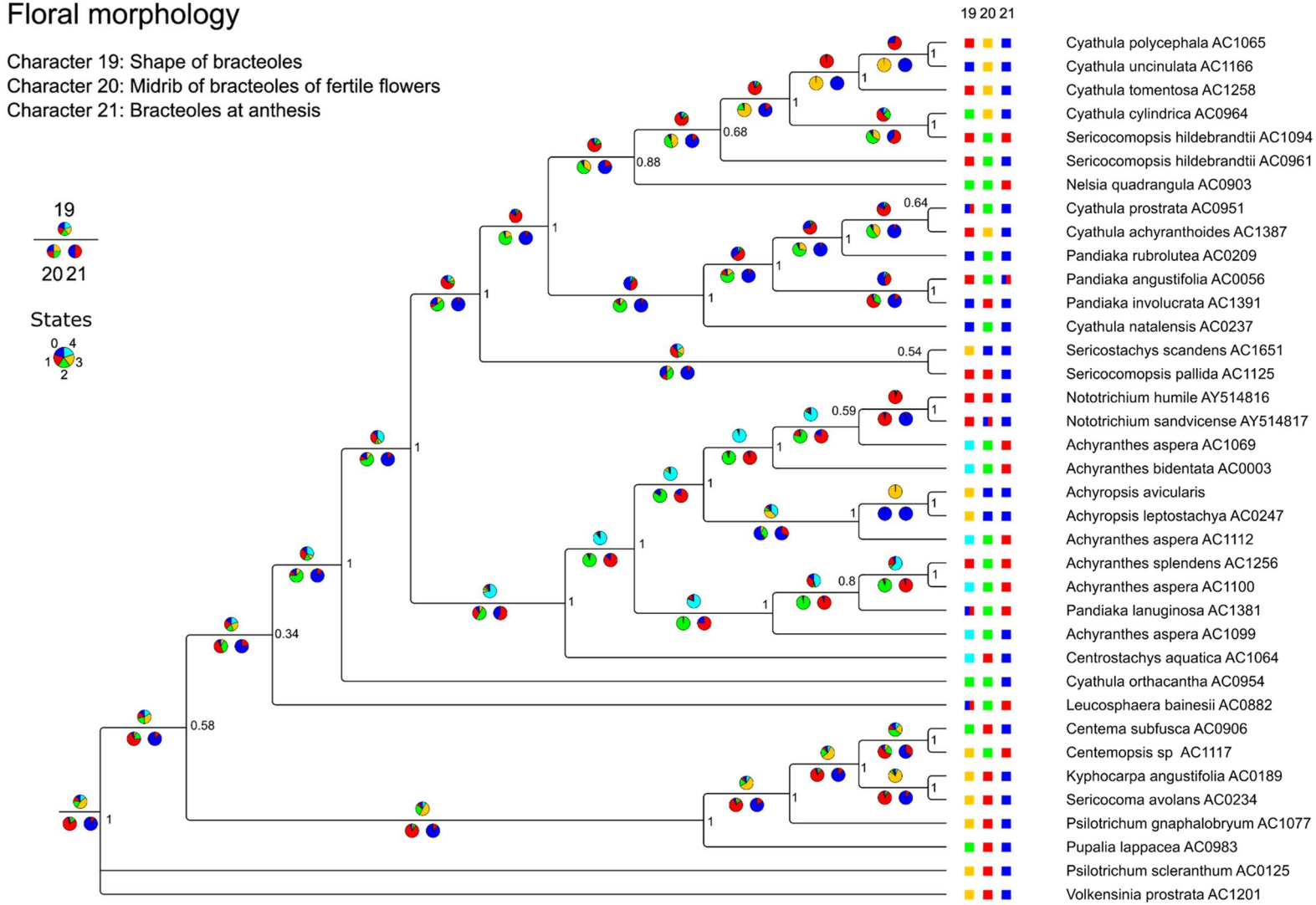

Figure 4.L: Evolution of floral morphology (bracteoles) in Achyranthoids with a focus on subclade II (character 19-21).
Pie charts depict the ancestral states reconstructed on the maximum clade credibility (MCC) tree of the plastid analysis. For character and state definitions see Appendix 4.E.

4.4 Discussion

4.4.1 Phylogenetic relationships with a focus on the achyranthoid subclade II

Compared to the phylogenetic analysis of the achyranthoid clade in Chapter 2 (Di Vincenzo et al., 2018), three further plastid intergenic spacers (*rps4-trnT-trnL*$_{UAA}$ and *rpl32-trnL*$_{UAG}$) and further species of the genera *Cyathula* and *Pandiaka* were added for this investigation. Since *Cyathula* had earlier been shown as polyphyletic (Chapter 2, Di Vincenzo et al., 2018), it was crucial to represent its morphological and geographical diversity at species level. Our increased character sampling of the plastid genome resulted in increased statistical confidence for most backbone nodes (maximum support throughout all three inference methods: Bayesian Inference, Maximum parsimony and maximum likelihood, Figure 4.B). *Cyathula orthacantha* is now established as an isolated lineage as is an *Achyranthes* subclade including *Centrostachys*, *Achyropsis*, *Nototrichium* and *Pandiaka lanuginosa* (clade A in Figure 4.B, Figure 4.C) that is sister to a clade comprising all the other genera including the majority of species of *Cyathula* and *Pandiaka* (clade B in Figure 4.B, Figure 4.C). The nrITS trees (Figure 4.C) largely agree with the plastid backbone and the composition of the subclades, although there is weak support for *C. orthacantha* as sister (0.93 PP, 81 ML-BS) to the otherwise well supported *Achyranthes* subclade (1.0 PP, 100 ML-BS, 99 MP-JK, clade A in Figure 4.B and Figure 4.C). Internally, relationships in the *Achyranthes* subclade are less clear and the nrITS topology deviates in some aspect from the mostly well supported plastid tree. *Centrostachys aquatica* appears on a long branch inside the clade in nrITS (Figure 4.C), whereas it is clearly supported as a distant sister group in the plastid tree (Figure 4.B). In nrITS this topology was not recovered with parsimony, and there could be effects of long branch attraction in the nrITS tree, in line with the weakly supported internal nodes of the nrITS tree, indicating a rather inconsistently different nuclear topology. Nevertheless, it is noteworthy that all phylogenetic evidence converges on the fact that the members of the morphologically rather homogeneous genus *Achyranthes* as currently circumscribed, apparently gave rise to entities currently recognized at genus level such as *Achyropsis* and *Nototrichium*.

The plastid tree now provides maximum support for a core of the clade comprising most species of *Cyathula* and *Pandiaka* (clade B), that was initially found in Chapter 2 (Di Vincenzo et al., 2018) and also includes *Nelsia* and the two different lineages of *Sericocomopsis hildebrandtii* (Figure 4.B), whereas *Sericocomopsis pallida* and *Sericostachys scandens* appear outside. Their sister group relationship suggested here (Figure 4.B) needs further testing. The nuclear tree (Figure 4.C) congruently depicts a sister group relationship of *Cyathula cylindrica* to one of the lineages of *Sericocomopsis hildebrandtii* as well as two lineages of *Cyathula*, one comprising *C. polycephala* and allies, and the other *C. achyranthoides* and *C. prostrata* appearing as sister species, *C. natalensis* and all species of *Pandiaka* except *P. lanuginosa* (Figure 4.B and Figure 4.C). There are short branches for the respective

relationships among these lineages, in both plastid and nuclear trees (highlighted in blue in Figure 4.B and Figure 4.C), which is in line with their suggested rapid diversification in the late Micocene (Chapter 2, Di Vincenzo et al. 2018).

The position of the African tropical woody liana *Sericostachys scandens* is not resolved within but unambiguously shown to be part of clade B (Figure 4.B and Figure 4.C). As indicated by results from BayesTraits, *Sericostachys* is morphologically outstanding, with several characters autapomorphic within Achyranthoids II, such as the life form, the arrangement of paracladia, the structure of cymes, and the shape of sterile flowers (Characters 2, 7, 8, and 11) or at least rare within Achyranthoids II (e.g., the venation on outer tepals; char 16). Additionally, stamens in *Sericostachys* are free and a very shallow androecial cup has short triangular interstaminal appendages according to Townsend (1985, 2000).

Remarkably, *Sericostachys* does not appear on a long branch in the molecular trees, indicating rapid anatomical and morphological evolution adapting to the life as rain forest liana flowering above treetops.

4.4.2 Evolution of morphological characters in Achyranthoids II

We did not aim at giving ancestral states for the whole clade of Achyranthoids or deeper nodes within the data set, due to the sampling bias within our data which is owing to the dense sampling of subclade II and the otherwise representative inclusion of members of subclade I and basal lineages of Achyranthoids. We used BayesTraits analyses mainly to illustrate the delimitation of taxa included into subclade II at genus level or at species level for the lineages of *Sericocomopsis hildebrandtii*.

4.4.2.4 Evolution of habit and vegetative morphology

Taxa with annual life cycle (Char 1, state 0) evolved several times from perennials in different lineages of the Achyranthoids (Figure 4.G). These results are in line with Ortuño & Borsch (2020) who showed for *Gomphrena* and relatives in Amaranthaceae vegetative characters to be homoplastic rather than to exhibit synapomorphies for individual lineages, and also found several independent transitions from perennials to annuals. In the case of *Gomphrena* the transition to an annual life cycle is connected to an adaptation to seasonally dry Inner-Andean valleys, where these plants survive the dry season as seeds. In the Achyranthoids such contrasts in habitat humidity are less evident, and *Cyathula orthacantha* is more a plant of overgrazed and disturbed places (Townsend 1985, 2000). The life form (Char 2,) shows transitions between woody shrubs and subshrubs and herbaceous plants. Interestingly, shrubs seem derived in species growing in dry bushland to semideserts like *Sericocomopsis pallida* (Fig. xx). This requires more precise insights in distribution and ecology, which are currently underway including the wealth of specimens collected during the course of our work on Amaranthaceae (M. Wondafrash and T. Borsch, pers. comm.). The treelets of *Nototrichium* clearly originated from herbs and exhibit an example of island gigantism and island woodiness in the Hawaiian archipelago in consequence of geographical isolation that led to an increase in size of species compared to their mainland relatives. It is known

already from Darwin (1859) that "[…] *islands often possess trees or bushes belonging to orders which elsewhere include only herbaceous species* […]". Our result also fits to the general pattern on the evolution of island woodiness, as documented e.g., by Lens et al. (2013) who found at least 38 independent shifts toward insular woodiness in the Canary Island flora, which they considered to "[…] *emphasize the remarkable lability in growth forms between herbaceous and woody lineages*". It is thus not surprising that the MRCA of *Nototrichium* plus its sister lineage was inferred as an herb. The woody liana in *Sericostachys scandens* is an autapomorphy for the genus within the Achyranthoids. *Sericostachys* is a rain forest plant that grows over tree tops and shows various adaptations as a liana (including diaspores that can be distributed by air, see below). In a broader context of the Amaranthaceae, lianas evolved in several distant lineages with species growing in tropical forests such as *Chamissoa* (Sohmer 1977), *Hebanthe* (Borsch & Pedersen 1997) or *Pedersenia* (Borsch et al. 2011). Also, the stellate indumentum of stem and leaves (char 3, state 1) is a derived apomorphy in *Sericocomopsis pallida* at the level of the core Achyranthoids but independently evolved in dry-adapted *Chionothrix latifolia* that is an early branch of the achyranthoid clade (Di Vincenzo et al., 2018) not included in this investigation, and within various genera of the neotropical Gomphrenoideae such as *Alternanthera* (Sánchez- del Pino 2012) or *Hebanthe* (Borsch & Pedersen 1997). The cauline leaf shape (char 4) is highly homoplastic but provides a useful feature for differentiating the two *Sericocomopsis hildebrandtii* lineages (compare Figure 4.F). The petiole length (char 5) is highly homoplastic within genera as is within single individuals (Appendix 4.I).

4.4.2.5 Evolution of inflorescence and floral morphology

We found several character states for Achyranthoids II, which differentiate it from Achyranthoids I or early branching lineages of Achyranthoids: All taxa of Achyranthoids II (except *Nototrichium*) possess androecial tube appendages, which presumably derived once in the common ancestor of subclade II but additionally within early branching lineages of Achyranthoids and in *Centemopsis* and *Kyphocarpa* of subclade I. Contrary, androecial tube appendages are missing in most genera outside subclade II (char 12; compare Figure 4.J and Appendix 4.I) , also in genera not sampled here such as *Chionothrix*, *Dasysphaera*, *Lopriorea*, *Rosifax* and *Marcelliopsis*, but which belong to the Achyranthoids (compare Chapter 2 and Di Vincenzo et al., 2018). The ovary in all taxa of Achyranthoids II is glabrous whereas it can be pilose or lanate in genera outside this clade, such as *Leucosphaera*, *Centemopsis*, *Kyphocarpa*, *Sericocoma* (char 24, compare Figure 4.J and Appendix 4.I), as well as in *Marcelliopsis*, and *Mechowia* (both Achyranthoids I; compare Chapter 2 and Di Vincenzo & al. 2018). The glabrous ovary probably was derived in the MRCA of Achyranthoids II from an ancestor with lanate or pilose ovary and evolved convergent in other lineages.

Morphological differences between achyranthoid II subclades A and B were found mainly in inflorescence and floral morphology e.g., in the structure of cymes (char 8, Figure 4.I). We earlier reported that sterile flowers appeared multiple times and were overrated so far as diagnostic characters

supporting the circumscription of genera and tribes (compare Chapter 2 and Di Vincenzo & al., 2018). In this investigation, we developed a set of inflorescence characters (chars 8-11, see Appendix 4.E for a detailed discussion on character definitions) that are largely based on paracladia as basic units of the inflorescences (Acosta & al., 2009), and using a reductive morphological character coding approach (Torres Montúfar & al., 2018).

Our results indicate that cymes with more than one order of branches (char 8, state 2 in Figure 4.I) are the ancestral state for achyranthoid subclade II (and maybe for the whole of Achyranthoids) and that these were reduced to cymes with first order branches or to single flowers several times (char 8, state 1 & 0). One of these reductions occurred in the ancestor of subclade II A, which only include taxa with solitary flowers (*Achyranthes*, *Nototrichium*, *Achyropsis*, *Centrostachys*). Additionally, deflexed cymes which are appressed to the inflorescence axis in maturity (char 9, Figure 4.I) depict the ancestral state for subclade II A and constitute a conspicuous feature of the genera *Achyranthes* and *Centrostachys* as currently circumscribed. However, our ancestral state reconstruction also shows that state 1 is plesiomorphic, and probably was reversed in plants no longer dispersed by large animals such as *Nototrichium* on the Hawaiian Islands, which also lost the spine-like excurrent midribs of bracteoles. Sterile flowers are completely missing in subclade II A, whereas they are present in most taxa of subclade II B (char 10, Figure 4.I). In our ancestral state reconstruction (Fig. xxx) there is a high probability that the common ancestor of subclade II A had no sterile flowers, implying a character state transformation (= loss of sterile flowers on the branch of the common ancestor of subclade II A) along with the reduction in flower number in the cymes. Di Vincenzo & al. (2018) found almost equal state probabilities for the presence (59%) and absence (41%) of sterile flowers as ancestral state in Achyranthoids under an unconstrained model, whereas best-fit BiSSE models favored independent gains of sterile flowers i) in a core of subclade II with a subsequent loss in the lineage of *Sericocomopsis hildebrandtii* sister to *C. cylindrica*, ii) in *C. orthacantha*, and iii) within Achyranthoids I in the terminal clades of *Pupalia* and relatives as well as in iv) *Kyphocarpa* and relatives. The latter terminal clades are just represented by a single individual in this investigation as we focus on Achyranthoids II. Future research will evaluate the effects of topological uncertainty, taxon sampling, models and (in-)dependent character evolution in the Amaranthaceae, but this goes beyond the scope of the present investigation. For our purposes of exploring possible synapomorphies to circumscribe taxa and of evaluating hitherto used diagnostic characters in a phylogenetic context we argue that the use of MCMC tree optimization as implemented in BayesTraits will provide adequate results, e.g., that the genus *Achyranthes* as currently understood is basically circumscribed with plesiomorphic character states. Another difference between subclade II A and II B is observed in the homogeinity of tepals (char 13, Figure 4.K). Tepals are all equal in the taxa of subclade II A (except in *Centrostachys aquatica*, in which the outermost tepal is different in shape and size, which depicts an autapomorphy for this species). In subclade II B most taxa exhibit two outer and three inner tepals, which are smaller but similar in shape, which is also the ancestral state for subclade II B (char 13, state 3 in Figure 4.K).

4.4.3 Monophyletic circumscription of *Cyathula* Blume and status of the genus *Sericocomopsis* Schinz

Most of the analyzed species of *Cyathula* appear within the core of clade B including the type species of the genus – *C. prostrata*. *Cyathula* is the oldest generic name present within subclade II B. According to the genus concept of *Cyathula* applied by Schinz (1894, 1935) and Townsend (1993, 2000), all species have cymes with sterile flowers with more or less excurrent midribs of bracteoles or tepals that are often forming hooks. Species of *Cyathula* have more or less quadrangular androecial tube appendages with a dentate or fringed distal margin. The presence of sterile flowers also applies to the genus *Nelsia* which has quadrangular fimbriate androecial tube appendages (Schinz 1911). *Nelsia* therefore falls well into the range of morphological variation of *Cyathula*, although the discussion on the diagnostic characters in Schinz (1911) rather focused on a comparison with the genera *Sericocoma* and *Kyphocarpa* since he separated *Nelsia quadrangula* (Engl.) Schinz as an own genus from *Sericocoma*, which was transferred to *Kyphocarpa* by Clarke (1909). However, none of the historical authors employed a comprehensive morphological comparison of all putatively related taxa, which can now be implemented in a much more straightforward way with a molecular phylogenetic perspective. According to our phylogenetic results, *Sericocoma* and *Kyphocarpa* are closely related, indeed, but do not belong to Achyranthoids II (Figure 4.B, Figure 4.C). Among its next relatives in clade B of Achyranthoids II, *Nelsia quadrangula* stands out by sterile flowers with bracteoles modified to conspicuous hairy, stiff and spine-like structures (character 11, state 3; Figure 4.I). However, this is a single specific feature in a series of modifications that convergently led to increased adhesive ability in diaspores serving zoochory in the Achyranthoids. Morphological analysis shows that the core of clade B is characterized by cymose partial florescences, with cymes of more than one order of branches and with sterile flowers in most cases present and modified into hooks (compare char. 8, 10, 11 in Figure 4.I). Although the remaining species of *Pandiaka* (approx. eight additional species) and *Nelsia* (1 additional species) were not included in this phylogenetic analysis, it is clear that that their generic names cannot be maintained since their type species (*P. involucrata* and *N. quadrangula*) are nested within *Cyathula*.

Sericocomopsis was described by Schinz (1895) as having only fertile flowers and broadly obovate to quadrangular androecial tube appendages with a fringed upper margin, a glabrous ovary with elongated style and inconspicuous stigma; these are otherwise only present in *Achyranthes*, *Centrostachys* and *Pandiaka* but the latter three genera have solitary flowers, and in *Nelsia*, which has sterile flowers.

Sericocomopsis pallida remains in a phylogenetically distant position from the other two lineages of *Sericocomopsis* and from the core of *Cyathula*. However, since the type species of the genus, *S. hildebrandtii* must be included into *Cyathula* Blume to make this genus monophyletic, a new genus has to be described for this lineage (for name details including etymology and diagnosis see the checklist in paragraph 4.5.1). Morphologically, this new genus is easily recognized within Achyranthoids by the stellate hairs of the indumentum of stem and leaves, and the unmodified sterile flowers. In order to make

Cyathula monophyletic we further suggest merging the genera *Sericocomopsis* (*S. hildebrandtii*, *S. meruensis*), *Nelsia*, and *Pandiaka* (except *P. lanuginosa*) with *Cyathula*, also considering that the inflorescence and floral characters originally used to define them turned out to be homoplastic.

Cyathula orthacantha is clearly established as a separate lineage (see discussion on phylogenetic relationships) constituting an early branch of subclade II of Achyranthoids and therefore must be excluded from *Cyathula.* It will be described as an own genus (for name details including etymology and diagnosis see the checklist in paragraph 4.5.1).

Cyathula lanceolata was resolved as one of the basal lineages of the Achyranthoids in Chapter 2 (Di Vincenzo & al., 2018) and therefore is clearly not part of *Cyathula* according to our new genus circumscription here. Therefore, it was not included in dataset A and B here and the name is listed under "excluded names" in the checklist part. The treatment of *C. lanceolata* is going to be part of a different publication.

4.4.4 Species limits of *Sericocomopsis hildebrandtii* Schinz

According to the widely used taxonomic accounts in Flora of Tropical East Africa and Flora of Ethiopia and Eritrea only two species of *Sericocomopsis* occur in East Africa: *S. pallida* is native to Kenya, Tanzania, Djibouti, Somalia and Ethiopia and *S. hildebrandtii* to Uganda, Kenya, Tanzania and Ethiopia (Townsend, 1985, 2000). Their typical habitats are semi-desert scrub and deciduous bushlands, dry thornbush, rocky or disturbed places, silty and stony plains, limestone ridges and slopes in elevations ranging from c. 40 up to c. 1900 m. And this view has been accepted in genus level treatments of the Amaranthaceae s.str. (Townsend 1993, Hernández-Ledesma & al. 2015). In contrast, Chapter 2 (Di Vincenzo & al., 2018) showed specimens identified as *S. hildebrandtii* to belong to two distant lineages in phylogenetic analyses or plastid and nuclear trees. In this investigation we broadened the sampling of individuals from *Sericocomopsis*, and added further regions from the plastid genome, what resulted in a corroboration of the distant position of these two lineages of *S. hildebrandtii* (Figs. 2, 3) with further increased node support in the plastid tree compared to our previous study (Di Vincenzo & al. 2018). Notably, all sequenced individuals were resolved in two highly supported terminal clades, one sister to *Cyathula cylindrica*, and the other appearing in an isolated position among different sublineages of *Cyathula*, suggesting the existence of two different, monophyletic species instead of one.

To find out which of our lineages resembled the type specimen of *S. hildebrandtii* we morphologically compared all individuals which were included in our phylogenies to type material of considered synonyms (compare the results part 4.3.2) and also checked the protologues of these. In his description of *S. meruensis* Suess. (Suessenguth 1950) already recognized the difference in the hairiness of the inflorescences between *S. hildebrandtii* and *S. meruensis* and stated that "[…] pili multo minores et nimius densi quam in *S. hildebrandtii*, qua re inflorescentia tota sine lente in hac specie glabra videtur […]" indicating that the hairs are less dense and shorter in *S. meruensis* compared *S. hildebrandtii* and

that observed without a lens, *S. meruensis* appears glabrous. We agree to this statement. During our field work, specimens collected as *S. hildebrandtii* could already be noticed to belong to two groups differing in the hairiness of inflorescences. However, Suessenguth (1950) did not recognize the differences in leaf shape and considered leaves to be similar in both species, what we do not confirm. By examining multiple specimens from each lineage, we found that leaves in the hairy specimens matching the type of *S. hildebrandtii* have a more ovate shape whereas leaves in the other lineage are obovate in shape. To the contrary, the type specimen of *S. grisea* Suess. shares the same states of the morphological characters with the type specimen of *S. hildebrandtii*. We therefore confirm the name *S. grisea* as a synonym of *S. hildebrandtii* as treated by Townsend (1985, 2000). The type specimen of *Sericocomopsis lindaviana* Peter, is also resembling the type specimen of *S. meruensis* and not that of *S. hildebrandtii* as treated by Townsend (1985, 2000). However, *S. lindaviana* is a nom. inval. according to Art 39.1 of the Code (Turland & al., 2018). We therefore propose to resurrect *Sericocomopsis meruensis* Suess. as this provides the oldest valid name for the more isolated lineage of *Sericocomopsis hildebrandtii* which is sister to the whole of *Cyathula cylindrica*, *C. uncinulata*, *C. polycephala*, *C. tomentosa*, *C. capitata*, and *S. hildebrandtii.* In summary, the morphological differences between *S. hildebrandtii* and *S. meruensis* reported by Suessenguth (1950) are confirmed here in a phylogenetic context. These results further underscore the need to carefully molecular phylogenetically evaluate entities put into synonymy by treatments that were carried out using a morpho-species approach.

4.4.5 Monophyletic circumscription of *Achyranthes* L.

Our results confirm the results from the second chapter (Di Vincenzo & al., 2018) in that *Achyranthes* is paraphyletic to *Achyropsis* and *Nototrichium,* and altogether they are strongly supported as sister to the monotypic *Centrostachys aquatica* which is first branching within subclade II A based on plastid regions. But this position is not supported based on nrITS, where *Centrostachys* was found as sister to some accessions of *Achyranthes aspera*, *A. splendens* plus *Nototrichium* and *Pandiaka lanuginosa* and all together are sister to a clade including *Achyranthes bidentata*, further accessions of *A. aspera* and *Achyropsis*. The fact that our molecular trees from both genomic compartments revealed *Pandiaka lanuginosa* as part of the *Achyranthes* clade is supported by morphological characters, which clearly distinguish it from other species of *Pandiaka*, e.g., the deflexed cymes at maturity (Char 9, state 1; Figure 4.I), the petiole length (Char 5, Appendix 4.I), and the life cycle (Char. 1; Figure 4.G). The deviant morphology was also noted by Townsend (1980), who, while maintaining *P. lanuginosa* within *Pandiaka*, described it as morphologically transitional between *Achyranthes* and *Pandiaka*. However, our taxonomic investigations revealed *Achyranthes schinzii* (Standl.) Cufod.[1] to be actually the correct

[1] ***Achyranthes schinzii*** (Standl.) Cufod. in Bull. Jard. Bot. État 23: 73. 1953 ≡ *Achyranthes lanuginosa* Schinz, Pflanzenw. Ost-Afrikas C: 174. 1895, nom. illeg. [Art. 53.1, non *Achyranthes lanuginosa* Nutt., in Trans. Amer. Philos. Soc., n.s., 5: 166. 1835] ≡ *Centrostachys schinzii* Standl. in J. Wash. Acad. Sci. 5(3): 76. 1915 ≡ *Pandiaka lanuginosa* Schinz in Engler & Prantl, Nat. Pflanzenfam., ed. 2, 16c: 64. 1934, nom. illeg. [Art. 52.1]

name for *Pandiaka lanuginosa*, even though this name is cited as synonym in the widely used taxonomic accounts in Flora of Tropical East Africa and Flora of Ethiopia and Eritrea.

Eventhough there are some characters making *Nototrichium* somehow unique compared to the remaining genera of subclade II A, the phylogeny clearly shows *Nototrichium* nested within *Achyranthes*, and in order to make this genus monophyletic we need to include the species of *Nototrichium*. Flowers in *Nototrichium* are described as four-parted while in all remaining genera of sublade II A they are five-parted. Furthermore, *Nototrichium* is a Hawaiian endemic geographically separated from the remaining genera and its species grow as treelets (compare char. 2 "Life form"), which is a derived feature associated to island gigantism that also appeared convergently in single species being endemics on islands within many other genera.

Centrostachys is the only genus within subclade II A and within the entire Achyranthoids which is adapted to an aquatic or subaquatic habitat, such as shore areas and marshy places but this alone is not a sufficient criterion to maintain it as separated monotypic genus. *Centrostachys* was shifted to the genus *Achyranthes* many times in classification history by different authors (or vice versa, as suggested by Standley (1915)) due to its morphological similarities (e.g. Bentham & Hooker, 1880; Schinz, 1893; Cavaco, 1962). As shown in our BayesTraits analyses, *Centrostachys aquatica* shares several morphological characters with *Achyranthes*: e.g., the absence of sterile flowers (char. 10), the shape of paracladia (char.6), the structure of cymes (char. 8), and, in particular the position/angle of cymes related to the inflorescence axis (char. 9) which is a unique feature within Achyranthoids and explains the pre-phylogenetic genus circumscription of *Achyranthes* and *Centrostachys*. Overall, molecularly, and morphologically, there is more evidence to include *Centrostachys* in *Achyranthes* than to retain it as a separate genus.

Achyropsis has also been the subject of controversy by several authors and has been included in the genus *Achyranthes* by some (e.g., Moquin-Tandon, 1849; Schinz, 1893). Townsend (1980) mentioned that both genera are remarkably close morphologically but considered their species as unlikely to be confused. According to him, flowers of *Achyropsis* are much smaller than in most species of *Achyranthes*, bracteoles are not long-aristate and the fruiting perianth is not deflexed in *Achyropsis*. However, these criteria can be considered for species delimitations within our new circumscribed genus *Achyranthes*, but phylogenies clearly show that the genus *Achyropsis* cannot be maintained if the genus *Achyranthes* is to be rendered monophyletic. Within subclade II A we therefore propose to maintain only one monophyletic genus, which has to be called *Achyranthes* L. as this is the oldest available generic name.

4.5 Taxonomic treatment

A commented checklist including complete homotypic and heterotypic synonymy of the genera and species within subclade II of the achyranthoid clade of Amaranthaceae is provided here according to the newly established genus circumscriptions, but excluding the genus *Achyranthes* according to its new circumscription, which corresponds to subclade II A (compare Figure 4.B and Figure 4.C). The complete checklist of the achyranthoid subclade II including also *Achyranthes* (subclade II A) will be published subsequently in the frame of publication of Chapter 4, which is already prepared for submission to the journal Taxon (Di Vincenzo & al., in prep.).

Newly established genus names, new combinations, and one species resurrection are indicated by "gen. nov.", or "comb. nov." followed by the planned taxon name in square brackets, the citation of authors, and complemented by the expression "[to be published]" (e.g.: gen. nov. ['*Evelynastra*'] Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]). Relevant provisions of the *International Code of Nomenclature for algae, fungi, and plants* (Turland & al., 2018), were applied and cited after the nomenclatural status (e.g., "nom. illeg. [Art. 53.1]"). In total we accept five genera within the achyranthoid subclade II. The genus *Achyranthes* L. is largely extended, now also including the species of the formerly accepted genera *Nototrichium* Hillebr., *Centrostachys* Wall. and *Achyropsis* (Moq.) Hook.f. plus the single species *Pandiaka lanuginosa* which was formerly accepted as a species of the genus *Pandiaka* by misapplication of an incorrect name but must be correctly cited as *Achyranthes schinzii* (Standl.) Cufod.[2]. As explained above, *Achyranthes* is excluded from the checklist here but is part of the upcoming publication Di Vincenzo & al. (in prep.). The second genus which is also largely extended is *Cyathula* Blume, which now also comprises the earlier accepted genera *Sericocomopsis* Schinz (of which one species was resurrected before inclusion into *Cyathula*), *Pandiaka* (Moq.) Hook.f. and *Nelsia* Schinz. The genus *Sericostachys* Gilg & Lopr. ex Lopr. remains as it was already accepted before. In addition, two monotypic genera are newly established here, each consisting of one species, one of which formerly was included into the genus *Cyathula* Blume and the other into *Sericocomopsis* Schinz, respectively.

