PHYLOGENETICS AND BIOGEOGRAPHY OF *EMILIA* CASS. (ASTERACEAE, SENECIONEAE).

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DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

R.J.Mapaya

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1st day of June 2017 in Braamfontein, Johannesburg

ABSTRACT

A molecular phylogenetic and biogeographic study of the palaeotropical genus *Emilia* Cass., tribe Senecioneae in the Asteraceae, was undertaken as very little was known about these aspects of the genus, which are informative for taxonomic and conservation practices and contribute to our understanding of its evolution. The investigation aimed to: (1) assess the species recognised by Jeffrey (1997) in the large-headed, morphologically variable and widespread *Emilia coccinea* complex using a phenetic approach, and evaluate how applicable the morphological and phenetic species concepts are to this complex; (2) elucidate the phylogenetic relationships of *Emilia* species, genera *Bafutia*, *Emiliella* and closely allied genera in the Senecioneae, using molecular DNA sequence data; (3) examine the pattern and timing of diversification in *Emilia* and correlate this pattern with morphological trends in the genus; and (4) identify centres of diversity and endemism for *Emilia* in southern Africa[1], and compare and assess the following spatial biodiversity indices: species richness (SR), corrected weighted endemism (CWE), phylogenetic diversity (PD), and phylogenetic endemism (PE), and their application to the conservation of *Emilia* in a chosen region (viz. Zimbabwe).

The phenetic study of the *E. coccinea* complex was based on 134 herbarium specimens spanning the altitudinal and geographical ranges of this complex and using multivariate analyses (cluster analysis and ordinations). Parsimony and Bayesian phylogenetic analyses, based on molecular plastid *trn*L-*trn*F and nuclear ITS sequence data, were conducted on a representative sample of *Emilia* species together with other closely related Senecioneae genera to provide the basis for a taxonomic revision of the genus. Phylogenetic relationships of *Emilia* species, including Jeffrey's sectional classification of *Emilia*, the distinctness of the morphologically similar species in the *E. coccinea* complex, and the generic status of similar genera *Bafutia* and *Emiliella* were then evaluated from reconstructed phylogenies. The biogeographic diversification history of *Emilia* was traced using the present distribution of species and a reconstructed nuclear ITS phylogeny. Dated molecular phylogenetic hypotheses constructed using BEAST were used to estimate the time of divergence in *Emilia*, and linked with optimized evolutionary patterns of morphological features to trace evolutionary trends in the genus. Centres of diversity and endemism were mapped and identified in southern Africa

[1] Southern Africa is defined here as the countries south of the Democratic Republic of Congo and Tanzania (Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland, Zambia, and Zimbabwe).

using distribution data obtained from PRECIS data sets, field work and herbarium specimens. Four spatial biodiversity indices (SR, CWE, PD, and PE), two of which incorporate evolutionary history of the genus (PD, PE), were examined for overlap in southern African countries and also evaluated for potential use in conservation planning in Zimbabwe by assessing the distribution/ranges of the ten Zimbabwean *Emilia* species and their occurrence in currently protected areas that include national parks and botanical reserves. Additionally, the conservation status of the species found in Zimbabwe was assessed using the 'IUCN Red Lists Categories and Criteria'.

Five of the eight species (viz., *Emilia emilioides, E. jeffreyana, E. praetermissa, E. subscaposa,* and *E. vanmeelii*) in the *E. coccinea* complex are phenetically and genetically distinct; *E. lisowskiana* is not distinct as three *E. coccinea sensu stricto* specimens clustered with it in the multivariate analysis and it is unresolved in the molecular analyses. Two species (*E. caespitosa* and *E. coccinea*) are indistinguishable from each other in both the phenetic and molecular analyses as they overlap significantly in many morphological characters, habitats, and co-occur in most areas suggesting that they are either indistinct and should be synonymized, or possibly that they have hybridized in areas where they co-occur. A key with useful combinations of morphological characters separating the eight species in the *E. coccinea* complex is provided.

The molecular phylogenetic analyses revealed that *Emilia* is not monophyletic, and that *Bafutia* and *Emiliella* are nested within it, indicating that these genera do not warrant separate generic status and should be combined with *Emilia*. Jeffrey's sectional classification is not supported by the reconstructed phylogenies and there are no distinguishable morphological patterns evident amongst the clades to warrant the proposal of any meaningful sectional delimitation. *Emilia baumii* and *E. graminea* are grouped outside *Emilia* in both the nuclear and plastic-based molecular analyses and exclusion of these species from the genus is recommended, although additional molecular markers are needed to support this exclusion. Well-supported topological incongruences are revealed between nuclear ITS and plastid *trn*L-*trn*F phylogenies suggesting that hybridization and/or introgression have played a role in the history of *Emilia*, as with many other senecionoid genera.

Emilia, a mainly tropical genus, is hypothesised to have originated in southern Africa during the Mid-Miocene (*ca.* 14.19 Mya) coinciding with a period of global climate cooling

following the mid-Miocene Climatic Optimum (*ca.* 15 Mya). Early diversification occurred northwards into diverse habitats in Africa with further diversification in most *Emilia* clades occurring during the Late Miocene and occupying various habitats such as savannas, grasslands, and forest edges. At least five independent dispersals out of southern Africa to Madagascar, ascribed to long distance wind dispersal, occurred during the Pliocene. The successful diversification of *Emilia* in Africa could have been enhanced by its prevalent annual life form postulated to be either ancestral or evolved early (*ca.* 13.32 Mya) in its history. Narrow leaves, radiate capitula, and non-yellow florets have all arisen independently several times in *Emilia*.

Emilia species are unevenly distributed in southern Africa with the highest number of species occurring in Zambia (12 species), followed by Zimbabwe (10 species) and Malawi (seven species). Centres of greatest diversity for *Emilia* species are found in northern and southern Malawi (including the Nyika and Zomba plateaus respectively) and Zimbabwe (Eastern Highlands and areas surrounding Harare). Two recognized centres of endemism, which are also part of the Austro-temperate Region, viz. the Chimanimani-Nyanga Centre in the Eastern Highlands and the Nyika Plateau Centre, are amongst the centres with the highest diversity of species of *Emilia*. Only six *Emilia* species are endemic or near endemic to southern Africa, a low number compared to other senecionoid genera in the Savanna and Austro-temperate Floras. With the exception of endemism, three of the spatial biodiversity indices (SR, PD, and PE) investigated in this study were congruent thus providing additional conservation information. These three biodiversity indices overlap in some of the following areas: northern Malawi, Harare region and eastern highlands of Zimbabwe and therefore should be prioritized for biodiversity conservation: thus fulfilling one of the mandates of the Convention on Biological Diversity. Phylogenetic diversity and PE provides further conservation information in eastern and north-western Zambia that could have been missed by using SR alone. These phylogenetic indices (PD and PE) should therefore be prioritized in the conservation of *Emilia* species, and other taxa in similar floras, thus mitigating the problems of climate change as areas that have an evolutionary history and contain geographically restricted traits are conserved.

In an assessment of the conservation status of species of *Emilia* in Zimbabwe, three species (*E. limosa*, *E. protracta*, and *E. tenellula*) are rare and/or threatened and are habitat specialists e.g. in swampy areas thus warranting protection and one (*E. baumii*) is Data

Deficient and should be investigated further. The majority of *Emilia* species are categorised as Least Concern. The current protected areas in Zimbabwe cover most areas where 70% of *Emilia* species occur, including those areas with high PD and PE, the exception being the Harare region where populations are unprotected. Conservation efforts should therefore be extended to these unprotected areas. In addition to traditional conservation approaches, it is recommended that conservation prioritization of *Emilia* species in southern Africa and possibly the whole of Africa, as well as other genera with similar distribution patterns in Savanna and/or Austro-temperate Floras, should integrate SR, PD, and PE since phylogenetic indices (PD and PE) provide information on evolutionary history and spatially restricted diversity which are necessary for understanding and maximizing conservation of evolutionary diversity.

DEDICATION

To Fungayi my beloved husband and children
Vimbai Blessing, Rejoice Rufaro, and Pride Kudzai

To God be the Glory

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CHAPTER 1

General Introduction

Background

The genus *Emilia* Cass. belongs to the tribe Senecioneae in the Asteraceae (Compositae), the largest family of flowering plants, comprising about 1 600 genera and more than 23 000 species (Nordenstam 2007). The Senecioneae is one of the largest tribes in the Asteraceae with *ca.* 3 500 species and 151 genera (Nordenstam 1978; Nordenstam 2009). The members of this tribe can be recognised most readily by their involucres, which comprise a single row of equal involucral bracts with or without a calyculus of smaller bracts (Jeffrey 1986; Bremer 1994; Nordenstam 2007). Senecioneae is chemically distinct from other tribes of the Asteraceae (Mabry and Bohlmann 1977; Robins 1977). Some members of this tribe (more than 150 species) contain pyrrolizidine alkaloids (Langel *et al*. 2011), which have hepatotoxic activity in mammals (Culvenor *et al.* 1976; Mabry and Bohlmann 1977). The tribe Senecioneae has an almost world-wide distribution, and exhibits remarkable morphological and ecological diversity. The variable growth habit in the tribe includes trees, shrubs, herbs, vines and epiphytes.

The genus *Emilia* was founded by Cassini in 1817. The source of the generic name *Emilia* is not known and the eponym has been suggested to be *Emile* or *Emilie*, possibly in reference to the name of a friend or family member (Nicolson 1980). The common name of the genus is 'tassel flower' based on the tassel-shaped flower heads composed of many florets. These florets are usually small and often brightly coloured red, orange, pink, purple, yellow, or white.

There are 117 species in the genus *Emilia* (The Plant List 2013). The genus is widely distributed with most species occurring in Africa (*ca.* 80) (Jeffrey 1986; Lisowski 1990, 1991; Beentje *et al*. 2005; Klopper *et al*. 2006), 14 in Madagascar, of which 11 are endemic (Humbert 1963), and two weedy species *E. fosbergii* Nicolson and *E. sonchifolia* (L.) DC., that have spread to the neotropics (Barkley 2006) possibly due to introduction (Jeffrey 1986). There is remarkable ecological diversity in *Emilia* as it occupies varied habitats. These range from moist areas (e.g. swampy areas) to dry and rocky areas, grasslands, woodlands (e.g. Miombo woodlands), mountainous and disturbed areas.

Some *Emilia* species are economically and/or medicinally important, and a phylogenetic study could elucidate whether their properties (e.g. life form, chemical

constituents, etc.) are due to a shared evolutionary history or have arisen independently (i.e. in parallel). Examples of weedy species are *E. sagittata* DC., *E. praetermissa* Milne-Redh. and *E. prenanthoidea* DC. Edible species include *E. lisowskiana* C.Jeffrey, the leaves of which are occasionally eaten as a vegetable either fresh in salads or cooked in West Africa and the Democratic Republic of Congo (DRC). Species used for medicinal purposes include *E. coccinea* (Sims) G.Don, which is extensively used for treatment of fever and convulsions in children in South-Eastern Nigeria (Edeoga *et al*., 2005). *Emilia sonchifolia* is an antipyretic and remedy for influenza, cough, and bronchitis. *Emilia amplexicaulis* Baker, *E. citrina* DC., and *E. graminea* DC., are also used in traditional medicine in Madagascar to treat scabies and syphilis (Pernet and Meyer 1957). The species with medicinal value have also been shown to contain pyrrolizidine alkaloids (PAs), which cause toxic reactions in humans, mainly veno-occlusive liver disease (Bremer 1994; Roeder and Wiedenfeld 2011). Other brightly coloured *Emilia* species such as *E. coccinea* and *E. sonchifolia* are cultivated as ornamental plants (Tadesse and Beentje 2004).

Emilia is morphologically diverse. The plants are herbaceous, annual, biennial or perennial and can be succulent or non-succulent. The leaves are cauline or subscapose, alternate, with lower leaves petiolate and upper ones sessile (Fosberg 1972; Bremer 1994). These leaves do not have a strongly developed indumentum. There is also great variation in the leaves of the same specimen, for instance, the lower leaves of *E. praetermissa* are broadly ovate, subcordate, and petiolate whereas upper leaves are almost deltoid, auriculate-cordate and sessile (Chung *et al*. 2009).

Additionally, there is much variation in the floral features within *Emilia*. The capitula are solitary or few to several, corymbose, radiate or discoid, and ecalyculate (Tadesse and Beentje 2004; Nordenstam 2007). The genus has few (usually 8–10), linear-oblong, uniseriate, and connate phyllaries (Fosberg 1972; Nordenstam 2007). The corolla is tubular and five-lobed, and anthers are ecalcarate (Jeffrey 1986; Tadesse and Beentje 2004). The style branches may be truncate to obtuse, unappendaged or appendaged with fused papillae (Jeffrey 1986). The cypsela are oblong, ribbed with five angles, and glabrous or pubescent (Fosberg 1972; Bremer 1994). The pappus is uniseriate and composed of numerous fine white bristles (Jeffrey 1986; Bremer 1994).

When distinguishing *Emilia* species, both Jeffrey (1986) and Lisowski (1990) emphasized the importance of the details of the style branch apices, viz. whether appendaged or not. If the style branch is unappendaged, the lengths and types of the fringing papillae were

noted, and if appendaged, the length and shape of the appendage and of the fused papillae of which it is composed.

Taxonomic history of genus *Emilia*

In 1817, Cassini (Bull. Sci. Soc. Philom. Paris 1817: 63) first described the genus *Emilia* [with the type *Cacalia sagittata* (Willd.)] as differing substantially from *Cacalia* L., a genus described by Linnaeus (1753). He then describes *Emilia flammea* Cass. in 1819 (Dict.Sci. 14: 406) based on *Cacalia sagittata*, the type for *Emilia*. In 1825, Cassini added two species to *E. flammea*, viz. *E. adenogyna* Cass. and *E. purpurea* Cass. *Emilia* is occasionally cited as '(Cass.) Cass., Dict. Sci. Nat. 34: 393 (1825)' but with no basionym citation, possibly because Cassini (1817) suggests that *Emilia* might be a genus or subgenus, although he goes on to treat it as a genus. In 1838, Candolle (Prodr. 6: 301) enumerated 13 species and synonymized *E. flammea* and *E. purpurea* with *E. sagittata* DC. and *E. sonchifolia* DC., respectively, as the former were superfluous, illegitimate names. *Emilia* was treated as a subgenus of *Senecio* L. by Hoffmeyer in 1890 [*Senecio* L. subgen. *Emilia* (Cass.) O.Hoffm. Pflanzenfam. 4, 5(54): 297]. Twenty three *Emilia* species were identified by Garabedian (1924), who gave a brief history of the genus but considered the genus *Emilia* to be 'an association of allied species' rather than a 'distinct genus' (Garabedian, 1924: 137).

Many *Emilia* species continued to be classified in *Senecio* L. or were not recognized (Tadesse and Beentje 2004) until Jeffrey's (1986) taxonomic work. He revised the taxonomy of East Tropical African Senecioneae and made some necessary nomenclatural changes prior to an account of the tribe for the 'Flora of Tropical East Africa'. The tropical African genus *Emilia* was redefined based on a shared basic chromosome number, x=5, and an ecalyculate involucre. Jeffrey (1986) also transferred *Senecio* sections *Spathulati* Muschl. and *Emilioidei* Muschl. to *Emilia* and provided a key to *Emilia* species. *Emilia* sect. *Spathulatae* (Muschl.) C.Jeffrey was characterised by species with radiate or discoid capitula, yellow florets, short corolla-lobes (about as long as broad), and exappendiculate style branches, usually with a central tuft of longer papillae (Jeffrey 1986). *Emilia* sect. *Emilia* (Muschl.) C.Jeffrey was characterised by having discoid capitula, white, yellow, orange, red, pink, lilac, mauve, magenta or purple florets, long, narrow corolla-lobes, and exappendiculate style branches or (more usually) with a short to long median apical appendage of fused obtuse papillae (Jeffrey 1986). Fifty-eight species were identified for East Tropical Africa (Uganda, Kenya, and Tanzania) by Jeffrey (1986) and he created 20 new combinations for species previously classified with *Senecio*. Seven species [*E. helianthella* C.Jeffrey, *E. abyssinica* (Sch.Bip. ex

A.Rich.) C.Jeffrey, *E. discifolia* (Oliv.) C.Jeffrey, *E. somalensis* (S.Moore) C.Jeffrey, *E. ukambiensis* (O.Hoffm.) C.Jeffrey, *E. tricholepsis* C.Jeffrey, and *E. hockii* (DeWild. & Muschl.) C.Jeffrey] were placed in *Emilia* sect. *Spathulatae*, together with four new combinations: *E. baberka* (Hutch.) C.Jeffrey, *E. brachycephala* (R.E.Fr.) C.Jeffrey, *E. fallax* (Mattf.) C.Jeffrey, and *E. tessmannii* (Mattf.) C.Jeffrey. The rest of the species were placed in *Emilia* sect. *Emilia*.

Regional revisions for *Emilia* have been done for northern and central Africa. Lisowski (1990) revised the species in central Africa (Congo, Rwanda, and Burundi) and recognised 41 species, 17 of which were newly described. Jeffrey (1997) revised the *E. coccinea* complex, and indicated that the name *E. coccinea* should be correctly applied to an eastern and southern tropical African *Emilia* species with bright orange flowers and long-appendaged style branches. Tadesse and Beentje (2004) gave an account of the genus in North-East Africa (Sudan, Ethiopia, Eritrea, Djibouti, and Somalia) in which they recognised 14 species, and out of these, five species (*E. herbacea* Mesfin & Beentje, *E. adamagibaensis* Mesfin & Beentje, *E. negellensis* Mesfin & Beentje, *E. serpentina* Mesfin & Beentje, and *E. arvensis* Mesfin & Beentje) were newly described. Cron (2014) produced a synopsis of six *Emilia* species in southern Africa, having previously removed the single misplaced Northern Cape species *E. hantamensis* J.C.Manning & Goldblatt which was then described as a new monotypic genus, *Bertilia* Cron based on phylogenetic and morphological evidence (Cron 2013).

The ‘revisionary gap’ in the southern tropical region of Africa is currently being addressed using morphology for the account of *Emilia* for *Flora Zambesiaca* (N. Hind, personal communication). For this reason, this study does not undertake a revision of the genus, but lays the foundation for such an undertaking by assessing the monophyly of both the genus and its sectional delimitations.

Thus, in the current study, a phylogenetic study of the genus *Emilia* is undertaken based on DNA sequence data, taking care to sample across putative groups and geographic regions. Closely related genera in the Senecioneae are sampled and serve as outgroups. Morphological features are mapped onto the phylogeny to provide information on evolutionary trends in the genus, as well as synapomorphic (shared derived) features that unite groups of taxa. Sectional delimitations are assessed and the monophyly of the genus and the sections proposed by Jeffrey (1986) are also tested. This phylogeny therefore provides a basis for a good future revision of *Emilia*.

The *Emilia coccinea* (Sims) G.Don complex and the species concepts debate

Emilia coccinea has traditionally been used to incorporate all variable and widely distributed African *Emilia* species with large capitula into one broadly-delimited polymorphic species (e.g. Hutchinson and Dalziel 1931; Agnew 1974). In 1997, Jeffrey revised the *E. coccinea* complex and noted the proper use of the name *E. coccinea*, to a species from eastern and southern tropical Africa with flowers coloured bright orange to red and long-appendaged style branches. *Emilia lisowskiana* C.Jeffrey to which Lisowski (1990; 1991) had misapplied the name *E. coccinea* when he applied it to the west African (Guinean-Congo) species with more leafy stems, truncate style branches and orange-yellow flowers (Jeffrey 1997) was described as a new species. Six species (*E. emilioides* (Sch.Bip.) C.Jeffrey, *E. jeffreyana* Lisowski, *E. praetermissa* Milne-Redh., *E. subscaposa* Lisowski, *E. caespitosa* Oliv., and *E. vanmeeli* Lawalrée) previously identified from African herbarium material by various authorities as *E. coccinea sensu lato* were also distinguished (Jeffrey 1997). The definition/concept of a species is thus crucial in such taxonomic work to understand the proper application of names and evaluate how species concepts have been applied within the *E. coccinea* complex.

Systematists have debated species concepts for a very long time and the definition of what might be termed a 'species' has not been agreed upon, thus the species problem still persists (Cracraft 2000). Mayden (1997) identified at least 22 species concepts within literature, and Wilkins (2009) listed 26 species concepts. Recently Zachos (2016) provided an annotated list of 32 species concepts based mainly on Mayden's (1997) and Wilkins' (2009) lists. Some of the commonly known species concepts include (in alphabetical order): the Biological Species Concept (Mayr 1942), Evolutionary Species Concept (Simpson 1951, 1961; Wiley 1978), Morphological Species Concept (Cronquist 1978), Phenetic Species Concept (Gilmour 1961; McNeill 1979; Sneath and Sokal 1973), and Phylogenetic Species Concept (various versions).

Plant species recognition has long been based on morphological characters (Duminil and Di Michele 2009) and the Morphological Species Concept is still useful today in terms of the practical recognition of various taxa (Mayden 1997; Judd *et al*. 2008). In the Morphological Species Concept, Cronquist (1988: 71) defines species as 'the smallest groups that are consistently and persistently distinct, and distinguishable by ordinary means'. Although taxonomists might disagree on the terms used in this definition, e.g. how consistent is 'consistent', and how ordinary are 'ordinary means', Cronquist (1988) argued that any group that does not meet each of these tests, at least to some reasonable degree, should not be

considered as a species. The definition emphasises morphological characters as specific criteria for recognising species. Anatomical, micro- and macro-morphological characters, cell ultrastructure, habitats, and other features may all be taken into account in delimiting species (Cronquist 1988). This is the basis on which much revisionary taxonomic work is performed.

Although also based on morphological and anatomical features, phenetic systematists (Gilmour 1961; McNeill 1979; Sneath and Sokal 1973) emphasize the criterion of overall similarity to determine relationships. The Phenetic Species Concept aims to be objective and uses as many features as possible and not just a few features deemed to be 'diagnostic' in the Morphological Species Concept. Some exact degree of phenetic similarity is specified by the Phenetic Species Concept and this similarity is measured by a phenetic distance statistic (Ridley 2003).Under the Phenetic Species Concept, species are treated as classes and traits of organisms are important in defining these classes (Ghiselin, 1974). As large a number of attributes as is feasible is analysed using multivariate statistical techniques, such as cluster analysis and ordinations, resulting in clusters or groups that are then considered as taxa (Dunn and Everitt 1982; Alderson 1985).

In contrast, phylogenetic systematists, e.g. Hennig (1966), Eldredge and Cracraft (1980) and Wiley (1981), are concerned with erecting hypotheses about the pattern of life's history so as to discover the genealogical relationships among the taxa being studied (Hennig 1966; Philips 1984). With the advent of molecular tools such as molecular sequence data for phylogenetic analyses and computer algorithms to assist therewith, phylogenetic systematics is now commonly used to show the evolutionary relationships amongst taxa (Yang and Rannala 2012). The Phylogenetic Species Concept is thus applicable in many fields such as systematics, evolution, genetics, and can also be applied to asexual organisms.

Different versions of the Phylogenetic Species Concept (PSC) were put forward by Eldredge and Cracraft (1980), Nelson and Platnick (1981), and Cracraft (1983). All the versions contain similar components that were amplified further by Nixon and Wheeler (1990: 218), when they defined species as: 'the smallest aggregation of populations (sexual) or lineages (asexual) diagnosable by a unique combination of character states in comparable individuals (semaphoronts)'. This version of the Phylogenetic Species Concept, which is character-based, simple, and easy to understand, is also called the Diagnosability Species Concept (Eldredge and Cracraft 1980; Nelson and Platnick 1981; Cracraft 1983; Judd *et al*. 2008). An alternative version of the Phylogenetic Species Concept (Apomorphy Species Concept; Donoghue 1985; Mishler 1985; Mishler and Brandon 1987; De Queiroz and Donoghue 1988; Mishler and Theriot 2000) requires that a species contains all the

descendants of one ancestral population and the species are identifiable by autapomorphies (i.e. synapomorphic to the individuals belonging to that species). Species recognition is strictly based on monophyletic groups. Finally, the third version of PSC is the Genealogical Species Concept (GSC; Baum and Donoghue 1995). Species description in this historically based GSC is established on 'basal exclusivity' (Baum and Shaw 1995). Members of an exclusive group of organisms are more closely related to each other than to outgroups (De Queiroz and Donoghue 1990; Baum 1992; Baum and Shaw 1995). Baum and Donoghue (1995: 566) explained that '…relatedness is viewed in terms of the genealogical descent of the genome as a whole rather than being based on descent from an ancestral organism'.

A globally accepted definition of what constitutes a species and how species arise has not emerged (Cracraft 2000; Hey 2006; De Queiroz 2007), and Templeton (1992) was of the view that a species concept should be evaluated in terms of one's goal or purpose. This study focuses on the application of the Morphological and Phenetic Species Concepts to the *E. coccinea* complex and the Phylogenetic Species Concept(s) is also considered for the genus as a whole.

Overview of phylogenetic relationships in *Emilia* and related species/genera in the Senecioneae

Phylogenetic studies in the Senecioneae using DNA sequences (e.g. Bain and Golden 2000; Pelser *et al*. 2002, 2003, 2007; Cron *et al*. 2008; Wang *et al*. 2009; Pelser *et al*. 2010; Calvo *et al*. 2013; Cron 2013) have enabled the systematic placement and generic circumscription of many Senecioneae genera. Three *Emilia* species (*E. coccinea*, *E. exserta* Fosberg, and *E. prenanthoidea* DC.) were included in phylogenetic analyses of the Senecioneae using plastid and nuclear data (Pelser *et al*. 2007; 2010), however the relationships amongst the genera in the Senecioneae were not fully resolved. Conclusions on the phylogenetic placement of these three *Emilia* species in relation to other genera such as *Cineraria* and *Pericallis* D.Don could therefore not be made. Well-supported incongruence between plastid and nuclear phylogenies was shown by certain clades with regard to their phylogenetic positions relative to other lineages, which further complicated interpretation of relationships (Pelser *et al*. 2010). Five species of *Emilia* from southern Africa were also included in a recent investigation into the inclusion of the Northern Cape-based *E. hantamensis* in *Emilia* and the placement in the Senecioneae using both plastid and nuclear markers by Cron (2013). Incongruence was also observed in the placement of *E. transvaalensis* (Bolus) Jeffrey between the two phylogenies, with *E. transvaalensis* being placed outside of *Emilia* in the plastid-based phylogeny. This

brings into question the monophyly of *Emilia* as currently circumscribed. Adequate and intensive sampling of *Emilia* species is thus clearly needed in order to produce a robust molecular phylogeny that would provide the foundation for a good future revision.

There is a possibility that other genera might belong in *Emilia* hence the monophyly of *Emilia* is disputable. Jeffrey (1986) suggested that all the tropical African species of the emilioid complex (including *Xyridopsis* B.Nord., *Emiliella* S.Moore, and *Bafutia* C.D.Adams) could be included within *Emilia*. The emilioid complex is one of three complexes (emilioid, synotoid, and othonnoid) of the tribe Senecioneae proposed by Jeffrey (1986), which has a basic chromosome number x=5 and is defined by the ecalyculate involucre. The genus *Xyridopsis* (composed of two species *X. welwitschii* B.Nord. and *X. newtonii* (O.Hoffm.) B.Nord.) was sunk into *Emilia* by Jeffrey (1986), and later transferred to *Psednotrichia* Hiern. by Anderberg and Karis (1995). *Psednotrichia*, endemic to Huila Plateau (Angola), shares the following characters with other Senecioneae genera: scapose peduncles, resiniferous corolla, and mucilaginous cypsela hairs hence its removal from *Emilia*. Further study might however show that the remaining two genera of the emilioid complex, *Emiliella* S.Moore (with eight species distributed in Angola, Democratic Republic of Congo (DRC; Kinshasa), and Zambia; Mendonça 1943; Lisowski 1989, 1991; Torre 1972, 1975; Hind and Frisby 2014) and *Bafutia* C.D.Adams, comprising one species with joint phyllaries (*B. tenuicaulis* C.D.Adams, found in Cameroon), should be placed in *Emilia* (Adams 1962; Jeffrey 1986). Chromosome numbers in these genera are not known (Jeffrey 1986; Nordenstam 2007). *Emilia*, *Emiliella,* and *Bafutia* all have ecalyculate capitula and differences among these three genera were highlighted by Hind and Frisby (2014). Although Hind and Frisby (2014) described *Emilia* as quite similar vegetatively to *Emiliella* species, they considered *Emiliella* not to be part of *Emilia*. The differences between *Emilia* and *Emiliella* include their involucre (which splits between adjoining phyllaries when the cypsela matures in *Emiliella*), pappus form (i.e. single scale pappus in *Emiliella* when present compared to the numerous and persistent pappus bristles in *Emilia*), and the cypsela, which is longer than the pappus in *Emiliella*. Jeffrey (1992) and Nordenstam (2006) supported recognition of *Bafutia* as a separate genus and it was placed in the Senecioneae subtribe Othonninae by Nordenstam *et al*. (2009).

Emilia has also been proposed to be related to genera *Gynura* Cass. and *Senecio* (Garabedian 1924), from which it is distinguished by very minor/few characters. Characters suggesting that *Emilia* is close to *Gynura* and *Senecio* include (respectively) the style-branches with subulate appendages (Garabedian 1924) and the disc corolla-lobes that are

equal in length and breadth (Jeffrey 1986). *Emilia* is distinguished from *Gynura* by the absence of a calyculus. However, this feature links *Emilia* with a few species of *Senecio*. In a recent molecular phylogeny based on plastid data (Pelser *et al*. 2010), Gynuroids were nested among core members of clade 1, whereas *Emilia* was a core member of clade 2 (Figure 1; Pelser *et al*. 2010). Although both Gynuroids and *Emilia* were core members of clade 2 in the ITS/ETS trees (Figure 2; Pelser *et al*. 2010), they are not sister taxa in this clade. Intensive sampling of *Emilia* species and related genera in a molecular phylogeny is needed to draw conclusions on the relationships of *Emilia*, *Senecio* and the Gynuroids.

Molecular evidence

The development of polymerase chain reaction (PCR) technology, has led to its wide use in DNA sequencing, which in turn has provided a major source of molecular data (Olmstead and Palmer 1994). DNA sequence data have been useful in providing an understanding into phylogenetic relationships of plants (e.g. Brown 2006; Hilu *et al*. 2008; Wang *et al*. 2014; Uncu *et al*. 2015). However this depends to some extent on identifying phylogenetically useful gene regions easy to amplify amongst various taxa (Hilu *et al*. 2008). In the current study, the phylogeny of the genus *Emilia* is inferred from chloroplast and nuclear DNA sequence data.

The use of several markers to resolve evolutionary relationships of groups greatly advanced the knowledge of plant systematics by shedding light on relationships that were problematic in previous studies that used only a few markers and limited data (Pelser *et al.* 2010). Also, congruence and incongruence between reconstructed phylogenies resulting from the analysis of different genes and gene regions have also been revealed by studies using multiple genes, thus enabling an understanding of macroevolution (Degnan and Rosenberg 2009). In this study, the nuclear internal transcribed spacer (ITS) regions of the 18S-5.8S-26S nuclear ribosomal DNA (Figure 1.1), the external transcribed spacer (ETS; preliminary assessment; Figure 1.1) and the more rapidly evolving and a frequently used plastid DNA markers in plants, *trn*L-*trn*F intron and spacer region (Figure 1.2; Hao *et al*. 2009), were explored to infer relationships in genus *Emilia*. Other advantages of plastid DNA are that it is abundant as thousands of plastid chromosomes are found in a typical plant leaf and most of the genes are single-copy in nature and thus free from problems associated with paralogy (Palmer 1987).

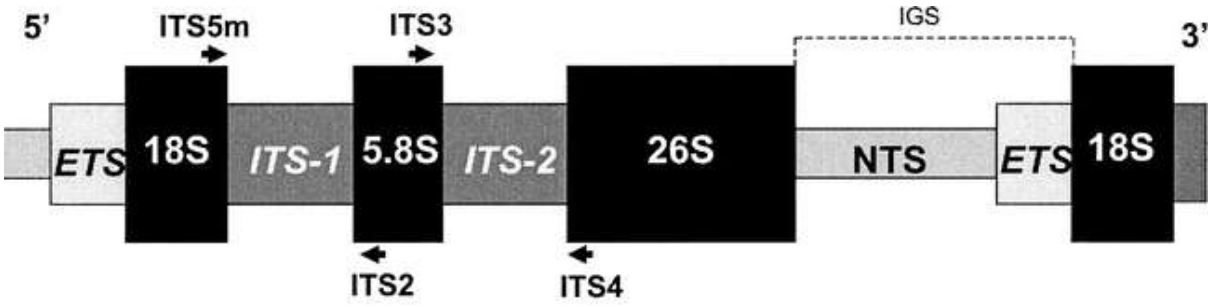

Figure 1.1. Nuclear DNA regions used in the phylogenetic reconstruction of *Emilia* species: Internal and external transcribed spacer regions: ITS 1, ITS 2, and ETS (Diagram source: Saar *et al.* 2003 p. 629). 18S, 5.8S, and 26S are genes for ribosomal subunits. NTS is the non-transcribed spacer, and IGS is the intergenic spacer. Arrows show the general location of primers and ITS 4 and ITS 5 were utilised in PCR amplification in this study.

Figure 1.2. Plastid DNA marker used here in the phylogenetic reconstruction of *Emilia* species: *trn*L-*trn*F intron and spacer region. Arrows show the general location of primers 'c', 'd', 'e', and 'f' utilised in PCR amplification in this study. (Diagram source: Lee *et al*. 2009 p. 1913).

Previous researchers have noted that nuclear regions usually provide more information at the species level than plastid ones. As a result, the ITS region has been extensively used to provide taxonomic characters in phylogenetic studies of closely related genera (Baldwin *et al*. 1995; Baldwin and Markos 1998; Soltis and Soltis 1998). The wide use of the ITS region in phylogenetic studies has been attributed to some of the following: (i) ITS sequences are biparentally inherited and this is valuable in revealing hybrid speciation, parentage of polyploids, and past cases of reticulation (Baldwin *et al*. 1995) (ii) it is rapidly evolving and the fairly high rate of nucleotide substitution in the transcribed spacers allows the ITS region to be used to differentiate newly diverged taxa, and (iii) it is easily sequenced with suitable primers using PCR technology, that is, universality (Liston *et al*. 1996; Álvarez and Wendel 2003). Nonetheless, there are some potential problems associated with using nuclear ITS region for phylogenetic analyses and these include history of gene duplication, leading to the duplicated sequences within and between lineages being paralogous (Álvarez and Wendel 2003). Also, homoplasy might be higher in ITS as compared to other DNA sequence data sets because of orthology/paralogy conflation, sequencing errors,

compensation base changes, alignment problems resulting from indel accumulation and/or some combination of these events (Álvarez and Wendel 2003).

The molecular markers used in the current study were selected on the basis of their merit in resolving phylogenetic relationships in studies of the senecionoid genera *Cineraria* L. and *Senecio* sect. *Crociseris* (Rchb.) Boiss., the othonnoid genus *Euryops* (Cass.) Cass., and family Polygonaceae (*Persicaria* (L.) Mill.) (Cron *et al*. 2008; Kim and Donoghue 2008; Devos *et al.* 2010; Calvo *et al.* 2013), and were also used in a preliminary study that included five southern African species of *Emilia* (Cron 2013). However, the use of both plastid and nuclear regions could reveal topological incongruence which might be a result of: (i) biological factors e.g. lineage sorting, hybridization and introgression, horizontal gene transfer (Zou and Ge 2008; Cron *et al*. 2008; Pelser *et al*. 2010; Cron 2013), (ii) sampling errors due to high numbers of uninformative characters (Wendel and Doyle 1998), and (iii) systematic errors that might result in long branch attraction (Bergsten 2005). Incongruent plastid and nuclear data sets therefore cannot be combined as the resultant phylogenetic tree might fail to track or might oversimplify the evolutionary history (Wiens 1998). Nonetheless combining congruent data sets has been shown to have the following advantages: (i) improved resolution, (ii) improved internal support for clades, and (iii) distinctively supported clades in comparison to separate data sets (Olmstead and Sweere 1994). A phylogenetic study on *Cineraria* has however shown that although resolution can be improved by combining two data sets, of the resultant homoplasy thus created means that the results are not very meaningful (Cron *et al*. 2008).

Biogeography and evolutionary trends in *Emilia*

Biogeography is defined as the study of biological life in a spatial and temporal context and is concerned with the analysis and explanations of patterns of distribution (Cox and Moore 1993). The basic elements in biogeography are areas of distribution and of endemism (Nihei 2008). Every species has its own geographic distribution and ecological limits (Cronquist 1988). When defining a taxon, the distribution pattern is one of the diagnostic characters (Van Wyk and Smith 2001).

The possible evolutionary history of a taxon and its habitat are important when interpreting biogeography patterns. The evolutionary processes over millions of years are dealt with in historical biogeography, which is deduced from phylogenetic relationships, current and/or past distributions (Crisci 2001). Deducing the biogeographic histories of plant lineages is important in comprehending the origin and evolution of current distribution of

biodiversity (Xiang and Thomas 2008). Although most *Emilia* species occur in Africa and a few in Madagascar, the origins and divergence times of these species are not known. The biogeographic history of the genus *Emilia* is therefore here hypothesized based on a dated molecular phylogeny and current distribution patterns. The origin of the genus and processes that might have contributed to the evolution of *Emilia* are also investigated. Molecular dating studies using fossil evidence have been utilised in family Asteraceae and indicate that the family originated in South America approximately 76–66 million years ago (Barreda *et al.* 2015), diversified early and occurs all over the world except Antarctica (Panero and Funk 2008; Barreda *et al.* 2015). For many millions of years, the Asteraceae have been dominant in many biomes world-wide, particularly the open habitat ecosystems (Raven and Axelrod 1974; Barreda *et al.* 2015), and during the Oligocene the family radiated and became an important component of many southern African biomes such as the savanna and afromontane biomes (Cowling 1983; Burgoyne *et al*. 2005).

The diversification of several genera and tribes in the Asteraceae has been studied (e.g. Bergh and Linder 2009; Devos *et al*. 2010; Pelser *et al*. 2010), although morphological character optimisations have mostly been done at the family level in Asteraceae (Panero *et al.* 2014). The reconstructed phylogenetic tree is used here to hypothesise the evolutionary history of some key morphological characters in *Emilia,* especially tracing those character changes that may have influenced diversification of *Emilia*.

There are various analytical methods of reconstructing ancestral state of characters and ancestral areas of distribution. Three common methods [parsimony, maximum likelihood (ML), and stochastic character mapping (SCM)] using MESQUITE are used to reconstruct ancestral character state. The parsimony method, was the most useful approach for tracking evolutionary history of morphological characters until the development of alternative methods (ML and SCM) that are more robust and overcome drawbacks of parsimony methods (e.g. phylogenetic uncertainty in ancestral states is not accommodated). Maximum likelihood and stochastic character mapping in MESQUITE 2.01 (Maddison and Maddison 2007) use stochastic models of character state change, thus accommodating phylogenetic uncertainty in ancestral states ‘by evaluating the ancestral character state on trees sampled from the posterior distribution’ (Xiang and Thomas 2008 p. 350). The ML method was therefore used in this study to trace evolutionary trends in *Emilia*.

For biogeographic analysis it is known that ‘geographic range ‘evolves’ differently than morphological and other characters, because species can colonise new areas while remaining in the old one, vicariance can split areas, and speciation can change geographic

ranges too'(N. Bergh, pers. comm.). In addition to ancestral state packages, specialist programs such as dispersal-vicariance analysis (DIVA), which is parsimony-based and LAGRANGE (Ree and Smith 2008), a maximum likelihood approach, were designed to model the geographic range. LAGRANGE is quite complex to implement and interpret the results, and recent studies still use DIVA and ancestral state packages (e.g. Xiang *et al*. 2006).

Conservation prioritization: a debate

Biodiversity conservation is restricted by limited resources, thus conservation investments should be prioritized (Wilson *et al*. 2009; Daru *et al*. 2015). There have been debates on conservation prioritization, i.e. whether to conserve species or an area (i.e. region or habitat), especially in the context of minimal conservation resources (Vane-Wright *et al*. 1991; Faith 1992; Linder 1995; Myers *et al*. 2000; Faith *et al*. 2004; Brooks *et al*. 2006; Mishler *et al*. 2014; Daru *et al*. 2015). Traditionally, areas with high species richness, high endemism, low species abundances, and those with species that are rare and threatened with extinction were prioritized in conservation (Dinerstein *et al*. 1995; Myers *et al*. 2000). Recently phylogenetic approaches using phylogenetic indices such as phylogenetic diversity (PD; Faith 1992) and phylogenetic endemism (PE; Rosauer *et al*. 2009) that take into account evolutionary components (Vane-Wright *et al*. 1991; Faith 1992) have been used together with traditional approaches to prioritize conservation areas and species. Phylogenetic diversity 'can be calculated for any subset of taxa on any cladogram, where the estimates of relative branch lengths are available' (Faith 1992 p. 4). Phylogenetic endemism on the other hand combines the geographic distribution of species and their phylogenetic diversity measure. Geographic regions with a high degree of unique evolutionary history are thus identified (Mooers and Redding 2009; Rosauer *et al*. 2009). The phylogenetic endemism index therefore prioritizes both species and area in conservation.

In the current study, the distribution of *Emilia* species in southern Africa is mapped and centres of diversity and endemism are identified. Various ways of conservation prioritization of *Emilia* species and their associated habitats are explored for a limited region of Africa (viz. Zimbabwe). Phylogenies based on nuclear DNA sequence data are used to enable PD and PE assessments. Additionally areas shown to be important for conservation of *Emilia* using the various biodiversity and phylogenetic indices are compared with the present identified conservation areas in Zimbabwe. In addition, the IUCN threat status of *Emilia* species sampled from Zimbabwe; (a country known to the author) are here evaluated. Red

Data listings are used to understand patterns and threats to biodiversity (Vié *et al*. 2009) and highlight species in need of conservation attention (Rodrigues *et al*. 2006). Conservation status (according to the IUCN 2001 criteria) assessments of 12 *Emilia* species in southern tropical Africa have been done to date (Tadesse and Beentje 2004; Raimondo *et al*. 2009; Cron 2014).

Problem statement

Phylogenetic relationships and the biogeography of the genus *Emilia* were not known despite its taxonomic history dating back as early as 1817. Jeffrey (1986) revised the taxonomy of east tropical African Senecioneae and regional revisions of *Emilia* for northern and central Africa have also been undertaken in recent years (Lisowski 1990; Tadesse and Beentje 2004). Nonetheless, no phylogenetic or phylogeographic, and biogeographic studies of *Emilia* have been done to date. Although *Emilia* was included in recent molecular phylogenies (e.g. Pelser *et al*. 2007, 2010; Cron 2013), it was not widely sampled, thus making it difficult to draw conclusions about phylogenetic relationships. It has been suggested that *Emilia* may be polyphyletic (Nordenstam 1978) and Jeffrey (1986) noted that *Emiliella* and *Bafutia* may be part of *Emilia* and match this genus in having ecalyculate capitula, unusual in the subtribe Senecioninae (Bremer 1994). However, Hind and Frisby (2014) are of the opinion that *Emiliella* is not part of *Emilia* citing differences in their phyllaries, pappus form, and cypsela length relative to the pappus. A robust, well-sampled molecular phylogeny of *Emilia* species is clearly needed to provide the foundation for a good revision and to address the questions around its generic circumscription, including the status of *Emiliella* and *Bafutia*. This phylogeny is also used to evaluate Jeffrey's (1986) sectional classification of *Emilia* and also propose, if justified, sectional delimitations. Hybridisation is known to occur in certain genera of Senecioneae (Pelser *et al*. 2010) but has not been investigated in *Emilia*, thus comparison of phylogenies based on both nuclear and plastid markers should be done to investigate whether hybridisation has played a role in the genus *Emilia*, that is, if phylogenies are congruent or incongruent. Issues such as species concepts are currently being debated in systematics but have never been applied to *Emilia* and thus their significance to this genus is not known. The large-headed and widely variable *Emilia coccinea* species complex is thus assessed according to the phylogenetic and/or phenetic species concepts. The lack of a reconstructed molecular phylogeny in *Emilia* meant that the biogeographic history of the genus could not be ascertained / hypothesised. A reconstructed molecular phylogeny of *Emilia* species is thus needed in order to date the divergence of species clades in *Emilia* and

hypothesise its biogeographic history. Patterns in character evolution are identified by optimising key morphological characters on the resultant phylogeny and linking them to the pattern and timing of diversification in *Emilia*. One of the three major aims of the Convention on Biological Diversity (CBD) is biodiversity conservation, a responsibility of humanity. There have been declines in plant biodiversity and rapid habitat loss locally, regionally, and globally (Shackleton 2000) and this is a concern to most countries as they are signatories to the CBD. One of the greatest challenges in conservation biology has been highlighted as, the provision of ways of prioritising effort to conservation planners (Forest *et al*. 2007). Debates on 'what to conserve' and how conservation efforts should be prioritized for regions and genera, including *Emilia*, in southern Africa have not been addressed. Apart from the traditional approaches to conservation, spatial biodiversity patterns crucial for conservation planning are not understood. Also phylodiversity measures (viz. PD and PE) are now prioritised in conservation in the light of global climate change (Mace *et al*. 2003; Forest *et al*. 2007). According to the IUCN 2009 criteria, the current conservation status assessments of only ten out of 24 *Emilia* species in southern Africa are known and have been evaluated. This study seeks to contribute to some of these debates, propose the IUCN threat categories of *Emilia* species from a selected region in Africa (viz. Zimbabwe) thereby contributing to the mandate for the assessment of all plant species under the Convention on Biological Diversity 'Global Strategy for Plant Conservation' (Secretariat of the Convention on Biological Diversity 2002). The results obtained from conservation evaluations and assessments of conservation prioritizations will also be applied to other groups of plants and thus inform/promote biodiversity conservation policies in Zimbabwe and other regions.

Aims of the study

In this study I therefore aim to (i) evaluate the species recognised by Jeffrey (1997) in the *Emilia coccinea* complex using a phenetic approach, assessing the applicability of the morphological and phenetic species concepts to the members of the *E. coccinea* complex. I also aim to (ii) to investigate the phylogenetic relationships of a representative sample of *Emilia* species, together with the genera *Emiliella, Bafutia,* and other closely related genera in the Senecioneae, using nuclear and plastid DNA sequence data. The resultant phylogeny serves to indicate whether or not *Emilia* is monophyletic, and assesses the generic status of *Emiliella* and *Bafutia*. It also provides a sound basis for future taxonomic revisions of the genus, including an assessment of Jeffrey's sectional classification of *Emilia.* Possible roles played by past hybridization, introgression, and/or incomplete lineage sorting in the

evolutionary history of *Emilia* are also investigated here by examining the anticipated incongruence between nuclear and chloroplast DNA phylogenies.

A third aim is (iii) to investigate the pattern and timing of diversification in *Emilia* using current geographic distributions of species and a dated molecular phylogenetic hypotheses, and to correlate this pattern with evolutionary (morphological) trends in the genus. Data from the fossil record and secondary calibrations are used to infer the diversification of *Emilia* across Africa and Madagascar. Finally, I aimed (iv) to identify areas of high species richness (centres of diversity) and areas of endemism for *Emilia* in southern Africa, and to contribute to current debates on conservation prioritization as they apply to *Emilia* for a selected region (viz. Zimbabwe) by comparing and evaluating various biodiversity indices — species richness (SR), phylogenetic diversity (PD), species endemism (CWE) and phylogenetic endemism (PE). In addition areas indicated as important for conservation of *Emilia* using these four indices are compared with currently designated conservation areas in Zimbabwe. Levels of threat as determined using the IUCN assessment categories for the relevant species are also used in assessing the current conservation capacity for conserving *Emilia*.

Outline of thesis

The thesis comprises six chapters: an introductory chapter (1), which is the current chapter; central chapters 2, 3, 4, and 5, and a concluding chapter (6). The central chapters are written as scientific papers, with one chapter published (chapter 2), and chapters 3, 4, 5 to be submitted to peer reviewed scientific journals in due course. The aims outlined above are each addressed in one of the four central chapters:

Chapter 2: A phenetic study of the *Emilia coccinea* complex (Asteraceae, Senecioneae) in Africa is published in *Plant Systematics and Evolution* 2016, volume 302(6), pages 703–720. In this chapter, a phenetic approach using multivariate analyses is used to evaluate eight species in the *E. coccinea* complex recognised by Jeffrey (1997).

Chapter 3: Molecular phylogenetic study of genus *Emilia* Cass. (Senecioneae, Asteraceae).

Chapter 4: Evolutionary patterns and biogeographic history of *Emilia* (Senecioneae, Asteraceae).

Chapter 5: Untangling conservation prioritization in the ‘tassel flower’: exploring biodiversity and phylogenetic indices.

The concluding chapter 6 discusses how each of the aims, objectives and key questions proposed in central chapters 2, 3, 4, and 5 have been addressed. Additionally, conclusions for the whole thesis are provided and directions for future studies are also indicated.

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CHAPTER 2

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ORIGINAL ARTICLE

A phenetic study of the *Emilia coccinea* complex (Asteraceae, Senecioneae) in Africa

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Abstract *Emilia coccinea* complex is a widespread and morphologically variable species in tropical and subtropical Africa. Jeffrey's (Kew Bull 52:205–212, 1997) revision of the African *Emilia* species with large capitula resulted in a complex of eight species with *E. coccinea* sensu stricto restricted to eastern and southern tropical Africa and characterised by long-appendaged style branches and bright orange flowers. To evaluate the delimitations within this complex, a morphological phenetic study based on 134 herbarium specimens spanning the geographical range of the *E. coccinea* complex was undertaken using cluster analysis and ordination (principal coordinates analysis and non-metric multidimensional scaling). Five of the eight species (*E. emilioides*, *E. jeffreyana*, *E. praetermissa*, *E. subscaposa*, and *E. vanmeelii*) formed distinct phenetic groups, whereas two species (*E. caespitosa* and *E. coccinea*) were indistinguishable because of variability in some key characters (viz., cypsela indumentum and shape of cauline leaves) suggesting that they are possibly one heterogeneous species. *Emilia lisowskiana* is not supported as a distinct species as three *E. coccinea* specimens group with it in the cluster analysis. *Emilia emilioides* with mostly long, narrow cauline leaves, narrow capitula, and unappendaged style branches apices is the most distinct taxon in all analyses. Univariate analyses of ten selected characters revealed that the reproductive features are able to distinguish some species, as well as a few vegetative ones. The application of various species concepts to this species complex is discussed. A key to the species in this complex is provided.

Keywords Cluster analysis · Ordination · Phenetics · Species concepts · Univariate analysis

Introduction

Emilia (Cass.) Cass. belongs to the tribe Senecioneae (Asteraceae) and is an economically important genus, with some species used for medicinal purposes, e.g., *E. coccinea* (Sims) G.Don (Edeoga et al. 2005), and others as vegetables, e.g., *E. lisowskiana* C.Jeffrey. The genus is indigenous to Africa (south of the Sahara), and Asia (e.g., South China and Philippines; Fosberg 1972). The plants are herbaceous, annual, biennial, or perennial, and can be distinguished mostly by the characteristics of their capitula, which are solitary to many, corymbose, discoid, or rarely radiate, with a single row of phyllaries and brightly coloured florets (Jeffrey 1986; Tadesse and Beentje 2004). The genus comprises about 117 accepted species (The Plant List 2013), and one of these species, *Emilia coccinea* sensu lato (s.l.), was considered to be widespread and morphologically variable, occurring in tropical and subtropical Africa (Jeffrey 1997; Fig. 1). Thus, most of the *Emilia* species from Africa with large capitula were previously placed in this one widely circumscribed species until Jeffrey's (1997) revision of the complex.

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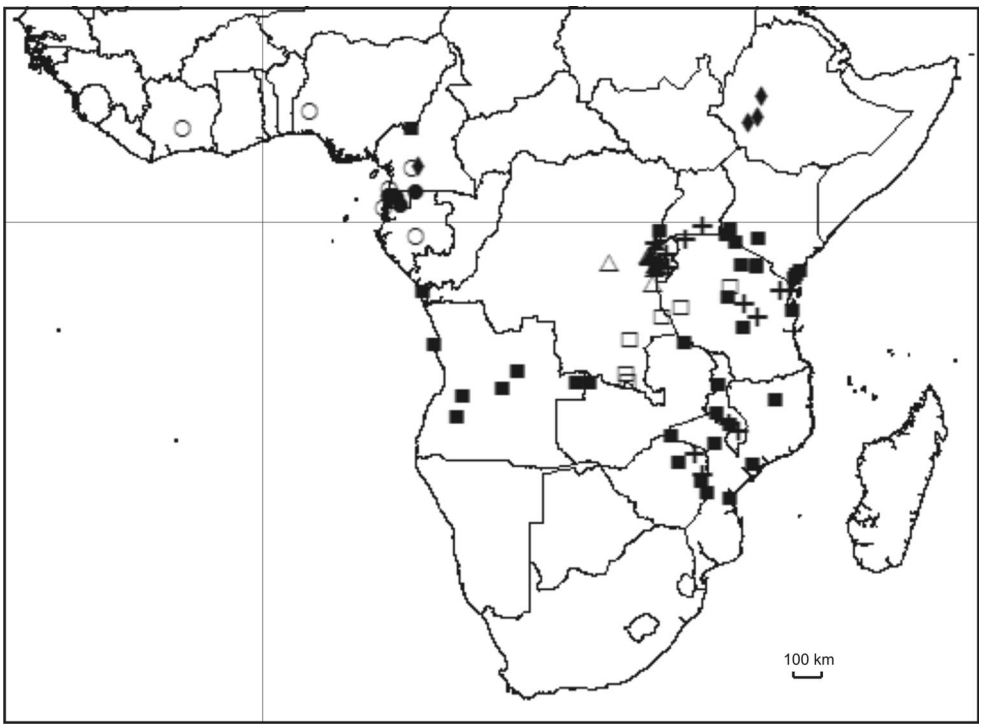

Fig. 1 Distribution of specimens of the eight species in the *E. coccinea* complex (locality information extracted from herbarium specimens) that were used in this phenetic study. *Emilia caespitosa* (*plus*), *E. coccinea* (*filled square*), *E. emilioides* (*filled diamond*), *E. jeffreyana* (*upright triangle*), *E. lisowskiana* (*filled circle*), *E. praetermissa* (*circle*), *E. subscaposa* (*filled upright triangle*), and *E. vanmeelii* (*square*)

Taxonomic History

Emilia coccinea (Sims) G. Don sensu stricto (s.s.) was first described as *Cacalia coccinea* Sims in Curtis's Botanical Magazine 16 t. 564 (1803) from a plant cultivated at Vauxhall, London (Jeffrey 1986, 1997; Fig. 2); however, no specimen was preserved. In 1839, the name *Cacalia coccinea* was changed to *Emilia coccinea* s.s. (Don 1839). The absence of physical type material of *E. coccinea* s.s. meant that the micromophorphological details, such as whether the style apices, are appendaged or not, important when distinguishing species in the genus *Emilia* (Jeffrey 1986; Lisowski 1990), could not be examined and were not included in the original description. This led to a lack of clarity when assigning specimens to this apparently widespread and variable species.

In 1986, Jeffrey distinguished specimens of *E. coccinea* based on their geographical variation and informally recognised two groups, one from West Africa with stems leafy throughout, long and narrow capitula, and yellow to orange florets; and the other from eastern and southern Africa (including Sudan) with short and broad capitula, and orange, bright red, or crimson florets (Jeffrey 1986). The eastern and southern African group was further divided into seven geographical groups (A–G; Jeffrey 1986), based on marginal differences in leaf shape, size of capitula, and floret colour. However, these groups proposed by Jeffrey (1986) were neither geographically nor morphologically distinct, for example, *E. coccinea* from Mozambique appeared in groups A, D, and E; and floret colour in group A was bright-orange to bright-red, group B was orange to bright orange, and group G was bright red. Jeffrey (1986) also suggested that further work might reveal that two species, the widespread *E. caespitosa* Oliv. and *E. emilioides* (Sch.) C.Jeffrey from Cameroon, Central African Republic, Ethiopia, and Sudan usually with white to creamy florets, should be treated as different forms of *E. coccinea*.

Lisowski (1990, 1991) revised *Emilia* in Central Africa [Burundi, Congo, Rwanda, and Democratic Republic of Congo (DRC)] and recognised 41 species within the region. However, he misapplied the name '*Emilia coccinea*' (now *E. lisowskiana*; Jeffrey 1997) exclusively to specimens from West Africa (Guinea and Congo) with truncate and unappendaged style branch apices, with or without a few short hairs (Fig. 3a). *Emilia caespitosa*, which had also been included in Jeffrey's (1986) key, and five other species (*E. emiliodes*, *E. jeffreyana* Lisowski, *E. praetermissa* Milne-Redh., *E. subscaposa* Lisowski, and *E. vanmeelii* Lawalreé) belonging to the *Emilia coccinea* complex were included in his key (Lisowski 1991). The style branches of *E. caespitosa* (Fig. 3d), *E. vanmeelii* (Fig. 3e), and *E. subscaposa* (Fig. 3f) were noted to be awl-shaped, strongly appendaged and differing in the length of the appendages as well as the presence/absence of fused sweeping hairs (papillae; Lisowski 1990), whereas those of *E. praetermissa* are truncate, unappendaged, and epapillose (Fig. 3b), and *E. jeffreyana* has shortly conical

Fig. 2 Lectotype of *Cacalia coccinea* selected by Jeffrey (1997); Source: plate t. 564 in Curtis Botanical Magazine 16 (1803)

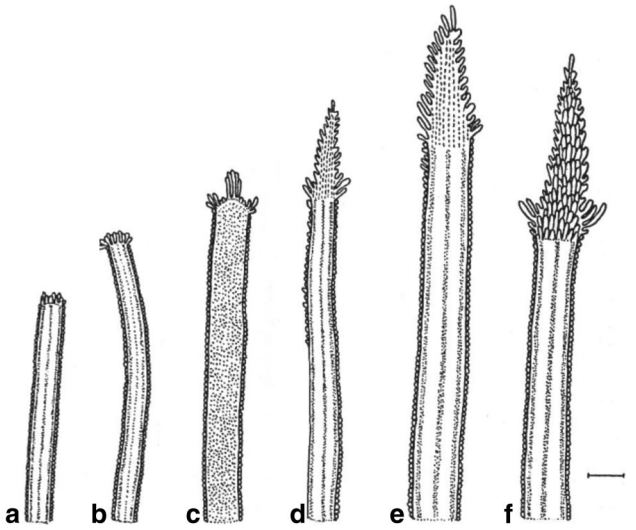

Fig. 3 Style branch apices of six species in the genus *Emilia*. **a** *E. lisowskiana* (as per Jeffrey 1997, misapplied to *E. coccinea* by Lisowski 1990). **b** *E. praetermissa*. **c** *E. jeffreyana*. **d** *E. caespitosa*. **e** *E. vanmeelii*. **f** *E. subscaposa*. *Scale bar* 0.12 mm. (Illustrations modified from Lisowski 1990)

style branch apices with a central tuft of long papillae (Fig. 3c; Lisowski 1990). The geographic ranges of *E. jeffreyana* and *E. subscaposa* include the DRC, Rwanda, and Burundi (Fig. 1), although *E. jeffreyana* is known to also extend into Kenya and Uganda (Lisowski 1991). *Emilia praetermissa* occurs in the DRC as well as in Côte d'Ivoire, Nigeria, Gabon, Cameroon, and Congo. *Emilia caespitosa* has a broad distribution in East and southeastern Africa, whereas *E. vanmeelii* has a relatively narrow geographic range—known only to occur in the DRC, Tanzania, and Zambia (Fig. 1).

