Gomphrena (Amaranthaceae, Gomphrenoideae) diversified as a C4 lineage in the New World tropics with specializations in floral and inflorescence morphology, and an escape to Australia

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Gomphrena (Amaranthaceae, Gomphrenoideae) diversified as a C₄ lineage in the New World tropics with specializations in floral and inflorescence morphology, and an escape to Australia

Abstract: The genus Gomphrena comprises about 120 species in the Americas and 35 in Australia. Previous research revealed that Gossypianthus, Lithophila and Philoxerus are closely related but the monophyly of Gomphrena remained unresolved. Our aim was to clarify phylogenetic relationships in Gomphrena and allies based on a thorough sampling of species and to reconstruct the evolution of morphological characters including C₄ photosynthesis, and to explore the disjunction of the Australian taxa. We generated datasets of plastid (matK-trnK, trnL-F, rpl16) and nrITS representing 45 taxa of Gomphrena plus relatives and analysed them with parsimony, likelihood and Bayesian methods. Ancestral states of phenotypic characters were reconstructed with BayesTraits. BEAST was employed for divergence time estimates using an extended Amaranthaceae–Chenopodiaceae dataset to place fossil calibration points. Gossypianthus is closely related to a Gomphrena radiata–G. umbellata–G. tomentosa clade and G. meyeniana, whereas Lithophila and Philoxerus appear as successive sisters of the Australian species of Gomphrena. The majority of Andean species appears in a large clade including annual and perennial species. The Cerrado species Gomphrena mollis and G. rupestris, which are C₃, constitute an early-branching lineage, whereas the core Gomphrena clade is C₄ and has the inner two sepals strongly compressed as synapomorphy. A major subclade evolved inflorescences with subglobose paracladia in a whorl, supported by pseudanthial leaves. Whereas the core Gomphrena clade started to diversify around 11.4 Ma (8.45–14.5 95% highest posterior density [HPD]) the Australian lineage split at only 4.8 Ma (2.61–7.18 HPD). Our detailed phylogenetic analysis of Gomphrena including Gossypianthus, Lithophila and Philoxerus, considering that these small segregate genera were based on states of vegetative characters exhibiting adaptations to specific habitats rather than phylogeny and overall morphology.

Key words: Amaranthaceae, androecium, C₄ photosynthesis, Caryophyllales, classification, Gomphrena, Gomphrenoideae, Gossypianthus, Lithophila, molecular phylogenetics, Philoxerus, pseudanthium

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regions continue to be discovered and described, many of them endemic (Ortuño & Borsch 2005, 2006). The Cerrado and Caatinga habitats in mid-western and northeastern Brazil also are diverse in Gomphrena (c. 50 spp.; Siqueira 1992), whereas Central America and the desert regions of Mexico and North America harbour only c. 20 species (Robertson & Clements 2003). A second and disjointed centre of diversity is in Australia, where c. 33 species predominantly occur in the arid and semi-arid regions of western, central and northeastern Australia (Palmer 1998).

Linnaeus (1753) formally described the genus Gomphrena and added further species in later publications (1756, 1762). From the eleven species he described only three remained in the genus, as it is currently widely accepted (Eliasson 1988; Townsend 1993), whereas others were transferred to Alternanthera, Froelichia and Philoxerus (= Blutaparon) by subsequent authors (e.g. Holzhammer 1956), Alternanthera and Froelichia are morphologically well-defined genera. Alternanthera is distinguished from Gomphrena and allies by the globose stigma on a more or less elongated style and the presence of entire or fringed androecium tube appendages (Vrijdaghs & al. 2014; previously called “pseudostaminalodia”, e.g. Eliasson 1988; Townsend 1993) alternating with the stamens, using the genus concept of Schinz (1893, 1934), adopted by Eliasson (1988) and Townsend (1993). The situation is different in Philoxerus, which cannot be easily separated from Gomphrena as it just differs by the general lack of stamen tube appendages or filament appendages, an androecium morphology that also occurs in some Australian species of Gomphrena. Froelichia differs from all other Gomphrenoideae by sepals fused for more than half, with this tube developing two lateral wings at maturity (Eliasson 1988). The morphology of the inner two sepals can differ and has been regarded as one of the diagnostic characters of Lithophila and Philoxerus (Townsend 1993). In these studies the term “tepals” was used for the perianth organs. More recent work converges on the opinion that these organs are in fact sepals (e.g. Ronse de Craene & Brockington 2013).

The currently widely used genus concept of Gomphrena (Townsend 1993; Hernández-Ledesma & al. 2015) is still pre-phyletogenetic and goes back to Schinz (1893). Based on this circumscription, Holzhammer (1955, 1956) provided the last full synopsis of Gomphrena at species level for the New World and Palmer (1998) treated the Australian taxa. Pedersen (1990) more recently resurrected the genus Xerosiphon Turcz. with two species and removed them from Gomphrena based on the difference that their sepals are united until the middle.

According to the resulting slightly narrower genus circumscription, Gomphrena is characterized by flowers with five free and symmetric sepals, a gynoecium with an inconspicuous to elongated style and two filiform stigma branches and the five filaments forming a tube to varying degrees, in some species almost completely fused for more than half, with this tube developing two lateral wings at maturity (Eliasson 1988). The morphology of the inner two sepals can differ and has been regarded as one of the diagnostic characters of Lithophila and Philoxerus (Townsend 1993; Eliasson 1988). Sánchez-del Pino & al. (2019) showed that this tube does not result from post-genital fusion but develops from a ring-like meristem. Most species of Gomphrena possess stamen appendages on each side of the filament in a terminal position, leaving the impression of anthers “sunken” between them (Borsch & Ortuño 2005, 2006; Pedersen 1997, 2000). Lithophila also shares these androecium characteristics but the number of filaments and anthers is reduced to two or three (Eliasson 1988), whereas Gossypianthus has a shortly fused androecial cup, lacks appendages but the androecium is fused to the sepals for the most part (Schinz 1934; Eliasson 1988). The gynoecia of the genera Gossypianthus and Lithophila are morphologically similar to Gomphrena.

Within the Amaranthaceae, Gomphrena belongs to subfamily Gomphrenoideae. This subfamily is characterized by the presence of unilocular anthers and has been shown as monophyletic with high statistical support (Müller & Borsch 2005; Sánchez-del Pino & al. 2009; Borsch & al. 2018). Following the matK-trnK study by Müller & Borsch (2005), who presented an overall phylogenetic analysis of the Amaranthaceae, Sánchez-del Pino & al. (2009) focused on the Gomphrenoideae with an increased taxon sampling of this clade. Based on the combined trnL-F and rpl16 sequence data Sánchez-del Pino & al. (2009), three highly supported subclades of the Gomphrenoideae were found, and informally called alternantheroids, gomphrenoids and iresinoids. The iresinoid clade contains the genus Iresine and is sister to the remaining Gomphrenoideae (Borsch & al. 2018). Alternantheroids and gomphrenoids, referred to as the core Gomphneoideae by Müller & Borsch (2005) share the presence of metaterticate pollen (Borsch 1998; Borsch & Barthlott 1998) as a synapomorphy. The alternantheroid clade consists of the monophyletic genera Alternanthera, Pedersonia and Tidestromia (Sánchez-del Pino & al. 2009, 2012; Borsch & al. 2011), whereas all remaining genera of the core Gomphneoideae are in the gomphrenoid clade.