[2] ***Achyranthes schinzii*** (Standl.) Cufod. in Bull. Jard. Bot. État 23: 73. 1953 ≡ *Achyranthes lanuginosa* Schinz, Pflanzenw. Ost-Afrikas C: 174. 1895, nom. illeg. [Art. 53.1, non *Achyranthes lanuginosa* Nutt., in Trans. Amer. Philos. Soc., n.s., 5: 166. 1835] ≡ *Centrostachys schinzii* Standl. in J. Wash. Acad. Sci. 5(3): 76. 1915 ≡ *Pandiaka lanuginosa* Schinz in Engler & Prantl, Nat. Pflanzenfam., ed. 2, 16c: 64. 1934, nom. illeg. [Art. 52.1]

4.5.1 Checklist of the taxa to be included into the achyranthoid subclade II of Amaranthaceae

Achyranthoid subclade II. Sec. Di Vincenzo & al. (2018)

Cyathula Blume, Bijdr. Fl. Ned. Ind. 11: 548. 1826, nom. cons. Sec. Di Vincenzo & al. (in prep.)

Type: *Cyathula prostrata* (L.) Blume ≡ *Achyranthes prostrata* L.

= *Achyranthes* sect. *Pandiaka* Moq. in de Candolle, Prodr. 13(2): 310. 1849 ≡ *Pandiaka* (Moq.) Hook.f. in Bentham G. & Hooker J. D. (ed.), Gen. Pl. 3: 35. 1880 — Type: *Pandiaka involucrata* (Moq.) B.D.Jacks.

= *Sericocomopsis* Schinz in Bot. Jahrb. Syst. 21: 184. 1895 syn. sec. Di Vincenzo & al. (in prep.). Lectotype: *Sericocomopsis hildebrandtii* Schinz [fide Regnum vegetabile: Vol. 129, NCU-3. Names in Current Use for Extant Plant Genera. 1993]

= *Argyrostachys* Lopr. in Bot. Jahrb. Syst. 30(1): 108. 1901 — Type: *Argyrostachys splendens* Lopr.

= *Nelsia* Schinz in Vierteljahrsschr. Naturf. Ges. Zürich 56: 247. 1911 syn. sec. Di Vincenzo & al. (in prep.) — Type: *Nelsia quadrangula* (Engl.) Schinz

– *Polyscalis* Wall., Numer. List: 233, n° 6939. 1832, nom. inval.

***Cyathula* comb. nov. [‘*heudelotii*’] (Moq.) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Non *Cyathula angustifolia* Moq. in de Candolle, Prodr. 13(2): 328 (1849). Sec. Di Vincenzo & al. (in prep.)**

≡ *Achyranthes heudelotii* Moq. in de Candolle, Prodr. 13(2): 310. 1849 ≡ *Pandiaka heudelotii* (Moq.) Benth. & Hook.f. ex B.D.Jacks., Index Kew. 2: 409. 1894 ≡ *Centrostachys heudelotii* (Moq.) Standl. in J. Wash. Acad. Sci. 5(3): 76. 1915 syn. sec. Di Vincenzo & al. (in prep.) − *Pandiaka heudelotii* (Moq.) Benth. & Hook.f., Gen. Pl. 3: 36. 1883, nom. inval. — Type: Senegal, Heudelot 280 (holotype: P! [fide Townsend 1980]).

= *Gomphrena angustifolia* Vahl, Symb. Bot. 3: 45. 1794 ≡ *Achyranthes angustifolia* (Vahl) Lopr. in Malpighia 14: 434. 1901, nom. illeg. ≡ *Pandiaka angustifolia* (Vahl) Hepper in Kew Bull. 25(2): 189. 1971 syn. sec. Di Vincenzo & al. (in prep.) — Type: ‘India orientali’ Koenig s.n. (holotype: C C10005385!).

= *Achyranthes angustifolia* Benth., Niger Fl.: 492. 1849 ≡ *Achyranthes benthamii* Lopr. in Bot. Jahrb. Syst. 30(1): 108. 1901 ≡ *Centrostachys angustifolia* (Benth.) Standl. in J. Wash. Acad. Sci. 5(3): 75. 1915 syn. sec. Di Vincenzo & al. (in prep.) ≡ *Pandiaka benthamii* (Lopr.) Schinz, Nat. Pflanzenfam., ed. 2, 16c: 64. 1934 — Type: Nigeria, On the Quorra, Vogel 98 (holotype: K [fide Townsend 1980]); Senegal, Lécart, T. 95 (holotype: M M0241537 [image!]).

= *Pandiaka heudelotii* f. *rubella* Suess. in Bull. Jard. Bot. État 15: 66. 1938 syn. sec. Di Vincenzo & al. (in prep.) — Type: Nigeria, Lely 641 (holotype: K).

= *Pandiaka heudelotii* var. *spicata* Suess. in Bull. Jard. Bot. État 15: 65. 1938 — Type: Ivory Coast, Brown 390 (syntype: M); Ivory Coast, Irvine 542 (syntype: M); Togo, prope Lome, Warnecke 182 (syntype: BR).

= *Pandiaka heudelotii* var. *subglobosa* Suess. in Bull. Jard. Bot. État 15: 66. 1938 — Type (statement): Syntypes: Congo (DRC): Distrikt des unteren Kongo: Benga, Vanderyst 14950 (BR); Kisantu, Vanderyst s.n. (BR); Kisantu, Gillet 1079 (BR); Kisantu, Vanderyst 30442 et 30875 (BR); Kipako-Sanda, Vanderyst 30407 (BR); Wulu-Kipako, Vanderyst 30524 et 30726 (BR); Mfulu-Kisantu, Vanderyst 40660 (BR); Miao, Dobbelaere 17 (BR); Kimuenza, Gillet 784. Congo (DRC): Distrikt des Kasai: Luluabourg, Vanderyst 20933 (BR). Nigeria: Katagum-District, Dalziel 154 (K). ANGOLA: Guinga, Welwitsch 6565. Soudan: Land der Djur, Schweinfurth 2309 (K) [fide Suessenguth 1938].

***Cyathula achyranthoides* (Kunth) Moq. in de Candolle, Prodr. 13(2): 326. 1849. Sec. Borsch (2001)**

≡ *Desmochaeta achyranthoides* Kunth, Nov. Gen. Sp. (quarto ed.) 2: 210. 1817 ≡ *Cyathula prostrata* var. *achyranthoides* (Kunth) Kuntze, Revis. Gen. Pl. 2: 542. 1891 — Type: Colombia, Mompox, Rio Magdalena, Bojorque, Apr 1801, A. Bonpland & F. Humboldt 1507 (syntypes: P P00670028 [image!], P P00670028 [image!]; isosyntypes: P P00136029 [image!], P P00136029 [image!]).

= *Desmochaeta densiflora* Kunth, Nov. Gen. Sp. (quarto ed.) 2: 211. 1817 ≡ *Cyathula achyranthoides* var. *densiflora* (Kunth) Moq. in de Candolle, Prodr. 13(2): 327. 1849

= *Achyranthes uncinata* Willd. ex Schult. in Roemer & Schultes, Syst. Veg. ed. 15[bis] 5: 554. 1819 ≡ *Desmochaeta uncinata* Schult. in Roemer & Schultes, Syst. Veg. ed. 15[bis] 5: 554. 1819

= *Pupalia densiflora* Mart., Nov. Gen. Sp. Pl. 2: 61. 1826

= *Achyranthes geminata* Thonn., Beskr. Guin. Pl.: 138. 1827 ≡ *Cyathula geminata* (Thonn.) Moq. in de Candolle, Prodr. 13(2): 330. 1849

= *Cyathula achyranthoides* var. *glabrescens* Moq. in de Candolle, Prodr. 13(2): 327. 1849

= *Achyranthes hirtiflora* A.Rich. in Hist. Fis. Cuba, Bot. 11: 175. 1850

***Cyathula biflora* Schinz in Vierteljahrsschr. Naturf. Ges. Zürich 76: 139. 1931. Sec. Cavaco (1954)**

Type: Madagascar, im Gebüsch bei Tamatave, Apr 1837, J. Goudot s.n. (holotype: Z ZT-00079984 [fide UTH / ETH: 6 jul 2022]).

***Cyathula braunii* Gilg ex Schinz in Vierteljahrsschr. Naturf. Ges. Zürich 76: 139. 1931. Sec. Townsend (1985)**

≡ *Pupalia braunii* (Gilg ex Schinz) Gilg ex Peter in Repert. Spec. Nov. Regni Veg. Beih. 40(2): 227. 1938 — Type: Tanzania, Rondo-Lutamba, 13 Jun 1906, K. Brown 1276 (unknown type categorys: EA, K K000243632 [image!]).

***Cyathula capitata* Moq. in de Candolle, Prodr. 13(2): 329. 1849. Sec. Bojian & al. (2003)**

= *Achyranthes hamata* H.Lév. & Vaniot, Fl. Kouy-Tchéou: 23. 1914

− *Polyscalis capitata* Wall., Numer. List: 233, n° 6940. 1832, nom. inval.

***Cyathula* comb. nov. [‘*carsonii*’] (Baker) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Di Vincenzo & al. (in prep.)**

≡ *Achyranthes carsonii* Baker in Bull. Misc. Inform. Kew 1897: 280. 1897 ≡ *Pandiaka carsonii* (Baker) C.B.Clarke in Thiselton-Dyer, Fl. Trop. Afr. 6(1): 70. 1909 syn. sec. Di Vincenzo & al. (in prep.) ≡ *Centrostachys carsonii* (Baker) Standl. in J. Wash. Acad. Sci. 5(3): 76. 1915 — Type: Zambia, Fwambo, Lake Tanganyika, alt. 5000-6000 ft., 1893, Carson [1893] 8 (holotype: K [fide Townsend 1988])

= *Argyrostachys splendens* Lopr. in Bot. Jahrb. Syst. 30(1): 109. 1901 — Type: Malawi, (Nyassaland) Unyka beim Dorfe Luembu, auf feuchten Wiesen, alt. 1600 m, Oct 1899, W. Goetze 1391 (isotype: E E00193546 [image!]; second step lectotype: BR BR0000008819727 [image!]).

= *Pandiaka obovata* Suess. in Bull. Jard. Bot. État 15: 67. 1938 — Type: Congo (DRC), District des oberen Katanga: Dembo de Katuba, Quarré 3222 (syntype: BR [fide Suessenguth 1938]); Congo (DRC), Distrikt des oberen Katanga: Katuba, Nov 1934, Quarré 4316 (syntype: BR [fide Suessenguth 1938]).

= *Pandiaka schweinfurthii* var. *glabra* Schinz ex Suess. in Bull. Jard. Bot. État 15: 68. 1938 ≡ *Pandiaka glabra* (Suess.) Hauman in Bull. Jard. Bot. État 18: 113. 1946 — Type: Congo (DRC), Distrikt des oberen Katanga: Elisabethville, Quarré 691 (holotype: BR [fide Suessenguth 1938]).

= *Achyranthes lanata* Schinz in Vierteljahrsschr. Naturf. Ges. Zürich 76: 141. 1931, nom. illeg. − *Pandiaka lanata* (Schinz) Hauman in Bull. Jard. Bot. État 18: 113. 1946, comb. inval. — Type: Congo (DRC), Katanga, Sep 1899, Verdick 204 [fide Schinz 1931].

***Cyathula carsonii* var. *milnei* (Suess. & Overkott) Di Vincenzo, Berendsohn, Wondafrash & Borsch [comb. nov. to be published]. Sec. Di Vincenzo & al. (in prep.)**

≡ *Pandiaka milnei* Suess. & Overkott in Bot. Arch. 41: 76. 1940 syn. sec. Di Vincenzo & al. (in prep.) ≡ *Pandiaka carsonii* var. *milnei* (Suess. & Overkott) Cavaco in Mém. Mus. Natl. Hist. Nat., B, Bot. 13(1): 139. 1962 syn. sec. Di Vincenzo & al. (in prep.) — Type: Congo (DRC),

Haut-Katanga, mines de Luiswiski, Quarré 5213 (syntypes: BR [fide Cavaco 1962], P [fide Cavaco 1962]). Type (statement): : Rhodesia septentrionalis, regio Mwinlunga, (Kalenda-Plain auf
Laterit); legit E. Milne-Redhead, Nr. 2835[fide Suessenguth & Overkott 1940].

= *Pandiaka carsonii* var. *linearifolia* Hauman ex C.C.Towns., Fl. Zambes. 9(1): 121. 1988, nom. illeg.

− *Pandiaka carsonii* var. *linearifolia* Hauman in Boutique, Flore du Congo Belge et du Ruanda-Urundi, Spermatophytes 2 2: 49. 1951, nom. inval.

***Cyathula ceylanica* Hook.f., Fl. Brit. India 4: 723. 1885. Sec. Townsend (1980)**

≡ *Cyathula capitata* Thwaites, Enum. Pl. Zeyl.: 249. 1861, nom. illeg. [non Cyathula capitata Moq.] — Type: Sri Lanka, Ooma-oya, Mar 1854, G. Thwaites s.n. (holotype: K [fide Townsend 1980]; isotype: PDA).

***Cyathula* comb. nov. [‘*confusa*’] (C.C.Towns.) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Di Vincenzo & al. (in prep.)**

≡ *Pandiaka confusa* C.C.Towns. in Kew Bull. 34(3): 427. 1980 syn. sec. Di Vincenzo & al. (in prep.) — Type: Zambia, Milne-Redhead 2841 (holotype: K).

= *Pandiaka schweinfurthii* var. *compacta* Suess. & Overkott in Bot. Arch. 41: 78. 1940 — Type: Zambia, Milne-Redhead 2841b (holotype: K [fide Townsend 1980]). Type (statement): Rhodesia septentríonalis, regio Mwinilunga; prope „Matonchi-Farm", inter Brachystegias. Legit E. Milne-Redhead, 18. X. 1937,
Nr. 2841 h [fide Suessenguth & Overkott 1940].

= *Pandiaka schweinfurthii* var. *minor* Suess. & Overkott in Bot. Arch. 41: 79. 1940 — Type: Zambia, Milne-Redhead 3358 (holotype: [fide Townsend 1980]).

= *Pandiaka schweinfurthii* var. *parvifolia* Suess. & Overkott in Bot. Arch. 41: 78. 1940 — Type: Zambia, Milne-Redhead 2841a (holotype: K [fide Townsend 1980]).

***Cyathula coriacea* Schinz in Vierteljahrsschr. Naturf. Ges. Zürich 76: 140. 1931. Sec. Townsend (1993)**

Type: Kenya, F. Thomas 79 (isotypes: BM BM000910351[image!], Z Z-000000278 [fide UTH / ETH]).

− *Cyathula paniculata* Hauman in Bull. Jard. Bot. État 18: 109. 1946, nom. inval. [Art. 39.1]

***Cyathula cylindrica* Moq. in de Candolle, Prodr. 13(2): 328. 1849. Sec. Townsend (1988)**

≡ *Pandiaka cylindrica* (Moq.) B.D.Jacks., Index Kew. 2: 409. 1894 — Type: Madagascar, Tananarivou [Tananarive, Antananarivo], W. Bojer s.n. (syntype: P P00487030!; isosyntypes: P P04021746!, C C10000107 [image!], M M0107354!); South Africa, Omtata,

Omsamwubo, 1840, C. Drège s.n. (syntype: S S07-12219!; isosyntypes: K K000243638!, K K000243641!, K K000243642!, S S07-12220!, S S07-12221!).

= *Cyathula schimperiana* Moq. in de Candolle, Prodr. 13(2): 328. 1849

= *Cyathula mannii* Baker in Bull. Misc. Inform. Kew 128-129: 278. Aug–Sep 1897 ≡ *Cyathula cylindrica* var. *mannii* (Baker) Suess. in Mitt. Bot. Staatssamml. München 1(8): 188. 1953 syn. sec. Di Vincenzo & al. (in prep.)

= *Desmochaeta distorta* Hiern, Cat. Afr. Pl. 1: 891. 1900 ≡ *Cyathula distorta* (Hiern) C.B.Clarke in Thiselton-Dyer, Fl. Trop. Afr. 6(1): 46. 1909 — Type (statement): Huilla. - In wooded thickets at Lopollo; fl. and fr. beginning of Feb. 1860. [Welwitsch] No. 6487.

= *Pupalia huillensis* Hiern, Cat. Afr. Pl. 1: 892. 1900 [as "*Pupal*"]

= *Cyathula cylindrica* var. *pilosa* Suess. in Bull. Jard. Bot. État 15: 62. 1938

= *Cyathula cylindrica* var. *orbicularis* Suess. in Mitt. Bot. Staatssamml. München 1(3): 77. 1951

= *Cyathula schimperiana* subvar. *subfusca* Suess. in Mitt. Bot. Staatssamml. München 1(6): 190. 1953 syn. sec. Di Vincenzo & al. (in prep.)

= *Cyathula albida* Lopr. in Bot. Jahrb. Syst. 27(1-2): 53. 1899 − *Cyathula albida* Lopr. in Malpighia 14: 445. 1901, nom. inval. — Type: Angola, Benguella: Huilla, May 1895, Antunes 289.

− *Pupalia schimperiana* Hochst. in Flora 24(1 Intell.): 22. 1841, nom. inval.

− *Achyranthes cylindrica* Bojer ex Moq. in de Candolle, Prodr. 13(2): 328. 1849, nom. inval.

− *Pupalia alopecurus* Fenzl ex Moq. in de Candolle, Prodr. 13(2): 328. 1849, nom. inval.

− *Trichinium latifolium* E.Mey. ex Moq. in de Candolle, Prodr. 13(2): 328. 1849, nom. inval.

Cyathula divulsa Suess. in Repert. Spec. Nov. Regni Veg. 51: 196. 1942. Sec. Sec. Townsend (1985)

Type: Tanzania, Dodoma Manyoni Distr., Manyoni Kopje, alt. 1280 m, 3 May 1932, B. Burtt 3663 (holotype: K K000243634 [image!]).

= *Cyathula schimperiana* var. *burttiana* Suess. in Mitt. Bot. Staatssamml. München 1(6): 189. 1953

Cyathula comb. nov. [‘*elegantissima*’] (Schinz) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Di Vincenzo & al. (in prep.)

≡ *Achyranthes elegantissima* Schinz in Bot. Jahrb. Syst. 21: 186. 1895 syn. sec. Di Vincenzo & al. (in prep.) ≡ *Centrostachys elegantissima* (Schinz) Standl. in J. Wash. Acad. Sci. 5(3): 76. 1915 syn. sec. Di Vincenzo & al. (in prep.) ≡ *Pandiaka elegantissima* (Schinz) Dandy, Fl. Pl. Sudan 1: 119. 1950 syn. sec. Di Vincenzo & al. (in prep.) — Type: Congo (DRC), Djur, Majob, 26 Apr 1869, G. Schweinfurth 1542 (holotype: S S07-12227 [image!]; isotype: K [fide Townsend 1980]).

= *Achyranthes oblanceolata* Schinz in Bot. Jahrb. Syst. 21: 187. 1895 ≡ *Pandiaka oblanceolata* (Schinz) C.B.Clarke in Thiselton-Dyer, Fl. Trop. Afr. 6(1): 70. 1909 ≡ *Centrostachys oblanceolata* (Schinz) Standl. in J. Wash. Acad. Sci. 5(3): 76. 1915 — Type: Sudan, G. Schweinfurth 2185 (isotype: K [fide Townsend 1980]).

= *Pandiaka cylindrica* Hook.f. ex Baker & C.B.Clarke in Thiselton-Dyer, Fl. Trop. Afr. 6(1): 68. 1909, nom. superfl. − *Cyathula cylindrica* Schweinf. ex Benth. & Hook.f., Gen. Pl. 3(1): 36. 1880, nom. inval. − *Pandiaka cylindrica* (Schweinf.) Benth. & Hook.f., Gen. Pl. 3(1): 36. 1880, comb. inval. — Type: Congo (DRC), Djur, Majob, 26 Apr 1869, G. Schweinfurth 1542 (holotype: K [fide Townsend 1980]).

***Cyathula erinacea* Schinz in Bot. Jahrb. Syst. 21: 189. 1895. Sec. Townsend (1985)**

≡ *Sericocomopsis erinacea* (Schinz) Peter in Repert. Spec. Nov. Regni Veg. Beih. 40(2): 231. 1938 — Type: Ostafrika, Fischer 70.

***Cyathula fernando-poensis* Suess. & Friedrich in Mitt. Bot. Staatssamml. München 1(6): 188. 1953. Sec. Suessenguth (1953)**

***Cyathula* comb. nov. [‘*hildebrandtii*’] (Schinz) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Di Vincenzo & al. (in prep.)**

≡ *Sericocomopsis hildebrandtii* Schinz in Bot. Jahrb. Syst. 21: 184. 1895 syn. sec. Di Vincenzo & al. (in prep.) ≡ *Cyphocarpa hildebrandtii* (Schinz) C.B.Clarke in Thiselton-Dyer, Fl. Trop. Afr. 6(1): 54. 1909 — Type: Kenya, Teita District, Ndi, Jul 1877, J. Hildebrandt 2584 (isotypes: K K000243587!, M M0107377!).

= *Sericocomopsis grisea* Suess. in Kew Bull. 4(4): 479. 1949 — Type: Tanzania, Kiruru, Upare, Moshi, alt. 780 m, May 1927, A. Haarer 499 (holotype: K K000243588!; isotype: M M0107376).

***Cyathula humbertiana* Cavaco, Notul. Syst. (Paris) 14: 265. 1953. Sec. Cavaco (1954)**

***Cyathula* comb. nov. [‘*involucrata*’] (Moq.) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Townsend (1985)**

≡ *Achyranthes involucrata* Moq. in de Candolle, Prodr. 13(2): 310. 1849 ≡ *Pandiaka involucrata* (Moq.) B.D.Jacks., Index Kew. 2: 409. 1894 ≡ *Centrostachys involucrata* (Moq.) Standl. in J. Wash. Acad. Sci. 5(3): 76. 1915 − *Pandiaka involucrata* (Moq.) Hook.f., Gen. Pl. 3: 35. 1880, comb. inval. — Type: Nigeria, Village of Pandiaki, 17 Sep 1841, J. Ansell 1042? (K K000243731 [image!], K K000243732 [image!]).

= *Pandiaka involucrata* var. *megastachya* Suess. in Repert. Spec. Nov. Regni Veg. 44: 46. 1938

− *Achyranthes capitata* Moq. in de Candolle, Prodr. 13(2): 310. 1849, nom. inval.

***Cyathula madagascariensis* Cavaco, Notul. Syst. (Paris) 14: 263–264. 1952. Sec. Cavaco (1954)**

***Cyathula* comb. nov. [‘*meruensis*’] (Suess.) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Di Vincenzo & al. (in prep.)**

≡ *Sericocomopsis meruensis* Suess. in Mitt. Bot. Staatssamml. München 1(1): 7. 1950 syn. sec. Di Vincenzo & al. (in prep.) — Type: Tanzania, Meru Crater, T.T., alt. 4700 m, Jan 1941, G. Babault B 1808 (holotype: K K000243589!; isotype: M M0107375 [image!]).

− *Cyathula lindaviana* Volkens ex Lopr. in Bot. Jahrb. Syst. 27: 62. 1900, nom. inval. [nomen nudum; Art. 38.1, 38.2 Ex. 1.] syn. sec. Di Vincenzo & al. (in prep.)

− *Achyranthes lindaviana* Schinz ex Peter in Repert. Spec. Nov. Regni Veg. Beih. 40(2): 230. 1938, nom. inval. [nomen nudum; Art. 38.1, 38.2 Ex. 1.] syn. sec. Di Vincenzo & al. (in prep.)

− *Sericocomopsis lindaviana* Peter in Repert. Spec. Nov. Regni Veg. Beih. 40(2): 229. 1938, nom. inval. [no latin description or reference to description; Art. 39.1] syn. sec. Di Vincenzo & al. (in prep.) — Syntypes: Tanzania, Kilimandscharo, Rahesteppe, Apr 1894, G. Volkens 2180 (Type: HBG HBG502344[image!]); Kenya, Meru: am Fl. Engare Nanjuki entlang, A. Peter P 2206; Tanzania, DOAfr., Kilimanscharo: Steppe zw. Pangani und Himo, Volkens 550; Kenya, Magadi: Olbussare-Fl. - Eyassi-See, Baum-Busch-Steppe über Gras, A. Peter P 43417; Tanzania, DOAfr., Kilimandscharo, Steppe Kwagogo - Moschi, Engler 1689; Tanzania, Engaruka: Ngaruka, Merker 446; Tanzania, Engaruka: Emugur Belekj, Strauchsteppe, A. Peter P 42753; DOAfr., Mserere: Maji ya Kuchimba - Emugur Oreteti, Steppe am Gunzert-Bg., A. Peter P 42691; Tanzania, Manyara "Winter-Hochland": Steppe südöstl. des Gelei, Jahn 22; Kenya, Magadi-Umbulu: Kambi ya Nyoka, A. Peter P 43505.

***Cyathula mollis* C.C.Towns. in Kew Bull. 40(1): 35. 1985. Sec. African Plant Database (version 4.0.0)**

Type: Central African Republic, Yalinga, Haute Kotto, 27 May 1921, G. Le Testu 2782 (holotype: BR BR0000008819833!; isotypes: B B100627694!, M M0107348, P P00466172!, P P00466173!, P P00466174!).

***Cyathula natalensis* Sond. in Linnaea 23: 97. 1850. Sec. Townsend (1988)**

= *Cyathula spathulata* Schinz in Bull. Herb. Boiss. 4: 421. 1896

= *Cyathula spathulifolia* Lopr. in Bot. Jahrb. Syst. 27(1-2): 54. 1899 − *Cyathula spathulifolia* Lopr. in Malpighia 14: 444. 1901, nom. inval. — Type: South Africa, Umzyniati Falls, an Waldrändern, M. Wood 1323 [fide Lopriore 1900].

− *Pupalia natalensis* Sond. in Linnaea 23: 97. 1850, nom. inval.

***Cyathula obtusifolia* Cavaco in Bull. Mus. Natl. Hist. Nat. II, 24: 575. 1953. Sec. Cavaco (1954)**

***Cyathula officinalis* K.C.Kuan in Acta Phytotax. Sin. 14(1): 60. 1976. Sec. Bojian & al. (2003)**

***Cyathula perrieriana* Cavaco in Bull. Soc. Bot. France 99: 130. 1952. Sec. Cavaco (1954)**

Type: Madagascar, Ouest: bois sablonneux, Manongarivo (Ambongo), Perrier de la Bâthie 1678.

***Cyathula pobeguinii* Jacq.-Fél. in Bull. Soc. Bot. France 96: 196. 1950. Sec. African Plant Database (version 4.0.0)**

Type (statement): Fouta-Djallon (Guinée française): Dabola, Pobéguin 987; Mali 1400 m. alt., Jacques-Félix 2017.

***Cyathula polycephala* Baker in Bull. Misc. Inform. Kew 1897(128-129): 278. 1897. Sec. Townsend (1985)**

≡ *Pupalia polycephala* (Baker) Chiov., Fl. Somala 2: 379. 1932 — Type: Kenya, Masai District, Kapiti, Sep 1884, J. Thomson s.n. (holotypes: K K000243627!, K K000243627 [image!]).

= *Cyathula cordifolia* Hochst. ex Chiov. in Ann. Bot. (Rome) 9(2): 130. 1911 — Type: Ethiopia, Semien: Debarek comune nei pascoli e macchie, 11 Jul 1909, E. Chiovenda 953.

= *Cyathula echinulata* Hauman in Bull. Jard. Bot. État 18: 107. 1946 — Type: Congo (DRC), District des Lacs Édouard et Kivu: versant sud du Karisimbi, alt. 2700 m, 7 Mar 1935, de Witte 2283 (holotype: BR BR0000008819703 [image!]).

= *Cyathula schimperiana* var. *tomentosa* Suess. in Mitt. Bot. Staatssamml. München 1(6): 190. 1953

***Cyathula* comb. nov. [‘*porphyrargyrea*’] (Suess. & Overkott) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Suessenguth & Overkott (1940)**

≡ *Pandiaka porphyrargyrea* Suess. & Overkott in Bot. Arch. 41: 77. 1940 syn. sec. Di Vincenzo & al. (in prep.) — Type: Angola, Between R. Ndamba (Mundamba) and R. Nkoki, on shallow soil overlying "laterite", in open with., 8 Jan 1938, Milne-Redhead 3985 (syntype: K K000243754 [image!]; isosyntypes: BM BM000910333 [image!], BR, M M-0107299 [image!]); Angola, Regio "Moxico", unter Ndamba (Mundamba) et Nkoki, in solo lateritico, Milne-Redhead 3986 (syntype: K [fide Suessenguth & Overkott 1940]); Angola, Regio "Moxico", unter Ndamba (Mundamba) et Nkoki, in solo lateritico, Milne-Redhead 3987 (syntype: K [fide Suessenguth & Overkott 1940]).

***Cyathula prostrata* (L.) Blume, Bijdr. Fl. Ned. Ind. 11: 549. 1826. Sec. Bojian & al. (2003)**

≡ *Achyranthes prostrata* L., Sp. Pl., ed. 2 1: 296. 1762 ≡ *Desmochaeta prostrata* (L.) DC., Cat. Pl. Horti Monsp.: 102. 1813 ≡ *Pupalia prostrata* (L.) Mart. in Nova Acta Phys.-Med. Acad.

Caes. Leop.-Carol. Nat. Cur. 13: 321. 1826 — Type: P. Bladh s.n. (lectotype: LINN LINN-HL287-13 [image!]).

***Cyathula prostrata* var. *lancifolia* (Merr.) Backer, Fl. Males. 4: 83. 1949. Sec. Backer (1949)**

≡ *Cyathula lancifolia* Merr. in Philipp. J. Sci., C 11: 179. 1916 — Type: Philippines, Samar, Catubig River at Tagibiran, in damp forest at low altitudes, Feb 1916, Ramos Bureau of Science no. 24553 (isotype: BM BM000950561 [image!]).