Since no type material of *Cacalia coccinea* was known to exist, Jeffrey (1997) selected the plate t. 564 in Curtis's Botanical Magazine 16 (1803) as the lectotype to establish the correct usage of the name *E. coccinea* s.s. (Fig. 2). The species were described as having a subscapose habit with short and broad phyllaries as well as scarlet flowers (Don 1839). The details of the style branch apices are not evident in the plate, nor whether the cypselas are glabrous or pubescent (Jeffrey 1997; RJ Mapaya personal observation; Fig. 2). The information that the plants were raised from seeds supplied by M. Thouin, national gardener at Paris (Sims 1803) enabled Jeffrey (1997) to trace similar specimens from 1800 at Paris (P), Berlin (B), and Uppsala (UPS). The cypsela indumentum and style branch apices could thus be investigated and Jeffrey (1997) concluded that *E. coccinea* s.s. had strongly appendaged style branch apices and cypselas pubescent throughout their length as well as stems that are leafy in the lower part.

Jeffrey (1997) restricted the application of the name *E. coccinea* s.s. to a species from eastern and southern tropical Africa with bright-orange or red florets and long-appendaged style branches (Fig. 4c). He described a new species, *E. lisowskiana* characterised by truncate style branches and orange-yellow florets (Fig. 3a), based on specimens from West Africa (Guinea and Congo) to which Lisowski (1990, 1991) had misapplied the name *E. coccinea* s.s.complex have cauline leaves that are

The species in the *E. coccinea* complex are thus herbaceous and annual, with much variation in floral features (Fig. 4a–d), although most have relatively large capitula (for the genus; 5–8 mm in diameter) with red, orange, or yellow florets. *E. emilioides* and *E. praetermissa* are the exceptions with white, pale-yellow, or pale-mauve florets, and *E. emilioides* also has smaller capitula. Morphological characters highlighted in Jeffrey's (1997) key, important when distinguishing species in this complex, include: leaf shape, ratio of leaf length to breadth, floret colour, details of the style branch apices (Fig. 4c), and glabrous versus pubescent cypselas (Fig. 4d). Most species

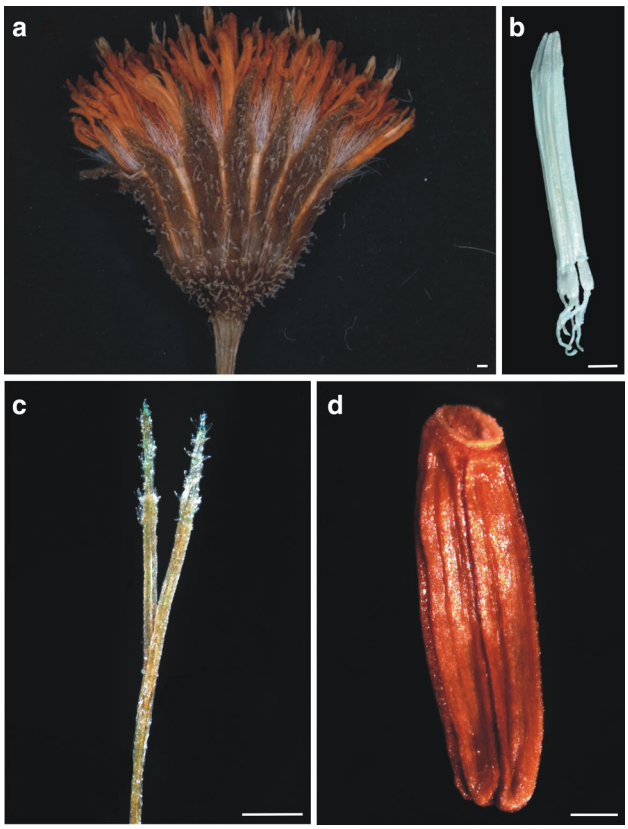

Fig. 4 Some of the morphological features used to distinguish species in the *Emilia coccinea* complex. **a** Pubescent involucral bracts on a capitulum of *E. coccinea* sensu stricto (Lovett 4106, MA). **b** Stamens of *E. coccinea* (*Kerfoot 5039*, MA) with balusterform filament collars and anther appendages. **c** Appendaged style branch apices of *E. coccinea* (Lovett and Kayombo 26, MA). **d** Glabrous cypsela of *E. caespitosa* (Festo and Bayona 1411, MA). *Scale bar* 3 mm. Photographs: **a**–**d**; R. Mapaya

in the *E. coccinea* complex have cauline leaves that are variable in terms of size and shape along the stem on the same specimen, which limits their taxonomic usefulness, and one (*E. subscaposa*) has leaves in a basal rosette. However, no suite of unique taxonomic characters that cannot be found in the rest of the species of this large genus defines the *E. coccinea* complex. Table 1 summarises the generally agreed morphological characters used by various authorities (Lisowski 1990, 1991; Jeffrey 1997; Tadesse and Beentje 2004) in distinguishing species of the *E. coccinea* complex.

Jeffrey (1997) appears to have applied the morphological species concept when delimiting the species in the widespread *E. coccinea* complex in East and Central tropical Africa, an approach used to practically recognise plant species based on morphological characters and still useful today (Mayden 1997; Judd et al. 2008; Duminil and Di Michele 2009). Although the phylogenetic species concept is the generally accepted approach nowadays to delimit species, it is not always practical/easy to apply to herbarium specimens (Hennig 1966; Eldredge and Cracraft 1980; Wiley 1981; Philips 1984).

A phenetic approach whereby the degree of similarity of members of a species is used to assess their relationships (Sokal and Crovello 1970; Ghiselin 1974; Sneath 1976; Jensen 2009) is, therefore, used here, using multivariate statistical techniques to assess this species complex. The strength of the phenetic species concept is that it considers many characters—both qualitative and quantitative—and can also be usefully applied to herbarium specimens (Sokal and Crovello 1970).

Aim of Study

The aim of this study was to evaluate the species recognised by Jeffrey (1997) in the *E. coccinea* complex using a phenetic approach and, thereby, compare the applicability of the phenetic and morphological species concepts to this group. Morphological characters most useful for the recognition of species in the *E. coccinea* complex were determined.

Materials and Methods

Herbarium Specimen-Based Study

A phenetic approach based on 134 herbarium specimens [*E. coccinea* s.s. (65), *E. caespitosa* (19), *E. emilioides* (8), *E. jeffreyana* (13), *E. lisowskiana* (7), *E. praetemissa* (9), *E. subscaposa* (5), and *E. vanmeelii* (8)] spanning the geographical range of the *E. coccinea* complex was undertaken (Online Resource 1). The number of specimens examined indicates their availabilities, and sampling was greater for those species that were more represented in herbaria and were more widespread. Types of three of the species (*E. jeffreyana*, *E. subscaposa*, and *E. vanmeelii*) and scanned images of the types for the rest of the species in the *E. coccinea* complex were examined, and specimen identities were checked against these types. Specimens were borrowed from the following herbaria: BR, EA, LISC, MA, MAL, MO, PRE, SRGH, and UZL (acronyms following Holmgren et al. 1990).

The specimens were examined, and the selected morphological characters were measured under a Zeiss Discovery V.12 dissecting microscope. Originally, 47 qualitative (binary and multistate) and quantitative characters were investigated (Table 2). Included among the characters used were those known to be useful in *Emilia* in general (Lisowski 1990, 1991), and those used by Jeffrey (1997) to key out the members of the *E. coccinea* complex.

Table 1 Summary of characters used by various authorities (Jeffrey 1997; Lisowski 1990, 1991; Tadesse and Beentje 2004) in distinguishing species of the *Emilia coccinea* complex

	E. caespitosa	*E. coccinea*	*E. emilioides*	*E. jeffreyana*	*E. lisowskiana*	*E. praetermissa*	*E. subscaposa*	*E. vanmeelii*
Stem indumentum	Glabrous or sparsely crispate-pubescent towards the base	Glabrous in upper part, crispate-pubescent in lower part	Glabrous	?	?	Glabrous to pilose	?	Crispate-pubescent in lower part
Leaves	Cauline	Cauline, stems leafy in the lower part	Cauline	Cauline	Cauline	Cauline	Basal rosette	Cauline, stems leafy in the lower part
Leaf shape	Broadly obovate to lanceolate	Rounded, spathulate, obovate to subpandurate	Lanceolate	Oblong-linear to narrowly lanceolate	Ovate-lanceolate to ovate	Ovate to deltoid	?	Obovate, to broadly lanceolate
Upper and median leaves	Circular to oblanceolate; cuneate or attenuate into a petioloid base		Lanceolate			Broad ovate, subcordate, with attenuate petioles	?	
Lower leaves		Spathulate to elliptic; cuneate or attenuate into a winged petioloid base	?	Oblanceolate-spathulate, attenuate to a petioloid base	?		?	Broadly elliptic to circular exauriculate petioloid base
Ratio, length to breadth	3–4 times				?	?		1.5 times
		three times		?				
Leaf margin	Shallowly sinuate-denticulate to sinuate-serrate	Subentire	Entire	?	Subentire or unequally toothed	Strongly sinuate/ broadly dentate	?	Shallowly sinuate-dentate
Upper leaves		Shallowly sinuate-dentate	?	?			?	
Lower leaves	?				?	?		Subentire
Capitula number and arrangement	2–4; terminal corymbs	1–6; ?	2; ?	4; ?	?; ?	1–7; open corymbs	?; ?	1–4; terminal corymbs
Phyllary: number	11–13 (8)	8–21 (13)	?	8–9	13	8–12 (10)	?	13–21
Length (mm)	?	?	?	6	?	8–11	?	4.5–7
Indumentum	Sparsely crispate-pubescent near apex	Glabrous	?	?	?	Pilose	?	Glabrous or finely crispate pubescent
Corolla colour	Bright-orange to orange-red	Bright-orange to orange-red, orange-yellow or yellow	White, pale-yellow, pale-mauve	Orange-yellow to scarlet	Orange-yellow to scarlet	White, pale-yellow, pale-mauve	Orange-yellow to scarlet	Crimson, bright-red, orange
Corolla length (mm)	5.5–12.5	5.3–9	?	7.5	?	8	9–9.5	6–10.5
Corolla lobes (mm)	1.5–2.8	1.3–2.2	?	1.3	?	2	?	?

Table 1 continued

	E. caespitosa	*E. coccinea*	*E. emilioides*	*E. jeffreyana*	*E. lisowskiana*	*E. praetermissa*	*E. subscaposa*	*E. vanmeetii*
Style branch length (mm)	1.5	?	?	?	?	1.2	?	1
Apex shape and extension	Flat, subulate appendaged, with papillae	Subulate, appendaged	Truncate, papillae	Truncate, unappendaged, epapillose or with tufts of long papillae, the central tufts longer than the others	Truncate, unappendaged, epapillose or short to long papillae at the apex	Curved backwards, unappendaged with minute hairs at apex.	Subulate, appendaged with dense papillae	Narrowly triangular appendaged with papillae
Cypsela indumentum	Glabrous or with a few hairs towards the apex	Shortly hairy in upper part	Pubescent throughout their length	Pubescent	Pubescent	Pubescent throughout their length	Pubescent	Glabrous
Cypsela length (mm)	2.2–3.8	2–4.7	?	3	?	3	3.7	2.8–3.5
Cypsela colour and shape	Pale-brown; subcylindrical	?; cylindrical	?; ?	?; narrowly oblong	?; ?	?; cylindrical, truncate-elliptic	Black; oblong	?; subcylindrical
Pappus length (mm)	3.5–7	3–5	?	3.5–6	?	7	?	3.5–6

? Represents missing information

These characters were, therefore, chosen to reveal the taxonomic variation shown by species in the *E. coccinea* complex (Chandler and Crisp 1998).

The cauline leaves were divided into two categories: middle to upper leaves and middle to lower leaves, as these differ in their shape as well as their attachment to the stem. Specimens with at least five leaves and an almost entire stem were selected for the analyses. Mature capitula were utilized for the floral measurements. Multiple measurements for the disc florets and cypselas within a specimen were taken from different capitula where possible, and a range of peduncle lengths were also measured. At least three measurements were taken for each character where possible and more when characters were noticeably very variable.

Style branches were examined by softening the florets using Glass Master®, dissected, observed under a Zeiss Discovery V.12 dissecting stereo microscope and photographed using the Zeiss AxioCam MRc attached to this dissecting microscope.

A data set with 42 characters was used in the final analyses as these characters were variant, informative, and mostly present on all specimens (Table 2). Five characters (life form, stem type, presence of trichomes on leaf surface, upper leaf attachment, and number of disc florets per capitulum) were excluded from the original data set either because they were invariant or because it was not possible to non-destructively determine the number of disc florets on most specimens, resulting in a lot of missing data for that character. This resulted in a matrix with 134 taxa and 42 characters: 24 quantitative (continuous), three binary (stem indumentum, lower leaf attachment, and style branch apex shape), and 15 multistate qualitative (ordered). (Data matrix is available upon request from the corresponding author.)

Multivariate Analyses

The multivariate techniques of the cluster analysis and ordinations [principal coordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS)] were used in the current study to reassess species limits in the widespread the *E. coccinea* complex. A cluster analysis seeks to establish similar subsets of taxonomic groups (Dunn and Everitt 1982) and is commonly used to study variation in geographic patterns (Thorpe 1983). Ordination techniques aim to summarise substantial information entirely in a few dimensions (Pimentel 1981) with a few assumptions regarding the nature of relationships in a data set. MDS is generally recommended for taxonomic studies (Pimentel 1981; Austin 1985) with the advantage that missing data do not cause computational problems (Rohlf 1972). The cluster analysis and ordinations (PCoA and NMDS) of the

Table 2 List of characters and states originally selected to be used in multivariate analysis of the *Emilia coccinea* complex

*1. Life form: annual (0); perennial (1)

2. Plant height (cm)

*3. Stem: erect (0); decumbent (1)

4. Stem: glabrous (0); sparsely to densely pubescent (1)

5. Stem diameter at base (mm)

6. Leaf type: basal rosette (at base) (0); cauline (1) both basal rosette and cauline (2)

7. Upper leaf shape: lanceolate (0); narrowly ovate (1); ovate (2); elliptic (3); oblanceolate (4); obovate (5); reniform (6)

8. Lower leaf shape: lanceolate (0); narrowly ovate (1); ovate (2); elliptic (3); oblanceolate (4); obovate (5); reniform (6)

9. Leaf margin: sub-entire (0); serrate (1); denticulate (2); dentate (3); strongly dentate (4); sinuate dentate (5)

*10. Leaf trichomes: absent (0); eglandular and unicellular (1); eglandular and bicellular (2) eglandular and multicellular (3); eglandular, multicelled base and apical wisp (4); glandular and multicelluar (5)

11. Presence of trichomes on leaf surface: ventral (0); dorsal (1); dorsal and ventral (2)

*12. Upper leaves: petiolate (0); sessile (1)

13. Lower leaves: petiolate/petioloid base (0); sessile (1)

14. Upper leaf apex shape: apiculate (0); acuminate (1); acute (2); rounded to obtuse (3)

15. Lower leaf apex shape: apiculate (0); acuminate (1); acute (2); rounded to obtuse (3)

16. Upper leaf base shape: attenuate (0); cuneate (1); obtuse (2); slightly cordate (3); cordate (4); deeply cordate (5); sagittate (6)

17. Lower leaf base shape: attenuate (0); cuneate (1); obtuse (2); slightly cordate (3); cordate (4); deeply cordate (5); sagittate (6)

18. Longest leaf length (mm; average of 3 measurements)

19. Longest leaf width (mm; average of 3 measurements)

20. Mid to upper leaf (M-UL) length (mm; average of 3 measurements)

21. Mid to lower leaf (M-LL) length (mm; average of 3 measurements)

22. M-UL width (mm; average of 3 measurements)

23. M-LL width (mm; average of 3 measurements)

24. Capitula: solitary (0); corymbose in 3′s (1); corymbose, 4 or more (2)

25. Terminal peduncle length (mm; average of 3 measurements)

26. Number of capitula per stem branch (average of 3 measurements)

27. Capitula diameter (mm; average of 3 measurements)

28. Phyllaries (involucral bracts): glabrous (0); sparsely pubescent (1); densely and persistently pubescent (2)

29. Number of phyllaries per capitulum: (average of 3 capitula)

30. Phyllary length: (mm; average of 3 capitula)

31. Phyllary width: (mm; average of 3 capitula)

*32. Number of disc florets per capitulum: (average of 3 capitula)

33. Floret colour: creamy to white (0); yellow (1); orange to red (2); pink to purple (3)

34. Corolla total length (mm; average of 3 measurements)

35. Corolla tube length: narrow part of tube (mm; average of 3 measurements)

36. Corolla lobe length (mm; average of 3 measurements)

37. Corolla lobe breath (mm; average of 3 measurements): measured at widest point

38. Anther appendage length (mm; average of 3 measurements)

39. Filament collar length (mm; average of 3 measurements)

40. Stamen total length (mm; average of 3 measurements)

41. Style branch apices shape: truncate (0); obtuse (1)

42. Style branch apices: appendaged with one or more groups of short to long papillae at the apex (0); unappendaged and epapillose (1); unappendaged with one or more groups of short to long papillae at the apex (2); small subulate appendage with papillae (3); appendaged narrowly or reduced triangular with papillae (4)

43. Cypsela\ovary: glabrous/very short hairs (0); pubescent all over (1); pubescent on ribs only (2)

44. Cypsela (shape): cylindrical/oblong (0); sub-cylindrical (1); truncate-elliptic (2)

45. Cypsela (mature) length (mm; average of 3 measurements)

46. Cypsela (mature) width\diameter (mm; average of 3 measurements)

47. Pappus (mature) length (mm; average of 3 measurements)

* Indicates characters excluded from the final analyses

morphological variation were performed using R version 3.2.1 (R Core Team 2015), and functions available in the following R packages: 'cluster' (Maechler et al. 2015), 'labdsv' (Roberts 2015), 'vegan' (Oksanen et al. 2015), and 'MASS' (Venables and Ripley 2002) were used. For the cluster analysis, the 'daisy' function in the 'cluster' package was used. Since we had a mixed data set with binary, multistate qualitative, and quantitative continuous characters, Gower's coefficient (Gower 1971) was used for generating the dissimilarity matrix after the data were standardised. The complete linkage clustering method was then used to hierarchically cluster the specimens, and the cophenetic correlation coefficient (*r*; Sokal and Rohlf 1962; Sneath and Sokal 1973) for the resultant trees and the dissimilarity matrix was used as a measure of 'goodness-of-fit' of the phenogram to the data set.

PCoA (Gower 1966), suitable for data sets with both quantitative and qualitative characters (Legendre and Legendre 2003) as well as some missing data (Rohlf 1972), was also performed. The function 'vegdist()' from the 'vegan' library, containing the Gower's coefficient, was also used to perform ordinations. The other functions available in the R packages 'labdsv' and 'MASS' were also used to perform ordinations. Ordination of characters was also performed to give an indication of the variables that were most important in explaining the distribution of the data in 3-dimensional (3D) space (i.e., character loadings similar to eigen vectors in principal components analysis). The dissimilarity matrix was computed across characters; otherwise, all other procedures were similar to the ordination described above.

To perform NMDS, the function 'metaMDS' from the 'vegan' package was used, and the dissimilarity matrix based on Gower's coefficient as in PCoA was computed. Two dimensions were specified, and to determine the 'stress' values used to assess 'goodness-of-fit' for optimising the analyses, the maximum iterations were set to 100. NMDS was run a few times until two convergent stress solutions were found.

Univariate Analyses

Univariate analyses (descriptive statistics and box and whisker plots) were employed here to show distributional character information for each species as well as to assess the variability and extent of overlap of ten selected quantitative characters (Streit and Gehlenborg 2014; Krzywinski and Altman 2014) amongst the eight species in the *E. coccinea* complex. All univariate analyses were done using STATISTICA version 12.0 (Inc StatSoft 2013). The ten quantitative characters (stem diameter, mid to lower leaf width, capitula diameter, phyllary length, corolla tube length, stamen total length, filament collar length, anther appendage length, cypsela width, and pappus length) were selected on the basis of being important in determining the distribution of specimens along the first and second axes of the ordination plots. The mean and standard deviations of these characters were computed, and they were tested for normality, skewness, and homogeneity of variance using the Kolmogorov–Smirnov test, skewness coefficient, and Levene's test, respectively. A nonparametric technique (Kruskal–Wallis test) was used to analyse two quantitative characters (corolla tube length and phyllary length) that failed tests of normality and homogeneity of variance after data transformation. Multiple comparisons of mean ranks for all groups were done to investigate the hypotheses involving means of individual species in the *E. coccinea* complex with respect to a particular selected quantitative character. One-way analysis of variance at 5 % level of significance was also used to determine whether there were differences between the means of each of the other eight characters that had met all the assumptions of parametric tests. This was followed by post hoc comparisons using Fisher's least significant difference (LSD) test (Snedecor and Cochran 1980; Howell 1999) for each of the selected quantitative characters.

Distribution of Species in the *Emilia coccinea* Complex

A distribution map of the eight species in the *Emilia coccinea* complex was created using ArcGIS® version 10.2.2 software (ESRI 2014) based on the available locality information on the herbarium specimens studied.

Results

Multivariate Analyses

A cluster analysis resulted in a phenogram (Fig. 5), in which five of the eight species in the *E. coccinea* complex form distinct groups, viz., *E. jeffreyana*, *E. praetermissa*, *E. emilioides*, *E. subscaposa*, and *E. vanmeelii*. Neither *E. caespitosa* nor *E. coccinea* s.s. form distinct clusters. *Emilia caespitosa* forms a cluster comprising 13 specimens, but two of its specimens from Uganda (114, 115) group next to the *E. jeffreyana* cluster, and four specimens are nested within two separate *E. coccinea* s.s. clusters (Fig. 5: clusters a, a1; two in each cluster). These two *E. coccinea* s.s. clusters (Fig. 5: clusters a, a1) comprise 35 and 27 specimens, respectively (excluding the *E. caespitosa* specimens). There are no particular morphological characters that distinguish the specimens in the two *E. coccinea* s.s. clusters. Although *E. lisowskiana* specimens all group together, three *E. coccinea* s.s. specimens [one

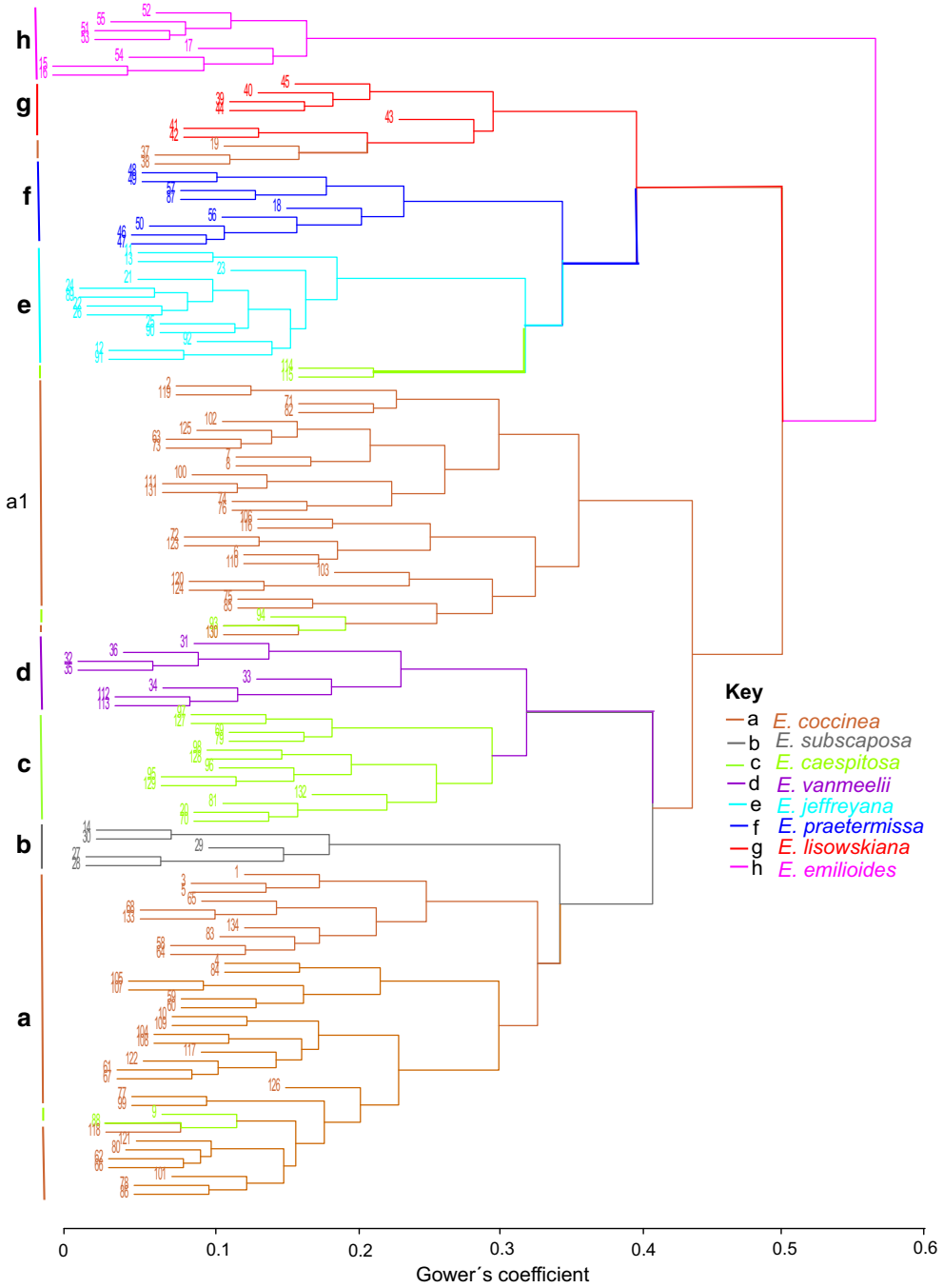

Fig. 5 Phenogram based on Gower's coefficient and complete hierarchical cluster method of specimens of the *Emilia coccinea* complex based on 42 morphological characters, $r = 0.70$. (Numbers of specimens as shown in Online Resource 1)

from Cameroon (19) and two from Equatorial Guinea (37, 38)] group with them (Fig. 5). The cophenetic correlation coefficient (r) is 0.7 for this phenogram, indicating a fairly good fit of the data to the tree.

The 2-dimensional (2D) scattergram resulting from NMDS (Fig. 6) of the same data set shows that four of the eight species in the *E. coccinea* complex create more or less distinct groups—*E. emilioides* (mid-right of plot), *E. subscaposa* (mid-top), *E. praetermissa* (mid-bottom), and *E. jeffreyana* (slightly right of the middle). *E. lisowskiana* is loosely grouped mainly left middle to lower corner, with two outliers (42, 43), and *E. vanmeelii* forms two distinct clusters—one in the upper middle and the other in the central region of the plot. *Emilia coccinea* s.s. is mainly to the left of the plot, and *E. caespitosa* towards the right, but their specimens intermingle with some of the other

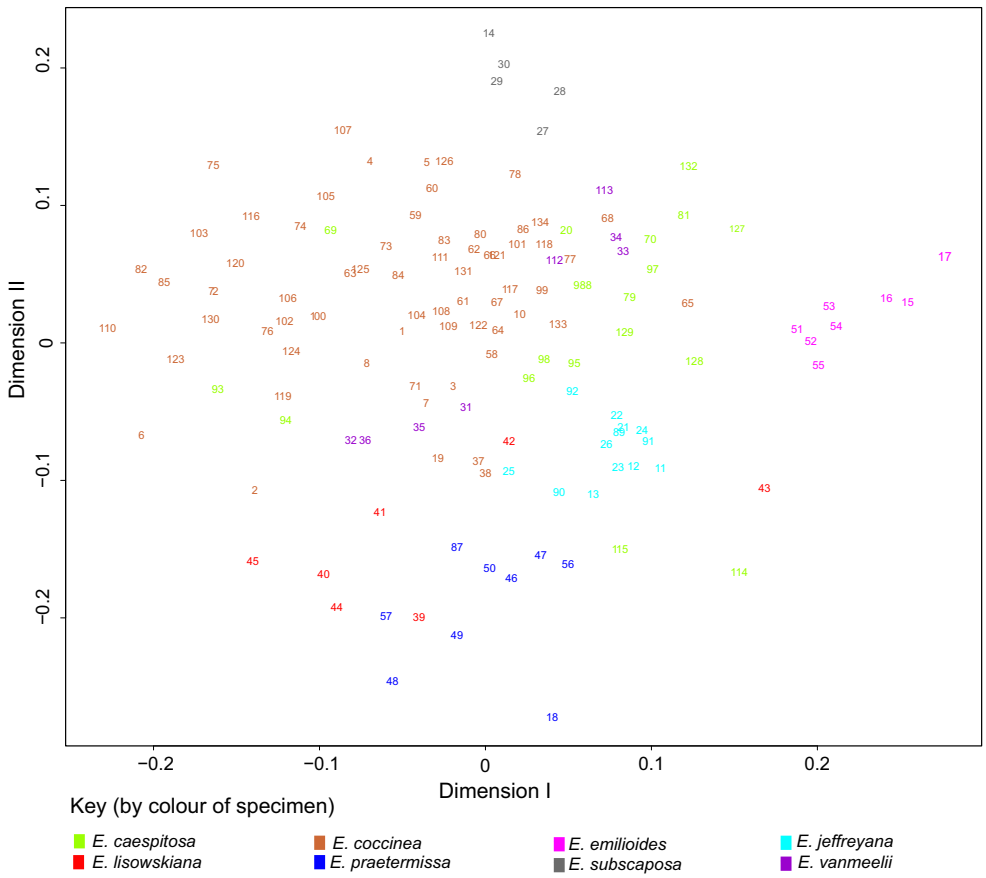

Fig. 6 Two dimensional NMDS scattergram for specimens of the *Emilia coccinea* complex based on 42 morphological characters. (Numbers of specimens as shown in Online Resource 1)

groupings; *E. caespitosa* is widely scattered across the 2D space. The 'stress' value for this analysis is 0.2539, indicating a fairly good fit of the data to the scattergram (Kruskal 1964). There is a similar overlap of all the specimens in the 3D plot (not shown).

The 2D plot of the PCoA using the same data set (not shown) is similar to that of the NMDS in that *E. emilioides* forms a distinct cluster, but the other seven species intermingle without showing any groupings. The first two coordinates of the PCoA account for 26.7 % of the variation in this data set. Pappus length, phyllary length, cypsela shape, and stem diameter (in the positive direction and in order of importance), and upper and lower leaf apex shape, anther appendage length, and cypsela width (in the negative direction) are the main determinants of the distribution of specimens along the first axis (Table 3). Capitula arrangement, upper leaf apex shape, style branch appendage, number of capitula per stem branch, and floret colour (in the positive direction), and upper leaf base shape, longest leaf width, number of phyllaries per capitulum, and mid to upper leaf width (in the negative direction) are important along the second axis. Lower leaf apex shape, longest leaf length, and mid to lower and upper leaf lengths (in the positive direction), and cypsela length, pappus length, corolla total length, and corolla tube length (in the negative direction) predominantly influence the placement of specimens along the third axis (Table 3).

Univariate Analyses

Two vegetative characters (stem diameter and mid to lower leaf width) and eight reproductive characters (capitula diameter, phyllary length, corolla tube length, stamen total length, filament collar length, anther appendage length, cypsela width, and pappus length) shown to be useful in distinguishing some of the eight species in the *E. coccinea* complex are represented in box and whisker plots (Fig. 7a–j). Although some characters are quite distinctive for certain species (e.g., mid to lower leaf width, capitula diameter, and stamen length distinguish *E. emilioides*), there is a considerable overlap for many of them. Generally, *E. coccinea* s.s. shows the greatest range in variation for all ten characters considered here (Fig. 7a–j), which is to be

Table 3 Character loadings resulting from PCoA of the 42 morphological characters along the first three principal coordinate (PC 1, PC 2, and PC 3) axes for the multivariate analysis of the *Emilia coccinea* complex

Characters	PC 1 (14.6 %)	PC 2 (12.1 %)	PC 3 (8.6 %)
Plant height	0.3569	**0.0180**	0.3050
Stem indumentum	−0.3775	0.0829	**−0.0220**
Stem diameter	0.4866	0.0696	0.1119
Leaf type	−0.3129	0.3036	−0.2414
Upper leaf shape	−0.2578	−0.2227	0.0397
Lower leaf shape	−0.0897	−0.3472	0.1696
Leaf margin	−0.2031	−0.3544	−0.0967
Leaf trichomes	0.3472	0.3472	0.3472
Lower leaf attachment	−0.0598	−0.1802	0.1762
Upper leaf apex shape	−0.6250	0.5180	0.2443
Lower leaf apex shape	**−0.6313**	0.2127	**0.4940**
Upper leaf base shape	0.0621	**−0.6568**	0.0990
Lower leaf base shape	**0.0415**	−0.1942	0.2777
Longest leaf length	0.3715	0.1329	0.4420
Longest leaf width	0.3145	−0.5043	0.3073
Mid to upper leaf length	0.2983	0.1552	0.3902
Mid to lower leaf length	0.4432	0.2320	0.3984
Mid to upper leaf width	0.2532	−0.4624	0.1583
Mid to lower leaf width	0.4228	−0.4436	0.3575
Capitula arrangement	**−0.0035**	**0.7166**	0.2477
Terminal peduncle length	−0.0518	0.0699	−0.1745
Number of capitula per stem branch	0.2116	0.5077	0.2909
Capitula diameter	−0.1392	−0.4085	−0.0754
Phyllary indumentum	−0.3809	−0.2261	0.3253
Number of phyllaries per capitulum	−0.3325	−0.4755	0.0341
Phyllary length	0.5581	0.0543	−0.3580
Phyllary width	−0.2253	**0.0043**	−0.2908
Floret colour	0.0885	0.4837	0.1499
Corolla total length	0.2913	−0.3148	−0.4235
Corolla tube length	0.4143	−0.2754	−0.3973
Corolla lobe length	−0.4716	−0.2701	−0.1207
Corolla lobe breath	−0.3493	0.0784	0.0309
Anther appendage length	−0.5815	−0.1376	−0.0294
Filament collar length	0.4097	−0.2485	−0.2485
Stamen total length	−0.2083	−0.2181	−0.2514
Style branch apex shape	−0.2875	0.1377	−0.1233
Style branch appendage	0.2551	0.5092	**−0.0012**
Cypsela indumentum	0.2983	−0.0904	−0.3585
Cypsela shape	0.5133	0.4463	−0.2770
Cypsela length	−0.3077	0.2093	**−0.4698**
Cypsela width	−0.4955	0.0818	−0.3711
Pappus length	**0.6306**	0.0869	−0.4483

Two highest and two lowest values for each axis are bolded

expected as this species is the most widespread geographically and was most extensively sampled. For all the vegetative and reproductive characters considered here (Fig. 7a–j), the means of *E. coccinea* s.s. and *E. caespitosa* are very similar, and the interquartile ranges (which measure variability of the features) overlap considerably (Fig. 7a–j). This explains why the specimens of these two species do not form distinct clusters in the multivariate analyses (Figs. 5, 6). The univariate tests of significance for all ten quantitative morphological characters used to

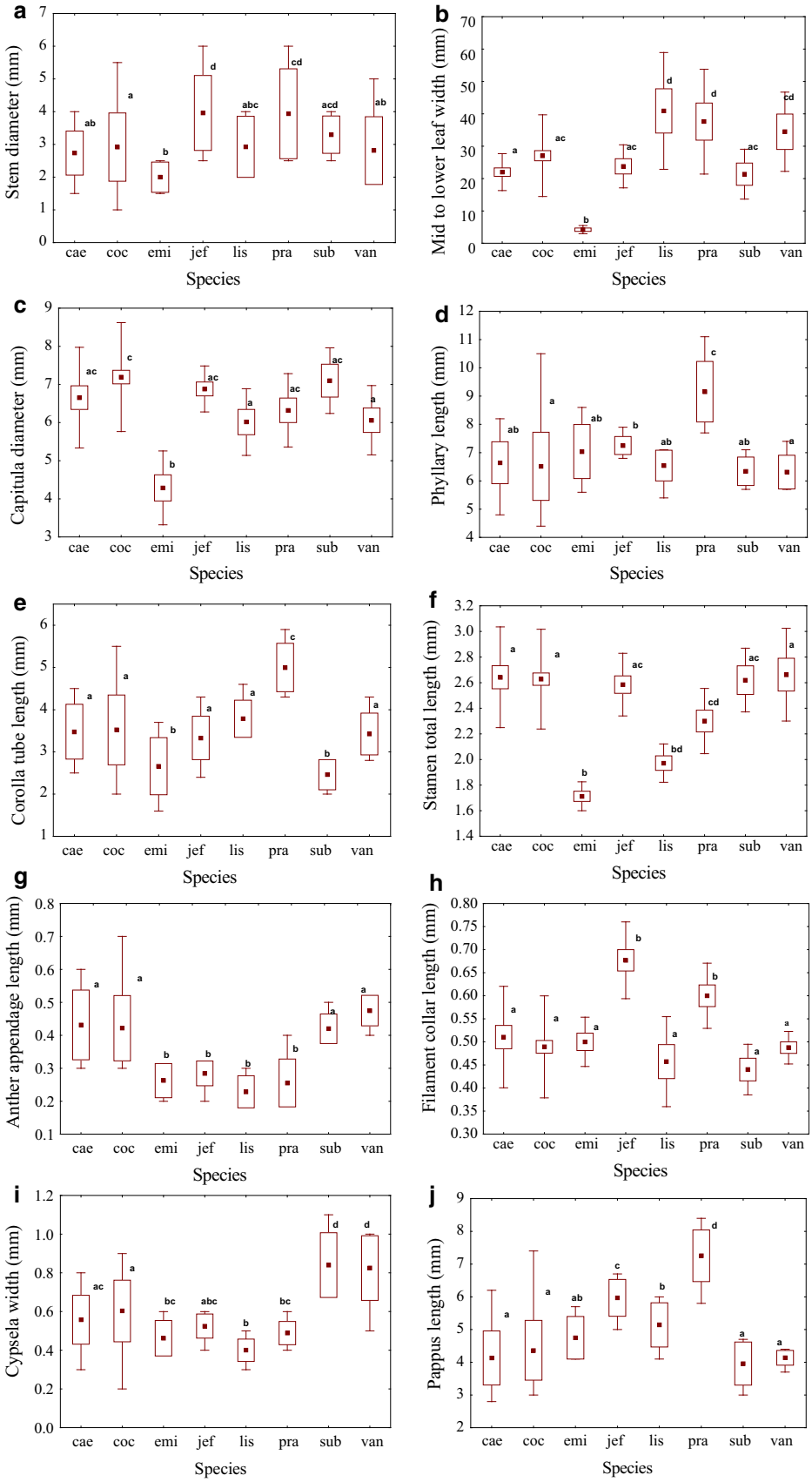

Fig. 7 a–j Ten selected quantitative characters (stem diameter, mid to lower leaf width, capitula diameter, phyllary length, corolla length, stamen total length, anther appendage length, filament collar length, cypsela width, and pappus length) that help to distinguish species in the *Emilia coccinea* complex. Abbreviations of species' names: *cae E. caespitosa*, *coc E. coccinea*, *emi E. emilioides*, *jef E. jeffereyana*, *lis E. lisowskiana*, *pra E. praetermissa*, *sub E. subscaposa*, and *van E. vanmeelii*. The same *letters* above *bars* indicate no significant difference ($p < 0.005$; nonparametric tests used for corolla tube length and phyllary length, and Fisher's LSD tests used for the other eight characters). *Box* represents the standard error, *whisker* represents the standard deviation, and the *solid rectangle* inside *box* represents the mean

Table 4 Differences in ten selected quantitative characters between the eight species in the *Emilia coccinea* complex

Character	Degrees of freedom	Sum of squares	Mean square	*F*/*H*-ratio	*p*
Stem diameter	125	29.54	4.22	4.32	0.0003
Mid to lower leaf width	115	4.17	0.60	18.98	0.000001
Capitula diameter	122	71.24	10.18	6.51	0.000002
Phyllary length	134	62.17	8.88	34.70	<0.0001
Corolla tube length	134	32.27	4.61	36.90	<0.0001
Stamen total length	126	9.06	1.30	10.78	<0.0001
Anther appendage length	126	0.08	0.11	14.67	<0.0001
Filament collar length	126	0.51	0.07	7.50	<0.0001
Cypsela width	125	1.31	0.19	9.88	<0.0001
Pappus length	126	99.73	14.25	21.79	<0.0001

Two of the characters (corolla tube length and phyllary length) were analysed using Kruskal–Wallis tests (H), and the remainder were analysed using one-way ANOVAs (F). Significant differences between species were determined using Fisher's (LSD) test

differentiate eight species in the *E. coccinea* complex are summarised in Table 4 and described below.

Stem diameter and the width of leaves from the middle to the lower part of stems of species in the *E. coccinea* complex differed significantly at the 5 % level (stem diameter $F_{7,125} = 4.22$, $p = 0.0003$; mid to lower leaf width $F_{7,115} = 6.61$, $p < 0.0001$; Table 4). The stems of *E. emilioides* are significantly thinner than four other species in the *E. coccinea* complex, whereas *E. jeffreyana* and *E. praetermissa* have thick stems (Fig. 7a). *Emilia emilioides* is also distinctly different from the other seven species in the *E. coccinea* complex as it generally has very narrow leaves and noticeably narrower capitula (Fig. 7b, c). On the other hand, *E. lisowskiana* and *E. praetermissa* have leaves that are much broader than the rest of the species in the *E. coccinea* complex (Fig. 7b). In addition, there is a considerable overlap in the capitula diameter of the seven of the eight species in the *E. coccinea* complex ($F_{7,122} = 6.51$, $p = 0.000002$), which all have broad capitula (Fig. 7c). However, the mean capitula diameters of *E. lisowskiana* and *E. vanmeelii* (6.01 and 6.06 mm, respectively) are slightly less broad.

Phyllary length (mean) of seven of the species in the *E. coccinea* complex overlaps considerably, with *E. praetermissa* having the longest (Fig. 7d). *Emilia coccinea* s.s. also shows the greatest range of variation for this character, with phyllaries ranging in length from 4.4 to 10.5 mm (Fig. 7d).

The Kruskal–Wallis test for the corolla tube length indicated a statistically significant result ($H_{7,134} = 36.90$, $p < 0.0001$; Table 4) for three (*E. emilioides*, *E. praetermissa*, and *E. subscaposa*) of the eight species in the *E. coccinea* complex. *E. praetermissa* differs significantly ($p < 0.0001$) by having long corolla tubes, whereas those of *E. emilioides* and *E. subscaposa* are short compared with the rest of the species in the complex (Fig. 7e).

The stamens of five out of eight species in the *E. coccinea* complex (*E. caespitosa*, *E. coccinea* s.s., *E. jeffreyana*, *E. subscaposa*, and *E. vanmeelii*; Fig. 7f) overlap with regard to their total lengths. However, the short stamens of *E. emilioides* differed significantly at the 5 % level ($F_{7,126} = 10.78$, $p < 0.0001$) from the rest of the species in the *E. coccinea* complex except *E. lisowskiana* (Fig. 7f). In addition, the length of stamens of *E. praetermissa* does not differ significantly from that of *E. lisowskiana*. The three species (*E. emilioides*, *E. lisowskiana*, and *E. praetermissa*) with short stamens also have short anther appendages, together with *E. jeffreyana* (Fig. 7g). The length of the anther appendages of these four species is statistically different (at the 5 % level of significance as indicated by the post hoc comparisons using Fisher's LSD test) from the other four species in the *E. coccinea*

complex (*E. caespitosa*, *E. coccinea* s.s., *E. subscaposa*, and *E. vanmeelii*; Fig. 7g). Although *E. jeffreyana* and *E. praetermissa* both have short anther appendages, they are the only two species in the *E. coccinea* complex with distinctly long filament collars (Fig. 7h).

Emilia subscaposa and *E. vanmeelii* have significantly broader cypselas than the rest of the species in the *E. coccinea*complex (Fig. 7i). The mean cypsela widths of the other six species overlap (Fig. 7i). Members of *E. praetermissa* have the longest pappus, and this character distinguishes it from the other seven species in the *E. coccinea* complex (Fig. 7j). One-way ANOVA results ($F_{7,126} = 21.79$, $p < 0.0001$) support that at least three species (*E. jeffreyana*, *E. lisowskiana*, and *E. praetermissa*) in the *E. coccinea* complex differ in terms of their pappus length (Fig. 7j).

Discussion

Species Recognition

Clear groupings for five (*E. emilioides*, *E. jeffreyana*, *E. praetermissa*, *E. subscaposa*, and *E. vanmeelii*) of the eight species in the *E. coccinea* complex were resolved by cluster and ordination analyses (NMDS) using morphological characters. However, *E. emilioides* is the only robust grouping recovered in both ordination analyses. This species is morphologically distinct from the other species in the *E. coccinea* complex due to its mostly long, very narrow cauline leaves (lanceolate; especially those in the mid to lower part of the stem), and unappendaged style branches. The NMDS analyses were more informative than the PCoA, with more groups (*E. emilioides*, *E. jeffreyana*, *E. praetermissa*, and *E. subscaposa*; Fig. 6) being recovered than in the PCoA, as has also been shown in other studies (e.g., Austin 1985; Chandler and Crisp 1998). The PCoA (not shown) only managed to convincingly recover the *E. emilioides* grouping, reflecting its poor ability to recover groupings in closely related taxa, as previously noted by Sneath (1976). *Emilia emilioides* is clearly distinguished from other species in the *E. coccinea* complex by having a narrow stem, narrow capitula, and very short stamens. The results of both multivariate and univariate analyses, therefore, support recognition of *E. emilioides* as a distinct species.

Emilia praetermissa, characterised by unappendaged style branches and pubescent cypselas, also forms a distinct cluster in the cluster analysis, although it is loosely grouped in NMDS. This species is distinguished from others in the *E. coccinea* complex by its strongly sinuate–dentate leaf margins compared with the shallowly sinuate–dentate or subentire to entire leaf margins in the other species. *Emilia praetermissa* is also distinctive in its large capitula, which result in it having significantly longer phyllaries, corolla tubes, and pappus than the other species in the complex. *Emilia subscaposa*, with its appendaged style branches with awl-shaped papillae at the apex and its leaves in a basal rosette, is also dissimilar. In addition, *E. subscaposa* differs from the other species by having broader capitula and cypselas, shorter corolla tubes, and a short pappus. The specimens of *E. jeffreyana* grouped together in both cluster analysis and NMDS (Figs. 5, 6); however, in cluster analysis, two specimens of *E. caespitosa* from Uganda group next to *E. jeffreyana*. *E. jeffreyana* has filament collars that are generally longer than the other species in the complex, as well as a longer pappus. All specimens of *E. vanmeelii* grouped together in cluster analysis, but with others (i.e., *E. caespitosa* and *E. coccinea* s.s) in NMDS resulting in two groups. Six of the *E. vanmeelii* specimens [the four grouping in the central part of the NMDS plot and two from the second group (33, 34)] are characterised by having corymbs of more than four capitula and predominantly red florets. However, the remaining two specimens in the second group (112, 113 from Tanzania) have solitary capitula with orange florets, characteristics that are rarely encountered in specimens of *E. vanmeelii* from East Africa (Beentje et al. 2005). *Emilia vanmeelii* is also mostly distinguished from other species in the *E. coccinea* complex by the shape of its cauline leaves i.e., obovate to broadly lanceolate (Jeffrey 1997; Tadesse and Beentje 2004; personal observation). *Emilia lisowskiana* is not supported as a distinct entity here, since three *E. coccinea* s.s. specimens group with it in the cluster analysis. Two of these *E. coccinea* s.s. specimens are from Equatorial Guinea, thus from the same geographical location as the *E. lisowskiana* specimens sampled here, whereas the third *E. coccinea* s.s. specimen is from Cameroon. *E. lisowskiana* is mostly distinguished from other species in the *E. coccinea* complex by its unappendaged style branch apices that are epapillose or with a few short hairs (Jeffrey 1997, personal observation).

The taxonomic distinctness of *E. caespitosa* and *E. coccinea* s.s. is not supported by this phenetic study. There was a sufficient overlap between these two species in multivariate and univariate analyses, such that they might be considered as one heterogeneous species (Figs. 5, 7a–j). Thus, past taxonomy may have incorrectly recognised these two *Emilia* species as distinct, and there exists only one species. However, further research needs to be done to verify this. These species differ mostly in the shape and colour of their cypselas—those of *E. caespitosa* being subcylindrical and pale brown, whereas *E. coccinea* s.s. has cylindrical and dark brown cypselas. *Emilia caespitosa* and *E. coccinea* s.s. were reported to be distinguishable by the shape of their cauline leaves (Jeffrey 1997), but they overlap being broadly obovate to lanceolate in *E. caespitosa*, and obovate to obovate-elliptic in *E. coccinea* s.s. (Jeffrey 1997; Tadesse and Beentje 2004; personal observation). Nonetheless, the species do differ slightly in that the leaves of *E. coccinea* s.s. are three times longer than broad compared with those of *E. caespitosa*, which are three to four times longer than broad (Jeffrey 1997; personal observation).

An alternate hypothesis is that *E. caespitosa* and *E. coccinea* s.s. are two distinct species that hybridize. Variable cypsela indumentum possibly reflects the mixing of these two species, as some specimens of *E. coccinea* s.s. have glabrous cypselas instead of pubescent ones, whereas some members of *E. caespitosa* (indicated by asterisk; Online Resource 1) have pubescent instead of glabrous cypselas. This could be possibly due to introgression with

E. coccinea s.s. (Jeffrey 1997), as *E. caespitosa* is not geographically or temporally isolated from *E. coccinea* s.s. However, removal of cypsela indumentum from the data set did not change the groupings in the phenogram resulting from the cluster analysis indicating that it is not the only character contributing to the mixing of these two species. They are also similar in their awl-shaped, appendaged style branches. In addition, they overlap in the following characters: stem diameter, phyllary length, corolla tube length, stamen total length (including anther appendages and filament collars), and pappus length. The character cypsela indumentum might be more phenotypically labile than the other characters considered in this study. It has been found to be unreliable in other senecioid genera, for example, the wide-ranging *Cineraria deltoidea* Sond. (Cron et al. 2007) and *C. erodoides* DC. (Cron et al. 2006). Cypsela indumentum has, however, been found to be taxonomically useful in the Eupatorieae (Wetter 1983).

Emilia caespitosa and *E. coccinea* s.s both have wide geographic ranges and cooccur in Angola, Burundi, DRC, Malawi, Mozambique, Uganda, Tanzania, Zambia, and Zimbabwe. Their habitats are also similar—abandoned and cultivated fields, along roadsides, mountainous areas, and miombo woodlands, and their altitudinal ranges coincide at 450–1700-m above sea level (a. s. l.). The range of habitats occupied by these annual species suggests that they act like weeds (Baker 1974), with *E. coccinea* s.s. being reported as a weed of roadsides, waste places, and unused land (Bosch 2004). Roadsides facilitate the dispersal of weeds, many of which are wind-dispersed as in the Asteraceae. Roadsides have been shown to provide corridors for gene flow in other weedy taxa (Spellerberg 1998), for example, in *Ageratina adenophora* (Dong et al. 2008) and *Raphanus raphanistrum* (Barnaud et al. 2013). Some specimens of *E. caespitosa* with pubescent cypselas occur along the roadsides in Tanzania (e.g., Kindeketa, Kayombo, and Laizer 2503 (EA), Mpwapwa District), and others are known from disturbed lands in the Kampala and Korogwe Districts of Uganda [e.g., Mwangoka 932 (MA); Rwaburindore 4082 (MO); Rwaburindore 4770 (MO)]. The other diverse habitats occupied by *E. coccinea* s.s. include dense forests and river valleys, whereas those of *E. caespitosa* include sandy river banks, forest reserves, and swamp areas. Hybridization might be occurring in *E. caespitosa* and *E. coccinea* s.s., since there is intergradation in some of their morphological characters as discussed above. Their flowering periods are known to overlap in Malawi, Mozambique, Tanzania, and Zimbabwe where they cooccur. Interspecific hybridization has been previously reported in *Emilia*, for example, *E. praetermissa* is a natural hybrid of *E. coccinea* and *E. sonchifolia* (Olorode and Olorunfemi 1973). A lack of phylogenetic congruence between plastid and nuclear data has provided the evidence of interspecific gene flow in the Senecioneae (e.g., Cron et al. 2008; Pelser et al. 2010; Yu et al. 2014), and it is possible that hybridisation and/or introgression have occurred or are occurring among some *Emilia* species. Phylogenetic and population-based studies (using molecular data) are needed to verify whether these species are indeed distinct species or not and if hybridization has possibly played a role in their origin or resulted in their lack of distinction.

Characters Useful in Recognition of *Emilia* Species

Generally, the vegetative characters used by Jeffrey (1997) in keying out species in the *E. coccinea* complex are too variable within a species to be useful taxonomically, thus mostly reproductive characters are useful in distinguishing species in the *E. coccinea* complex. Nonetheless, stem diameter and mid to lower leaf width were useful in separating groups/distinguishing species in the *E. coccinea* complex, and leaf apex shape influenced distribution of specimens along the first axis in the ordinations. Reliable reproductive characters that distinguished most species in this complex include capitula size, arrangement and diameter, phyllary length, corolla tube length, stamen total length, filament collar length, anther appendage length, style branch appendage, cypsela shape and width, and pappus length. The style branch appendage has also been found by other researchers to be an important diagnostic feature for distinguishing species in the subtribes Senecioninae and Astereae of Asteraceae, for example, *Senecio* and *Laestadia* Knuth ex Less., respectively (Nelson 1994; Riva et al. 2009). This is also true for *Emilia* (Jeffrey 1986, 1997; Lisowski 1990, 1991; Tadesse and Beentje 2004) and is confirmed by this study. Floret colour in general is also important but could not be scored reliably on the herbarium specimens. Nonetheless, it is useful in distinguishing species in the *E. coccinea* complex and influenced distribution of species in the ordinations. Colour characters are considered by many taxonomists as unstable and unreliable, since their measurement is dependent on the colour vision of the observer and is very hard to quantify (Chandler and Crisp 1998).

Application of Species Concepts to the *Emilia coccinea* Complex

Although the morphological species concept applied by Jeffrey (1997) is often useful for practically recognising taxa (Stuessy 1990; Judd et al. 2008), some of the morphological characters he used, such as cypsela indumentum, are inconsistent within the species of the *Emilia coccinea* complex, making some of the groups ambiguous, especially when other supporting characters (e.g., leaf shape) overlap. The phenetic species concept applied to the

E. coccinea complex supports the recognition of at least five of the eight species (*E. emilioides*, *E. jeffreyana*, *E. praetermissa*, *E. subscaposa*, and *E. vanmeelii*) as they form distinct groups in the cluster and/or ordination analyses, suggesting possible relationship based on similarity (Moss 1972; Moss and Hendrickson 1973; Stuessy 1990). Two species, *E. caespitosa* and *E. coccinea* s.s. not recognisable by this approach, are possibly not 'good' species as highlighted above, although this needs to be tested using a molecular approach, since only morphological similarity was considered in the current phenetic study. In addition, the phenetic approach emphasises that one should use as many variable and available characters as possible as overall similarity is important (Stuessy 1990; Jensen 2009), yet it is not always possible to obtain and utilize all characters (Ghiselin 1966; Johnson 1970; Moss 1972; Duncan and Baum 1981; Sokal 1986; Jensen 2009).

Therefore, a phylogenetic study to elucidate relationships of species in *Emilia* based on DNA sequence data is currently being undertaken by the authors to augment the results of this phenetic study. Phylogenetic analyses are more commonly accepted, since they are based on evolutionary relationships where homology is considered, whereas the phenetic approach considers overall similarity of features without taking into consideration how these features evolved (Cain and Harrison 1960; Sneath 1976). Therefore, convergence could result in different species acquiring analogous features (e.g., in response to environmental pressures) and, thus, becoming phenotypically similar.

The integrative approach of Damm et al. (2010), in which multiple components of the taxonomic circle are utilised (DNA, morphology, reproduction, ecology, and geography; DeSalle et al. 2005), provides additional tools to distinguish species in the *E. coccinea* complex. In addition to morphology and reproduction discussed above, two other components, ecology (habitats) and geography (geographic distribution), add to our understanding of these species. The geographic distribution, altitudinal ranges, and habitats of five species in the *E. coccinea* complex (*E. emilioides*, *E. jeffreyana*, *E. lisowskiana*, *E. praetermissa*, and *E. subscaposa*) suggest that they are allopatric as they do not coincide in most cases. Although two of these species (*E. jeffreyana* and *E. subscaposa*) both occur in Central Africa (Burundi, DRC, and Rwanda) with *E. jeffreyana* extending further into Ethiopia, Uganda, and Kenya, their habitats differ (Jeffrey 1986, 1997; Tadesse and Beentje 2004). *Emilia subscaposa* is confined to grasslands and cassava fields between 700 and 1030 m a. s. l., whereas *E. jeffreyana* has varied habitats (e.g., disturbed open rainforest, dense humid forests, and along roadsides) and occurs at 780–2200 m a. s. l. (Lisowski 1990, 1991). *E. praetermissa* occurs mostly as a weed of cultivation and along roadsides in West Africa (e.g., Cameroon, Nigeria, and Gabon), whereas *E. emilioides* has been recorded in Sudan and Ethiopia in habitats that include marshy lands and areas with black clay soils (Lisowski 1991; Jeffrey 1997). The habitats of *E. lisowskiana* have not been noted on specimens examined in this study, nor are they mentioned in the literature. However, this species is found in West Africa extending southwards to Angola and Zambia, and occurs at an altitudinal range of 575–1120 m a. s. l. (Jeffrey 1997).