Molecular trees of Sánchez-del Pino & al. (2009), Sage & al. (2007) and Bena & al. (2017) show that the gomphrenoid clade contains species of Gomphrena in two different lineages: The first lineage comprises Lithophila, Gossypianthus and Philoxerus (= Blutaparon) along with most species of Gomphrena (= Gomphrena s.str.), and including G. globosa as the type species of the genus in a terminal clade, with Xerosiphon, Froelichia and Gual­liminea appearing as successive sisters. Phylogenetic results thus confirmed the view of Pedersen (1990) to treat Xerosiphon as different from Gomphrena. The second lineage is composed of Hebanthe, Pjoflia and some species of Gomphrena such as G. vaga and G. elegans. Interestingly, the two different lineages containing species of Gomphrena are also characterized by pollen morphology. The first lineage (“clade a” in Sánchez-del Pino & al. [2009]) is characterized by metaterticate pollen
with strongly reduced tectum (Borsch 1998), whereas the second lineage has metarcticulate pollen with the tectum completely covering the mesoporia and possessing just small perforations or foveolae (Borsch 1998; Borsch & Pedersen 1997). However, in all these studies relationships within each of these two lineages remained unresolved.

This investigation focuses on the first lineage. Sánchez-del Pino & al. (2009) published the hitherto most resolved trees with plastid trnL-F+rpl16 sequences but included just 11 species. They found three lineages in a polytomy, among which one depicted Gossypianthus sister to Gomphrena boliviana, another Gomphrena flaccida sister to Blutaparon+Lithophila, and the third the remaining species of Gomphrena. Recently, Bena & al. (2017) added trnL-F sequences of a dozen further species from Argentina, but their trees remained statistically unsupported. Here we improve the sampling of Gomphrena from the different ecoregions (such as Andean dry valleys, Caatinga, Prepuna and Puna ecosystems in south America, and tropical and subtropical ecosystems in Australia) and of the different morphologically allied species groups present in the genus (Holzhammer 1955, 1956) using a set of genomic regions that have been shown to harbour high levels of phylogenetic signal in the plastid (e.g. Borsch & Quandt 2009; Korotkova & al. 2011) as well as nuclear ITS to test for congruence between genomic partitions.

Regarding morphology, Eliasson (1988) provided a comparative assessment of floral morphology for all genera of Gomphrenoideae then accepted, but he did not carry out any analysis of character evolution in a phylogenetic context. Nevertheless, hypotheses on androecium evolution within Gomphrena were put forward by Fries (1920) who proposed that Gomphrena comprises groups of species in which the complexity of the androecium was reduced, so in G. tomentosa and allies which lack stamen appendages. Eliasson (1988) hypothesized a stepwise character state transformation in the androecium from species with “pseudostaminodes” alternating with the stamens to species with apical filament lobes such as Gomphrena spp. through varying degrees of fusion. Vrijdaghs & al. (2014) examined the ontogeny of the androecium in Gomphrenoideae and concluded that the androecial tube develops from a circular intercalary meristem, from which also the alternating appendages arise as androecial tube appendages which are thus not homologous to residual stamens, and which therefore cannot be called “pseudostaminodia”. However, Vrijdaghs & al. (2014) only examined Alternanthera, Iresine and Tidestromia. More recently, Sánchez-del Pino & al. (2019) studied the ontogeny of Blutaparon, Guilleminnea and several species of Gomphrena and Pfaffia which have lateral appendages on both sides of the filament but no alternating stamen tube appendages, and concluded that these lateral appendages are de novo organs not homologous to the stamen tube appendages. Acosta & al. (2009) compared the structure of synflorescences across Amaranthaceae, and found that Gomphrenoideae possess thyrsoid structures with paracladia reduced to solitary flowers but they did not examine Gomphrena in more detail. We therefore selected a set of 21 vegetative and floral characters to assess the morphological variation of Gomphrena and allies, the matrix of which was then used to reconstruct character evolution in a phylogenetic context.

As a further character we investigated the distribution of C₄ photosynthesis in Gomphrena and allies, which was previously analysed in the whole Amaranthaceae s.str. by Sage & al. (2007). The authors determined carbon isotope data for three quarters of the species of Amaranthaceae among which were also most species of Gomphrena from the Americas and Australia. However, Sage & al. (2007) only included five Gomphrena species to map the evolution of C₄ photosynthesis on the tree of Amaranthaceae and revealed a common origin of C₄ in species of Gomphrena belonging to “clade a” alongside with Philoxerus, Guilleminnea and Froelichia. This result was confirmed by Bena & al. (2017) who tested if the evolution of the C₄ pathway correlated with changes in macroclimatic niches and found that C₄ Gomphrenoideae specialized to dryer regions compared to their C₃ relatives and then expanded into colder environments. That is consistent with the current distribution of C₄ species of Gomphrena growing at high elevations of the Andes in Argentina and Bolivia (Sage & al. 2007; Borsch & al. 2015b). Here we use a representative sampling of C₃ and C₄ species of Gomphrena and allies, to more accurately reconstruct the evolution of photosynthetic pathways.

The disjunct distribution of Gomphrena between the Americas (majority of species) and Australia and the hypothesis that Australian species (e.g. G. flaccida) could be closely related to segregate genera with a Caribbean (Lithophila) and neotropical to Pacific distribution (Philoxerus) underscores the need to consider intercontinental plant migration between South America and Australia to understand the diversification of Gomphrena and allies. South American-Australian disjunct distribution patterns can be explained by two main hypotheses. The first is vicariance resulting from Gondwanan land connections up to terrestrial “Austral-Antarctic” migration routes during the Palaeocene-Eocene thermal maximum (Barker & al. 2007a; Pennington & Dick 2004; Upchurch 2008). The second is long-distance dispersal (LDD) between remote continental land masses, for which suitable means of dispersal such as winds or sea currents must be present (Cook & Crisp 2005; Barker & al. 2007b). Using data from published phylogenetic and biogeographic analyses, Sammartín & al. (2007) tested if directional dispersal can explain diversity among Southern Hemisphere plant groups. The authors could not detect any significant pattern, which, however, has to be viewed in light of the then available dated phylogenies. The clade of Gomphrena and allies therefore also offers an interesting case to illuminate South American-Australian biogeographic relationships.
Our goal is to better understand the evolutionary history of Gomphrena and allies and to develop a modern phylogeny-based taxonomic treatment for this group of plants. Therefore, this investigation has the specific objectives to (i) analyse the phylogenetic relationships of Gomphrena and closely allied genera Gossypianthus, Guilleminea, Lithophila and Philoxerus; to (ii) assess the variation of morphological characters and reconstruct their evolution, in particular of those characters that have been used or potentially are diagnostic to delimit these genera; to (iii) clarify overall phylogenetic relationships within Gomphrena s.str. using extended matK-trnK and ITS datasets; to (iv) determine the phylogenetic position and divergence time of the disjunct Australian taxa of Gomphrena. Moreover, our aim was to (v) illuminate the evolution of C₄ photosynthesis on the basis of our new phylogenetic results and to test in how far a realigned genus Gomphrena is characterized by C₄ photosynthesis.

Material and methods
Taxon sampling and composition of datasets
We used four datasets to complete the objectives of this investigation. The first dataset comprises major entities of Gomphrena (12 species) and allied genera (Lithophila, Gossypianthus, Guilleminea, Lithophila and Philoxerus) and also covers the other lineages of the gomphreneoid clade sensu Sánchez-del Pino & al. (2009) such as Xerosiphon, Froelichia, Hebanthe and Pfajia. Pedersenia as a representative of the Alternantheroid clade served as outgroup. We selected 27 taxa to generate a dataset of plastid regions (rpl16 intron, matK gene and trnK intron as well as the trnL intron and the trnL-F spacer; dataset A) and a corresponding matrix of morphological characters. In most cases, molecular and morphological character data were obtained from the same individuals. For Froelichia, Hebanthe, Lithophila, Philoxerus, and Xerosiphon and some species of Gomphrena s.str. (G. boliviana, G. haenkeana, G. pulchella) the trnL-F and rpl16 data came from Sánchez-del Pino & al. (2009). In two exceptions, data from two closely related taxa were concatenated to represent the respective lineages: Pfajia fruticulosa (matK-trnK, morphology, this study), and P. tuberosa (rpl16 and trnL-F from Sánchez-del Pino & al. 2009), as well as Gomphrena mandonii (matK-trnK, morphology, this study), and G. elegans (rpl16 and trnL-F from Sánchez-del Pino & al. 2009).