= *Cyathula lancifolia* var. *stenophylla* Merr. in Philipp. J. Sci. 29: 478. 1926 ≡ *Cyathula prostrata* var. *stenophylla* (Merr.) Kanis in Kew Bull. 31(2): 340. 1976

***Cyathula prostrata* var. *pedicellata* (C.B.Clarke) Cavaco, Fl. Cameroun 17: 46. 1974. Sec. Townsend (1985)**

≡ *Cyathula pedicellata* C.B.Clarke in Thiselton-Dyer, Fl. Trop. Afr. 6(1): 46. 1909 ≡ *Cyathula prostrata* f. *pedicellata* (C.B.Clarke) Hauman, Fl. Congo Belge 2: 64. 1951

***Cyathula prostrata* (L.) Blume var. *prostrata*, Bijdr. Fl. Ned. Ind. 11: 549. 1826. Sec. Borsch (2001)**

= *Achyranthes alternifolia* L.f., Suppl. Pl.: 159. 1782 ["1781"], nom. illeg. [is replaced synonym for *Achyranthes globosa* Pers.] ≡ *Cyathula alternifolia* Druce in Rep. Bot. Soc. Exch. Club Brit. Isles 4(6): 618. 1917 ≡ *Achyranthes globosa* Pers., Syn. Pl. 1: 259. 1805 ≡ *Desmochaeta globosa* (Pers.) Schult. in Roemer & Schultes, Syst. Veg. ed. 15[bis] 5: 533. 1819 ≡ *Cyathula globosa* (Pers.) Moq. in de Candolle, Prodr. 13(2): 330. 1849

= *Cyathula geniculata* Lour., Fl. Cochinch.: 102. 1790

= *Achyranthes diffusa* Moench, Methodus: 315. 1794

= *Achyranthes debilis* Poir., Encycl. Suppl. 2: 10. 1811 ≡ *Cyathula prostrata* var. *debilis* (Poir.) Moq. in de Candolle, Prodr. 13(2): 326. 1849

= *Desmochaeta micrantha* DC., Cat. Pl. Horti Monsp.: 102. 1813

= *Desmochaeta repens* Schult. in Roemer & Schultes, Syst. Veg. ed. 15[bis] 5: 552. 1819 ≡ *Achyranthes repens* B.Heyne ex Roth, Nov. Pl. Sp.: 167. 1821, nom. illeg. [non Achyranthes repens L. non Achyranthes repens Elliott] ≡ *Cyathula repens* Moq. in de Candolle, Prodr. 13(2): 330. 1849

= *Cyathula prostrata* f. *rubella* Suess. in Bull. Jard. Bot. État 15: 63. 1938 — Type: Distrikt des zentralen Regenwaldes: Yangambi, alt. 470 m, Louis 1030 (BR [fide Suessenguth 1938]).

− *Achyranthes mollis* Lepr. ex Seub., Fl. Bras. 5(1): 230. 1875, nom. inval.

***Cyathula* comb. nov. [‘*quadrangula*’] (Engl.) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Townsend (1988)**

≡ *Sericocoma quadrangula* Engl. in Bot. Jahrb. Syst. 10: 7. 1889 ≡ *Sericocomopsis quadrangula* (Engl.) Lopr. in Bot. Jahrb. Syst. 27(1-2): 42. 1899 ≡ *Cyphocarpa quadrangula* (Engl.) C.B.Clarke in Thiselton-Dyer, Fl. Trop. Afr. 6(1): 54. 1909 ≡ *Nelsia quadrangula* (Engl.) Schinz in Vierteljahrsschr. Naturf. Ges. Zürich 56: 247. 1911 syn. sec. Di Vincenzo & al. (in prep.) — Type: Namibia, Hereroland, Usakos, alt. 900 m, May 1886, H. Marloth 1255 (holotype: PRE PRE0381229-0!).

= *Sericocoma nelsii* Schinz, Nat. Pflanzenfam. 3(1a): 107. 1893 — Type (statement): No specimen cited. Fig. 48L, p. 94.

= *Sericocoma welwitschii* Baker in Bull. Misc. Inform. Kew 1897: 278. 1897 ≡ *Sericocomopsis welwitschii* (Baker) Lopr. in Bot. Jahrb. Syst. 27(1-2): 42. 1899 ≡ *Cyphocarpa welwitschii* (Baker) C.B.Clarke in Thiselton-Dyer, Fl. Trop. Afr. 6(1): 53. 1909 — Type: Angola, Province of Mossamedes, Welwitsch 6501 ().

Cyathula comb. nov. [‘_ramulosa_’] (Hiern) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Baker & Clarke (1909)

≡ *Pandiaka ramulosa* Hiern, Cat. Afr. Pl. 1: 894. 1900 syn. sec. Di Vincenzo & al. (in prep.)

= *Pandiaka polystachya* Suess. in Bull. Jard. Bot. État 15: 67. 1938 — Type: Congo (DRC), District des oberen Katanga: In jugo Manika dicto, prope Katentania, in planitie aperta, 1921, Homblé 799 (syntype: BR [fide Suessenguth 1938]); Congo (DRC), Distrikt des oberen Katanga: Kapanga, Nov 1933, Overlaet 704 (syntype: [fide Suessenguth 1938]).

= *Pandiaka incana* Suess. & Overkott in Bot. Arch. 41: 75. 1940 ≡ *Pandiaka polystachya* var. *incana* (Suess. & Overkott) Cavaco, Notul. Syst. (Paris) 16: 98. 1960 — Type: Zimbabwe, Regio Mwinilunga. (S.W. Dobeka Bridge, auf Termitenhügeln, Milne-Redhead 2738 (unknown type category: K).

Cyathula comb. nov. [‘_richardsiae_’] (Suess.) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Townsend (1988)

≡ *Pandiaka richardsiae* Suess. in Mitt. Bot. Staatssamml. München 1(6): 192. 1953 — Type: Zambia, Abercorn District. By old Katwe Road above Inono Valley. Escarpment above Chilongowelo. In sandy soil in open, alt. 5000 ft., H. Richards 65 (holotype: Knot designated).

Cyathula comb. nov. [‘_rubrolutea_’] (Lopr.) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Townsend (1985)

≡ *Achyranthes rubrolutea* Lopr. in Bot. Jahrb. Syst. 27(1-2): 57. 1899 ≡ *Pandiaka rubrolutea* (Lopr.) C.C.Towns. in Kew Bull. 34(3): 428. 1980 syn. sec. Di Vincenzo & al. (in prep.) — Type: Congo (DRC), Lualaba Lumbarazy, Descamps s.n. (syntype: BR BR0000008820143 [image!]); Congo (DRC), M'Pueto, Mar 1896, Descamps s.n. (lectotypes: BR

BR0000008820150 [image!] designated by Townsend 1980: 428, Z designated by Townsend 1980; isolectotype: BR BR0000008820150 [image!]).

= *Pandiaka andongensis* Hiern, Cat. Afr. Pl. 1: 895. 1900 — Type: Angola, Welwitsch 8567 (holotype: BM [fide Townsend 1980]; isotype: K).

= *Pandiaka aristata* Suess. in Bull. Jard. Bot. État 15: 64. 1938 — Type: Tanzania, Iringa, auf Granit, Lynes 1h.215e (holotype: K [fide Townsend 1980]).

= *Pandiaka lindiensis* Suess. & Beyerle in Repert. Spec. Nov. Regni Veg. 44: 46. 1938 — Type: Tanzania, H. Schlieben 6083 (isotype: K [fide Townsend 1980]).

= *Pandiaka andongensis* var. *gracilis* Suess. in Mitt. Bot. Staatssamml. München 1(8): 341. 1953 — Type: Tanzania, Tanner 767 (holotype: K [fide Townsend 1980]).

***Cyathula semirosulata* Masam. in Trans. Nat. Hist. Soc. Taiwan 33: 111. 1943. Sec. POWO (2022)**

Notes: Not listed in Flora of Taiwan 2nd ed. vol. 6, Checklist https://www.researchgate.net/publication/320263417_Flora_of_Taiwan_Vol_1 nor in Digital Flora of Taiwan http://www.efloras.org/flora_page.aspx?flora_id=100

***Cyathula tomentosa* (Roth) Moq. in de Candolle, Prodr. 13(2): 327. 1849. Sec. Bojian & al. (2003)**

≡ *Achyranthes tomentosa* Roth, Nov. Pl. Sp.: 167. 1821

= *Desmochaeta tomentosa* Schult. in Roemer & Schultes, Syst. Veg. ed. 15[bis] 5: 554. 1819

= *Achyranthes sequax* Wall., Fl. Ind. 2: 506. 1824 ≡ *Desmochaeta sequax* (Wall.) A.Braun in Flora 24: 288. 1841 − *Polyscalis sequax* Wall., Numer. List: 233, n° 6939. 1832, nom. inval. − *Cyathula sequax* (Wall.) Moq. in de Candolle, Prodr. 13(2): 327. 1849, nom. inval.

***Cyathula* comb. nov. [‘*trichinioides*] (Suess.) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Hauman (1951)**

≡ *Pandiaka trichinioides* Suess. in Bull. Jard. Bot. État 15: 68. 1938 syn. sec. Di Vincenzo & al. (in prep.) — Type: Congo (DRC), Distrikt des Uele: circa Bambesa, Uele, 1936, Pittery 136 (lectotype: BR BR0000008820174 [image!]).

***Cyathula triuncinata* Moq. in de Candolle, Prodr. 13(2): 327. 1849. Sec. Cavaco (1954)**

***Cyathula triuncinella* Schinz in Bot. Jahrb. Syst. 21: 189. 1895. Sec. Cavaco (1954)**

Type: Madagascar, Ostküste, 1853, Boivin.

***Cyathula uncinulata* (Schrad.) Schinz, Pl. Bequaert. 5: 386. 1932. Sec. Townsend (1988)**

≡ *Achyranthes uncinulata* Schrad., Index Seminum (GOET) 1833: 1. 1833 ≡ *Desmochaeta uncinulata* (Schrad.) Hiern, Cat. Afr. Pl. 1: 890. 1900

= *Alternanthera lappulacea* Schltdl. ex Moq. in de Candolle, Prodr. 13(2): 329. 1849

= *Cyathula globulifera* var. *abyssinica* Moq. in de Candolle, Prodr. 13(2): 329. 1849 ≡ *Cyathula uncinulata* var. *abyssinica* (Moq.) Pic.Serm., Miss. Stud. Lago Tana 7: 37. 1951 − *Saltia globosa* Hochst. & Steud. ex Moq. in de Candolle, Prodr. 13(2): 325. 1849, nom. inval.

= *Cyathula sphaerocephala* Baker in J. Linn. Soc., Bot. 20: 238. 1883 — Type: Madagascar, Central Madagascar, Baron 1135 ().

= *Cyathula uncinulata* var. *pleiocephala* Suess. in Mitt. Bot. Staatssamml. München 1(8): 338. 1953

= *Cyathula uncinulata* var. *ceylanica* Cavaco, Fl. Madag. 67: 28. 1954

= *Cyathula globulifera* Moq. in de Candolle, Prodr. 13(2): 329. 1849 − *Achyranthes globulifera* Bojer ex Moq. in de Candolle, Prodr. 13(2): 329. 1849, nom. inval.

− *Paronychia capensis* Eckl. ex Steud., Nomencl. Bot., ed. 2, 2: 269. 1841, nom. inval.

− *Pupalia globosa* Hochst. & Steud. in Flora 24(1 Intell.): 22. 1841, nom. inval.

− *Pupalia holosericea* Fenzl ex Moq. in de Candolle, Prodr. 13(2): 329. 1849, nom. inval.

Cyathula comb. nov. [‘_welwitschii_’] (Schinz) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Townsend (1988)

≡ *Achyranthes welwitschii* Schinz, Pflanzenw. Ost-Afrikas C: 174. 1895 ≡ *Pandiaka welwitschii* (Schinz) Hiern, Cat. Afr. Pl. 1: 894. 1900 syn. sec. Di Vincenzo & al. (in prep.) ≡ *Centrostachys welwitschii* (Schinz) Standl. in J. Wash. Acad. Sci. 5(3): 76. 1915 syn. sec. Di Vincenzo & al. (in prep.) — Type: Angola, Welwitsch 6488 (isotypes: BM [fide Townsend 1980], K [fide Townsend 1980]; unknown type category: Z Z-000056078 [fide UTH / ETH]).

= *Achyranthes schweinfurthii* Schinz in Bull. Herb. Boiss. 4: 421. 1896 ≡ *Pandiaka schweinfurthii* (Schinz) C.B.Clarke in Thiselton-Dyer, Fl. Trop. Afr. 6(1): 69. 1909 ≡ *Centrostachys schweinfurthii* (Schinz) Standl. in J. Wash. Acad. Sci. 5(3): 76. 1915 syn. sec. Di Vincenzo & al. (in prep.) — Type: Sudan, Dem Gudju, Dar Fertit, 26 Jun 1871, G. Schweinfurth ser. 3, 66 (holotype: Z Z-000056075 [fide UTH / ETH]).

= *Psilotrichum debile* Baker in Bull. Misc. Inform. Kew 128-129: 279. Aug–Sep 1897 ≡ *Pandiaka debilis* (Baker) Hiern, Cat. Afr. Pl. 1: 894. 1900 ≡ *Pandiaka welwitschii* var. *debilis* (Baker) Suess. in Repert. Spec. Nov. Regni Veg. 44: 47. 1938 — Type: Angola, Welwitsch 6570 (holotype: K [fide Townsend 1980]).

= *Pandiaka kassneri* Suess. in Bull. Jard. Bot. État 15: 66. 1938 — Type: Congo (DRC), Shaba, Kasanga, 23 Mar 1908, Kässner 2665 (isotypes: BR BR0000008820082 [image!], K K000243740 [image!] [fide Townsend 1980], P P00466162 [fide MNHN: 9 jul 2022], Z Z-000056076 [fide UTH / ETH]).

− *Achyranthes kassneri* Schinz ex Peter in Repert. Spec. Nov. Regni Veg. Beih. 40(2): 240. 1938, nom. nud.

Gen. nov. [‘*Evelynastra*’] Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Di Vincenzo & al. (in prep.)

Type: Species comb. nov. [‘*Evelynastra pallida*’] (S.Moore) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published] ≡ *Sericocoma pallida* S.Moore

Etymology: The genus name ‘*Evelynastra’* is dedicated to my beloved daughter Evelyn Alicia Di Vincenzo. The ending ‘astra’ points to the main characteristic of the genus – the stellate hairs of the indumentum of stem and leaves – which is an almost unique character within Achyranthoids.

Diagnosis: perennial shrubs or subshrubs; leaves distinctly petiolate and within single individuals varying from elliptic to obovate, spathulate or oblanceolate; indumentum of stem and leaves constituted of branched hairs: stellate hairs on leaf surfaces and condensed-dendroid on inflorescence axes, upper branches and sometimes leaf veins stellate (see Figure 4.D, photo A & B); paracladia globose or elongate to cylindric with cymes densely aggregated; paracladia solitary on main axes as well as on lateral branches; cymes with more than one order of branches, and sometimes with very few sterile flowers on uppermost branches in relation to many fertile flowers per cyme; sterile flowers always unmodified [not modified to hooks or spines as common in other achyranthoid genera]; androecial tube appendages quadrate to oblong, furnished with fimbriate dorsal scales and the apex denticulate or fringed; tepals: 2 outer lanceolate with an acuminate tip, up to 3 veins, and the midrib mucronate to shortly excurrent, 3 inner smaller but similar in shape; texture of tepals remaining herbaceous or scarious at maturity; bracteoles: ovate with an acuminate tip, the midrib mucronate to shortly excurrent; bracts: ovate with an acuminate tip, the midrib not excurrent; ovary glabrous.

Species comb. nov. [‘*Evelynastra pallida*’] (S.Moore) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Di Vincenzo & al. (in prep.)

≡ *Sericocoma pallida* S.Moore in J. Bot. 15: 70. 1877 ≡ *Sericocomopsis pallida* (S.Moore) Schinz in Bot. Jahrb. Syst. 21: 185. 1895 syn. sec. Di Vincenzo & al. (in prep.) ≡ *Cyphocarpa pallida* (S.Moore) C.B.Clarke in Thiselton-Dyer, Fl. Trop. Afr. 6(1): 54. 1909 Townsend (1985) — Type: Somalia, Meid, Ahl Mountains, alt. 1200 m, Apr 1875, J. Hildebrandt 1521 (holotype: K K000243585, K000243584).

= *Sericocomopsis pallida* (S.Moore) Schinz var. *parvifolia* Suess. in Kew Bull. 4(4): 480. 1949 — Type: Somalia, [British Somaliland] Wanad, Protection Plot, Burao, 6 Oct 1944, P. Glover & H. Gilliland 70 (holotype: K K000243586 [image!]; isotype: EA EA000002257!).

= *Sericocomopsis pallida* (S.Moore) Schinz var. *grandis* Suess. in Mitt. Bot. Staatssamml. München 1(8): 343. 1953 — Type: Kenya, Northern Province, Moyale, alt. 780 – 1200 m, 22 Jul 1952, J. Gillett 13614 (isotypes: BR BR0000008820921 [image!], EA EA000002256!, M M0107374 [image!], PRE PRE0824140-0, S S07-12210 [image!]).

Gen. nov. [‘*Sebsebia*’] Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Di Vincenzo & al. (in prep.)

Type: Species comb. nov. [‘*Sebsebia orthacantha*’] (Hochst. ex Asch.) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published] ≡ *Pupalia orthacantha* Hochst. ex Asch.

Etymology: In honor of the Ethiopian botanist Sebsebe Demissew, who was already honoured with the Kew International Medal (2016), the Cuatrecasas Medal for Excellence in Tropical Botany (Smithsonian National Museum of Natural History, USA, 2021), elected as a member of the Royal Society (2018) for “outstanding contributions to research and innovation”, and recently received the Linnean Society’s award in recognition of his dedication to work on the Ethiopian flora (April 2022).

Diagnosis: annual herbs; leaves distinctly to long petiolate and within single individuals varying from ovate to elliptic or lanceolate; indumentum of stem and leaves constituted of uniseriate hairs; paracladia globose or elongate to cylindric with cymes densely aggregated; paracladia solitary on main axes as well as on lateral branches; cymes with more than one order of branches, in which fertile and sterile flowers are arranged together; sterile flowers and their bracteoles modified to spines serving animal dispersal; in fruit the axis and branches of the lateral cymes become indurate and inseparably connate, so that each cyme falls as a complete burr; androecial tube appendages broadly cuneate-obovate with a dentate to fimbriate apex and a furcate dorsal scale; tepals all similar, lanceolate with an acute tip, up to 3-5 veins, and the midrib mucronate to shortly excurrent; tepals becoming slightly indurate at their base at maturity; bracteoles: broadly ovate with an acuminate tip, the midrib distinctly to long excurrent; bracts: lanceolate with an acuminate tip, the midrib mucronate to shortly excurrent; ovary glabrous.

Species comb. nov. [‘*Sebsebia orthacantha*’] (Hochst. ex Asch.) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]. Sec. Di Vincenzo & al. (in prep.)

≡ *Pupalia orthacantha* Hochst. ex Asch., Beitr. Fl. Aethiop.: 181. 1867 ≡ *Cyathula orthacantha* (Hochst. ex Asch.) Schinz, Nat. Pflanzenfam. 3(1a): 108. 1893 syn. sec. Di Vincenzo & al. (in prep.) ≡ *Cyphocarpa orthacantha* (Hochst. ex Asch.) C.B.Clarke in Thiselton-Dyer, Fl. Trop. Afr. 6(1): 55. 1909 ≡ *Sericocomopsis orthacantha* (Hochst. ex Asch.) Peter in Repert. Spec. Nov. Regni Veg. Beih. 40(2): 230. 1938 − *Pupalia orthacantha*

Hochst. ex Hohen. in Bot. Zeitung (Berlin) 14: 597. 1856, [nomen nudum; Art. 38.1, 38.2 Ex. 1.] syn. sec. Di Vincenzo & al. (in prep.) — Syntypes: Ethiopia, Goelleb and Dschadsche, alt. 3000 – 6000 m, 24 Aug 1854, G. Schimper 2154 (S S07-12491!); Ethiopia, Goelleb and Dschadsche, alt. 4000 – 6000 m, Aug–Sep 1854, G. Schimper 2153 (isosyntypes: S S07-12231!, BR BR0000008357076!, JE JE00026022, P P00482810!, GOET GOET005657).

= *Cyathula kilimandscharica* Suess. & Beyerle in Repert. Spec. Nov. Regni Veg. 44: 44. 1938 — Type: Tanzania, Deutsch-Ost-Afrika, Kilimandscharo, Kil. N. Soitokilok, Buschsteppe, alt. ca. 1700 m, 23 Apr 1934, H. Schlieben 5122 (neotype: M M0107349).

= *Pupalia erecta* Suess. in Repert. Spec. Nov. Regni Veg. 44: 47. 1938 — Type: Tanzania, Bezirk Iringa, Ruahatal zw. Dodoma und Iringa, Akaziensteppe, alt. 700 m, 7 Mar 1934, C. Troll 5212 (holotype: B 10 0154186!).

= *Cyathula orthacanthoides* Suess. in Mitt. Bot. Staatssamml. München 1(1): 4. 1950 — Type: Tanzania, Mpwapwa, 28 Mar 1945, I. Rensburg 28 (K [fide Suessenguth 1950]).

Sericostachys Gilg & Lopr. ex Lopr. in Bot. Jahrb. Syst. 27(1-2): 50. 1899. Sec. Townsend (1993)

Lectotype: *Sericostachys scandens* Gilg & Lopr. designated by Cavaco 1962:61

Notes: Iamonico (2018) lectotypified the genus Sericostachys but he overlooked that lectotypification had already been done by Cavaco (1962), and thus Iamonico's designation is superfluous.

Sericostachys scandens Gilg & Lopr. in Bot. Jahrb. Syst. 27(1-2): 51. 1899. Sec. Townsend (1985)

First-step-lectotype: Cameroon. Yaoundé, 1897, Zenker 1420 designated by Cavaco 1962:63

Second-step-lectotype: S S07-12218 designated by Iamonico 2018: 151 (isolectotypes: K K000243691, L L.1691315, P P00466163!, P P00466164!, WAG WAG0000066 designated by Iamonico 2018: 151; further isolectotypes: GOET GOET005663!, HBG HBG502314!, M M0107369!). Cameroon, Buea, Lehmbach 140 (syntype: B 10 0154172! designated by Iamonico 2018: 151).

= *Sericostachys tomentosa* Gilg & Lopr. in Bot. Jahrb. Syst. 27(1-2): 51. 1899 ≡ *Sericostachys scandens* var. *tomentosa* (Gilg & Lopr.) Cavaco in Mém. Mus. Natl. Hist. Nat., B, Bot. 13(1): 62. 1962 — Type: Uganda, Kiwala in Yoro, alt. 6000-9000 ft., Ruwenzori-Expedition, 1893-1894, G. Scott Elliot 7657 (holotype: K K000243692 [image!]).

= *Sericostachys scandens* f. *maior* Chiov. in Atti Accad. Ital. 11: 55. 1940 — Type: Ethiopia, Zone umide del bosco di Tabor (Saio), alt. 1800, 18 Jan 1938, G. Giordano 2468 (holotype: FT FT001000).

Notes: Iamonico (2018) designated the lectotype of S. scandens out of the two collections mentioned in the protologue (Kamerun: Yaúnde, Bongolloberg (ZENKER 1420); Buea (LEHMBACH 140)) which he considered as syntypes. He chose the specimen Zenker 1420 located in the Swedish Museum of natural history (S) as the lectotype of the name S. scandens. All remaining specimens are considered as isolectotypes and the specimen collected by Lembach remaining as syntype. However, he overlooked that lectotypification had already been done by Cavaco (1962) who designated the collection from Zenker 1420 as the lectotype of the name S. scandens. Thus, lectotypification by Cavaco (1962) is considered here as the first-step-lectotyification and Iamonico (2018) in his publication subsequently designated only the second-step-lectotype: Zenker 1420 (S07-12218).

Unplaced names

***Cyathula triuncinata* var. *managuensis* Ram.Goyena, Fl. Nicarag. 2: 727. 1911**

***Nelsia angolensis* Bamps in Garcia de Orta, Sér. Bot. 2: 71. 1975. Sec. African Plant Database (version 4.0.0)**

≡ *Cyathula* comb. nov. [‘*angolensis*’] (Bamps) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published].

Distribution (general): Angola.

***Nelsia tropidogyna* C.C.Towns. in Kew Bull. 34(2): 235. 1979**

≡ *Cyathula* comb. nov. [‘*tropidogyna*’] (C.C.Towns.) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published] — Type: Angola, Huíla, Lubango, nos plainos do alto de Tundavala, 12 May 1964, O. Azancot de Meneses 1109 (holotype: K; isotype: PRE).

Distribution (general): Angola.

Unresolved names

***Cyathula gregoryi* (S.Moore) Schinz. Sec. African Plant Database (version 4.0.0)**

≡ *Cyphocarpa gregoryi* S.Moore

***Pandiaka metallorum* P.A.Duvign. & Van Bockstal, Rapp. Sol Vég.: 130. 1960. Sec. African Plant Database (version 4.0.0)**

≡ *Cyathula* comb. nov. [‘*metallorum*’] (P.A.Duvign. & Van Bockstal) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published].

Notes: Basionym publication: Viennot Bourgin (G.) et divers auteurs. — Rapports du sol et de la végétation. Premier colloque de la Societé botanique de France. Paris. Masson et Cie éditeurs, 1960. 184p.

Excluded names

***Cyathula lanceolata* Schinz in Bot. Jahrb. Syst. 21: 188. 1895. Sec. Di Vincenzo & al. (in prep.)**

≡ *Species* comb. nov. (Schinz) Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published] ≡ *Sericocomopsis lanceolata* (Schinz) Peter in Repert. Spec. Nov. Regni Veg. Beih. 40(2): 229. 1938 ≡ *Pandiaka lanceolata* (Schinz) C.B.Clarke in Thiselton-Dyer, Fl. Trop. Afr. 6(1): 68. 1909 — Type: East Africa, Tanzania, G. Fischer 561 (BM BM000910348); East Africa, 14 Nov 1893, G. Fischer 256 (lectotype: Z Z-000000282; isolectotype: HBG HBG502324 [image!]).

Notes: *Cyathula lanceolata* was resolved as sister to *Volkensinia prostrata* within a grade of three early diverging achyranthoid lineages (compare Di Vincenzo & al., 2018). Even though relationships were not fully resolved there, it is clear that *C. lanceolata* must be excluded from the here newly circumscribed genus *Cyathula*.

= *Cyathula crispa* Schinz in Bot. Jahrb. Syst. 21: 188. 1895 — Type: South Africa, Transvaal: Makapansberge, Rehmann 5420 ().

= *Cyathula lanceolata* var. *scabrida* Schinz in Bot. Jahrb. Syst. 21: 188. 1895 — Type: Namibia, Amboland, Rautanen (syntype: loc. ign.); Namibia, Hereroland, Höpfner 91 (syntype: loc. ign.).

= *Cyathula merkeri* Gilg in Bot. Jahrb. Syst. 36: 207. 1905 ≡ *Sericocomopsis lanceolata* var. *merkeri* (Gilg) Peter in Repert. Spec. Nov. Regni Veg. Beih. 40(2): 229. 1932 ≡ *Cyathula lanceolata* var. *merkeri* (Gilg) Schinz − *Cyathula merkeri* Gilg in Notizbl. Königl. Bot. Gart. Berlin 3: 196. 1902, nom. nud.

= *Pandiaka deserti* N.E.Br. in Bull. Misc. Inform. Kew 1909: 134. 1909 ≡ *Cyathula deserti* (N.E.Br.) Suess. in Repert. Spec. Nov. Regni Veg. 44: 46. 1938

= *Cyathula hereroensis* Schinz in Vierteljahrsschr. Naturf. Ges. Zürich 66: 222. 1921

= *Cyphocarpa kuehlweiniana* Peter in Repert. Spec. Nov. Regni Veg. Beih. 40(2, Anh.): 26. 1932

= *Cyathula merkeri* var. *strigosa* Suess. in Repert. Spec. Nov. Regni Veg. 44: 45. 1938

= *Cyathula strigosa* Suess. in Mitt. Bot. Staatssamml. München 1(1): 5. 1950

= *Pandiaka wildii* Suess. in Mitt. Bot. Staatssamml. München 1(2): 63. 1950

= *Cyphocarpa kuehlweiniana* var. *melanacantha* Peter

4.6 Appendix 4.A-G Supplementary material for Chapter 4

Appendices

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Appendix 4.A: List of analysed taxa of the Achyranthoids, including geographic origin, voucher information and ENA/GenBank accession numbers.