Conclusions and Recommendations for Further Study

The phenetic approach applied to the *E. coccinea* complex in this study has shed light on whether or not species in this complex are distinguishable. Multivariate analyses of the *E. coccinea* complex support the recognition of five of the eight species (*E. emilioides*, *E. jeffreyana*, *E. praetermissa*, *E. subscaposa*, and *E. vanmeelii*) as morphologically distinct species. *Emilia lisowskiana* is not supported as a distinct species in the cluster analysis as three *E. coccinea* s.s. specimens group with it. The two species *E. caespitosa* and *E. coccinea* s.s. are clearly not phenetically distinct as many of their morphological characters (e.g., phyllary length, corolla tube length, stamen length, anther appendage length, and filament collar) overlap, and they do not separate into distinct clusters in either cluster analysis or ordination analysis. Furthermore, *E. caespitosa* and *E. coccinea* s.s. coincide in some areas, and occupy similar habitats. A phylogenetic approach using molecular markers and/or a population based approach using AFLPs or microsatellites is required to confirm the taxonomic status of these two species in the *E. coccinea* complex. A key to the eight species in the *E. coccinea* complex is presented below:

Key to species in the *Emilia coccinea* complex

1a. Leaves cauline; distribution not restricted to DRC, Burundi, Rwanda 2
1b. Leaves in a basal rosette; distribution restricted to DRC, Burundi, Rwanda ..... ***E. subscaposa***
2a. Mid to lower leaves broad (9.5–62.3 mm wide); capitula broad (5.0–11.7 mm wide) 3
2b. Mid to lower leaves narrow (2.5–6.0 mm wide); capitula narrow (3.2–6.1 mm wide) ... ***E. emilioides***
3a. Leaf margins strongly sinuate dentate; florets white, pale yellow to mauve ..... ***E. praetermissa***
3b. Leaf margins entire to subentire, shallowly sinuate dentate to serrate; florets orange-yellow, bright-orange, orange-red, bright-red, scarlet, or purple 4

4a. Stems leafy in the lower part; habitat strictly miombo woodland ..... ***E. vanmeelii***
4b. Stems not leafy in the lower part; habitat varied including disturbed places, and roadsides 5
5a. Lower leaf apex mostly acute; phyllaries mostly glabrous throughout ..... ***E. jeffreyana***
5b. Lower leaf apex apiculate to acuminate, phyllaries sparsely to densely and persistently…pubescent throughout c6
6a. Style branches truncate, unappendaged, epapillose or short papillae at apex ..... ***E. lisowskiana***
6b. Style branches awl-shaped, appendaged, papillose .. 7
7a. Cypsela often glabrous, subcylindrical, pale brown ..... ***E. caespitosa***
7b. Cypsela often pubescent, cylindrical, dark brown ..... ***E. coccinea***

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Information on Electronic Supplementary Material

Online Resource 1. Supplementary data detailing the 134 specimens of *Emilia coccinea* complex used in this phenetic study. Identification code, collector and collection number, herbarium, locality information, altitude, and month of flowering are noted.

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CHAPTER 3

A molecular phylogenetic study of *Emilia* Cass. (Senecioneae, Asteraceae).

Abstract

Emilia is a widely distributed palaeotropical genus in the tribe Senecioneae (Asteraceae), comprising 117 species, mostly annuals. Although the taxonomic history of *Emilia* dates back as early as 1817, no phylogenetic study has been done to date. Bayesian and parsimony phylogenetic analyses were therefore performed on a representative sample of *Emilia* species together with other closely related genera in the Senecioneae using nuclear ITS and plastid *trn*L-*trn*F sequence data to provide the foundation for a taxonomic revision of the genus. We address questions around the generic circumscription of *Emilia* including the status of similar genera *Emiliella* and *Bafutia*, assess Jeffrey's sectional classification of *Emilia*, and evaluate the distinctness of the morphologically similar species in the large-headed *Emilia coccinea* complex. The resultant phylogenies reveal *Emilia* to be paraphyletic and polyphyletic, with *Bafutia* and *Emiliella* nested within it, and Jeffrey's sectional classification is not supported. Two of three doubtful species in the *E. coccinea* complex grouped together in both the nuclear and plastid phylogenies suggesting they may be synonymous. Well-supported topological incongruences between nuclear and plastid phylogenies suggest that hybridization and/or introgression have played a role in the history of *Emilia*, as with many other senecionoid genera.

Introduction

Emilia Cass. is a palaeotropical genus belonging to the tribe Senecioneae in the Asteraceae and comprises 117 species (The Plant List 2013), most of which are annuals. It is widely distributed with most species occurring in Africa (*ca.* 80), 14 in Madagascar with 11 of these endemic (Humbert 1963), and two weedy species *E. fosbergii* Nicolson and *E. sonchifolia* (L.) DC. that have spread to the neotropics (Barkley 2006). The genus is distinguished mostly by reproductive features, including solitary or corymbose, discoid or radiate capitula with small florets of various colours (white, yellow, orange, red, pink, or purple), ecalyculate and uniseriate involucres, and oblong to oblong-elliptic cypselas, glabrous or pubescent with persistent pappus bristles. A variety of chromosome numbers (n = 5, 8, 10, 11, 15, 20) are reported for six species of *Emilia* (Baldwin 1946, Jeffrey 1992, Nordenstam 2007), suggesting polyploidy and aneuploidy are present in the genus.

The taxonomic history of *Emilia* dates back as early as 1817 when Cassini first described the genus as differing from *Cacalia* L., a genus described by Linnaeus (1753). In 1819 he then describes *Emilia flammea* Cass. (Dict.Sci.14: 406) based on *Cacalia sagittata*, the type for *Emilia*. Cassini added two species to *E. flammea*, viz. *E. adenogyna* Cass. and *E. purpurea* Cass. in 1825. Candolle (1838; Prodr. 6: 301) enumerated 13 species and synonymized *E. flammea* and *E. purpurea* with *E. sagittata* DC. and *E. sonchifolia* DC., respectively, as the former were superfluous, illegitimate names. *Emilia* was treated as a subgenus of *Senecio* L. by Hoffmeyer in 1890 [*Senecio* L. subgen. *Emilia* (Cass.) O.Hoffm. Pflanzenfam. 4, 5(54): 297]. Garabedian (1924), in one of the earliest revisions of *Emilia*, recognised 23 species for the genus and also provided a brief history. She considered *Emilia* 'more as an association of allied species than as a distinct genus' (Garabedian 1924: 137). Nonetheless, most of its members continued to be classified in *Senecio* L. or remained undetermined until the work of Jeffrey in 1986 (Cufondontis 1967; Tadesse and Beentje 2004).

Jeffrey (1986) recognised 58 species for East Tropical Africa (Uganda, Kenya, and Tanzania) and also made 20 new combinations for species previously placed in *Senecio*. Two sections of *Senecio, Spathulati* Muschl. and *Emilioidei* Muschl. were transferred to *Emilia*. *Emilia* sect. *Spathulatae* (Muschl.) C.Jeffrey comprised 11 species characterised by discoid or radiate capitula with yellow florets, and short corolla lobes (Jeffrey 1986). *Emilia* sect.

Emilia (Muschl.) C.Jeffrey with 47 species was characterised by discoid capitula, florets of various colours, and long, narrow corolla lobes (Jeffrey 1986).

Jeffrey (1986) also suggested that all the tropical African species belonging to the emilioid complex (viz. *Xyridopsis* B.Nord., *Emiliella* S.Moore, and *Bafutia* C.D.Adams) could be placed in *Emilia*. The emilioid complex is characterised by a basic chromosome number x=5 and an ecalyculate involucre. He thus included the genus *Xyridopsis* into *Emilia* although it was later transferred to *Psednotrichia* Hiern. by Anderberg and Karis (1995) the decision also upheld by Nordenstam (2007). The separation of *Xyridopsis* from the large and heterogenous *Emilia* was based on some characters shared by *Xyridopsis* with other Senecioneae genera. These characters include: mucilaginous cypsela hairs, resiniferous corolla, ecalyculate involucres, and scapose peduncles. *Psednotrichia xyridopsis* (O.Hoffm.) Ander. & P.O.Karis, and *P. newtonii* (O.Hoffm.) Ander. & P.O.Karis are not sampled here since specimens could not be obtained. For the remaining two genera of the emilioid complex (viz. *Emiliella* S.Moore and *Bafutia* C.D.Adams) not sunk into *Emilia*, Jeffrey (1986: 875) also noted similarities between *Emilia* and these genera, as they all have ecalyculate capitula and suggested that *Emiliella* and *Bafutia* 'may also have to be united with *Emilia*'. *Emiliella* is a genus of eight species (Hind and Frisby 2014) and is geographically restricted to Angola, Democratic Republic of Congo (DRC), and Zambia (Mendonça 1943; Lisowski 1989, 1991; Torre 1972, 1975; Hind and Frisby 2014). *Emilia* is 'somewhat similar vegetatively to some species of *Emiliella*, although a far greater range of leaf form is seen in *Emilia*' (Hind and Frisby 2014: 9550). Based on Moore's (1918) generic diagnosis of *Emiliella*, the similarity of its small capitula to those seen in *Emilia* could be the basis of the derivation of the name '*Emiliella*' (Hind and Frisby 2014). However, Hind and Frisby (2014) are of the opinion that *Emiliella* is not synonymous with *Emilia* for three reasons, firstly, when the cypsela matures, the phyllaries of *Emiliella* split or rend between one pair of adjoining phyllaries, but in *Emilia* the phyllaries split along their hyaline margins and curl backwards; secondly, the pappus when present in *Emiliella* is a single scale, whereas in *Emilia* pappus bristles are numerous and persistent; and thirdly, the cypsela is longer than the pappus (when present) in *Emiliella* but shorter than the pappus in *Emilia*. In contrast to *Emiliella*, *Bafutia* is monotypic and *Bafutia tenuicaulis* C.D.Adams, an erect, small annual herb about 30 cm tall, with connate phyllaries, occurs only in Cameroon (Adams 1962). *Bafutia* was retained as a separate genus by Jeffrey (1992) because of the connate phyllaries, which are the major distinguishing feature in the subtribe Othonninae (Bremer 1994) and Nordenstam (2007) supported this position, placing it in subtribe Othonninae of the Senecioneae (Nordenstam *et al.* 2009).

Neither *Bafutia* nor *Emiliella* have been previously included in molecular phylogenetic analyses of the Senecioneae and this study will elucidate their generic status.

Regional revisions of *Emilia* for northern and central Africa have also been undertaken in recent years (Lisowski 1990, 1991; Tadesse and Beentje 2004). Jeffrey (1997) revised the morphologically variable *Emilia coccinea* complex in Africa and recognised eight species. In addition, Cron (2014) produced a synopsis of *Emilia* in southern Africa, having previously removed the single Northern Cape species *E. hantamensis* J.C.Manning & Goldblatt to a new monotypic genus, *Bertilia* Cron based on phylogenetic and morphological evidence (Cron 2013). The delimitations of the eight species within the *E. coccinea* complex were recently evaluated using a morphometric approach (Mapaya and Cron 2016; Chapter 2). Five out of eight species (*E. emilioides* (Sch.) C.Jeffrey, *E. jeffreyana* Lisowski, *E. praetermissa* Milne-Redh., *E. subscaposa* Lisowski, and *E. vanmeelii* Lawalreé) were recognised as morphologically distinct, whereas *E. caespitosa* Oliv. and *E. coccinea* (Sims) G.Don were indistinguishable because of overlap in many of their morphological characters and the similar habitats they occupy. *Emilia lisowskiana* C.Jeffrey was also not distinct since three specimens of *E. coccinea* grouped with it in the cluster analysis. This molecular phylogenetic study therefore facilitates confirmation of the taxonomic status of these eight species, especially the three that could not be distinguished morphologically in the phenetic study.

Despite the taxonomic history of *Emilia* dating back to 1817, no phylogenetic or phylogeographic study of the genus has been done to date. *Emilia* was included in phylogenetic studies of the Senecioneae using DNA sequences (Pelser *et al*. 2002, 2003, 2007, 2010), although it was not widely sampled. Only three *Emilia* species (*E. coccinea*, *E. exserta* Fosberg, and *E. prenanthoidea* DC.) were sampled by Pelser *et al*. (2007), however relationships between several lineages in the ITS cladogram, including the *Austrosynotis*–*Cineraria* clade in which *Emilia* was placed, were poorly resolved.

A more inclusive phylogenetic analysis of the Senecioneae using plastid data (Pelser *et al.* 2010), suggested that *Emilia* and *Bethencourtia* (Nees) Choisy are sister taxa in a clade together with *Senecio hollandii* Compton and *S. lineatus* (L.f.) DC., and this clade is sister to the 'New World 1 group' taxa which includes *Lomanthus fosbergii* (Cuatrec.) B.Nord. & Pelser, *Monticalia abietina* (Willd. Ex Wedd.) C.Jeffrey, *Pentacalia arborea* (Knuth) H.Rob., and *Pseudogynoxys haenkei* (DC.) Cabrera. These relationships were not supported by the nuclear data. The ITS/ETS phylogeny placed *E. coccinea* sister to *S. saxatilis* Lomak., in a clade also comprising sister taxa *S. deltoideus* Less. and *S. scandens* Buch.-Ham. ex D.Don,

which were positioned in the *Senecio* segregates group in the plastid analysis (Pelser *et al*. 2010). In the nuclear analysis, *Bethencourtia* is in an unresolved clade with *Jacobaea vulgaris* Gaertn. and *S. lineatus* group together with other species from the *Senecio* segregates assemblage. *Pericallis* D.Don is sister to 'New World 2 group' taxa (which includes *Lundinia plumbea* (Griseb.) B.Nord., *Elekmania picardae* (Krug and Urb.) B.Nord., *Senecio adamantinus* Bong., and *Zemisia discolour* (SW.) B.Nord.) in the nuclear analysis (Pelser *et al*. 2010). Well-supported incongruence between plastid and nuclear phylogenies was thus shown by these clades with reference to their placement relative to other lineages (Pelser *et al*. 2010). Since only one species of *Emilia*, *E. coccinea* (not the type species for the genus) was included in these analyses, the relationships hypothesised here need to be tested by more inclusive sampling of *Emilia* and its putative close relatives.

Five southern African *Emilia* species, viz. *E. schinzii* O.Hoffm. (previously *E. ambifaria* (S.Moore) C.Jeffrey), *E. discifolia* (Oliv.) C.Jeffrey, *E. marlothiana* (O.Hoffm.) C.Jeffrey, *E. protracta* Moore, and *E. transvaalensis* (Bolus) C.Jeffrey, were included in Cron's (2013) phylogenetic analysis in the reassessment of *Bertilia hantamensis* (J.C.Manning & Goldblatt) Cron. The placement of *E. transvaalensis* was found to be incongruent in the nuclear- and plastid-based phylogenies — it grouped with the other *Emilia* species in a strongly supported clade in the ITS phylogeny, but in the *trn*L-*trn*F phylogeny it was placed sister to *Kleinia galpinii* Hook.f. and *Oresbia heterocarpa* Cron & B.Nord. in an earlier diverging clade. This suggests that hybridization, introgression, and/or possibly incomplete lineage sorting (ILS) are occurring in *Emilia*. Further studies are needed to confirm this.

Incongruence between plastid and nuclear-based phylogenies is a fairly common occurrence in the Asteraceae and possible reasons are the biological processes hybridization and incomplete lineage sorting (e.g. Cron *et al*. 2008; Pelser *et al*. 2010; Cron 2013; Naciri and Linder 2015). Hybridization is defined in a strict sense as mating between unrelated individuals, although the term usually applies to mating between species (Mallet 2005). The transfer of genetic material between species results in gene trees ('trees of a group of homologous genes each sampled from a different species'; Pamilo and Nei 1988: 368) tracking various speciation histories (Petri *et al*. 2013), for example, when the same species are retrieved as sister to different species in the plastid and nuclear-based phylogenies (Baack and Rieseberg 2007). Hybridization has been reported in many senecionoid genera including *Cineraria* L., *Emilia*, *Euryops* Cass., and *Senecio* (Nordenstam 1963; Chapman and Jones 1971; Olorode and Olorunfemi 1973; Cron *et al*. 2008). Introgression, which occurs as a

result of hybridization and repeated backcrossing, is 'the permanent incorporation of genes from one set of differentiated populations into another' (Petit and Excoffier 2009: 386). The process of introgression can either be unidirectional – genes transferred solely from one species to another, or bidirectional – genes exchanged between two species (Judd *et al*. 2008). Three possible consequences of introgression are: firstly, different species unite, secondly, genetic material is transferred from one species to another without species uniting — resulting in the introgressed species having increased genetic diversity, and thirdly, formation of new species from stabilised introgressants (Seehausen 2004; Baack and Rieseberg 2007). The process of introgression can be difficult to determine since the results of gene flow can be contradicted by the existence of ancestral polymorphism (Baack and Rieseberg 2007; Naciri and Linder 2015). Lineage sorting on the other hand is 'a random process of retention and extinction of alleles in a lineage over time' (Devos *et al*. 2010: 63). Incomplete lineage sorting takes place when diverging species inherit alleles for which the genealogy does not reveal the order of speciation events (Doyle 1992; Maddison 1997). It mostly occurs in large populations and when species have recently diverged (Maddison 1997; Pelser *et al*. 2010). There is lack of effective and widely applicable methods for distinguishing hybridization and ILS (Joly *et al*. 2009). One of the methods used by Pelser *et al*. (2010) to distinguish between hybridization and ILS as causes of the incongruent patterns observed in Senecioneae was based on coalescence (when two lineages merge into a single individual in a specific generation some time in the past; Kingman 2000). This coalescent-based method is problematic in that it requires large effective population sizes.

Adequate and intensive sampling of *Emilia* species using nuclear and plastid DNA sequence data is thus needed in order to produce a robust molecular phylogeny to provide a foundation for a good future revision and to address the questions raised above. In the current study, the nuclear internal transcribed spacer (ITS) regions of the 18S-5.8S-26S nuclear ribosomal DNA and most frequently used plastid DNA markers in plants, *trn*L-*trn*F intron and spacer regions (Hao *et al*. 2009), were utilised to infer relationships in *Emilia*. These molecular markers have proven useful in resolving phylogenetic relationships in *Euryops* (Asteraceae; Devos *et al.* 2010), and in the phylogeny that included five southern African species of *Emilia* (Cron 2013).

The aim of this study was therefore to investigate the phylogenetic relationships of a representative sample of *Emilia* species, together with the genera *Emiliella*, *Bafutia* and other closely related genera in the Senecioneae, using nuclear and plastid DNA sequence data. The resultant phylogeny serves to indicate whether *Emilia* is monophyletic or not, and assesses

the generic status of *Emiliella* and *Bafutia*. It also provides a sound basis for future taxonomic revisions of the genus, including assessment of Jeffrey's sectional classification of *Emilia.* Possible roles played by past hybridization, introgression, and/or incomplete lineage sorting in the evolutionary history of *Emilia* are also investigated here.

Three objectives were therefore proposed for the current study. The first objective was to reconstruct a molecular phylogeny of *Emilia* species and closely related genera in the Senecioneae using nuclear and plastid DNA sequence data. The research questions associated with this objective are: (i) Is the genus *Emilia* monophyletic, and if not, which species of *Emilia* should be excluded from the genus based on the molecular phylogeny?; (ii) Are Jeffrey's (1986) sections *Spathulatae* and *Emilia* supported? Should other sectional delimitations be proposed from the reconstructed phylogeny?; and (iii) Are all eight species recognised by Jeffrey (1997) in the *E. coccinea* complex distinct, or does the molecular phylogeny corroborate the findings of the phenetic study (Chapter 2), i.e. that five species in the *E. coccinea* complex are distinct, but two (*E. coccinea sensu stricto* (*s.s.*) and *E. caespitosa*) or possibly three (*E. lisowskiana*) are indistinguishable?

The second objective was to test the hypothesis that genera *Emiliella* and *Bafutia* are part of *Emilia*. Thus, should the genera *Emiliella* and *Bafutia* be combined with *Emilia* as suggested by Jeffrey (1986), but opposed by Hind and Frisby (2014)? The third objective was to examine the anticipated incongruence between nuclear and chloroplast phylogenies in order to infer the role played by past hybridization, introgression, or incomplete lineage sorting in the evolutionary history of genus *Emilia*.

Materials and Methods

Taxon sampling and DNA sequence data

Sixty six *Emilia* species, including representatives of sections *Emilia* and *Spathulatae* (seven out of 11 species) and the type of *Emilia*, *Emilia sagittata* DC., as well as three *Emiliella* species and *Bafutia tenuicaulis* were sampled but not all amplified for both the nuclear ITS and plastid *trn*L-*trn*F regions. Multiple accessions were sampled whenever possible so as to assess species monophyly (e.g. in the widespread *E. coccinea s.s.*). Much of the sampled material was obtained (with permission) from herbarium specimens.

Field work to collect fresh material of *Emilia* in Zimbabwe was undertaken during various months when the species were known to be flowering over the period 2012 to 2014.

Voucher specimens were deposited at C. E. Moss Herbarium at the University of the Witwatersrand (J) and duplicates placed at SRGH. Leaf material preserved in silica gel was available for three previously collected *Emilia* species from Namibia, one from South Africa and two from Cameroon, with vouchers at J and YA respectively.

In preliminary laboratory work, two nuclear regions (ITS and ETS) and three plastid DNA regions (*trn*L-*trn*F, *mat*K, and the *trn*K intron) were tested. The ETS region and two plastid regions (*mat*K and *trn*K intron) proved difficult to amplify among the *Emilia* species (especially when herbarium samples were used) and due to time and budget constraints were not utilised in the present study[1]. The nuclear ITS and plastid *trn*L-*trn*F regions amplified fairly well (even from herbarium specimens) and exhibited sufficient variation to be useful for a species-level phylogenetic analysis. For the ingroup, newly obtained sequence data is available for the following specimens: 52 accessions representing 43 *Emilia*, two *Emiliella* and one *Bafutia* species for the ITS regions, and 67 accessions representing 58 *Emilia*, three *Emiliella* and one *Bafutia* species for the *trn*L-*trn*F region. In addition, sequences of five *Emilia* species for the nuclear ITS and three for plastid *trn*L-*trn*F were obtained from GenBank (Table A1, Appendix 3.1). Three species (*E. juncea*, *E. infralignosa*, and *E. tenera*) partially amplified for the ITS regions, and *E. myriocephala* for the *trn*L-*trn*F region resulting in some missing data.

Outgroup comparisons

Tephroseris integrifolia subsp. *integrifolia* (L.) Clairv. of the cacalioid subtribe Tussilagininae was used as a definitive outgroup to root the trees. *Tephroseris* is distant to the senecioid genera sampled; it is retrieved at the base of the Tussilagininae clade in the reconstructed cladograms of the Senecioneae using morphological and molecular characters (Bremer 1994; Pelser *et al*. 2010) and this genus has also been used to root trees in previous studies (e.g. Cron *et al*. 2008; Cron 2013).

Genbank sequences for 22 species from 16 genera representing 14 major groups in the tribe Senecioneae (Table 3.1) were also included in the phylogenetic analyses, based on previous molecular phylogenetic studies (e.g. Pelser *et al*. 2007, 2010; Cron 2013). These outgroup taxa from the Senecioneae were selected due to their close relationship with the ingroup – representatives from sister clades in both nuclear and plastid phylogenies were included in the analysis. Representatives of the major senecionoid lineages were included

[1] Seventeen and seven samples for the ETS region and the 5′ trnK intron respectively were also successfully amplified and sequenced and these sequences will be deposited in GenBank.

based on availability. Only partial sequences for five outgroup species (*Jacobaea vulgaris*, *Othonna capensis*, *S. flavus*, *S. lineatus*, and *S. scandens*) were available for the *trn*L-*trn*F region, resulting in some missing data. Voucher information for the samples used and GenBank accession numbers are indicated in Appendix 3.1 (Table A1). The final nuclear and plastid data sets used in the phylogenetic analyses comprised 80 and 95 sequences respectively, including outgroups.

Table 3.1 Senecioneae outgroup taxa included in the phylogenetic analyses of *Emilia* using plastid *trn*L-*trn*F and nuclear ITS markers

Senecioneae groups sampled	Representative species from each group
Tussilagininae s.s.	*Tephroseris integrifolia* subsp. *integrifolia*
Othonninae	*Euryops pectinatus* Cass., *Othonna capensis* L.H.Bailey
Gynuroids	*Kleinia galpinii*, *Solanecio biafrae* (Oliv. & Hiern) C.Jeffrey
Synotoids	*Dauresia alliariifolia* (O.Hoffm.) B.Nord. & Pelser
Senecio s.s.	*Senecio elegans* L., *Senecio flavus* (Decne.) Sch. Bip., *Senecio ilicifolius* L., *Senecio pinnatifolius* var. *lanceolatus* (Benth.) I.Thomps.
Senecio segregates	*Senecio deltoideus* Less., *Senecio scandens*
Emilia-Bethencourtia group	*Bethencourtia palmensis* (Nees) Choisy, *Senecio lineatus*
Dendrosenecio	*Dendrosenecio kilimanjari* subsp. *cottonii* (Hutch. & G.Taylor) E.B.Knox.
Oresbia	*Oresbia heterocarpa*
Jacobaea	*Jacobaea vulgaris*
Steirodiscus	*Steirodiscus tagetes* (L.) Schltr.
Bolandia-Mesogramma-Stilpnogyne clade	*Bolandia pedunculosa* (DC.) Cron, *Stilpnogyne bellidioides* DC.
Cineraria	*Cineraria mollis* E.Mey ex DC.,
Pericallis	*Pericallis murrayi* (Bornm.) B.Nord.

DNA extraction

Total genomic DNA was extracted from approximately 20 – 30 mg leaf material (dried in silica gel) or taken with permission from herbarium specimens (EA, LISC, MA, MAL, MO, P, SRGH) using either the GenElute™ Plant Extraction Minikit (Sigma-Aldrich, Missouri, USA) or the DNeasy Plant Mini kit (QIAGEN®, Venlo, Netherlands) following manufacturers' protocol. For herbarium specimens, the protocol was modified by increasing the incubation time from 10 to 30 min. at 65 ºC and also doubling the elution time from five to 10 min. at room temperature, otherwise standard procedures were followed. Extracted DNA was purified using the One Step™ PCR Inhibitor Removal Kit (Zymo Research Corporation®, California, USA). Additional DNA sample extractions of two species of *Emilia*

(*E. myriocephala* C.Jeffey, *E. pammicrocephala* (S. Moore) C. Jeffrey) and two of *Emiliella* (*E. zambiensis* Torre, and *E. luwiikae* D. J. N. Hind & Frisby) were acquired from the Royal Botanic Gardens, Kew.

PCR amplification and sequencing

The Polymerase Chain Reaction (PCR) was used to amplify the entire ITS region using the primer combinations AB101 and AB102 (Sun *et al*. 1994; Table 3.2) or ITS5 and ITS4 (White *et al*. 1990; Table 3.2). Internal primer combinations were used for a few specimens, viz. ITS int1 and ITS int2 (Cron *et al*. 2008) together with AB102 and AB101 respectively (Table 3.2). The *trn*L-*trn*F intron and spacer were amplified using the 'c' and 'f' primers with internal primers 'd' and 'e' used for some difficult samples (Taberlet *et al*. 1991; Table 3.2). Finally, in the preliminary trials, the ETS region was amplified using the AST1 and 18S-ETS primers (Baldwin and Markos 1998; Markos and Baldwin 2001; Table 3.2), the entire *mat*K gene was amplified using *trn*K-3914F and *trn*K-2R primers (Johnson and Soltis 1994; Table 3.2), and the *trn*K intron (5' and 3'), flanking both sides of the *mat*K gene were also amplified using primers 39F-546R for 5'*trn*K and 1023F-1559R for 3'*trn*K (Table 3.2) designed by Pelser *et al*. (2002).

Table 3.2 Primers used in PCR and sequencing of the genera *Emilia*, *Emiliella*, and *Bafutia* and their sources

DNA region	Primer name	Sequence (5'-3')	Reference
ITS	AB101 (forward)	ACG AAT TCA TGG TCC GGT GAA GTG TTC G	Sun *et al*. 1994
	AB102 (reverse)	TAG AAT TCC CCG GTT CGC TCG CCG TTA C	Sun *et al*. 1994
	ITS 5 (forward)	GGA AGT AAA AGT CGT AAC AAG G	White *et al*. 1990
	ITS 4 (reverse)	TCC TCC GCT TAT TGA TAT GC	White *et al*. 1990
	ITS int 1 (forward)	CGG CAG GCA TGT CCC AAG GA	Cron *et al*. 2008
	ITS int 2 (reverse)	GCT TCG GGC GCA CTT GCG TTC	Cron *et al*. 2008
*trn*L-*trn*F	c (forward)	CGA AAT CGG TAG ACG CTA CG	Taberlet *et al*. 1991
	d (reverse)	GGG GAT AGA GGG ACT TGA AC	Taberlet *et al*. 1991
	e (forward)	GGT TCA AGT CCC TCT ATC CC	Taberlet *et al*. 1991
	f (reverse)	ATT TGA ACT GGT GCA CGA G	Taberlet *et al*. 1991
ETS	AST1 (forward)	CGT AAA GGT GCA TGA GTG GTG	Markos and Baldwin 2001
	18S-ETS (reverse)	ACT TAC ACA TGC ATG GCT TAA TCT	Baldwin and Markos 1998
*mat*K	*trn*K-3914F (forward)	TGG GTT GCT AAC TCA ATG G	Johnson and Soltis 1994
	*trn*K-2R (reverse)	AAC TAG TCG GAT GGA GTA G	Johnson and Soltis 1994
*trn*K intron	39F (forward)	TGC GGC TAG GAT CTT TTA CAC A	Pelser *et al*. 2002
	546R (reverse)	TTT TTC AAC CCA ATC GCT CTT T	Pelser *et al*. 2002

1023F (forward)	GAT TTG GGC CGA TTT CTC	Pelser *et al*. 2002
1559R (reverse)	GCA CAC GGC TTT CCC TCT G	Pelser *et al*. 2002

Double-stranded DNA amplifications were performed mostly in a 20 µl volume containing 13.4 µl sterile water, 1.0 µl DMSO (5% or 10 %), 4.0 µl 10X DNA polymerase buffer, 0.4 µl 10 mM each deoxynucleotide triphosphate (dNTP), 0.25 µl of each primer (10 µM), 0.2 µl 5U/µl Phusion Fast Hot Start II taq DNA polymerase, and 0.5–1.0 µl template DNA (adjusted with de-ionized water as necessary). Thermocycling was conducted on a Bio-Rad T100™ DNA thermal cycler with the sets of parameters for each region and/or primer set as shown in Table 3.3. Annealing temperatures were optimised within the ranges shown (Table 3.3) for species that were difficult to amplify. Cycles were increased to 35 for herbarium material. Negative controls (all components except DNA) were included in each set of samples to check for contaminants.

Table 3.3 Thermal cycling conditions for plastid (*trn*L-*trn*F) and nuclear markers (ITS and ETS)

Primer combinations	Premelt	Denature	Annealing	Extension	Final extension	Cycles
AB101, AB102	98 ºC, 30 s	98 ºC, 30 s	54 ºC–58 ºC, 20 s	72 ºC, 30 s	72 ºC, 7 min.	30
ITS5, ITS4	98 ºC, 30 s	98 ºC, 30 s	54 ºC–58 ºC, 20 s	72 ºC, 30 s	72 ºC, 7 min.	35
AB101, ITS int 2, ITS int 1, AB102	98 ºC, 30 s	98 ºC, 30 s	54 ºC–58 ºC, 20 s	72 ºC, 30 s	72 ºC, 7 min.	35
*trn*c, d, e, f	98 ºC, 30 s	98 ºC, 30 s	55 ºC, 20 s	72 ºC, 30 s	72 ºC, 7 min.	30/35
AST1, ETS 18S	98 ºC, 30 s	98 ºC, 30 s	55 ºC, 20 s	72 ºC, 30 s	72 ºC, 7 min.	30

The resulting PCR products were run on a 1% (w/v) agarose gel stained with SYBR®-safe DNA gel stain and visualised on a Molecular Imager® Gel Doc™ XR+ system with Image Lab™ Software (Bio-Rad, U.S.A). The PCR products were then cleaned of excess primers and dNTPs by following the ExoSAP-IT™ PCR cleaning protocol of Werle *et al*. (2004).

Purified PCR products were sequenced using ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit Version 3.0 (Applied Biosystems, Warrington, UK) on an Applied Biosystems 3130xl Genetic Analyser at the Central Sequencing Facility, University of Stellenbosch, using the same primers for sequencing as for the PCR. Cycle sequencing was conducted on an Applied Biosystems Gene Amp® PCR system 2700 machine programmed as follows: 25 cycles of 96 ºC for 30 s, 50 ºC for 15 s, and 60 ºC for 4 min.

Electropherograms of all DNA sequences were assembled into contiguous sequences by checking for agreement between the two strands i.e. the base positions in the forward (5'-3') and reverse (3'-5') sequences and these were proof-read manually, edited and consensus sequences assembled using Sequencher 5.1 (Genecodes Corporation). These sequences were then aligned using BioEdit Sequence Alignment Editor (Hall 1999) and adjusted manually where necessary. The resultant alignments were coded manually for insertions or deletions (indels). Gaps in all the data sets were coded as separate binary characters (presence/absence) according to the simple indel coding method of Simmons and Ochoterena (2000). Indels coded were added as an extension to the DNA sequence matrices. Data matrices for coded indels are included in Appendix 3.1 (Tables A2, A3).

Phylogenetic analyses

Maximum Parsimony and Bayesian Inference analyses

Phylogenetic analyses of the aligned matrices were carried out to reconstruct the interspecific phylogenetic relationships of *Emilia, Emiliella, Bafutia,* and related genera in the Senecioneae. Maximum Parsimony (MP) and Bayesian Inference (BI) analyses for the *trn*L-*trn*F and ITS regions (separately) including and excluding coded indels were performed.

Parsimony analyses were conducted using the program TNT 1.1 (Goloboff *et al*. 2008) with all characters equally weighted and unordered. The Traditional Search option with 100 random addition sequences and TBR (tree bisection-reconnection) branch swapping was used. Multiple most parsimonious trees were combined as a strict consensus tree. Support for internal branches was evaluated using the Bootstrap method (Felsenstein 1985), with 1000 replicates and 10 random addition sequences. Standard measures used to assess the quality of trees were: tree length (L), consistency index (CI; used to reveal the amount of homoplasy in the most parsimonious trees), and retention index (RI; measures the amount of similarity in a character that can be interpreted as a synapomorphy in the most parsimonious trees).

Prior to Bayesian analysis, the best-fitting model for each DNA region was selected with jModelTest v.0.1.1 (Posada 2008) with default settings and employing the Akaike information criterion (AIC; Akaike, 1974). The general time reversible model with a proportion of invariable sites and a gamma distribution (GTR+I+G) and the general time reversible model with a gamma distribution (GTR+G) models were selected for the ITS and *trn*L-*trn*F regions respectively.

Bayesian inference was performed using MrBayes (v. 3.2.1; Ronquist and Huelsenbeck 2003) via the CIPRES Science Gateway v. 3.3 (Miller *et al*. 2010) on four data sets (ITS and *trn*L-*trn*F; including and excluding coded indels). Three independent Bayesian inference runs were performed, each comprising two independent and simultaneous runs of four Markov chain Monte Carlo (MCMC) chains (one cold and three hot) each for 10 million generations (Geyer 1991). The sampling frequency was one tree saved per 1000 generations. The effective sample size of parameter estimates, stability of likelihood values, and number of burn-in trees (initial 25% of sampled trees were discarded as burn-in) was analysed using Tracer v.1.5 (Rambaut and Drummond 2007). Consensus tree topology was viewed and edited in FigTree v.1.3.1 (Rambaut 2009). Posterior probabilities ≥ 0.95 and < 0.95 were regarded as strongly and weakly supported respectively.

Statistical tests of Incongruence

Congruence between nuclear and plastid DNA data sets was assessed via the partition homogeneity test (PHT; Farris *et al.* 1995) as implemented in PAUP* v.4.0b10 using the heuristic search option with random sequence addition (1000 random replicates) and TBR branch-swapping. Partition homogeneity test P-values below 0.01 are considered as evidence of significant incongruence (Cummingham 1997). Assessment of incongruent patterns was also done by comparing the plastid and nuclear–based trees directly, taking branch support into account. The plastid and nuclear data sets were not combined because of the results of the PHT (P = 0.001) and also well supported observed conflict, as discussed later.

Results

Nuclear ITS and plastid *trn*L-*trn*F phylogenies

The *trn*L-*trn*F alignment for 95 accessions including 22 outgroup Senecioneae comprised 993 characters, whereas the nuclear ITS alignment for 80 accessions with 22 outgroups had 868 base pairs. The ITS region was more informative than the generally conserved *trn*L-*trn*F region with 340 versus 100 parsimony informative characters respectively (Table 3.4), and with 61 and 35 parsimony informative coded indels in the ITS and *trn*L-*trn*F data sets respectively (Table 3.4). Similar clades were generally recovered in both *trn*L-*trn*F analyses (i.e. including and excluding coded indels) and overall relationships of species were more strongly supported in the phylogeny including coded indels (Figure 3.1). However, the

number of trees increased from 42 equally most parsimonious (EMP) trees to 250 EMP trees when coded indels were included. Including coded indels in the ITS analysis generally improved the resolution in the consensus tree even though it also increased the number of EMP trees from 14 to 56. Amongst the outgroup taxa, previously unsupported sister relationships (i.e. when indels are not coded), for example, *Cineraria mollis*, *Bolandia pedunculosa*, and *Stilpnogyne bellidioides* were also supported with the inclusion of coded indels (bootstrap support (BS) = 73%; posterior probalility (PP) = 1.00; Figure 3.2). Three species *E. limosa*, *E. lopollensis*, and *E. tenellula* form a weakly supported clade (BS = 54%) in a data set with coded indels (Figure 3.2), whereas they form part of the polytomy with *E. helianthella*, *E. subscaposa*, and *E. humifusa* when indels are excluded (not shown). Tree statistics and information of data matrices are summarised in Table 3.4.

The PHT for congruence between nuclear ITS and plastid *trn*L-*trn*F data sets revealed significant incongruence (P = 0.001) and incongruence amongst well supported clades was observed; therefore the data sets were not combined for further analyses but were dealt with separately.

The inclusion of indels in the Bayesian analyses using plastid *trn*L-*trn*F and nuclear ITS data also generally resulted in more strongly supported clades compared to the phylogenies retrieved when indels were not included, although similar topologies were retrieved in respective analyses. Some relationships in the nuclear parsimony analysis differed from the nuclear Bayesian inference phylogeny, for example, the following sister relationships in the MP tree: (i) *Jacobaea vulgaris* and *Steirodiscus tagetes* (ii) *Bethencourtia palmensis* and *Senecio lineatus* (iii) *Euryops*, *Othonna* to *E. graminea* and *E. baumii*; are not recovered in the topology resulting from the Bayesian analysis (Figure 3.2; Appendix 3.1, Figure A2). The strict consensus trees including coded indels with Posterior probabilities and bootstrap support values indicated on the branches are presented in this study (Figures 3.1, 3.2) and the Bayesian consensus trees are presented in Appendix 3.1 (Figures A1, A2).

Table 3.4 Tree statistics and character information for maximum parsimony analyses of nuclear ITS and plastid *trn*L-*trn*F DNA regions for *Emilia* and related Senecioneae. Abbreviations: incl./excl. coded indels = including/excluding coded insertions/deletions

	ITS	*trn*L-*trn*F
Number of taxa	80	95
Total number of characters (including coded indels)	929	1028
Total number of parsimony informative indels coded	61	35
Variable characters (excluding coded indels)	413	177

Parsimony informative characters (incl./excl. coded indels)	401/340	135/100
% Parsimony informative characters (incl./excl. coded indels)	43.2/39.2	13.1/10.1
Tree length (incl./excl. coded indels)	1471/1378	294/236
Consistency index (incl./excl. coded indels)	0.51/0.50	0.80/0.85
Retention index (incl./excl. coded indels)	0.82/0.81	0.96/0.97

Ingroup taxa: *Emilia, Emiliella* and *Bafutia*

Plastid data/trees

Two main clades (A and B) were retrieved in both the *trn*L-*trn*F parsimony and Bayesian inference analyses including coded indels (Figure 3.1; Appendix 3.1, Figure A1). However the sister relationship of clades A and B in the plastid parsimony analysis is not recovered (collapsed; indicated by arrow in Figure 3.1) in the topology resulting from the Bayesian analysis although the two main clades (A and B) were retrieved (Appendix 3.1, Figure A1). Clade A is strongly supported (BS = 100%; PP = 1.00) and Clade B is weakly supported (BS = 59%; PP = 0.95; Figure 3.1). The *Emilia* species group in two strongly supported clades (A and E) separated by the senecionoid outgroup taxa, indicating that the genus is not monophyletic. Seventeen *Emilia* species from various countries group together in Clade A, with *E. decaryi* sister to the remaining 16 which form a strongly supported polytomy (BS = 100%; PP = 1.00). Two of the 17 *Emilia* species in Clade A also have representatives in the 'main' *Emilia* clade E, viz. *E. violacea* and *E. humifusa*, even though the provenance for both accessions of each species is the same (viz. Tanzania and Madagascar, respectively). *Emilia violacea* also occurs in Burundi, DRC, and Zambia. *Bethencourtia* and *Senecio lineatus* are sister to Clade E, which comprises the other 52 *Emilia* samples, as well as *Bafutia* and the three *Emiliella* species (BS = 92%; PP = 1.00; Figure 3.1). The clade (F2) comprising the *Emiliella* species, *E. drummondii*, *E. luwikae*, and *E. zambiensis*, and *Bafutia tenuicaulis* are part of an unsupported polytomy (Clade F; PP = 0.97) comprising 29 *Emilia* species (38 *Emilia* samples). Relationships within the clade where *Bafutia* is placed (Clade F1; BS = 93%; PP = 1.00) are mostly unresolved and it groups with nine *Emilia* species (15 *Emilia* samples), including six members of the *E. coccinea* complex. *Emilia transvaalensis* does not group with either of the *Emilia* clades, but with the gynuroids (*Solanecio biafrae* and *Kleinia galpinii*) in a strongly supported (BS = 78%; PP = 1.00) sister relationship (Clade C; Figure 3.1).

ITS data/trees

Emilia is also not monophyletic in the phylogeny based on the ITS data (Figure 3.2), however, only two species (*E. baumii* and *E. graminea*) group outside of the main *Emilia* clade (E), together with *Euryops pectinatus* and *Othonna capensis* in a fairly weakly supported clade (Clade A; BS = 71%). These relationships were not recovered in the topology resulting from the Bayesian analysis: in Clade A, *E. graminea* and *E. baumii* are still sister taxa and are sister to the other Senecioneae, but are not in a clade with *Euryops* and *Othonna* (Appendix 3.1, Figure A2). Only eight of the 17 *Emilia* species in Clade A of the plastid consensus tree (Figure 3.1) were included in the nuclear data set as the others failed to amplify for the ITS region.

Pericallis is sister to the main *Emilia* clade (Clade C; BS = 100%) comprising 53 *Emilia* samples, although the relationship is only well-supported in the BI analysis (BS = 67%; PP = 0.95; Figure 3.2). The placement of the *Bethencourtia-Senecio lineatus* clade sister to the *Senecio* segregrates group is not supported. Within *Emilia*, three southern African species form the earliest diverging clade with *E. transvaalensis* sister to *E. marlothiana* and *E. schinzii* (Clade D, BS = 100%; PP = 1.00). The remaining *Emilia* species are located in a weakly supported clade (E; BS = 70%; PP = 0.78). *Bafutia tenuicaulis* and the two *Emiliella* species are placed in clade F2, which is unsupported (BS < 50%; PP < 0.5; Figures 3.2, A2) and also includes 13 *Emilia* species (14 *Emilia* samples). The *Emiliella* species group together (BS = 100%; PP = 1.00) in an unresolved relationship with *E. leucantha*, *E. longipes*, *E. adscendens*, *E. integrifolia*, and *E. emilioides* (BS = 86%; PP = 1.00; Figure 3.2). When indels are excluded, *E. cenioides* and *E. protracta* also form part of this subclade (E1) together with *E. tenera* (not shown). *Bafutia tenuicaulis* is sister to *E. juncea* (BS = 64%; PP = 1.00) together with *E. violacea* and *E. jeffreyana* in a strongly supported clade (BS = 88%; PP = 1.00), and sister to a clade with *E. sagittata* and *E. longiramea* (BS = 100%; PP = 1.00). The second *Emilia* subclade (E2) is well supported (BS = 99%; PP = 1.00) and comprises 14 *Emilia* species (16 samples; Figure 3.2). Seven of these *Emilia* species are from section Spathulatae and they group together with seven from section *Emilia*.

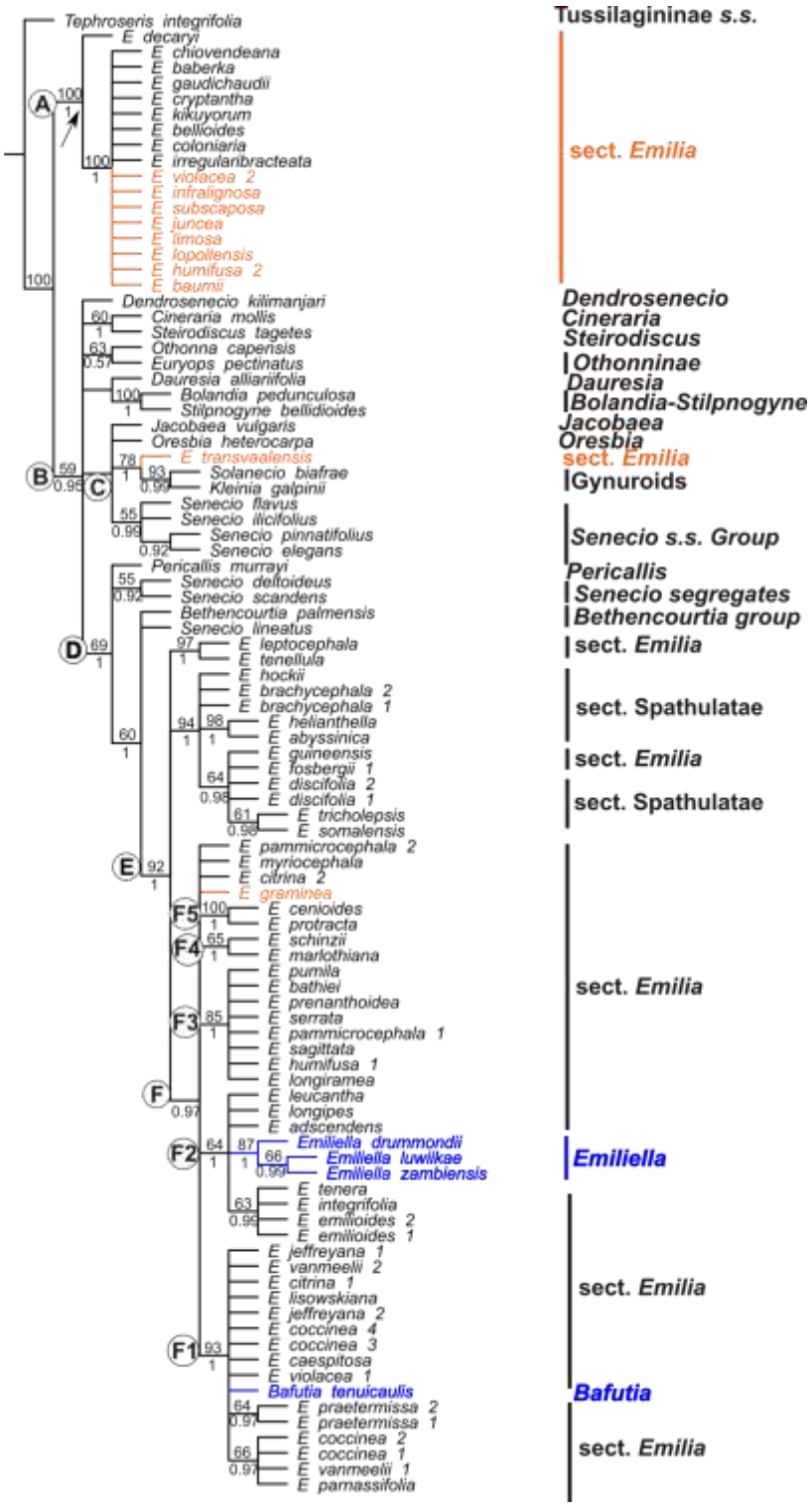

Figure 3.1. Strict consensus of 250 most parsimonious trees based on the plastid *trn*L-*trn*F dataset including indels (CI = 0.80, RI = 0.96). Bootstrap support and Posterior probabilities are indicated above and below branches respectively. Arrow shows the clade that collapsed in the Bayesian inference tree (Appendix, Figure A1). Clades labelled A-F are discussed within the text. Genera *Bafutia* and *Emiliella* are highlighted in blue. *Emilia* species incongruent with the nuclear ITS dataset are highlighted in orange. Note: only eight of the 17 species in Clade A (highlighted in orange) were included in the ITS analysis.

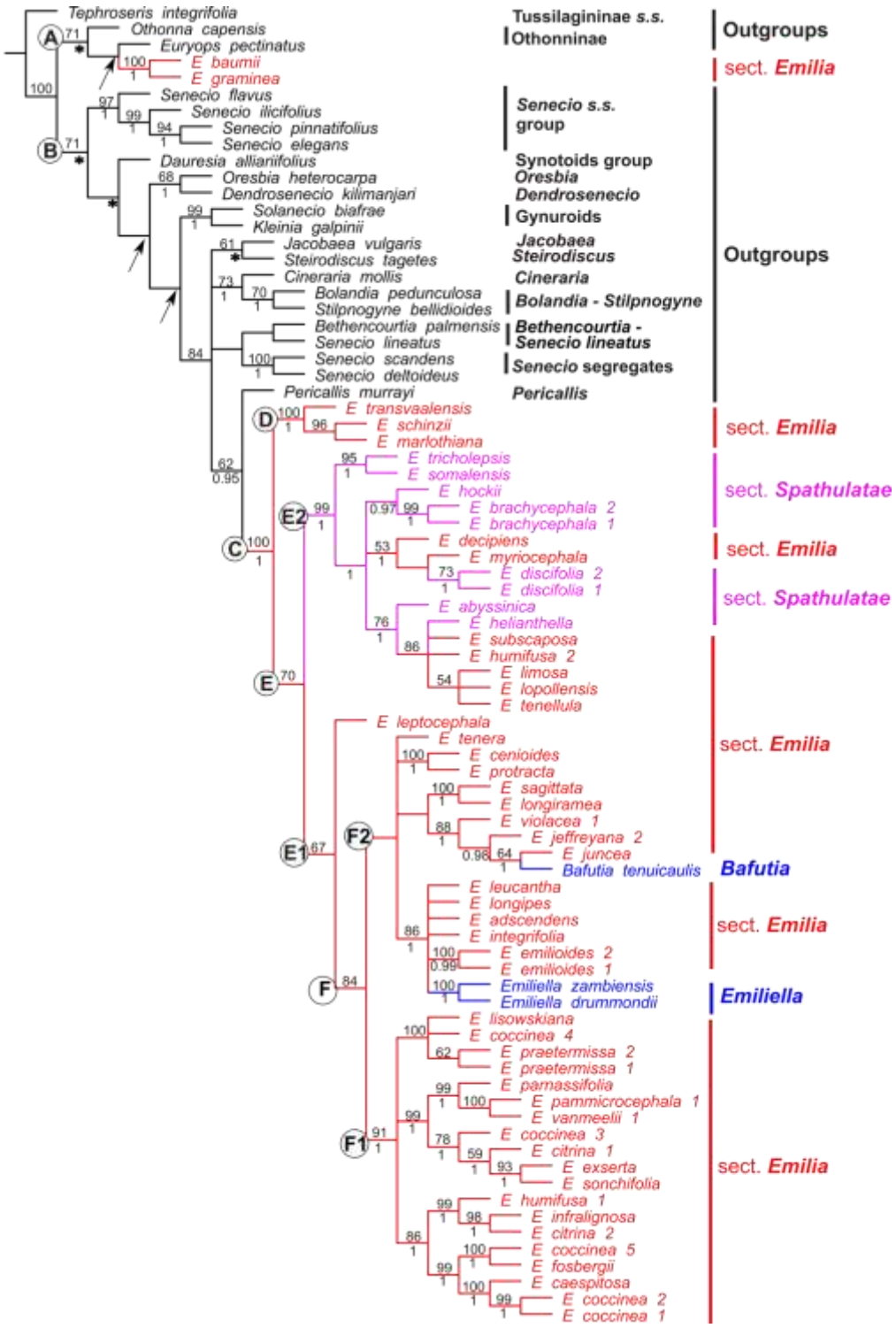

Figure 3.2. Strict consensus of 56 most parsimonious trees based on the nuclear ITS data set including indels (CI = 0.51, RI = 0.82). Bootstrap support and Posterior probabilities are indicated above and below branches respectively. Arrows show clades that collapsed and asterisk (*) show clades that differ in the Bayesian inference tree. Clades labelled A-F are discussed within the text. Genera *Bafutia* and *Emiliella* are highlighted in blue. *Emilia* sect. *Emilia* species are highlighted in red and *Emilia* sect. *Spathulatae* species are highlighted in fuschia.

Sections Emilia *and* Spathulatae *of Jeffrey (1986)*

Jeffrey's (1986) sections *Spathulatae* and *Emilia* do not form monophyletic groups in either the plastid or the nuclear phylogenies (Figures 3.1, 3.2). Seven species from section *Spathulatae* (*E. hockii*, *E. brachycephala*, *E. helianthella*, *E. abyssinica*, *E. discifolia*, *E. tricholepsis*, and *E. somalensis*) group together (Clade F) with two species (*E. fosbergii* and *E. guineensis*) from section *Emilia* in a strongly supported subclade (BS = 94%; PP = 1.00; Figure 3.1) in the plastid phylogeny. In the nuclear phylogeny, these same seven species group with seven other species from section *Emilia* in a strongly supported clade (E2; BS = 99%; PP = 1.00; Figure 3.2).

Emilia coccinea complex

The eight species in the *E. coccinea* complex do not all group together in either the plastid or the nuclear phylogenies. In the *trn*L-*trn*F phylogeny, six out of eight species in the *E. coccinea* complex (i.e. not *E. subscaposa* and *E. emilioides*) occur in Clade F1 (BS = 93%; PP = 1.00) comprising 15 *Emilia* samples and *Bafutia*, but the relationships within this clade are mostly unresolved (Figure 3.1). *Emilia subscaposa* is placed in a basal, strongly supported (BS = 100%; PP = 1.00) clade (Clade A) with 16 other *Emilia* species (Figure 3.1). *Emilia emilioides* occurs in Clade F2 (BS = 64%; PP = 1.00) together with five *Emilia* species and the three *Emiliella* species.

On the other hand, in the ITS phylogeny, seven out of the eight species in the *E. coccinea* complex (i.e. all except *E. subscaposa*) occur in Clade E1 comprising 34 *Emilia* samples, two *Emiliellas*, and *Bafutia* (Figure 3.2). Five of these seven species (*E. caespitosa*, *E. coccinea*, *E. lisowskiana*, *E. praetermissa*, *E. vanmeelii*) group together in a strongly supported clade (F1; BS = 91%; PP = 1.00). *Emilia emilioides* and *E. jeffreyana* occur in two separate subclades of F2 together with two *Emiliella* species and *Bafutia tenuicaulis* respectively. Only *E. subscaposa* is located in subclade E2 together with five other *Emilia* species (BS = 86%; PP < 0.95) in a clade with *E. abyssinica* sister to it (BS = 76%; PP = 1.00; Figure 3.2).

Five accessions of *E. coccinea* occur in Clade F1 (BS = 91%; PP = 1.00; Figure 3.2) together with nine other *Emilia* species, but do not form a monophyletic group. *Emilia caespitosa* is sister to a pair of *E. coccinea* accessions from Zimbabwe (BS = 100%; PP = 1.00) in a subclade of F1 that also includes a cultivated accession of *E. coccinea* and four other *Emilia* species (BS = 86%; PP = 1.00; Figure 3.2). The other two accessions of *E. coccinea* (*E. coccinea*3 and *E. coccinea*4 both from Cameroon) occur in two different

subclades of Clade F1 (Figure 3.2). Additionally, the two accessions of *E. humifusa* do not form a monophyletic group — one occurs in Clade F1 sister to *E. infralignosa* and a sample of *E. citrina* (BS = 99%; PP = 1.00) and the other in a subclade of Clade E2 with five other *Emilia* species (BS = 86%; PP < 0.95). In addition, the two accessions of *E. citrina* do not form a monophyletic group as they occur in two separate subclades of Clade E1 (Figure 3.2).

Outgroup relationships

Plastid phylogenies

The various representatives of recognised groups within the Senecioneae form distinct clades (e.g. Othonninae, *Cineraria–Steirodiscus*, and *Bolandia–Stilpnogyne* clades), however relationships among them are unresolved. The gynuroids group together (BS = 93%; PP = 0.99) and are strongly supported (BS = 78%; PP = 1.00) as sister to *E. transvaalensis* when indels are included (Clade C; Figure 3.1), whereas when indels are excluded the relationship between them is unresolved (BS = 88%; PP = 1.00; not shown). The *Senecio s.s.* group (BS = 55%, PP = 0.99), *Jacobaea*, and *Oresbia* also occur in the same clade (C) with the gynuroids and *E. transvaalensis* (Figure 3.1). The *Senecio* segregates group (BS = 55%, PP < 0.95) and *Pericallis* are placed sister to a clade comprising *Bethencourtia* and *Senecio lineatus*, which is sister to the *Emilia* clade (E; Figure 3.1).