The second dataset includes a much higher number of samples (80 for plastid matK-trnK = dataset B-1 and 82 for ndITS = dataset B-2) with the aim to illuminate the overall tree space of the “Gomphrena clade” (= 45 taxa of Gomphrena s.str. including Gossypianthus, Lithophila, and Philoxerus, plus Guilleminea). Sampling was guided by morphological diversity, the sectional classification recognized so far (Holzhammer 1956) and the distribution of species in different biogeographical regions of the Americas and Australia. Since species limits in many cases are not yet well understood, plastid and nuclear sequences were obtained from the same individuals. In addition, some previously published matK-trnK sequences (e.g. Müller & Borsch 2005) were used for some species (Gomphrena ferruginea, G. jucipellita, G. pulchella, Guilleminea densa, Philoxerus vermicularis). Voucher information and EMBL/GenBank accession numbers are provided in Appendix 1.

A third, extended matK-trnK dataset (dataset C) of the Amaranthaceae–Chenopodiaceae alliance was used for molecular clock dating in order to accommodate fossil calibration points. The sequence matrix employed the same representatives as in Di Vincenzo & al. (2018) for Chenopodiaceae, other Caryophyllales and eudicot lineages. For the Amaranthaceae, the representation of the achyranthoids was reduced here whereas Gomphrenoeae were sampled as in dataset B-1 of this investigation with some terminals belonging to the same species not included.

A fourth dataset (D-1 for matK-trnK and D-2 for ITS) was created by extending datasets B to include the further C₄ genera of Gomphrenoeae (species of Alternanthera and Tidestromia) into the ancestral character reconstruction of photosynthesis types. The sampling followed Borsch & al. (2018), from which most of the sequences were taken, with additions in Alternanthera from Sage & al. (2007) and Sánchez-del Pino & al. (2012). The ITS sequence of Pseudoplantago was newly generated. Chamissoa was used to root the trees as it is an early branching lineage (Müller & Borsch 2005) with Pitolitus and Pandia representing the aervoid and achyranthoid clades of the Amaranthoideae, respectively. However, the greater distance in particular of the sequences from Alternanthera and Tidestromia led to unalignability in two hotspots (which therefore needed to be excluded); thus this dataset is inferior for calculating precise relationships within Gomphrena as it has fewer characters.

DNA isolation and sequencing
Genomic DNA was isolated from silica-gel-dried leaf tissue or herbarium specimens using a triple CTAB extraction method (Borsch & al. 2003). The matK-trnK, trnL-F and rpl16 regions were selected because of their high phylogenetic structure (Borsch & Quandt 2009; Korotkova & al. 2011) and to achieve consistency with other Amaranthaceae datasets (Müller & Borsch 2005; Di Vincenzo & al. 2018; Borsch & al. 2018). Primers were used as in Borsch & al. (2018) to amplify two overlapping halves of the matK-trnK region, whereas DNAs isolated from herbarium specimens often required to amplify even shorter fragments. Also the additional Gomphrena-specific primers ACmatK442F (5′-AGTCAAAAAGAGCGATTTGGG), ACmatK602F (5′-CTTGGTTTTGACTGTA TCGC-3′), AC-
Maximum Parsimony (MP) analyses were performed as forward sequencing primer to complement the pherograms made with ACmatK1400R that could not read over a large microsatellite located in the trnK intron in several species of *Gomphrena* about 90 nt upstream of the matK start codon. The rpl16 intron was amplified with flanking primers rpl16-1216F and rpl16-18R that were also used for sequencing along with the additional internal forward primer GOMrpl16-495F (Borsch & al. 2018). The trnL-F region was either amplified as a whole using primers trnTc and trnTf (Taberlet & al. 1991) or in two parts when DNA was isolated from herbarium material with primers trnTc and trnTd as well as tmL460F (Worberg & al. 2007) and trnTf. Primers trnTc and tmL460F were used for sequencing.

The ITS region was amplified and sequenced with the universal primers ITS4 and ITS5 (White & al. 1990). For some difficult samples from herbarium specimens, the new internal primers ACITS3F (5′-CGGGATTCTGC-3′) and AC-ITS2R (5′-GATGGTTCAACAAAAGTAAAA TAGAGG-3′) and 10 random addition cycles. The command files were generated in PAUP with 10,000 replicates, using a TBR branch swapping algorithm with 56.788% of characters deleted and one tree held during each replicate, following Müller (2005b).

The substitution models for the individual data partitions were determined with jModelTest 2 (Darriba & al. 2012) and using the Akaike information criterion (Akaike 1974). The best-fitting model was TVM+Γ for trnL-F and rpl16 and GTR+Γ for matK-trnK (datasets A and B-1). The best-fitting substitution model found for ITS was SYM+Γ.

Bayesian inference (BI) was carried out using MrBayes v. 3.2 (Ronquist & al. 2012) using the specifications from the best fitting models. A sampling frequency of 1000 was applied with the first 25% discarded as burn-in, four independent runs were performed with 4 chains each and 10 million MCMC generations. The convergence and effective sample size (ESS) of each replicate were checked using Tracer v. 1.5.0 (Rambaut & al. 2013). The remaining population of trees was summarized as majority rule consensus tree also using MrBayes 3.2.

Maximum Likelihood (ML) analysis was performed using RAxML GUI 1.3 v. 7.2.8 with 1000 bootstrap replicates Stamatakis (2006). Searches were performed using the general time-reversible (GTR) model with among-site rate heterogeneity modelled by a GAMMA distribution with 25 rate categories based on the available model choice in RAxML.

### Assessment of morphological characters and ancestral character state reconstruction

A set of 21 morphological characters was defined covering vegetative, inflorescence and floral morphology. Therefore, characters used in previous studies of *Amaranthaceae* (e.g. Holzhammer 1955; Pedersen 1976, 1990, 1999; Eliasson 1988; Borsch & Pedersen 1997) were re-analysed and also new features and compared with the observed variation in the plant specimens in order to arrive at clear definitions of characters and their states reflecting hypotheses of homology (see Appendix 2). Also new characters on vegetative and inflorescences morphology were assessed. The selection of characters also considered their previous use as diagnostic features for the various generic concepts that were applied to *Gomphrena* and allies. Since the purpose of this investigation was not species delimitation, we did not include the usually much more variable characters with often many states (e.g. shape or texture of sepals) or quantitative data (e.g. length of sepals). These will be dealt with in future studies.

The 21 morphological characters were scored for the same species as in the 27-taxon combined plastid dataset based on herbarium specimens corresponding to the sam-
ples used for molecular analyses. To depict the ancestral states we used the maximum clade credibility tree from the Bayesian analysis, which was identical in topology to the Bayesian Majority rule consensus and the best ML trees. Ancestral states were reconstructed using BayesTraits, v. 2.0 (Pagel & al. 2004) sampling 1000 randomly selected trees after the burn-in. Commands for BayesTraits were generated by TreeGraph2 (Stöver & Müller 2010). Ancestral state probabilities were imported into TreeGraph2 to simultaneously visualize them on the branches.