Taxon	Voucher/Herbarium	Field/ Garden Origin	Lab No	*trnK/matK*	*rpl16*	*rpl32-trnL-UAG*	*rps4-trnT-trnL*	*trnL-F*	ITS
***Achyranthes aspera* L.**	M. Wondafrash 4076 (ETH, B)	Ethiopia	AC1069	LT992592 Di Vincenzo & al. (2018)	LT992953 Di Vincenzo & al. (2018)		this study	LT993043 Di Vincenzo & al. (2018)	LT992992 Di Vincenzo & al. (2018)
***Achyranthes aspera* L.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 281 (EA, B, ETH)	Kenya	AC1099	LT992595 Di Vincenzo & al. (2018)	LT995259 Di Vincenzo & al. (2018)	this study	this study	LT993046 Di Vincenzo & al. (2018)	LT992995 Di Vincenzo & al. (2018)
***Achyranthes aspera* L.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 287 (EA, B, ETH)	Kenya	AC1100	LT992596 Di Vincenzo & al. (2018)	LT995260 Di Vincenzo & al. (2018)	this study	this study	LT993047 Di Vincenzo & al. (2018)	LT992996 Di Vincenzo & al. (2018)
***Achyranthes aspera* L.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 308 (EA, B, ETH)	Kenya	AC1112	LT992597 Di Vincenzo & al. (2018)	LT995261 Di Vincenzo & al. (2018)	this study	this study	LT993048 Di Vincenzo & al. (2018)	LT992997 Di Vincenzo & al. (2018)
***Achyranthes aspera* L.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 325 (EA, B, ETH)	Kenya	AC1119	LT992598 Di Vincenzo & al. (2018)	LT992955 Di Vincenzo & al. (2018)	this study	this study	LT993049 Di Vincenzo & al. (2018)	LT992998 Di Vincenzo & al. (2018)
***Achyranthes aspera* L.**	V. Di Vincenzo, N. Korotkova 360 (B, ETH)	Ethiopia	AC1165	LT992602 Di Vincenzo & al. (2018)		this study	this study	LT993053 Di Vincenzo & al. (2018)	LT993002 Di Vincenzo & al. (2018)
***Achyranthes aspera* L.**	V. Di Vincenzo, A. Hailu 85 (B, ETH)	Ethiopia	AC1206	LT992607 Di Vincenzo & al. (2018)	LT995255 Di Vincenzo & al. (2018)	this study	this study	LT993057 Di Vincenzo & al. (2018)	LT993006 Di Vincenzo & al. (2018)
***Achyranthes bidentata* Blume**	T. Borsch s. n.	China, Kunming	AC0003	LT992574 Di Vincenzo & al. (2018)	LT992944 Di Vincenzo & al. (2018)	this study	this study	LT993014 Di Vincenzo & al. (2018)	LT992958 Di Vincenzo & al. (2018)
***Achyranthes splendens* Mart. Ex Moq.**	S. Perlman 5803	Hawaii	AC1256	LT999872 Di Vincenzo & al. (2018)		this study	this study		LT995187 Di Vincenzo & al. (2018)
***Achyropsis avicularis* (E.Mey. ex Moq.) Benth. & Hook.f. ex B.D.Jacks.**	Venter 9671 (NY)	South Africa			EF688660 Sanchez del-Pino et al. (2009)			EF688731 Sanchez del-Pino et al. (2009)	
***Achyropsis leptostachya* (E. Mey. ex Meisn.) Baker & C.B. Clarke**	K. Müller 876 (B, PRE)	South Africa	AC0247	AY998117 Müller & Borsch (2005a)	LT995234 Di Vincenzo & al. (2018)	this study	this study	LT993028 Di Vincenzo & al. (2018)	LT992975 Di Vincenzo & al. (2018)
***Centema subfusca* (Moq.) T. Cooke**	P. Bamps (Moq.) Lopr. (BR)	South Africa	AC0906	LT999833 Di Vincenzo & al. (2018)	LT995193 Di Vincenzo & al. (2018)	this study	this study	LT995131 Di Vincenzo & al. (2018)	LT995161 Di Vincenzo & al. (2018)
***Centemopsis* sp.**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 318 (EA, B, ETH)	Kenya	AC1117	LT999856 Di Vincenzo & al. (2018)	LT999821 Di Vincenzo & al. (2018)	this study	this study	LT996933 Di Vincenzo & al. (2018)	LT999806 Di Vincenzo & al. (2018)
***Centrostachys aquatica* (R. Br.) Wall.**	K. Balkwill & M.-J. Balkwill 4356 (B)	Nigeria	AC1064	LT992591 Di Vincenzo & al. (2018)	LT992952 Di Vincenzo & al. (2018)	this study	this study		LT992991 Di Vincenzo & al. (2018)

Appendix 4.A – continued

Taxon	Voucher/Herbarium	Field/ Garden Origin	Lab No	*trnK/matK*	*rpl16*	*rpl32-trnL-UAG*	*rps4-trnT-trnL*	*trnL-F*	ITS
***Cyathula achyranthoides* (Kunth) Moq.**	I. Friis, M.G. Gilbert & K. Vollesen 3598	Ethiopia, Bale	AC1387	this study	this study	this study	this study	this study	this study
***Cyathula achyranthoides* (Kunth) Moq.**	Meier, W. 13874	Venezuela	AC1388	this study	this study	this study	this study	this study	this study
***Cyathula capitata* Moq.**	(KUN)	China	AC1257	LT999873 Di Vincenzo & al. (2018)	LT995219 Di Vincenzo & al. (2018)	this study	this study	LT995155 Di Vincenzo & al. (2018)	LT995188 Di Vincenzo & al. (2018)
***Cyathula cylindrica* Moq.**	K. Müller 877 (B, PRE)	South Africa	AC0241	LT992575 Di Vincenzo & al. (2018)	LT995233 Di Vincenzo & al. (2018)	this study	this study	LT993027 Di Vincenzo & al. (2018)	LT992974 Di Vincenzo & al. (2018)
***Cyathula cylindrica* Moq.**	M. Wondafrash 3605A (ETH, B)	Ethiopia	AC0964	LT992587 Di Vincenzo & al. (2018)	LT992949 Di Vincenzo & al. (2018)	this study	this study	LT993039 Di Vincenzo & al. (2018)	LT992987 Di Vincenzo & al. (2018)
***Cyathula cylindrica* Moq.**	Reekmans M. 10647, BR0000013742072 (BR)	Burundi, prov: Bubanza,	AC1652	this study	this study	this study	this study	this study	this study
***Cyathula divulsa* Suess.**	J.B. Phipps & L.D.E.F. Vesey-Fitzgerald 3237	Northern Rhodesia (Sambia)	AC1394						this study
***Cyathula natalensis* Sond.**	K. Müller 879 (B, PRE)	South Africa	AC0237	LT999825 Di Vincenzo & al. (2018)	LT995189 Di Vincenzo & al. (2018)	this study	this study	LT995127 Di Vincenzo & al. (2018)	LT995156 Di Vincenzo & al. (2018)
***Cyathula orthacantha* (Hochst. ex Asch.) Schinz**	K. Müller 872 (B, PRE)	South Africa (RSA), Northern Province	AC0930	LT999836 Di Vincenzo & al. (2018)	LT995195 Di Vincenzo & al. (2018)	this study	this study	LT995133 Di Vincenzo & al. (2018)	LT995163 Di Vincenzo & al. (2018)
***Cyathula orthacantha* (Hochst. ex Asch.) Schinz**	M. Wondafrash 3461 (ETH, B)	Ethiopia	AC0954	LT999840 Di Vincenzo & al. (2018)	LT995196 Di Vincenzo & al. (2018)	this study	this study	LT995134 Di Vincenzo & al. (2018)	LT995164 Di Vincenzo & al. (2018)
***Cyathula orthacantha* (Hochst. ex Asch.) Schinz**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 330 (EA, B, ETH)	Kenya	AC1122	LT999857 Di Vincenzo & al. (2018)	LT995208 Di Vincenzo & al. (2018)	this study	this study	LT995145 Di Vincenzo & al. (2018)	LT995176 Di Vincenzo & al. (2018)
***Cyathula polycephala* Baker**	M. Wondafrash 3394 (ETH, B)	Ethiopia	AC1065	LT999845 Di Vincenzo & al. (2018)	LT995201 Di Vincenzo & al. (2018)	this study	this study	LT995139 Di Vincenzo & al. (2018)	LT995169 Di Vincenzo & al. (2018)
***Cyathula prostrata* (L.) Blume**	M. Wondafrash 3434 (ETH, B)	Ethiopia	AC0951	LT992586 Di Vincenzo & al. (2018)	LT995244 Di Vincenzo & al. (2018)	this study	this study		LT992986 Di Vincenzo & al. (2018)
Cyathula prostrata (L.) Blume	E. Russo 114 (B, Herb. Russo)	Peru	AC0022	AY514862 Müller & Borsch (2005b)	LT995221 Di Vincenzo & al. (2018)	this study	this study		LT992959 Di Vincenzo & al. (2018)

Appendix 4.A – continued

Taxon	Voucher/Herbarium	Field/ Garden Origin	Lab No	*trnK/matK*	*rpl16*	*rpl32-trnL-UAG*	*rps4-trnT-trnL*	*trnL-F*	ITS
***Cyathula tomentosa* (Roth.) Moq.**	(KUN)	Tibet	AC1258	LT999875 Di Vincenzo & al. (2018)			this study		
***Cyathula uncinulata* (Schrad.) Schinz**	V. Di Vincenzo, N. Korotkova 361 (B, ETH)	Ethiopia	AC1166	LT999865 Di Vincenzo & al. (2018)	LT995214 Di Vincenzo & al. (2018)	this study	this study	LT995150 Di Vincenzo & al. (2018)	LT995182 Di Vincenzo & al. (2018)
***Cyathula uncinulata* (Schrad.) Schinz**	V. Di Vincenzo, A. Hailu 86 (B, ETH)	Ethiopia	AC1207	LT999869 Di Vincenzo & al. (2018)	LT995218 Di Vincenzo & al. (2018)	this study	this study	LT995154 Di Vincenzo & al. (2018)	LT995186 Di Vincenzo & al. (2018)
***Kyphocarpa angustifolia* (Moq.) Lopr.**	K. Müller 860 (BONN, PRE)	South Africa	AC0189	AY998111 Müller & Borsch (2005a)	LT995225 Di Vincenzo & al. (2018)	this study	this study	LT993017 Di Vincenzo & al. (2018)	LT992963 Di Vincenzo & al. (2018)
***Leucosphaera bainesii* (Hook.f.) Gilg**	K. Müller 866 (B, PRE)	South Africa (RSA), Northern Province	AC0882	LT992581 Di Vincenzo & al. (2018)	LT995238 Di Vincenzo & al. (2018)	this study	this study	LT993033 Di Vincenzo & al. (2018)	LT992980 Di Vincenzo & al. (2018)
***Nelsia quadrangula* (Engl.) Schinz**	E. Coppejam (BR)		AC0903	LT992582 Di Vincenzo & al. (2018)	LT995239 Di Vincenzo & al. (2018)		this study	LT993034 Di Vincenzo & al. (2018)	LT992981 Di Vincenzo & al. (2018)
***Nototrichium divaricatum* Lorence**									AY174422
***Nototrichium humile* Hillebr.**	B. A. Prigge 15249 (LA)	USA, Hawaii		AY514816 Müller & Borsch (2005b)					
***Nototrichium sandwicense* Hillebr.**	VanNeste 216	USA	AC9DD17						MF348854
***Nototrichium sandwicense* Hillebr.**	B. A. Prigge 15250 (LA)	USA, Hawaii		AY514817 Müller & Borsch (2005b)					
***Pandiaka angustifolia* (Vahl) Hepper**	J. Müller 324 (FR)	Burkina Faso	AC0056	AY514818 Müller & Borsch (2005b)	LT995224 Di Vincenzo & al. (2018)	this study	this study	LT993016 Di Vincenzo & al. (2018)	LT992961 Di Vincenzo & al. (2018)
***Pandiaka involucrata* (Moq.) B.D. Jacks**	U. Scholz, P. Koumassi, H. Kühn & H. Peuker 451	Togo	AC1391	this study	this study	this study	this study		this study
***Pandiaka lanuginosa* Schinz**	WRQ Luke 15344 (EA)	Kenya	AC1381	this study		this study	this study	this study	this study
***Pandiaka rubrolutea* (Lopr.) C.C.Towns.**	Greenway & Kanuri 14399 (PRE)		AC0209			this study	this study		this study

Appendix 4.A – continued

Taxon	Voucher/Herbarium	Field/ Garden Origin	Lab No	*trnK/matK*	*rpl16*	*rpl32-trnL-UAG*	*rps4-trnT-trnL*	*trnL-F*	ITS
***Pandiaka welwitschii* (Schinz) Hiern**	M. Reekmans 90325 (EA)	Burundi	AC1384						this study
***Psilotrichum gnaphalobryum* (Hochst.) Schinz**	M. Wondafrash 2200 (ETH, B)	Ethiopia	AC1077	LT992593 Di Vincenzo & al. (2018)	LT992954 Di Vincenzo & al. (2018)	this study	this study	LT993044 Di Vincenzo & al. (2018)	LT992993 Di Vincenzo & al. (2018)
***Psilotrichum scleranthum* Thwaites**	K. Müller 878 (B, PRE)	South Africa	AC0125	AY514822 Müller & Borsch (2005b)	LT999811 Di Vincenzo & al. (2018)	this study	this study	LT996924 Di Vincenzo & al. (2018)	LT999809 Di Vincenzo & al. (2018)
***Pupalia lappacea* (L.) A. Juss.**	M. Wondafrash 3922 (ETH, B)	Ethiopia	AC0983	LT992590 Di Vincenzo & al. (2018)	LT995262 Di Vincenzo & al. (2018)	this study	this study	LT993042 Di Vincenzo & al. (2018)	LT992990 Di Vincenzo & al. (2018)
***Sericocoma avolans* Fenzl**	K. Müller 859 (B, PRE)	South Africa	AC0234	AY998103 Müller & Borsch (2005a)	LT995232 Di Vincenzo & al. (2018)	this study	this study	LT993026 Di Vincenzo & al. (2018)	LT992973 Di Vincenzo & al. (2018)
***Sericocomopsis hildebrandtii* Schinz**	M. Wondafrash 3500 (ETH, B)	Ethiopia	AC0961	LT999842 Di Vincenzo & al. (2018)	LT995198 Di Vincenzo & al. (2018)	this study	this study	LT995136 Di Vincenzo & al. (2018)	LT995166 Di Vincenzo & al. (2018)
***Sericocomopsis hildebrandtii* Schinz**	M. Wondafrash 3462 (ETH, B)	Ethiopia	AC0955	LT999841 Di Vincenzo & al. (2018)	LT995197 Di Vincenzo & al. (2018)	this study	this study	LT995135 Di Vincenzo & al. (2018)	LT995165 Di Vincenzo & al. (2018)
***Sericocomopsis hildebrandtii* Schinz**	M. Wondafrash 2065 (ETH, B)	Ethiopia	AC1084	LT999850 Di Vincenzo & al. (2018)	LT995205 Di Vincenzo & al. (2018)	this study	this study	LT995143 Di Vincenzo & al. (2018)	LT995173 Di Vincenzo & al. (2018)
***Sericocomopsis hildebrandtii* Schinz**	M. Wondafrash 2213 (ETH, B)	Ethiopia	AC1094	LT999851 Di Vincenzo & al. (2018)	LT995206 Di Vincenzo & al. (2018)	this study	this study	LT995144 Di Vincenzo & al. (2018)	LT995174 Di Vincenzo & al. (2018)
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 339 (EA, B, ETH)	Kenya	AC1130	LT999859 Di Vincenzo & al. (2018)	LT995210 Di Vincenzo & al. (2018)	this study	this study	LT995147 Di Vincenzo & al. (2018)	LT995178 Di Vincenzo & al. (2018)
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, A. Hailu 261 (B, ETH)	Ethiopia	AC1230	this study		this study	this study		this study
***Sericocomopsis hildebrandtii* Schinz**	A.F.H. Linsen & W.B.I.T. Giesen 24 (B)	Kenya	AC1310		this study	this study	this study	this study	
***Sericocomopsis hildebrandtii* Schinz**	A. Peter 2206 (B)	Kenya	AC1311	this study	this study	this study	this study	this study	
***Sericocomopsis hildebrandtii* Schinz**	A.A. Pesambili 6222 (B)	Tanzania	AC1312	this study	this study		this study		

Appendix 4.A – continued

Taxon	Voucher/Herbarium	Field/ Garden Origin	Lab No	*trnK/matK*	*rpl16*	*rpl32-trnL-UAG*	*rps4-trnT-trnL*	*trnL-F*	ITS
***Sericocomopsis hildebrandtii* Schinz**	E. Milne-Redhead & P. Taylor 11306 (B)	Tanzania	AC1313	this study	this study	this study	this study		
***Sericocomopsis hildebrandtii* Schinz**	A. & P. Hiepko 2611 (B)	Kenya	AC1314	this study	this study	this study	this study		
***Sericocomopsis hildebrandtii* Schinz**	A. Hemp 4007 (B)	Tanzania	AC1315	this study	this study		this study		
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, A. Hailu 175 (B, ETH)	Ethiopia	AC1319	this study	this study	this study	this study	this study	this study
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, A. Hailu 179 (B, ETH)	Ethiopia	AC1320	this study	this study	this study	this study	this study	this study
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, A. Hailu 185 (B, ETH)	Ethiopia	AC1321	this study	this study	this study	this study	this study	this study
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, A. Hailu 190 (B, ETH)	Ethiopia	AC1322	this study	this study	this study	this study	this study	this study
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, A. Hailu 192A (B, ETH)	Ethiopia	AC1323	this study	this study	this study	this study	this study	this study
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, A. Hailu 198 (B, ETH)	Ethiopia	AC1324	this study	this study	this study	this study	this study	
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, A. Hailu 204 (B, ETH)	Ethiopia	AC1325	this study	this study	this study	this study	this study	
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, A. Hailu 211 (B, ETH)	Ethiopia	AC1326	this study	this study	this study	this study	this study	this study
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, A. Hailu 213 (B, ETH)	Ethiopia	AC1327	this study	this study	this study	this study	this study	this study
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, A. Hailu 257 (B, ETH)	Ethiopia	AC1328	this study	this study	this study	this study	this study	
***Sericocomopsis hildebrandtii* Schinz**	V. Di Vincenzo, A. Hailu 258 (B, ETH)	Ethiopia	AC1329	this study		this study	this study	this study	

Appendix 4.A – continued

Taxon	Voucher/Herbarium	Field/ Garden Origin	Lab No	*trnK/matK*	*rpl16*	*rpl32-trnL-UAG*	*rps4-trnT-trnL*	*trnL-F*	ITS
Sericocomopsis pallida (S. Moore) Schinz	M. Wondafrash 3928 (ETH, B)	Ethiopia	AC1080	LT999849 Di Vincenzo & al. (2018)	LT995204 Di Vincenzo & al. (2018)		this study	LT995142 Di Vincenzo & al. (2018)	LT995172 Di Vincenzo & al. (2018)
***Sericocomopsis pallida* (S. Moore) Schinz**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 334A (EA, B, ETH)	Kenya	AC1125	LT999858 Di Vincenzo & al. (2018)	LT995209 Di Vincenzo & al. (2018)	this study	this study	LT995146 Di Vincenzo & al. (2018)	LT995177 Di Vincenzo & al. (2018)
***Sericocomopsis pallida* (S. Moore) Schinz**	V. Di Vincenzo, P. Kamau, M. Wondafrash, M. Mbale 334B (EA, B, ETH)	Kenya	AC1126	this study	this study	this study	this study	this study	this study
***Sericocomopsis pallida* (S. Moore) Schinz**	V. Di Vincenzo, M. Wondafrash 42 (B, ETH)	Ethiopia	AC1203	LT999867 Di Vincenzo & al. (2018)	LT995216 Di Vincenzo & al. (2018)	this study	this study	LT995152 Di Vincenzo & al. (2018)	LT995184 Di Vincenzo & al. (2018)
***Sericocomopsis pallida* (S. Moore) Schinz**	R.B. Drummond & J.H. Hemsley 2305 (B)	Tanzania	AC1317	this study	this study			this study	
***Sericocomopsis pallida* (S. Moore) Schinz**	N. Kilian 1767 & W. Lobin 6621 (B)	Somalia	AC1318	this study	this study				
***Sericostachys scandens* Gilg & Lopr.**	E. Fischer s.n. (B)	Rwanda	AC0042	AY514819 Müller & Borsch (2005b)	this study	this study	this study	this study	this study
***Sericostachys scandens* Gilg & Lopr.**	Ntaowira Niranoa 563, BR0000013741808 (BR)	Democratic Republic of the Congo, Tshivanga PNKB	AC1651	this study		this study	this study	this study	this study
***Volkensinia prostrata* (Volkens ex Gilg) Schinz**	M. Wondafrash 4200 (ETH, B)	Ethiopia	AC1201	LT999866 Di Vincenzo & al. (2018)	LT995215 Di Vincenzo & al. (2018)	this study	this study	LT995151 Di Vincenzo & al. (2018)	LT995183 Di Vincenzo & al. (2018)

Appendix 4.B: Primers used for amplification and sequencing of the plastid genomic regions.
For the regions *rpl32-trnL-UAG*, *rps4-trnT-trnL* and for difficult samples of *trnK/matK*, *rpl16* and *trnL-F* the following primers were used. For amplification and sequencing of the nuclear ITS genomic region, the same primers as listed in the Appendix 2.C. of Chapter 2 were used.

primer name	sequence 5' → 3'	dir	source	region
rpl32-F	CAGTTCCAAAAAAACGTACTTC	F	Shaw et al. 2007	*rpl32-trnL-UAG*
AMAR-70-rpl32F	TCGTTTGAACAATAGATGTCTTTC	F	This study	
AMARrpl32-trnL-420F	TAACAACTCTTAYGCCAACCC	F	This study	
trnL-UAG_46R	ACTGCTTCCTAAGAGCAGCG	R		
trnL-UAG-90R	GATACCATGCCGCCACTC	R		
AMARrpl32-trnL-500R	AGAAGGGTGATAAAATAKTTTC	R	This study	
AMARrps4-F	TGGTAAAGACCCTAGACGG	F	This study	*rps4-trnT-trnL*
AMARrps4-5F	ACGAGGTCCTCGGTAACGTG	F	This study	
AMARrps4-10F	GGTAACGTGACATAAAGACTC	F	This study	
AMARtrnT-358F	TTCAGAGTTGSAAGTTTCTATG	F	This study	
AMARtrnT-360F	CAGAGTTGSAAGTTTCTATGAC	F	This study	
AMARtrnT-470F	GGCTATCGGAATCGAACCG	F	This study	
AMARtrnT-L-50F	CGTCGAGAACAATTAGATTC	F	This study	
AMARtrnT-L-370F	TTCTTCGGCTTTCATTCATAG	F	This study	
trnL34R	CTCCATACTAAGGCTCAAGC	R		
AMARtrnT-520R	GCTTAGCTCAGAGGTTAGAG	R	This study	
AMARtrnT-410R	GAAGAACAAGGAAAATAMTAAAGG			
AMARtrnT-L-320R	GACCCRGAGTTCATTTTTATYG	R	This study	
AMARtrnT-L-550R	GTTTCATCTCTATCGGAARTC	R	This study	
rpl16F	CTATGCTTAGTGTGTGACTC	F	Löhne & al. 2007, they cited it as Campagna & Downie 1998 but their primer sequence is different	*rpl16*
AMARrpl16-700F	GATTCCAATATGTAAGGTTTTTG	F	This study	
AMARrpl16-950R	ATCGCCTCTTGATTAATGGTTAG	R	This study	
rpl16R	TCTTCCTCTATGTTGTTTACG	R		
trnT-AC1	AARCATTTTTCGGTATAG	F	Borsch 2006	*trnT-F*
AMARtrnL-F-400F	CTGTGCTGTGTTGTTCTAAG	F	This study	
AMARtrnL-F-900F	CGGACGAGAATAAAGATAGAGTC	F	This study	
trnTf	ATTTGAACTGGTGACACGAG	R	Taberlet et al. 1991	
AMARtrnL-F-500R	GGTGTCATTAATCATTTG	R	This study	
AMARtrnL-F-1000R	GGATAGAGGGACTTGAACC	R	This study	
trnKF	GGGTTGCTAACTCAATGGTAGAG	F	Wicke & Quandt 2009	trnK/matK
ACmatK490F	CTGGTTGAAAGATGCTTCKTC	F	Müller & Borsch 2005a	
ACmatK500F	TTCTTCTTTGCATTTATTACG	F	Müller & Borsch 2005a	
ACmatK574F		F		
ACmatK1250F	CTCATTATTATAGTGGCTCYTC	F	Müller & Borsch 2005a	
trnK2R	AACTAGTCGGATGGAGTAG	R	Johnson & Soltis 1995	
ACmatK871R		R		
ACmatK632R		R		
ACmatK1300R	TRCGAGACAAAGTTCTAGCAC	R	Müller & Borsch 2005a	
ACmatK1400R	GGATTCGTATTCACATACA	R	Müller & Borsch 2005a	
ACmatK1401R	ATGGATTCGTATTCACATAC	R		
ACmatK2231R		R		
psbA5'R	AACCATCCAATGTAAAGACGGTTT	R	Shaw et al. 2005	

Appendix 4.C: Partitions and best-fitting nucleotide substitution models of dataset A (combined plastid marker).
Results were obtained from model selection in ModelTest-NG v0.1.7 via AICc (Akaike Information Criterion corrected for small sample sizes).

Partition name	Model
trnk1	GTR+G
matk_gen	GTR+G
trnk2	GTR+G
rpl16	GTR+G
trnT_spacer_incomplete	HKY+G
trnL_5_Exon	JC
trnL_Intron	GTR+G
trnL_3_Exon	JC
TrnL_F_spacer	HKY+G
TrnF_Exon	JC
rpl32	GTR+I+G
rps4_trnT_IGS	GTR+G
trnT_UGU_gen	F81+G
trnT_trnLIGS	GTR+G

Appendix 4.D: Partitions and best-fitting nucleotide substitution models of dataset B (Nuclear ITS).
Results were obtained from model selection in ModelTest-NG v0.1.7 via AICc (Akaike Information Criterion corrected for small sample sizes).

Partition name	Model
18S	JC
ITS1	SYM+G
5.8S	JC
ITS2	SYM+G
28S	JC

Appendix 4.E: Morphological character definitions in Achyranthoids and their states applied for ancestral character state reconstructions (ACSR).

Character group	No.	Character definition
Habit and vegetative morphology	1	**Life cycle** Comprises annual herbs or perennials which live for several years (including herbs, subshrubs, shrubs, lianas, or trees). In some cases (e.g., in *Achyranthes aspera*) the distinction between annual and perennial is difficult as young plants may not yet have developed the final vegetative branching although they exhibit axillary buds or short lateral branches (in these cases coded as "perennial"). ***States**: annual **(0)**, perennial **(1)***
	2	**Life form** Lianas are rare in Amaranthaceae and are here only understood to occur in *Sericostachys* which has long-twining stems growing to the top of canopy trees (stems not self-sustaining); the sometimes scandent and slender stems in species of *Cyathula* are included in the state "subshrub". ***States**: herb **(0)**, subshrub **(1)**, shrub **(2)**, treelet **(3)**, liana **(4)***
	3	**Indumentum of stem and leaves** The stem and leaf surfaces of Amaranthaceae often exhibit an indumentum composed of different types of trichomes. In Achyranthoids most of the species possess uniseriate (so called "simple hairs"; Townsend 1985) whereas very few species exhibit branched hairs. In this dataset this is the case in *Sericocomopsis pallida* with stellate hairs on leaf surfaces and condensed-dendroid on inflorescence axes, upper branches and sometimes leaf veins (compare Figure 4.D, photo A & B). ***States**: simple **(0)**, stellate and condensed-dendroid **(1)***
	4	**Shape of cauline leaves** ***States**: ovate **(0)**, elliptic **(1)**, lanceolate **(2)**, linear -narrowly lanceolate **(3)**, obovate-spathulate-oblanceolate **(4)***
	5	**Petiole length of cauline leaves** [in cm] ***States**: 0 = sessile **(0)**, ≤ 0.5 **(1)**, 0.6-1.5 **(2)**, 1.6-2 **(3)**, ≥2.1 **(4)***

Appendix 4.E – continued

Character group	No.	Character definition
Inflorescence morphology	6	**Shape of paracladia** Amaranthaceae possess a cymose inflorescence architecture (Acosta & al. 2009). In Achyranthoids the cymes are present in most taxa but can be reduced to solitary flowers (in *Achyranthes*, *Achyropsis* and *Nototrichium*; see character 8), like also in Gomphrenoideae (Ortuño & Borsch 2020). The shape of paracladia ranges from globose to long spiciform with cymes or flowers clearly separated from each other (state at anthesis since inflorescences elongate during development). In some species (*Cyathula uncinulata*) the synflorescences appear as a single paracladium but the irregular subglobose shape is caused by the agglomeration of short paracladia on short lateral branches. This character corresponds to character 7 in Ortuño & Borsch (2020). ***States**: spiciform with cymes or solitary flowers clearly separated during anthesis **(0)**, globose or elongate to cylindric with cymes densely aggregated **(1)**, strongly condensed with cymes closely aggregated and paracladia agglomerated, thus synflorescences appear as a single subglobose to irregularly and thickly cylindrical paracladium **(2)***
	7	**Arrangement of paracladia / Synflorescence structure** The complex synflorescence structures in the Amaranthaceae cosnsist of paracladia (= partial florescences) arranged in different ways (Acosta & al. 2009). This character defines the respective structures of the branching system that is constituted by the axes bearing paracladia (corresponds to character 8 in Ortuño & Borsch (2020). The species of *Achyropsis* exhibit richly branched synflorescences with branches still supported by smaller cauline leaves but not scales, so that they are coded with state 0. This is in contrast to the branched inflorescences in *Sericostachys* where branches are supported by brownish scales. The globose paracladia in *Pandiaka involucrata* appear on the main axis and very short terminal branches supported by smaller cauline leaves but contrary to the cyathia in several species of Gomphrena (Ortuño & Borsch 2020) these branches are still visible (here coded as state 0). ***States**: paracladia solitary on main axes as well as on lateral branches **(0)**, paracladia arranged in a complex thyrsoid structure (a terminal paracladium as main florescence and lateral paracladia are co-florescences, with up to two orders of branching **(1)***
Floral morphology	8	**Structure of cymes** Amaranthaceae bear cymose inflorescence structures which are varying in the degree of branching in different genera and species. Cymes with only 1st order branches (simple cymes) are rare and found in two species: in *Sericostachys* where the lateral flowers are sterile and subtending the fertile main flower, and in *Centemopsis*, where only one of the lateral branches is developed and thus cymes are not fully symmetric. More richly branched cymose structures (with 2nd order branches and higher) are often not fully symmetric as well and can contain sterile flowers in various proportions (e.g., all flowers of 2nd order branches sterile and very reduced as in *Cyathula prostrata* or only very few sterile flowers on uppermost branches in relation to many fertile flowers present as in *Sericocomopsis pallida*). ***States**: cymes reduced to single flowers **(0)**, cymes with first order branches **(1)**, cymes with more than one order of branches **(2)***

Appendix 4.E – continued

Character group	No.	Character definition
Floral morphology	9	**Position (angle) of cymes in relation to the inflorescence axis during maturity** In *Achyranthes* and *Centrostachys* the reduced cymes are deflexed and closely appressed to the inflorescence axis in fruit ripening. ***States**: cymes not deflexed and closely appressed to the inflorescence axis **(0)**, cymes deflexed and appressed to the inflorescence axis **(1)***
	10	**Presence or absence of sterile flowers** ***States**: absent **(0)**, present **(1)***
	11	**Shape of sterile flowers** In Achyranthoids several species exhibit unique inflorescence structures in which fertile flowers are arranged together with sterile flowers in the cymes of partial florescences. In several species tepals and partially also the bracteoles of sterile flowers are modified to hooks, scales or bristles serving animal dispersal or rarely to hairs serving wind dispersal (Di Vincenzo & al., 2018). Since modifications of sterile flowers are diverse, the different states account for different kinds of modified sterile flowers. In *Nelsia* the at maturity conspicuous stiff and spine-like hairy structures appear opposite on the 1st order branch of cymes and are thus interpreted as bracteoles whereas tepals are very rudimentary (state 3). ***States**: unmodified **(0)**, hooks **(1)**, straight spines **(2)**, with dominating plumose-pilose spine-like bracteoles **(3)**, filiform hairs rapidly elongating and feathery during fruit from modified linear tepals **(4)***
	12	**Androecial tube appendages** Androecial tube appendages, formerly known as "interstaminodial appendages" or "pseudostaminodes" (e.g. Eliasson, 1988; Townsend, 1993), are found in a wide range of shape and size in several Amaranthaceae species. In accordance with Limarino & Borsch (2020), I follow the approach of Vrijdaghs & al. (2014) and Sánchez-Del Pino & al. (2019) and consider androecial tube appendages (originating from primordia on the androecial tube rim) to be non-homologous with the appendages on the filaments (originating from the bases of the filaments). ***States**: absent **(0)**, present **(1)***
	13	**Homogeneity of tepals** I focus only on size and shape of the tepals. State 1 is limited to the monotypic genus *Centrostachys*. ***States**: all equal **(0)**, outermost tepal different in size and shape, remaining tepals almost equal **(1)**, 2 outer & 3 inner distinctly different in size and shape **(2)**, 2 outer, 3 inner smaller but similar in shape **(3)***