Nuclear phylogenies

The relationships among the outgroup taxa are better resolved in the ITS phylogeny than in the plastid one – especially when indels are included. Clade B comprises representatives of previously recognised groups within the Senecioneae forming distinct clades, although relationships among them are not well supported and some collapse in the Bayesian consensus tree (Figure A2). The *Senecio s.s.* group is strongly supported (BS = 97%; PP = 1.00) and sister to the other outgroups in Clade B (i.e. excluding the Othonninae; Figure 3.2), but this relationship is unresolved in the Bayesian analysis. *Oresbia* and *Dendrosenecio* form a well-supported clade in the BI analysis (BS = 68%; PP = 1.00), and the relationship of the Gynuroids *Kleinia* and *Solanecio* is strongly supported (BS = 99%; PP = 1.00). *Jacobaea* and *Steirodiscus* are sister to each other (BS = 61%) in a polytomy comprising the (*Cineraria* (*Bolandia*, *Stilpnogyne*)) clade, *Bethencourtia – Senecio lineatus* group, and the *Senecio* segregates group (Figure 3.2). This relationship is not recovered in the Bayesian consensus tree (Appendix 3.1, Figure A2). *Pericallis*, as noted previously, is weakly to fairly well

supported (BS = 62%; PP = 0.95) as sister to the main *Emilia*, *Emiliella*, and *Bafutia* clade (C; Figure 3.2).

Incongruence between plastid *trn*L-*trn*F and nuclear ITS phylogenies

Well-supported topological incongruence is revealed when the plastid and nuclear consensus trees are compared. Notable conflicts are in the placement of some *Emilia* species, for example, in Clade A of the ITS analyses, *E. baumii* and *E. graminea* are strongly supported (BS = 100%; PP = 1.00) as sister to the Othonninoid taxa, *Othonna capensis* and *Euryops pectinatus* (BS = 71%; Figure 3.2; relationship not recovered in Bayesian tree; Appendix 3.1, Figure A2), whereas in the *trn*L-*trn*F analyses only *E. baumii* is placed in a basal polytomy of Clade A with 16 other *Emilia* species and *E. graminea* is in Clade F (Figures 3.1). The position of *E. infralignosa* also differs between the phylogenies, being sister to *E. citrina*2 in the ITS analyses (BS = 98%; PP = 1.00; Figure 3.2) but placed in the polytomy in Clade A in the *trn*L-*trn*F phylogeny (Figure 3.1). *Emilia transvaalensis*, which groups with *E. schinzii* and *E. marlothiana* in a strongly supported relationship (BS = 100%; PP = 1.00) in the ITS phylogeny (Clade D; Figure 3.2), occurs in the same clade as *Solanecio biafrae* and *Kleinia galpinii* (BS = 78%; PP = 1.00) in the trees resulting from *trn*L-*trn*F analyses (Figure 3.1). *Emilia myriocephala* is retrieved in subclade E2 resolved as sister to *E. decipiens* and *E. discifolia* in the ITS phylogeny (BS = 53%; PP = 1.00; Figure 3.2), but is placed unresolved in Clade F (BS < 50%; PP = 0.97) which comprises 30 *Emilia* species (37 *Emilia* samples), *Emiliella*, and *Bafutia tenuicaulis* in the *trn*L-*trn*F phylogeny (Figure 3.1). Amongst the other outgroups, the incongruence in the related Senecioneae matches that reported in Pelser *et al*. (2010). For example, the (*Cineraria* (*Bolandia*, *Stilpnogyne*)) clade in the ITS phylogeny is incongruent with relationships depicted in the plastid phylogeny where *Cineraria* groups with *Steirodiscus* and the sister genera *Bolandia* and *Stilpnogyne* are sister to *Dauresia*.

Discussion

Comparison of the nuclear ITS and plastid *trn*L-*trn*F regions

The nuclear ITS region was more variable and had more synapormorphic indels than the plastid *trn*L-*trn*F region. These results are similar to previous studies where it has been noted that nuclear regions usually provide more information at the species level than plastid ones (e.g. Cronn *et al*. 2002; Small *et al*. 2004; Kainulainen *et al*. 2010; Calvo *et al*. 2013; Kim *et*

al. 2015). Consequently, the ITS region has been extensively used to provide taxonomic characters in phylogenetic studies of closely related genera (Baldwin *et al*. 1995; Baldwin and Markos 1998; Soltis and Soltis 1998) and has been shown to be a useful marker in the study of evolutionary relationships in Senecioneae (e.g. Pelser *et al*. 2007, 2010; Cron *et al*. 2008, Cron 2013).

Monophyly of *Emilia*

The current generic circumscription of *Emilia* is not supported by either the plastid *trn*L-*trn*F or the nuclear ITS analyses and the genus is shown to be both paraphyletic and polyphyletic, thus supporting Nordenstam's (1978) assertion that *Emilia* may be polyphyletic. The genera *Emiliella* and *Bafutia* are nested within *Emilia* in both analyses, thus supporting Jeffrey's (1986) suggestion that they should be united with *Emilia* but disputing Hind and Frisby's (2014) argument that *Emiliella* is not part of *Emilia*. These three genera all have ecalyculate capitula that are mostly small and discoid. Other genera in the Senecioninae (e.g. *Bolandia*, *Euryops*, and *Phaneroglossa* B.Nord.) also have ecalyculate capitula. The type species of *Emilia*, *E. sagittata*, is retrieved in a weakly supported subclade comprising most *Emilia* species, *Bafutia*, and *Emiliella* in the ITS phylogeny (Clade E1; Figure 3.2), thus confirming the paraphyly of *Emilia*. This presents the problem, as in other phylogenetic systematic studies (e.g. *Helichrysum* Mill.; Galbany-Casals *et al.* 2014), of whether to split a large genus in order to make the various components monophyletic or to lump previously circumscribed genera together to avoid paraphyly. The clades within *Emilia* lack clear morphological synapomorphies that facilitate/support it being split into smaller monophyletic groups. Lumping of *Bafutia* and *Emiliella* in *Emilia* on the other hand would involve new nomenclatural combinations resulting in an increase in the number of species in an already large genus.

Emilia is a morphologically diverse genus/assemblage, with species being mostly annual or perennial herbs with ecalyculate, uniseriate phyllaries, and radiate or discoid capitula, either solitary or few to several in corymbs with florets of various colours, viz. white, yellow, pink, orange, red, or purple. Style branch apices in *Emilia* are truncate or obtuse, with or without sweeping hairs. The 5-ribbed cypsela is elliptic-oblong to cylindrical and light to dark brown in colour, with a uniseriate pappus consisting of many persistent bristles (Jeffrey 1986; Nordenstam 2007; Cron 2014).

As noted above, most *Emilia* species are similar to *Emiliella* and *Bafutia* as they comprise mostly annual herbs, with cauline leaves, corymbose small, discoid, and ecalyculate

capitula that are solitary or few (Nordenstam 2007). The colour of florets in both *Emiliella* (pink) and *Bafutia* (pink or reddish-purple) fit well into the suite of variously coloured florets of *Emilia*. *Emiliella* differs from *Emilia* in the following ways: (i) splitting of the phyllaries at cypsela maturity; (ii) pappus length relative to the cypsela; and (iii) pappus form sometimes absent in *Emiliella* (Hind and Frisby 2014). The style branch apices in *Emiliella* are unappendaged and penicillate whereas in *Emilia* they can be unappendaged and epapillose, unappendaged with few hairs, or appendaged and papillose. *Bafutia* also differs from *Emilia* by having connate phyllaries, and obtuse, club-shaped, penicillate style branches, whereas, as noted above, *Emilia* has free or basally connate phyllaries and truncate or obtuse style branch apices with or without sweeping hairs (Tadesse and Beentje 2004). The oblong cypselas in *Bafutia* are shallowly ribbed and smooth and the few minute pappus-setae shed at anthesis are shorter than the cypsela (Adam 1962; Nordenstam 2007), also different from *Emilia*.

Although morphological differences between *Emilia*, *Emiliella* and *Bafutia* are important, the molecular analyses place *Emiliella* and *Bafutia* firmly nested within *Emilia* in both the plastid and nuclear phylogeny. If either *Emiliella* or *Bafutia* were to be retained as separate genera, *Emilia* would be paraphyletic.

In the plastid phylogeny, 17 species from sect. *Emilia* (Clade A) group sister to the other senecionoid genera included here, i.e. outside the *Emilia* clade (where the type *E. sagittata* is located), thus further confirming the non-monophyly of *Emilia*. These seventeen *Emilia* species are from various geographic regions including East, West, Central and southern Africa — thus there is no geographic relationship among them. However, they all belong to section *Emilia* and share morphological synapomorphies that include discoid capitula and variously coloured florets (viz. white, yellow, orange, red, pink, and purple) with long corolla lobes. In contrast, in the ITS phylogeny, only one of the 17 species that groups outside of *Emilia* in the plastid phylogeny, viz. *E. baumii* (from southern Africa), is placed outside *Emilia*, together with *E. graminea* (from Madagascar). (It should be noted that only eight of these 17 species were included in the ITS analysis). *Emilia baumii* and *E. graminea* group with *Othonna* and *Euryops* in the parsimony ITS analysis, although this relationship with the Othonninae is not recovered in the Bayesian inference analysis. This grouping of *E. baumii* and *E. graminea* with the Othonninae is surprising as neither *E. baumii* nor *E. graminea* has connate involucral bracts or undivided styles, characteristic features of the Othonninae (Bremer 1994; Sykes 2004). *Emilia baumii* is the only species placed outside of *Emilia* in both the plastid and nuclear phylogenies, suggesting that it does not belong in *Emilia*. However, its narrow, entire, cauline leaves, yellow florets and unappendaged style

branches are typical of many *Emilia* species. *Emilia graminea* also has narrow cauline leaves that are mostly elliptic-oblong, and ecalyculate, solitary, radiate capitula, but with pink to purple florets. According to the phylogeny based on the nuclear data, which often reflect morphological evolution more closely than plastid data (Soltis and Kuzoff 1995; Yu *et al*. 2013), *E. graminea* should possibly also be excluded from *Emilia*, although additional molecular markers are needed to verify the exclusion and placement of both *E. baumii* and *E. graminea*.

Jeffrey's sections *Emilia* and *Spathulatae*

The two sections of *Emilia* (sections *Emilia* and *Spathulatae*) are not supported as monophyletic groups in either the plastid or nuclear phylogeny. *Emilia guineensis* from Guinea and a weedy species, *E. fosbergii*, that has spread to the neotropics (Barkley 2006), both belonging to sect. *Emilia*, are nested within sect. *Spathulatae* in the plastid phylogeny (sampled only there). Seven species from sect. *Emilia* also group with those from sect. *Spathulatae* in the nuclear ITS phylogeny. Thus the synapomorphies for sect. *Spathulatae* (discoid or radiate capitula, yellow florets, and short corolla lobes) recognised by Jeffrey (1986) are not upheld, since the species from sect. *Emilia* grouping with those from sect. *Spathulatae* have discoid capitula and florets of various colours. Capitula type and floret length thus show convergence in *Emilia* and yellow floret colour arose independently a number of times.

Since only 69% and 47% of species in *Emilia* were sampled in the *trn*L-*trn*F and ITS phylogenies respectively, no sectional delimitations can be proposed from these reconstructed phylogenies and there are no clear morphological patterns apparent in the various clades. Nonetheless, the current study serves as a foundation for future taxonomic revisions of *Emilia*.

***Emilia coccinea* complex**

Six out of eight species recognised by Jeffrey (1997) in the *E. coccinea* complex (except *E. subscaposa* and *E. emilioides*) occur in the same subclade, together with a few other species not from the *E. coccinea* complex in both plastid and nuclear analyses. Although *E. subscaposa* has a large capitulum characteristic of the *E. coccinea* complex, these molecular phylogenetic analyses confirm the phenetic findings that it is the most dissimilar member of this complex (Mapaya and Cron 2016). *Emilia subscaposa* differs from the other species in the *E. coccinea* complex by having leaves in a basal rosette. In the nuclear phylogeny, *E.*

subscaposa occurs in the same clade (E2) with two other species (*E. lopollensis*, *E. hockii*) also with leaves in a basal rosette, whereas in the plastid phylogeny *E. subscaposa* and *E. lopollensis* are placed outside of *Emilia* (in Clade A), together with 15 other *Emilia* species mostly with cauline leaves. Similarly, *E. emilioides* is morphologically distinct from the other species in the complex (Chapter 2) due to its narrow leaves, narrow capitula, short stamens and white to pale yellow flower colour. *Emilia subscaposa* and *E. emilioides* are clearly distinct species and not closely related to the other members of the *E. coccinea* complex. In addition, three other species in the *E. coccinea* complex (viz. *E. jeffreyana*, *E. praetermissa*, and *E. vanmeelii*) are supported by the nuclear ITS analysis as distinct species, although relationships are unresolved in the plastid analysis. Five sspecies in the *E. coccinea* complex are accepted as distinct – leaving out three (*E. caespitosa*, *E. coccinea*, and *E. lisowskiana*).

The five accessions of *E. coccinea* (two from Zimbabwe, two from Cameroon, one of unknown origin) included here do not form a monophyletic group in either plastid or nuclear phylogeny. They nonetheless all match scanned images of the type specimen of *E. coccinea* [*Cacalia coccinea* Sims; Bot. Mag. 16: t. 564 (1803)]. Based on the phenetic results (Figure 5; Chapter 2) and the strongly supported clade (F1; BS = 91%; PP = 1.00; Figure 3.2) in which the five accessions of *E. coccinea* occur but not all sister to each other and with other species present, it can be concluded that *E. coccinea* is not a monophyletic species. Either *Emilia coccinea* sensu stricto should be further subdivided or many more species would need to be synonymised to address the issue of monophyly.

Emilia caespitosa (from Zimbabwe) is strongly supported as sister to the two accessions of *E. coccinea*, also from Zimbabwe, in the ITS phylogeny, but this relationship is unresolved in the same clade in the plastid phylogeny. *Emilia coccinea* and *E. caespitosa* are very likely synonymous, as indicated by the phenetic analyses (Chapter 2; Mapaya and Cron 2016), where *E. caespitosa* grouped with clusters of *E. coccinea*. There was also considerable overlap in morphological features in the univariate analyses indicating that they are one variable species (Mapaya and Cron 2016). An alternative scenario is that these two species are distinct but hybridizing and/or introgressing where they co-occur. *Emilia lisowskiana*, which grouped together with *E. coccinea* specimens in the cluster analysis, is here unresolved together with *E. coccinea*4 and *E. praetermissa* in the nuclear analysis and is also unresolved in Clade F1 of the plastid analysis; thus there is no conclusive evidence as to whether it is a distinct species or not. Morphologically, *E. lisowskiana* differs from *E. coccinea* by having upper leaves that are ovate-lanceolate to mostly ovate and style branch apices that are unappendaged and epapillose (Jeffrey 1997).

The Morphological Species Concept applied by Jeffrey (1997) to the *E. coccinea* complex is not supported here – either by the phenetic analysis (especially by two species - *E. caespitosa* and *E. coccinea* that were phenetically indistinguishable because of morphological character intergradation), or by the phylogenetic analysis. The *E. coccinea* complex is not monophyletic and there are no clear morphological synapomorphies recognised for this group - thus the Phylogenetic Species Concept is not applicable to this complex. The requirement of the Phylogenetic Species Concept (sensu Cracraft 1983) is that taxa are monophyletic, diagnosable clusters of individuals and species are the smallest diagnosable clusters. Diagnostic characters are used to indicate independent evolutionary histories and delimit species boundaries. With the additional funding and time, adding more *Emilia* species (that failed to amplify) to the plastid and nuclear data sets might result in resolution and better support of the *E. coccinea* clade resulting in informed decisions of whether to merge some of the other *Emilia* species into a single more broadly delimited species that includes *E. coccinea* and *E. caespitosa* specimens, or whether to split species in the *E. coccinea* complex. Nonetheless an expanded *E. coccinea* and *E. caespitosa* that are synonymised would still be polyphyletic with other species nested in it.

The lack of resolution within the *E. coccinea* complex clade in the phylogeny based on the plastid data and the incongruence between the nuclear and plastid phylogenies makes it difficult to apply the Phylogenetic Species Concept, since species boundaries are estimated by concordant clades of multi-gene genealogies (Taylor *et al*. 2000). The same challenge of incongruence between plastid and nuclear data sets has also been reported in the phylogenetic analyses and species delimitation among members of the Asteraceae viz. *Centaurea* sect. *Jacea* (Mill.) Pers. and sect. *Phrygia* Pers. where hybridization and the occurrence of shared ancestral polymorphisms were observed (López-Alvarado *et al*. 2014).

Outgroup relationships

Outgroup relationships included in this study are more clearly resolved when nuclear data are used and the groupings recovered are consistent with previous molecular studies (e.g. Pelser *et al*. 2007, 2010; Cron *et al*. 2008; Cron 2013). *Pericallis* and the *Senecio* segregates group are sister to *Bethencourtia*, *Senecio lineatus* and the main *Emilia* clade (E) in the *trn*L-*trn*F analysis. In the ITS analysis, *Pericallis* is sister to the clade (C) comprising most *Emilia* species, *Emiliella*, and *Bafutia*. *Pericallis* (endemic to the Canary Islands) is similar to *Emilia* in having ecalyculate capitula that are corymbose, and florets with truncate style branches, although it has radiate capitula and most *Emilia* species have discoid capitula. *Bethencourtia*

is also from the Canary Islands, and is also similar to *Emilia* in having radiate capitula (although the numerous yellow flowerheads are mostly small), acute to obtuse papillose style branches, and balusterform filament collars (Nordenstam 2006). *Bethencourtia* mostly differs from *Emilia* in having a shrubby habit, caudate anthers, and distinct carpopodium of several cell layers (Nordenstam 2006).

Incongruence between plastid *trn*L-*trn*F and nuclear ITS phylogenies

Incongruence between plastid and nuclear phylogenies has been previously shown in the Senecioneae (e.g. Cron *et al*. 2008, 2013; Pelser *et al*. 2010) and hybridization, introgression, and ILS have been proposed as the most common biological explanations for incongruence (Maddison 1997; Baack and Riesberg 2002; Degnan and Rosenberg 2009; Jones *et al*. 2014; Roy *et al*. 2015). In the phylogenies reconstructed here, incongruence is clearly evident in the placement of the large basal clade of 17 *Emilia* species from sect. *Emilia* (Clade A) outside the main *Emilia* clade in the plastid phylogeny, compared to only *E. baumii* and *E. graminea* in the nuclear parsimony phylogeny. Chloroplast capture, i.e. introgression of the chloroplast from one species into another after a hybridization event and subsequent backcrossing of first filial (F1) – generation of offspring with parental types (Wolfe and Elisens 1995; Yu *et al*. 2013), is a likely explanation for the phylogenetic discord observed here (Tsitrone *et al*. 2003). Chloroplast capture has also been documented in various taxa including *Helianthus* of tribe Heliantheae of the Asteraceae, as well as *Pinus*, and *Quercus* (Rieseberg and Soltis 1991). In *Helianthus* sect. *Helianthus*, two species *H. bolanderi* and *H. debilis* subsp. *silvestris* had discordant positions in nuclear vs. plastid phylogenies and this was attributed to chloroplast capture through introgression or hybrid speciation (Rieseberg and Soltis 1991).

Hybridization and/or introgression are also evident in the history of *Emilia*, as shown by the different positions of *E. transvaalensis* in the plastid vs. nuclear analyses, supporting earlier findings by Cron (2013). *Emilia transvaalensis* does not seem to share many morphological features with the gynuroids (except discoid capitula), but shows significant similarity to *Emilia* in the following features: herbaceous and erect habit, lilac florets, rounded to truncate style branches with short papillae, balusterform filament collars, and cylindric five-angled pubescent cypselas (Hilliard 1977; Cron 2013, 2014). *Kleinia* on the other hand, is succulent and has phyllaries that are mostly calyculate whereas the scrambling non-succulent *Solanecio* is also distinguished from *E. transvaalensis* by its calyculate phyllaries (Halliday 1988; Bremer 1994). Since *E. transvaalensis* is morphologically similar

to *Emilia* species it can be concluded that *E. transvaalensis* is possibly of hybrid origin and/or introgression might have occurred (Cron 2013).

Hybridization has also previously been reported in other *Emilia* species: Olorode and Olorunfemi (1973) suggested that *E. praetermissa* is an allopolyploid originating from hybridization between *E. coccinea* (n = 5) and *E. sonchifolia* (n = 5) followed by a doubling of chromosomes. Here, *E. praetermissa* (from Cameroon) occurs in an unresolved clade (F1) together with *E. coccinea* in the plastid analysis (for which *E. sonchifolia* var. *sonchifolia* was not sampled). In the nuclear analysis, *E. praetermissa* is in a polytomy comprising *E. coccinea* (also from Cameroon) and *E. lisowskiana*. Single base pair (bp) insertions (positions 667, 717) in the nuclear ITS data support the relationship of *E. praetermissa* and *E. coccinea*4 from Cameroon, as do two unique point mutations (at positions 628 and 663). On the other hand, no unique mutations are shared with *E. sonchifolia*. Further research using other molecular markers, such as amplification fragment length polymorphisms (AFLPs) or simple sequence repeats (SSRs) is therefore needed to confirm whether *E. praetermissa* is of hybrid origin.

The placement of the two accessions of *E. citrina* at different positions in the plastid and nuclear phylogenies possibly suggests that they may be distinct taxa. In the nuclear phylogeny, *E. citrina*2 from Madagascar, groups with two other species *E. humifusa* and *E. infralignosa* also from Madagascar, whereas the other accession, *E. citrina*1 from Malawi, groups with *E. coccinea* from Cameroon and *E. sonchifolia* and *E. exserta* (GenBank sequences from specimens of unreported origin). This could possibly suggest that one of the accessions (probably *E. citrina*1 from Malawi) has been misidentified since *E. citrina*2 is endemic to Madagascar. Two accessions of *Emilia humifusa* (1 and 2) also occur at different positions in both the nuclear ITS and plastid *trn*L-*trn*F phylogenies (Figures 3.1, 3.2) and appear to be distinct lineages. The other scenario is that one of these species could have been misidentified by past taxonomy. *Emilia humifusa* is endemic to Madagascar and the two accessions both occur in Antsiranana and Fianarantsoa Provinces. Nonetheless *E. humifusa* (1 and 2) are herbs of almost the same height (up to 60 cm) with glabrous stems. They are morphologically similar in the following characters: leaves that are sessile, auriculate, and amplexicaul with shallowly dentate leaf margins, solitary, campanulate capitula with 10–12 phyllaries, and orange florets. The scanned specimen *E. humifusa*1 was used to determine the morphological characters thus it was impossible to diagnose some reproductive characters and its habitat was not recorded on the specimen. However, *E. humifusa*2 is characterised by having truncate, unappendaged style branch apices with few minute hairs and pubescent

cypselas and occurs together with mosses amongst granite outcrops. *Emilia humifusa* (1 and 2) also occur at different altitude of 500 m and 1 374 m (respectively) above sea level. The taxonomic statuses of *E. citrina* (1 and 2) and *E. humifusa* (1 and 2) need further investigation.

Incongruence is also observed amongst certain outgroup taxa (e.g. *Cineraria*, *Steirodiscus*, *Oresbia*, *Dendrosenecio*) in their positions in the plastid versus nuclear phylogenies. These corroborate Pelser *et al*.'s (2010) findings, for which incongruence, hybridization, and ILS were proposed as reasons. The processes of hybridization and lineage sorting may be difficult to distinguish from each other since they result in similar gene tree topologies (Holder *et al*. 2001; Holland *et al*., 2008; Joly *et al*. 2009), thus it is possible that ILS could explain the observed incongruence here, although ILS is not widespread in the Senecioneae (Pelser *et al*. 2010). Coalescent-based approaches are also used to distinguish ILS from hybridization by testing for randomness (ILS) and non-randomness (hybridization) in patterns of incongruence using more than two unlinked DNA data sets (Buckley *et al*. 2006; Pelser *et al*. 2010). This approach could not be applied here since we only had two unlinked data sets (plastid and nuclear), and so hybridization and ILS could not be distinguished with certainty as explanations of the observed incongruence.

Conclusions and recommendations for future study

Emilia is strongly supported as both paraphyletic and polyphyletic in the nuclear and plastid phylogenetic analyses reported here. Seventeen *Emilia* species are placed outside '*Emilia*' in the plastid phylogeny, whereas in the nuclear phylogeny only one of these species, *E. baumii*, together with *E. graminea* is outside '*Emilia*'. Chloroplast capture through hybridization and introgression may have occurred resulting in the erroneous placement of these seventeen species outside of *Emilia*. *Emilia baumii*, however, is placed outside '*Emilia*' in both plastid and nuclear phylogenies which indicates that it (at least) should be removed from this genus. The genera *Emiliella* and *Bafutia* are both nested within *Emilia* suggesting that they do not warrant separate generic status and should be placed in *Emilia*. Jeffrey's (1986) sections *Spathulatae* and *Emilia* are not upheld here as they do not form monophyletic groups in either plastid or nuclear phylogenies. *Psednotrichia xyridopsis* (O.Hoffm.) Ander. & P.O.Karis, and *P. newtonii* (O.Hoffm.) Ander. & P.O.Karis) not sampled here should be included in further investigatations to verify the monophyly of *Emilia*.

The molecular phylogenies also revealed that five out of eight species recognised by Jeffrey (1997) in the *E. coccinea* complex are distinct, but two (*E. coccinea* and *E. caespitosa*) are indistinguishable, corroborating the findings of the phenetic study (Chapter 2) that *E. coccinea* and *E. caespitosa* might be considered as one heterogeneous species because of considerable overlap in their morphological features, habitats, and geographical distribution. To fully resolve relationships among these closely related species in the *E. coccinea* complex (including the status of *E. lisowskiana*), further investigations using additional DNA regions need to be carried out.

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Appendix 3.1

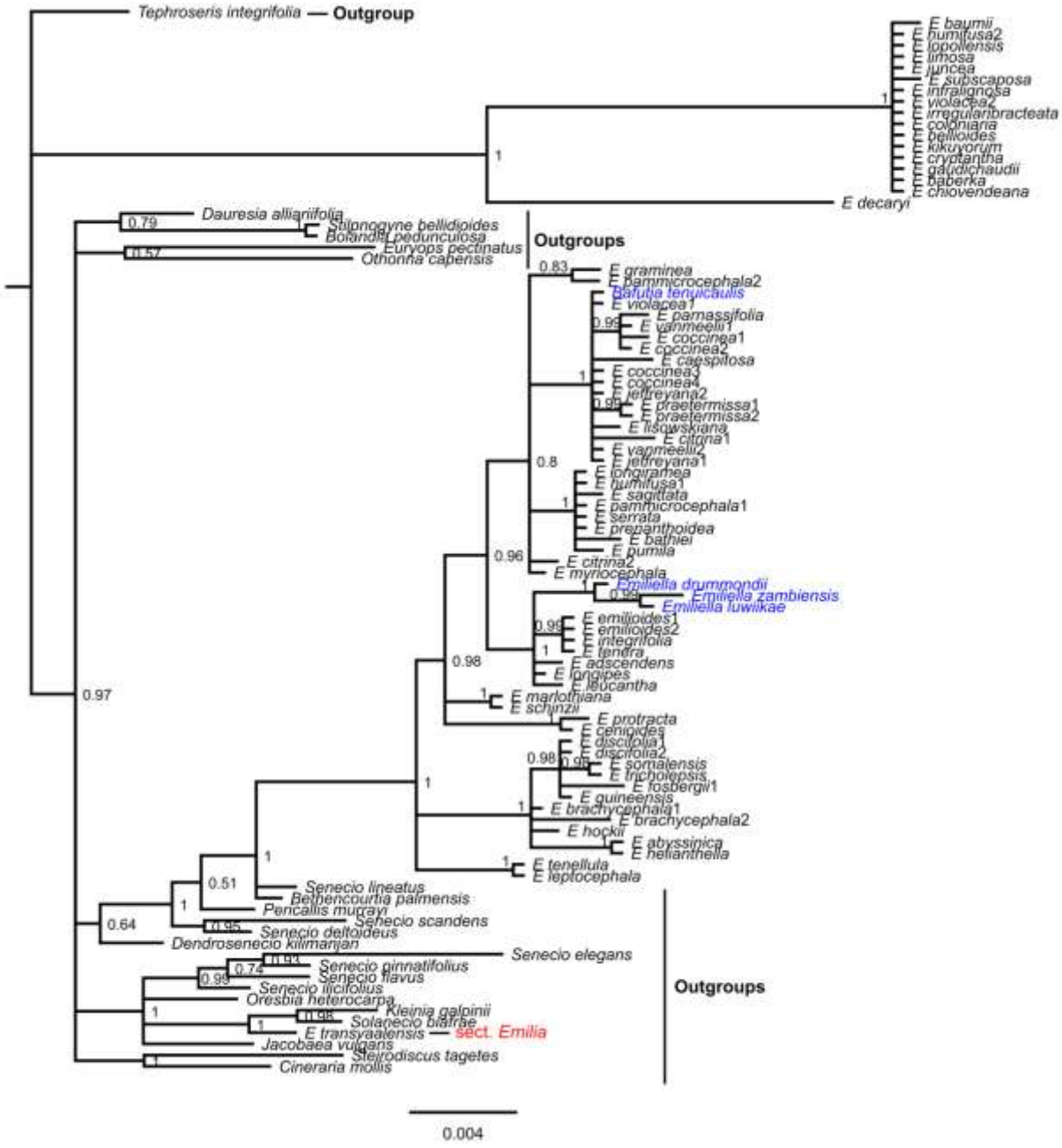

Figure A1. The 50 % majority rule consensus tree in the Bayesian analysis of the plastid *trn*L-*trn*F data including indels. Posterior probabilities are indicated on the branches. Genera *Bafutia* and *Emiliella* are highlighted in blue.

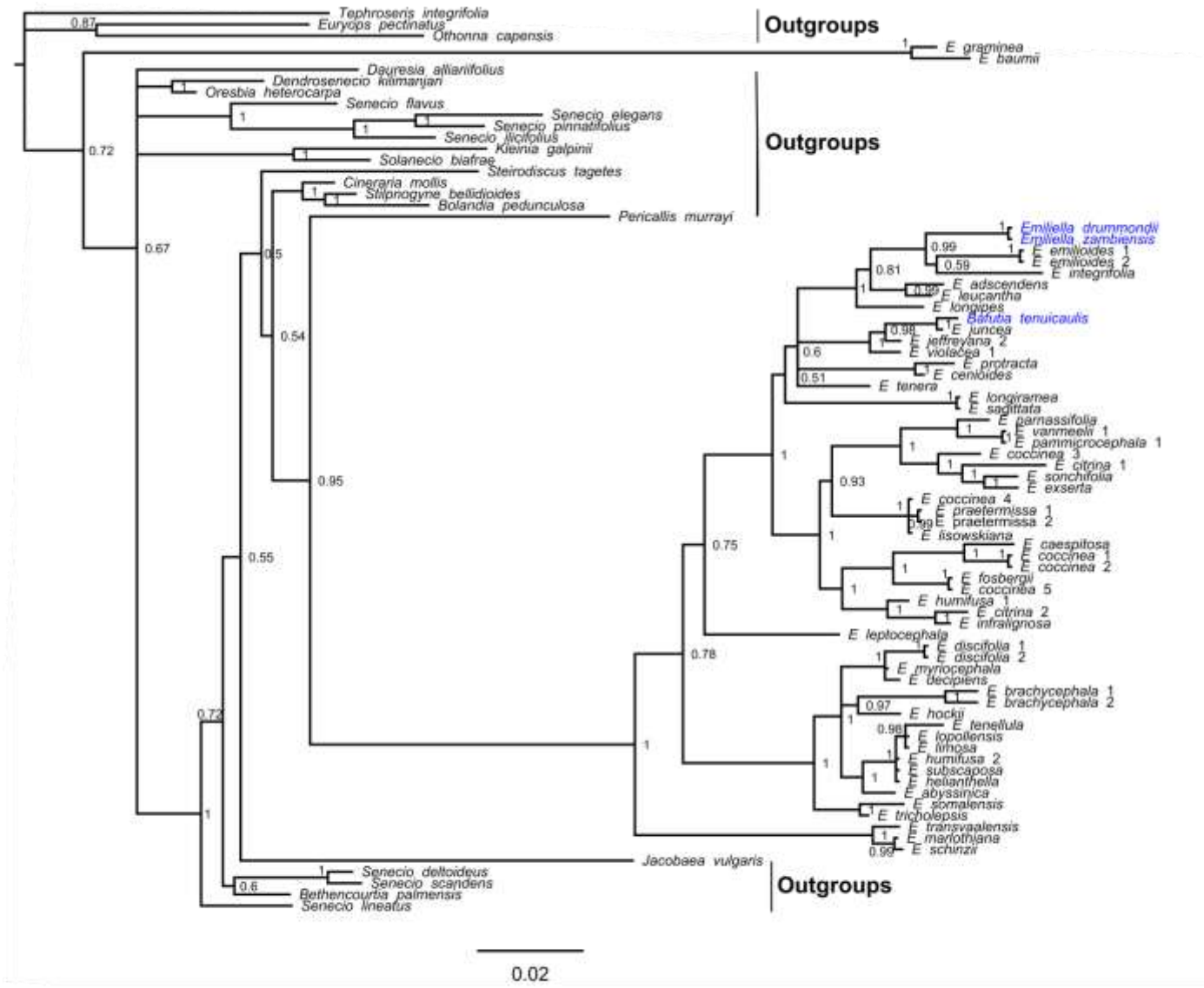

Figure A2. The 50 % majority rule consensus tree in the Bayesian analysis of the nuclear ITS data including indels. Posterior probabilities are indicated on the branches. Genera *Bafutia* and *Emiliella* are highlighted in blue.

Table A1. Voucher specimens (*Emilia*, *Emiliella*, *Bafutia*, and closely related genera in Senecioneae), GenBank accession numbers (*ETS*, *ITS*, trnL-trnF; -, + denotes a missing and present sequence respectively).

Species	Voucher/Herbarium	Locality/Origin	ETS	ITS	*trn*L-F
E. abyssinica (Sch. Bip. ex A.Rich.) C.Jeffrey	R. J. Mapaya M41 **(J)**	Zimbabwe, Mrehwa, Nyanduri village	+	-	-
E. abyssinica (Sch. Bip. ex A.Rich.) C.Jeffrey	R. J. Mapaya M49 **(J)**	Zimbabwe, Mrehwa, Nyanduri village	-	+	+
E. adscendens DC.	A. Anderberg, J. Smedmark *et al.* AS193 **(MO)**	Madagascar, Antananarivo **(Endemic)**	-	+	+
E. schinzii (S.Moore) C.Jeffrey	G. V. Cron & M. Goodman 789 **(J)**	Zimbabwe	-	+	+
E. baberka (Hutch.) C.Jeffrey	R. Letouzey L3500 **(MA)**	Cameroon, Dang Haoussa	-	-	+
E. bathiei Humbert	H. Perrier de la Bathei B17564 **(P)**	Madagascar **(Endemic)**	-	-	+
E. baumii (O.Hoffm.) S.Moore	N. C. Chase NC5235 **(LISC)**	Zimbabwe, Nyanga Distr.	-	+	+
E. bellioides					
E. bracycephala (R.E.Fr.) C.Jeffrey	R. J. Mapaya M06 **(J)**	Zimbabwe, Mutasa Distr.	-	+	+
E. bracycephala (R.E.Fr.) C.Jeffrey	R. J. Mapaya M46 **(J)**	Zimbabwe, Domboshava Heritage Centre	-	+	+
E. caespitosa Oliv.	A. Ntemi Sallu N167 **(MA)**	Tanzania, Tanga, Muheza	-	+	+
E. cenioides C.Jeffrey	P. Kuchar K23373 **(MO)**	Tanzania, Singida Distr.	-	+	+
E. chiovendeana (Muschl.) Lisowski	H. G. Msiska HM223 **(MAL)**	Malawi, Mulanje Distr.	-	-	+
*E. citrina*1 DC.	Nangoma & Patel NP111 **(MAL)**	Malawi, Thyolo Distr.	+	+	+
*E. citrina*2 DC.	L. Nusbaumer & P. Ranirison NR1611 **(MO)**	Madagascar, Antsiranana	+	+	+
E. coccinea (Sims) G.Don	R.J. Mapaya & C. Chapano M38 **(J)**	Zimbabwe, Mazowe Botanic Reserve	-	-	+
*E. coccinea*1 (Sims) G.Don	R.J. Mapaya & C. Chapano M47 **(J)**	Zimbabwe, Mazowe Botanic Reserve	-	+	+
*E. coccinea*2 (Sims) G.Don	R.J. Mapaya M48 **(J)**	Zimbabwe, Mrehwa, Koga village	+	+	+
*E. coccinea*3 (Sims) G.Don	E. Biye B313 **(YA)**	Cameroon	-	+	+
*E. coccinea*4 (Sims) G.Don	B821 **(YA)**	Cameroon	-	+	+
E. cryptantha C.Jeffrey	K. A. Lyle LK5824 **(EA)**	Uganda, Masaka Distr.	-	+	+
E. decaryi Humbert	J. Leandri LJ1784 **(P)**	Madagascar **(Endemic)**	-	-	+

E. decipiens C.Jeffrey	G. Pope & A.R. Smith PS2245 **(LISC)**	Malawi, Dedza Distr	-	+	-
*E. discifolia*1 (Oliv.) C.Jeffrey	R.J. Mapaya M08 **(J)**	Zimbabwe, Mutasa, Bonda	+	+	+
*E. emilioides*1 (Sch. Bip.) C.Jeffrey	Friis, Aweke *et al*. FA2061 **(BR)**	Ethiopia, Addis Ababa	-	+	+
*E. emilioides*2 (Sch. Bip.) C.Jeffrey	W.J.J.O & de Wilde *et al.* OW 4640 **(BR)**	Cameroon	-	+	+
E. fosbergii Nicolson	Unknown Sn03 **(P)**	Unknown	-	-	+
E. gaudichaudii Gagnep.	Unknown Sn05 **(P)**	Unknown	-	-	+
E. graminea DC.	Phillipson, Burki *et al.* PB5792 **(MO)**	Madagascar, Fianaratsoa **(Endemic)**	-	+	+
E. guineensis Hutch. & Dalziel	A.J.B. Chevalier C18276 **(P)**	Guinea	-	-	+
E. helianthella C.Jeffrey	S.Bigwood, K. Hoeselaar *et al.* BH6225 **(MO)**	Tanzania, Manyoni Distr.	-	+	+
E. hockii (De Wild. & Muschl.) C.Jeffrey	Q. Luke & P. Luke LL12901 **(EA)**	Tanzania, Makete Distr.	-	+	+
*E. humifusa*1 DC.	P. Derleth DP36 **(P)**	Madagascar, Antsiranana	-	+	+
*E. humifusa*2 DC.	S.T. Malcomber *et al.* MS1369 **(MA)**	Madagascar, Fianarantsoa	-	+	+
E. infralignosa Humbert	B. Lewis, S. Rasoavimbahoaka *et al.* LR1238 **(MO)**	Madagascar, Antsiranana **(Endemic)**	-	-	+
E. integrifolia Baker	Malombe & Fisher MF1288 **(EA)**	Kenya, Kakamega Distr.	+	+	+
E. irregularibracteata (De Wild.) C.Jeffrey	P. Bamps & F. Malaisse BM8133 **(MO)**	DRC	+	-	+
*E. jeffreyana*1 Lisowski	P. Auquier AP3623 **(BR)**	Rwanda, Cyangugu	-	+	+
*E. jeffreyana*2 Lisowski	B. Bytebier & WRQ Luke BL2750 **(BR)**	DRC, Maniema Prov.	-	+	+
E. juncea Robyns	P.K. Rwaburindore R4813 **(MO)**	Uganda, Buhweju, Bushenyi	-	-	+
E. kikuyorum R.E.Fr.	L. Ojiambo OL420 **(EA)**	Kenya, Trans Nzoia S. S. National Park	-	+	+
E. leptocephala (Mattf.) C.Jeffrey	P.H. Smith SP1463 **(LISC)**	Botswana, Northern	-	+	+
E. leucantha C.Jeffrey	Bigwood *et al.* BD5361 **(EA)**	Tanzania, Shumbawanga Distr.	-	+	+
E. limosa (O.Hoffm.) C.Jeffrey	E. A. Robinson RE3597 **(SRGH)**	Zambia, Mwinilunga Distr.	-	+	+
E. lisowskiana C.Jeffrey	F. Cabezas *et al*. C1342 **(MA)**	Equatorial Guinea, Kie Ntem	-	+	+
E. longipes C.Jeffrey	P. Kuchar K23938 **(MA)**	Tanzania, Singida Distr.	-	+	+
E. longiramea (S.Moore) C.Jeffrey	A. F. Bradley, G. Walters *et al.* BW1174 **(MO)**	Gabon	-	+	+
E. lopollensis (Hiern) C.Jeffrey	Teix & M. M. TM8710 **(LISC)**	Angola: BIE Distr.	-	+	+
E. marlothiana (O.Hoffm.) C.Jeffrey	G. V. Cron & M. Goodman 781 **(J)**	Zimbabwe	-	+	+
*E. pammicrocephala*1 (S.Moore) C.Jeffrey	F. L. Hendrickx HF406 **(P)**	DRC	-	+	+
E. parnassifolia (De Wild. & Muschl) S.Moore	Pope, Smith & Goyder PS2166 **(LISC)**	Zambia, Kaputa Distr.	-	+	+

E. pumila DC.	H. Humbert H24450 **(P)**	Madagascar, Ambatobiribiry	-	+	+
*E. praetermissa*1 Milne-Redh.	E. Biye B310 **(YA)**	Cameroon	+	+	+
*E. praetermissa*2 Milne-Redh.	E. Biye B311 **(YA)**	Cameroon	-	+	+
E. prenanthoidea DC.	Unknown Sn83 **(P)**	Unknown	-	-	+
E. sagittata (Vahl) DC.	A.F. Bradley, G. Walters *et al.* BW1065 **(MA)**	Gabon	-	+	+
E. serrata Humbert	H. Perrier de la Bathie B15900 **(P)**	Madagascar **(Endemic)**	-	-	+
E. somalensis (S.Moore) C.Jeffrey	J.A. Mlangwa *et al.* MG844 **(MA)**	Kenya, Kajiado Distr.	-	+	+
E. subscaposa Lisowski	M. Reekmans RM374 **(BR)**	Burundi, Bujumbura	-	+	+
E. tenellula (S.Moore) C.Jeffrey	P. A. Smith P1463 **(SRGH)**	Botswana, Northern Distr.	-	+	+
E. tenera (O.Hoffm.) C.Jeffrey	J & J. Lovett LJ774 **(MO)**	Tanzania	-	-	+
E. tricholepsis C.Jeffrey	R.E. Gereau G6408 **(MO)**	Tanzania	-	+	+
*E. vanmeelii*1 Lawalrée	A. Bodenghien BA340 **(BR)**	DRC, Koapamitono	-	+	+
*E. vanmeelii*2 Lawalrée	Bamps & Malaisse BM8506 **(BR)**	DRC, Mitwaba-Manono	-	-	+
*E. violacea*1 Cronquist	Bigwood, Mbago *et al.* BM2713 **(EA)**	Tanzania, Mpanda Distr.	-	+	+
*E. violacea*2 Cronquist	D. Sitoni S1121 **(LISC)**	Tanzania, Mwanza, Magu Distr.	-	+	+
Em. drummondii var. *drummondii* Torre	Bingham, M.G. B9747 **(BR)**	Zambia, Western Province, Kasuka village	-	+	+
Em. luwiikae D.J.N. Hind & Frisby			-	-	+
Em. zambiensis Torre			-	+	+
Bafutia tenuicaulis C.D.Adams	Munyenyeni & Sileshi MS825 **(YA)**	Cameroon, N-W Province	-	+	+
*E. coccinea*5 (Sims) G.Don	P. Pelser	GenBank	-	AF459966.1	-
*E. discifolia*2	Cron *et al.*	GenBank	-	AY953930.1	AY952920.1
E. exerta Fosberg	Pelser *et al.*	GenBank	-	EF538195.1	-
E. fosbergii Nicolson	Wiezorek AMW3171	GenBank	-	GQ478091.1	-
E. protracta S.Moore	G.V. Cron & M. Goodman 490	GenBank	-	KC900104.1	KC900114.1
E. sonchifolia var. *javanica* (L.) DC	Hsieh *et al*.	GenBank	-	EF108405.1	-
E. transvalensis (Bolus) C.Jeffrey	D. McCallum 1050 **(J)**	GenBank	-	KC900105.1	KC900115.1
Be. palmensis (Nees) Choisy	Pelser *et al.*	GenBank	-	EF538160.1	GU817975.1
Bolandia pedunculosa (DC.) Cron	G. V. Cron	GenBank	-	AY953925	AY952915.1
C. mollis E. Mey. ex DC.	G. V. Cron	GenBank	-	AY953923	AY952913.1
Dauresia alliarifolia (O.Hoffm.) B.Nord. & Pelser	Coleman (ITS); P. Pelser (*trn*L-F)	GenBank	-	AF457413.1	GU817991.1
D. kilimanjari subsp.	Cron *et al.*	GenBank	-	AY953933.1	AY952923.1

cottonii (Hutch. & G.Taylor) E.B.Knox					
Euryops pectinatus	Devos *et al*.	GenBank	-	EU667514.4	EU670134.1
Jacobaea vulgaris Gaertn.	Pelser *et al.*	GenBank	-	GU818567	EF028725.1
Kleinia galpinii	Cron *et al.*	GenBank	-	AY953934.1	AY952924.1
Othonna capensis L.H.Bailey	Pelser *et al*.	GenBank	-	AF459960.1	EF028727.1
Oresbia heterocarpa Cron & B.Nord	Cron *et al*.	GenBank	-	AY953935.1	AY952925.1
P. murrayi (Bornm.) B.Nord.	Pelser *et al.*	GenBank	-	EF538285.1	EF538115.1
Senecio deltoideus Less.	Cron *et al*.	GenBank	-	AY953927.1	AY952917.1
Senecio elegans L.	Pelser *et al.*	GenBank	-	GU818642.1	GU818064.1
Senecio lineatus (L.f.) DC.	Pelser *et al.* (ITS); Bayer *et al*. (trnL-F)	GenBank	-	AF459939.1	AF100515.1
Senecio flavus (Decne.) Sch. Bip.	Pelser *et al*.	GenBank	-	GU818648.1	EF028729.1
Senecio ilicifolius L.	Pelser *et al*.	GenBank		GU818662.1	GU818074.1
Senecio pinnatifolius var. *lanceolatus* (Benth.) I.Thomps.	Pelser *et al*.	GenBank	-	GU818680.1	GU818081.1
Senecio scandens Buch.-Ham.	Chen & Han	GenBank	-	FJ980344.1	-
Solanecio biafrae (Oliv. & Hiern) C.Jeffrey	Pelser *et al.*	GenBank	-	GU818711.1	GU818093.1
Steirodiscus tagetes (L.) Schltr.	P. Pelser	GenBank	-	GU818720.1	GU818094.1
Stilpnogyne bellidioides DC.	G. V. Cron	GenBank	-	KC900101.1	KC900111.1
Tephroseris integrifolia subsp. *integrifolia* (L.) Clairv.	Pelser *et al.*	GenBank	-	GU818724.1	GU818100.1

Table A2. Data matrix for insertions / deletions (indels) using ITS data set. **Key:** 0 = gap absent, 1 = gap present, - = inapplicable, ? = missing data.

	Position of coded indel
	1111111111222222222233333333334444444444555555555566
Accession	1234567890123456789012345678901234567890123456789012345678901
Tephroseris integrifolia	1010000111110111101110110111101110011111110011011011000 1001
Euryops pectinatus	1010000111110111001110110111101110011111110011011011000 1001
Othonn capensis	1010000111110111001110110111101110011111110011011011010 1001
E. graminea	1010110110111110110111000101010111111111011100110001010011001
E. baumii	1010110110111110110111000101010111111111011100011000101001 1001
Dauresia alliariifolius	1010000111110111101110110111101110111111110011011011000 1011
Dendrosenecio kilimanjari	1010000111110111111110110111101110111111110011011011000 1011
Oresbia heterocarpa	1010000111110111111110110111101110111111110011011011000 1011
Senecio flavus	1010000111100111100110110101101111101111111100110110110101010
Senecio elegans	1010000111000111100110110111-1111101111011100110110110101010
Senecio pinnatifolius	1010000111000111100110110111101110111011100110110110101010
Senecio ilicifolius	1010000111100111100110110111101110111111110011011011010 1010
Kleinia galpinii	1010000111110111101110110111101011101111111100110110110001011
Solanecio biafrae	1010000111110111101110110111101011101111111100110110110101011
Steirodiscus tagetes	1010000111110111101110110111101110111111110011011011000 1011
Cineraria mollis	1010000111110011101110110111101110111111110011011010000 1011
Stilpnogyne bellidioides	1010000111110011101110110111101110111111110011011010000 1011
Bolandia pedunculosa	1010000111110011101110110111101110111111110011011010000 1011
Pericallis murrayi	1010000111110111101111110111101110111111110011011011000 1011
Senecio deltoideus	1010000111110111101110110111101110111111110011011011000 1011
Senecio scandens	1010000111110111101110110111101110111111110011011011000 1011
Senecio lineatus	1010000111110111101110110111101110111111110011011011000 1011
Jacobaea vulgaris	1010000111110111101110110111101110111111110011011011000 1011
Bethencourtia palmensis	1010000111110111101110110111101110111111110011011011000 1011
Emiliella drummondii	1000000111110110101111110101101111101111111100111110110001011
Emiliella zambiensis	1000000111110110101111110101101111101111111100111110110001011
Bafutia tenuicaulis	1000000111110111101111110101101111101111111100111110110001011
E. transvalensis	1100001101110111101111110101101111101011101000111110110001011
E. marlothiana	1100001101110111101111110101100111101111101000111110110001011
E. schinzii	1100001101110111101111110101100111101111101000111110111001011
E. emilioides 1	1000000111110111101111110101101111101101110100111110110101011
E. emilioides 2	1000000111110111101111110101101111101101110100111110110101011
E. integrifolia	1000000111110111101111110101101111101111111100111110110101011
E. discifolia 1	1000000111110111101101110101101111101111111100101110111101111
E. discifolia 2	1000000111110111101101110101101111101111111100101110111101111
E. brachycephala 1	1000000111110111101101110101101111101111111100111110111101111
E. brachycephala 2	1000000111110111101101110101101111101111111100111110111101111
E. tenellula	1000000011110111101101110101101111100-1111110111111011110111 1111
E. somalensis	1000000111110111101101110101111111101111111100111110111101111
E. leptocephala	1000000111110111101111110101101111101111111100111110111000011
E. protracta	0001000111110111101111110101101111101111111100011110111000011
E. cenioides	0001000111110111101111110101101111101111111101011110111000011
E. violacea 1	1000000111110111101111110101101111101010111100111110111000011
E. parnassifolia	1000000111110111101111110101101111101111111100111110111000011
E. vanmeelii 1	1000000111110111101111110101101111101111111000111110111000011
E. caespitosa	1000000111110111101111110101001101100111111010111110111000011
E. coccinea 1	1000000111110111101111110101001101100111111010111110111000011
E. coccinea 2	1000000111110111101111110101001101100111111010111110111000011
E. coccinea 3	1000000111110111101111110101101111101111111100111110111000011
E. coccinea 4	1000000111110111101111110101101111101111111100111110111000011
E. jeffreyana 2	1000000111110111101111110101101111101111111100111110111000011
E. praetermissa 1	1000000111110111101111110101101111101111111100111110101000011
E. praetermissa 2	1000000111110111101111110101101111101111111100111110101000011

E. lisowskiana	10000001111101111011111101011011111011111111001111101110000 11
E. adscendens	10000001111101111011111101011011111011111111001111101110000 11
E. humifusa 1	10000001111101111010111010110111110111111110011111011100001 1
E. humifusa 2	10000000111101111011101101101111100-1111110011111011110011 1
E. citrina 1	10000001111101111011111101011011111010101110001111101110000 11
E. citrina 2	10000001111101111010111010110111110111111110011111011100001 1
E. longiramea	10000001111101111011111101011011111011111111001111101110000 11
E. sagittata	10000001111101111011111101011011111011111111001111101110000 11
E. longipes	10000001111101111011111101011011111011011111001111101110000 11
E. juncea	10000001111101111011111101011 01?? ???????????????????????? ????
E. tenera	10000001111101111011011010110 1??????????????????????????????????
E. infralignosa	10000001111101111010111010110 1??????????????????????????????????
E. leucantha	10000001111101111011111101011011111011111111001111101110000 11
E. lopollensis	10000000111101111011011010110 1111100-1111110111111011110011 1
E. limosa	10000000111101111011011010110 1111100-1111110111111011110011 1
E. subscaposa	10000000111101111011011010110 1111100-1111110011111011110011 1
E. hockii	10000001111101111011011010110 1111110111111110011111011110011 1
E. tricholepsis	10000001111101111011011010111 1111011111111001111101110011 1
E. abyssinica	10000001111101111011011010110 1111011111111001111101110011 1
E. helianthella	10000000111101111011011010110 1111100-1111110011111011110011 1
E. myriocephala	10000001111101111011011010110 1111011111111001010111011110011 1
E. decipiens	10000001111101111011011010110 1111011111111001010111011110011 1
E. pammicrocephala 1	10000001111101111011111101011011111011111111001111101110000 11
E. sonchifolia	10000001111101111011111101011111111011111111001111101110000 11
E. fosbergii	10000001111101111011111101011011101011111111001111101110000 11
E. coccinea 5	10000001111101111011111101011011101011111111001111101110000 11
E. exserta	10000001111101111011111101011111111011111111001111101110000 11

Table A3. Data matrix for insertions / deletions (indels) using *trn*L-*trn*F data set. **Key:** 0 = gap absent, 1 = gap present, - = inapplicable, ? = missing data.

	Position of coded indel
	1111111111222222222233333
Accession	1234567890123456789012345678901234 5
Tephroseris integrifolia	11011010001100000111001111111110001
Dauresia alliariifolia	11011010001100000111001111111110001
Euryops pectinatus	??01101000110010011100111011110001
Othonna capensis	???? ?? ???? ??????01110011101111100 -1
E. graminea	11000100000100000111101111111110001
E. baumii	11011011001110010111101111011010011
Senecio elegans	11011010001100101111011101111111101
Senecio pinnatifolius	11011010001100101111001001111110001
Senecio ilicifolius	11011010001100101111001001111110001
Senecio flavus	?? ?? ?? ??????????1111001011111110001
Senecio scandens	???? ?? ???????????110111111111110001
Senecio lineatus	?? ? ??? ???????????111001111111110001
Senecio deltoideus	11011010001100000111001111111110001
Dendrosenecio kilimanjari	110?10100011000001110011111111110001
Oresbia heterocarpa	11011010001100100111001111111110001
Steirodiscus tagetes	11011010001100100111001111111110001
Cineraria mollis	11011010001100000111001111111110001
Stilpnogyne bellidioides	11011010111101000111001111111110101
Bolandia pedunculosa	11011010111101000111001111111110101
Kleinia galpinii	11011010001100100111000111111100001
Solanecio biafrae	11011010001100101111000111111100001
Pericallis murrayi	11011010001100000111001111111110001
Jacobaea vulgaris	?? ? ??? ??????????0111000111111110001
Bethencourtia palmensis	11011010001100000111001111111110001
Emiliella drummondii	01010110000010000 00-1001111111110001
Emiliella zambiensis	10010110000010000 00-1001111110110101
Bafutia tenuicaulis	11100110000100000111001111111110000
E. transvalensis	11011010001100100111001111111110001
E. marlothiana	11010110001100000111001111111110001
E. schinzii	11010110001100000111001111111110001
E. emilioides 1	11010110000100000111001111111110001
E. emilioides 2	11010110000100000111001111111110001
E. integrifolia	11010110000100000111001111111110001
E. discifolia 1	11010110001100000111001111111110001
E. discifolia 2	11010110001100000111001111111110001
E. brachycephala 1	11010110001100000111001111111110001
E. brachycephala 2	11010110001000000111001111111110001
E. tenellula	11010110001000000110001111111110001
E. leptocephala	11010110001000000110001111111110001
E. somalensis	11010110001100000101001111111110001
E. protracta	11010100001100000111001111111111001
E. cenioides	11010100001100000111001111111111001
E. violacea 1	11100110000100000111001111111110000
E. parnassifolia	11100110000100000111001111111110100
E. vanmeelii 1	11100110000100000111001111111110000
E. caespitosa	11100110001100000111001111111110000
E. coccinea 1	11100110000100000111001111111110000
E. coccinea 2	11100110000100000111001111111110000
E. coccinea 3	11100110000100000111001111111110000
E. coccinea 4	11100110000100000111001111111110000
E. jeffreyana 2	11100110000100000111001111111110000
E. praetermissa 1	11100110000100000111001111111110000
E. praetermissa 2	11100110000100000111001111111110000

E. lisowskiana	1110011000010000011101111111110000
E. adscendens	1101011000010000011100111111110001
E. longiramea	1100011000010000011100111111110001
E. humifusa 1	1100011000010000011100111111110001
E. humifusa 2	1101101100111001011111111011010011
E. citrina 1	1110011000010000011100111111110000
E. citrina 2	1100011000010000011100111111110001
E. sagittata	1100011000010000011100111111110001
E. longipes	1101011000010000011100111111110001
E. leucantha	1101011000010000011100111111110001
E. tenera	1101011000010000011100111111110001
E. lopollensis	1101101100111001011111111011010011
E. limosa	1101101100111001011111111011010011
E. juncea	1101101100111001011111111011010011
E. subscaposa	1101101100111001011111111011010111
E. infralignosa	1101101100111001011111111011010011
E. hockii	1101011000110000011100111111110001
E. fosbergii 1	1101011000110000011100111111110001
E. tricholepsis	1101011000110000010100111111110001
E. abyssinica	1101011000110000011100111101110001
E. helianthella	1101011000110000011100111101110001
E. pammicrocephala 1	1100011000010000011100111111110001
E. myriocephala	?? ? ?? ??????????0011100111111110001
Emiliella luwiikae	0001011000010000000-1001111110110001
E. serrata	1100011000010000011100111111110001
E. violacea 2	1101101100111001011111111011010011
E. vanmeelii 2	1110011000010000011100111111110000
E. jeffreyana 1	1110011000010000011100111111110000
E. prenanthoidea	1100011000010000011100111111110001
E. pammicrocephala 2	1100010000010000011100111111110001
E. bathiei	1100011000010000011100111111110101
E. pumila	1100011000010000011100111111110001
E. decaryi	1101101000111000011111111011010001
E. irregularibracteata	1101101100111001011111111011010011
E. coloniaria	1101101100111001011111111011010011
E. bellioides	1101101100111001011111111011010011
E. kikuyorum	1101101100111001011111111011010011
E. cryptantha	1101101100111001011111111011010011
E. gaudichaudii	1101101100111001011111111011010011
E. baberka	1101101100111001011111111011010011
E. chiovendeana	1101101100111001011111111011010011
E. guineensis	1101011000110000011100111111110001

Table A4: List of the currently recognised *Emilia* species and their distribution.