**Molecular clock dating**

Dating was carried out with BEAST v. 1.8.0 (Drummond & al. 2012) using the broad Amaranthaceae—Chenopodiaceae matK-trnK dataset C. Because no fossils for the Gomphrenoideae are known, the same three fossil calibration points used by Di Vincenzo & al. (2018) in Chenopodiaceae were also employed here. A maximum age of 125 Ma was assumed which corresponds to the most likely age of the crown group of core eudicots (Bell & al. 2010). Age distribution priors for fossil primary calibration points were set as “exponential” (Ho & Philips 2009), whereas the three secondary calibration points (using age estimates of Bell & al. 2010) were included with “normal” age distributions equal to the 95% highest posterior densities interval (HPD) of Bell & al. (2010). Thus, our priors and calibration points for the dating of the gomphrenoid clade in Amaranthaceae were equal to the dating of Amaranthaceae with a focus on the achyranthoid clade as recently carried out by Di Vincenzo & al. (2018). A birth-death model was employed to model lineage diversification, using a random starting tree. Trees were sampled every 1000th generation after a burn in of 50%, calculating a total of 50 million generations for two MCMC runs. Adequate parameter sampling was checked with Tracer v. 1.4.0 (Rambaut & Drummond 2007). The combined post-burn-in tree distribution of both runs was then summarized as a maximum clade credibility tree using TreeAnnotator v. 1.8.0 (Drummond & al. 2012).

**Evolution of C₄ photosynthesis**

To assess the type of the photosynthetic pathway, δ¹³C values were taken from Sage & al. (2007). Many of the specimens studied there are also included into our phylogenetic analysis. Some additional specimens (out of the mostly Andean clade of Gomphrena) were examined for Kranz anatomy, which all were close relatives of taxa previously studied for isotopes. Presence of C₄ versus C₃ was then coded as a binary character. Ancestral states were reconstructed as reported for the other morphological characters. An analysis of ancestral states was implemented both for the plastid and the ITS trees to account for the incongruence in the position of the G. mollis—G. rupestris clade using datasets D-1 and D-2.

**Generation of the distribution map**

The distribution map of the coastal species (Philoxerus portulacoides and P. verrucularis) was produced in ArcGIS 10.3, based on the data obtained from the label of herbarium specimens reviewed in the National Herbarium of Bolivia (LPB), the Instituto Darwinion (SI) in Buenos Aires, and Berlin (B). In addition, the data entries from the online registers of Tropicos-MOBOT (MO), Jardim Botânico do Rio de Janeiro (JABOT) specimens from Brazil; and the African Plant Database (https://www.ville-ge.ch/musinfo/bd/cjb/africa/recherche.php) were considered. The georeferenced data was used in “DECIMAL DEGREES” format and all coordinates were verified with Google Earth. To assess the distribution in Australia occurrence data were obtained by querying the GBIF Portal (GBIF 2016).

**Results**

**Characteristics of the sequence datasets**

Statistics of the multiple sequence alignments for the 27 samples of trnL-F, rpl16, matK-trnK, the individual and consensus information are in Table 1. The length of the alignment of 27 samples of the regions trnL-F, rpl16, matK-trnK (dataset A) had 1082, 1150, 2499 characters, respectively, with eight hotspots (HS) of unclear homology excluded (most being poly A/T stretches). The number of parsimony informative characters were 91 in trnL-F, 118 in rpl16, and 178 in matK-trnK. The alignment of matK-trnK in the larger dataset B-1 had 2510 characters including 45 indels, excluding three HS. The ITS alignment (dataset B-2) had 722 characters including 81 indels, of which 269 were parsimony informative (See Table 1).

**Molecular phylogenetic trees**

The combined plastid tree (matK-trnK, trnL-F, rpl16; Fig. 1) depicts a basal split into a clade of Pafejia and allies on one hand and Gomphrena s.str. and relatives on the other. Almost all nodes receive maximum support under MP, BI and ML. Several lineages were identified within a major clade that is here called “Gomphrena clade”: the first two branches are a Gomphrena prostrata–Guilleminia clade and a Gomphrena mollis–G. rupestris clade whereas all other lineages form the “core C₄ Gomphrena clade” (Fig. 1–3). Within the latter, a clade comprising G. meyeniana sister to G. radiata plus G. tomentosa, Gossypianthus and Gomphrena boliviana; a clade of Lithophila, Philoxerus and G. flaccida from Australia; a clade of G. haenkeana and G. celosioides; and a clade with G. agrestis, G. lanigera and G. pulchella were found. Xerostiphon and Froelichia are successive sisters to the “Gomphrena clade”.

The trees inferred from the extended matK-trnK dataset B-1 (Fig. 2) show the same principal lineages but several of them are revealed with more diverse crown
groups. These are a central and southern Andean dry Puna and Prepuna Gomphrena clade, and a G. boliviana–G. martiana clade. All species sampled from Australia are resolved together with G. flaccida in a Philoxerus + Australian Gomphrena clade. The clade with G. agrestis and G. lanigera from the Cerrado of Bolivia and Brazil, G. pulchella ranging from the Cerrado to the Chaco is extended by G. cardenasii, an endemic from the Cerrado-Chiquitania of Bolivia and thus comprises species from the lowlands of southeastern South America. The most noteworthy result is that G. haenkeana represents a species-rich Andean clade with two well supported subclades (A and B), whereas the Mexican G. nitida appears unresolved to them (Fig. 2).

The ITS data (Fig. 3) recover the same principal clades and also largely the same topology. Nevertheless, the position of the Gomphrena mollis–G. rupestris clade is incongruent in the ITS trees, where it diverges before the G. prostrata–Guilleminea clade. The Australian species of Gomphrena clade are also retrieved with high support as close relatives to Philoxerus and Lithophila (Fig. 3).

### Morphological characters
Six characters describe the habit and vegetative morphology in Gomphrena and allies as defined in Appendix 2. Character 1 (life cycle) is a complex trait, which is relevant because of many species being annuals. Characters 7 to 10 relate to inflorescences. The paracladia in Gomphrena

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**Table 1. Sequence statistics.** Taxon datasets: A: 27 taxa combined matK-trnK+rpl16+trnL-F; B: 80 sequences of matK-trnK of Gomphrena and relatives; C: 82 sequences of ITS of Gomphrena and relatives; D: matK-trnK of Amaranthaceae–Chenopodiaceae with representation of Gomphrena for molecular-clock dating.

<table>
<thead>
<tr>
<th>Dataset A</th>
<th>alignment</th>
<th>HS positions</th>
<th>sequence chars</th>
<th>indel chars</th>
<th>variable</th>
<th>informative</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpl16</td>
<td>1284</td>
<td>477–517, 576–591, 1015–1024, 1065–1104**</td>
<td>1150</td>
<td>54</td>
<td>249</td>
<td>117</td>
</tr>
</tbody>
</table>


| Dataset B-2 | ITS | 647**** | – | 647 | 87 | 394 | 270 |


* The matK-trnK dataset lacks the first 14 nucleotides in the trnK-5′ intron and the downstream 67 nucleotides of the trnK-3′ intron were trimmed because the sequences were incomplete at this end in most samples; those samples that were amplified and sequenced with primer psbA-5R at a later stage of the study are longer and also include the trnK-3′ exon and the trnK-psbA spacer (submitted to ENA but included into the matrix). Two inversions are found in the matK CDS (AC0105 alignment pos. 1054–1062 and AC0051, AC0465, AC0931 and AC1214 alignment pos. 1421–1423).

** The first 12 nucleotides of the trnL intron are not included into the matrix.

*** The first 27 nucleotides of the rpl16 dataset are not included into the matrix; the sequences are trimmed at the end of the matrix shortly before the rpl16-3′ exon but in some samples the sequences extend some 300 nucleotides further due to different primers used for PCR and sequencing (submitted to ENA but not included into the matrix).