Appendix 4.E – continued

Character group	No.	Character definition
Floral morphology	14	**Shape of outer tepals of fertile flowers** As tepals in Achyranthoids can be different within single flowers (compare character 9). I focus on the outer tepals here since they offer a higher variability compared to inner tepals. For *Centrostachys aquatica* the outermost tepal is coded here which is different from all remaining tepals (compare character 9). I distinguish between the following states regarding to the flat part of the tepal (differences of the excurrent midrib are coded in the subsequent character): ***States**: lanceolate with acute tip **(0)**, lanceolate with acuminate tip **(1)**, narrow lanceolate with acute tip **(2)**, narrow lanceolate with acuminate tip **(3)**, elliptic-oblong or lanceolate-oblong with a blunt tip **(4)***
	15	**Midrib of outer tepals** The midribs of the outer tepals within achyranthoid species can be excurrent to various degrees. Also, they can differ in their quality, by forming hooks. The latter state has evolved supporting animal dispersal (Di Vincenzo et al. 2018). Therefore, I defined the following states. ***States**: not excurrent **(0)**, mucronate to shortly excurrent **(1)**, distinctly / long excurrent **(2)**, uncinately hooked **(3)***
	16	**Veination of outer tepals** In some species with indurate tepals the number of veins is difficult to assess and may also differ among different flowers. I coded therefore the presence of up to 3 or up to 5 veins if in any flower of a given individual this vein number could be observed. ***States**: 1-veined **(0)**, up to 3 veins **(1)**, up to 5 veins **(2)**, ≥5 veins **(3)***
	17	**Veination of inner tepals** Inner tepals with only one vein where not observed in any taxon. Thus, I coded the following states. ***States**: up to 3 veins **(0)**, up to 5 veins **(1)**, ≥5 veins **(2)***
	18	**Texture of tepals at maturity** Several Achyrantoid taxa exhibit indurate tepals. In *Sericocomopsis meruensis* the tepals are becoming indurate at base between the abaxially prominent veins. There are also some species of *Centemopsis* where tepals become conspicuously indurate in the lower half at maturity or even at anthesis (in *Lopriorea*, not included in this dataset). ***States**: remaining herbaceous or scarious **(0)**, becoming slightly indurate at base **(1)**, becoming conspicuously indurate in lower part **(2)***

Appendix 4.E – continued

Character group	No.	Character definition
Floral morphology	19	**Shape of bracteoles** As bracteoles of fertile and sterile flowers can be different and not all achyranthoid species bear sterile flowers, here I focus on the bracteoles of the fertile flowers. I distinguish between the following states. ***States**: lanceolate with acuminate tip **(0)**, ovate with acuminate tip **(1)**, broadly ovate with acuminate tip **(2)**, ovate- broadly ovate with obtuse tip **(3)**, orbicular with an emarginate/retuse tip **(4)***
	20	**Midrib of bracteoles of fertile flowers** The midribs of the bracteoles within achyranthoid species can be excurrent to various degrees. Also, they can differ in their quality, by forming hooks. The latter state has evolved supporting animal dispersal (Di Vincenzo et al. 2018). Therefore, I defined the following states. ***States**: not excurrent **(0)**, mucronate to shortly excurrent **(1)**, distinctly / long excurrent **(2)** uncinately hooked **(3)***
	21	**Bracteoles at anthesis** In some taxa bracteoles become strongly recurved during anthesis. Since bracteoles fall with the fruits and therefore are part of the dispersal units during fruit fall, this might be correlated with a function in dispersal in taxa where bracteoles become spiny (e.g., in *Nelsia quadrangula*, *Centemopsis kirkii*, *Achyranthes aspera* and *Sericocomopsis hildebrandtii)*. ***States**: bracteoles not or only slightly recurved in most fertile flowers at anthesis **(0)**, bracteoles strongly recurved in all fertile flowers at anthesis **(1)***
	22	**Shape of bracts** ***States**: lanceolate with acuminate tip **(0)**, ovate with blunt tip **(1)**, ovate with acuminate tip **(2)**, broadly ovate with obtuse tip **(3)**, broadly ovate with acuminate tip **(4)***
	23	**Midrib of bracts** ***States**: not excurrent **(0)**, mucronate to shortly excurrent **(1)**, distinctly / long excurrent **(2)***
	24	**Hairiness of ovary** ***States**: glabrous **(0)**, pilose **(1)**, lanate (2)*

Appendix 4.F: Morphological character matrix applied for ancestral character reconstructions

Taxa for which the same individual was used for morphological and molecular analyses are marked with an asterisk and voucher information is given in Appendix 4.A. For the remaining taxa barcodes are given and voucher data can be assessed from the Virtual herbarium of the Herbarium Berolinense (http://ww2.bgbm.org/herbarium/default.cfm).

Taxon with sample number or Barcode	Char 1	Char 2	Char 3	Char 4	Char 5	Char 6	Char 7	Char 8	Char 9	Char 10	Char 11	Char 12	Char 13	Char 14	Char 15	Char 16	Char 17	Char 18	Char 19	Char 20	Char 21	Char 22	Char 23	Char 24
Achyranthes_aspera_AC1069*	1	0	0	1, 2	3	0	0	0	1	0	n.a.	1	0	3	1	1	0	1	4	2	1	0	2	0
Achyranthes_aspera_AC1099*	1	1	0	1, 4	2	0	0	0	1	0	n.a.	1	0	2	0	1	0	1	4	2	0	0	1	0
Achyranthes_aspera_AC1100*	1	1	0	0, 2	2	0	0	0	1	0	n.a.	1	0	3	0	1	0	1	4	2	1	0	2	0
Achyranthes_aspera_AC1112*	1	0	0	0	3	0	0	0	1	0	n.a.	1	0	3	0	1	0	1	4	2	1	0	2	0
Achyranthes_bidentata_B101074034	1	0	0	0, 2	3	0	0	0	1	0	n.a.	1	0	3	0	1	0	1	4	2	1	4	1	0
Achyranthes_splendens_B101077184	1	2	0	0, 4	2	0	0	0	1	0	n.a.	1	0	0	0	1	0	1	1	2	1	2	0	0
Achyropsis_avicularis_B101074120	1	0	0	0, 2	1	0	0	0	0	0	n.a.	1	0	0	0	1	0	1	3	0	0	2	1	0
Achyropsis_leptostachya_AC0247*	1	0	0	0, 2	1	0	0	0	0	0	n.a.	1	0	0	0	1	0	1	3	0	0	2	1	0
Centema_subfusca_B101076970	1	1	0	3	0, 1	1	0	2	0	1	2	0	2	4	0	3	1	2	2	1	0	4	1	0
Centemopsis_sp_AC1117*	0	0	0	3	0	1	0	1	0	0	n.a.	1	3	4	1	1	0	2	3	2	1	0	1	1
Centrostachys_aquatica_AC1064*	1	0	0	2	2	0	0	0	1	0	n.a.	1	1	2	2	1	2	1	4	1	0	2	2	0
Cyathula_achyranthoides_AC1387*	1	0	0	1, 2, 4	1	0	0	2	0	1	1	1	0	1	2, 3	2	0	1	1	3	0	0	1	0
Cyathula_cylindrica_AC0964*	1	0	0	0, 1	4	1	0	2	0	1	1	1	3	3	1	1	1	0	2	3	0	1, 2	1	0
Cyathula_natalensis_AC0237*	1	1	0	1, 4	1	2	0	2	0	1	1	1	3	4	1	1	0	1	0	2	0	4	2	0
Cyathula_orthacantha_AC0954*	0	0	0	0, 1, 2	3	1	0	2	0	1	2	1	0	0	1	1	1	1	2	2	0	0	1	0
Cyathula_polycephala_AC1065*	1	0	0	0, 2	2	0	0	2	0	1	1	1	2	1	3	0	0	0	1	3	0	2	1	0
Cyathula_prostrata_MW 4087	1	0	0	4	1	0	0	2	0	1	1	1	0	1	1	1	0	1	0, 1	2	0	0	1	0
Cyathula_tomentosa_B101077186	1	0	0	0, 1	2	0	0	2	0	1	1	1	2	1	3	1	0	0	1	3	0	2	1	0
Cyathula_uncinulata_AC1166*	1	0	0	1	4	2	0	2	0	1	1	1	2	3	3	0	0	0	0	3	0	2	1	0
Kyphocarpa_angustifolia_AC0189*	0	0	0	3	0	1	0	2	0	1	2	1	2	2	1	1	0	2	3	1	0	2	2	1
Leucosphaera_bainesii_AC0882*	1	2	0	4	1	2	0	2	0	1	3	0	0	1	2	1	0	1	0, 1	2	1	2	2	2
Nelsia_quadrangula_B101073864	1	0	0	0, 2	2	0	0	2	0	1	3	1	3	0	2	1	0	1	2	2	1	4	2	0
Nototrichium_humile_B101073894	1	3	0	0	1	0	0	0	0	0	n.a.	0	0	0	0	1	0	1	1	1	0	1	1	0
Nototrichium_sandwicense_B101073908	1	3	0	0	2	1	0	0	0	0	n.a.	0	0	0	0	1	0	1	1	0, 1	0	2	0	0
Pandiaka_angustifolia	0	0	0	3	0	1	0	0	0	0	n.a.	1	3	0	0	3	0	2	1	2	0, 1	4	1	0
Pandiaka_involucrata_AC1391*	1	1	0	1, 2	1	1	0	0	0	0	n.a.	1	3	2	0	2	0	0	0	1	0	2	0	0
Pandiaka_lanuginosa_B101074059	1	0	0	0, 2	2	0	0	0	1	0	n.a.	1	0	3	0	2	1	1	0, 1	2	1	2	1	0
Pandiaka_rubrolutea_B101073949	0	0	0	4	0, 1	1	0	0	0	0	n.a.	1	3	3	2	2	0	1	0	2	0	0	1	0
Psilotrichum_gnaphalobryum_AC1077*	1	1	0	0	2	0	1	0	0	0	n.a.	0	2	4	0	1	0	0	3	1	0	1	1	0
Psilotrichum_scleranthum_AC0125*	1	2	0	0	1	0	0	0	0	0	n.a.	0	3	0	0	3	2	2	3	1	0	2	1	0
Pupalia_lappacea_AC0983*	1	1	0	0	2	0	0	2	0	1	1	0	0	4	0	1	0	0	2	1	0	0	1	0
Sericocoma_avolans_AC0234*	1	2	0	3	0	1	0	0	0	0	n.a.	0	0	0	0	1	0	0	3	1	0	2	2	2
Sericocomopsis_meruensis_AC0961*	1	2	0	4	1	0	0	2	0	0	n.a.	1	3	0	1	1	0	1	1	2	0	1	1	0
Sericocomopsis_hildebrandtii_AC1094*	1	2	0	0, 1	1	1	0	2	0	0	n.a.	1	3	0	1	1	0	0	1	2	1	1, 2	1	0
Sericocomopsis_pallida_AC1125*	1	2	1	1, 4	2	1	0	2	0	1	0	1	3	1	1	1	0	0	1	1	0	2	0	0
Sericostachys_scandens_AC1651*	1	4	0	0	2	0	1	1	0	1	4	1	3	0	0	3	2	0	3	0	0	1	0	0
Volkensinia_prostrata_B101077233	1	0	0	0	2	0	0	2	0	1	3	1	0	2	0	1	0	0	3	1	0	3	1	0

Appendix 4.G: Alignment statistics of the plastid dataset A

	trnK/matK		*rpl16*	*trnL-F*							*rpl32-trnL$_{UAG}$*		*rps4-trnT-trnL$_{UAA}$*		
Dataset including hotspots	*trnK* intron (I; II)	*matK*	*rpl16*	*trnT-L* spacer	*trnL-F* exon 5`	*trnL-F* intron	*trnL-F* exon 3`	*trnL-F* spacer	*trnF*	*rpl32*	*rpl32-trnL$_{UAG}$* IGS	*trnL$_{UAG}$*	*rps4-trnT* IGS	*trnT* gen	*trnT-trnL$_{UAA}$* IGS
Position in the alignment	1-885; 2470-3108	886-2469	3109-4584	4585-4728	4729-4759	4760-5630	5631-5677	5678-6063	6064-6131	6132-6359	6360-7138	7139-7247	7248-7670	7671-8004	8005-8932
Length range	0-784; 0-608	0-1536	0-1081	0-132	0-31	0-667	0-47	0-358	0-68	0-219	0-619	0-108	0-354	0-267	0-705
Mean length (SD)	681.9 (183.4); 402.2 (202.2)	1409.8 (334.1)	871.7 (352.7)	74.1 (45.4)	23.1 (13.4)	468.2 (247.3)	36.9 (19.2)	273.7 (136.9)	36.1 (19.3)	160.6 (69.2)	501.4 (208.5)	74.4 (32.6)	291.5 (87.5)	225.6 (61.4)	600.8 (166.8)
GC content (%)	29.4; 35.9	31,5	29,7	35,6	48,3	27,2	31	34,2	48,3	30,3	23	47	25,3	32,3	24,9
Hotspots	3; 0	0	6	1	0	5	0	2	0	0	1	0	4	2	2
Hotspot postion in the aligment	681-687, 830-836,867-883; 0	-	3302-3307, 3433-3438, 3895-3902, 4010-4083, 4127-4147, 4329-4332	4715-4721	-	4898-4914, 4942-4951, 5082-5100, 5180-5183, 5347-5420	-	5734-5739, 5991-6000	-	-	6837-6841	-	7337-7349, 7546-7553, 7576-7585, 7636-7643	7743-7757, 7841-7846	8106-8146, 8416-8434
Inversions	1	1	1	0	0	1	0	0	0	0	0	0	0	0	2
Inversion postion in the aligment	97-101	1500-1502	3920-3958	-	-	5232-5261	-	-	-	-	-	-	-	-	8286-8333; 8363-8370
Dataset excluding region incomplet endings and hotspots; and reinverted inversions															
Position in the alignment	1-794; 2377-2589	795-2376	2590-3683	3684-3778	3779-3809	3810-4532	4533-4579	4580-4949	4950-4971	4972-5159	5160-5933	5934-6013	6014-6319	6320-6632	6633-7390
Length range	0-722; 0-206	0-1537	0-954	0-93	0-31	0-616	0-47	0-352	0-22	0-183	0-617	0-79	0-294	0-255	0-670
Mean length (SD)	654.5 (175.2); 184.9 (49.7)	1409.8 (334.1)	787.6 (317.9)	61.9 (37.4)	23.1 (13.4)	431.8 (227.9)	37 (19.2)	268.0 (134.1)	17.6 (8.8)	146.0 (61.6)	500.4 (208.1)	65.6 (28.1)	258.1 (76.2)	218.7 (59.0)	567.3 (156.8)
Variable characters (%)	20.0; 20.2	17,76	17,7	18,9	0	10,7	8,5	18,1	22,7	13,3	23,4	2,5	17,6	13,1	19,8
Informative characters (%)	9.6; 7.5	8,3	8,8	9,5	0	4,4	4,2	8,2	4,5	6,9	11,1	0	9,5	6,1	9,6
Number of coded indels	52; 11	12	70	8	0	62	0	10	0	2	66	1	19	19	85

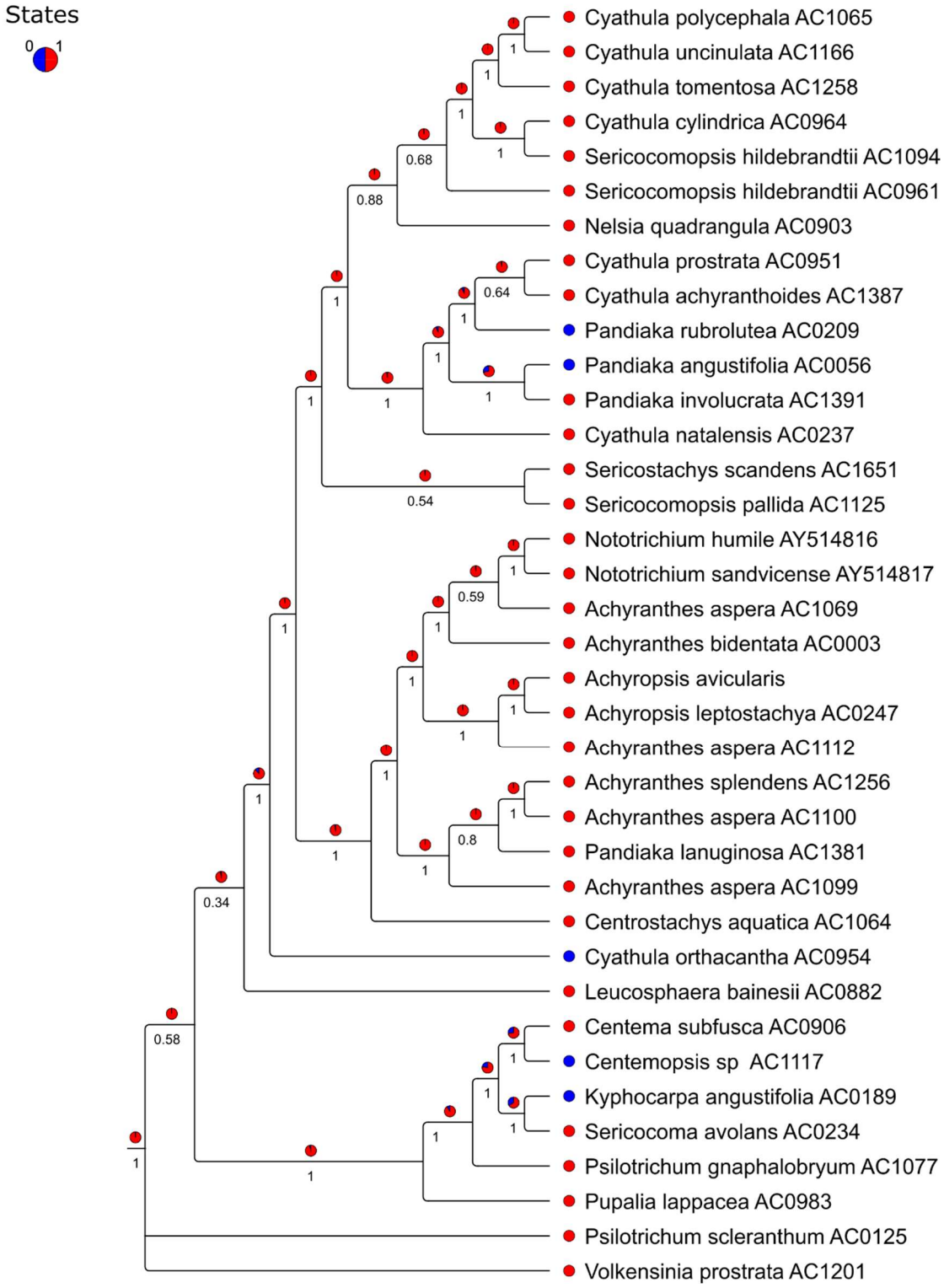
Appendix 4.H: Alignment statistics of the nuclear dataset B

	ITS1	*5.8 S*	*ITS2*
Dataset including hotspots			
Position in the alignment	1-358	359-514	515-834
Length range	0-309	85-156	199-285
Mean length (SD)	272.4 (63.1)	152 (13.3)	253.6 (21)
GC content (%)	51,5	53,8	56
Hotspots	0	0	1
Hotspot postion in the aligment	-	-	558-572
Dataset excluding region incomplet endings and hotspots			
Position in the alignment	1-295	296-451	452-704
Length range	0-272	85-156	192-229
Mean length (SD)	254.2 (57.1)	152 (13.3)	216.5 (5.8)
Variable characters (%)	54,6	5,8	51,8
Informative characters (%)	44,1	4,5	39,9
Number of coded indels	60	2	54

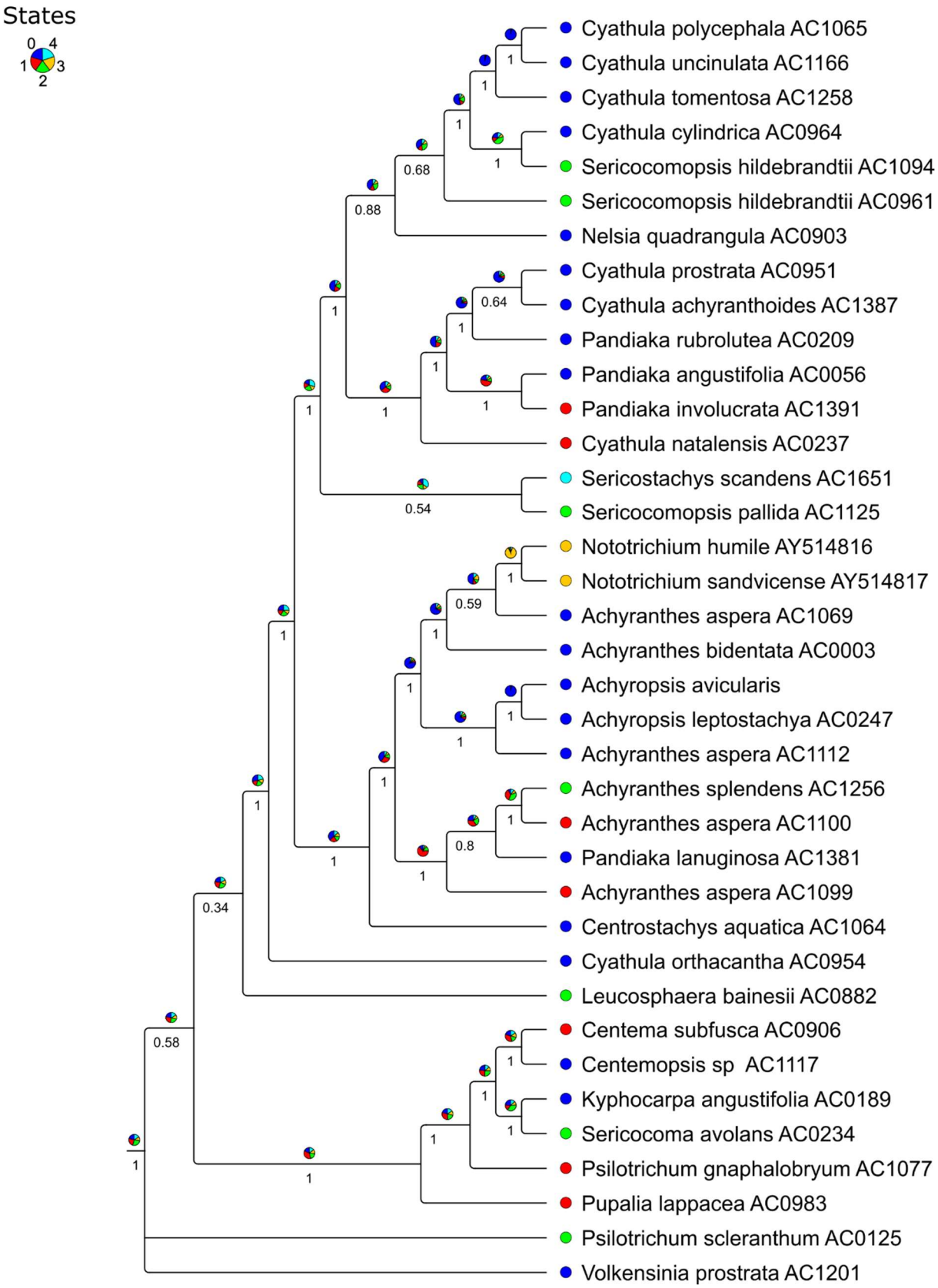
Appendix 4.I: Ancestral states of characters 1-24 for the achyranthoid subclade II.

The pie charts represent probabilities for ancestral states as reconstructed with BayesTraits and plotted on the maximum clade credibility tree obtained by Bayesian Inference of the combined plastid dataset. See Appendix 4.E for the definition of characters and their states as well as the full matrix in Appendix 4.F.