Species and synonyms	Distribution / Country
EMILIA Cass.	
abyssinica (Sch.Bip. ex A.Rich) C.Jeffrey var. **abyssinica** *Senecio abyssinicus* Sch.Bip. ex A.Rich. *Senecio bellidifolius* A.Rich., non Kunth (nom.illegit) *Senecio quartinianus* Asch.	Nigeria, Cameroon, Central African Republic, Sudan, Democratic Republic of Congo (DRC), Rwanda, Burundi, Uganda, Djibouti, Ethiopia, Kenya, Tanzania, Mozambique, Malawi, Zambia, and Zimbabwe
abyssinica (Sch.Bip. ex A.Rich) C.Jeffrey var. **macroglossa** C.Jeffrey *albocostata* Hiern = **E. marlothiana** (O.Hoffm.) C.Jeffrey	Tanzania
adamagibaensis Mesfin & Beentje	Southern Ethiopia
adscendens DC.	Madagascar
alstonii Fosberg	India
arvensis Mesfin & Beentje	Southern and eastern Ethiopia
amplexicaulis Baker	Madagascar
aurita C.Jeffrey	Tanzania
baberka (Hutch.) C.Jeffrey	Cameroon, Nigeria, Sudan
baldwinii Fosberg	Sri Lanka
bampsiana Lisowski	DRC
basifolia Baker	DRC, Tanzania, Mozambique, Malawi, Zambia
bathiei Humbert	Madagascar
baumii (O.Hoffm) S.Moore *Senecio baumii* O.Hoffm.	D.R. C., Angola
bellioides (Chiov.) C.Jeffrey *Senecio bellioides* Chiov.	Somalia, Kenya
bianoensis Lisowski	DRC
brachycephala(R.E.Fr.) C.Jeffrey	Zambia, Zimbabwe
caespitosa Oliv. *E. macaulayae* Garab. *E. humberti* Robyns	Angola, Burundi, DRC, Kenya, Malawi, Mozambique, Sudan, Rwanda, Tanzania, Uganda, Zambia, Zimbabwe
capillaris Humbert	Madagascar
cenioides C.Jeffrey	Tanzania
chiovendeana (Muschl.) Lisowski [T]*Senecio chiovendeanus* Muschl. [T]*Senecio pammicrocephalus* auct., Maquet, non S.Moore	DRC, Rwanda
citrina DC.	Madagascar
coccinea (Sims) G.Don *E. flammea* auct., non Cass. *E. javanica* auct., non Cass. *E. sagittata* auct., non DC. *coccinea* auct., saltem p.p. quoad plantas Africae occid., et sensu Lisowski, non (Sims) G.Don = **E. lisowskiana** C.Jeffrey	Guinea, Sierra Leone, Liberia, Côte d'Ivore, Ghana, Togo, Benin, Nigeria, Cameroon, Fernando Po, Gabon, Central African Republic, Cabinda, Angola, DRC, Burundi, Uganda, Sudan, Kenya, Tanzania, Mozambique, Zambia, Zimbabwe
coloniaria (S.Moore) C.Jeffrey *Senecio coloniarius* S.Moore	Angola

crepidioides Garab.	Madagascar
crispata C.Jeffrey	DRC, Tanzania,
cryptantha C.Jeffrey	Uganda, Tanzania
debilis S.Moore	Burundi, DRC, Kenya, Uganda
decaryi Humbert	Madagascar
decipiens C.Jeffrey	Tanzania
discifolia (Oliv.) C.Jeffrey *Senecio discifolius* Oliv. *Senecio hoffmannianus* Muschl.	DRC, Rwanda, Burundi, Uganda, Sudan, Ethiopia, Kenya, Tanzania, Zambia, Zimbabwe
djalonensis Lisowski	Guinea
duvigneaudii Lisowski	DRC
emilioides (Sch. Bip) C. Jeffrey	Sudan
exserta Fosberg	India
fallax (Mattf.) C.Jeffrey	Cameroon, Central African Republic
flaccida C.Jeffrey *flammea* auct., non Cass = **E. coccinea** (Sims) G.Don	Tanzania
flammea Cass.	Mozambique
fosbergii Nicolson	Bahamas, Tahiti, Hawaii, eastern Asia
fugax C.Jeffrey	Tanzania, Zambia
gaudichaudii Gagnep.	Tropical Asia, Vietnam, Indo-China
gossweileri (S.Moore) C.Jeffrey *Crassocephalum gossweileri* S.Moore	Angola
graminea DC.	Madagascar
guineensis Hutch. & Dalziel *Senecio schimperi* auct., C.D.Adams in F.W.T.A. II, non Sch. Bip. Ex A.Rich.	Guinea, Malawi
helianthella C.Jeffrey	Tanzania
herbacea *Mesfin & Beentje*	Ethiopia
hiernii C.Jeffrey *Othonna gracilis* Hiern	Angola
hockii (De Wild. & Muschl.) C.Jeffrey *Senecio hockii* De Wild. & Muschl. *Senecio rogersii* S.Moore	DRC, Tanzania, Malawi, Zambia
homblei (De Wild.) C.Jeffrey *Senecio homblei* De Wild. *humbertii* Robyns = **E.caespitosa** Oliv. *humbertii* Robyns var. *angustifolia* Robyns = **E.caespitosa** Oliv.	DRC
humifusa DC. var. humifusa	Madagascar
humifusa DC. var. puberula	Madagascar
infralignosa Humbert	Madagascar
integrifolia Baker	DRC, Uganda, Kenya, Tanzania, Malawi, Zambia, Madagascar
irregularibracteata (De Wild.) C.Jeffrey *Senecio irregularibracteatus* De Wild.	DRC
jeffreyana Lisowski	DRC, Rwanda, Burundi, Uganda, Ethiopia, Kenya

juncea Robyns var. **iringensis** C.Jeffrey	Tanzania
juncea Robyns var. **juncea**	DRC, Rwanda, Burundi, Uganda
kasaiensis Lisowski *kikuyorum* R.E.Fr. = **E.debilis** S.Moore	DRC
khaopawtaensis H.Koyama	?
kilwensis C.Jeffrey T*kivuensis* (Muschl.) C.Jeffrey = **E. debilis** S. Moore	Tanzania
kivuensis C.Jeffrey	Kenya, Tanzania, Uganda, Rwanda
lejolyana Lisowski	DRC
leptocephala (Mattf.) C.Jeffrey	Cameroon
leucantha C.Jeffrey	Tanzania, Zambia
libeniana Lisowski	DRC, Rwanda, Burundi
limosa (O. Hoffm.) C.Jeffrey *Senecio limosus* O.Hoffm.	Angola, DRC, Tanzania, Malawi, Zambia, Zimbabwe, South Africa
lisowskiana C.Jeffrey *E. coccinea* auct., saltem p.p. quoad plantas Africae occid., et sensu Lisowski, non (Sims) G.Don	Rio Muni, Guinea, Fernando Po, Sierra Leone, Ivory Coast, Liberia, Ghana, Togo, Nigeria, Cameroon, Gabon, Central African Republic, Angola, Congo, DRC, Uganda, Sudan, Zambia
longifolia C.Jeffrey	Uganda, Tanzania
longipes C.Jeffrey	Tanzania
longiramea (S.Moore) C.Jeffrey *Crassocephalum longirameum* S.Moore	Congo, Angola, DRC
lopollensis (Hiern) C.Jeffrey *Senecio lopollensis* Hiern	Angola
lubumbashiensis Lisowski	DRC
lyrata (Cass.) C.Jeffrey	Mauritius, La Réunion
malaisseana Lisowski	DRC
marlothiana (O.Hoffm.) C.Jeffrey *E. albocostata*Hiern *Othonna glauca* Klatt *Senecio marlothianus* O.Hoffm. *Senecio viridiflorus* Hutch.	Angola, Namibia
mbagoi Beentje & Mesfin	Tanzania
micrura C.Jeffrey	Tanzania
moutsamboteana Lisowski	Congo - Brazzaville
myriocephala C.Jeffrey *newtonii* (O.Hoffm.) C.Jeffrey = **Psednotrichia newtonii** (O.Hoffm.) Anderb. & P.O.Karis	Tanzania
negellensis Mesfin & Beentje	Southern Ethiopia
pammicrocephala (S.Moore) C.Jeffrey *Senecio chiovendeanus* auct., Robyns p.p. quoad de Witte (1869) *Senecio pammicrocephalus*	DRC, Rwanda, Uganda, Tanzania

parnassiifolia (De Wild. & Muschl.) S.Moore *Senecio parnassiifolia* De Wild. & Muschl.	DRC, Zambia
perrieri Humbert	Madagascar
petitiana Lisowski	DRC
pinnatifida Merr.	?
praetermissa Milne-Redh.	Côte d'Ivore, Guinea, Sierra Leone, Ivory Coast, Ghana, Nigeria, Cameroon, Gabon, Congo, DRC
prenanthoidea DC.	India, Indonisea, Malaysia, New Guinea, Philippines, Thailand, Vietnam
protracta S.Moore *Senecio protractus* (S.Moore) Eyles	Zambia, Zimbabwe, Namibia
pseudactis C.Jeffrey var. **major** Lisowski	DRC
pseudactis C.Jeffrey var. **minor** Lisowski	DRC
pseudactis C.Jeffrey var. **pseudactis** *Pseudactis emilioides* S.Moore	DRC, Rwanda, Burundi
pumila DC.	Madagascar
ramulosa Gamble	India
rehmanniana Lisowski	DRC
rigida C.Jeffrey	Tanzania
robynsiana Lisowski *sagittata* auct., non DC. = **E. coccinea** (Sims) G.Don	DRC, Tanzania
sagittata DC.	Mozambique
scabra DC.	India
schinzii (S.Moore) C.Jeffrey *Othonna ambifaria* S.Moore *Othonna polycephala* Klatt *Othonna rosea* Klatt *Senecio dinteri* Muschl. ex Dinter *Senecio schinzii* O.Hoffm.	Angola, Zimbabwe, Botswana, Namibia, South Africa
schmitzii Lisowski	DRC
serpentine Mesfin & Beentje	South and south-eastern Ethiopia
serrata Humbert	Madagascar
shabensis Lisowski	DRC
simulans C.Jeffrey	Tanzania
somalensis (S.Moore) C.Jeffrey *Euryops somalensis* S.Moore *Senecio discifolius* Oliv. var. *scaposus* O.Hoffm *Senecio megamontanus* Cufod.	Somalia, Ethiopia, Kenya
sonchifolia (L.) DC. ex Wight	Senegal, Guinea, Sierra Leone, Liberia, Cote d'Ivore, Burkina Faso, Togo, Ghana, Nigeria, Fernando Po, Cameroon, Tchad, Gabon, Central African Republic, Congo, DRC, Kenya, Tanzania, Malawi, South Africa, Madagascar, Seychelles, Tropical Asia, Tropical America
speeseae Fosberg	Sri Lanka
subscaposa Lisowski	DRC, Burundi, Rwanda
tenellula (S.Moore) C.Jeffrey *Senecio tenellulus* S.Moore	Angola, Zambia, Zimbabwe, Botswana
tenera (O.Hoffm.) C.Jeffrey *Senecio tenera* O.Hoffm.	Uganda, Tanzania
tenuipes C.Jeffrey	Tanzania
tenuis C.Jeffrey	Tanzania
tessmannii (Mattf.) C.Jeffrey [T]*Senecio tessmannii* Mattf.	Cameroon, DRC, Burundi, Uganda
transvaalensis (Bolus) C.Jeffrey *Senecio thermarum* Bolus *Senecio transvaalensis Bolus*	Mozambique, Zimbabwe, Botswana, Swaziland, South Africa
tricholepis C.Jeffrey	Kenya

ukambensis (O.Hoffm.) C.Jeffrey *Senecio ukambensis* O.Hoffm.	Kenya, Tanzania
ukingensis (O.Hoffm.) C.Jeffrey *Senecio ukingensis* O.Hoffm.	Tanzania
vanmeelii Lawalrée	DRC, Tanzania, Mozambique, Malawi, Zambia
violacea Cronquist	DRC, Burundi, Tanzania, Zambia
zairensis Lisowski	DRC
zeylanica C.B.Clarke	India

CHAPTER 4

Evolutionary patterns and biogeographic history of *Emilia* (Senecioneae, Asteraceae).

Abstract

The genus *Emilia* (*ca.* 117 species) is widely distributed with most species occurring in tropical Africa (*ca.* 80), and a few in Madagascar (14) and Asia (12). The origin of *Emilia* and its migration patterns were unknown, i.e. whether the genus arose *in situ* or migrated northwards or southwards from its place of origin. We used the current geographic distributions of species mapped onto a reconstructed nuclear ITS phylogeny and dated molecular phylogenetic hypotheses to investigate the pattern and timing of diversification in *Emilia*, and correlated this pattern with evolutionary trends in the genus. *Emilia* appears to have originated in southern Africa during the Mid-Miocene (*ca.* 14.19 Mya) and diversified northwards into varied habitats in Africa. The timing of *Emilia'*s origin coincides with a period of global climate cooling following the mid-Miocene Climatic Optimum (*ca.* 15 Mya) when more open vegetation systems such as the African grassland ecosystems and savannas first appeared and became widespread in the Late Miocene (*ca.* 8 Mya). Most *Emilia* species originated during the Pliocene and Pleistocene epochs, with at least five independent dispersals out of southern Africa to Madagascar occurring during the Pliocene. Most *Emilia* species are annual and a few are perennial. This annual life form is hypothesised to have either been ancestral or evolved early (*ca.* 13.32 Mya) in *Emilia's* history and is likely linked to its successful diversification in Africa. Narrow leaves, radiate capitula, and non-yellow florets (e.g. purple, white, and orange) have all arisen independently numerous times in *Emilia*.

Introduction

The genus *Emilia* Cass. of the tribe Senecioneae in the Asteraceae is widely distributed with most species occurring in Africa (*ca.* 80), 14 in Madagascar (Humbert 1963), and 12 in Asia. Some species in *Emilia* have a large geographical range, for example, two weedy species, *E. fosbergii* Nicolson and *E. sonchifolia* (L.) DC., have spread to the neotropics (Fosberg 1972), while others are geographically restricted to a small local region. Of the African *Emilia* species, the largest number (41) occur in Central Africa (Congo, Rwanda and Burundi; Lisowski 1991), followed by 38 species in East Tropical Africa (Uganda, Kenya and Tanzania; Jeffrey 1986), 24 species in southern Africa[2], 14 species in North-East Africa (Tadesse and Beentje 2004), and 12 species occur in West Africa (distribution data gathered from Adams 1963; Jeffrey 1986; Tadesse and Beentje 2004).

Emilia species occur in a variety of habitats including moist, wooded grassland, dense or open mixed woodland, montane forest, savannas, mountain summits or tops of ridges, rocky places, ruderal sites and roadsides, as well as in moist habitats such as marshy areas, vleis, and shallow standing water. The geographic distribution, varied habitats of *Emilia*, and the association of most *Emilia* species with many woody genera in African savannas suggest that this genus fits well into the Savanna flora, which is one of the six African floras proposed by Linder (2014; the others being the Austro-temperate flora, Lowland forest flora, Tropic-montane flora, Tropic-alpine flora, and Arid flora). The Savanna flora is widely distributed extending to the seasonally arid parts of the continent and with its centre of species richness along the high ground forming the watershed between the Congo, Zambezi, and Ruaha River systems (Linder 2014). The most common vegetation characterising this flora is woodland, mixed with grass in the understory and fire is a common occurrence (White 1983). The southern African savanna biome includes the miombo and mopane woodlands, shrublands, grasslands, and grassy dambos (Huntley 1982; Burgess *et al*. 2004). A few *Emilia* species also occur in some of the elements of the Austro-temperate flora, i.e. outliers of the Chimanimani mountains of Zimbabwe (viz. *E. caespitosa*, *E. coccinea*; Phipps and Goodier 1962; Linder 2014), the Huila Plateau in Angola (*E. integrifolia*), and the Nyika Plateau in Malawi (*E. coccinea*, *E. guineensis*, *E. integrifolia*, *E. limosa*) (Van Wyk and Smith 2001).

[2] Southern Africa is defined here as the countries south of the Democratic Republic of Congo and Tanzania, viz. Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland, Zambia, and Zimbabwe. This definition differs from other definitions of southern Africa, e.g. Cowling and Hilton-Taylor's (1994) definition as the region south of the Cunene and Limpopo rivers.

Tracing the biogeographic and evolutionary history of *Emilia* provides the opportunity to contribute to our understanding of how these African floras evolved.

Role of climate change in influencing past distribution of Asteraceae in Africa

The Asteraceae is the largest family of flowering plants with about 23 000 species, most of which are economically important, for example, sunflowers, lettuce and ornamentals such as *Chrysanthemum* L., *Dahlia* Thunb., *Gerbera* L. and *Osteospermum* L. This family has a world-wide distribution except for Antarctica (Panero and Funk 2008). The Asteraceae originated *ca.* 76–66 million years ago (Mya) in South America as shown by recently discovered fossil evidence (Barreda *et al.* 2015), diversified early, and has been dominant in many biomes around the world, especially in open habitat ecosystems (Raven and Axelrod 1974). The family's early diversification in South America was followed by an African explosion (DeVore and Stuessy 1995; Stuessy 2010), and in southern Africa the family radiated in all recognized biomes during the Oligocene and became one of the dominant families in most southern African biomes, for example, savanna and afromontane biomes (Raven and Axelrod 1974; Cowling 1983; Burgoyne *et al*. 2005).

Climate has influenced vegetation patterns in Africa in the past, including in the family Asteraceae. There have been significant vegetation changes in Africa during the Miocene epoch (23.03–5.332 Mya). In the early Miocene, northern Africa was covered by tropical trees and vegetation, which changed into a more open-habitat vegetation towards the end of the Miocene (Nei *et al*. 2015). Climatic changes in the late Miocene-Pliocene resulted in an 'arid track' in Eastern Africa, characterised by open grassland along which many species migrated (De Winter 1971; Jürgens 1997). Southern Africa experienced a colder and drier climate from the late Miocene onwards with the replacement of dense thickets in the interior by more open savanna and grassland by the end of the Pliocene (Vrba 1985). The present African biomes were established during the Pleistocene with climatic fluctuations resulting in longer cold and dry conditions fluctuating with shorter warmer and wetter spells, and the contraction and expansion of thicket vegetation (Burgoyne *et al*. 2005; Cowling *et al*. 2005).

Emilia is a tropical genus for which the origin and general migration trends were not known, that is, whether the genus arose *in situ* or migrated northwards or southwards from its place of origin. The 'African track' was hypothesised for a tropical African origin of the Cape flora with migration southwards via the Afromontane region (Levyns 1938, 1952, 1964; Axelrod and Raven 1978), as the number of taxa in the Cape with tropical links exceeds those

with Gondwanan affinities (Levyns 1964). In contrast, a post-Gondwanan (late Miocene-Pliocene) migration northwards from the Cape to the tropics of members of the Asteraceae, such as *Metalasia* R.BR., *Relhania* L'Hér. emended K.Bremer, and *Stoebe* L. in the tribe Gnaphalieae and also *Senecio* L. (Senecioneae), has been based on fossil evidence and also supported by dated phylogenies (Coleman *et al*. 2003; Bergh and Linder 2009). A northward migration from South Africa to the Mediterranean region and diversification that started in the late Eocene and intensified during the Oligocene was also suggested for the geophytic genus *Androcymbium* Willd. (Colchicaceae) based on chloroplast DNA and karyological data analyses (Caujapé-Castells *et al*. 2002; Procheş *et al*. 2006). The idea of a predominantly north to south migration has been rejected by e.g. Linder (1994) and Galley and Linder (2006) based on cladistic studies of Cape-centred genera (e.g. *Erica* L., *Phylica* L., and *Ehrharta* Thunb.). Linder (2014) also suggested that much evolution in Africa is by *in situ* speciation rather than migration. Another view is that of Adamson (1958) and Wild (1968) who proposed vicariance where the flora present in each region represents remnants of an African flora that was once more widespread and retreated with climatic changes.

Although the diversification of various genera and tribes in the Asteraceae has been studied (e.g. Bergh and Linder 2009; Devos *et al*. 2010; Pelser *et al*. 2010), phylogenetic relationships and evolutionary trends linked with e.g., habitat changes and pollination syndromes have not previously been investigated in *Emilia*. Morphological character reconstruction has previously been done at the family level in Asteraceae (Panero *et al.* 2014) and also in a few genera such as *Helichrysum* Mill. (Galbany-Casals *et al.* 2014). The key diagnostic morphological features of *Emilia* species, viz. life history, growth form, stem and leaf features, and reproductive features, e.g. capitula arrangement, style apex shape and cypsela pubescence, together with the availability of a nuclear ITS phylogeny (Chapter 3) enable us to reconstruct the ancestral features of this genus and trace character changes of interest, for example, those that may have influenced the diversification of *Emilia*.

Madagascar

Eleven of the fourteen *Emilia* species occurring in Madagascar are endemic to the island, which has a rich endemic flora and is a global hotspot for biodiversity conservation (Ganzhorn *et al*. 2008). Many endemic lineages in Madagascar originated from the overseas dispersal of their founder African species during the Cenozoic (65.5 Mya; Rabinowitz *et al*. 1983; Agrawal *et al*. 1992; Cowie and Holland 2006; Yodar and Nowak 2006) and experienced extensive *in situ* diversification (Strijk e*t al*. 2012). A number of studies (e.g.

Morley 2003; Yodar and Nowak 2006; Trénel *et al*. 2007) have also shown that the origin of many groups in Madagascar post-date the isolation of Madagascar. Due to its proximity, Africa appears to be the most important source for plants dispersing to Madagascar, for example, *Helichrysum*, which appears to have colonised the island at least five times (Galbany-Casals *et al*. 2014). Most phylogenetic studies of Malagasy biota have indicated a pattern of sister group relationships to African taxa (Wild 1965, 1968; Meve and Liede 2002; Yodar and Nowak 2006). The island has also been reported to have floristic relationships with East Africa where related taxa (e.g. *Coleochloa setifera* (Ridl.) Gilly and *Myrothamnus flabellifolius* Welw.) are present in both areas on inselbergs (Barthlott and Porembski 1998). In addition, some Madagascan inselberg flora is similar to that found on inselbergs in southern Africa (notably the Karoo-Namib region), for example, genera *Euphorbia* L. and *Kalanchoe* Adans. (Barthlott and Porembski 1998). Madagascar has been shown to be the main source of colonizing plant lineages for surrounding islands (e.g. the Mascarene Archipelago comprising Réunion, Mauritius and Rodrigues) with founder species mostly belonging to widespread and species-rich genera (Strijk *et al*. 2012). Furthermore, Madagascar could have served as a source of species for Africa, for example, the family Velloziaceae is postulated to have migrated to Africa across the ocean and then used inselbergs as stepping stones to spread through Africa (Barthlott and Porembski 1998).

Phylogenetic dating

Within the Asteraceae, molecular dating analyses have been problematic due to the family's poor fossil record (McKenzie and Barker 2008). In recent years, new fossil discoveries have facilitated a more satisfactory estimation of divergence times of some basal lineages within the family, for example, the split between subfamily Barnadesioideae and the rest of family Asteraceae occurred either during the early Paleogene or late Cretaceous (Barreda *et al.* 2012; Nei *et al.* 2015). Recently Barreda *et al.* (2015) concluded that the assumed origin of Asteraceae should be pushed back by approximately 20 million years (Myr) and is now more reliably dated to *ca.* 76–66 Mya because of the discovery of several fossil pollen grains assigned to an extinct clade of Asteraceae conserved in Antarctic deposits for more than 65 Myr together with other extinct groups, including dinosaurs (e.g. *Brachiosaurus*, *Tyrannosaurus*) and ammonites.

When deducing dates from a molecular phylogeny, it is crucial to know how the initial dates were calibrated (Heads 2005; Sauquet *et al.* 2012). When there are no fossils known for the chosen taxa, secondary calibration points obtained from a previous study are

commonly used (Sauquet *et al.* 2012), for example, Bergh and Linder (2009) fixed the root age of Gnaphalieae by secondarily deriving it from previous dating exercises of Kim *et al.* (2005).

Aim and objectives

The aim of this study was to investigate the pattern and timing of diversification in *Emilia* using current geographic distributions of species and dated molecular phylogenetic hypotheses, and to correlate this pattern with evolutionary (morphological) trends in the genus.

Three objectives were proposed for the current study. The first objective was to examine patterns of geographic distribution in *Emilia*, by optimising the distribution of included species onto one of the equally most parsimonious (EMP) nuclear ITS phylogenetic trees, and to answer the question: What is the mostly likely area of origin of *Emilia* based on the current distributions and the reconstructed phylogeny?

The second objective was to estimate the ages of extant *Emilia* lineages as well as the times of divergence of the main *Emilia* clades and other closely related species sampled in this study using data from the fossil record and secondary calibrations and ultimately infer the diversification pattern of *Emilia* across Africa and Madagascar. The research questions associated with this objective are: (i) When did *Emilia* originate and are the estimated divergence times in this genus associated with any past climatic changes/events in Africa?; (ii) What role has past climate change (and the associated changes in vegetation) played in the speciation and distribution of *Emilia* species in Africa?; (iii) Does the dated phylogeny show evidence of one or more dispersal events of *Emilia* from Africa to Madagascar? Or alternately, has Madagascar served as a source of species for Africa?

The third objective was to investigate evolutionary (morphological) trends in *Emilia* as inferred from the reconstructed molecular phylogeny with morphological characters mapped onto it. A range of characters were investigated for ancestral versus derived states, for evidence of homoplasy and were also linked to vegetation/habitat changes, development of pollination syndromes, and photosynthetic efficiency where possible.

Materials and Methods

Tracing the biogeographic history of *Emilia*

To investigate the biogeographic history of *Emilia*, the current distributions of the eighty accessions, including 22 Senecionoid outgroups, 45 species (55 accessions) of *Emilia*, two *Emiliella* and one *Bafutia* species were optimised onto one of the most parsimonious (MP) nuclear ITS phylogenetic trees produced from an analysis including insertion-deletion events (indels) with the tussilaginoid *Tephroseris integrifolia* rooting the tree. The genus *Emilia* was treated here as a monophyletic entity by excluding the two species outside *Emilia* (*E. baumii* and *E. graminea*) and by including *Emiliella* and *Bafutia*). The ITS phylogeny was used here instead of the plastid phylogeny because it is better resolved, with more informative characters, it appears to reflect morphological patterns more closely than the plastid region, and is biparentally inherited. Geographic areas were defined and coded as multistate characters as follows: 0–Europe; 1–widespread; 2–Eurasia; 3–Australasia; 4–Canary Islands; 5–Madagascar; 6–East Africa; 7–West Africa; 8–Central Africa; and 9–southern Africa; Table A1, Appendix 4.1). These defined geographic areas were based on the origin of the specimen material sampled as well as the general distribution of the species. It should be noted that in cases where the general distribution of species was broad (i.e. species occurring across more than four geographical regions), it was coded as widespread. Distribution data for all *Emilia*, *Emiliella*, *Bafutia* and other Senecionoid species included in this study were obtained from field observations, herbarium specimens borrowed from the following nine herbaria: BR, EA, LISC, MA, MAL, MO, PRE, SRGH, and UZL (acronyms following Holmgren *et al*. 1990), databases [e.g. the National Herbarium, Pretoria Computerized Information System (PRECIS) data obtained from Malawi, Mozambique, South Africa, Zambia, and Zimbabwe], and the literature — Jeffrey (1986), Lisowski (1991), Arnold and De Wet (1993), Herman (2003), Da Silva *et al*. (2004), Mapaura and Timberlake (2004), Burrows and Willis (2005), Phiri (2005), Setshogo (2005), Germishuizen *et al*. (2006), Klopper *et al*. (2006), Raimondo *et al*. (2009), and Cron (2014). Mapping of geographic areas onto the selected MP tree was done using the Ancestral State Reconstruction Package in Mesquite v.2.01 (Maddison and Maddison, 2009).

Estimating the time of divergence in *Emilia*

A dated molecular phylogeny was created using the Bayesian approach with the programme BEAST v.1.8 and the BEAST.xml input file was created with BEAUti v.1.8 (Drummond *et al*. 2012). Several short BEAST runs were initially performed to check the MCMC performance. Optimal operator adjustments were done as suggested using the output diagnostics and finally BEAST analyses were performed in triplicate using the ITS dataset with indels. To estimate the posterior distribution, each chain was allowed to run 50 million generations and the trees saved every 5000 generations. Each analysis was provided with a random starting tree. The best-fitting substitution model for the ITS region, selected with jModelTest v.0.1.1 (Posada 2008) and employing Akaike Information Criterion (AIC; Akaike 1974), was GTR+I+G. The gamma distribution for this substitution model was modelled with four categories. The relaxed Bayesian clock with the rate of molecular evolution assumed to vary between branches and drawn independently from a lognormal distribution was implemented (Drummond and Rambaut 2007). The Yule tree prior was assigned for branch lengths, specified in BEAST, and used with a normal prior distribution. A uniform prior between 0 and 0.1 was set for the 'mean.Rate' parameter, the 'coefficient of variation' prior was uniform between 0 and 1.0, and the 'covariance' had a uniform prior between −1.0 and 1.0. Convergence of the results of the three BEAST analyses (runs) was assessed by using the program Tracer v1.5 to confirm that effective sampling size (ESS) of all parameters estimated from the posterior distribution of trees was greater than 200. The first 10% of samples were discarded as burnin and the BEAST utility program LogCombiner v.1.8.0 was used to combine the parameter estimates from the three runs. TreeAnnotator v.1.8.0 was utilised to summarise the sample of plausible trees together with the sample of parameter estimates resulting in the maximum clade credibility tree, i.e. the tree with the highest sum of posterior probabilities on all its internal nodes (Drummond *et al*. 2007). The posterior probability limit was set to 0.5 and the mean node heights were summarised. These were visualised using FigTree v.1.4.2 (Drummond *et al.* 2007). The dated phylogenetic tree was plotted with a geological (stratigraphic) time scale using R package strap (Bell and Lloyd 2014). The plastid analysis whose best-fitting substitution model was GTR+I was also run for comparison following the same procedure.

Secondary calibrations were utilised in this study to date the reconstructed *Emilia* phylogeny. Suitable monophyletic taxon sets (Table A4, Appendix 4.1) were designated and calibration nodes were defined via these. Calibration was achieved using three nodes: the root node, *Bethencourtia,* and *Pericallis*. The root node was provided with an age constraint

derived from secondary calibration for the age of subfamily Asteroideae, and constrained to be monophyletic. Results obtained in a study by Strijk *et al*. (2012) were used to calibrate the root node as advised by Luis Palazzesi (personal communication). Our prior for the root node was a normal distribution with a mean at 39.4 Myr and 95% confidence interval (CI) of 27.89–50.91 Myr (node a in red; Figures 4.2, 4.3). The priors for the other two root nodes, *Bethencourtia* (node c in red—*Bethencourtia–Senecio* clade origin) and *Pericallis* (node b in red—*Pericallis* node) (Figures 4.2, 4.3) were also a normal distribution. *Bethencourtia* and *Pericallis* are both endemic to the Canary Islands, and dates for the origins of the islands based on age estimates from literature of the oldest islands in the distribution ranges of *Bethencourtia* and *Pericallis* were applied in calibrating these root nodes. The means were at 11.6 Myr (age of Tenerife) with 95% CI of 5.02–18.18 Myr for *Bethencourtia* (Ancochea *et al*. 1990; *Bethencourtia–Senecio* clade origin; Figures 4.2, 4.3) and 16.0 Myr (age of Gran Canaria) with 95% CI of 9.42–23.84 Myr for *Pericallis* (Izquierdo *et al*. 2001; *Pericallis* node; Figures 4.2, 4.3).

Morphological character evolution

Evolutionary trends for sampled taxa were evaluated by optimising thirteen selected morphological characters, eight binary (life history, stem character, stem pubescence, leaf type, capitula type (radiate or discoid), capitula grouping, phyllary indumentum, and cypsela indumentum), three multistate qualitative (floret colour, leaf margin type, and style branch apex shape), and two quantitative continuous (leaf width and capitulum width) (Table A2, Appendix 4.1) onto the maximum clade credibility (MCC) tree resulting from BEAST analysis of the nuclear ITS data including indels. These characters are frequently used in the identification of *Emilia* species and were scored from descriptions in the literature (Candolle 1838; Garabedian 1924; Humbert 1963; Jeffrey 1986, 1992, 1997; Lisowski 1991; Tadesse and Beentje 2004), observations in the field, as well as from specimens borrowed from the following herbaria: BR, EA, J, LISC, MA, MAL, MO, PRE, SRGH, and UZL.

All the characters considered here vary among *Emilia* species. For the two continuous characters, leaf and capitulum width, measurements done on mature leaves and capitula were obtained from literature, verified from field observations where possible and also from specimens borrowed from various herbaria as above. These were coded using gap-coding and the differences in the mean values for the characters were considered in creating gaps (Archie 1985). The cut-off for narrow leaves measured at the broadest point was 10 mm and broad leaves were regarded as those above 15 mm in width (Table A2, Appendix 4.1). The narrow

Emilia leaves are mostly linear to narrowly elliptic or oblanceaolate, oblong-narrowly ovate to narrowly ovate and sessile (Tadesse and Beentje 2004; Cron 2014). Capitula diameter was also considered at the broadest point and narrow and wide capitula were regarded as those up to 4 mm and those above 6 mm in diameter respectively (Table A2, Appendix 4.1). The capitula in *Emilia* are cylindric to campanulate (Tadesse and Beentje 2004).

The data matrix for these thirteen characters was prepared for 71 species, i.e., 46 species of *Emilia*, two species of *Emiliella*, as well as *Bafutia* and 22 outgroup Senecioneae (Table A3, Appendix 4.1). The selected outgroups were considered representatives in terms of morphology and/or distribution for their genus. Multiple accessions, where available for a single species, were pruned in the BEAST reconstructed tree. Floret colour was polymorphic and style branch apex shape had missing data for some species. Mapping of morphological characters onto a BEAST tree was done using the Ancestral State Reconstruction Package in Mesquite v.2.71 (Maddison and Maddison 2009). The maximum likelihood (ML) approach using the Markov k-state 1 (Mk1) parameter model of evolution (Pagel 1999) with the same probability for each character state was used to reconstruct ancestral states. The Mk1 model is a generalization of the Jukes-Cantor model and equivalent to Mk model of Lewis (2001). Branch lengths are considered and the rate of change between character states is estimated in the Mk1 model. Equal probability is given to any character state change. Ancestral states reconstructions for two characters: floret colour (polymorphic for 18 taxa) and style branch apex shape (missing data for seven outgroup taxa) were not supported/allowed by the ML approach. Therefore the parsimony reconstruction method was used for these two characters and the character states were 'unordered'.

Results

Tracing the biogeographic history of *Emilia*

The optimised reconstructed phylogeny indicates that ancestors of many of the senecionoid outgroup taxa included here originated in southern Africa with a few dispersing to e.g. Australia — *Senecio pinnatifolius*, the Canary Islands — *Bethencourtia palmensis* and *Pericallis*, Eurasia — *Jacobaea vulgaris*, West Africa — *Solanecio biafrae*, and East Africa — *Dendrosenecio* (Figure 4.1). The ancestor of *Emilia* (node E) is also hypothesised as southern African, and the earliest diverging *Emilia* clade (Clade 1), comprises the southern African species *E. transvaalensis*, *E. marlothiana*, and *E. schinzii* (Figure 4.1). Thus, despite

its mainly tropical distribution, *Emilia* appears to have had a southern African origin. Most of the clades also originated in southern Africa and then dispersed to various regions: *Emilia* species in Clade 2 — to East Africa, then to West and Central Africa, while *E. brachycephala*, *E. tenellula*, *E. lopollensis*, *E. discifolia* and *E. decipiens* remained in southern Africa and the latter two species also dispersed to East Africa; Clade 4 — to East Africa where species diversified with at least three dispersals back to southern Africa (*Emiliella zambiensis* and *Em. drummondii*; *Emilia leucantha* and *E. protracta*; Clade 4a; Figure 4.1) and also spread to West Africa (*E. emilioides*), or to Central and West Africa (Clade 4b; Figure 4.1); Clade 5a — to West Africa (viz. *E. coccinea*3&4, *E. praetermissa*1&2); to Central and West Africa (*E. lisowskiana*); to Central and East Africa (*E. vanmeelii* and *E. pammicrocephala*); whilst *E. sonchifolia* became widespread and *E. exserta* dispersed to Tropical Asia (viz. India and Sri Lanka); Clade 5b — to East, Central, and West Africa (*E. caespitosa*); with *E. coccinea*5 and *E. fosbergii* becoming widespread; and to Madagascar (*E. citrina*, *E. humifusa*, and *E. infralignosa*; (Figure 4.1).

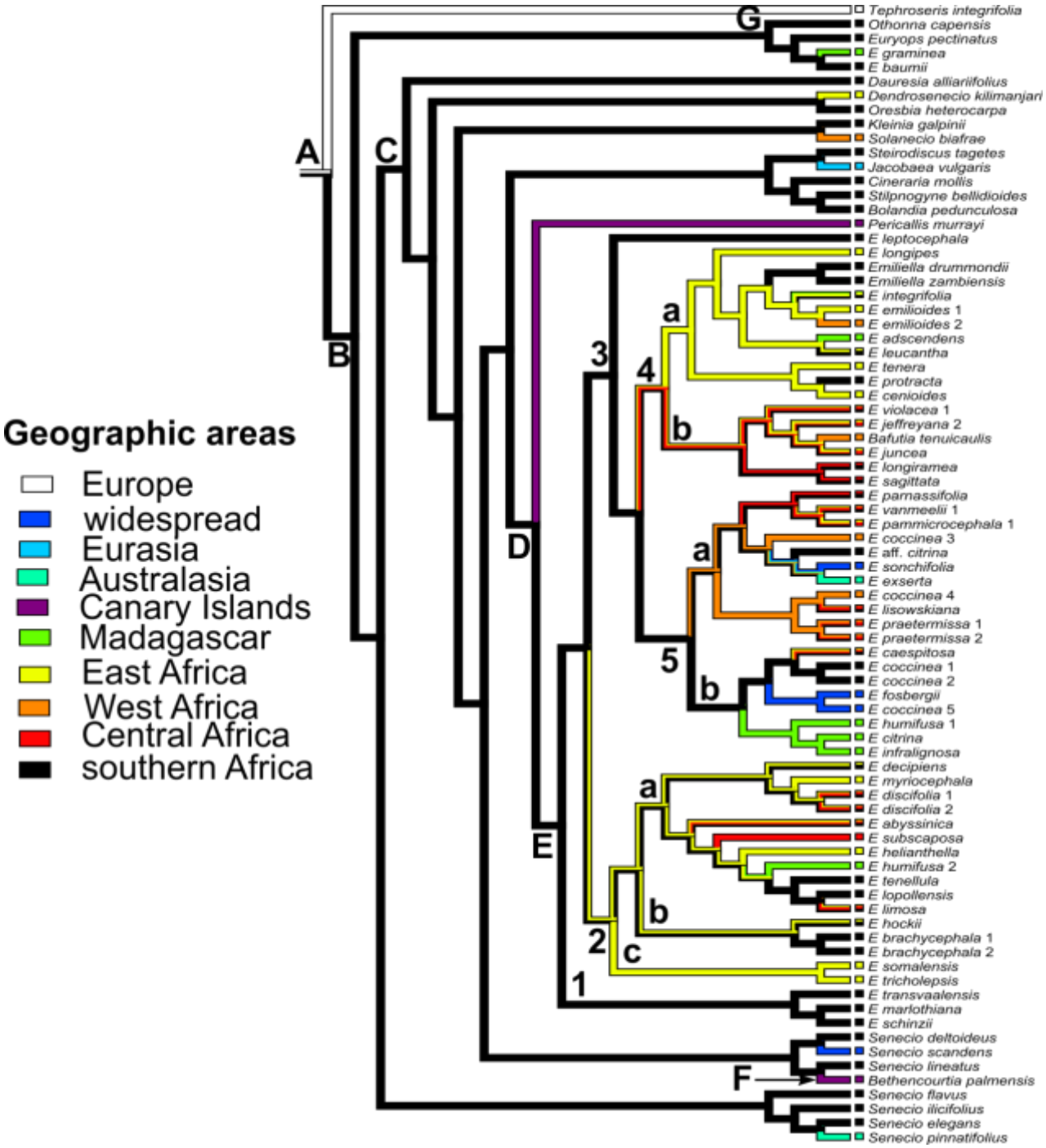

Figure 4.1. Optimisation of geographic areas for *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. Upper-case letters identifying nodes and clades labelled 1-5 are discussed within the text.

There are at least five independent dispersals out of southern or East Africa to Madagascar for the six Madagascan *Emilia* species included here. Three of the endemic Madagascan species (*E. citrina*, *E. humifusa*1, and *E. infralignosa*) share a common ancestor

and a single dispersal event, while endemics *E. adscendens* and *E. humifusa*2 each dispersed independently. (Note: the occurrence of *E. humifusa*1 and *E. humifusa*2 at different positions on the nuclear ITS phylogeny suggests that they are distinct species). *Emilia integrifolia* also dispersed to Madagascar, but still occurs in East and southern Africa. It should be noted that the Madagascan species *E. graminea* is placed outside of the *Emilia* clade in the reconstructed nuclear ITS phylogeny but not in the plastid one (Chapter 3, Figure 3.1). In addition, *E. baumii* (from southern Africa) is not part of the *Emilia* clade in either phylogeny, and is sister to *E. graminea* in the ITS phylogeny (Chapter 3, Figure 3.1).

Estimates of time of origin and diversification of *Emilia*

BEAST analysis parameters

The mean number of new lineages arising from a single parent lineage per million years (Yule.birth rate) was 0.135 (0.087–0.191). The mean number of substitutions per site per million years (mean.Rate) across the whole tree was estimated to be 0.0037 (0.0026–0.0051). The mean branch rate under the relaxed clock model (ucld.mean) across all data partitions was 0.0041 (0.0027–0.0057) substitutions per site per million years and the standard deviation of this parameter (ucld.stdev) was 0.73 (0.5914–0.8672). This estimate is relatively close to 1.0 suggesting rate heterogeneity across lineages (Drummond *et al.* 2007). The 'coefficient of variation' was estimated to be 0.818 (0.6697–0.9947) and the non-zero value for this parameter also confirms rate heterogeneity across lineages (Drummond *et al.* 2007). The parameter 'covariance' which measures the average autocorrelation of rates of evolution from parent to daughter lineages was estimated to be 0.0191 (−0.1289–0.1808). Since the value of 'covariance' spans zero, this suggests that daughter branches have rates which are very different from parent branches (Drummond *et al.* 2007).

Mean node age estimation

The ages of the majority of lineages included in this study fall in the Pleistocene, Pliocene, and Miocene epochs (Table 4.1; Figures 4.2, 4.3). When *Tephroseris* is excluded, the 95% HPD for the age of origin of the 'ingroup taxa' comprising *Emilia*, *Emiliella*, *Bafutia* and the Othonninae and Senecioninae clades spans the Miocene and Eocene and the estimated origin of the senecionoid clade has a mean in the Oligocene (Table 4.1; Figures 4.2, 4.3).

The estimated age of origin of the main *Emilia* clade including *Bafutia* and *Emiliella* is 14.19 Mya (9.49–18.94 Mya), i.e. in the Miocene (Table 4.1; Figures 4.2, 4.3). Most

Emilia species originated recently and their estimated ages fall in the Pliocene/Pleistocene epochs (Figures 4.2, 4.3). The results of the plastid analyses (not shown here) also show that most *Emilia* species originated during the Pliocene and Pleistocene epochs. There are two major clades within the main *Emilia* clade (E): Clade 1 and a large clade consisting of small clades labelled (2, 3, 4, and 5) for discussion purposes (Figures 4.2, 4.3). The lineage that gave rise to the three southern African species (*E. transvaalensis*, *E. marlothiana*, and *E. schinzii*; Clade 1) and the rest of *Emilia* is estimated to have originated *ca.* 14.19 Mya (9.49–18.94 Mya). The southern African clade then diversified much later *ca.* 2.58 Mya (0.5–5.22 Mya) (Figures 4.2, 4.3). Clade 4, which includes *Bafutia* and *Emiliella*, is estimated to have originated *ca.* 10.1 Mya (8.58–17.42 Mya) (Figures 4.2, 4.3). Clade 5, comprising five of the eight species in the *E. coccinea* complex (Mapaya and Cron 2016), three species from Madagascar (*E. citrina*, *E. humifusa*, and *E. infralignosa*), three widespread species (*E. exserta*, *E. fosbergii*, and *E. sonchifolia*), as well as *E. pammicrocephala* and *E. parnassifolia*, is estimated to have originated *ca.* 9.48 Mya (5.91–13.13 Mya), whereas the estimated age of origin of Clade 2 is *ca.* 8.12 Myr (4.49–11.98 Myr). The ancestor of the Madagascan lineage (comprising three endemic species) is hypothesized to have dispersed to Madagascar *ca.* 7.82 Mya (4.61–11.35 Mya) with diversification as relatively recently as *ca.* 4.0 Mya (1.35–6.99 Mya), i.e. in the Pliocene (5.332–1.806 Mya; Figures 4.2, 4.3).

Table 4.1. Divergence age estimates of selected nodes in millions of years before present. Values represent the mean and 95% HPD.

Node	Description	Mean (Myr)	95% HPD (Myr)
A	Root node	33.02	22.44–44.21
B	Ingroup taxa (excluding *Tephroseris*)	29.14	19.29–39.03
C	Senecioninae clade (excluding *Tephroseris*, Othonninae)	24.85	16.39–33.18
D	*Pericallis* - main *Emilia* clade	18.2	12.68–24.1
E	Main *Emilia* clade including *Bafutia* and *Emiliella*	14.19	9.49–18.94
F	*Bethencourtia* - *S. lineatus*	8.37	3.35–13.45
G	Othonninae clade	22.01	12.16–32.13

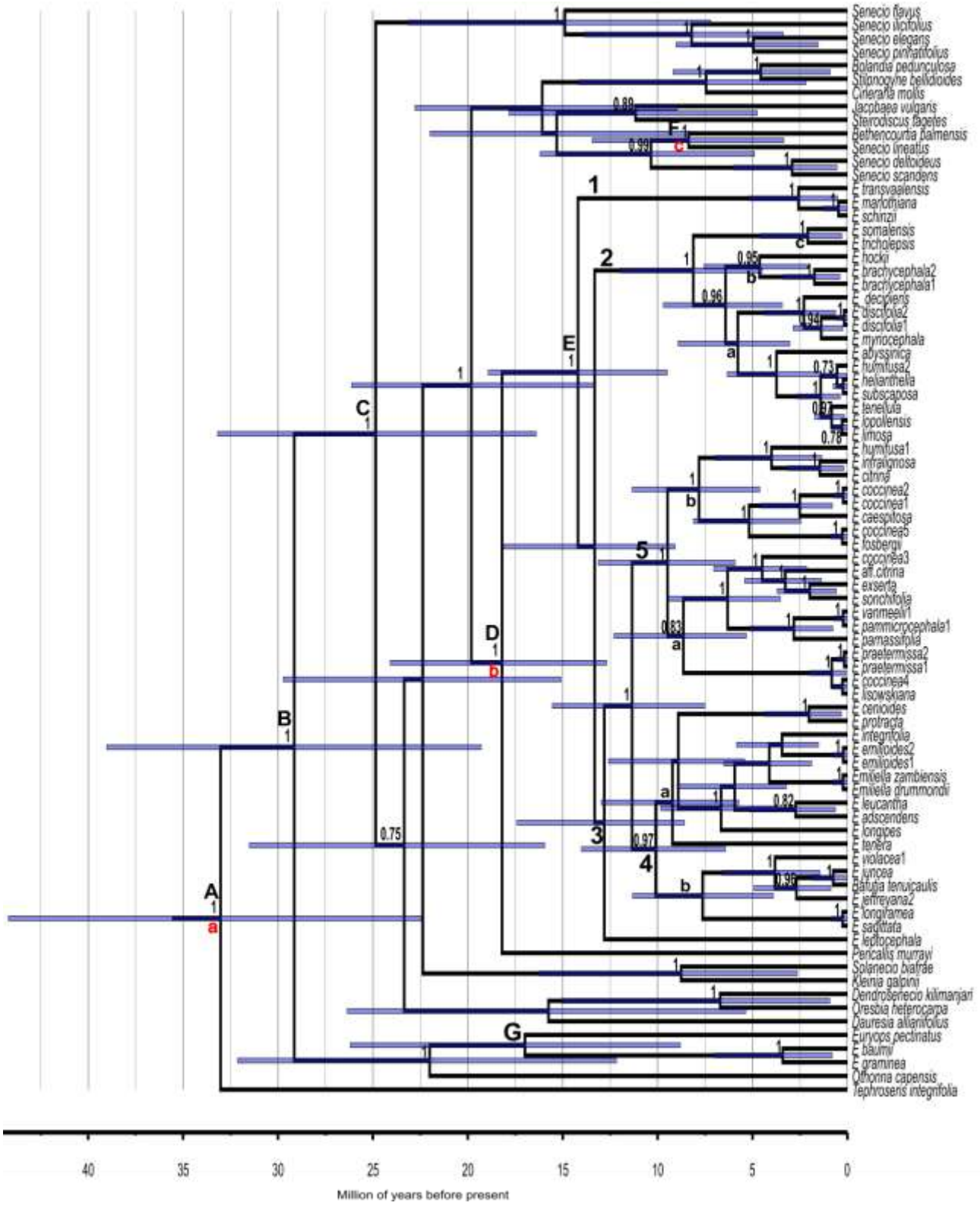

Figure 4.2. The maximum clade credibility tree summarized by TreeAnnotator and plotted with a time scale using FigTree v1.3.1. Posterior probability (PP) values ≥ 0.7 are indicated above branches. The 95% credible intervals for node ages are shown with horizontal bars. Upper-case letters identifying nodes are discussed in the text. Lower-case letters (red) show the calibration nodes: a – the root node; b – *Pericallis* node; c – *Bethencourtia* node.

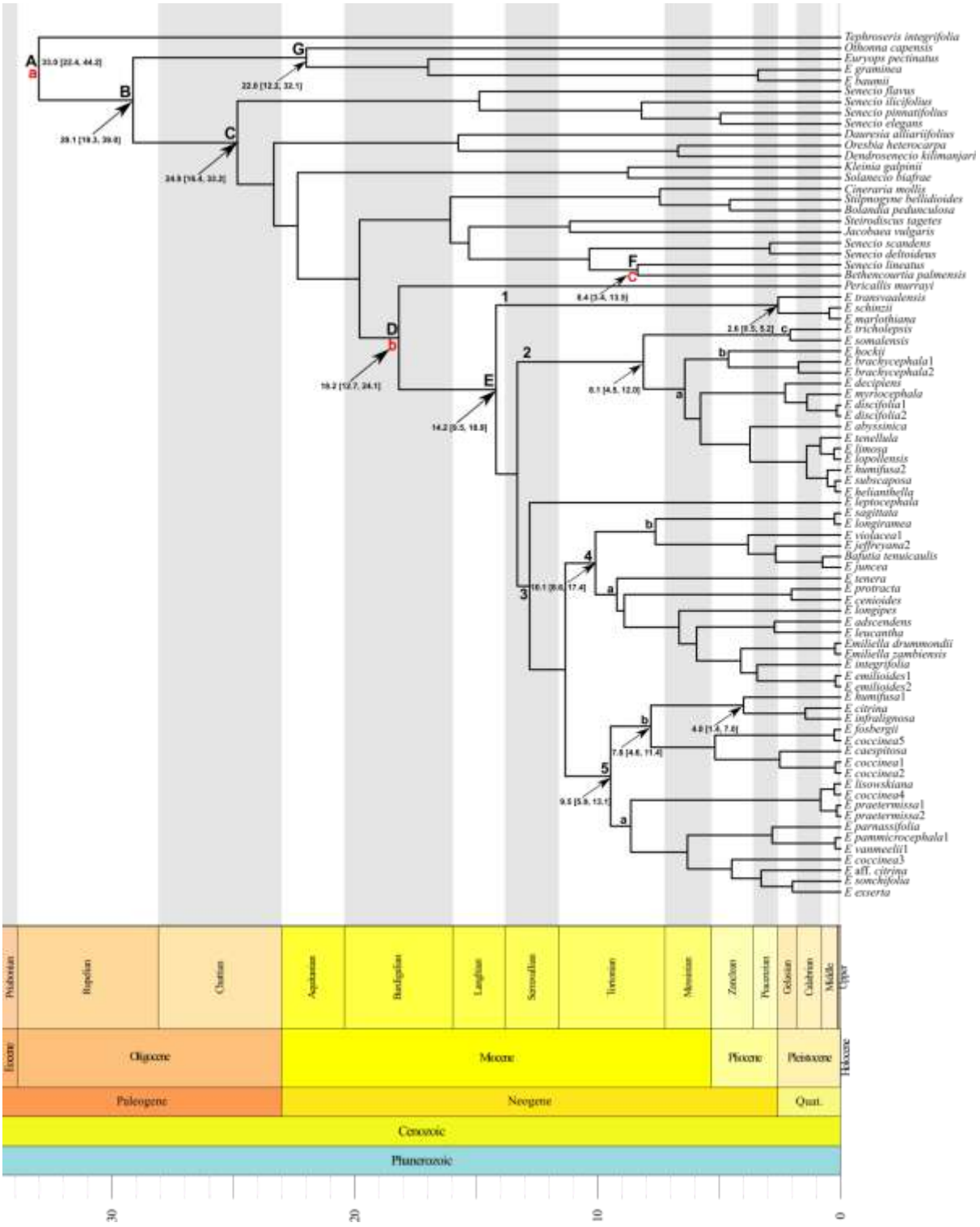

Figure 4.3. The maximum clade credibility tree summarized by TreeAnnotator and plotted with a geological (stratigraphic) time scale using the strap package in R. Upper-case letters identifying nodes are discussed in the text. Lower-case letters (red) show the calibration nodes: a – the root node; b – *Pericallis* node; c – *Bethencourtia* node. Clades labelled 1-5 are discussed within the text. Divergence time estimates [mean (95% HPD)] on selected nodes are indicated in bold font.

Morphological evolution in *Emilia*

Thirteen selected morphological characters were optimised onto the MCC tree resulting from BEAST analysis of the nuclear ITS data including indels (Figures 4.4 – 4.16) to investigate the evolutionary trends in these features. The ML and parsimony approaches were used as outlined above. All the characters except floret colour and style branch apex shape are equivocal. The following vegetative character states: an annual life history, an erect growth form, and cauline leaves are very likely ancestral (plesiomorphic) in *Emilia* since they have high proportional likelihoods at the *Emilia* ancestral node.

As noted above, life history is equivocal, however an annual life history is most likely to be ancestral (proportional likelihood of annual state = 0.74; node A, Figure 4.4) in *Emilia*, although perennial life history is evident in some of the earliest diverging southern African species. Most *Emilia* species are annual (Figure 4.4). Nonetheless, interpreting life history based on the *Pericallis* node, perennial life history is likely to be ancestral (proportional likelihood of perennial state = 0.67). Considering this scenario, the annual life history is therefore hypothesised to have evolved only once in *Emilia* (Figure 4.4). However, there have been reversals to a perennial life history five times in the *Emilia* species included here (viz. in *E. somalensis*, *E. pammicrocephala*, *E. hockii*, *E. infralignosa*, and *E. adscendens*; Figure 4.4). Most species exhibit the plesiomorphic erect growth form (proportional likelihood of erect growth form = 0.9997; Figure 4.5), and a decumbent or sprawling growth form appears to have arisen independently at least four times in *Emilia* (viz. in *E. protracta*, *E. pammicrocephala*, *E. decipiens*, and *E. hockii*; Figure 4.5). Glabrous and pubescent stems are equivocal and have equal proportional likelihoods (0.5) at the ancestral node and node (A) (Figure 4.6). The glabrous state is nonetheless synapomorphic for some relationships of sister taxa (e.g. *E. protracta* and *E. cenioides*; *E. humifusa*, *E. citrina*, and *E. infralignosa*; Figure 4.6).

Convergence in phyllotaxy is seen in five of the *Emilia* species sampled here (*E. tenera*, *E. longipes*, *E. hockii*, *E. lopollensis*, and *E. subscaposa*) that have leaves forming a basal rosette (i.e. are subscapose; Figure 4.7), with basal rosetted leaves having arisen independently in each species. The proportional likelihood of the cauline leaf arrangement is 0.999 (node A, Figure 4.7) because most *Emilia* species sampled here have cauline leaves, also likely to have been present in the ancestor. Broad leaves have a high proportional likelihood (0.768) at the ancestral *Emilia* node (A) and narrow leaves are hypothesised to have evolved independently at least nine times in *Emilia* (Figure 4.8). This character state appears synapomorphic for the clade comprising six *Emilia* species including *E. emilioides*

and *Emiliella*, athough there is a reversal to broad leaves in *E. cenioides* and *E. protracta*. Narrow leaves are also present in sister taxa, *E. brachycephala* and *E. hockii*, as well as *E. graminea* and *E. baumii* — both outside of '*Emilia*' in the ITS phylogeny (Figure 4.8). There has also been convergence to narrow leaves in *Bafutia tenuicaulis* and *E. longiramea*, which occur in the same clade (Figure 4.8). The following leaf margin types: entire, sinuate-dentate, crenate to dentate, and serrate considered here have proportional likelihoods of 0.25 at both the ancestral node and node A (Figure 4.9). Sinuate-dentate leaf margin is most common in *Emilia*, whereas crenate to dentate leaf margins arose several times in *Emilia* and are present, for example, in a clade comprising *Bafutia*, 'the type *E. sagittata*' and two other *Emilia* species (Figure 4.9). Serrate margins arose independently eight times in *Emilia*.