**** In ITS, the flanking 18S and 26S are trimmed from the alignment and matrix but included in the sequences submitted to ENA.
(Fig. 4) and allies are complex structures that resemble different levels of expansion of a complex synflorescence architecture, including the reduction of internodes, and a specific whorl-like arrangement of the paracladia (character 8, state 3; Fig. 4). In particular, character 9 (apical leaves subtending paracladia) is grouped into different states that resemble different forms of transition of cauline-like to very specialized pseudanthial leaves (state 2). Characters 12 and 13 refer to floral morphology, whereas characters 14 to 21 describe the variation of the androecium in detail (Appendix 2). The androecia of all 27 species included in dataset A are also illustrated schematically in Fig. 6. The matrix of morphological characters and their states is found in Appendix S7.

Evolution of morphological characters

Ancestral state probabilities for vegetative characters are depicted as pie charts (chars 1–6, 8, 9, 12, 13 are illustrated in Fig. 5; characters 7 and 10 see Appendix S8). Note that heteromorphic sepal shapes with the inner two smaller than the outer and strongly compressed in fruit (character 12, state 1 in grey) is a synapomorphy for the core C, Gomphrena clade. *Pseudantha* (in Fig. 5 character 8, state 3 in blue for the stellately arranged paracladia; character 9 state 2 in green for the specialized leaves) is derived three times, in *G. meyeniana*, *G. boliviana* and *G. haenkeana*. The evolution of the androecium (characters 14, 16, 17, 19; for chars 15, 18, 20, 21 see Appendix S9) is shown in Fig. 6. The size of the androecial tube varies considerably. Long androecial tubes appear in two lineages and are associated with showy, often colourful inflorescences. Staminal tube appendages alternating with the filaments only appear in *Pedersenia* (alternantheroids) and *Froelichia* but otherwise are completely lacking in the gomphrenoid clade (Fig. 6).

Diversification of *Gomphrena* and allies in time

The age of the crown group corresponding to the monophyletic subfamily Gomphrenoideae was inferred to be 30.9 Ma (Fig. 7; 21.16–43.22 95% HPD, node number 14 in Appendix S10), whereas the *Gomphrena* clade (see Fig. 1, 2) (15.1 Ma, 8.92–23.6 95% HPD, node 21) and the less inclusive core C, Gomphrena clade (node 23; 11.4 Ma, 6.69–18.78 95% HPD) are much younger. The Australian subclade has a stem age of 10 Ma (5.7–16.9 95% HPD) and thus started to diversify at a similar time to the speciose Andean subclades A and B (Fig. 1, 3, 7).

Evolution of C4 photosynthesis

The reconstruction of ancestral states using the Bayesian trees inferred from the extended datasets D-1 (for *matK-trnK*) and D-2 (for ITS) is depicted for all nodes in Appendices S11 and S12, respectively. The plastid trees show C4 photosynthesis to have originated in the common ancestor of the Gomphrena clade plus Froelichia, and two more times independently in a sublineage of *Alternanthera* and in *Tidestromia* (Fig. 7). The plastid topology also suggests a reversal from C3 to C1 (Fig. 7). To the contrary, the nuclear ITS tree, that resolves the *G. mollis*–*G. rupestris* clade in a different position not as second but as first branch of the Gomphrena clade (Fig. 3), suggests a single gain of C4 photosynthesis in Gomphrena and allies (Appendix S12).

Discussion

Phylogeny of the Gomphrena clade and relationships of Lithophila, Gossypianthus, Guilleminea and Philoxerus

All plastid trees converge on a deep split into a lineage comprising *Hebanthe*, *Pfaffia* and allies (the *Pfaffia* clade) and a lineage including *Gomphrena* and allies, with *Xerosophion* and *Froelichia* branching as successive sisters to the remaining taxa (Fig. 1, 2). The deep nodes received maximum support under MP, BI, and ML in *matK-trnK* alone (Fig. 2) as well as plastid regions combined (Fig. 1). The same topology for the principle lineages of the gomphrenoids as one of the tree major clades of the monophyletic subfamily Gomphrenoideae (in addition to alternantheroids and iresinoids) was found by Sánchez-del Pino & al. (2009) based on *trnL-F* and *rpl16* sequences alone. The trees recovered from nrITS show the same branching order of Froelichia and Xerosophion (Fig. 3) although support of the respective nodes is less. The clade that includes the majority of Gomphrena species in addition to Xerosophion and Froelichia depicts Guilleminea, Gossypianthus, Philoxerus and Lithophila nested among other species of Gomphrena (Fig. 1–3). This major clade is characterized by metareticulate pollen with the tectum reduced to distal bands (see also Borsch & al. 2018 for a detailed analysis of pollen evolution where this clade is represented by Froelichia and Gomphrena lanigera). The clade of Gomphrena including Guilleminea, Gossypianthus, Lithophila and Philoxerus is here annotated as the “Gomphrena clade” (Fig. 1–3) and receives maximum support in all analyses both from plastid and nrITS.

The taxon sampling of the Gomphrena clade in this investigation is several times higher and now includes a representative sampling of Gomphrena compared to the only 13 species in Sánchez-del Pino & al. (2009). Our trees inferred from plastid and nrITS sequence data congruently reveal ten lineages within the Gomphrena clade (see Fig. 1–3). The earliest diverging lineages are a Gomphrena prostrata–Guilleminea clade and a Gomphrena mollis–*G. rupestris* clade. These are inferred as successive sisters from the plastid data, although the combined analysis of *trnK-matK+rpl16-trnL-F* sequence data shows only moderate support for the second-branching position of the *G. mollis–G. rupestris* clade under MP
(71% JK) and ML (89% BS; Fig. 1). The signal for this topology of the trnK-matK partition alone (Fig. 2) is much stronger. The nrITS partition depicts the G. mollis–G. rupestris clade in a switched position, branching before the G. prostrata–Guilleminnea clade. Further analysis with a spectrum of loci from nuclear and organellar genomic partitions will be needed to test if this is hard incongruence, being the result of a reticulate evolutionary event.

The relationship of Guilleminnea and Gomphrena prostrata receives maximum support in all plastid trees, whereas nrITS is less conclusive (0.89 PP, 80% JK, 69% BS). Guilleminnea densa has a procumbent habit and is characterized by perigonous flowers in terminal racemidium, with the androecium united in the sepal, and the five sepal of similar size, like the other species in the genus (Eliasson 1988, Pedersen 1990). Whereas most of the six species occur in the Chaco, Puna, and Andean dry valleys of Bolivia, Peru, Argentina and Paraguay (365 to 5000 m), G. densa ranges throughout the neotropics to the southern United States, and can be found as an introduced plant in Africa. Gomphrena prostrata has procumbent to ascending stems, flowers in terminal racemidium, a largely fused androecial tube, the anthers attached between two small filament appendages, and five sepal of similar size, being densely pubescent in the dorsal part. The plant occurs in Caatinga and Cerrado habitats of Brazil, where it is adapted to sandy soils. Gomphrena mollis and G. rupestris have detrital trichomes in the leaves and stems, while the rest of species in the Gomphrena clade have uniseriate and linear trichomes. The genera Philoxerus and Lithophila are deeply nested within the Gomphrena clade and appear closely related to the Australian species of Gomphrena in a well-supported clade in all analyses (Fig. 1–3).