Character 01 - Life cycle

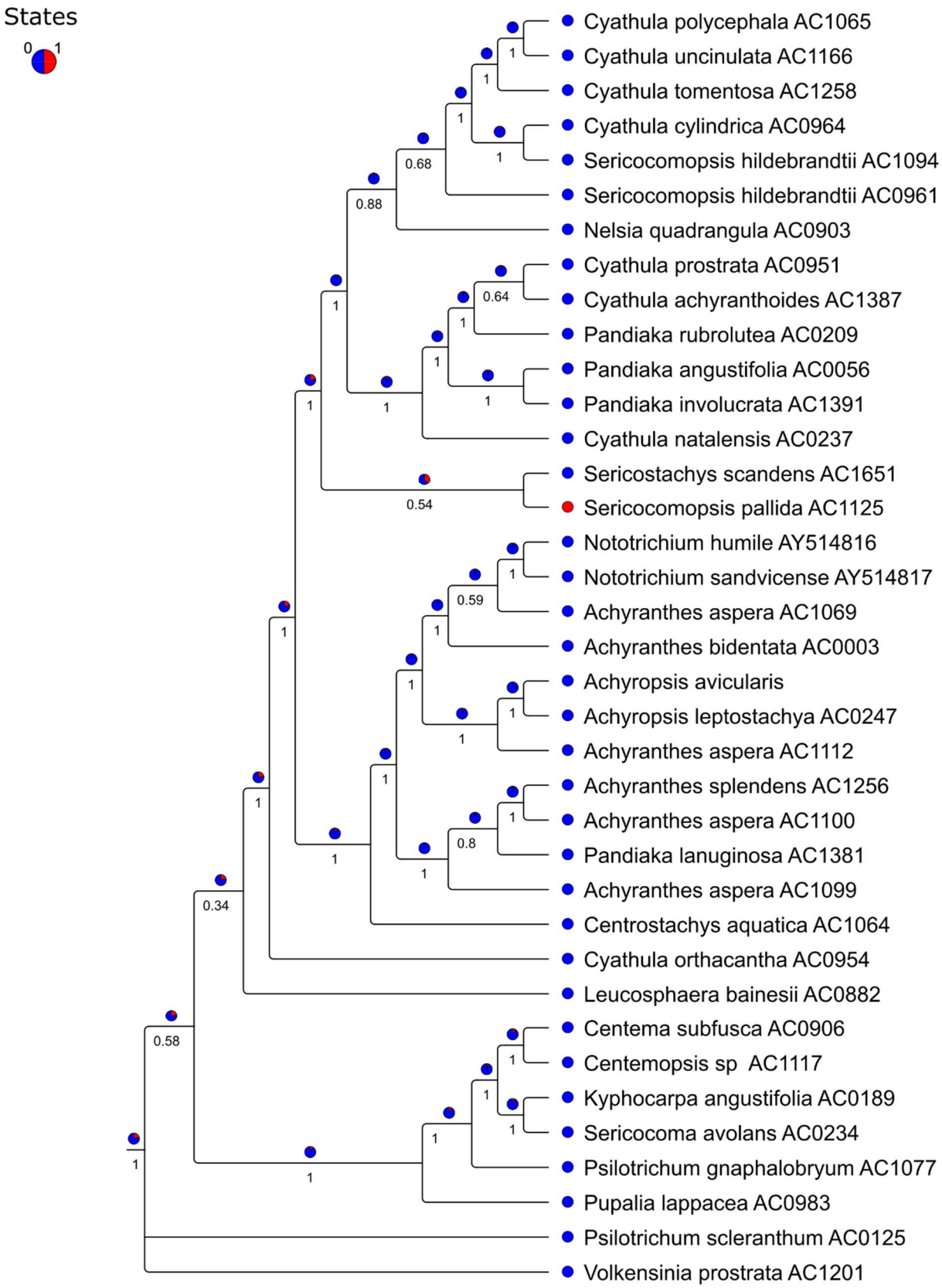


Appendix 4.I – continued

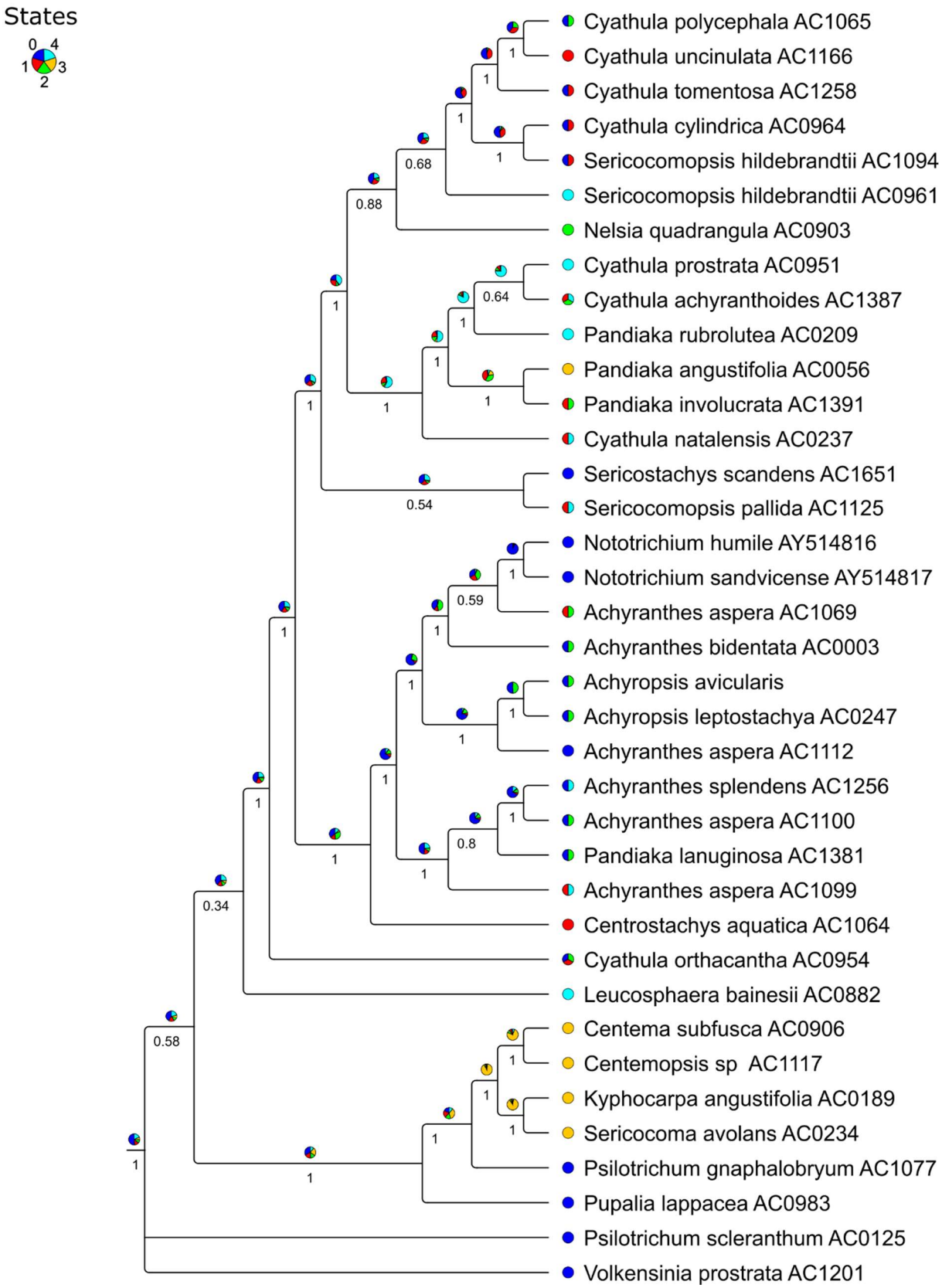


Appendix 4.I – continued

Character 03 - Indumentum of stem and leaves

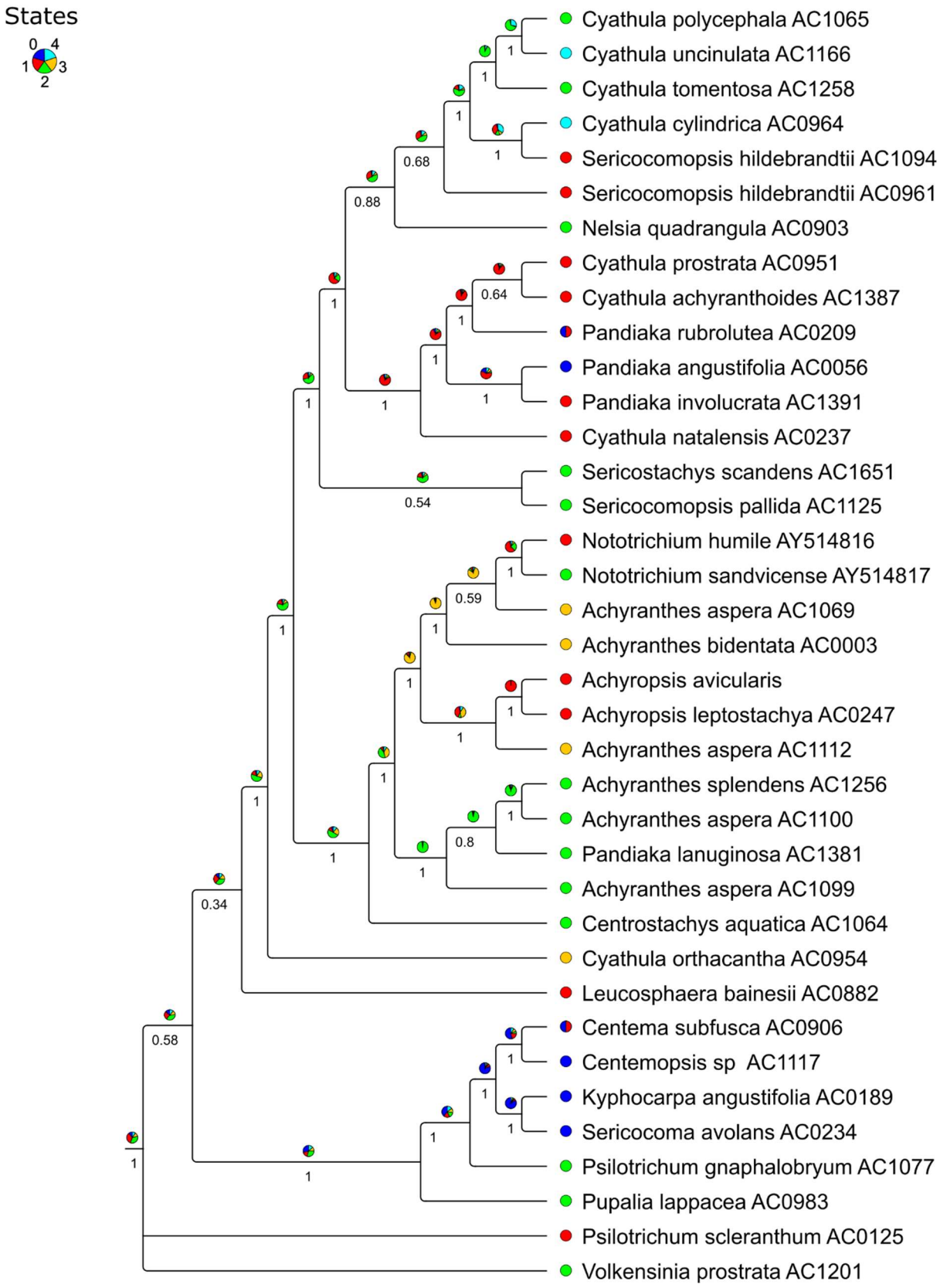


Appendix 4.I – continued

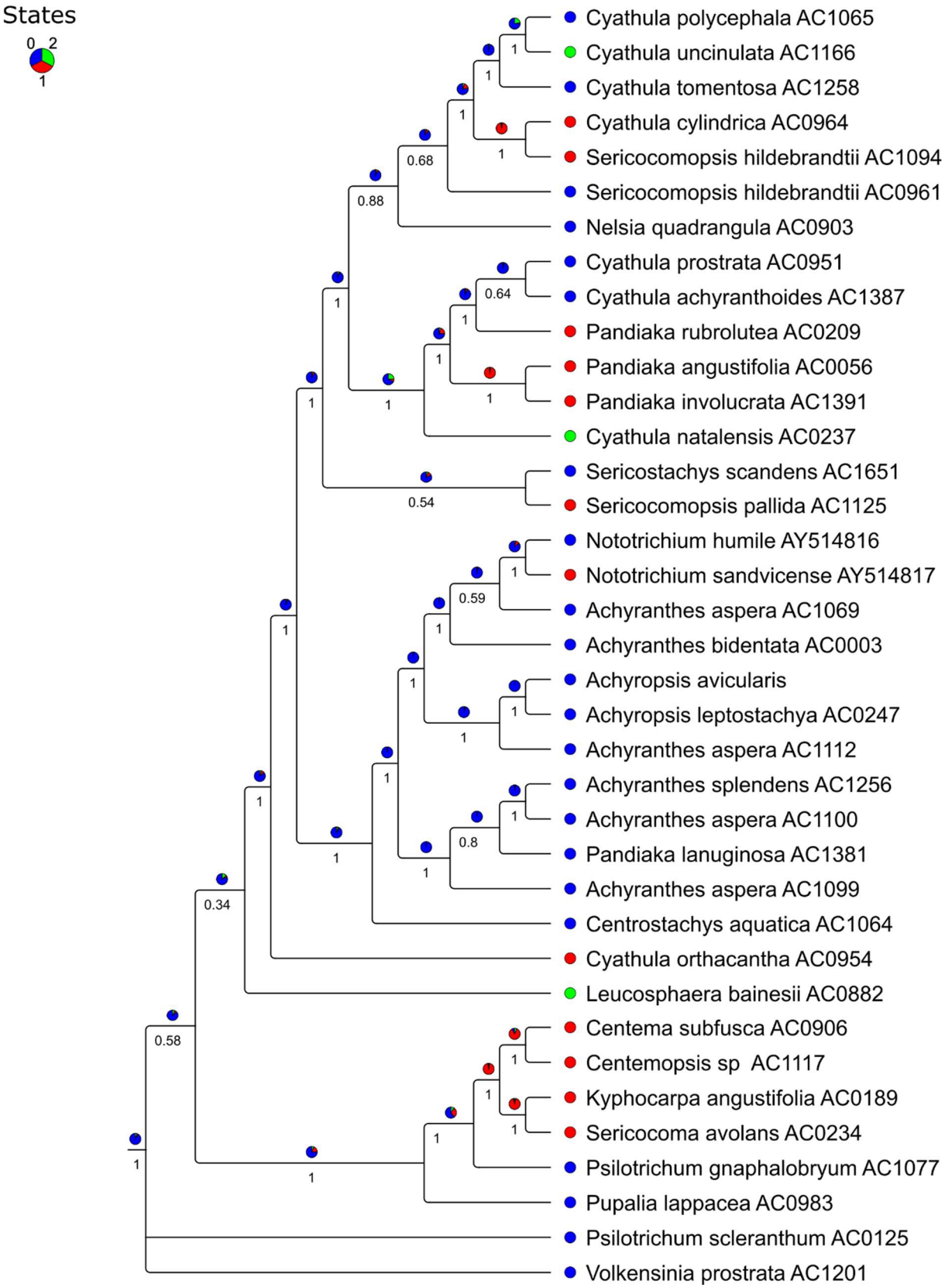


Appendix 4.I – continued

Character 05 - Petiole length of cauline leaves

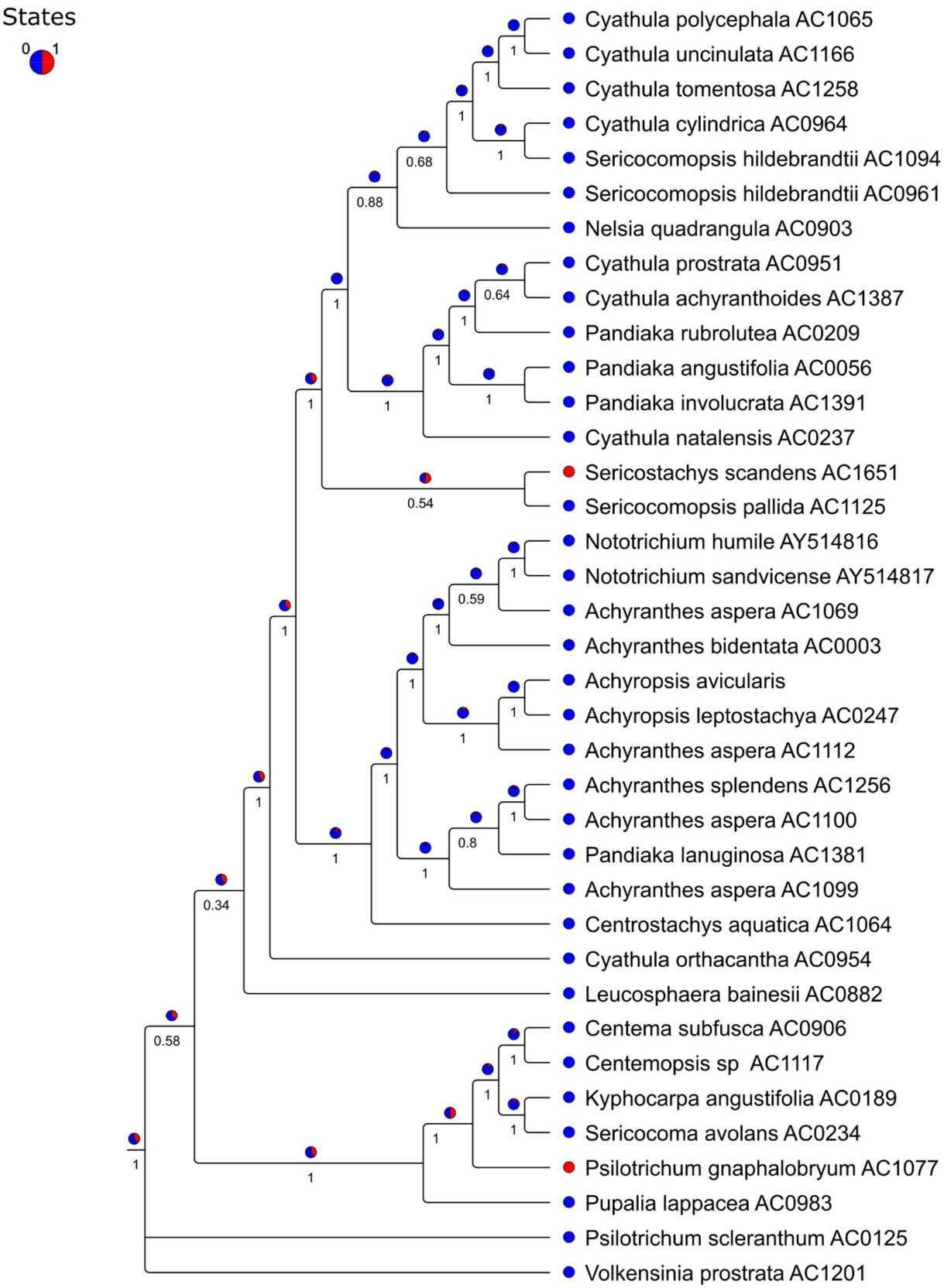


Appendix 4.I – continued

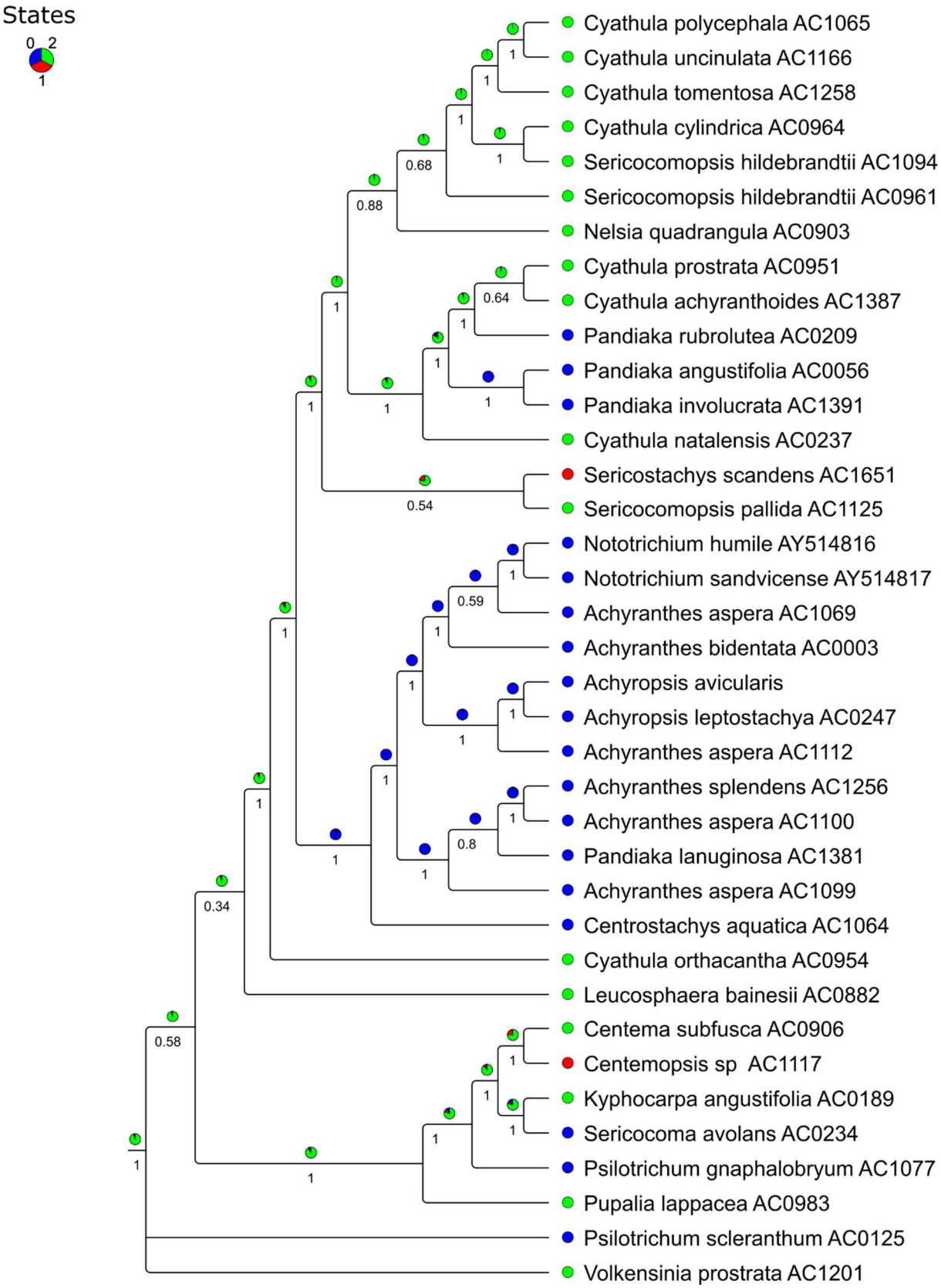


Appendix 4.I – continued

Character 07 - Arrangement of paracladia / Synflorescence structure

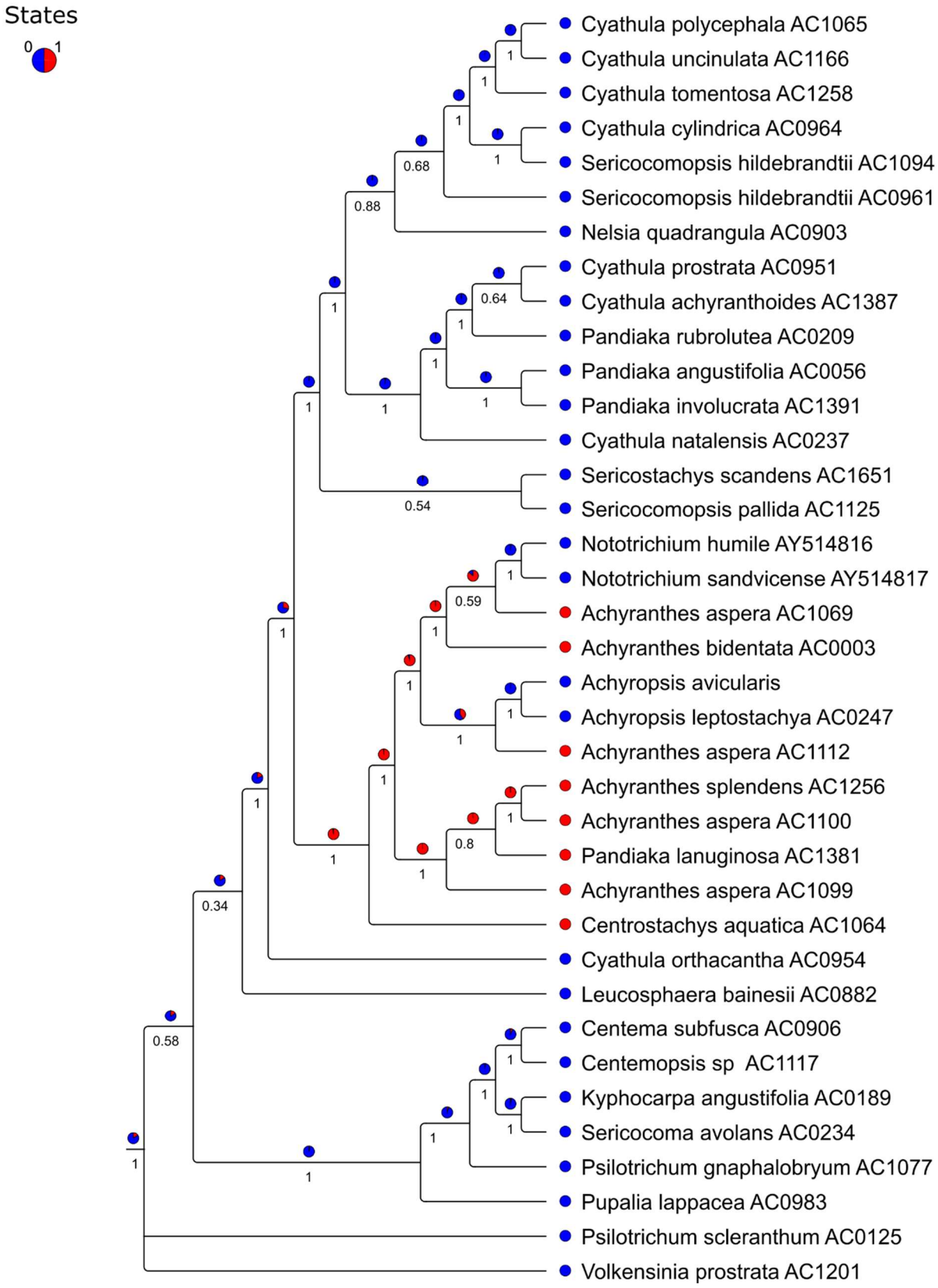


Appendix 4.I – continued



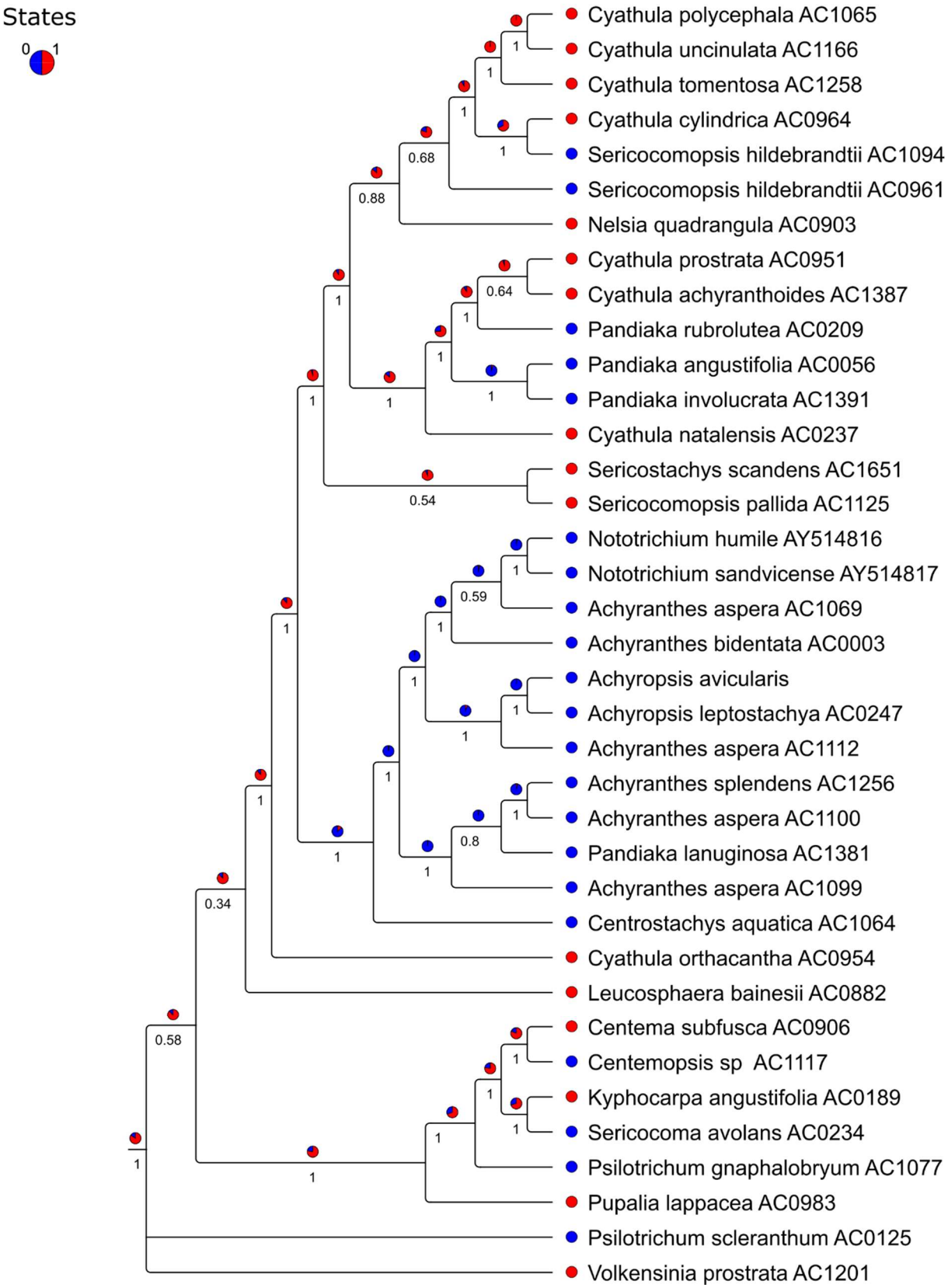
Appendix 4.I – continued

Character 09 - Position (angle) of cymes in relation to the inflorescence axis during maturity