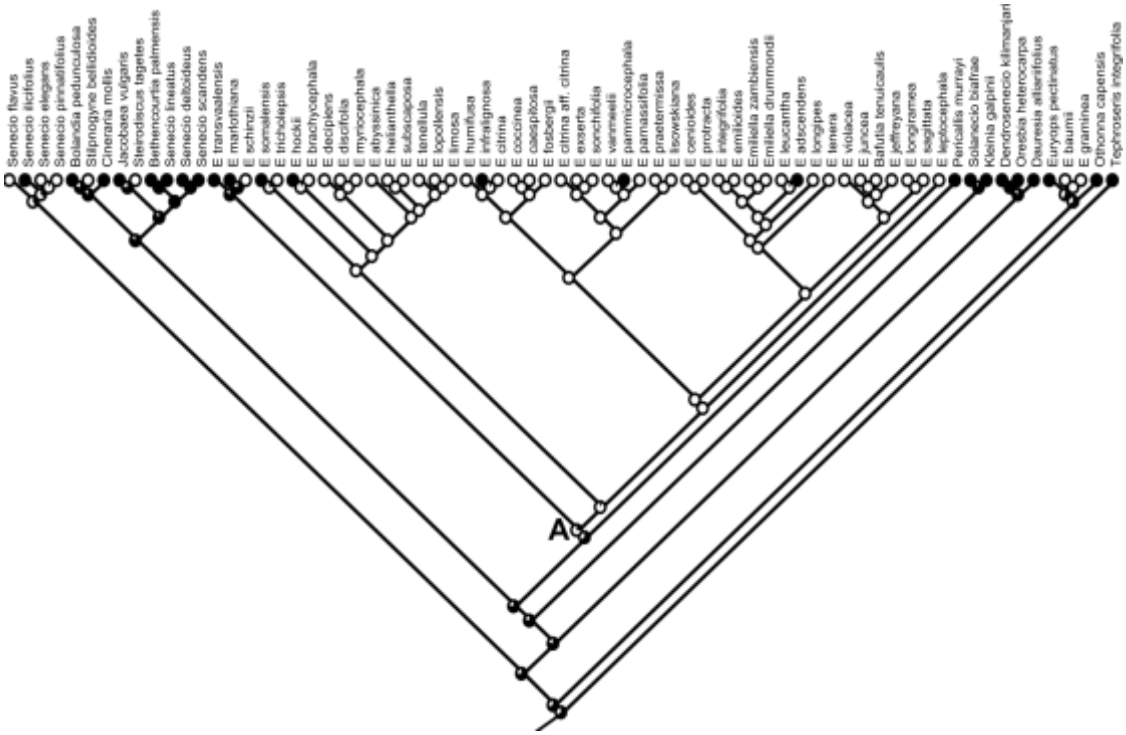

Figure 4.4. Optimisation of life history onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. White = annual; black = perennial. Letter A indicates the ancestral node of *Emilia*. Proportional likelihoods at outgroup (*Tephroseris*) and *Emilia* nodes respectively: annual = 0.338, 0.739; perennial = 0.662, 0.261.

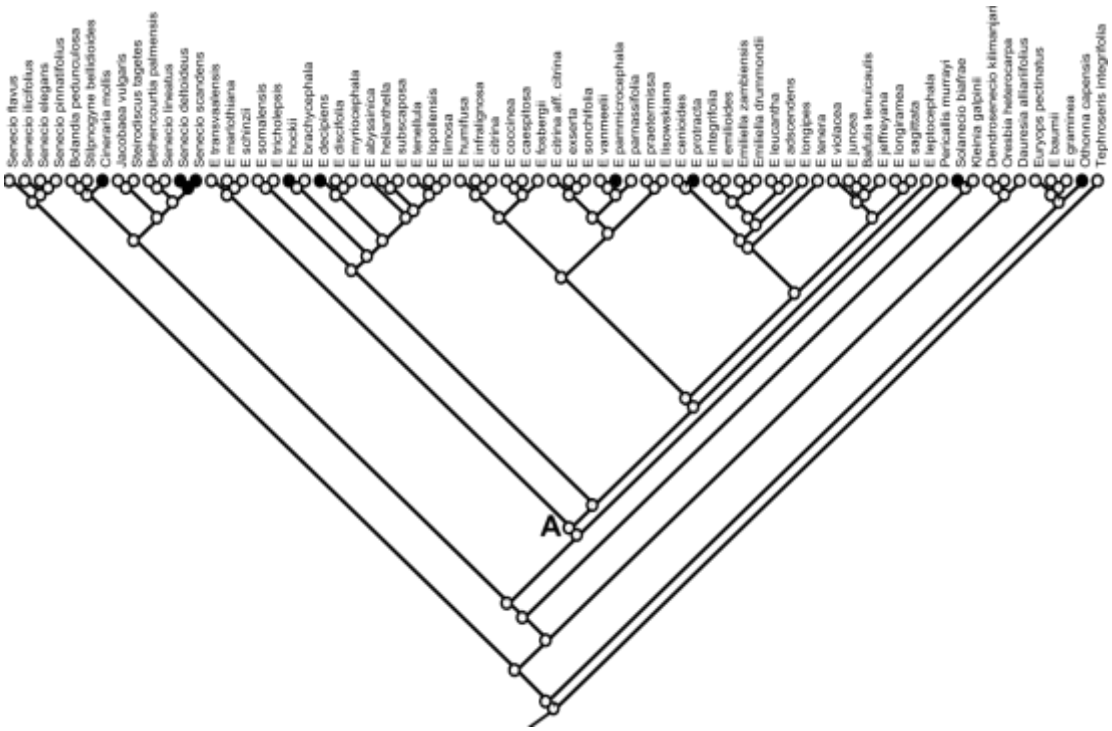

Figure 4.5. Optimisation of plant growth form onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. White = erect; black = decumbent. Letter A indicates the ancestral node of *Emilia*. Proportional likelihoods at outgroup (*Tephroseris*) and *Emilia* nodes respectively: erect = 0.947, 0.9997; decumbent = 0.053, 0.0003.

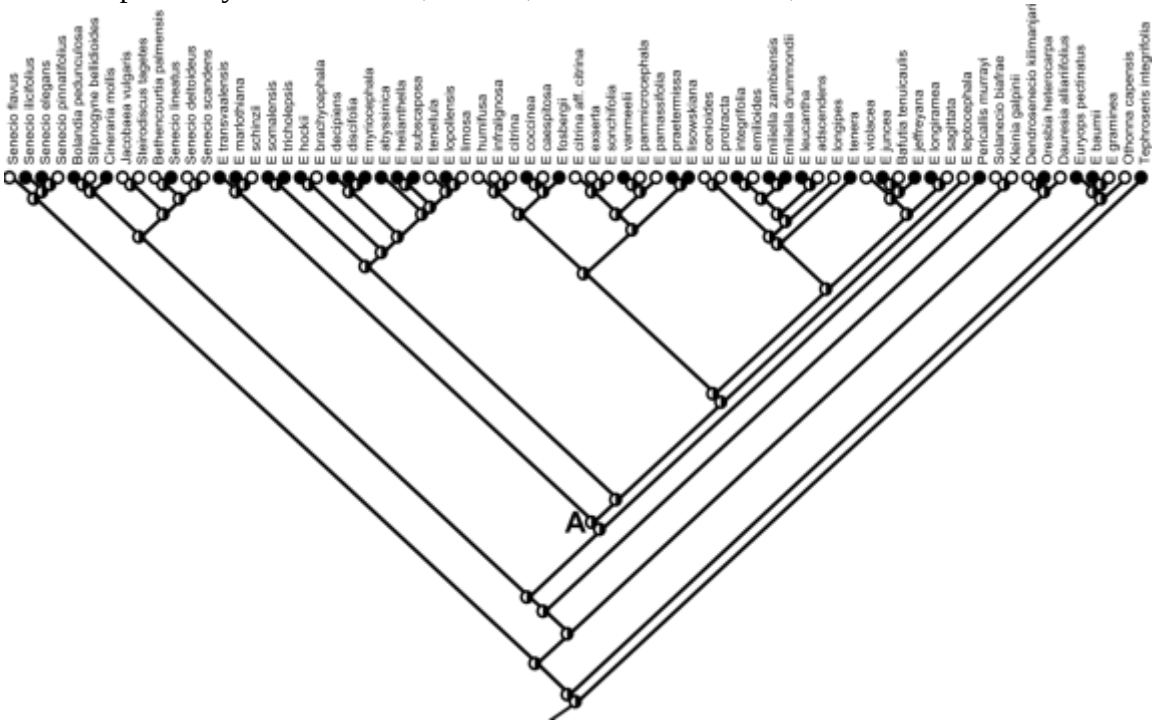

Figure 4.6. Optimisation of stem indumentum onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. White = glabrous; black = pubescent. Letter A indicates the ancestral node of *Emilia*. Proportional likelihoods at outgroup (*Tephroseris*) and *Emilia* nodes respectively: glabrous = 0.5, 0.5; pubescent = 0.05, 0.5.

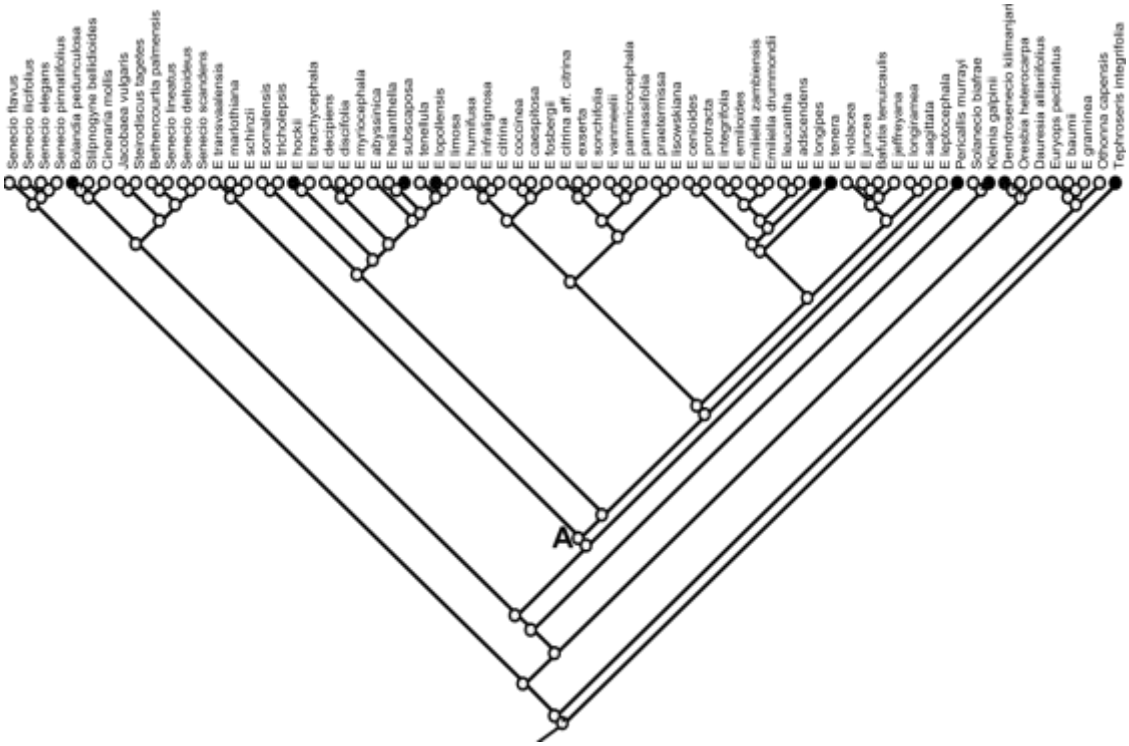

Figure 4.7. Optimisation of phyllotaxy onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. White = cauline; black = basal rosette. Letter A indicates the ancestral node of *Emilia*. Proportional likelihoods at outgroup (*Tephroseris*) and *Emilia* nodes respectively: cauline = 0.846, 0.999; basal rosette = 0.154, 0.001.

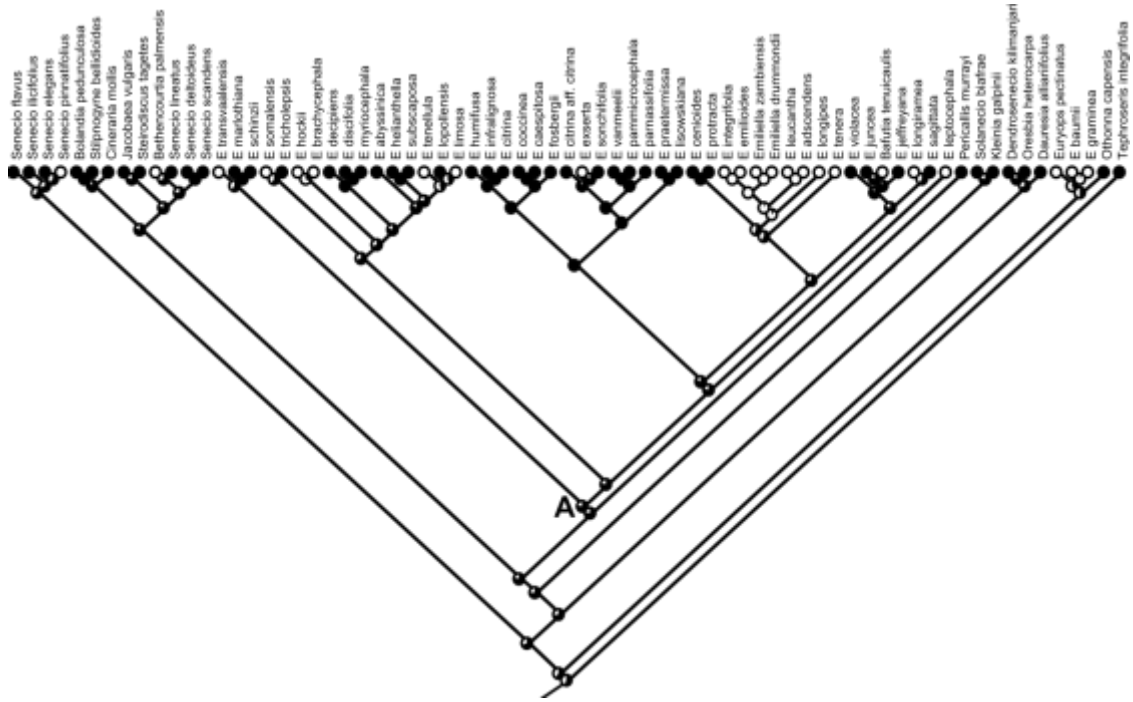

Figure 4.8. Optimisation of leaf width onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. White = narrow; black = broad. Letter A indicates the ancestral node of *Emilia*. Proportional likelihoods at outgroup (*Tephroseris*) and *Emilia* nodes respectively: narrow = 0.445, 0.232; broad = 0.555, 0.768.

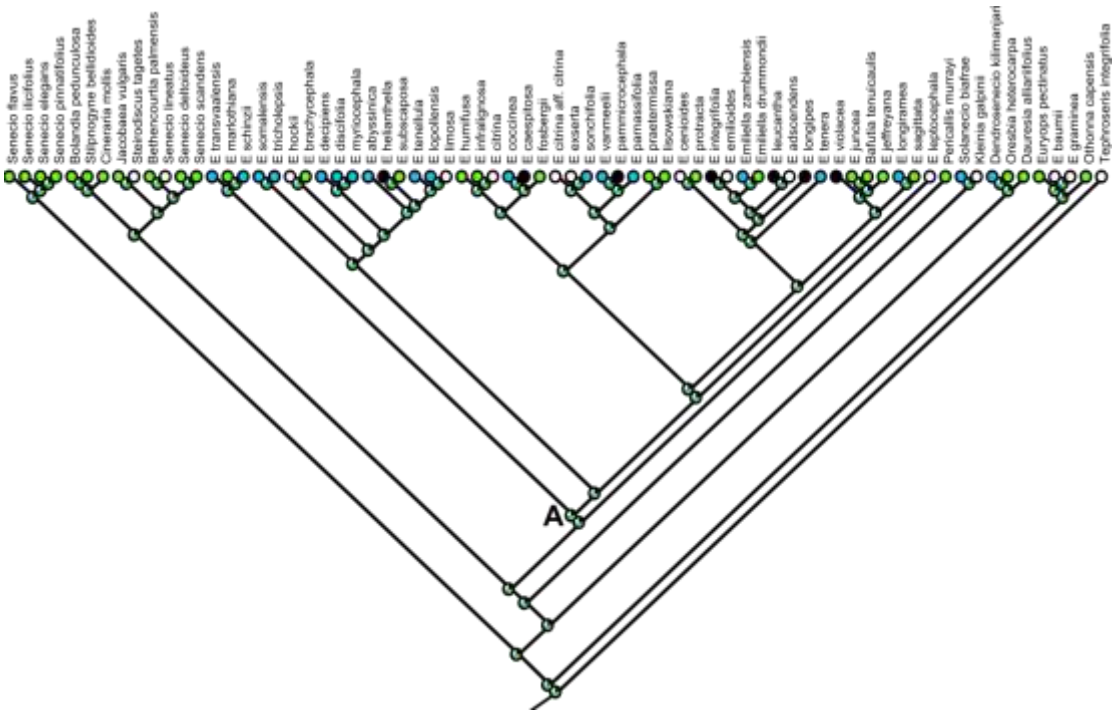

Figure 4.9. Optimisation of leaf margin type onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia,* and Senecionoid outgroups. White = entire; blue = sinuate-dentate; green = crenate to dentate; black = serrate. Letter A indicates the ancestral node of *Emilia*. Proportional likelihoods at outgroup (*Tephroseris*) and *Emilia* nodes for all character states = 0.25.

The reproductive characters are also equivocal at the ancestral node, except for two — floret colour and style branch apex shape. The character state ‘discoid capitula’ is also likely to be ancestral (plesiomorphic) in *Emilia* since it has a high proportional likelihood (0.917; Figure 4.11). Interpretations based on the *Pericallis* node suggests that the character state ‘radiate capitula’ is also likely to be ancestral (plesiomorphic) since it has a high proportional likelihood (0.79). Seemingly most outgroup taxa have radiate capitula (Figure 4.11).

Emilia species commonly have many capitula, with solitary capitula having arisen independently in *Bafutia*, *Emiliella*, and in 10 *Emilia* species included here (Figure 4.10). Discoid capitula (with a proportional likelihood of 0.917 at node A) appears to be a synapomorphy for *Emilia* with reversals to ‘radiate capitula’ six times in *E. helianthella, E. abyssinica, E. discifolia, E. brachycephala* and sister taxa *E. somalensis* and *E. tricholepsis* (Figure 4.11). Capitula width has equal proportional likelihoods of 0.5 at both the ancestral node and node A, that is, both ‘narrow’ and ‘broad’ states are equally represented across *Emilia* (Figure 4.12), while *Pericallis* and 11 other related outgroups have narrow capitula. Twenty seven *Emilia* species have broad capitula whereas 18 have narrow capitula together with *Emiliella* and *Bafutia* species (Figure 4.12). Broad capitula occur mostly in the clade

comprising five species from the *E. coccinea* complex and three Madagascan species although narrow capitula appear in four *Emilia* species (*E. parnassifolia*, *E. pammicrocephala*, *E. exserta* and *E. fosbergii*) in the same clade. Pubescent phyllaries are postulated to have arisen several times independently in *Emilia* and the character states 'glabrous'and 'pubescent' phyllaries also have equal proportional likelihoods of 0.5 at both the ancestral node and node A (Figure 4.13).

Pink to purple and cream to white florets (seen in 18 *Emilia* species included here, including the earliest diverging clade of southern African species) are shared with the sister group *Pericallis*, but this differs from the other closely related Senecioneae (Figure 4.14). The character state 'orange to red' is synapomorphic for a clade comprising 13 *Emilia* species, including e.g. *E. infralignosa*, *E. coccinea*, *E. praetermissa*, *E. longiramea*, and *E. parnassifolia*, however two species (*E. citrina*, *E. vanmeelii*) have yellow florets and three have pink to purple florets in the same clade. Cream to white florets are rare in *Emilia* and have arisen only twice in the *Emilia* species included here (viz. *E. marlothiana* and *E. emilioides*).

Most *Emilia* species have style branch apices that are unappendaged with few sweeping hairs and this is also the inferred ancestral state. Appendaged and papillose style branches appear to have arisen eight times in *Emilia* and are synapomorphic for sister taxa *E. caespitosa* and *E. coccinea* and also *E. vanmeelii*, *E. pammicrocephala*, and *E. parnasifolia* (Figure 4.15).

The majority of *Emilia* species have densely hairy cypselas (proportional likelihood = 0.952; node A, Figure 4.16). Glabrous to very sparsely hairy cypselas appears to have evolved independently at least twice in *Emilia* and is synapomorphic for a clade comprising two *Emiliella* and four *Emilia* species with reversals to densely hairy cypselas in the other four species (*E. cenioides*, *E. emilioides*, *E. leucantha*, and *E. longipes*) in this same clade. Glabrous to very sparse cypselas is also synapomorphic for a clade comprising *E. lopollensis*, *E. tenellula*, and *E. limosa* (Figure 4.16).

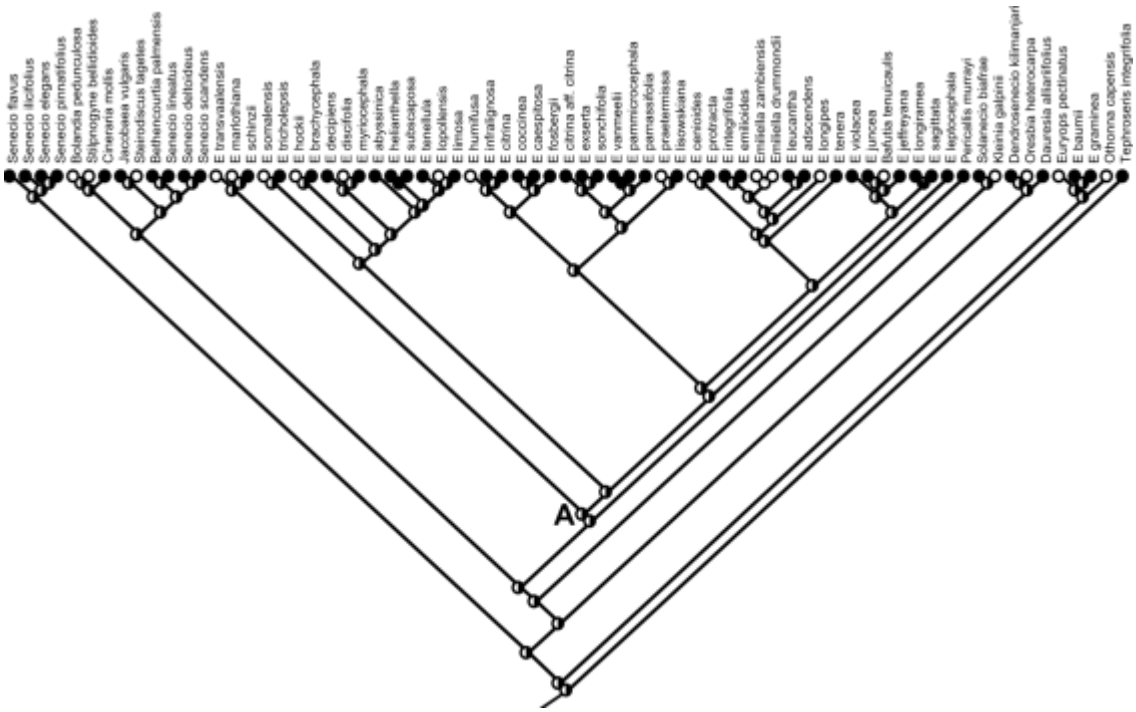

Figure 4.10. Optimisation of capitula grouping onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. White = solitary, occasionally up to two; black = in groups of three or more. Letter A indicates the ancestral node of *Emilia*. Proportional likelihoods at outgroup (*Tephroseris*) and *Emilia* nodes respectively: solitary, occasionally up to two = 0.5, 0.5; in groups of three or more = 0.5, 0.5.

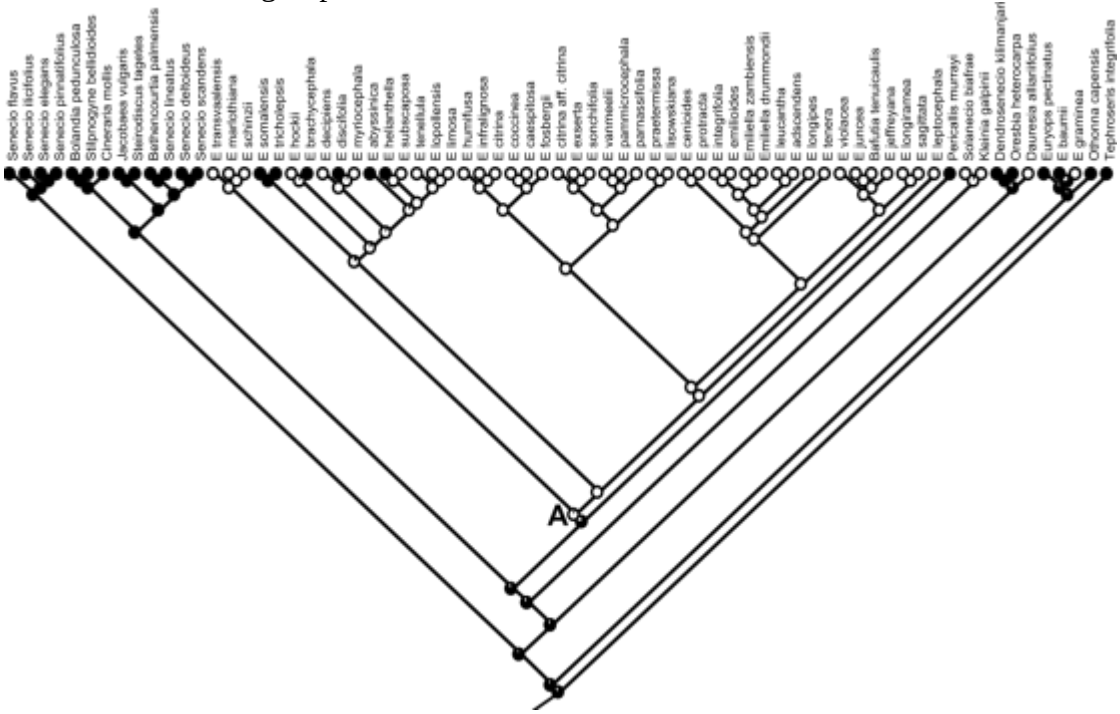

Figure 4.11. Optimisation of capitula type onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. White = discoid; black = radiate. Letter A indicates the ancestral node of *Emilia*. Proportional likelihoods at outgroup (*Tephroseris*) and *Emilia* nodes respectively: discoid = 0.101, 0.917; radiate = 0.899, 0.083.

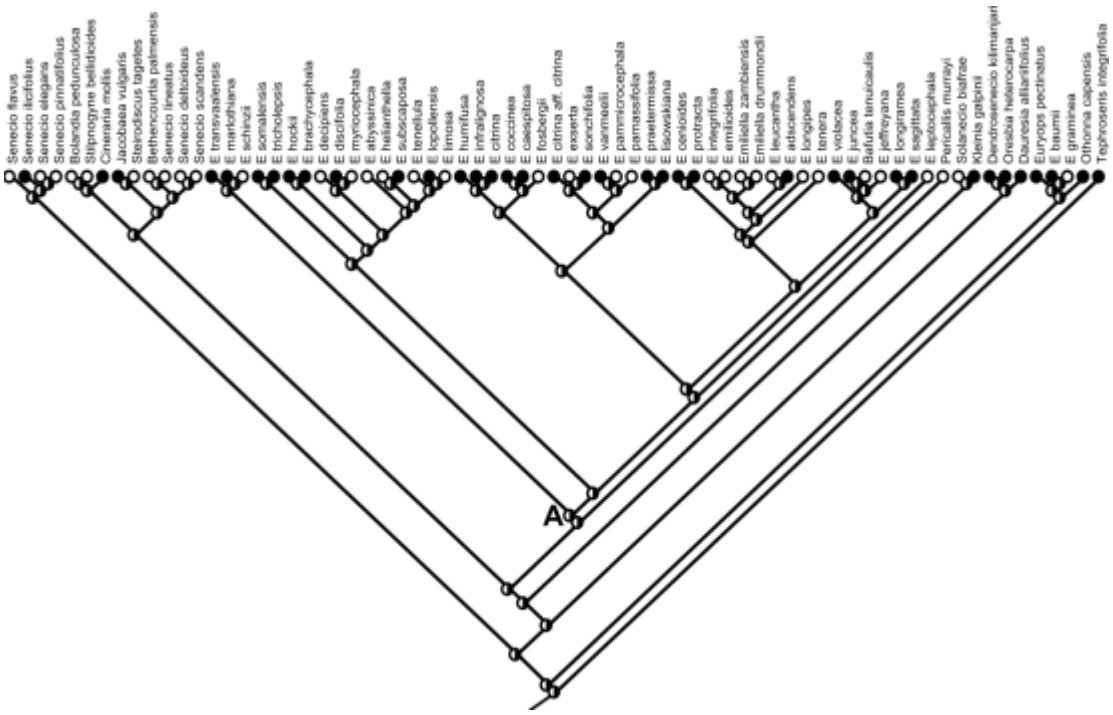

Figure 4.12. Optimisation of capitula width onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. White = narrow; black = broad. Letter A indicates the ancestral node of *Emilia*. Proportional likelihoods at outgroup (*Tephroseris*) and *Emilia* nodes respectively: narrow = 0.5, 0.5; broad = 0.5, 0.5.

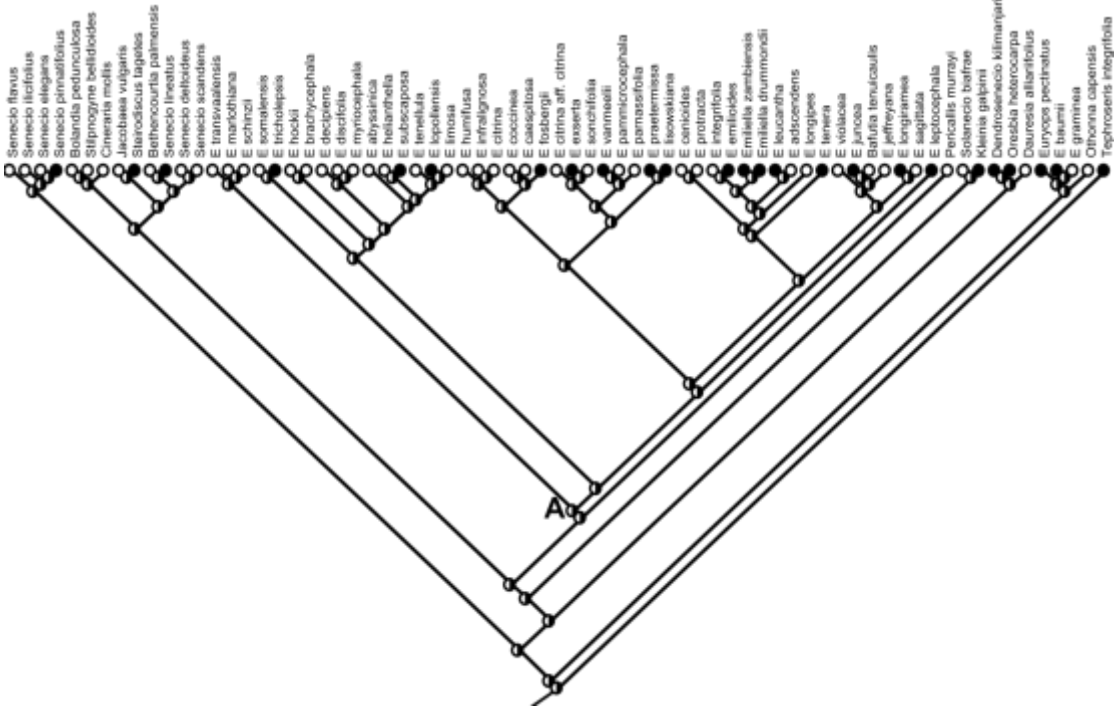

Figure 4.13. Optimisation of phyllary indumentum onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. White = glabrous; black = pubescent. Letter A indicates the ancestral node of *Emilia*. Proportional likelihoods at outgroup (*Tephroseris*) and *Emilia* nodes respectively: glabrous = 0.5, 0.5; pubescent = 0.5, 0.5.

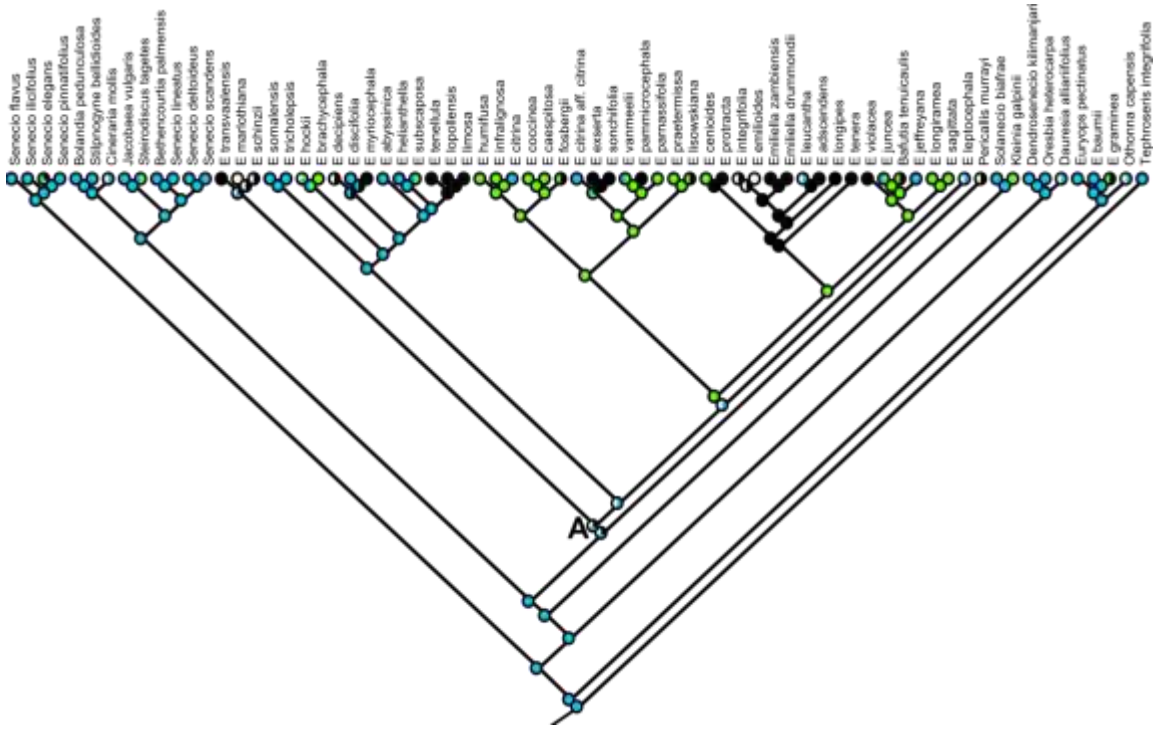

Figure 4.14. Parsimony optimisation of floret colour onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. White = cream to white; blue = yellow; green = orange to red; black = pink to purple. Letter A indicates the ancestral node of *Emilia*.

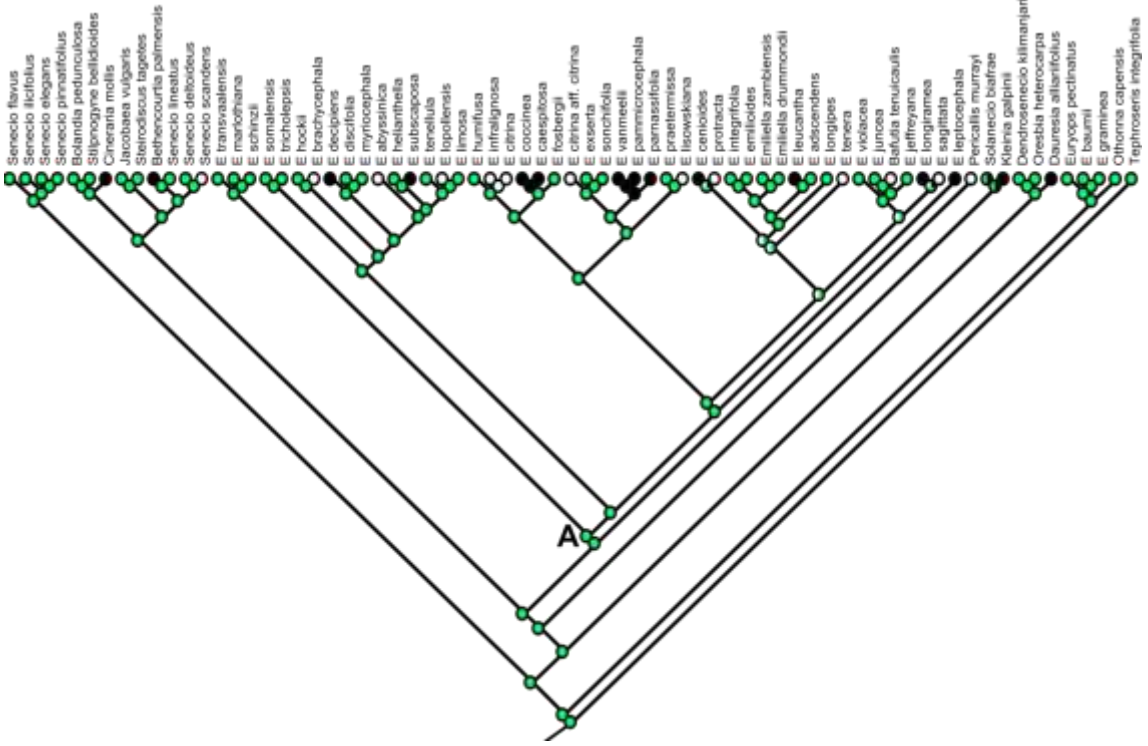

Figure 4.15. Parsimony optimisation of style branch apex shape onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. White = unappendaged and epapillose; green = unappendaged and few hairs; black = appendaged and papillose. Letter A indicates the ancestral node of *Emilia*.

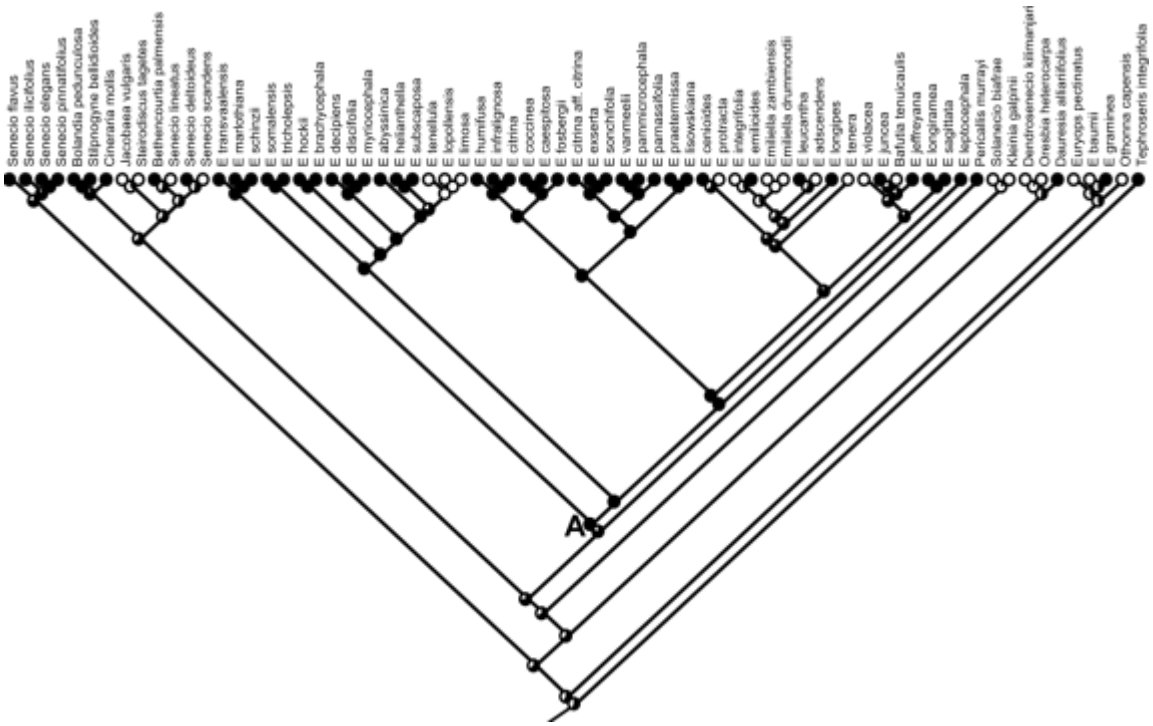

Figure 4.16. Optimisation of cypsela indumentum onto the maximum clade credibility tree resulting from BEAST analysis of the nuclear ITS data including indels in *Emilia*, *Emiliella*, *Bafutia*, and Senecionoid outgroups. White = glabrous to very sparsely hairy; black = densely hairy. Letter A indicates the ancestral node of *Emilia*. Proportional likelihoods at outgroup (*Tephroseris*) and *Emilia* nodes respectively: glabrous to very sparsely hairy = 0.443, 0.048; densely hairy = 0.557, 0.952.

Discussion

Biogeographic origin and diversification of *Emilia* in Africa

The subtribes Othonninae and Senecioninae are hypothesized to have originated in southern Africa and/or diversified there (Funk *et al*. 2005; Pelser *et al*. 2007), and the findings of this study corroborate this with the sampled outgroup taxa originating in southern Africa (Figure 4.1). The tribe Senecioneae has had a strong African influence throughout its evolutionary history, mainly in the subtribes Othonninae and Senecioninae and the lineage that gave rise to these two subtribes is also from southern Africa (Pelser *et al*. 2007). The age of the Senecioninae clade is here estimated to be 24.85 Mya (16.39–33.18 Mya) and that of the Othonninae clade 22.01 Mya (12.16–32.13 Mya), i.e. at the beginning of the Miocene, although other tribes of the Asteraceae, e.g. the Gnaphalieae (Bergh and Linder 2009), have been estimated to originate in the Oligocene (33.9–23.03 Mya). According to Pelser *et al*. (2010) age estimations of some species in subtribes Othonninae and Senecioninae are younger than the dates obtained here for the same subtribes. Four outgroups sampled here

dispersed out of southern Africa to Eurasia (*Jacobaea vulgaris*), Australia (*Senecio pinnatifolius*) and the Canary Islands (*Bethencourtia* and *Pericallis*). This study corroborates Pelser *et al*.'s (2007) findings that Africa served as the source area for the Senecioneae and other continents appear to have been colonised several times independently. Our findings are also in agreement with the notion that the southern African region has served as "an important cradle of diversification for a large part of the daisy family, as well as an 'evolutionary springboard' from which multiple lineages colonised the rest of the world" (Bergh and Linder 2009 p. 14).

Despite *Emilia* being a mainly tropical genus, it appears to have originated in southern Africa as shown by the early diverging lineages, for example, the lineage comprising *E. marlothiana* (southern Angola and Namibia), *E. transvaalensis* (Botswana, South Africa, Swaziland, and Mozambique), and *E. schinzii* (Angola, Botswana, Namibia, South Africa, and Zimbabwe) and also the lineage leading to *E. leptocephala* from Botswana, Zambia, and Angola. According to the dated ITS phylogeny, *Emilia* originated during the Mid-Miocene and the age of this genus is estimated to be 14.19 Myr (9.49–18.94 Myr) (Table 4.1). (Note: This agrees with the results of the analysis of the reconstructed dated plastid *trn*L–*trn*F phylogeny (not shown here), as it is incongruent with the nuclear phylogeny. The Miocene epoch (23.03–5.332 Mya) was a time of warmer global climate than during the earlier Oligocene or the later Pliocene and the African continent was widely covered by tropical forest (McRae 1999). The idea of an extensive rain-forest cover is contradicted by evidence from palaeosols and dry country fossil-floras from Maboko Formation of Maboko Island and Majiwa Bluffs, southwestern Kenya during the Middle Miocene (16 Mya) (Retallack 1992; Retallack *et al*. 2002). The more open vegetation systems such as the grassland ecosystem first appeared during the Middle Miocene, replacing the diminishing forests, and the grass-dominated savanna biome began to expand and became widespread in the Late Miocene (*ca.* 8 Mya), as shown by pollen and carbon isotopes from West and East Africa (Jacobs 2004). Using evidence of fire regimes, Bytebier *et al*. (2011) also dated grasslands to *ca.* 12.4 Myr implying that they were present in Africa from the Middle Miocene. Most *Emilia* clades diversified further during the Late Miocene (Figures 4.2, 4.3) and occupied varied habitats in Africa that include savannas, grasslands, and forest edges.

Although the origin of *Emilia* coincided with the evolutionary significant Benguela cold-water upwelling system, established along the southwest coast of Africa about 10 Mya (Sieser 1980), which contributed to the dry summers along the southern African west coast, the genus did not originate or diversify in the Cape Floristic Region (CFR). This is in contrast

to the many other species-rich tribes of the Asteraceae (e.g. Gnaphalieae, Arctotideae; McKenzie and Baker 2008; Bergh and Linder 2009) and large Senecioneae genera (e.g. *Euryops*, *Othonna*, and *Senecio*; Linder 2003; Devos *et al*. 2010). *Emilia* is a palaeotropical genus, with only a few species (mostly perennials) from the early diverging clade showing similar aridity adaptations (e.g. succulence) to other members of the Asteraceae, for example, in the CFR. *Emilia marlothiana* is adapted to arid conditions, occurring in rocky areas, on sandy flats, in dry river beds, and savanna thornveld (Cron 2014) mainly in the Central, North and South regions of Namibia, where it occupies the Nama Karoo and Desert Biomes with an average annual rainfall of 50–100 mm (Burke 2004). *Emilia schinzii* also occurs in some fairly arid regions in Botswana and central and northern Namibia, as well as in the grasslands of South Africa, western parts of Zimbabwe and Angola (Cron 2014). Later diversification in *Emilia* may have been promoted by the prevalent annual habit in *Emilia* species that occupied and proliferated in various habitats and dispersed to other parts of Africa. The annual growth habit ancestral in *Emilia* is possibly linked to the success in diversification of *Emilia* species in these various parts of Africa since annual plants tend to reproduce quickly and produce more seeds that enable the survival of species in varied habitats (Espeland and O'Farrell 2010). Annual growth habit has also been predicted by theoretical models to evolve as an adaptive response to unpredictable environments, including frequently disturbed habitats and aridity (Sterns 1992). In some cases, empirical data has shown that a shift to an annual life history occurs in hot and dry conditions that would affect adult perennial plants (Evans *et al.* 2005; Cruz-Mazo *et al*. 2009). Ancestral state reconstruction studies in *Nemesia* Vent. have shown that annuals are derived from perennial ancestors and annual growth habit arose multiple times within this genus (Datson *et al*. 2008). Most of the annual species in *Nemesia* (*ca.* 75% of the genus) occur in the Greater Cape Floristic Region (Goldblatt 1978) and these annual plants are able to avoid long periods of aridity through the production of dormant seeds (Datson *et al*. 2008). This could be similarly true for *Emilia*.

In southern tropical Africa, some *Emilia* lineages, for example, the widely distributed *E. caespitosa* and *E. coccinea*, occur in varied habitats at altitudes of up to 2400 m above sea level (a.s.l) including mountainous areas such as the afromontane forest patches in the Chimanimani-Nyanga Centre in Zimbabwe and the Nyika Plateau (Malawi). It is possible that these two species took refuge in the Afromontane floras resulting from Pleistocene climatic fluctuations and then possibly spread into the mountains. *Emilia caespitosa* and *E. coccinea* also occur in other habitats: miombo woodlands, abandoned and cultivated fields, and along roadsides (Mapaya and Cron 2016). The habitats (e.g. miombo woodlands) and

association of these two *Emilia* species with many woody genera associated with African savannas suggests that they might have originated in the Afromontane forest patches. *Emilia coccinea*'s distribution extends to West Africa (mountains of Cameroon) where the presence of the outlier of Afromontane flora has been attributed to long-distance dispersal (Lovett and Friis 1996), although this has been debated since *E. coccinea* could have just spread into the mountains as it is one of its many habitats (Burgoyne *et al*. 2005).

Emilia lineages (and/or species) dispersed from southern Africa to Central, East, and West Africa during the Late Miocene with subsequent diversification in these regions. The development of the Late Miocene-Pliocene arid track in East Africa (Zachos *et al*. 2001) resulted in open grasslands that could have provided suitable habitats for *Emilia* species in that region. However, the majority of these East African lineages diversified into wet/moist habitats (Clade 4; Figure 4.1), with some of the species growing in moist/swampy grasslands, e.g. *E. cenioides*, *E. longipes*, and *E. tenera*. The two *Emiliella* species and *Emilia protracta* in this same clade but with a southern African distribution, are also found in wet habitats, including floodplains, river banks, and swampy grassland. Two other species occurring in East Africa, *Emilia somalensis* and *E. tricholepsis* (Clade 2), originated during the Pleistocene epoch, and during this time Africa experienced a global reduction in temperature and associated changes in rainfall resulting in the spread of open grassland (Vrba 1985). *Emilia somalensis* and *E. tricholepsis* occupy habitats that include short and wooded grassland, rocky slopes, and mountain summits.

Some *Emilia* lineages, for example, the clade comprising *E. decipiens*, *E. discifolia*, and *E. myriocephala*, occur in open habitat ecosystems and have diversified in more than one region, that is, in Central and East Africa. *Emilia discifolia* is the most widely distributed in this clade and some of its habitats include grassland, degraded woodland especially miombo woodlands, ruderal places, and roadsides at altitude of 450–850 m a.s.l.

Out of Africa to Madagascar

There were multiple dispersal events out of Africa to Madagascar in *Emilia*, mainly from southern and East Africa. At least five independent colonisations have occurred (Figure 4.1) for the six Madagascan species (out of 14) included here. Long distance dispersal by wind to Madagascar is very possible since *Emilia*'s cypselas are relatively small, light and the bristled pappus would have enhanced wind dispersal capability. The exceptionally rich biodiversity in Madagascar is connected to East Africa by lineages such as *Anthospermum* L. (Rubiaceae), *Coleochloa setifera* (Cyperaceae), and *Xerophyta* Juss. (Velloziaceae), which occur on

inselbergs in both countries (Barthlott and Porembski 1998). Most phylogenetic studies of Malagasy biota have similarly indicated a pattern of sister group relationships with African taxa; thus Africa is suggested as the most important source of plant dispersal to Madagascar (Yodar and Nowak 2006). Genera which occur both in Africa and Madagascar include *Adansonia* L. and *Anisopappus* Hook & Arn. (Wild 1964, 1968) and species in sect. *Anisopappus* are common in both Africa and Madagascar (Wild 1964).

Our analyses date the diversification of the Madagascan *Emilia* lineage comprising *E. citrina*, *E. humifusa*1, and *E. infralignosa* as relatively recent (4.0 Mya; Figure 4.2), that is, in the Pliocene (5.332–1.806 Mya). *Emilia citrina* is endemic to Madagascar and found along river banks, waterfalls, forest edges and road sides (Humbert 1963). The natural habitats of *E. humifusa* and *E. infralignosa* include forest edges, river banks or shorelines, and rocky bushy areas. The two accessions of *Emilia humifusa* (1 and 2) appear to be distinct lineages as they are not placed sister to each other in the nuclear ITS phylogeny even though they both occur in the adjacent Antsiranana and Fianarantsoa Provinces of Madagascar where they are endemics. These species also occur at different positions in the plastid *trn*L-*trn*F phylogeny (Figure 3.1). The other possibility is that one of these two species could have been misidentified. *Emilia humifusa* (1 and 2) are morphologically similar in the following: they are herbs of almost the same height (up to 60 cm); have glabrous stems; sessile, auriculate, amplexicaul leaves with shallowly dentate leaf margins; solitary, glabrous, campanulate capitula slightly longer than broad with 10–12 phyllaries; and orange florets. *E. humifusa*2 has pubescent cypsela and truncate, unappendaged style branch apices with few minute hairs, however these characters were not determined in *E. humifusa*1 due to unavailability of the physical specimen resulting in the scanned specimen being used. *E. humifusa*2 mostly occurs in hollows of granite outcrops in association with mosses at an altitude of 1 374 m above sea level (a.s.l.), the habitat of *E. humifusa*1 is not noted on the specimen although it occurs at an altitude of 500 m a.s.l. Further studies using sensitive molecular markers are needed to verify the taxonomic status of *Emilia humifusa* (1 and 2) in order to provide more insight in dating the diversification of the Madagascan *Emilia* species.

Morphological character evolution

Adaptations to different habitats

A wide range of growth habits and life forms occur among the Senecioneae and are adaptations to the diversity of habitats occupied by members of this tribe (Nordenstam *et al*. 2009). In the Old World senecioids, annual herbs are postulated to have evolved repeatedly (Nordenstam *et al*. 2009). The annual life form is hypothesised to have either been ancestral or evolved early in *Emilia* [13.32 Mya (9.08–18.11 Mya)], and is also evident in the genera *Bafutia* and *Emiliella*, which are both annuals and are nested within *Emilia*. Annuals are short-lived plants adapted to exploiting habitats with ephemeral resources. They also repeatedly go through cycles of fast population growth, and are subject to *r*-selection (Gadgil and Solbrig 1972), hence their proliferation in terms of number of species in a variety of habitats. An annual growth habit could have enabled rapid diversification into habitats as the climate and vegetation changed across Africa during the late Middle Miocene. Some annuals (e.g. *E. brachycephala*, *E. limosa*, and *E. protracta*) also tend to have distinct habitat preferences, such as moist habitats at edges of rivers or dams (Cron 2014; personal observation). Other species occupy varied habitats including disturbed lands and along roadsides (e.g. *E. caespitosa*, and *E. coccinea*). On the other hand, the few perennial *Emilia* species (e.g. *E. transvaalensis*) have the option of vegetative reproduction which could mean a greater probability of survival and the succulent or semi-succulent habit also gives these perennial species the opportunity to survive dry periods and therefore to 'over-winter'. Perennial life form is also often associated with islands, for example, all *Echium* L. species (but two) in the Canary Islands are perennial with a woody rootstock, whereas the mainland European *Echium* species are all herbaceous (Bramwell 1972; Böhle *et al*. 1996; Whittaker and José Maria 2007). Similarly, island endemics *Emilia adscendens* and *E. infralignosa* have reverted to the perennial life form in Madagascar. *Emilia* species with pubescent stems are also adapted to survive in harsh environments (e.g. *E. somalensis* occurs on rocky slopes and dry sandy soil; Tadesse and Beentje 2004), as the hairs protect the plants from extreme weather conditions such as heat and drought by reducing evaporation (Johnson 1975). *Emilia* species with glabrous stems occur mostly in moist habitats (viz. *E. brachycephala*, *E. limosa*, *E. leptocephala*, and *E. protracta*). Hairs are also occasionally present on phyllaries of *Emilia* and may serve as a defence against insect herbivory, as well as protection of the florets from drought by conserving moisture. Phyllary indumentum like most other optimised characters

in our study is homoplasious and *Emilia* species with pubescent phyllaries occur in varied habitats that are not necessarily arid.

Cypsela morphology (shape, size, colour, and surface) is taxonomically important in the classification of many genera and species of Asteraceae, including *Emilia* (Bremer 1994; Swelankomo *et al*. 2007). Most *Emilia* species have elliptic-oblong five-ribbed cypselas, their width ranging from about 0.2–1.1 mm, mostly light to dark brown with glabrous or hairy surfaces. In *Emilia*, most species have densely hairy cypselas and glabrous to sparsely hairy cypselas are derived. Hairs on the cypselas are biseriate, hence called 'duplex' or 'twin hairs' (Hess 1938) and are widespread in the Senecioneae. These hairs play an important role in imbibition and germination. The twin hairs are often mucilaginous (e.g. *Emilia*, *Euryops* (Cass.) Cass, *Jacobaea* Mill., *Senecio*, and *Cineraria* L.) and the cypselas of these species were characterized as 'myxocarpic diaspores' (Nordenstam *et al*. 2009; De-Paula *et al*. 2015). Myxodiaspory is also common in several other families (e.g. Acanthaceae, Brassicaceae, Lamiaceae, and Poaceae) and is not restricted to species in arid environments, although it has been shown to aid seed germination in osmotically stressful and saline habitats of the desert environment in *Artemisia sphaerocephala* Krasch. (Yang *et al*. 2010). Mucilage was also shown to play a role in the dispersal of *Alyssum minus* (L.) Rothm. (Brassicaceae) seeds by forming a dry papery mucilage wing, facilitating seed hydration by increasing surface contact with the substrate, and helping as a water reserve for germination (Sun *et al*. 2012). Most *Emilia* species with glabrous cypselas are found in moist habitats (viz. very damp soils, shallow wetlands (dambos), swampy grasslands) and therefore no longer need mucilaginous hairs for seed germination.

Narrow leaves in *Emilia* are predominantly linear to narrowly elliptic or oblanceaolate, oblong-narrowly ovate to narrowly ovate, slightly fleshy, and sessile characteristics suitable for survival under harsh environmental conditions (Tadesse and Beentje 2004; Cron 2014). The change from broad to narrow leaves in *Emilia* is similar to a general trend of plants in arid or harsh environments and previous research has shown that narrow leaves reduce transpiration by decreasing the size of the boundary layer (Xu *et al*. 2009). Amongst those species with narrow leaves, *E. adscendens* and *E. hockii* have entire leaf margins whereas the others have toothed margins (e.g. *E. leucantha*, and *E. integrifolia*). Leaves with toothed margins have been shown to be disadvantageous in dry environments because the teeth increase water loss and rates of sap flow (Xu *et al*. 2009).

Leaf arrangement and photosynthetic efficiency

The common leaf arrangement in *Emilia* is cauline whereby the leaves overlap minimally on the usually erect stems. These leaves are positioned so as to maximise the surface area to intercept sunlight, thereby enhancing the plant's ability to photosynthesise and thus influencing the success of the plants. On the other hand, basal rosetted leaves shade each other, reducing photosynthetic rate, which might explain why few *Emilia* species have leaves arranged in a basal rosette. The advantage of a basal rosette is mainly in the protection from extremes of temperature, and as the leaves are near the ground, and because of their limited exposure are protected from drying winds and browsing animals (Tyler 1902). *Emilia* species with a rosette habit included in this study mostly occur in swampy areas and basal rosettes possibly serve a protective function against herbivory and hence improve their survival since these plants are hard to pull from the ground and their leaves can come off leaving the roots firmly attached to the ground.

Capitula and pollination

Capitula in *Emilia* are either solitary or in terminal corymbs and 'capitula grouping' is equivocal for the ancestor in our study. Arrangement of capitula in cymose corymbs, as found in the majority of species in the Asteraceae, is a derived condition in the family and is indicative of larger seed sets that could have contributed to its early success in the colonization of extensive areas of the Old World (Panero *et al*. 2014). In the Asteraceae, a solitary and large capitulum has been shown to be mechanically vulnerable, whereas a corymb of smaller capitula is less easily damaged and a few broken branches do not have much effect on the inflorescence as a whole (Proctor *et al*. 1996). Capitula 'in groups of three or more' in *Emilia* are more conspicuous than solitary capitula and are likely to be visited more often by insects. It is thus easy for the insects to move from one head to another and pollinate more florets in reduced time than from one solitary capitulum to another (Leppick 1977).