Evolution of vegetative morphology in Gomphrena and allied genera

Vegetative characters tend to be homoplastic rather than exhibiting synapomorphies for major clades (Fig. 5). The annual life cycle (character 1, state 0) thereby was derived multiple times from perennial ancestors and is depicted as an independent transition in four lineages of the core C. Gomphrena clade, so in G. haenkeana, G. flaccida, G. radiata, G. boliviana, but also in Froelichia floridanca. The annual life form therefore is mostly associated with plants occurring in dry (G. flaccida, G. radiata) or seasonally dry environments (G. haenkeana) indicating the adaptive nature with plants only appearing in the wet season. Multiple origins of annuals have also been observed in other lineages of angiosperms radiating in areas with specific geographical patterns of dry environments such as Nemesis Vent. (Scrophulariaceae; Datson & al. 2008). The evolution of root types shows a similar homoplastic adaptive pattern, linked to different survival strategies in the different environments where species grow. For example, G. meyeniana is distributed along the Andes (1890–4800 m) and presents taproots that represent 82% of its total biomass (Patty & al. 2010), whereas aerial parts can be largely lost during the dry season. These features occur in many unrelated plant lineages and are an adaptation to high mountain ecosystems (Körner 2003). In a similar way,
taproots of *Guilleminea* or *G. tomentosa* and relatives are tuberose as a drought adaptation strategy. Whereas taproots originated in the ancestor of the *Gomphrena* clade, this state got lost again with shifts to annual life forms (e.g. *G. radiata*). The taproots of Cerrado species like *G. pulchella* or *G. lanigera* have been observed to store of fructanose (Fank-de-Carvalho & al. 2015), which is a further response to the environmental stress of the dry season and to fires where the soil temperature can raise to > 70°C. The adventitious roots of *Philoxerus* (character 2, state 2) evolved independently, and allow the plants to spread in the wet sand of coastal habitats. Roots of this species show tolerance to salinity (Bove et al. 2011), and Cordazzo and Seeliger (2003) have proven the regeneration capacity of *P. portulacoides* from fragments of the adventitious root.

Basal leaves have evolved independently three times in different lineages of the *Gomphrena* clade. We coded two different states regarding to their different morphology in plants with prostrate versus erect stems. *Gomphrena tomentosa* and relatives as well as *Guilleminea* have prostrate or decumbent stems, but a few leaves develop from the reduced main axis. On the other hand, species such as *G. lanigera*, *G. agrestis* and *G. flaccida* have erect stems which are hardly branched, but develop a radical rosette of long-lived leathery leaves, often with a dense indumentum.

Ancestral character state reconstruction shows that procumbent and decumbent stems are derived from erect ones, and evolved two times independently within the core C4 *Gomphrena* clade (in the Lithophila+*Philoxerus* clade; and in the ancestor of *G. tomentosa*, *G. meyeniana* plus *Gossypianthus*) and a third time in the ancestor of *Gomphrena prostrata* and *Guilleminea* (Fig. 5). In the latter two clades plants grow in dry and hot environments where procumbent and decumbent stems protect them from wind, and reduce evapotranspiration. This corresponds to the evolution of radical leaves. In *Guilleminea* they are small and lost when the plants grow (Mears 1967), whereas in *Gossypianthus* they are persistent and more prominent, a similar quality also present in *Gomphrena tomentosa*, *G. radiata* and

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Fig. 2. Extended phylogeny of *Gomphrena* based on matK-trnK sequence data including indels (dataset B-1). The Bayesian topology is shown as phylogram. Support values shown above branches are posterior probabilities; below are JK percentages from parsimony (left) and BS percentages from likelihood reconstruction (right). Major lineages are annotated as well as the geographic origin (country, department) of the respective samples. Note that there is a diverse clade that is mostly constituted by Andean species.
where it seems to be associated with plants possessing a tertiary branch also occur in perennial or annual herbs, whereas elongate to cylindric paracladia with remote subtending leaf organs (character 9, state 1; Fig. 5). The ancestral inflorescence in Gomphrenoideae (Fig. 5). Nevertheless, stems with secondary and tertiary branches also occur in perennial or annual herbeaceous plants. In the latter this is a strategy to produce numerous axes that can quickly develop flowers and thus foster reproduction. Those species that only have unbranched stems generally possess a paracladia or at or near the terminal or node of the stem. This feature (state 0) has evolved independently several times (in *Xerosiphon aphyllus*, *Froelichia floridana*, *Gomphrena flaccida*, *G. mollis*, *G. agrestis* and *G. meyeniana*). Among the species of *Gomphrena*, *G. tomentosa* presents indeterminate growth, and as well *Gossypianthus* and *Guilleninea*. According to our ancestral character state reconstruction, this feature (character 6, state 0) in only a few cases, where it seems to be associated with plants possessing a tap root and procumbent stems.

**Evolution of inflorescence and floral morphology, especially the androecium in *Gomphrena* and allied genera**

The ancestral inflorescence in *Gomphrenoideae* was a globose to subglobose paracladia with densely adjacent solitary flowers (character 7, state 0) appearing terminal on a more or less elongated axis, without any specialized subtending leaf organs (character 9, state 1; Fig. 5). The head-like shape was maintained in the *Gomphrena* clade whereas elongate to cylindric paracladia with remote flowers were independently derived in *Hebanthe* and *Froelichia* (not illustrated). The arrangement of paracladia into more complex synflorescences (character 8; partial synflorescences) is variable in *Gomphrenoideae* (Acosta & al. 2009) and also considerably differs in *Gomphrena* and allies depending on the branching system of more or less complex synflorescences. Because no detailed insights on the development or anatomy of inflorescences in *Amaranthaceae* are available that would support further hypotheses on homology, we coded the different arrangements of paracladia as unordered multiple

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**Fig. 3.** Extended phylogeny of *Gomphrena* based on nITS including indels (dataset B-2). The Bayesian topology is shown as phylogram. Support values shown above branches are posterior probabilities; below are JK percentages from parsimony (left) and BS percentages from likelihood reconstruction (right). Major lineages are annotated as well as the geographic origin (country, department) of the respective samples.
Fig. 4. Morphological diversity in flowers and inflorescences of *Gomphrena* and allies. – A: inflorescence of *Gomphrena* spec. 7 with less prominently visible paracladia but bright yellow pseudanthial leaves (Ortuño & al. 1677); B: *Gomphrena meyeniana* with pseudanthial leaves from lower surface (Borsch & Ortuño 3561); C: inflorescence of *G. fuscipellita* with six paracladia supported by broadly rounded, green pseudanthial leaves (Borsch & Ortuño 3594); D: *Gomphrena haenkeana* showing long, pink sepals and androecial tubes with reflexed pale yellow filament appendages, whereas stamens are dark yellow (Borsch & Ortuño 3627); E: *Philoxerus vermicularis* without pseudanthial leaves in the terminal inflorescence and two opposite leaf-like ones in the lateral inflorescence, and a pair of succulent cauline leaves (Borsch & al. 3444); F: *Lithophila muscoides*, part of a cushion-like plant in a limestone crevice (Greuter & al. 26915); G: Terminal part of flowering stem of *Guilleminea densa* (Borsch & al. 3437). – Photo credits: A: T. Ortuño; B–G: Th. Borsch.
Fig. 5. Evolution of vegetative (characters 1 to 6), inflorescence (characters 8 and 9) and floral morphology (characters 12 and 13) in Gomphrena and relatives (see Appendix 2 for the definition of characters and their states as well as the full matrix in Appendix S7). The pie charts represent probabilities for ancestral states as reconstructed with BayesTraits and depicted on the maximum clade credibility tree obtained by Bayesian inference of the combined plastid dataset. Pie charts were omitted for some deep nodes with minor changes and for characters 7 and 10 where changes appeared not to be relevant for L. Brockington & al. 2009). Gomphrena pulchella represents a further specialization (autapomorphy, character 9, state 3), where an increased number of up to 10 leaves subtend a terminal solitary paracladium. The flowers in this species are the largest in Gomphrenoideae (>45 mm in length), and also androecial tube is very long (Fig. 6), leading to very conspicuous flower heads. These specialized leaves could also serve as a protection against fire before anthesis and during fruit development in G. pulchella which is a typical Chaco species (Pedersen 1976). This pattern of inflorescence evolution indicates that there is a trend toward the formation of pseudanthia in the core C_4 Gomphrena clade. Compared to several other families of flowering plants such as Apiaceae, Asteraceae, Euphorbiaceae (their cyathium), or Rubiaceae, the formation of pseudanthia in Anaranthaceae so far did not receive much attention. Classen-Bockhoff (1990) just mentioned their existence in Gomphrena and Pililotus.