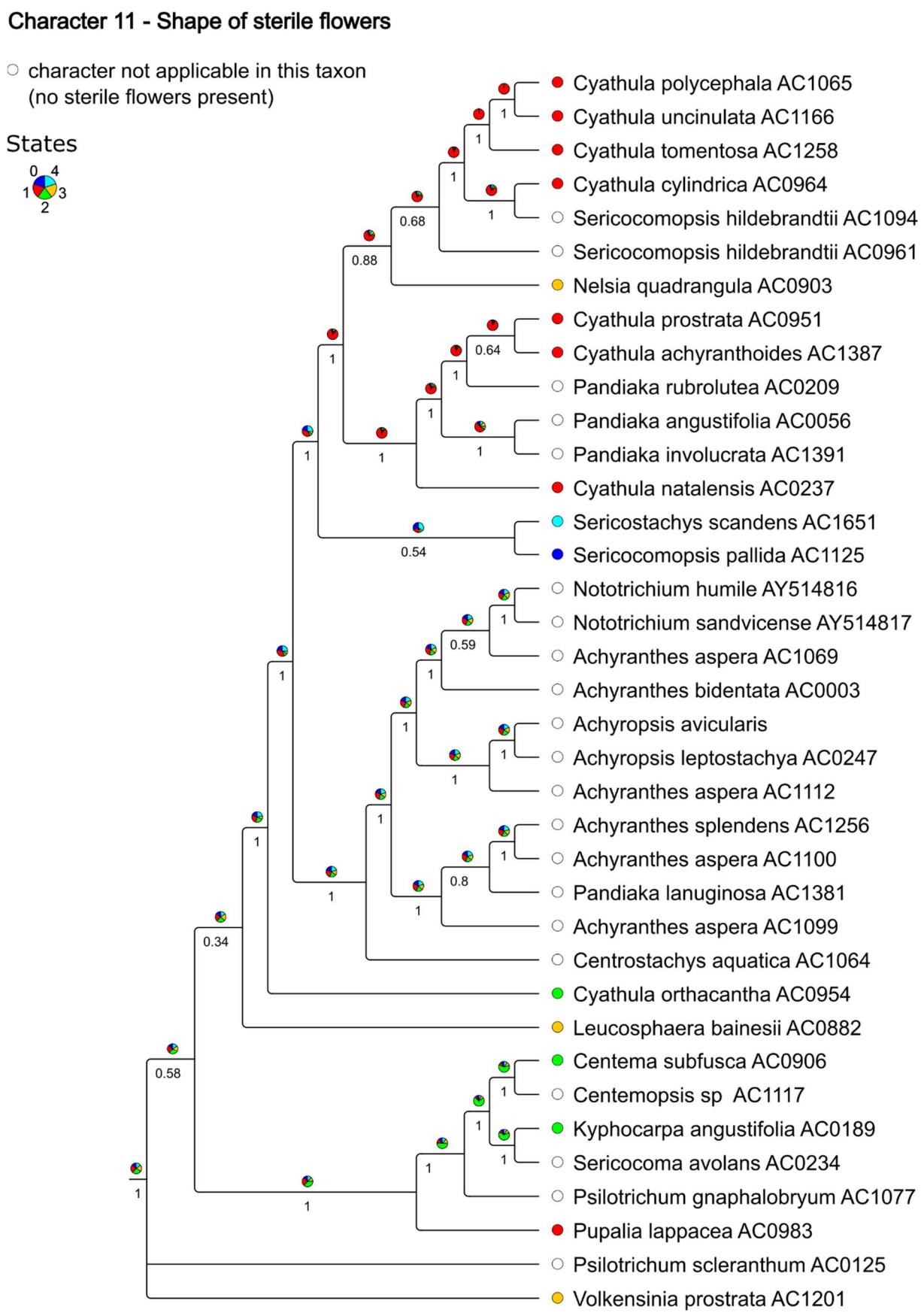


Appendix 4.I – continued

Character 10 - Presence or absence of sterile flowers

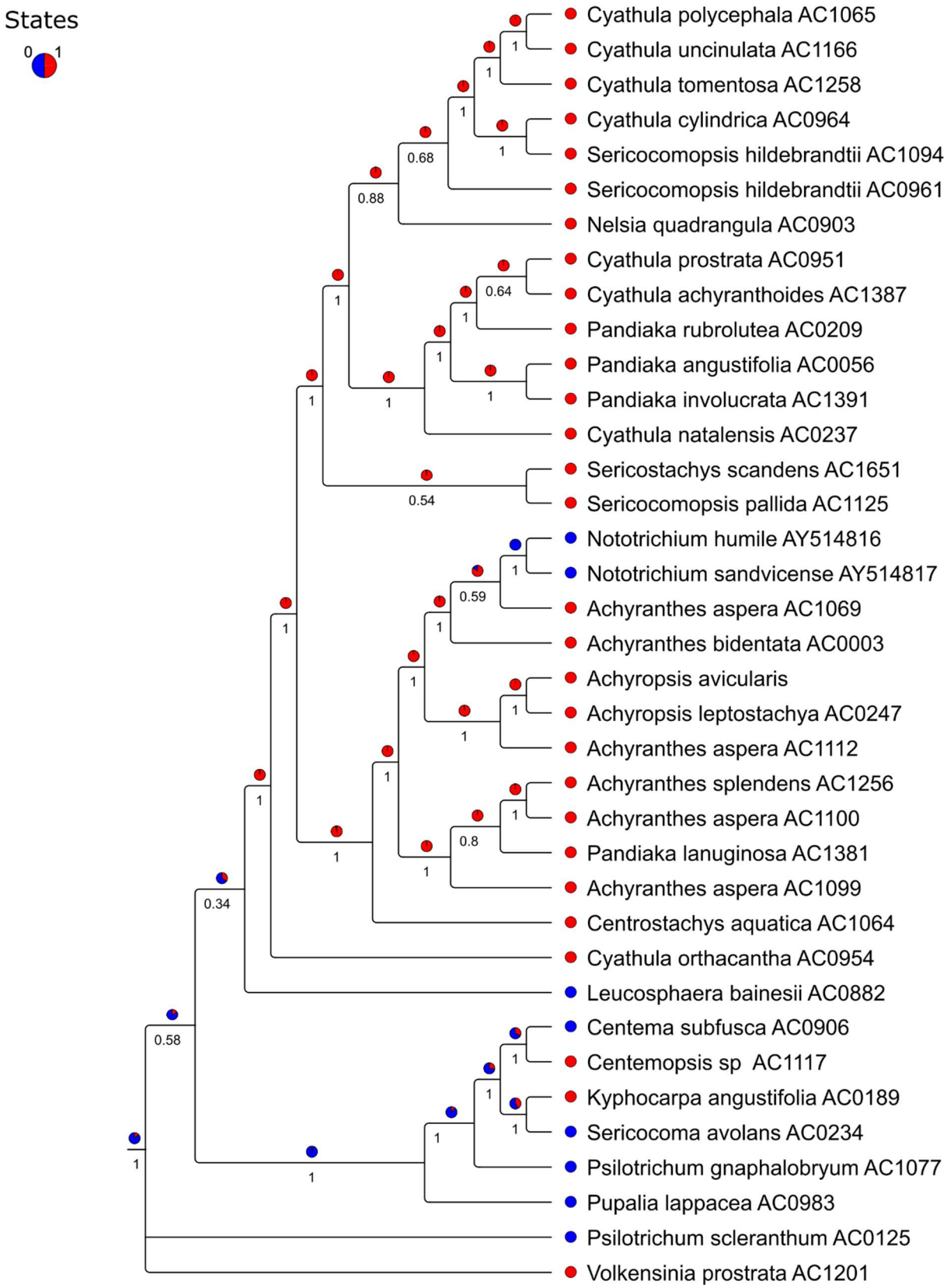


Appendix 4.I – continued



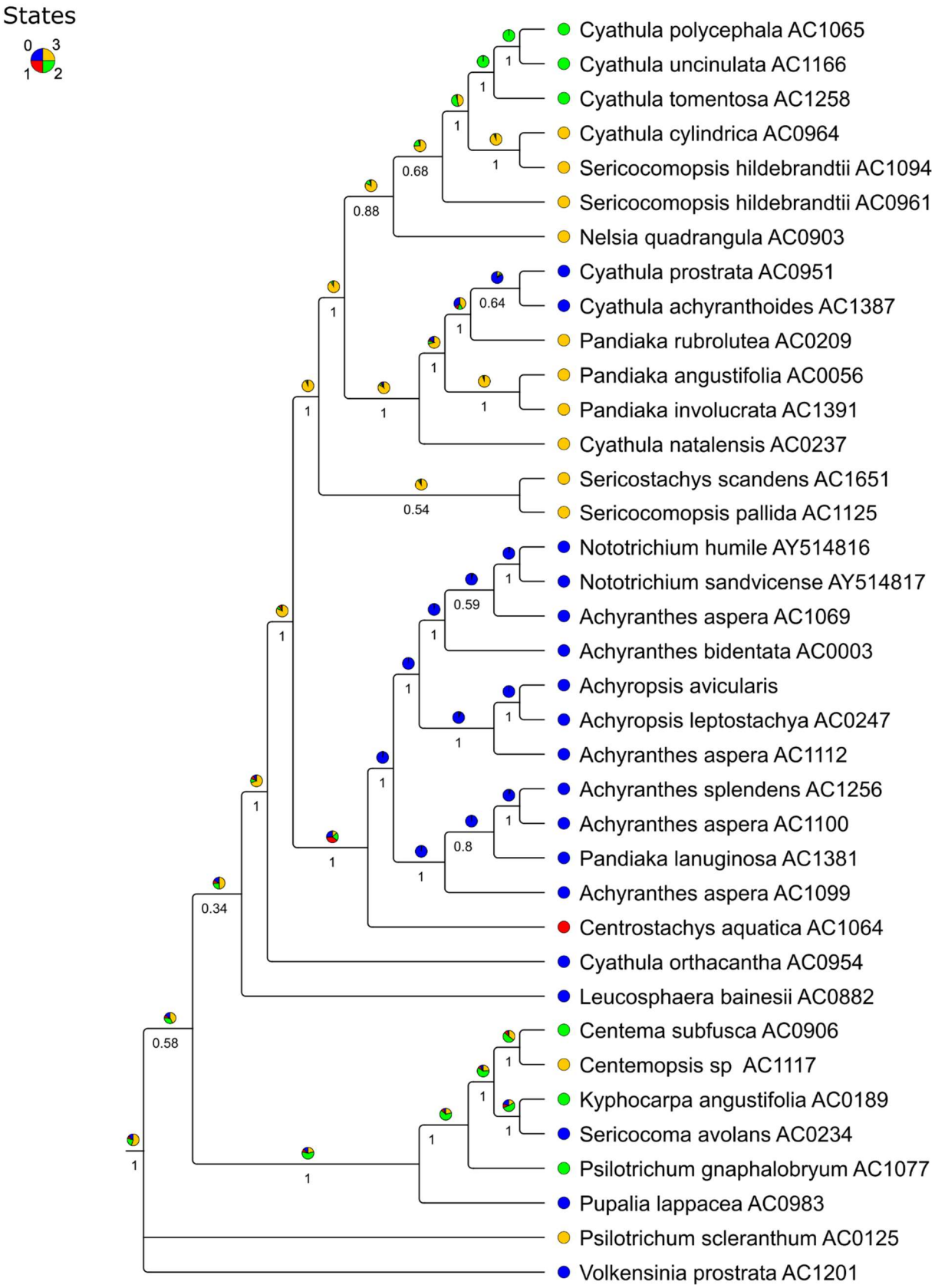
Appendix 4.I – continued

Character 12 - Androecial tube appendages



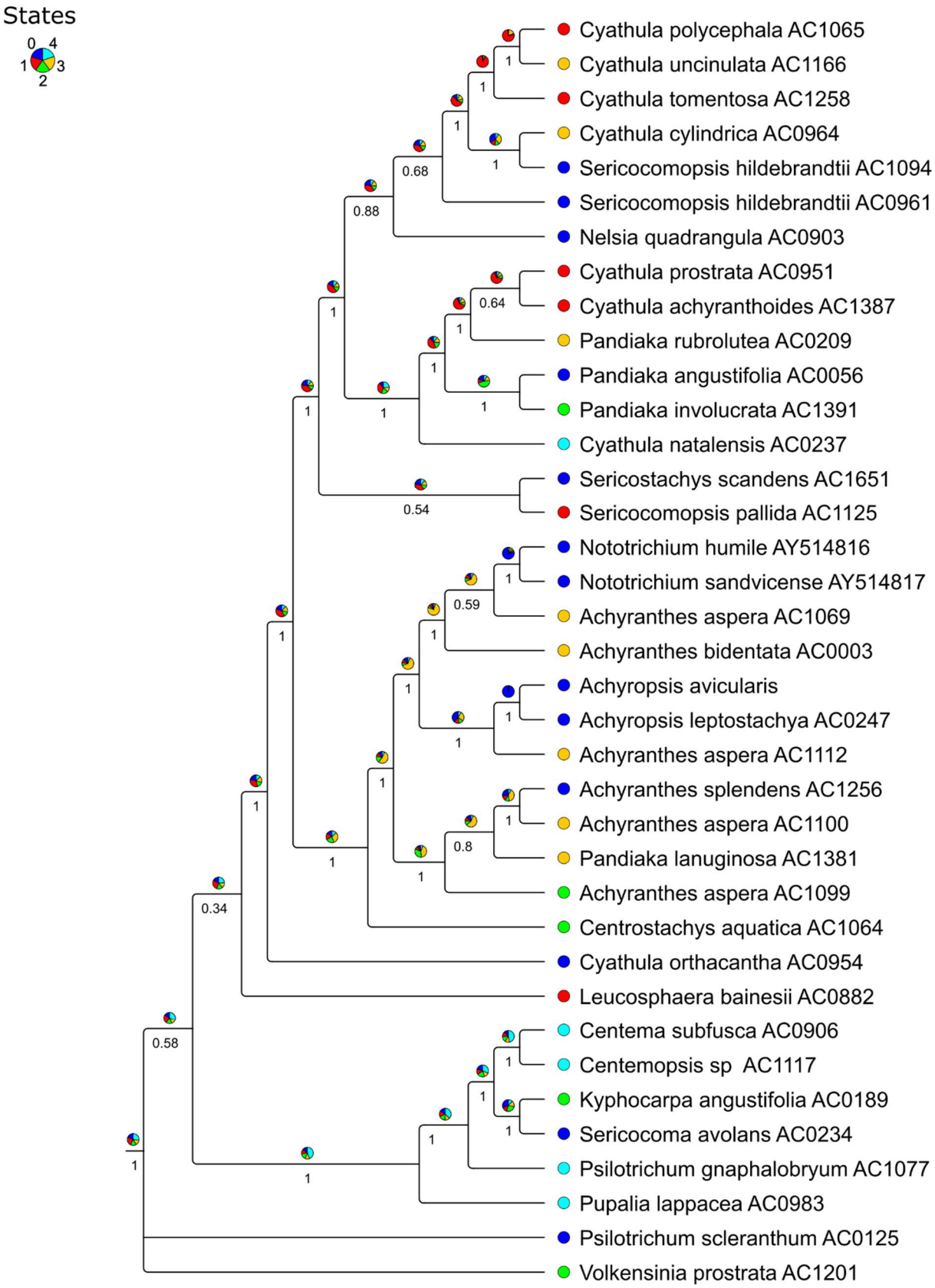
Appendix 4.I – continued

Character 13 - Homogeneity of tepals



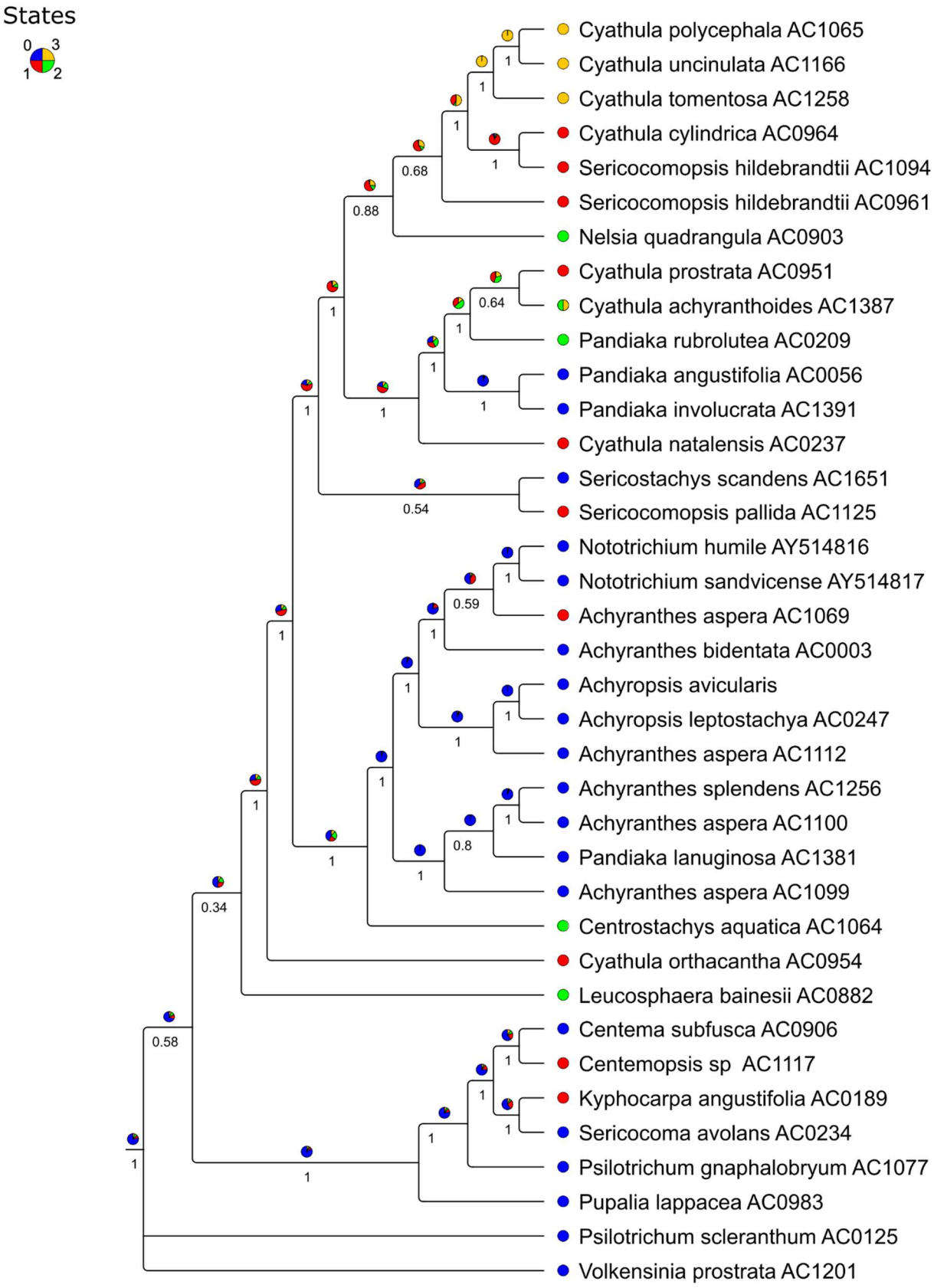
Appendix 4.I – continued

Character 14 - Shape of outer tepals of fertile flowers



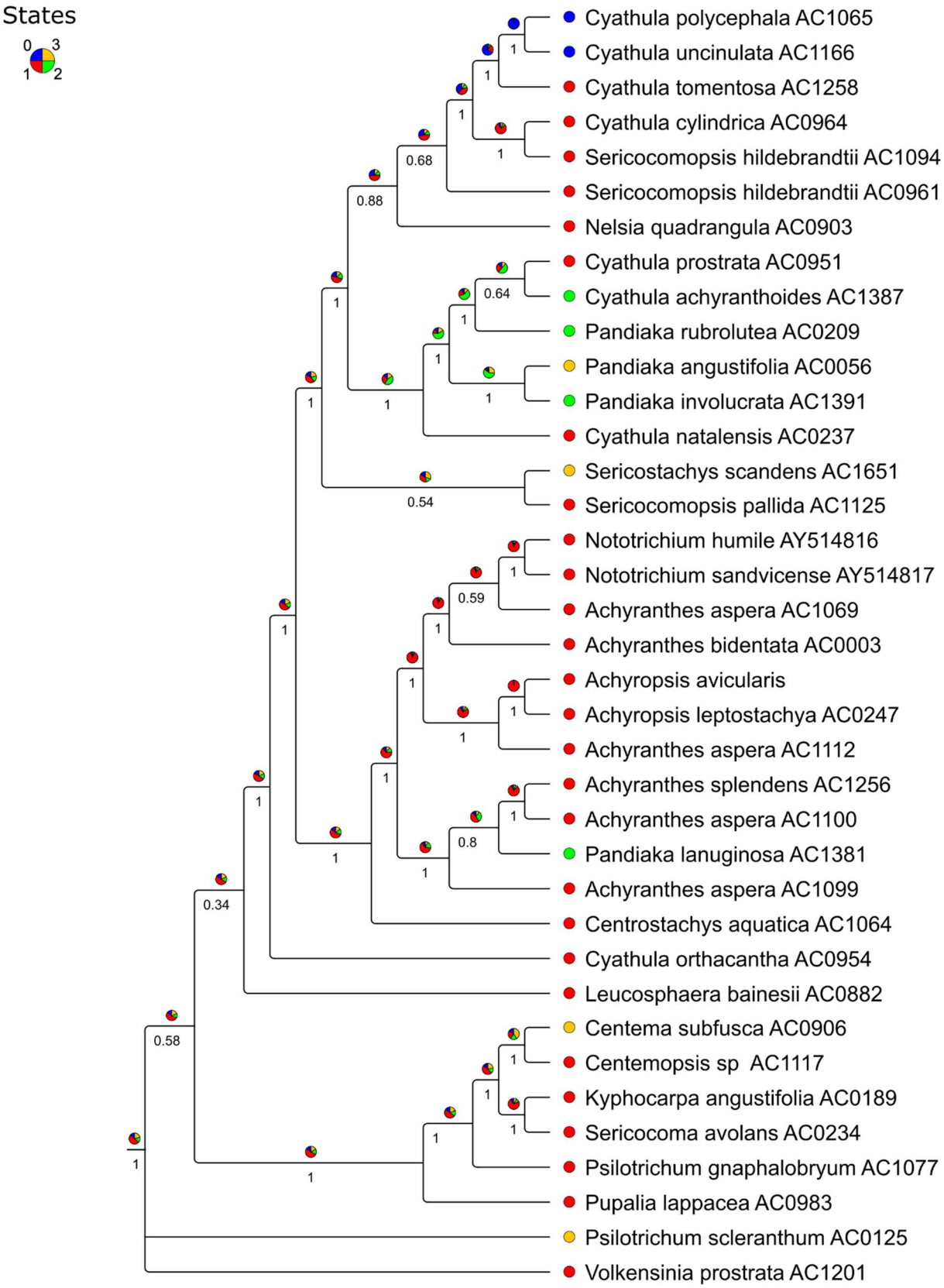
Appendix 4.I – continued

Character 15 - Midrib of outer tepals



Appendix 4.I – continued

Character 16 - Veination of outer tepals



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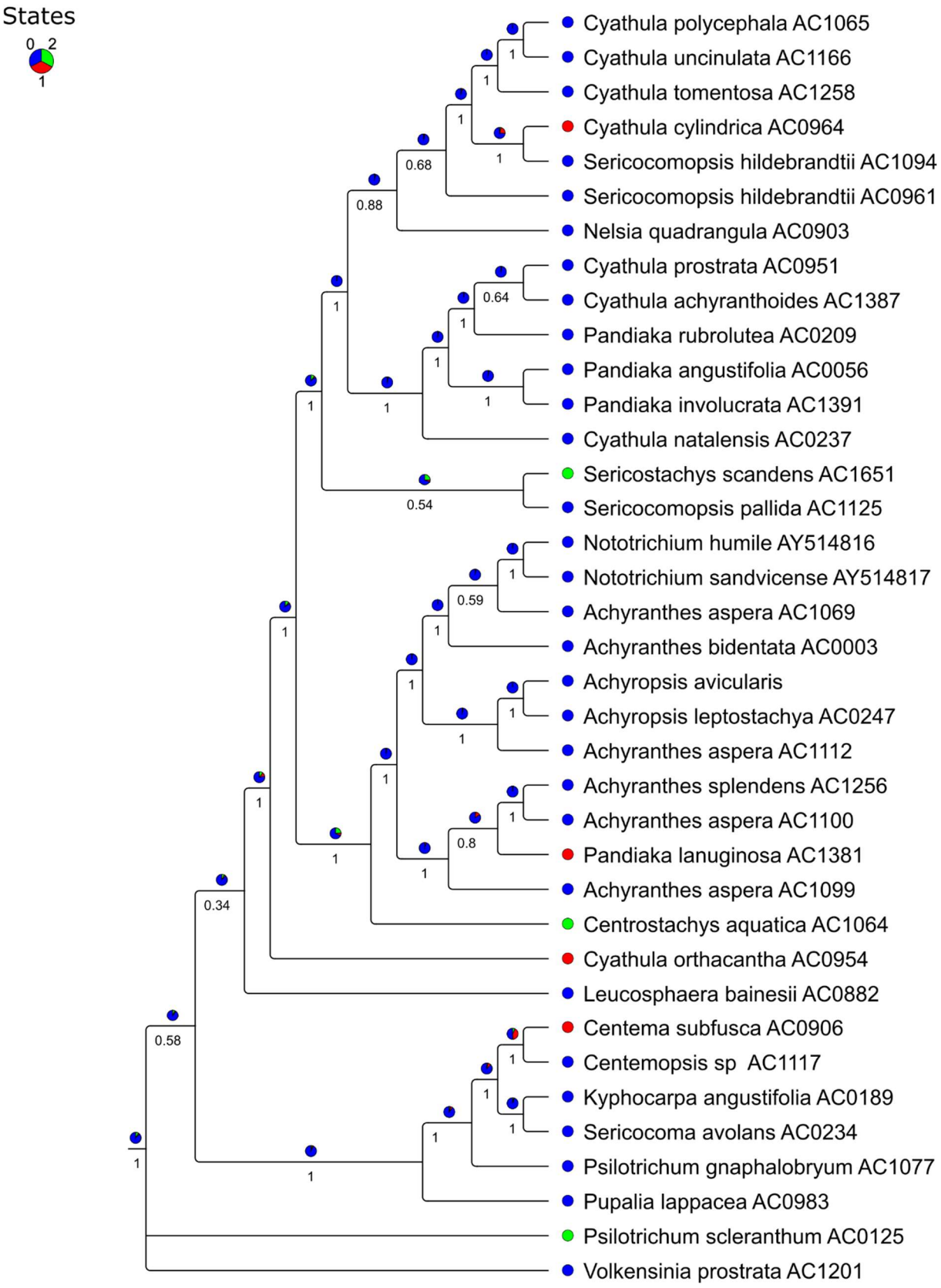
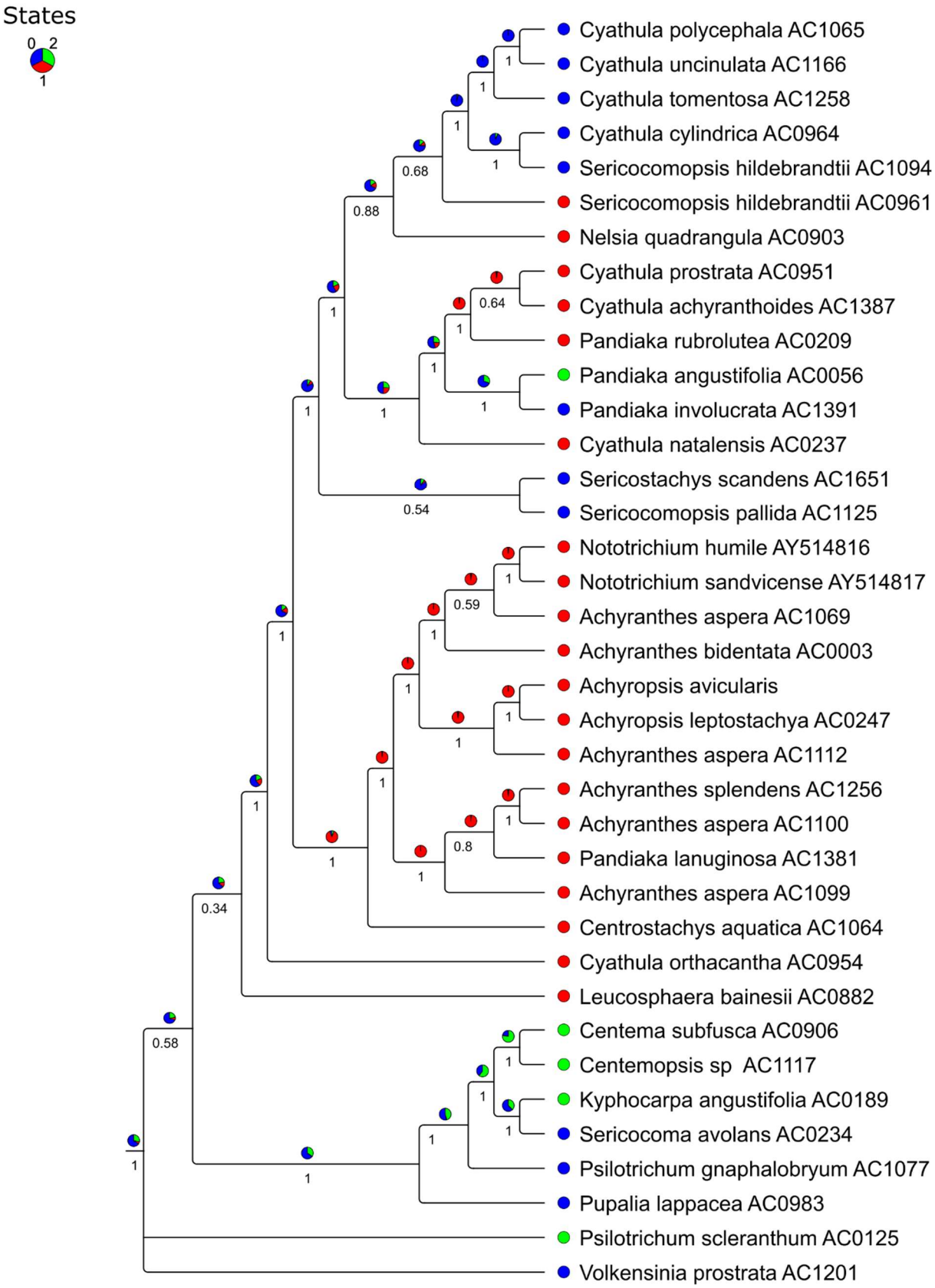


Appendix 4.I – continued

Character 18 - Texture of tepals at maturity



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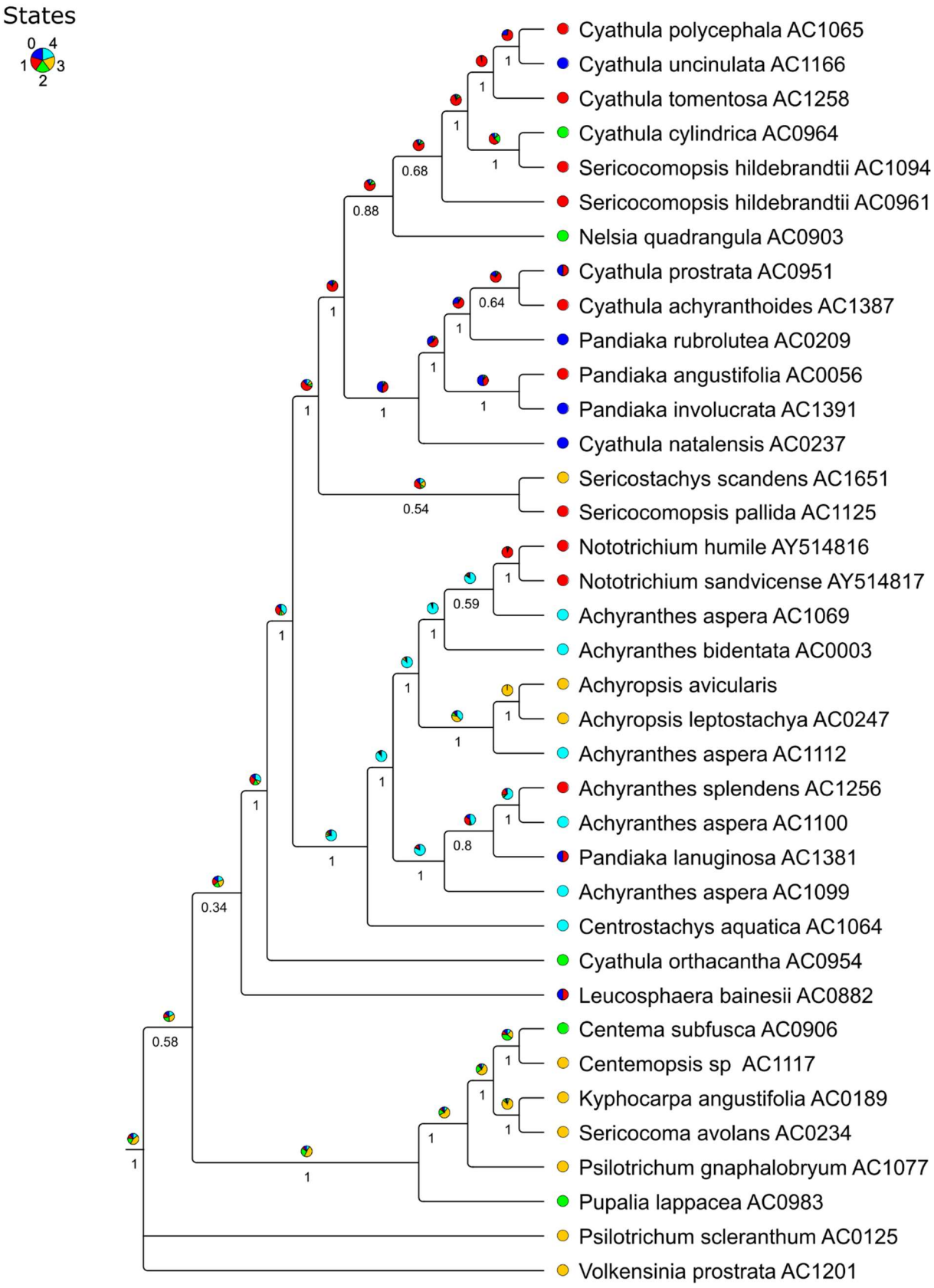
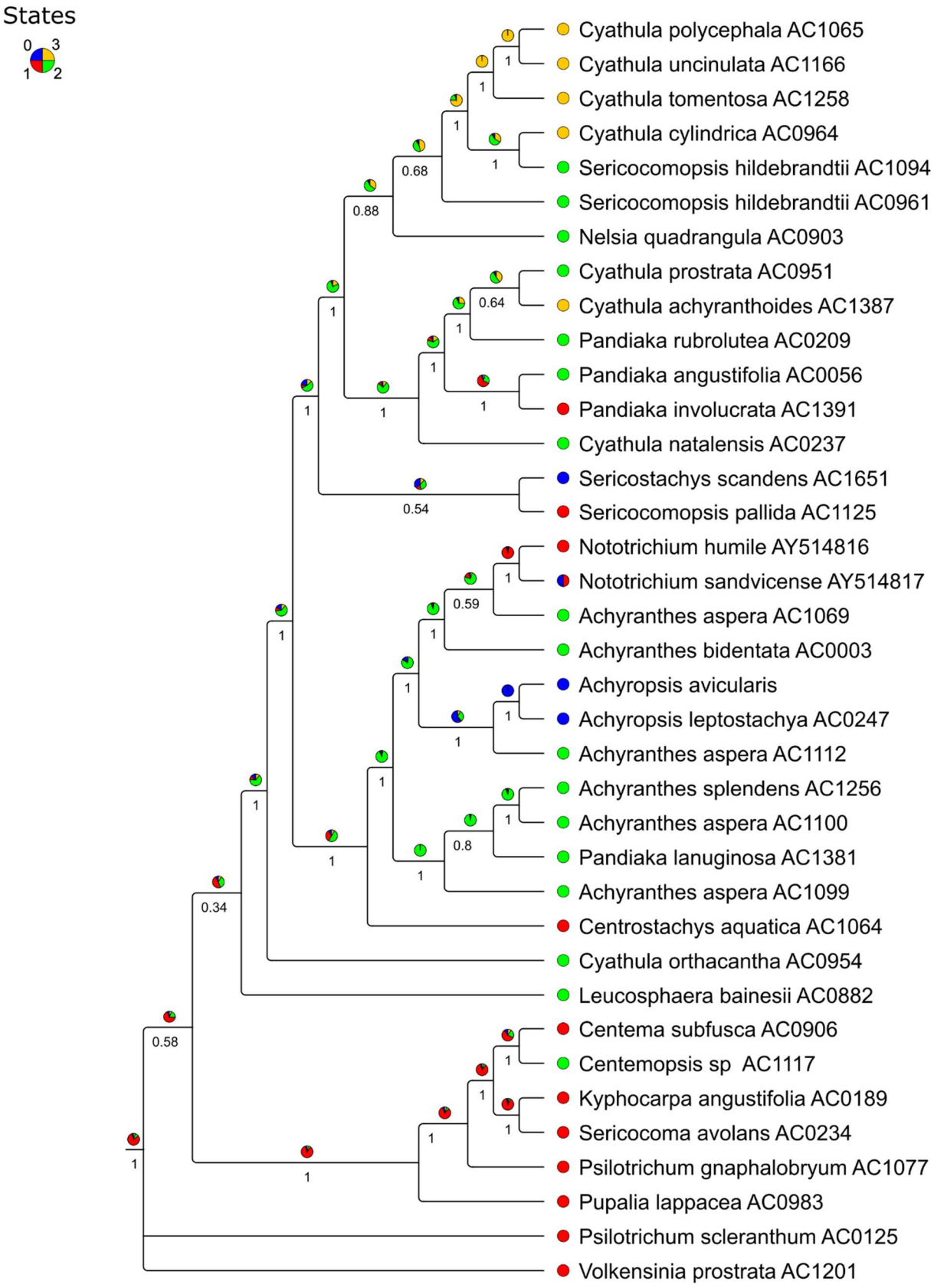