The capitula in *Emilia*, as in other Asteraceae, are functionally protogynous and possibly self-incompatible (Burtt 1977; Ladd 1994), thereby promoting cross pollination (Burtt 1977). Outcrossing (cross pollination) results in genetic variation within species which could facilitate populations adapting to new or changing environments and colonising new places (Proctor *et al*. 1996). It is important for *Emilia* to adapt to modified habitats resulting from fluctuations in the world's climate from the Mid-Miocene (when the genus originated) up to present time. Generally the capitula in the Senecioneae are yellow-flowered, although

other colours including white, orange, red, pink, and purple do occur (Nordenstam 2007). The predominant yellow colour serves as a pollinator attraction mechanism in the family Asteraceae and various researchers (e.g. Kevan 1983; Stuessy *et al.*1986) have shown that a variety of insects, notably, flies and butterflies seem to prefer yellow flowers as they are often highly reflective. Asteraceae are pollinated by specific pollinators and the most important insect pollinators in Tropical areas are butterflies, which predominate, are highly specialized and exclusively feed on nectar; solitary bees, which play a minor role as pollinators and gather mainly pollen; and hoverflies (syrphids) (Mani and Saravanan 1999; Jeffrey 2007). Insect pollination by solitary bees and beetles is likely in *Emilia* and visitation by butterflies also occurs (personal observation). In addition to yellow, orange or red florets are common in *Emilia* and these colours visually attract pollinators together with other rewards such as pollen and nectar (Jeffrey 2009). The character state 'discoid capitula' is a synapomorphy for *Emilia*, however there are several reversals to radiate capitula (mostly yellow) in six *Emilia* species. The reason could be that ray florets significantly increase the conspicuousness of the capitula and are associated with attracting pollinators (especially butterflies) and/or providing a landing platform for them (Burtt 1977; Leppick 1977; Stuessy *et al*. 1986). In both discoid and radiate capitula, the disc florets provide a flat surface comprising several protruding pistils and stamens, over which some insect pollinators crawl (Leppick 1977). Discoid and radiate capitula also attract these insect pollinators by their heads being conspicuous in terms of size. In *Emilia* most species have broad discoid capitula and a few have broad radiate capitula (e.g. *E. tricholepsis*, *E. somalensis*, and *E. discifolia*). In addition some *Emilia* species (e.g. *E. praetermissa*, *E. fosbergii*, and *E. sonchifolia*) also have florets that exceed the phyllaries making the heads more conspicuous to pollinators. *Emilia* species with broad capitula (thus conspicuous flower heads containing larger cypselas) are more successful in colonizing varied habitats compared to those with narrow capitula, as larger seeds provide large quantities of nutrients available for a long time during the slow development of the embryos (Burtt 1977).

Style morphology is an important character in the delimitation of genera in the tribe Senecioneae (Bremer 1994). The styles vary in terms of branch appendage and type and arrangement of sweeping hair. In the Senecioneae, style branch appendages when present are extended above the stigmatic areas and sweeping hairs are concentrated at the apex of style branches (Bremer 1994). Additionally, together with anthers, style branches play a role in secondary pollen presentation, which is important in limiting the quantity of pollen withdrawn by a pollinator during a single visit — thereby increasing the chances of

successfully transferring pollen and enhancing the pollination process (Ladd 1994; Jeffrey 2009). In the Senecioneae, secondary pollen presentation is achieved when pollen shed into the anther-tube is removed by sweeping hairs located on the outside of the style branches or on the style branch apex (Jeffrey 2009), or pumped out by sweeping hairs on style branch apices. In *Emilia*, the appendaged, papillose style branches that appear to have evolved independently several times, are probably important in secondary pollen presentation (as outlined above), thus contributing to the success of *Emilia* in colonising varied habitats.

Conclusions and recommendations for future study

The dated phylogeny and biogeographic study have provided valuable information regarding the origin and diversification of *Emilia* in Africa and its dispersals to Madagascar. *Emilia* originated in southern Africa during the Mid-Miocene (*ca.* 14.19 Mya) and diversified into varied habitats in Africa. This origin coincided with the expansion of open habitats following the mid-Miocene Climatic optimum (*ca.* 15 Mya). Past climatic and vegetation changes over time in Africa (e.g. global reduction in temperature and associated changes in rainfall that resulted in the spread of open grassland during the Pleistocene epoch) have been considered in hypothesising likely causes of speciation in *Emilia*. Multiple dispersal events out of Africa to Madagascar have been hypothesised to have occurred recently in *Emilia*, that is, in the Pliocene epoch.

The annual growth form, narrow and cauline leaves, discoid capitula, and floret colour are some of the morphological trends that could have influenced diversification and adaptation of *Emilia* species to various habitats. The morphological character evolution has here been linked to adaptations to different habitats, photosynthetic efficiency (e.g. cauline leaf arrangement on erect stems) and pollination syndromes (e.g. conspicuous capitula in groups of three or more, and brightly coloured florets). The annual habit in *Emilia* likely resulted in rapid diversification into varied habitats as the climate and vegetation changed across Africa during the late Middle Miocene.

If the dated plastid phylogeny is used and the incongruent patterns between the plastid and ITS phylogenies are taken into consideration, similar conclusions regarding the biogeographic history, origin, and character evolution of *Emilia* are generally supported.

In order to obtain further insights into the diversification of the Madagascan *Emilia* species, additional *Emilia* species from Madagascar not sampled here should be added to the

phylogeny. In addition, the taxonomic status of *E. humifusa* (1 and 2) should be confirmed by further studies using sensitive molecular markers such as microsatellites.

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Appendix 4.1

Table A1. Data matrix for geographic areas used in optimisations. Geographic areas were coded as follows: 0–Europe; 1–widespread; 2– Eurasia; 3–Australasia; 4–Canary Islands; 5–Madagascar; 6–East Africa; 7–West Africa; 8–Central Africa; and 9–southern Africa.

Species	Geographic area states
Tephroseris integrifolia	0
Euryops pectinatus	9
Othonna capensis	9
E graminea	5
E baumii	9
Dauresia alliariifolius	9
Dendrosenecio kilimanjari	6
Oresbia heterocarpa	9
Senecio flavus	9
Senecio elegans	9
Senecio pinnatifolius	3
Senecio ilicifolius	9
Kleinia galpinii	9
Solanecio biafrae	7
Steirodiscus tagetes	9
Cineraria mollis	9
Stilpnogyne bellidioides	9
Bolandia pedunculosa	9
Pericallis murrayi	4
Senecio deltoideus	9
Senecio scandens	1
Senecio lineatus	9
Jacobaea vulgaris	2
Bethencourtia palmensis	4
Emiliella drummondii	9
Emiliella zambiensis	9
Bafutia tenuicaulis	7
E transvaalensis	9
E marlothiana	9
E schinzii	9
*E emilioides*1	6
*E emilioides*2	7
E integrifolia	5&6&9
*E discifolia*1	6&8&9
*E discifolia*2	6&8&9
*E brachycephala*1	9

*E brachycephala*2	9
E tenellula	9
E somalensis	6
E leptocephala	9
E protracta	9
E cenioides	6
*E violacea*1	6&8&9
E parnassifolia	8&9
*E vanmeelii*1	6&8&9
E caespitosa	6&7&8&9
*E coccinea*1	9
*E coccinea*2	9
*E coccinea*3	7
*E coccinea*4	7
*E jeffreyana*2	6&8
*E praetermissa*1	7&8
*E praetermissa*2	7&8
E lisowskiana	7&8&9
E adscendens	5
*E humifusa*1	5
*E humifusa*2	5
*E citrina*1	9
*E citrina*2	5
E longiramea	8&9
E sagittata	8&9
E longipes	6
E juncea	6&8
E tenera	6
E infralignosa	5
E leucantha	6&9
E lopollensis	9
E limosa	6&8&9
E subscaposa	8
E hockii	6&9
E tricholepsis	6
E abyssinica	6&7&8&9
E helianthella	6
E myriocephala	6
E decipiens	6&9
*E pammicrocephala*1	6&8
E sonchifolia	1
E fosbergii	1
E coccinea 5	1
E exserta	3

Table A2. Morphological characters and states used in character optimization for the genus *Emilia* and related genera in the Senecioneae.

1. Life history	(0) annual, (1) perennial
2. Plant growth form	(0) erect, (1) decumbent / sprawling
3. Stem indumentum	(0) glabrous, (1) pubescent
4. Leaf arrangement on stem / Phyllotaxy	(0) cauline, (1) basal rosette / subscapose
5. Leaf width (at broadest point)	(0) narrow (up to 10 mm), (1) wide (> 15 mm)
6. Leaf margin type	(0) entire, (1) sinuate dentate, (2) dentate–crenate, (3) serrate
7. Capitula types	(0) discoid, (1) radiate
8. Capitula grouping	(0) solitary, occasionally up to two, (1) in groups of three or more
9. Capitula diameter (at broadest point)	(0) narrow (up to 4 mm diameter), (1) wide (> 6 mm diameter)
10. Phyllary indumentum	(0) glabrous, (1) pubescent
11. Floret colour	(0) cream to white, (1) yellow, (2) orange to red, (3) pink to purple
12. Style branches apex	(0) unappendaged & epapillose, (1) unappendaged & few hairs at apex, (2) appendaged & papillose
13. Cypsela indumentum	(0) glabrous to very sparsely hairy, (1) densely hairy

Table A3. Morphological data matrix for characters used in optimisations for *Emilia* and related Senecioneae. ? represents inapplicable or unknown states.

Species/Character number	0 1	0 2	0 3	0 4	0 5	0 6	0 7	0 8	0 9	1 0	1 1	1 2	1 3
Tephroseris integriifolia	1	0	1	1	1	0	1	1	1	1	1	1	1
Dauresia alliariifolius	1	0	0	0	1	2	0	1	1	0	0&1	2	1
Euryops pectinatus	1	0	1	0	0	2	1	0	1	1	1	1	0
Othonna capensis	1	1	0	0	1	2	1	0	1	0	0&1	?	0
E. graminea	0	0	0	0	0	0	0	1	0	0	2&3	1	1
E. baumii	0	0	1	0	0	0	1	1	1	1	1	1	0
Senecio deltoideus	1	1	0	0	1	2	1	1	0	0	1	1	1
Senecio scandens	1	1	0	0	1	2	1	1	0	0	1	0	0
Senecio lineatus	1	0	1	0	1	0	1	1	0	1	1	1	0
Dendroseneciokilimanjari	1	0	0	0	1	2	1	1	1	1	1	1	0
Oresbia heterocarpa	1	0	1	0	1	2	1	0	1	1	1	1	0
Senecio flavus	0	0	0	0	1	2	1	1	0	0	1	?	1
Senecio elegans	0	0	1	0	1	2	1	1	0	0	1&2&3	?	1
Senecio pinnatifolius	0	0	0	0	0	2	1	1	0	1	1	?	1
Senecio ilicifolius	1	0	1	0	1	2	1	1	1	0	1	?	1
Steirodiscus tagetes	0	0	0	0	1	0	1	0	0	1	1&2	1	0
Cineraria mollis	1	1	1	0	1	2	0	1	1	0	0&1	2	1
Stilpnogyne bellidioides	0	0	0	0	1	2	1	0	0	0	1	1	1
Bolandia pedunculosa	1	0	1	1	1	2	1	0	0	0	1	1	1
Kleinia galpinii	1	0	0	1	1	0	0	0	1	1	2	2	0
Solanecio biafrae	1	1	0	0	1	1	0	1	0	0	1	?	0
Pericallis murrayi	1	0	1	0	1	2	1	1	0	0	0&3	0	1
Jacobaea vulgaris	1	0	0	0	1	2	1	1	1	0	1	1	0
Bethencourtia palmensis	1	0	0	0	0	2	1	1	0	0	1	2	1
Emiliella drummondii	0	0	1	0	0	2	0	0	0	1	3	1	0
Emiliella zambiensis	0	0	1	0	0	1	0	0	0	1	3	1	0

Bafutia tenuicaulis	0	0	0	0	0	2	0	0	0	0	2&3	0	0
E. transvaalensis	1	0	1	0	0	1	0	0	1	0	3	1	1
E. marlothiana	1	0	1	0	1	2	0	0	1	0	0	1	1
E. schinzii	0	0	0	0	1	1	0	1	0	0	0&3	1	1
E. emilioides	0	0	0	0	0	0	0	1	0	1	0	1	1
E. integrifolia	0	0	1	0	0	3	0	1	0	0	0&3	1	0
E. discifolia	0	0	1	0	1	1	1	0	1	0	1	1	1
E. bracycephala	0	0	0	0	0		1	1	1	0	2	0	1
E. tenellula	0	0	0	0	0	1	0	1	0	0	3	1	0
E. leptocephala	0	0	0	0	0	0	0	1	0	1	0&1	2	1
E. somalensis	1	0	1	0	0	1	1	0	1	0	1	1	1
E. protracta	0	1	0	0	1	2	0	1	1	0	3	0	0
E. cenioides	0	0	0	0	1	0	0	0	1	0	2	2	1
E. violacea	0	0	0	0	1	3	0	1	1	0	3	1	0
E. parnassifolia	0	0	0	0	1	1	0	1	0	0	2	2	1
E. vanmeelii	0	0	1	0	1	1	0	1	1	1	1&2	2	1
E. caespitosa	0	0	0	0	1	3	0	1	1	0	2	2	1
E. coccinea	0	0	1	0	1	1	0	1	1	0	2	2	1
E. jeffreyana	0	0	1	0	1	2	0	1	0	0	1	1	1
E. praetermissa	0	0	1	0	1	2	0	0	1	1	2	1	1
E. lisowskiana	0	0	1	0	1	2	0	1	1	1	2&3	0	1
E. adscendens	2	0	0	0	0	0	0	1	1	0	3	1	0
E. longiramea	0	0	1	0	0	1	0	1	1	1	2	2	1
E. humifusa	0	0	0	0	1	2	0	0	1	0	2	1	1
E. aff. *citrina*	0	0	0	0	1	0	0	1	1	0	1	0	1
E. citrina	0	0	0	0	1	0	0	1	1	0	1	0	1
E. sagittata	0	0	0	0	1	2	0	1	1	0	2	0	1
E. longipes	0	0	0	1	0	3	0	0	0	0	3	1	1
E. leucantha	0	0	1	0	0	3	0	1	0	1	0&1	2	1
E. tenera	0	0	1	1	0	1	0	1	0	1	3	0	0

E. lopollensis	0	0	1	1	1	1	0	0	1	1	3	0	0
E.limosa	0	0	0	0	0	0	0	1	0	0	3	1	0
E. juncea	0	0	1	0	1	2	0	1	1	1	1&2	1	1
E. subscaposa	0	0	1	1	1	2	0	1	1	1	1&2	2	1
E. infralignosa	2	0	0	0	1	2	0	1	1	0	2	0	1
E. hockii	2	1	1	1	0	0	0	0	1	0	0&1&2	1	1
E. fosbergii	0	0	1	0	1	2	0	1	0	1	2&3	1	1
E. tricholepsis	0	0	1	0	1	1	1	1	1	1	1	1	1
E. abyssinica	0	0	1	0	1	1	1	1	0	0	1	0	1
E. helianthella	0	0	1	0	1	3	1	1	0	0	1	1	1
E. pammicrocephala	1	1	0	0	1	3	0	1	0	0	3	?	1
E. myriocephala	0	0	1	0	1	1	0	1	0	0	3	?	1
E. decipiens	0	1	1	0	1	1	0	1	0	0	0&3	2	1
E. sonchifolia	0	0	0	0	1	1	0	1	1	0	3	1	1
E. coccinea 5	0	0	1	0	1	1	0	1	1	0	2	2	1
E. exserta	0	0	0	0	0	0	0	1	0	1	3	?	1

Table A4. Taxon sets created in the XML file in BEAUti v.1.8. and used to define calibration nodes in *Emilia* and related Senecioneae.

Taxon set	Included taxa	Monophyletic
Ingroup	All ingroup taxa (excluding *Tephroseris*)	Yes
Emilia	Main *Emilia* clade including *Bafutia* and *Emiliella*	Yes
Senecioninae	(excluding *Tephroseris*, Othonninae)	Yes
Othonninae	*E. baumii*, *E. graminea*, *Euryops pectinatus*, *Othonna capensis*	Yes
Bethencourtia-S. lineatus	*Bethencourtia*, *S. lineatus*	Yes
Pericallis-Emilia	*Pericallis*, main *Emilia* clade	Yes

CHAPTER 5

Untangling conservation prioritization in the 'tassel flower': exploring biodiversity and phylogenetic indices.

Abstract

This study seeks to contribute to debates on conservation prioritization as they apply to *Emilia* species and their associated habitats in southern Africa, with an additional focus on current conservation capacity for conservation of *Emilia* in Zimbabwe. This is achieved by using geographic distribution data for the 24 southern African *Emilia* species to identify centres of diversity and areas of endemism in *Emilia*, and a reconstructed nuclear ITS phylogeny to assess and compare four biodiversity indices (species richness—SR, corrected weighted endemism—CWE, phylogenetic diversity—PD, and phylogenetic endemism—PE) for conservation prioritization. The effectiveness of current designated conservation areas in Zimbabwe in representing biodiversity using these various indices is also evaluated. Furthermore the conservation status of Zimbabwean *Emilia* species is determined using the IUCN Red Lists assessment categories. The results highlight that in southern Africa the number of *Emilia* species is highest in Zambia (12 species), whereas Botswana, Namibia, and South Africa have the least species (three species in each country). Centres of diversity for *Emilia* species are found in northern and southern Malawi (including the Nyika and Zomba plateaus respectively) and Zimbabwe (Eastern Highlands and areas surrounding Harare). Areas with high SR generally coincide with areas with high PD in both Malawi and Zimbabwe in the assessment across southern Africa. Phylogenetic endemism overlaps to some extent with SR and PD in its centres of highest diversity in Malawi and Zimbabwe, whereas CWE does not coincide in most cases with these indices as there are very few (six) endemic *Emilia* species in southern Africa. The current protected areas in Zimbabwe sufficiently cover most areas where *Emilia* species occur, including those with high PD and PE, except for the Harare Region where extension of conservation efforts is therefore needed. To inform conservation decisions around species and areas, I recommend that in addition to traditional conservation approaches (e.g. IUCN Red Lists assessment categories and use of SR and endemism), conservation prioritization should integrate a variety of biodiversity indices. These indices should include those that provide information regarding the

evolutionary history of taxa and thus of floras (e.g. PD, PE) and thereby facilitate the maximizing conservation of evolutionary diversity as well as of species diversity.

Introduction

Conservation is defined as the preservation of biological diversity, which in turn is the sum total of life in a given region (Simpson 2010). Mishler *et al*. (2014 p. 2) argued that biodiversity should not only consider species but 'the full set of nested clades representing phylogenetic relationships among organisms at all levels'. To conserve biodiversity, especially if resources for conservation such as funding (financial) and human capital are limited — as in most developing countries, it is important to prioritize conservation efforts (Myers *et al*. 2000; Faith *et al*. 2004; Brooks *et al*. 2006; Daru *et al*. 2015). Conservation prioritization issues or 'the choice of what to conserve' have been debated widely (e.g. Vane-Wright *et al*. 1991; Faith 1992; Linder 1995; Mishler *et al*. 2014; Daru *et al*. 2015). Questions have been asked whether a species-based or an area-based (region or habitat) focus should be implemented in prioritizing conservation schemes (Margules and Usher 1981; Vane-Wright *et al*. 1991). It has been further argued that even if a species- or area-based focus is considered, habitat and species are not equal, thus there is still need to prioritize between them.

Traditionally, conservation areas have been selected based on concentrations of species, i.e. species richness (SR) defined as number of species per unit area (Brown *et al*. 2007), endemism, rarity, and degree of threat to species (Stattersfield *et al*. 1998; Myers *et al*. 2000; Groves *et al*. 2002; Daru *et al*. 2015). The term 'endemic' is defined as a taxon limited in its range to a specific geographical area or found nowhere else (Anderson 1994; Van Wyk and Smith 2001). This might be due to historical (e.g. dispersal, evolution and longevity of taxon), ecological or physiological reasons (Major 1988). Narrow endemic taxa are those that consist of one or a few small populations (Drury 1980) and hence are confined to a single domain or a few localities. A centre of endemism is determined by a high concentration of endemic taxa (Van Wyk and Smith 2001; Laffan and Crisp 2003). Furthermore, species with very narrow ranges, low abundances, and those that are rare and under threat of becoming extinct are assigned Threat categories and prioritized in conservation (Myers *et al*. 2000; Ceballos and Ehrlich 2006). Rare species are regarded as an important component of endemicity and species diversity (Kruckeberg and Rabinowitz 1985), whereas species that are

rare and threatened with extinction may point to highly impacted habitats that urgently need protection (Rebelo and Tansley 1993).

The use of traditional approaches to conservation, for example, species richness and endemism may not be adequate to recognise 'concentrations of spatially restricted evolutionary diversity' (Rosauer *et al*. 2009 p. 4061). The other limitations of these traditional biodiversity metrics are that they fail to consider the 'diversity of traits and amount of evolutionary history' in species, which might have conservation implications (Schmidt-Lebuhn *et al*. 2015 p. 1115). Species that are evolutionarily distinct (evolutionarily diverse) should therefore be prioritized in conservation (Vane-Wright *et al*. 1991) and by maximizing the conservation of evolutionary diversity, the genotypic, phenotypic and functional diversity will be maximised (Vane-Wright *et al*. 1991; Faith 1992). Faith (1992) contended that prioritization of species for conservation should preferably be based on phylogenetic relationships and phylogenetic diversity (PD), a quantitative approach to the assignment of priorities to taxa in conservation evaluation. Phylogenetic diversity measures maximum feature diversity in a reserve and can be calculated for any subset of taxa on any cladogram, given some estimate of relative branch lengths therein (Faith 1992). Phylogenetic diversity has been used for locating priority areas for plant and animal conservation (Forest *et al*. 2007; Davies *et al*. 2008; Faith 2008; Mishler *et al*. 2014). Another phylogenetic index proposed for conservation prioritization of species and areas is phylogenetic endemism (PE) (Rosauer *et al*. 2009). Phylogenetic endemism combines geographic distribution and PD of species thus enabling identification of geographic regions with a 'high degree of restricted evolutionary history' (Rosauer *et al*. 2009; Daru *et al*. 2015 p. 770). Based on the above arguments, it can be concluded that the best biodiversity indice should be used to assess conservation priorities and allocate limited conservation resources to maximise conservation returns.

In the current study, a variety of biodiversity indices are evaluated to facilitate conservation prioritization of *Emilia* species and their associated habitats in southern Africa[3], with a particular focus on Zimbabwe for conservation assessments of *Emilia* and current conservation capacity. The reconstructed *Emilia* phylogeny based on nuclear DNA sequence data, together with distribution data, are used to enable PD and phylogenetic endemism assessments, while species richness (SR) and species endemism (CWE, defined below) are

[3]Southern Africa is defined here as, the countries south of the Democratic Republic of Congo and Tanzania (Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland, Zambia, and Zimbabwe). This definition differs from other definitions of southern Africa, e.g. Cowling and Hilton-Taylor's (1994) definition as the region south of the Cunene and Limpopo rivers.

also evaluated using distribution data for the chosen regions of study. Corrected weighted endemism (CWE), referred to here as species endemism, is 'a measure of endemism that is least related to species richness and this index corrects for the species richness effect by measuring the proportion of endemics in a grid cell' (Crisp *et al*. 2001 p. 186) and is calculated as outlined in the Methods below. Corrected weighted endemism quantifies the distribution of endemic or narrow-ranged species (Crisp *et al*. 2001).

Twenty four of the 117 species in *Emilia* Cass. (Senecioneae, Asteraceae; The Plant List 2013) occur in southern Africa. The genus is mostly part of the Savanna flora, although eleven of the 24 southern African *Emilia* species have Austro-temperate affinities (Linder 2014) — occurring in the eastern highlands of Zimbabwe (including the Chimanimani-Nyanga Centre of endemism), as well as Mount Mulanje and the Nyika Plateau and other centres of plant diversity and endemism in Malawi linked to the Austro-temperate 'biome'. The distribution patterns of these southern African *Emilia* species were investigated to reveal areas of high species richness and areas of endemism. Geographical distribution information is also essential for Red Data listings often used to indicate threats to biodiversity (Vié *et al*. 2009) and highlight species in need of conservation attention (Rodrigues *et al*. 2006). Spatial analyses of four biodiversity and phylogenetic indices: SR, CWE, PD, and PE using Biodiverse v.0.19 (Laffan *et al*. 2010) were also done using 38 *Emilia* species from sub-Saharan Africa. These 38 *Emilia* species from sub-Saharan Africa were included in a nuclear ITS-based phylogeny (Chapter 3)—needed for such spatial analyses. Nonetheless, the focus in these analyses was southern Africa.

The conservation status assessments according to the International Union for Conservation of Nature and Natural Resources (IUCN) 2001 criteria of 12 out of the 24 southern African *Emilia* species have been done (Tadesse and Beentje 2004; Raimondo *et al*. 2009), while Cron (2014) commented on the conservation status of six of these species. The IUCN Red List categories of vulnerability for these species categorised them all as Least Concern, except for *E. protracta* S.Moore which is data deficient (DD). In the current study, the threat status of *Emilia* species sampled from one southern African region (viz. Zimbabwe) is evaluated for conservation purposes. Ten *Emilia* species occur in Zimbabwe and their distribution patterns and conservation status have not been previously investigated. Some of these Zimbabwean *Emilia* species occur in protected areas offering varying levels of protection and these include: National and Recreational Parks, Botanical Gardens and Reserves, and private conservancies (Timberlake and Müller 1994). Protected areas have been shown to be key to global conservation strategies as they preserve ecosystems against

loss of biodiversity and promote sustainable management (Myers *et al.* 2000; Clerici *et al.* 2007; Lopoukhine *et al.* 2012). Zimbabwe was selected for this study because of the availability of distributional data for *Emilia* species and author's in-depth knowledge of the region.

The results obtained from evaluation of a range of approaches and phylogenetic indices for *Emilia* species could be applicable to many taxonomic groups in similar floras and thus inform biodiversity conservation policies in southern Africa and specifically for Zimbabwe, thereby promoting conservation of African biodiversity. They will thus contribute to target 2, objective 1 of the Global Strategy of Plant Conservation 2011–2020, which requires that 'An assessment of the conservation status of all known plant species should be done as far as possible to guide conservation action' (www.cbdint/gspc/targets.shtml, retrieved 6 August 2016).

Aims

The aims of this study were to (i) identify areas of high species richness (centres of diversity) and areas of endemism for *Emilia* in southern Africa, and also to (ii) contribute to current debates on conservation prioritization as they apply to *Emilia* for southern Africa and a selected region therein (viz. Zimbabwe) by comparing and evaluating various biodiversity indices — species richness (SR), species endemism (CWE), phylogenetic diversity (PD), and phylogenetic endemism (PE). The distribution and biodiversity indices and IUCN Red Data assessments are used to assess the effectiveness of conservation of *Emilia* in the current protected areas in Zimbabwe.

Objectives

The objectives associated with these aims are: (i) to map the distribution and identify areas/centres of diversity and endemism of *Emilia* species in southern Africa, and investigate whether they coincide; (ii) to investigate whether the approaches of maximising species richness, endemism richness, phylogenetic diversity, and phylogenetic endemism prioritize similar or different regions to conserve *Emilia* species in southern Africa. Thus - should identified areas that ensure the maximum preservation of evolutionary potential be prioritized for conservation? Or should areas with *Emilia* species that are threatened and/or rare be prioritized in conservation schemes?; and (iii) to evaluate how conservation efforts should be prioritized for *Emilia* species in a specific region of southern Africa, viz. Zimbabwe, by

identifying occurrence of endemic, rare, and threatened species and also exploring congruence among various biodiversity indices and thereby contribute to the current debate on 'what to conserve'. Questions linked to this objective include: (i) Are there any endemic, rare and threatened *Emilia* species in Zimbabwe that need to be prioritized in conservation efforts and where do they occur?, (ii) Do current designated conservation areas in Zimbabwe protect all or most species of *Emilia*? If they do not, what criteria should be used in prioritizing conservation efforts in Zimbabwe to ensure effective conservation of *Emilia* species?

Materials and Methods

Distribution patterns of *Emilia* — identifying areas of diversity and endemism

Distribution data of the 24 *Emilia* species occurring in southern Africa were obtained from the following databases: Global Biodiversity (GBIF) and National Herbarium, Pretoria Computerized Information System (PRECIS; Edwards and Leistner 1971; Morris and Glen 1978) for Malawi, Mozambique, South Africa and Namibia, Zambia, and Zimbabwe, and from herbarium specimens of the following ten herbaria: BR, EA, J, LISC, MA, MAL, MO, PRE, SRGH, and UZL (abbreviations as per Holmgren *et al*. 1990). In addition, distribution information was acquired from the literature — Jeffrey (1986), Lisowski (1991), Tadesse and Beentje (2004) and Cron (2014), and fieldwork done in Zimbabwe over the period 2012 – 2014. Information concerning habitat, altitude, and abundance of plants was obtained during fieldwork and also compiled from notes on herbarium specimen labels. A distribution map of the 24 species of *Emilia* from southern Africa was created using ArcGIS® version 10.2.2 software (ESRI 2014) based on the available locality information from the sources detailed above. Distribution patterns of *Emilia* species were then used to produce a chorological map of *Emilia* species in southern Africa.

Comparing biodiversity indices

Spatial analyses using four commonly used diversity indices SR, PD, CWE, and PE (e.g. Mishler *et al*. 2014; Daru *et al*. 2015; Schmidt-Lebuhn 2015) were performed using Biodiverse v.0.19 (Laffan *et al*. 2010). The spatial analysis results of these four indices were exported as ArcInfo floatgrid files and mapped in ArcGIS® version 10.2.2 software (ESRI 2014). The distribution data of the 41 species used in these analyses were compiled as

outlined below. Species richness was defined as the total number of species in each grid cell. Species endemism (CWE) was calculated as follows: Weighted endemism (WE) / Species richness (SR), where WE is the sum of species in each grid 'weighted by the inverse of its range' (Crisp *et al*. 2001 p. 186; Daru *et al*. 2015).

Phylogenetic Diversity (PD) and PE, respectively, were calculated as:

$$\mathrm{PD} = \sum_{\{b \in B\}} L_b$$

and

$$\mathrm{PE} = \sum_{\{b \in B\}} \frac{L_b}{R_b}$$

'where $\{B\}$ summarises the set of branches joining taxa to the root of the phylogenetic tree, b is a branch in the spanning path $\{B\}$, L_b is the length of branch b, expressed as proportion of the total length of the tree and R_b is the range size of the clade' (Rosauer *et al*. 2009 p. 4063).

The phylogeny on which PD and PE were based was created using a dataset with thirty eight *Emilia* species, two *Emiliella*[4] species, and the monotypic *Bafutia*[5]. This dataset differed from the one used in Chapter 3 as it consisted only of *Emilia*, *Emiliella*, and *Bafutia* species from sub-Saharan Africa and excluded the Senecioneae outgroups, all multiple accessions of *Emilia* species, *E. graminea* DC. and *E. baumii* (O.Hoffm.) S.Moore which grouped outside *Emilia*, *E. fosbergii* Nicolson and *E. exserta* Fosberg with distributions outside sub-Saharan Africa, and species from Madagascar. Initially 23 taxa, i.e. all Senecioneae outgroups (except *Pericallis* the genus sister to *Emilia*) and *E. graminea* and *E. baumii*, were excluded from the original nuclear ITS dataset with 80 taxa, thus leaving a dataset comprising 57 taxa. An analysis was then rerun using this dataset with 57 taxa including *Pericallis murrayi* (Bornm.) B.Nord. as outgroup rooting the tree and the Bayesian approach using MrBayes v. 3.2.1 (Ronquist and Huelsenbeck 2003) and the evolutionary model GTR+I+G. *Emilia* species from Madagascar, *E. fosbergii*, *E. exserta*, and all multiple accessions of *Emilia* species were then pruned in Mesquite v.2.71 (Maddison and Maddison 2009) from the reconstructed nuclear ITS tree with 57 taxa. The topology of the resultant consensus tree (41 species) was the same as that obtained when all 80 species were included. The analysis was done via the CIPRES Science Gateway (Miller *et al*. 2010) (see Chapter 3 for the detailed description of the phylogenetic reconstruction method).

[5, 6] Genera *Emiliella* S.Moore and *Bafutia* C.D.Adams were nested within *Emilia* and were included in the analysis.

Conservation assessment in Zimbabwe

Chorological analyses were done at a local scale for *Emilia* species occurring in Zimbabwe and the numbers of species and endemics were accordingly mapped. The specimen entries in the PRECIS database were georeferenced using a quarter-degree square (1/16th degree) referencing system (Leistner and Morris 1976). The number of *Emilia* species in Zimbabwe occurring in each one-degree square and quarter-degree square was counted using the distribution data obtained from specimen records and other sources (noted above). Centres of diversity were determined from this chorological map. Distributions were also compared with Centres of Endemism according to Van Wyk and Smith (2001) and Beentje *et al*. (1994) and these are summarised in Table 5.1. Species were considered endemic to a region if they are found nowhere else or if they occur in a specific Centre of Endemism (Major 1988) and near-endemic when they are marginally shared with a neighbouring region or marginally present elsewhere (Matthews *et al*. 1993).

Comparison of the occurrences of *Emilia* species in Zimbabwe's protected/conserved areas were done by superimposing shapefiles made in ArcView (ESRI 2000) of these protected areas onto the mapped distributions of *Emilia*. Shapefiles showing Zimbabwean administrative boundaries were also overlaid onto these protected areas. Zimbabwe's protected areas are Government owned and managed by the Zimbabwe Parks and Wildlife Management Authority. These protected areas include 11 National Parks, 14 Recreational Parks, 16 Botanic Gardens and Reserves, and 43 State Forests covering 49000 km^2 of the country (www.protectedplanet.net/country/ZWE, retrieved 28 November 2016). Conservancies on the other hand mostly serve to protect endangered wildlife. There are five major conservancies in Zimbabwe, which are mostly privately owned. All the Zimbabwean conservancies occupy less than two percent of the country's land area.

To assess the conservation status of *Emilia* species occurring in Zimbabwe, the IUCN (2001) criteria for Red Data listing of species and Guidelines version 6.2 (IUCN 2006, retrieved 10 October 2016) were used. The wider distribution of these *Emilia* species in southern Africa was considered when they were assessed for Zimbabwe. Assessments of *Emilia* species were compiled from published and unpublished information and included expert input by Chapano C, Cron G. V., and Mapaura A (personal communication). Rationale for assigning *Emilia* species to different categories is supported by data on population size and trend, distribution, habitat preferences, threats, and conservation actions in place or needed (Rodrigues *et al*. 2006).

Results

Distribution of *Emilia* and centres of diversity in southern Africa

The centre of diversity for *Emilia* in southern Africa is in the landlocked countries of Malawi, Zambia, and Zimbabwe (Figure 5.1). There are four centres of diversity according to the concentrations of *Emilia* species in Malawi, three in the northern region (viz. Mzimba District; Nkhata Bay; Rumphi District – Nyika Plateau) and one in the southern region (Zomba District – Zomba Plateau) (Figure 5.1). The number of *Emilia* species is highest in Zambia (15 species, only 12 mapped here due to lack of distribution records/data availability), followed by Zimbabwe (10 species, all species mapped), and Malawi (seven out of nine species mapped). Angola has eight species of which six species – including *E. protracta* on the banks and floating islands of the Kavango river are mapped here and Mozambique also has eight species with only four species mapped. The remaining southern African countries have fewer species — three (all species mapped) in each of Botswana, Namibia and South Africa (Figure 5.1). In addition to the three species outstanding for Zambia (*E. brachycephala* (R.E.Fr.) C.Jeffrey, *E. hockii* (De Wild. & Muschl.) C.Jeffrey, and *E. vanmeelii* Lawalrée), lack of locality data for some *Emilia* specimens from Zambia undoubtedly contributed to the low number of populations recorded in this country in contrast to e.g. Malawi, which has adequate distributional data for *Emilia* specimens collected. Angola and Mozambique also have very limited locality data of *Emilia* species available as a result of political/civil wars which made most areas inaccessible for botanical research in these countries.

There are few endemic or near-endemic *Emilia* species in the Centres of Endemism recognised by Van Wyk and Smith (2001) and Beentje *et al*. (1994) and each of these centres has only one species present (Table 5.1). Four Centres of endemism in Malawi (one), Zambia (two) and Zimbabwe (one) do not have species of *Emilia* present. Two other areas viz. Mzimba District and Victoria Falls not recognised as Centres of endemism each has one endemic and near-endemic species (Table 5.1).

Table 5.1. Centres of endemism with corresponding endemic and near-endemic (NE) species of *Emilia* in southern Africa

Centre of endemism		*Emilia* species
Botswana:	Okavango Delta (swamp)	*E. tenellula*
Malawi:	Mount Mulanje	None
	Nyika Plateau	*E. sagittata*
	Mzimba District*	*E. hockii*
Namibia:	Kavango Region	*E. protracta* (NE)
South Africa:	Wolkberg	*E. limosa*
Zambia:	Luangwa Valley	None
	Zambezi source area	*E. leptocephala*
	Nyika	None
Zimbabwe:	Chimanimani-Nyanga	None
	Great Dyke	*E. baumii* (NE)
	Victoria Falls*	*E. protracta* (NE)

* Not recognised Centres of endemism, but have an endemic and a near-endemic species.

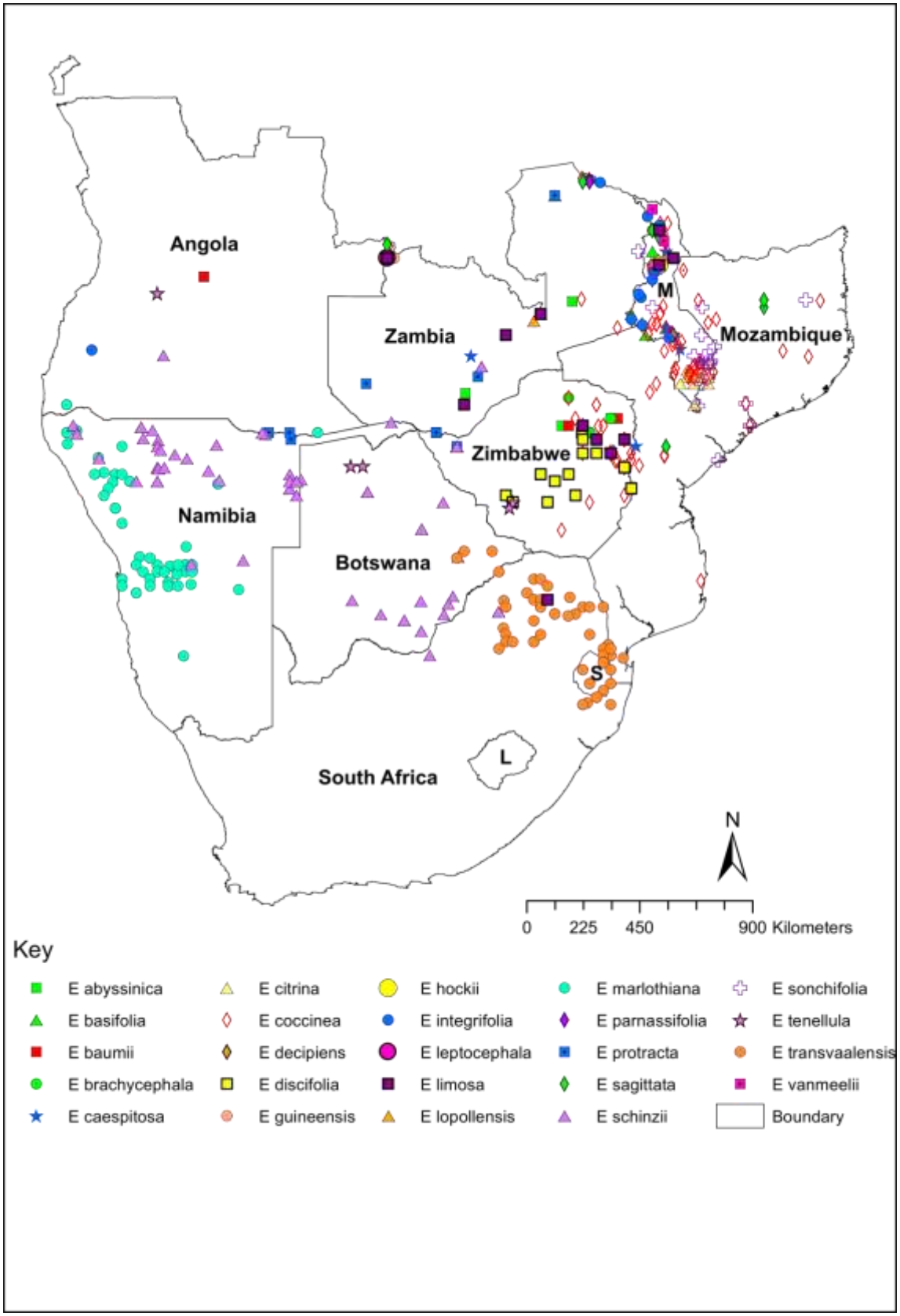

Figure 5.1. Distribution of 24 *Emilia* species in southern Africa. Abbreviations for country letters: L = Lesotho, M = Malawi, and S = Swaziland.

Biodiversity analyses in sub-Saharan Africa: SR, PD, CWE, and PE

The four biodiversity indices (SR, PD, CWE, and PE) are mapped for *Emilia, Bafutia and Emiliella* in Figures 5.2–5.5. Areas of highest species richness (similar to the above-mentioned centres of diversity) are confirmed for Northern Malawi: Mzimba District (seven species), Central Region: Dedza District (four species), Mashonaland West Province and the Eastern highlands of Zimbabwe (five and four species respectively) (Figure 5.2).

Phylogenetic diversity (Figure 5.3) is highest in Mzimba District (Northern Malawi), and generally high in a few areas in Dedza District (Central Region Malawi); Mbala and Mpulungu Districts (at the southern tip of Lake Tanganyika) in Northern Province, Zambia; Harare District (Harare Province, Zimbabwe); and Mazowe District (Mashonaland Central Province, Zimbabwe). Areas with a medium PD are found in Rumphi District (Nyika National Park) in the Northern Region, Malawi; Chama District (Nyika National Park) in the Eastern Province, Zambia; Mwinilunga District in the North-Western Province, Zambia; and Mutare and Mutasa Districts in Manicaland Province, Zimbabwe. Most of the areas in sub-Saharan countries have low PD (Figure 5.3). Areas with high PD generally coincide with areas with high species richness (Figures 5.2, 5.3).

Most areas in western Tanzania and two in central Tanzania, two areas in north-western and western Zambia (Zambezi and Mongu respectively) and single areas in Kenya (Kajiado County), western Uganda (Kashoya-Kitomi Central Forest Reserve) and northwest Cameroon (Menchum) have high mean species endemism (CWE; Figure 5.4) and the list of endemics in these countries is shown in Table 5.2. Nearly all these areas with a greater proportion of species with a restricted distribution range (CWE) are low in species richness (Figures 5.2, 5.4).

Phylogenetic endemism has centres of highest value in Tanzania, Kenya, and Malawi (Figure 5.5). This phylogenetic index is also high in north-western and western Zambia, and the Harare Region, Zimbabwe (Figure 5.5). Phylogenetic endemism coincides with CWE in most areas (Figures 5.4, 5.5). Phylogenetic indices (PD and PE) are more informative than SD and CWE as they show additional areas not recognised by SD and CWE.

Countries in sub-Saharan Africa e.g. DRC, Kenya, and Uganda were under-represented in these biodiversity analyses because of lack of locality data from herbarium specimens as they were not part of the current study and this would need to be addressed for a more comprehensive study of biodiversity in sub-Saharan Africa.

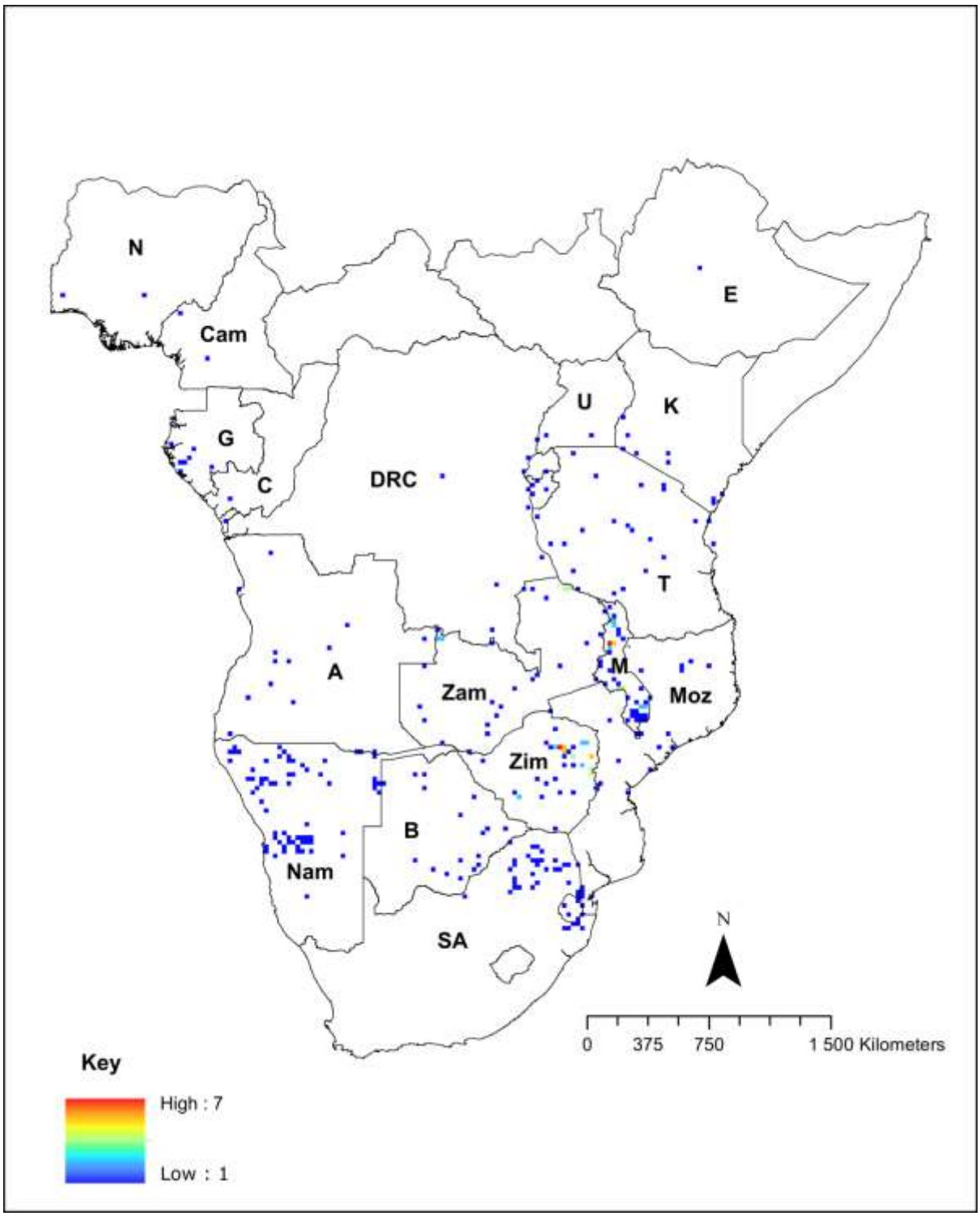

Figure 5.2. Biodiversity patterns of southern African species of *Emilia* and *Emiliella* — based on species richness (SR). Abbreviations of country letters in alphabetical order: A = Angola; B = Botswana; Cam = Cameroon; C = Congo DRC = Democratic Republic of Congo; E = Ethiopia; G = Gabon; K = Kenya; M = Malawi; Moz = Mozambique; N = Nigeria; Nam = Namibia; SA = South Africa; T = Tanzania; U = Uganda; Zam = Zambia; Zim = Zimbabwe.

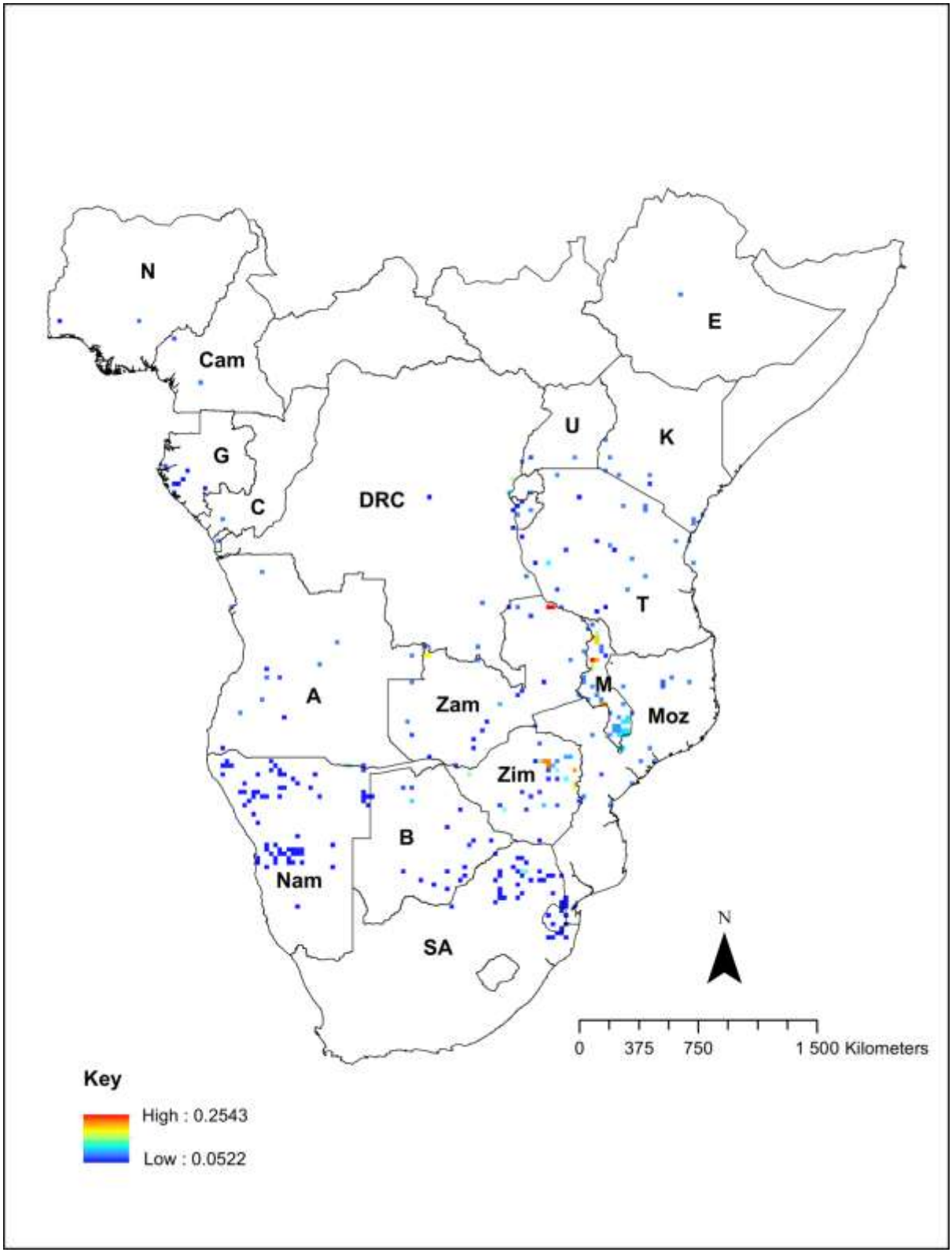

Figure 5.3. Biodiversity patterns of southern African species of *Emilia* and *Emiliella* — based on phylogenetic diversity (PD). Abbreviations of country letters are given in Figure 5.2.

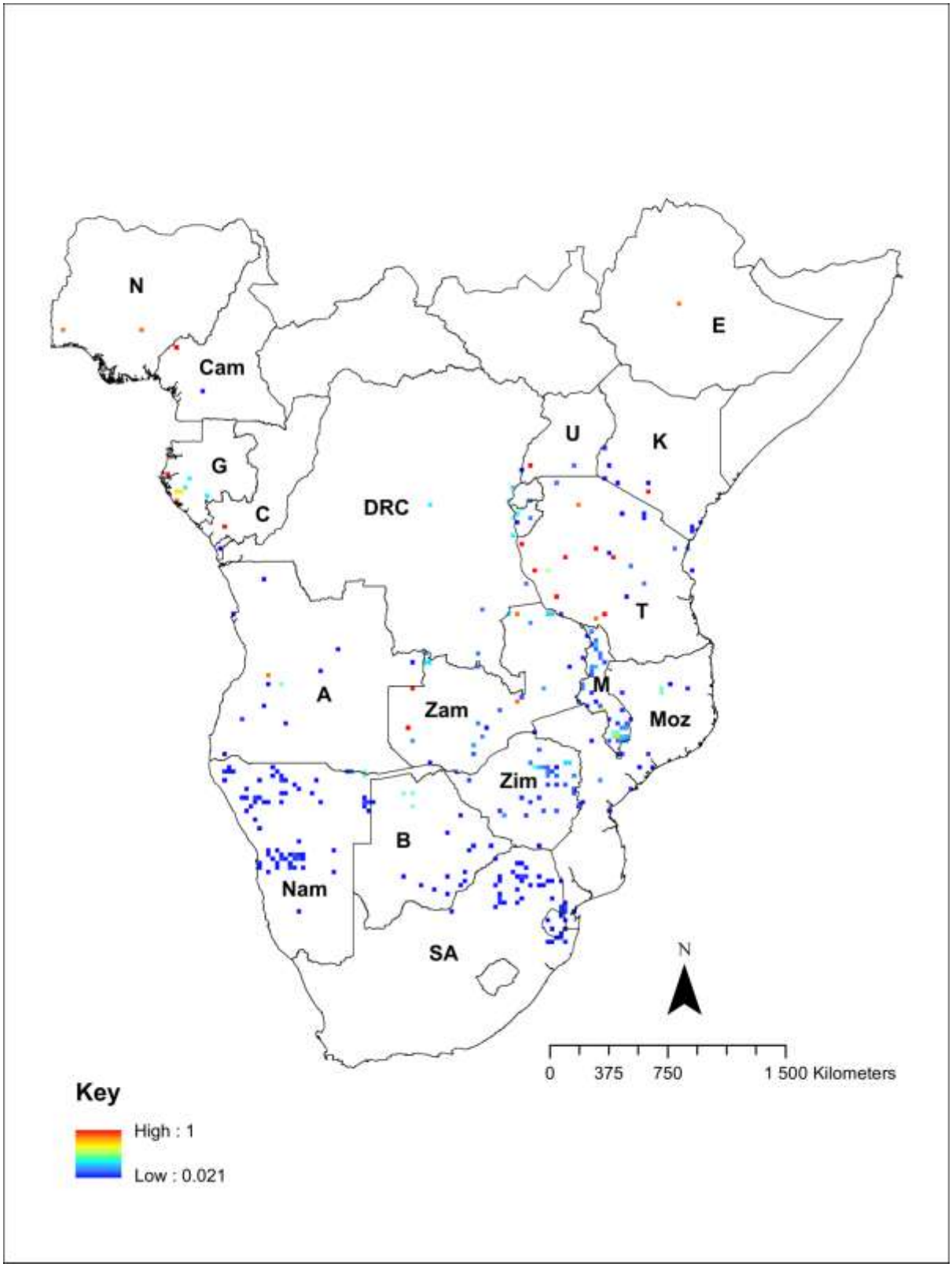

Figure 5.4. Biodiversity patterns of southern African species of *Emilia* and *Emiliella* — based on species endemism (CWE). Abbreviations of country letters are given in Figure 5.2.

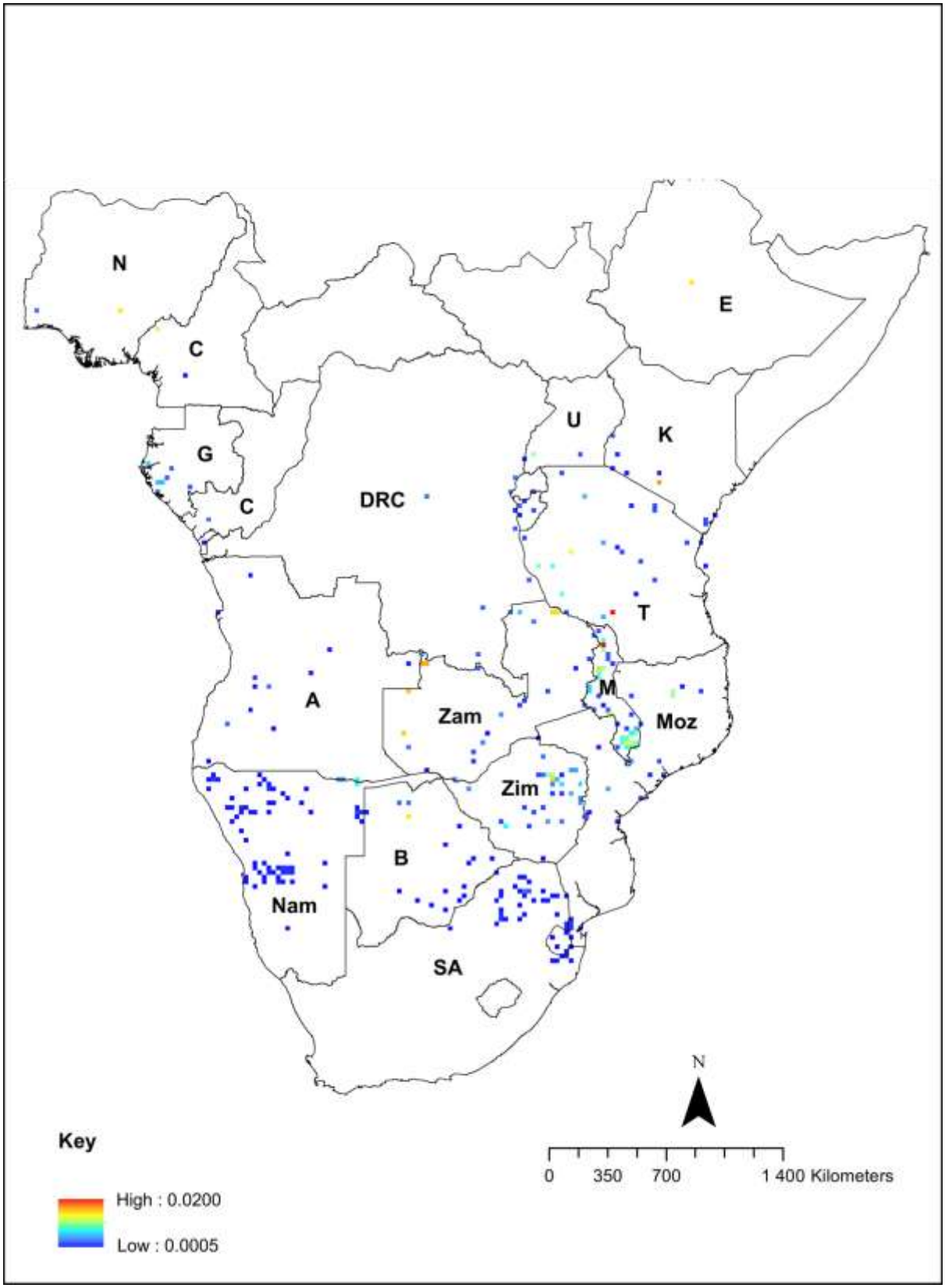

Figure 5.5. Biodiversity patterns of southern African species of *Emilia* and *Emiliella* — based on phylogenetic endemism (PE). Abbreviations of country letters are given in Figure 5.2.