Gomphrena and allies as all Caryophyllales possess five perianth parts with opposite stamens, located in individual primordia in quincuncial order independently of the stamens (Vrijdaghs & al. 2014). Their arrangement results in two external, one intermediate, and two inner sepals, the latter of which can vary strongly in size and texture (Character 12). The state with the inner two sepals smaller than the outer two, which are cymbiform, compressed and carinate in fruit is resolved as synapomorphy for the core C_4 Gomphrena clade (Fig. 5). The G. mollis-–G. rupestris clade, which also exhibits smaller inner sepals differs in that these inner sepals do not become thickened at maturity (state 2), which could represent a transitional state if the G. mollis–G. rupestris clade is assumed to be the sister of the core C_4 Gomphrena clade. Further analyses using reductive coding of this complex

states (character 8 with 6 states). Whereas all states reach nearly equal probabilities to be ancestral at deeper nodes (Fig. 5), it can be shown that the very specific architecture of a terminal florescence surrounded by usually 5 paracladia in a whorl-like structure (character 8, state 3) evolved two times independently in G. boliviana and G. haenkeana (Fig. 5). In fact, all relatives of G. boliviana (the G. boliviana–G. martiana clade) and of G. haenkeana in the two Andean subclades A and B (see Fig. 2, 3) possess the same states, so that the two species depicted in Fig. 5 are good representatives for their respective clades with a whorl-like arrangement of paracladia. It will be interesting to study the ontogeny of this structure to determine in how far it is the result of a strong condensation of a thyrsoid branching system or also involves collateral duplication of buds developing into paracladia.

This feature is correlated with the presence of pseudanthial leaves that are arranged in a whorl (character 9, state 2), earlier noted by Holzhammer (1956) and Siqueira (1992) as “involucral leaves”. Whereas two opposite, small subtending cauline leaves (state 1) is reconstructed as the ancestral state in the core C_4 Gomphrena clade, whorl-like pseudanthial leaves are derived even three times. Once in the speciose clade of G. haenkeana and relatives to which also G. fascipeltita and G. pallida belong, in the G. boliviana–G. martiana clade, and a third time in G. meyeniana that grows with a short upright stem at high elevations in the Andes (Fig. 4, 5). In the case of G. meyeniana pseudanthial leaves surround a single main florescence with relatively large white flowers appearing in good contrast to the dark pseudanthial leaves. Some populations of G. pallida have even been found to possess yellow-coloured pseudanthial leaves (Fig. 4). Conspicuously coloured leaves also serve as optical attractants in inflorescences of other Caryophyllales lineages such as Bougainvillea Comm. ex Juss. (Nyctaginaceae; Brockington & al. 2009). Gomphrena pulchella represents a further specialization (autapomorphy, character 9, state 3), where an increased number of up to 10 leaves subtend a terminal solitary paracladium. The flowers in this species are the largest in Gomphrenoideae (>45 mm in length), and also androecial tube is very long (Fig. 6), leading to very conspicuous flower heads. These specialized leaves could also serve as a protection against fire before anthesis and during fruit development in G. pulchella which is a typical Chaco species (Pedersen 1976). This pattern of inflorescence evolution indicates that there is a trend toward the formation of pseudanthia in the core C_4 Gomphrena clade. Compared to several other families of flowering plants such as Apiaceae, Asteraceae, Euphorbiaceae (their cyathium), or Rubiaceae, the formation of pseudanthia in Anaranthaceae so far did not receive much attention. Classen-Bockhoff (1990) just mentioned their existence in Gomphrena and Pililotus.
character (Torres-Montúfar & al. 2018) will be interesting but will require detailed anatomical analyses of the tissues. In *Guilleminea* perigynous flowers are derived as the only genus in *Gomphrenoideae* (character 13, state 0).

Differences in the morphology of the androecium were used in the circumscription of *Gomphrena* and other genera of the *Amaranthaceae* (Schinz 1893; Eliasson 1988; Townsend 1993). Nevertheless, the homology of interstaminal and staminal appendages has been a subject of debate. Eliasson (1988) suggested a stepwise character state transition from so-called “pseudostaminodes” alternating with stamens as present in *Alternanthera* and *Pedersenia* (Fig. 6) to “apical filament lobes” present in many species of *Gomphrena* (e.g. *G. haenkeana*), *Pfaffia* and *Xerosiphon* (Fig. 6). Vrijdaghs & al. (2014) investigated the ontogeny of the androecium in *Gomphrenoni-
Fig. 7. Overview on the evolution of *Gomphrena* in time. The tree represents the clade of the *Gomphrenoideae* from the overall divergence time estimation of *Amaranthaceae* and *Chenopodiaceae* calculated with BEAST and 95% HPD intervals as grey bars (see Appendix S10 for the full tree with precise divergence times). Pie charts depict the evolution of C_4 photosynthesis in *Gomphrena*, with the core *Gomphre­noideae* shaded in red. The core *Gomphrenoideae* clade is nested within a representative way and also did not provide the necessary resolution of nodes to make more specific conclusions, but their tree did not sample the genus *Philoxerus*.

The evolution of lateral filament appendages (character 19) shows strong homoplasy. They have arisen twice within the core C_4 *Gomphrena* clade but were lost again in the common ancestor of the *Philoxerus*+Australian *Gomphrena* clade in a similar range than the mostly Andean clade. Moreover, Sánchez-del Pino & al. (2019) concluded that the lateral filament appendages in *Gomphrena* and *Pfaffia* are de novo organs, thus not being homologous to the stamen tube appendages. This is consistent with the observation that the *Amaranthaceae*-*Chenopodiaceae* clade generally has a single whorl of antencespalous stamens without any trace of staminodes (Ronse de Craene 2013). Our reconstruction of ancestral states shows that the presence of androecial tube appendages (character 17, state 0; Fig. 6) is ancestral in *Gomphrenoideae*. They were lost in the gomphrenoid clade but apparently re-gained in *Froelichia*. In line with our results (Fig. 6), Eliasson (1988) illustrated organs like stamen tube appendages in *Froeli­chia*. However, in the absence of any ontogenetic information for *Froelichia*, their homology may be further investigated as we cannot exclude any lateral fusion of de novo lateral stamen appendages that may have occurred together with the evolution of a completely closed androecial tube in that genus. Sánchez-del Pino & al. (2019) annotated the presence of stamen tube appendages on a tree of *Gomphrenoideae* and consistently show their absence in the gomphrenoid clade, indicating their loss after the divergence of *Pseudoplantago*. However, their character coding of *Froelichia* differs from ours. Other major lineages of the *Amaranthaceae* also exhibit similar variation patterns in the androecium such as the eervoid clade (Hammer & al. 2019).
contrary, our results show that the gain of filament appendages (character 19, Fig. 6) in species of *Gomphrena* coincides with either the evolution of large and showy flowers or pseudanthia (e.g. *G. haenkeana*, *G. mollis*; see Fig. 5).