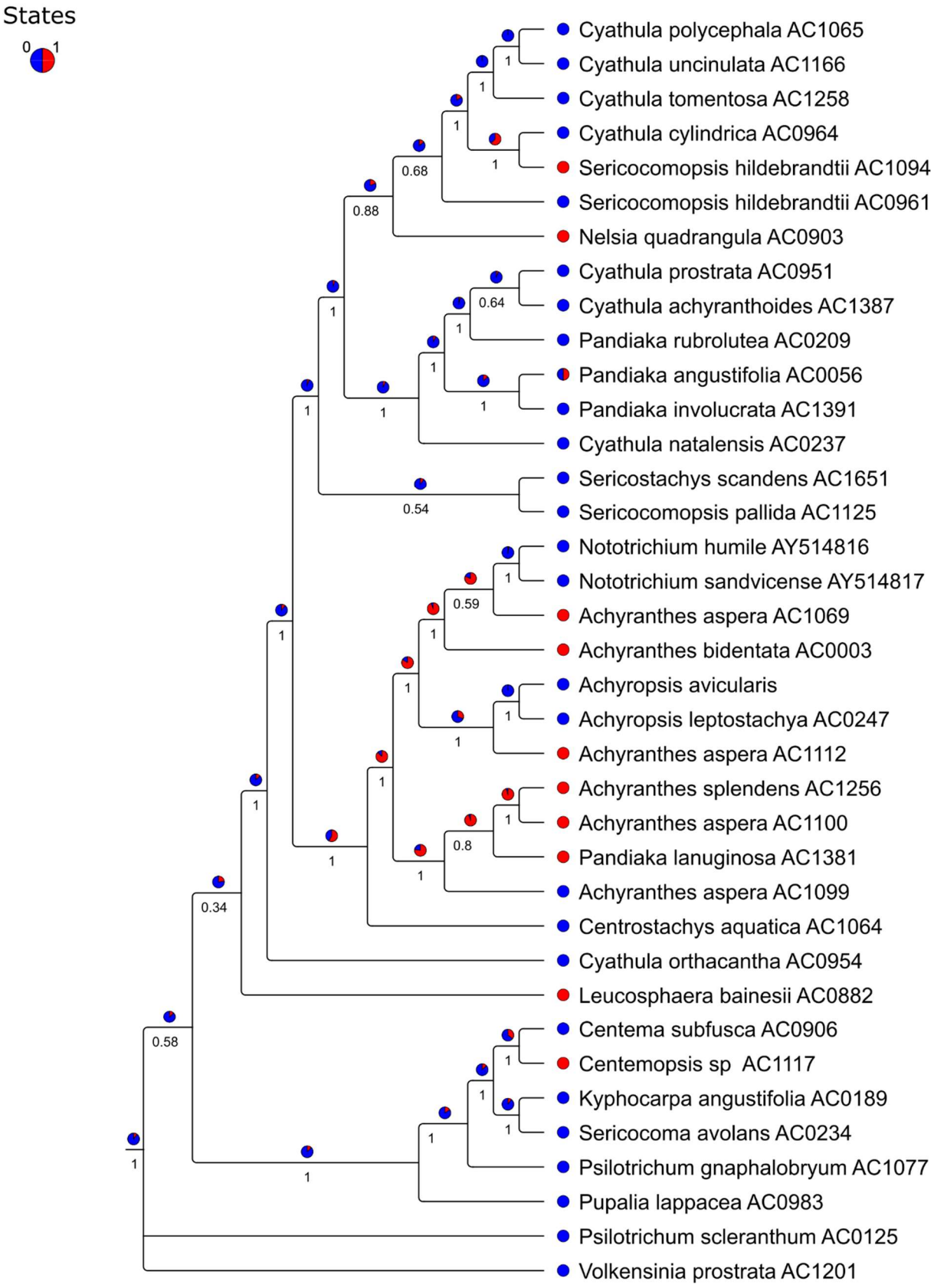
Appendix 4.I – continued

Character 20 - Midrib of bracteoles of fertile flowers



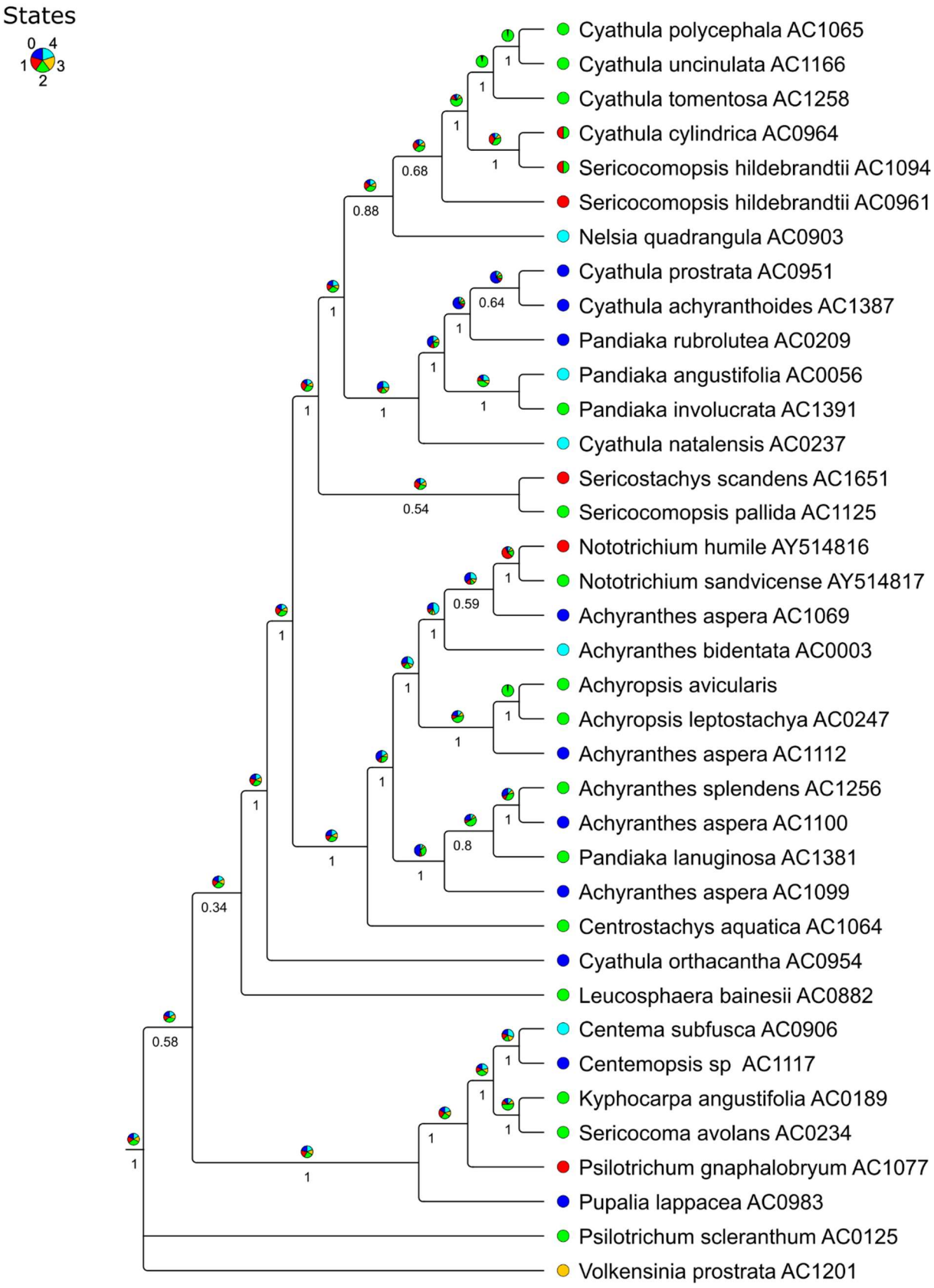
Appendix 4.I – continued

Character 21 - Bracteoles at anthesis

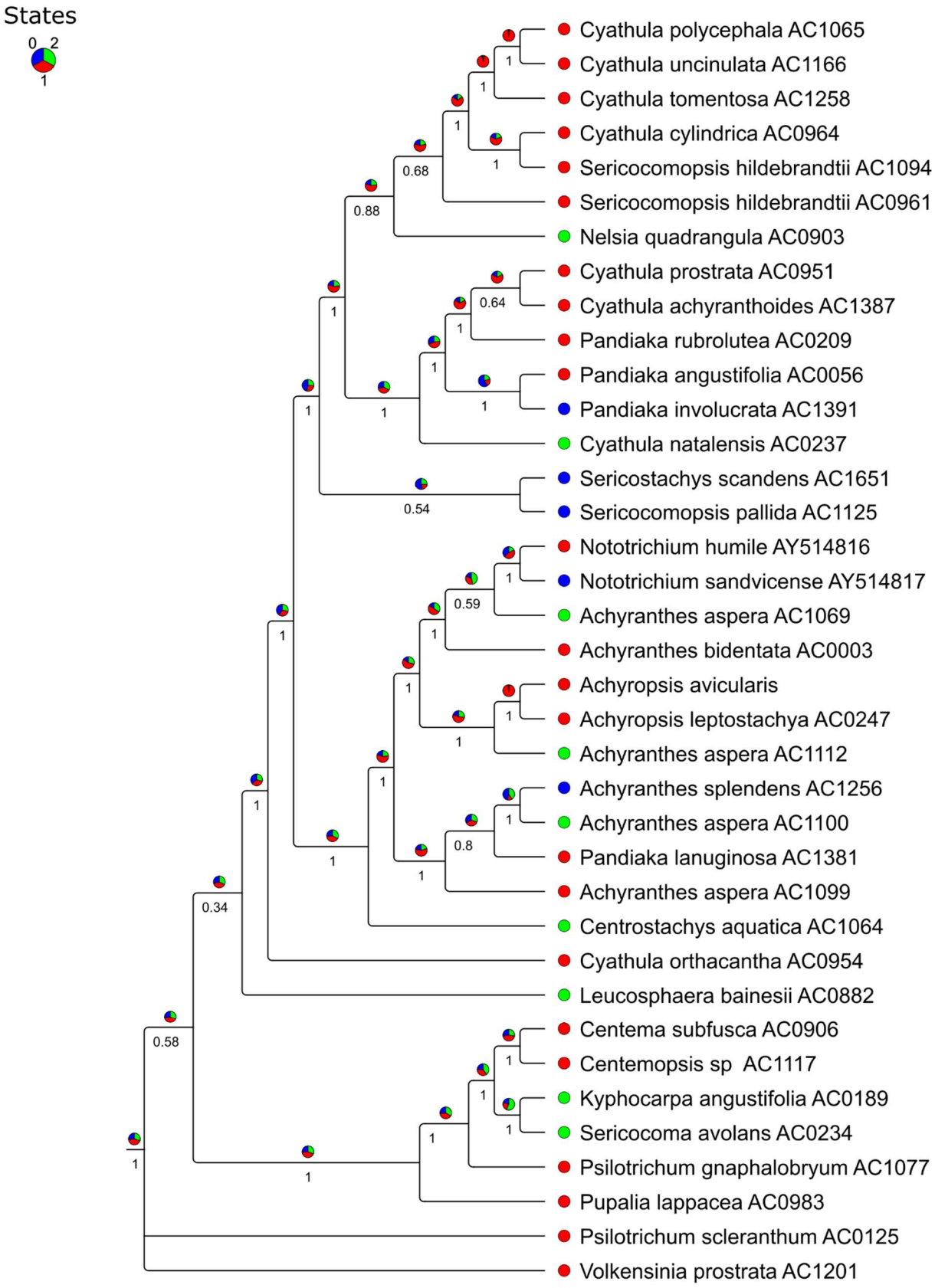


Appendix 4.I – continued

Character 22 - Shape of bracts

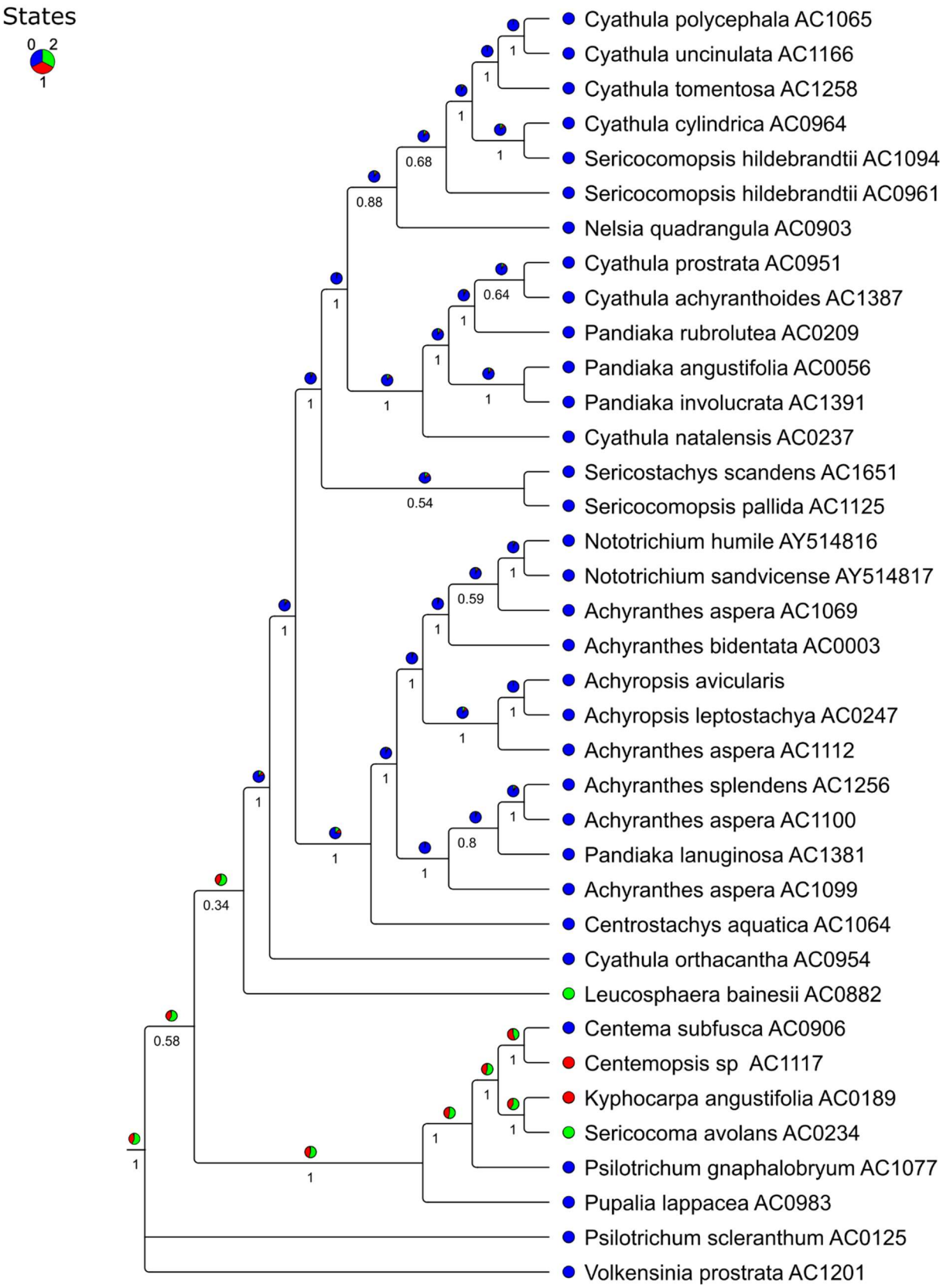


Appendix 4.I – continued



Appendix 4.I – continued

Character 24 - Hairiness of ovary



– Chapter 5 –

General discussion

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5.1 Phylogeny and diversification of the achyranthoid clade

One major aim of the doctoral thesis was to validate the existence of the earlier proposed achyranthoid clade (Müller & Borsch, 2005) and evaluate its composition and structure. Thus, phylogenetic relationships within the achyranthoid clade, as well as its position within Amaranthaceae needed to be investigated based on a denser taxon sampling and DNA sequences of selected genomic regions with a high phylogenetic signal. In particular introns and spacers of the plastid genome (*trnK/matK*, *rpl16* and *trnL-F*) were used for molecular analyses but also nuclear regions (nrITS) were implemented. The concatenated data set of the plastid genomic regions considerably improved the resolution and statistical support of the Amaranthaceae phylogenies, compared to previous investigations (e.g., Müller & Borsch, 2005). The phylogenies of this study supported the five lineages (Celosioids, Amaranthoids, Aerovoids, Gomphrenoids, and Achyranthoids) reconstructed by Müller & Borsch (2005), and at the same time, in fulfillment of the second major aim, resolved two new highly supported major subclades within the achyranthoid clade (see Figure 2.B in Chapter 2).

Based on the comprehensive and highly supported phylogenies it was further possible to explore the diversification times for Amaranthaceae and sublineages, especially focusing on the Achyranthoids, which was another major aim of this study. During the course of the doctoral thesis, the existence of the achyranthoid clade was confirmed as the second major radiation of Amaranthaceae besides the neotropical Gomphrenoids to which is was resolved as sister with maximum support (Figure 2.B in Chapter 2). BEAST analyses (Drummond et al. 2012) revealed the age of the crown group of Amaranthaceae, which was estimated to 51.25 Ma (Eocene). The Achyranthoids split from their sister lineage, the Gomphrenoids, in the Oligocene, c. 27.7 million years ago and started to diversify in the early Miocene, 22.2 Mya. The diversification of its two major subclades I and II was inferred to c. 20 Mya during the Miocene (see Figure 2.C in Chapter 2). Furthermore, several genera (e.g., *Pupalia*, *Psilotrichum*, *Sericocomopsis, Cyathula*, *Achyranthes*, *Calicorema*) and species (e.g. *Sericocomopsis hildebrandtii*, *Achyranthes aspera*) were shown to be non-monophyletic and inner relationships of the achyranthoid clade could be mostly resolved, especially in subclade II, where further plastid intrageneric spacer sequence data (*rpl32-trnL*$_{UAG}$, *rps4-trnT-trnL*$_{UAA}$) and additional taxa were employed in the analyses of Chapter 4 (see Figure 4.B). The new phylogeny presented in Chapter 4 resolved five supported lineages within subclade II, and helped to clarify internal relationships of genera within Achyranthoids II, and thus depicts an important mile stone for the overall phylogeny of Amaranthaceae. However, there are still some insecurities remaining e.g., for the position and relationship of the monotypic genus *Sericostachys* and the isolated species *Sericocomopsis pallida* which depicts the only species in the here newly established monophyletic genus '*Evelynastra*' which is to be published (the respective manuscript is currently in the process of submission). Nevertheless, those for subclade II additionally sequenced plastid regions (Chapter 4) were informative and need to be complemented for the remaining taxa of subclade I in subsequent studies, in order to further unravel the composition and

to facilitate species delimitation in new genus circumscriptions which render all genera of subclade I monophyletic, as it was achieved for subclade II. Remaining uncertainties within Achyranthoids, might probably be clarified by the application of phylogenomic approaches and the inclusion of remaining unsampled species of Achyranthoids. However, this was beyond the scope of this project.

5.2 Morphology/ Pollen morphology

Based on the well resolved and statistically supported phylogenetic trees, the evolution of morphological characters was investigated. Diversification rates and state-dependent speciation and extinction were tested extensively for the Achyranthoids in relation to the presence and absence of modified sterile flowers. State-independent methods (BAMM; Rabosky et al., 2014) and state-dependent speciation and extinction models (as implemented in BiSSE; Maddison et al. 2007, Beaulieu and O'Meara 2016) were used, in order to reveal a possible correlation between the occurrence of modified sterile flowers (hooks and spines; see Figure 2.A in Chapter 2) within taxa of Achyranthoids and the diversification of this clade. The fact that adhesive sterile flowers were found to have evolved multiple times in Achyranthoids, rejected the initial hypothesize of being a key innovation, that boosted diversification of the African clade. However, multiple convergent occurrences of this trait suggest an adaptive value for epizoochory. These results are in line with several studies showing epizoochory as an effective mechanism of short- and long-distance dispersal in African ecosystems (Ridley, 1930; Agnew & Flux, 1970; Stebbins, 1971; Sorensen, 1986; Mori & Brown, 1998; Lobova & Mori, 2004).

Furthermore, a total of twenty-four morphological characters were investigated for subclade II in Chapter 4, using a Bayesian approach (BayesTraits; Pagel & al., 2004) to test which of them are homoplastic and which synapomorphic. Three of them are unique for Achyranthoids and twenty-one were chosen according to Ortuño & Borsch (2020), in order to make both studies comparable and thus contribute to an overall Amaranthaceae character matrix which could then be applied for extensive analyses of morphological evolution on family level. Character states were defined according to the phenotypic diversity observed in Achyranthoids II (see Appendix 4.E in Chapter 4). Whereas the BayesTraits results showed the habit and vegetative characters to be largely homoplastic and thus not helpful for delimitation of taxa, the inflorescences and floral morphology yielded synapomorphies for Achyranthoids and internal lineages through the combination of different characters. Consequently, a key to genera and species needs to be written, based on morphological results and in accordance with the here newly established genus concept. Likewise, this approach needs be applied for the taxa included in subclade I, to complement the analyses from Chapter 4 in which only representatives of subclade I were included.

Considering that pollen morphology is highly variable within Amaranthaceae, also several pollen morphological characters where reconstructed with the same aims as outlined for morphological characters above (see Chapter 3). Stellate pore ornamentation (see Appendix 3.C, character 15 state 2,

in Chapter 3) had already been shown to be derived multiple times within the achyranthoid clade by Müller & Borsch (2005b). Here, their definition of it as a complex character syndrome was accepted and the evolution of the complete syndrome within Achyranthoids could be further located to subclade I, through a better resolved phylogeny and better representation of achyranthoid taxa (see Figure 3.E in Chapter 3).

However, in this study main emphasize was put on the complementation of recent pollen morphological studies by Borsch et al. (2018) and in line with their results, several pollen character states were found autapomorphic for major lineages as well as for single genera, which greatly enriches the morphological treatments of this study and helps to understand the evolution of pollen within the group. Especially for *Centemopsis* of Achyranthoids I, several autapomorphies were discovered in pollen morphology including its unique pollen shape which was not recognized or described in detail in any Amaranthaceae pollen study before and Borsch (1998) described it as almost spheroidal, whereas here the shape was rated as a truncated icosahedral, which resembles the geometry of a football (see the results of Chapter 3 and Figure 3.B).

The SSE models, which were used for analyses in Chapter 2, are currently the subject of controversial discussions and are either criticized (e.g., Louca&Pennell, 2020), or recommended and still widely used (e.g., Gonzalez et al., submitted; Helmstetter et al., 2022; O'Meara & Beaulieu, 2021). This implies the need of further testing of those methods in detail. However, this requires a detailed comparison for which analyses need to be replicated and compared among different models and programs using the same data sets. But this needs to be considered in subsequent studies.

Additionally, morphological results could be complemented by geographical/ecological approaches, e.g., distribution maps, which could be generated for genera and species, which will then contribute to a comprehensive knowledge on the whole achyranthoid clade.

5.3 Taxonomy

A further main objective was the development of a new and modern classification system of the achyranthoid clade which is based on a well resolved and highly supported phylogeny on the one hand, and on the other hand, is substantiated and complemented with (pollen-) morphological investigations. This was necessary based on the high degree of polyphyly which was confirmed in Chapter 2, and already indicated in previous studies. Based on the five monophyletic major lineages reconstructed within subclade II (see Figure 4.B in Chapter 4), a new genus concept was suggested for Achyranthoids II which rendered all genera within subclade II monophyletic. Specially to mention here, is the establishment of two new monotypic genera of which one (gen. nov. ['*Evelynastra*'] Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]) is constituted by the currently accepted species *Sericocomopsis pallida*, and the other (gen. nov. ['*Sebsebia*'] Di Vincenzo, Berendsohn, Wondafrash & Borsch [to be published]) by *Cyathula orthacantha*, respectively. Two new circumscriptions were

established for *Cyathula* and *Achyranthes,* whereas *Sericostachys* remains as presently accepted (see Figure 4.B and Discussion in Chapter 4). These taxonomic innovations are currently in the process of submission as part of the publication of Chapter 4 and depict an important contribution to the taxonomic treatment of the whole Amaranthaceae. This indicates the importance of the development of an equivalent treatment for the taxa of subclade I, including the genera *Pupalia*, *Psilotrichum*, and *Calicorema* all of which have been shown to be polyphyletic in Chapter 2. The position of *Cyathula lanceolata* as an early-diverging taxon (see Figure 2.B in Chapter 2), resulted in its exclusion from *Cyathula* Blume according to the new genus circumscription in Chapter 4, but at the same time raised the question of its classification, which subsequently needs to be addressed. However, detailed investigations of this taxon will be necessary, for a deeper understanding of the phylogenetic relationships, and in order to elucidate what this species is morphologically and were it is best classified.

5.4 General impact of the study

Besides the scientific aspects, the work of this doctoral thesis largely contributed to strengthening the collaboration with partners from developing countries, in particular the already established collaboration with partners in Ethiopia. In addition, new collaborations were established, and an agreement (MoU) was signed in the frame of this doctoral project with partners from the National Museum of Kenya / East African herbarium (EA), including joint field work in Kenya as well as a stay to work in the EA herbarium. These collaborations in biodiverse regions are of great importance in order to assess and better understand the diversity and evolution of the achyranthoid clade, which is largely composed of African taxa. This is in line with overall biodiversity conservation efforts in Africa to prevent further biodiversity losses, which depicts a problem with major global implications. Considering that, a comprehensive understanding of the species based on modern techniques and scientific expertise is required to enable an appropriate and sustainable management of the high biodiversity of the region.

Additionally, this project contributes to the development of the genus level classification of the Amaranthaceae which was published in 2015 (Borsch et al., 2015; Hernández-Ledesma et al., 2015), based on a collaborative international network, and is dynamically updated online using the EDIT platform for cybertaxonomy (www.caryophyllales.org).

Contributions to chapters

Contributions to Chapter 2:

Di Vincenzo, V., Gruenstaeudl, M., Nauheimer, L., Wondafrash, M., Kamau, P., Demissew, S., & Borsch, T. 2018. Evolutionary diversification of the African achyranthoid clade (Amaranthaceae) in the context of sterile flower evolution and epizoochory. *Ann. Bot.* 122: 69–85. https://doi.org/10.1093/aob/mcy055.

Own contributions: Study design (together with T. Borsch); organization of field work, collection and identification of most of the material during joined field work (together wih M. Wondafrash and Peris Kamau); set up and carrying out of laboratory work including primer design; set up/carrying out of data analyses (together with M. Gruenstaeudl and L. Nauheimer); layout of all figures; microscope photography (with assistance from Julia Dietrich); writing of the first draft of the manuscript which was finalized with contributions from all authors.

Contributions to Chapter 3:

Di Vincenzo, V. & Borsch, T. (in prep.) Pollen morphology of the achyranthoid clade (Amaranthaceae).

Own contributions: Study design (together with T. Borsch); scanning electron microscopy and the preparation of pollen samples (with some assistance from Juliane Bettig in the final phase); generation of molecular datasets, character matrix and set up/carrying out of data analyses; layout of figures; writing of the chapter and the first draft of the manuscript.

Contributions to Chapter 4:

Di Vincenzo, V., Berendsohn, W., Wondafrash, M. & Borsch, T. (in the process of submission to *Taxon*). Phylogenetics, morphological character evolution and taxonomy of the achyranthoid clade (Amaranthaceae): Evidence to make the genus *Cyathula* Blume monophyletic and to resurrect a third species of the former genus *Sericocomopsis* Schinz in East Africa.

Own contributions: Study design (together with T. Borsch); collection and identification of most of the material during joined field work (together with M. Wondafrash and Peris Kamau); set up of laboratory work and primer design (with assistance in the lab from Bettina Giesicke); generation of the character list and analyses of herbarium specimen (together with T. Borsch); generation of molecular datasets, character matrix and set up/carrying out of data analyses; compilation of the checklist in the EDIT platform (together with W. Berendsohn); elaboration of a draft for the revised circumscription of taxa, with further contributions by the other authors; writing of the first draft of the manuscript which was finalized with contributions from all authors.

The whole study was supervised by T. Borsch.

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