Table 5.2. List of endemic species of *Emilia*, *Emiliella*, and *Bafutia* occurring in sub-Saharan Africa and obtained using CWE in Biodiverse v.0.19

Country	Endemics
Cameroon (northwest, Menchum)	*Bafutia tenuicaulis*
Kenya (Kajiado county)	*E. somalensis*
Tanzania (western, Ugalla River Game Reserve, Kigoma)	*E. myriocephala*, *E. tricholepsis*, *E. longipes*, *E. leucantha*
Tanzania (south-western, Njombe; central-western, Tabora)	*E. tenera*, *E. cenioides*
Tanzania (central, Manyoni)	*E. helianthella*
Uganda (western, Bushenyi)	*E. juncea*
Zambia (north–western, Zambezi; western, Mongu)	*Emiliella zambiensis*, *Emiliella drummondii*

Centres of diversity of *Emilia* in Zimbabwe

Two centres of diversity for *Emilia* are revealed in Zimbabwe, the first is the Nyanga Region, and the second is the area surrounding Harare such as Cleveland Dam, Domboshava, and Mazowe (grid squares 1731 and 1832 respectively) with five species each (Figure 5.6). Other areas in the Eastern Highlands (Rusape, Mutare) and Marondera also have a high diversity of *Emilia* species with four species each in grid squares 1831, 1832, and 1932 (Figure 5.6). In the Western part of Zimbabwe, only two *Emilia* species, *E. protracta* and *E. schinzii* (O.Hoffm.) Cron, occur in the Victoria Falls and Hwange National Parks (grid squares 1725 and 1826 respectively) and *Emilia* species are conspicuously absent from the surrounding areas and also from the north-west of Zimbabwe. There are no endemic or near-endemic *Emilia* species in the Chimanimani-Nyanga Centre of Endemism although the Nyanga area is one of the centres of diversity for *Emilia*. However, *E. baumii* is found in the Great Dyke Centre of Endemism where it is near-endemic, although it also occurs at two other localities (Mtoko and Nyanga Districts) in Zimbabwe.

Conservation capacity in Zimbabwe

My study has revealed that most areas in Zimbabwe with high species richness in *Emilia* do not fall within the designated protected areas, thus they are not accorded any formal

protection. Nonetheless, six *Emilia* species occur in one or more of the six National Parks: Victoria Falls (*E. protracta*), Hwange (*E. schinzii*), Matopos (*E. tenellula* (S.Moore) Jeffrey), Mutirikwi (*E. brachycephala* and *E. discifolia*), Chimanimani (*E. coccinea* (Sims) G.Don and *E. discifolia*), and Nyanga (*E. discifolia*); one species is protected in the Cleveland Dam Recreational Park (*E. limosa* (O.Hoffm.) Jeffrey), and another species in a Heritage preservation centre, Domboshava Heritage Centre (*E. brachycephala*) close to Harare (Figure 5.7). *Emilia* species usually occur in small clusters of viable, healthy populations scattered in these protected areas. The distribution of *E. tenellula* is restricted in Zimbabwe and is known from the type locality (Matopos National Park; Moore 1906) and from the nearby Besna Kobila Private Conservancy in south western Zimbabwe, although it could not be relocated despite numerous searches over four wet seasons. In addition to the national parks, *E. discifolia* is also protected at Besna Kobila Private Conservancy and *E. coccinea* in the Stapleford State Forest and Chipinge Safari Area (Figure 5.7). Seventy percent of *Emilia* species including the rare and endangered and two widely distributed species are therefore protected in Zimbabwe, although very few locales are in these protected areas with most populations occurring outside protected areas. It is not known whether the management of these protected areas is suitable for *Emilia* species, a factor to be considered in their conservation. Only 30% of the species (viz., *E. abyssinica* (Sch. Bip. ex A.Rich.) C.Jeffrey, *E. baumii*, and *E. caespitosa* Oliv.) are not in protected areas (Figure 5.7). These three species, together with other already protected species, occur in areas with high PD (Harare and surrounding areas, Mutare Region, and Nyanga Centre) and high PE (Harare and surrounding areas), which are therefore evolutionarily distinct and should be prioritised for conservation. Furthermore *E. baumii* occurs on the Great Dyke Mountain Pass (Mashonaland West) and is also found in Mashonaland East (viz. Mtoko) and Manicaland (viz. Nyanga), although there are limited collections for this species and further data are needed to fully assess its threat status (Figure 5.1).

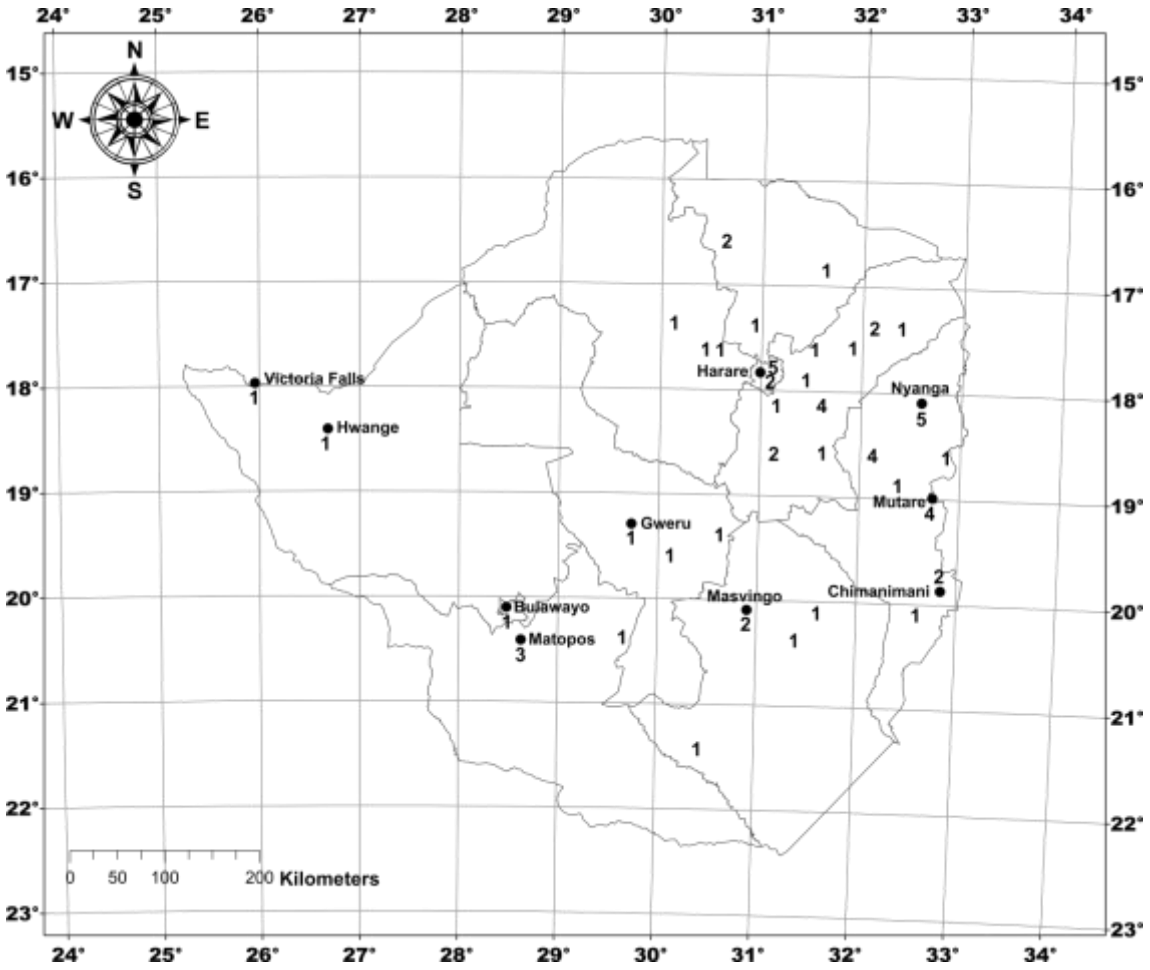

Figure 5.6. The number of *Emilia* species occurring in each Quarter-Degree Square (QDS) in Zimbabwe.

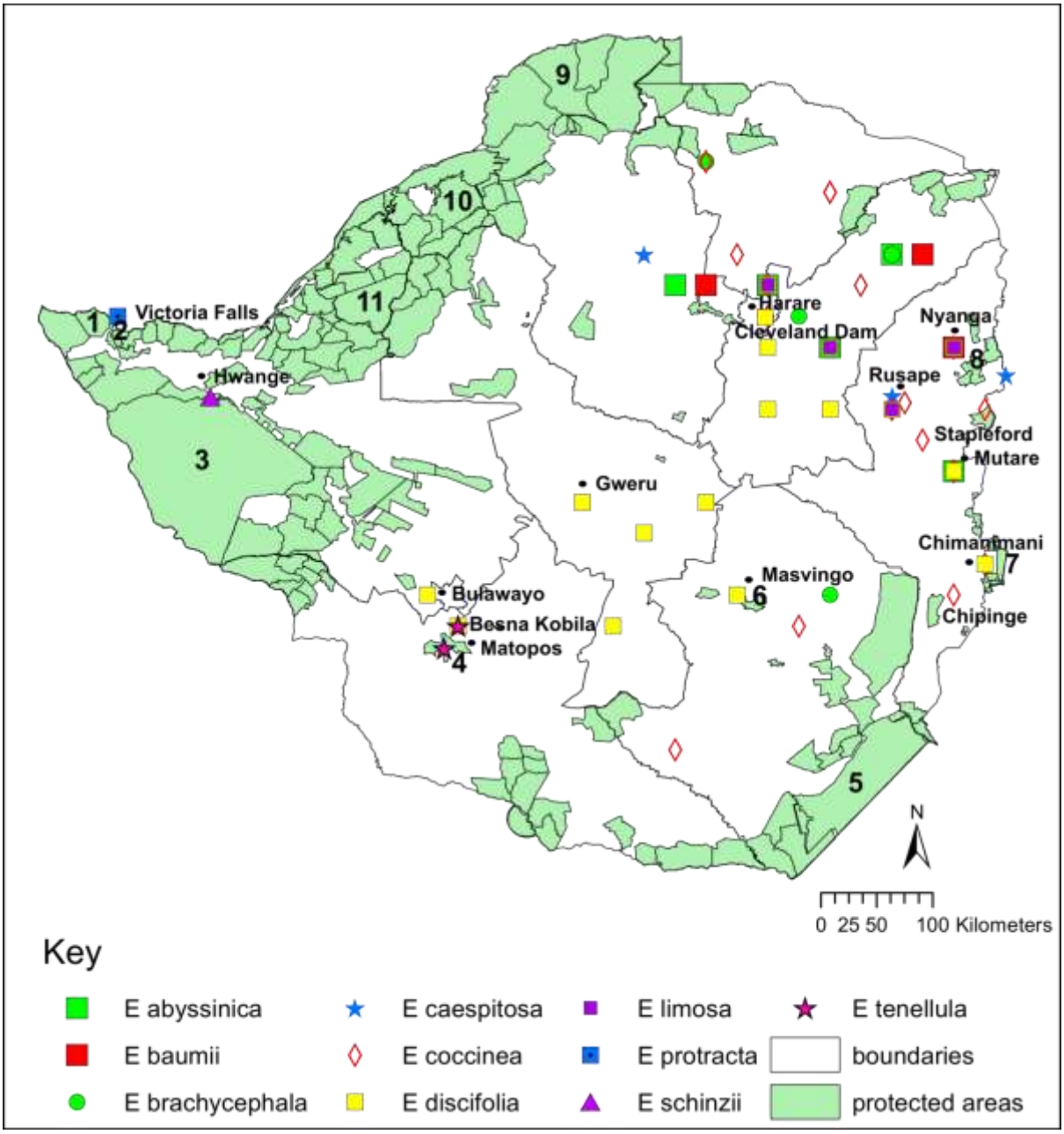

Figure 5.7. Distribution of *Emilia* species in the existing protected areas of Zimbabwe. Administrative boundaries are shown=; Protected areas include National Parks, Recreational Parks, Botanical Gardens, and State forests. Numbers refer to National Parks: 1 = Zambezi; 2 = Victoria Falls; 3 = Hwange; 4 = Matopos; 5 = Gonarezhou; 6 = Mutirikwi; 7 = Chimanimani; 8 = Nyanga; 9 = Mana Pools; 10 = Matusadonha; 11 = Chizarira.

Conservation status of *Emilia* species in Zimbabwe

According to the IUCN Red Data List Criteria v.3.1 (2012) (www.iucnredlist.org/static/categories_criteria_3_1, retrieved 27 November 2016), six out of ten (60 %) *Emilia* species in Zimbabwe have been categorised as least concern because of their wide distribution, common habitats, and because they are also protected in Conserved areas/National Parks (Table 5.3). The population sizes of all *Emilia* species are usually very small since they do not grow in clusters and are usually single, scattered plants. The population trends in *Emilia* could not be determined because of lack of data and are therefore not reported here. Two species (20 %) are considered rare (*E. limosa*, *E. protracta*) because they are habitat specialists that are also restricted in distribution (Table 5.3). *Emilia limosa* occurs mostly in moist swampy areas and was last collected in Nyanga (Zimbabwe) in November 1956. This species was recorded from four sites in Zimbabwe (Figures 5.1, 5.7) and further efforts to recollect the plant from these localities were unsuccessful. *Emilia tenellula* is possibly rare and endangered since it has not been collected extensively and not found again and occurs in specific habitats e.g. moist swampy areas, although it has also been previously classified as data deficient (Cron 2014). *Emilia baumii* is also regarded as data deficient (Table 5.3) and is known only from the type locality in Angola (Am Longa aberh. Nimesera) and three other localities in Zimbabwe.

Table 5.3 Red data assessments for the Zimbabwean *Emilia* species – based on their southern African distributions. (EN B2ab = Endangered, small range, severe fragmentation and/or few locations (1, ≤5, ≤10), continuing decline; EA = East Africa; N = North, W = West, C = Central, E = East, S = South; a.s.l. = above sea level).

Taxon name	Recommended /suggested Red list category/ Status	Criteria	Distribution/ Locality	Habitat	Conservation	Threat(s)	Notes	Previous assessments/ Publication
E. abyssinica (Sch.Bip. ex A.Rich) C.Jeffrey	Least Concern (LC)	Widespread and found in common habitats	Malawi: Dedza, Mzimba and Mzuzu Districts; Mozambique: Niassa, Marrupa; Zambia: Serenje and Choma Districts; Zimbabwe: North, East	Miombo woodlands; open disturbed places; cultivated areas; sandy soils. Altitude 650–1220 m a.s.l. (EA); 1402–1510 m a.s.l. (Zimbabwe)	Populations of *E. abyssinica* protected in Mukuvisi woodlands, Zimbabwe; Chongoni forest Reserve, Malawi	Cultivation		LC – EA (Beentje *et al*. 2005)
E. baumii (O.Hoffm) S.Moore	Data Deficient (DD)	Recorded from three localities in Zimbabwe and has not been recently found at these sites. Few populations known	Angola: Am Longa aberh. Nimesera; Zimbabwe: Mpingi pass,-Great Dyke, Nyanga District, Mtoko District, Mudzi Dam	Short thickets, pasturage, Summit of whaleback rock, serpentine grassland, moist area near dam Altitude 1250 m a.s.l.	Not protected	Small scale mining	Further investigation required for this Data Deficient species	Not evaluated (NE)
E. brachycephala (R.E.Fr.) C.Jeffrey	Least Concern (LC)	Widespread and restricted to moist habitats. Noted to be common on granite *Smith A* and *Moll E.J 492*	Zambia: Kabwe District; Zimbabwe (N, W, C, E, S)	Granite kopje, sandveld, very damp and wet areas, among grass Altitude 1500–1800 m a.s.l. (Zimbabwe)	Protected in Zimbabwe at Domboshava Heritage Centre, Matopos National Park, Kyle National Park	None		Not evaluated (NE)

E. caespitosa Oliv.	Least Concern (LC)	Widespread and found in many common habitats.	Malawi: Mzimba, Dedza, Ncheu, and Zomba Districts; Mozambique: Manica, Zambezi District; Zimbabwe: Mutasa, Mutare heights, Mtoko	Miombo woodlands, forest reserves, mountainous areas, abandoned and cultivated fields, foot paths, sandy river banks, swampy areas Altitude 300–1900 m a.s.l.	Not protected	Cultivation		LC – North East tropical Africa (Tadesse and Beentje 2004)
E. coccinea (Sims) G.Don	Least Concern (LC)	Widespread and found in many common habitats. Recorded as common on Nyika plateau (Malawi) *Jones* and *Binns B 41*	Angola: Huambe District, Chianga; BIE, Cuemba, Huíla, Alto Chicapa, Luanda; Mozambique: Manica, Sofala, Tete, Zambezia - Namacurra; Malawi: Blantyre, Chiradzulu, Dedza, Dowa Karonga, Lilongwe, Mchinji, Mzimba, Ncheu, Nkhatabay, Ntchisi, Rumphi, Salima, Zomba Districts; Zambia: Mwinilunga, Abercorn Districts, Luangwa valley; Zimbabwe: N, E	Miombo woodlands, dense forests, forest margins, disturbed riverine forests, mountainous areas, short swampy grassland, abandoned and cultivated areas, roadside Altitude 0 – 1800(–2400 m) a.s.l. EA	Protected in Zimbabwe: Haroni-Makurupini forests; Malawi: Perekezi forest reserve	Cultivation		Not evaluated (NE)
E. discifolia (Oliv.) C.Jeffrey	Least Concern (LC)	Widespread and common throughout Zimbabwe. Small populations occur at several localities around Zimbabwe.	Zimbabwe (N, E, C, W, S); Zambia.	Roadsides, Miombo woodlands amongst rocks, open area in wooded grassland, rocky outcrop in grassland, hill slopes in valley, farm near rocks,	Zimbabwe: protected at Mutirikwi National Park (Masvingo), Mukuvisi Woodlands, Chimanimani	None	No threatening processes to cause actual potential population decline.	LC – North East tropical Africa (Tadesse and Beentje 2004)

				short heavily grazed grassland, grassland, degraded woodland, roadsides, ruderal situations Altitude 450–2500 m a.s.l.	mountains, Besna Kobila farm, Matopos, Nyanga National Park.			
E. limosa (O.Hoffm.) C.Jeffrey	Rare (R, southern Africa)	Restricted in distribution in southern Africa. Widespread in East Africa. Habitat specialist. Recorded from four sites in Zimbabwe.	South Africa: Limpopo Province; Angola Zambia: Northern Province, Mwinilunga District; Zimbabwe: central and eastern; Malawi: Nyika District–Nyika plateau, Mzimba District	Moist areas, vlei, permanent swamps, peat bogs, edges of dam, different substrates such as granite, sandstone and laterite Altitude 1300 – 1920 m a.s.l. in southern Africa; 990m – 2800 m a.s.l. in East Africa	Zimbabwe: protected at Cleveland dam (Harare). Malawi: Lake Kaulime, Nyika National Park.	Flooding – might remove plants on river banks, leads to decrease in soil oxygen levels thus plant roots suffocate and the species die.	Cron (2014) noted that *E. limosa* was recorded from two sites in southern Africa and thus classified it as rare.	LC – EA (Beentje *et al*. 2005; Kamundi 2005); Rare – southern Africa (Cron 2014)
E. protracta Moore	Rare (R)	Restricted in distribution in southern Africa. Very specific wetland habitat. Few populations known.	Namibia: Northern region along the Kavango river; Zambia: southern part – Lochinvar National Park; Zimbabwe: western part – Victoria Falls	Victoria Falls rainforest in *Oryza – Setaria* open grassland; Zambia (Livingstone District), rainforest; banks and marshy ground and alluvial flats of Okavango River Altitude 869 -1000 m a.s.l.	A large population is protected in the Victoria Falls National Park. Also protected in Lochinvar National Park (Zambia) although there is data deficient.	Grows on large river banks that are prone to flooding and damming of rivers could possibly eliminate this species.	Flooding – might remove plants on river banks, leads to decrease in soil oxygen levels thus plant roots suffocate and the species die.	Rare (R) – southern Africa (Cron 2014)
E. schinzii (S.Moore) C.Jeffrey	Least Concern (LC)	Widespread	Botwana; Namibia: central and northern parts; South Africa: Waterberg region; Zimbabwe: western	Grows in cracks in base rocks, dry sand around the margin of pans, white Kalahari sand on	Protected within the Etosha National Park (Namibia), and Hwange	Grazing by wild animals Flooding		LC – southern Africa (Foden and Potter 2005; Raimondo *et al*. 2009)

			parts; Angola	level ground, moist sands with sparse short grass, along roadsides, river banks, catchment areas of water courses, cattle ranch Altitude 950-1250 m a.s.l.	National Park (Zimbabwe)			
E. tenellula (S.Moore) C.Jeffrey	EN B2ab (ii or iv)	Occurrence < 5000 km^2 5 or fewer locations in southern Africa. Only four populations known. Habitat specialist.	Angola: Huambo District: Novo Lisboa, near da Caóla; Botswana: Northern District: Island on Ngogtia River downstream of Xaenga, Xobega river game crossing; Zimbabwe: western part - Besna Kobila Farm, Matopos	Very damp soil in vleis, marshes, bog, swampy area, in shallow standing water , along banks of flooded rivers Altitude: Angola: 1700 m a.s.l. Botswana: 900–1000 m a.s.l. ; Zimbabwe: 1430–1465 m a.s.l.	Besna Kobila Farm (a private conservancy in Matopos) and World's View – Matopos National Park (type locality).	Draining or clearing of wetlands for agriculture, mining, and urbanisation. Climate change e.g. changes in rainfall patterns. Alien and invasive plant species.	Limited collection: (only two plants collected at Besna Kobila Farm and type specimen in marshland near World's View). The species has a very specific habitat requirement. Efforts to recollect it in western Zimbabwe were futile. Flowering periods difficult to predict due to unpredictable rainfall.	EN B2ab (ii or iv), Data Deficient (DD) – southern Africa (Cron 2014)

Discussion

Diversity and endemism centres for *Emilia*

Emilia species are unevenly distributed in southern Africa with some areas showing high species richness and these coincide with a few recognised centres of endemism. Two of the identified centres of greatest species diversity for *Emilia* fall within the Chimanimani-Nyanga and Nyika Plateau Centres of endemism in Zimbabwe and Malawi respectively, both part of the Afromontane Region (Van Wyk and Smith 2001) or Austro-temperate Region (Linder 2014). However the areas surrounding Harare (Zimbabwe) do not fall in any identified centre of endemism. The high species richness in these identified areas could be a result of discrepancies in collecting effort by various botanists. Several collecting expeditions to Malawi (Nyika Plateau and Mount Mulanje) and Zimbabwe (Chimanimani-Nyanga area) could have led to the documentation of many species including *Emilia* in these areas, possibly explaining the high species richness recorded there. The location of the Zimbabwean National Herbarium in Harare (SRGH) could have contributed to high plant collection intensity in the Harare region, because of the availability of good collectors at SRGH. The Harare region provides suitable and diverse habitats for *Emilia* species, including *E. limosa* found in specialised and restricted damp habitats at Cleveland Dam Recreational Park and *E. discifolia* found in the area comprising *Brachystegia* woodland in the same park (Figure 5.7). The mountainous eastern highlands of Zimbabwe (including the Chimanimani-Nyanga area) have high species richness but no endemic species. This is similar to findings by Linder (2001) in a study based on plant species of sub-Saharan Africa, who also identified the Chimanimani-Nyanga area as a centre of species richness but hardly distinct in terms of endemism. Rainfall was attributed to be a predictor of species diversity/richness (Linder 2001) in the Eastern Highlands, similar to Linder's (1991), and Craven and Vorster's (2006) findings of a positive relationship between precipitation and species richness in the floristically diverse south-western Cape, South Africa and the north-east region of Namibia respectively. In contrast, Lovett and Friis (1996) postulated that high endemism is determined by climatic stability instead of high rainfall. They further argued that consistently arid and wet areas are likely to have similar number of endemics.

Only seven of the 24 *Emilia* species (excluding *Emiliella*) occurring in southern Africa (29 %) are endemic or near-endemic to the southern African countries where they occur. Four of these endemic and near-endemic *Emilia* species occur in recognised centres of

plant diversity (Van Wyk and Smith 2001), i.e. the Great Dyke (*E. baumii*), Nyika Plateau (*E. sagittata*), the Zambezi source area (*E. leptocephala*), which falls under the Zambezian Regional Centre of Endemism (Van Wyk and Smith 2001) and the Wolkberg (*E. limosa*). *Emilia protracta* is a near-endemic in the Kavango Region of Namibia and Angola where it is found on floating islands of mostly sedges and along the Kavango river banks. It also occurs in a microhabitat in the rainforest associated with the Victoria Falls (Zimbabwe). No endemic *Emilia* species are found in the mountainous Chimanimani-Nyanga Centre, a region documented to have many endemics, including seven endemic species in the Asteraceae, three of which are *Helichrysum* species (Wild 1964). This area is composed of quartzite soils and occurs in the wetter part of Zimbabwe, which receives rainfall throughout the year and moderate temperatures thus offering microclimatic conditions ideal for many endemics. Nonetheless, the widely distributed *E. coccinea* and *E. discifolia* do occur on the Chimanimani-Nyanga Centre, identified as one of the centres of diversity for *Emilia* species. There are also no *Emilia* endemics on Mount Mulanje, which is composed of granite formations and known to have fewer endemics as compared to the Chimanimani-Nyanga Centre (Wild 1964).

Twelve (50%) of the species of *Emilia* found in southern Africa (as defined here) extend to East Africa and nine of them also occur in the DRC. Only three *Emilia* species (12.5%, *E. marlothiana*, *E. schinzii* and *E. transvalensis*) are near-endemic to southern African region — defined strictly as south of the Limpopo, Kavango, and Kunene rivers. This figure is very low when compared to other senecioid plant groups in the same region [e.g. *Cineraria* (77%) and *Euryops* (94 %); Nordenstam 1969; Cron *et al*. 2009].

In southern Africa, centres of diversity and endemism for *Emilia* do not coincide in most cases except for the Nyika Plateau in Malawi; thus there is minimal overlap in species richness and endemism. Little or no overlap in species richness and endemism has also been shown by Mendelsohn *et al*. (2002) for the combined flora and fauna of Namibia and by Orme *et al*. (2005) using global data on breeding distribution of extant bird species. Nonetheless correlation between endemicity and species richness has been shown by other researchers (e.g. Rebelo 1994; Kerr 1996). Incongruence between species richness and endemism has implications in that selecting species rich locations for conservation of *Emilia* species will result in endemics not being protected, and conversely, selecting areas with high endemism might miss species rich locations. The accurate information on the distribution of endemic species obtained here can contribute to informing decisions to promote their conservation.

Analyses using the four biodiversity indices, SR, PD, CWE, and PE, have shown some overlap in southern Africa. A few areas with high species diversity correspond with Centres of Endemism as recognised by Van Wyk and Smith (2001). Species richness and PD also coincide in most areas, similar to the positive correlation between these indices in a study investigating 'phylogenetic measures of biodiversity' in Australian *Acacia* species (Mishler *et al*. 2014). However, CWE is low in most areas in southern Africa (as defined here – i.e. south of the DRC) as many of the *Emilia* species occurring here (such as *E. cenioides*, *E. helianthella*, *E. tenera*, and *E. somalensis*) are also present in African countries further northwards, for example, Kenya and Tanzania (Table 5.2) and possibly also in the DRC. The *Emilia* species found in East Africa and the DRC are underrepresented here since the focus was on southern Africa, thus there is need to improve collection records/data to get a more accurate picture of distribution patterns for these more northerly countries.

Patterns of plant endemism in sub-Saharan Africa also differ from those of species diversity. Phylogenetic endemism and CWE overlap in most areas, although CWE coincides minimally with SR in Malawi. Phylogenetic diversity is congruent with PE and CWE in most areas in southern Africa. Spatial differences revealed in the present analyses of SR, threat, and endemism have been reported in previous studies (e.g. Orme *et al*. 2005), and these differences make it difficult to select areas to be prioritized for conservation (Daru *et al*. 2015). The different phylogenetic approaches (PD and PE) capture different aspects of biodiversity (Faith 1992; Daru *et al*. 2015) and this study has shown that PD and PE seem to provide more information than just SR and CWE respectively. Additionally PE seems to provide the greatest levels of distinction as this phylogenetic index combines species geographical distributions and evolutionary history of the species under study (Rosauer *et al*. 2009; Daru *et al*. 2015); thus geographic regions containing a large amount of 'restricted evolutionary history' viz. Tanzania, Kenya, Malawi, north-western and western Zambia, and the Harare Region, Zimbabwe were identified (Figure 5.5).

Application of the various biodiversity and phylogenetic indices investigated in this study would result in different conservation schemes. Because of the low number of endemic species in *Emilia* across Africa, protection of species endemism and/or rare or threatened species is not worth focussing on in southern Africa. This study was extended to sub-Saharan Africa, although these countries were not as extensively sampled. In East Africa, Tanzania's endemism with respect to *Emilia* has also been shown by previous studies to be high (11.2 %; Beentje *et al*. 1994), thus possibly warranting maximisation of conservation of species endemism in Tanzania. These *Emilia* species appear to fall in Tanzania's unprotected areas,

and so this information is useful for conservation planning in Tanzania in terms of which 'new'/additional areas to protect. Conservation prioritisation in *Emilia* using the various biodiversity indices should therefore rather focus on regions than the whole of Africa. Daru *et al*. (2015) in a study of biodiversity hotspots (based on trees) in southern Africa, focussed on PD to highlight where conservation efforts should be concentrated in southern African flora, i.e. where PE and PD were high. This information was also useful in highlighting the need for protection of centres of past refugia or evolutionary radiations, e.g. in the Cape Floristic Region (CFR). Daru *et al.* (2015) also showed that in southern Africa, SR and PD were generally well represented in protected areas (viz. National Parks) whereas high PE and CWE were located mostly outside protected areas (Daru *et al*. 2015). A focus on PD in southern Africa means that conservation priority areas would include the following: central and northern Malawi; northern Zambia; the Harare region and the eastern highlands of Zimbabwe. These areas also show high PE thus providing additional information required in conservation planning as these indices (PD and PE) are used to identify centres of plant biodiversity with 'geographic concentrations of evolutionary isolated and spatially restricted biota' (Rosauer and Jetz 2015 p. 168). Areas with high PE have been shown to support biodiversity elements with minor/small representation elsewhere, which when lost, would affect PD (Rosauer and Jetz 2015). Phylogenetic diversity and PE indices have also been shown to be useful in augmenting the conservation prioritization decision-making process, where the assessment of phylogeny instead of species numbers alone can be used in reserve design and biodiversity areas 'with unique evolutionary history and traits in need of conservation' are identified (Mishler *et al*. 2014 p. 4473).

Conservation evaluations of *Emilia* and their protected areas in Zimbabwe

The assessment of the conservation status of Zimbabwean *Emilia* species showed that sixty percent are widely distributed and occur in common habitats (e.g. grasslands and/or miombo woodlands). The other thirty percent of *Emilia* species are rare or endangered and occur in specialised habitats such as swampy areas. Two species, *E. baumii* and *E. protracta,* are near-endemic in Zimbabwe — to the Great Dyke and Victoria Falls rainforest respectively. However, the rare and endangered species in Zimbabwe (as assessed per country) also occur elsewhere in southern Africa, where they are endemic to specific regions or countries – e.g. to tributaries of the Okavango Delta (*E. tenellula*), the Woodbush area of Limpopo Province, South Africa (*E. limosa*), and near-endemic to the Kavango Region (*E. protracta*). These species are restricted in distribution occurring in specialised habitats with few small

populations known in these particular regions and/or countries although some of these species are abundant and widespread elsewhere, e.g. *E. limosa* in Malawi and Tanzania. Recognition of these rare species is important in prioritising their areas of occurrence as conservation areas. Further investigation is required for *E. baumii* which is data deficient and has only been collected in Angola and three other sites around Zimbabwe. The Red Data assessment categories for *Emilia* would provide information that is necessary to guide conservation efforts focussed on species of *Emilia*.

There are 25 protected sites in Zimbabwe occupying 7.86 % of the country's land surface area (www.biodiversitya-z.org/content/zimbabwe, retrieved 16 November 2016). Each of these protected areas 'covers an area of more than 10 km^2, and is in IUCN Management Categories I-V and is managed by the Zimbabwe Parks and Wildlife Management Authority (www.iucn.org/content/1990-united-nations-list-national-parks-and-protected-areas, retrieved 16 November 2016). The current protected areas in Zimbabwe adequately cover most areas with high PD and PE except the Harare Region, thus *Emilia* species in these areas are generally sufficiently protected. In most areas/habitats in Zimbabwe with high SR in *Emilia*, many populations fall outside the currently identified protected/conserved areas, and are therefore not protected. Conservation efforts should therefore be extended to include parts of the Harare Region where *Emilia* species occur and other areas where most populations are unprotected. Timberlake and Müller's (1994) proposed botanical conservation approach for Zimbabwe considered three categories (viz. international areas with high vegetation diversity; national areas with specific vegetation types and ecosystems; local areas with sites of botanical interest e.g. where threatened species are protected). Fourteen conservation areas in Zimbabwe were then identified and these also cover areas with high species diversity and endemism. Five of these 14 conservation areas are mountainous areas [viz. Chimanimani, Nyanga, Mount Wedza (south of Marondera), Mount Buhwa (Zvishavane), and the Nyoni hills (south of Masvingo)] and their surroundings. Species occurring in these mountainous areas are inaccessible and therefore more naturally protected (Timberlake and Müller 1994). However *Emilia* species mostly occur on lowland/flat areas surrounding these mountainous areas and are conserved, for example, in National Parks and Botanical Reserves.

The conservation approach being proposed for Zimbabwean *Emilia* species should combine three of the four biodiversity and phylogenetic indices investigated in this study (viz. SR, PD, and PE). Species endemism (CWE) is not included here since it has been shown to be low in southern Africa. However the endemism component is included in PE which also

considers the phylogeny (Laity *et al*. 2015). This conservation approach is also applicable to other taxonomic groups that might have similar distribution patterns and occur in similar floras e.g the widespread genera *Crotalaria* L. and *Kirkia* Oliver in Savanna Flora; and *Kniphofia* Moench, and *Helichrysum* Mill. in Afromontane Flora or Austro-temperate Flora (Linder 2014). Therefore, conservation evaluations in these taxonomic groups using biodiversity indices should be done and compared as this could reveal a pattern in these floras helpful in promoting the conservation of African biodiversity more generally. I also recommend an integrative approach of using these various biodiversity and phylogenetic indices to inform species and areas conservation decisions. Phylogenetic approaches provide additional information, notably evolutionary history and spatial distribution of biodiversity, not captured by traditional biodiversity indices and should therefore be utilised in biodiversity conservation (Laity *et al*. 2015). Combinations of various biodiversity indices in conservation assessments have been successfully used to recommend creation of/prioritization of conservation areas in previous studies (e.g. Faith *et al*. 2004; Mishler *et al*. 2014; Daru *et al*. 2015). In addition to the conservation approach used, a collaborative approach is also needed amongst individuals, non-governmental organisations and Government in order to effectively conserve Zimbabwe's vegetation and its associated wildlife in general.

Conclusions and recommendations

This study has provided additional information on conservation prioritization issues in *Emilia* that will help in strategizing biodiversity conservation in southern Africa and other regions. The biodiversity approaches of maximising SR, PD and PE could generally prioritize similar regions and genera with a specific distribution pattern similar to *Emilia*, i.e. those that are part of the Savanna and/or Austro-temperate floras. The areas identified as needing additional protection using SR, PD and PE would ensure that maximum preservation of evolutionary potential and distinctiveness is prioritized for conservation, as well as the traditional accounting for high species richness. Thus, I recommend integrating SR and the phylogenetic indices (PD and PE). These provide 'additional information to policy makers about the spatial distribution of biodiversity. This can enhance the assessment of conservation value, leading to a more complete and sophisticated understanding of the biodiversity of an area or region, how it evolved and why it is important to conserve' (Laity *et al*. 2015 p. 133). In Zimbabwe, conservation efforts aimed at protecting *Emilia* species should incorporate some unprotected

areas around the Harare Region with a high SR (five species), and high phylogenetic indices (PD and PE). Most populations of *Emilia* occurring in areas with high SR in Zimbabwe are unprotected and should also be prioritised in conservation.

In order to understand and interpret patterns of species distribution accurately in countries such as Angola, Mozambique, and Zambia, greater access to locality data is required – this might involve greater collaboration and collecting effort and/or increased computerization of existing records. Also urgent assessment of the threat status of *E. baumii,* which is data deficient, is needed.

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CHAPTER 6

General Discussion

A phenetic, phylogenetic and biogeographic study of genus *Emilia* Cass. was undertaken here, with four major aims and a number of associated objectives for each aim, which are addressed in the central chapters (2, 3, 4, and 5) of the thesis. Each of these aims is outlined below together with a brief discussion of the significant findings, with synthesis of results where relevant.

(1) *To evaluate the species recognised by Jeffrey (1997) in the* Emilia coccinea *complex using phenetic and molecular phylogenetic approaches, assess the applicability of the morphological, phenetic, and phylogenetic species concepts to this* E. coccinea *complex.*

A multivariate approach was used to address this aim based on 134 herbarium specimens of the eight species in the *Emilia coccinea* complex and a final data set with 42 morphological characters. Five *Emilia* species (*E. emilioides* (Sch. Bip.) C.Jeffrey, *E. jeffreyana* Lisowski, *E. praetermissa* Milne-Redh., *E. subscaposa* Lisowski, and *E. vanmeelii* Lawalrée) in the *E. coccinea* complex were recognized as distinct, while three were taxonomically indistinguishable: *E. caespitosa* Oliv., *E. coccinea* (Sims) G.Don and *E. lisowskiana* C.Jeffrey. *Emilia lisowskiana* grouped with three of the *E. coccinea sensu stricto* specimens, and two of these are from the same geographical location (Equatorial Guinea) with *E. lisowskiana* although the third is from Cameroon. This species is characterised by having epapillose unappendaged style branch apices (Jeffrey 1997), a character that separates it from other species in the *E. coccinea* complex. *Emilia coccinea* and *E. caespitosa* are similar in their reproductive characters, e.g. possessing appendaged, awl-shaped style branches, and also in the vegetative character, stem diameter. The lack of distinction between *Emilia caespitosa* and *E. coccinea* was confirmed by the nuclear molecular phylogenetic analysis (Chapter 3), where *E. caespitosa* grouped within a clade with *E. coccinea*. Their relationship was unresolved within the same clade in the plastid phylogeny, but these two species should probably be synonymised. *Emilia lisowskiana*, however, which grouped together with *E. coccinea* specimens in the cluster analysis, is unresolved in the nuclear ITS and plastid *trn*L-*trn*F–based phylogenies. Lack of resolution, especially in the plasid phylogeny, limits our ability to definitively interpret species boundaries, and the possibility of past hybridization

influencing the phylogenetic pattern in the nuclear marker must be considered. *Emilia caespitosa* and *E. coccinea* could be one heterogeneous species, however the *E. coccinea* accessions do not all group together and some seem to be geographically positioned. Another possibility is that *E. caespitosa* and *E. coccinea* are distinct species, but hybridize as suggested by the presence or absence of cypsela indumentum on some members of *E. caespitosa* and *E. coccinea* respectively (instead of being glabrous or pubescent). However cypsela indumentum might be an unreliable character as in other senecioid genera (e.g. *Cineraria deltoidea* Sond. and *C. erodoides* DC.; Cron *et al.* 2006, 2007), although this character was found to be taxonomically useful in Eupatorieae (Wetter 1983). Hybridization and/or introgression between *E. caespitosa* and *E. coccinea* are a distinct possibility as the species are not geographically or temporally (i.e. flowering periods overlap in four countries) separated, co-occurring in nine countries and in similar habitats.

The vegetative characters used by Jeffrey (1997) could not differentiate all eight species in the *E. coccinea* complex, since these characters are variable within species. However, eleven reproductive characters, including features of the capitula, phyllaries, style branch apices, and pappus, are shown here to reliably distinguish species in the *E. coccinea* complex. Style branch appendage, confirmed here as a significant diagnostic feature in *Emilia* noted by other authors, e.g. Jeffrey (1986, 1997), Lisowski (1990, 1991) and Tadesse and Beentje (2004), to be important in *Emilia* is also a useful character for differentiating species in the subtribes Astereae and Senecioninae (Nelson 1994; Riva *et al.* 2009).

The morphological species concept applied by Jeffrey (1997) to the eight species in the *E. coccinea* complex and practically used by most researchers to distinguish species has weaknesses in it due to the variability of some of the diagnostic characters used to separate species and the phenetic approach is thus helpful as it uses many equally weighted characters simultaneously. The phenetic and phylogenetic species concepts applied to this *E. coccinea* complex revealed that five out of eight *Emilia* species are distinct suggesting that they are phenetically and genetically similar (Moss 1972; Stuessy 1990; Mishler and Theriot 2000). The weakness of the phenetic species concept is that 'overall similarity' important in the phenetic approach requires the use of many characters (Stuessy 1990; Jensen 2009) and some of these are difficult to measure (Duncan and Baum 1981; Sokal 1986). Nonetheless the phenetic species concept was useful in distinguishing most species in the *E. coccinea* complex. Additionally, the evolutionary relationships amongst the species in the *E. coccinea* complex have been revealed by the molecular approach, although some individual *Emilia* species did not form a monophyletic group, a requirement of the apomorphy species concept

(e.g. Mishler and Theriot 2000). A key distinguishing the eight species of the *E. coccinea* complex is also presented here, in which useful combinations of characters (i.e. quantitative and qualitative) are used (as compared to Jeffrey's (1997) key).

(2) *To investigate the phylogenetic relationships of a representative sample of* Emilia *species, together with the genera* Emiliella S.Moore, Bafutia C.D.Adams, *and other closely related genera in the Senecioneae, using nuclear and plastid DNA sequence data. The resultant phylogeny serves to indicate whether* Emilia *is monophyletic or not, and assesses the generic status of* Emiliella *and* Bafutia*. It also provides a sound basis for future taxonomic revisions of the genus, including assessment of Jeffrey's sectional classification of* Emilia*. Possible roles played by past hybridization, introgression, and/or incomplete lineage sorting in the evolutionary history of* Emilia *are also investigated here by examining the anticipated incongruence between nuclear and chloroplast DNA phylogenies.*

Molecular phylogenies comprising *Emilia*, *Emiliella*, and *Bafutia* species and 22 closely related genera in the Senecioneae were reconstructed using nuclear ITS (80 accessions) and plastid *trn*L-*trn*F (95 accessions) DNA sequence data. Both nuclear and plastid phylogenies indicated that the genus *Emilia* is not monophyletic. Seventeen *Emilia* species are grouped outside *Emilia* in the plastid *trn*L-*trn*F phylogeny and one species *E. baumii* (O.Hoffm.) S.Moore is placed outside of *Emilia* in both the plastid and nuclear phylogenies, indicating that it is not part of *Emilia*. However in the nuclear ITS phylogeny both *E. baumii* and *E. graminea* DC. surprisingly group with the Othonninae, although neither species has the diagnostic traits of the Othonninae, viz., undivided styles or connate phyllaries (Bremer 1994; Sykes 2004). I therefore recommend the exclusion of both *E. baumii* and *E. graminea* from *Emilia*, although additional molecular markers are needed to support this exclusion. Genera *Emiliella* and *Bafutia* are nested within *Emilia* in both the nuclear and plastid molecular phylogenies further supporting that *Emilia* is not monophyletic and that *Emiliella* and *Bafutia* are not distinct/do not warrant their current generic status. I thus propose that these two genera should be placed in *Emilia* as suggested by Jeffrey (1986), and are united by having mostly small discoid ecalyculate capitula. Jeffrey's (1986) sections *Spathulatae* and *Emilia* are not supported in this study as there are no matching clades. No distinguishable morphological patterns are evident in the various clades, thus no meaningful sectional delimitations are proposed from the phylogenies reconstructed here. However the present study contributes the groundwork for future taxonomic revisions of *Emilia* and future work using more molecular markers and an increased sampling of species is recommended to produce more complete, better resolved and supported phylogenies and thereby possibly

enable a sectional classification of *Emilia.* The use of additional molecular markers is not expected to solve the problem of incongruence amongst different sets of data, but better resolution and support for some clades would strengthen the phylogenetic hypotheses and facilitate interpretation of the results.

Comparison of the nuclear ITS and plastid *trn*L-*trn*F phylogenies revealed well-supported topological incongruencies suggesting that chloroplast capture, hybridization, introgression, and/or incomplete lineage sorting (ILS) might have occurred in the evolutionary history of genus *Emilia*. Incongruence was observed in the placement of the large clade comprising 17 *Emilia* species from sect. *Emilia* (Clade A; Chapter 3) outside the main *Emilia* clade in the plastid *trn*L-*trn*F phylogeny compared to *E. baumii* and *E. graminea* in the nuclear ITS phylogeny. Further incongruence was shown by the different positions of *E. transvaalensis* (Bolus) C.Jeffrey in the plastid versus nuclear analyses suggesting that this species might be of hybrid origin, confirming earlier findings by Cron (2013). Hybridization has also been shown to occur in other *Emilia* species (Olorode and Olorunfemi 1973). Topological incongruencies between plastid and nuclear phylogenies have previously been observed in the Senecioneae (e.g. Cron *et al*. 2008, 2013; Pelser *et al*. 2010) and hybridization, introgression, and ILS were suggested as the causes of incongruence (e.g. Jones *et al*. 2014; Roy *et al*. 2015). Further investigations possibly using a coalescent-theory based approach (Pelser *et al*. 2010) and other molecular markers such as amplification fragment length polymorphisms (AFLPs) are recommended in order to establish the causes of incongruence in *Emilia*.

(3) *To investigate the pattern and timing of diversification in* Emilia *using current geographic distributions of species and dated molecular phylogenetic hypotheses, and to correlate this pattern with evolutionary (morphological) trends in the genus. Data from the fossil record and secondary calibrations are used to infer this diversification pattern of* Emilia *across Africa and Madagascar.*

Emilia appears to have originated in southern Africa based on the current species' distributions and the phylogeny reconstructed here. This hypothesis is supported by the early diverging lineages such as that comprising *E. marlothiana*, *E. transvaalensis*, and *E. schinzii*, which occur in Namibia, South Africa, Botswana, Zimbabwe and southern Angola. Most of the *Emilia* clades that originated in southern Africa dispersed to various regions of Africa such as East Africa, then to West and Central Africa, while a few remained in southern Africa and later dispersed to East Africa, together with further diversification of species and at least

three dispersals back to southern Africa, and then also spreading to West Africa. Some species such as *E. sonchifolia* became widespread and *E. exserta* dispersed to Tropical Asia (India and Sri Lanka).

The age of *Emilia* is estimated to be 14.19 Myr (95% HPD: 9.49–18.94 Myr) and the genus is hypothesised to have originated during the Mid-Miocene. The estimated time of origin in *Emilia* corresponds to significant cool and warm climatic events such as the Middle-Miocene Climatic Optimum (*ca.* 15 Mya; Flower and Kennett 1994), after which there was an expansion of open habitats. Later diversification of most *Emilia* clades in Central, East, and West Africa occurred during the Late Miocene as the climate and vegetation changed across Africa and the species occupied diverse habitats in Africa such as grasslands, savannas, and forest edges. Some *Emilia* lineages, such as the clade comprising *E. decipiens*, *E. discifolia*, and *E. myriocephala*, have diversified in more than one region, that is, in Central and East Africa and occur in open habitat ecosystems.

Several dispersal events occurred mainly from southern and East Africa to Madagascar in *Emilia* and at least five independent dispersals were noted for the six out of the 14 Madagascan species included in this study. Long distance wind dispersal to Madagascar is quite likely as *Emilia*'s cypselas are suited to wind dispersal by being relatively small, light, and having a bristled pappus. Africa has been shown to have served as a source of species for Madagascar because of sister group relationships between Malagasy biota and African taxa (Yodar and Nowak 2006).

Eleven of the thirteen morphological characters (excluding floret colour and style branch apex shape) optimised onto the reconstructed molecular phylogeny were equivocal in *Emilia*. Three characters were shown to have a high probability of being ancestral (plesiomorphic), viz. annual life history, erect growth form, and cauline leaves.

The annual life form predominant in *Emilia* and also present in genera *Bafutia* and *Emiliella*, is postulated to have either been ancestral or evolved early in this genus [13.32 Mya (9.08–18.11 Mya)] and is very likely associated with the success in diversification of *Emilia* species in various parts of Africa where there was a change in climate and vegetation during the late middle Miocene. The survival of *Emilia* species in diverse habitats could have been enhanced by their rapid reproduction as well as production of many seeds — characteristic of annual plants (Espeland and O'Farrell 2010). The few perennial *Emilia* species can reproduce vegetatively, thus increasing their survival chances and they are also adapted to survive dry periods and/or in arid areas because of their succulent or semi-succulent habit.

Pollination is enhanced in *Emilia* by certain characters of the capitula, e.g. capitula in groups of more than three and broad capitula with flower heads exceeding the phyllaries, which make them conspicuous to pollinators. In addition, the highly reflective yellow florets (a common colour in the genus) attract a variety of insects. The state 'discoid capitula' is synapomorphic for *Emilia*, although the conspicuous radiate capitula (mostly yellow) attract pollinators (especially butterflies) and provides a landing stage for them (Stuessy *et al*. 1986). Appendaged, papillose style branches appear to have evolved independently several times in *Emilia* and are probably important in secondary pollen presentation, thus contributing to the success of *Emilia* in colonising diverse habitats.

(4) *To identify areas of high species richness (centres of diversity) and areas of endemism for* Emilia *in southern Africa. To contribute to current debates on conservation prioritization as they apply to* Emilia *for a selected region (viz. Zimbabwe) by comparing and evaluating various biodiversity indices — species richness (SR), phylogenetic diversity (PD), species endemism (CWE) and phylogenetic endemism (PE). The distribution and biodiversity indices and IUCN Red history information are used to assess the effectiveness of conservation of* Emilia *in the currently protected areas in Zimbabwe.*

The distribution of *Emilia* species in southern Africa is uneven with high species concentrations occurring in northern and southern Malawi, and in eastern and north-eastern Zimbabwe (viz. the Nyanga Region and areas surrounding Harare respectively). In contrast, only a few species have been recorded in Botswana, Namibia and South Africa. The uneven species distribution in *Emilia* could be attributed to among other factors, climatic regimes — rainfall, and habitat requirements – moist versus arid/seasonally arid habitats, but also to different collecting efforts by botanists and availability of locality data from various countries. Three centres of greatest species diversity have been identified for *Emilia* — two fall within the Chimanimani-Nyanga and Nyika Plateau Centres of endemism, which are part of the Austro-temperate Region (Linder 2014) and the third is in areas surrounding Harare, Zimbabwe. Very few endemic or near-endemic *Emilia* (seven) and *Emiliella* (two) species occur in southern Africa compared to other plant taxonomic groups in similar areas/habitats in the Savanna and/or Austro-temperate regions. The low number of *Emilia* endemics in southern Africa is due to some of the species being widely distributed and also occurring in East Africa (EA) and the Democratic Republic of Congo (DRC). Species richness and endemism overlap minimally in southern Africa as centres of diversity and endemism for *Emilia* do not largely coincide except the Nyika Plateau (Malawi). Similar research done by

Mendelsohn *et al*. (2002) using data on the combined flora and fauna in Namibia and Orme *et al*. (2005) using global data on breeding distribution of living bird species showed little to no overlap between species richness and endemism. Incongruence between species richness and endemism means that choosing habitats with high endemism for conservation prioritization in *Emilia* would leave out areas with high species richness, and similarly, choosing these species rich areas would miss endemics, rare, and threatened species.

Nonetheless, three biodiversity indices (SR, PD, and PE; i.e. excluding CWE) overlap and are shown here to mostly prioritise the same areas and associated habitats, namely northern and central Malawi, Harare region and eastern highlands of Zimbabwe for *Emilia* conservation in southern Africa. Species richness and PD (for *Emilia*) coincide in most southern African areas, similar to the positive correlation between these indices in Australian *Acacia* (Mishler *et al.* 2014). The evaluation of PD provides additional information on conservation prioritisation of *Emilia* species that are evolutionarily distinct, thus maximising the preservation of evolutionary potential in these identified areas that could be missed by SR alone. Furthermore when SR and PD are used together, a more detailed picture of conservation importance of an area is provided (Moritz 2002; Laity *et al*. 2015). Phylogenetic diversity and PE are also congruent in certain areas, e.g. central and northern Malawi, and northern Zambia as well as the regions in Zimbabwe highlighted by SR viz. Harare region and the eastern highlands, thus providing further information for identification of geographical regions with restricted evolutionary history. Assessment of biodiversity indices in *Emilia* can be extended to other taxonomic groups in similar floras (i.e. the Savannah and Austro-temperate floras), and I recommend that additional similar assessments be done and compared, as this might reveal a pattern in these floras which would greatly assist in promoting the conservation of biodiversity in Africa.

In an assessment of how conservation efforts should be prioritized for *Emilia* species in a specific region of southern Africa, viz. Zimbabwe, three out of ten Zimbabwean *Emilia* species were found to be rare and endangered and occur in specialised habitats such as marshy areas. No endemic *Emilia* species are recorded in Zimbabwe, but two near endemics occur here — *E. baumii* (also Data Deficient) in the Great Dyke Centre of endemism and *E. protracta* in a specialised microhabitat in the Victoria Falls rainforest where it is conserved. Although the current conservation areas in Zimbabwe including National Parks and Botanic Gardens protect the majority of *Emilia* species including the rare and endangered ones, very few populations occur in these areas and are thus protected. Conservation prioritization and

efforts should thus focus on these near endemic and rare and/or endangered *Emilia* species viz. *E. limosa*, *E. protracta* and *E. tenellula* together with their associated habitats.

Phylogenetic diversity and PE both have high values in the Harare region and the eastern highlands of Zimbabwe indicating that these regions should be prioritized in conservation as they might also have a distinct evolutionary history and geographically restricted traits that should be conserved (Mishler *et al*. 2014; Laity *et al*. 2015). Most areas with high PD and PE fall within the current and proposed protected areas (Timberlake and Muller 1994) except for Harare and its surrounding area. Therefore, to ensure effective conservation of *Emilia* species, conservation efforts should also be extended to cover areas or habitats surrounding the Harare region where there are few protected areas. I therefore recommend an integrative approach using SR, PD, and PE for conservation prioritization of *Emilia* species in Zimbabwe and southern Africa in general, as these indices have been shown to indicate the same priorities for *Emilia* species conservation.

Recommendations for future studies

The genus *Emilia* is not monophyletic and there is need to intensively sample the rest of *Emilia* species and other missing species of *Emiliella* to answer the questions around monophyly. Other related genera such as *Gynura* Cass. linked to *Emilia* by having style-branches with subulate appendages and *Psednotrichia* Hiern. of the emilioid complex should also be included in a molecular phylogenetic study to confirm their relationships with *Emilia*, *Emiliella*, and *Bafutia*. Most *Emilia* species are annual and it is difficult and not guaranteed to find them at the same place as they flower according to the rains which are also not predictable thus they were not included in the study in cases where herbarium material were unavailable and/or were difficult to amplify. With the availability of funding, extensive field work should therefore be done in other African countries to collect fresh leaf material to be used as the source of DNA for phylogenetic study especially for the species that were difficult to amplify using herbarium specimens and those that produced faint bands that could not be sequenced.

Further research using additional molecular markers e.g. the external transcribed spacer (ETS) region trialled in the preliminary study is required to investigate the phylogenetic relationships which were not resolved in this study using the nuclear ITS and plastid *trnL-trn*F markers. The ETS region was difficult to amplify for herbarium samples and therefore not used here due to budget and time constraints although it was apparently

variable enough to be useful for a species-level phylogenetic analysis. Phenetic studies (Mapaya and Cron 2016) supported by the molecular phylogenetic studies, have highlighted that three species in the *E. coccinea* complex (*E. caespitosa*, *E. coccinea*, and *E. lisowskiana*) are not distinct and should probably be synonymised. A comparison of the type specimens of the synonymous species *E. caespitosa* and *E. coccinea* and careful matching to specimens assigned to these species should be done in order to assist in this decision. The possible exclusion of both *E. baumii* and *E. graminea* from *Emilia* also needs to be investigated further. Other molecular markers, such as simple sequence repeats (SSRs) or amplification fragment length polymorphisms (AFLPs) are also needed to confirm whether *Emilia* species that were incongruent between the nuclear and plastid phylogenies, e.g. *E. praetermissa* and *E. transvaalensis* are of hybrid origin. Cytological studies need to be undertaken to supplement data from molecular studies as chromosome numbers are not known in most *Emilia* species and are diagnostic at the species level in the senecionoid genera. These chromosome numbers might be important in delimiting groups within *Emilia* as well as determining the systematic positions of the species in dispute.

In order to understand and interpret patterns of species distribution accurately further information on distribution/locality data is required for some countries such as Zambia, Mozambique, and possibly Angola where there is incomplete data. Also urgent assessment of IUCN threat status is needed for *E. baumii*, which is data deficient. Several field trips are needed to relocate this species at Zimbabwean localities where it was initially found when it is known to be flowering in order to update our knowledge about its occurrence. Conservation studies of *Emilia* should be extended to include East Africa and the DRC, where *Emilia* species are also known to occur and are not included in this study since the focus was on southern Africa. Specimen locality data for these countries could be compiled from relevant literature (e.g. Lisowski 1990, 1991), other data bases with the respective countries information as well as herbarium specimens requested from various herbaria.

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