Androecial tubes have extended in length two times during the evolution of *Gomphrena* (character 16, Fig. 6), to the extreme of the up to 30 mm long tubes in *G. pulchella*. Floral evolution in the *G. mollis*–*G. rupes- tris* lineage is therefore convergent to *G. haenkeana*, *G. pulchella* and allies (mostly Andean clade; see Fig. 1). Long androecial tubes also correlate to showy flowers, which appears to indicate a specialized pollination syndrome. However, the pollination biology of *Gomphrena* is not well known. Based on scattered observations, *Gomphrena* and allies seem to be pollinated by insects. During field work for this study, moths were observed visiting flowers of *G. pallida* that introduced their proboscis through the androecium tube, and butterflies were reported to pollinate the Australian *G. splendidia* (Barrett & Palmer 2015). The position, length and shape of filament appendages (characters 20 and 21) is highly homoplastic (Appendix S9). On the other hand, *Lithophila musco- ides* has true staminodes, which result from the reduction of the androecium to two or three functional stamens (Fig. 6; Eliasson 1988). This species was classified as an own genus based on this feature and its specific Caribbean habitat (Swartz 1788) but it is deeply nested within *Gomphrena*.

**Overall phylogenetic relationships and diversification of the core C, *Gomphrena* clade**

The extended taxon sampling based on plastid *matK-trnK* (Fig. 2) and *nrITS* (Fig. 3) sequence data revealed the same principal lineages of *Gomphrena* and allies as in the analysis of combined plastid markers (Fig. 1) but indicates that the majority of the more extensive taxon set belong to a large “mostly Andean clade” (Fig. 2, 3) that receives maximum statistical support in all analyses. In the 27-taxon set this clade is just represented (Fig. 2, 3) that receives maximum statistical support in all analyses. In the 27-taxon set this clade is just represented (Fig. 2, 3) that receives maximum statistical support in all analyses. In the 27-taxon set this clade is just represented (Fig. 2, 3) that receives maximum statistical support in all analyses.

Trees inferred from plastid and nuclear partitions converge in depicting two sublineages (A and B) of a clade of mostly Andean species (Fig. 2, 3), both of which predominately comprise perennial and annual species from dry valleys and Prepuna habitats (from 2500 to 4000 m a.s.l.) of the central Andes, with the exception of *Gomphrena perennis*, which is a morphologically variable species extending from the Chaco and Bosque Tucumano-Boliviano to the Andean dry valleys (Borsch & al. 2015b). *Gomphrena cf. nitida* from Oaxaca in Mexico appears in a relatively isolated position unresolved in a polytomy. However, further individuals representing the central American populations (Borsch 2001) need to be sampled to clarify if the morphologically allied plants from the region are closely related.

Our trees include samples of the annual *Gomphrena phaeotricha* from the South of Bolivia and the North of Argentina, depicted in sublineage A. The species was described by Pedersen (1976) based on differences in floral morphology in comparison to *G. pallida*. Our results agree with Pedersen (1976) by providing molecular evidence that *G. phaeotricha* and *G. pallida* are only distantly related, essentially belonging to two different sublineages A and B. However, the circumscription of both species in the sense of Pedersen (1976) and as currently accepted may not reflect natural entities. Pedersen recognized several infraspecific taxa in *G. pallida* to accommodate some of the morphological variation but at his time did not apply any evolutionary method. Further work is therefore underway on species limits in the mostly Andean clade (Ortuño & Borsch, unpubl. data). One other annual species was already described based on its deviant floral morphology (Ortuño & Borsch 2005), *G. mizqueensis* from dry valleys in the province of Mizque.
(department of Cochabamba). The matK-trnK tree depicts it as closely related to samples of *G. pallida* within subclade B but also the perennial *G. fuscipellita*, a morphologically easily recognizable species described from rocky outcrops with herbaceous vegetation in the same area (Ortuño & Borsch 2005) but apparently extending to the Toro Toro National Park in northern Potosí (T. Ortuño, pers. obs.). Other perennials also belong to subclade B such as *G. oligocephala*, *G. bicolor* and *G. potosiana* according to both ITS and matK-trnK (Fig. 2, 3). On the other hand, subclade B comprises the perennials *G. perennis*, *G. trollii* and the annualls *G. haenkéana* and *G. furruginea* alongside with *G. pheotricha*. Our earlier hypothesis that the Andean annuals could represent a lineage that has adapted to survive the dry season with seeds as diaspores and then radiated in the inner Andean dry valleys (Ortuño & Borsch 2005) can thus not be fully accepted. The annual species of *Gomphrena* grow and flower in March and April after the rains (López 2003). Both Andean sublineages A and B comprise annuals and perennials, indicating multiple shifts in life forms, which in the case of *Gomphrena* could have occurred in conjunction with the origin of dry valleys, resulting in a strongly geographically governed pattern of diversity. The two Andean subclades of *Gomphrena* started to diversify about 2.6 Ma (0.7–7.1 and 0.2–5.0 95% HPD, respectively; Fig. 7, Appendix S10) and the annual lineages appear to be not much older than 1 Ma.

The *Gomphrena meyeniana* clades comprises plants growing at high altitudes that all possess a characteristic and unique morphology with leaves arranged in a rosette that arises from a reduced main axis, flower heads few to solitary on inflorescence axes arising from a stout, almost invisibly short stem, bracteoles without crest and stamens almost completely fused into a staminal tube lacking filament appendages Holzhammer (1955, 1956; see Fig. 4, 6). Our molecular phylogenetic data that depict the *G. meyeniana* clade as a highly supported lineage with a crown group diverging on a long stem (Fig. 2, 3) are in line with this. The latest treatment at species level is by Pedersen (1990), who only recognized *G. meyeniana* with several infraspecific taxa that reflect the morphological and ecological variation within the clade. Our results indicate significant phylogenetic diversity within the *G. meyeniana* clade but strongly diverging ITS ribotypes visible as polymorphic sites in our ITS pherograms within most individuals (Ortuño & Borsch pers. obs.) show that hybridization occurs within this clade that is likely to contribute to the phenotypic variation observed. Nevertheless, all ITS copies found within *G. meyeniana* and allies are clearly different from all other lineages of *Gomphrena*, underscoring that the role of reticulate patterns and incomplete lineage sorting needs to be analysed to delimit species within the clade but will not influence our view on the composition of the *G. meyeniana* clade.

The *Gomphrena radiata–G. umbellata–G. tomentosa* clade appears with maximum support in plastid and nuclear trees (see Fig. 2, 3) and encompasses species adapted to dry habitats. Trees show two subclades, one of which includes all the *G. tomentosa* specimens, whereas the other subclade includes *G. umbellata* and *G. radiata*. The main morphological difference between these subclades is the life form present. *Gomphrena tomentosa* and close relatives are perennials (Fries 1920; Hunziker & Subils 1977), whereas *G. radiata* and *G. umbellata* are annuals (Pedersen 1976). *Gomphrena umbellata* is extremely adapted to sandy places at high elevations (2800 to 4400 m a.s.l.; Borsch & al. 2015b). Its leaves are reduced to the uppermost cauline leaves and the apical leaves subtending paracladia whereas the stems are under the sand. In all species of the *G. radiata–G. umbellata–G. tomentosa* clade the filaments are fused into a tube for their most part lacking lateral filament appendages (Fig. 6). Based on the absence of these appendages Fries (1920) proposed the “*Chnoanthus* group”, later formally described as a section by Holzhammer (1956). Fries (1920) hypothesized that *Gomphrena* comprises a series of species, where the filament appendages were reduced in steps during the evolution of the genus, and *G. tomentosa* appears at the end of this series. Although the evolution of lateral filament appendages is homoplastic (Fig. 6, Appendix S9), the section *Chnoanthus* appears as a natural group. Recently, Bena & al. (2017) generated trnL-F sequences of an individual of each *G. umbellata*, *G. radiata*, *G. cladotrichoides* and *G. mendocina*, the latter two of which are close relatives of *G. tomentosa*. They also recovered these four taxa in a clade, albeit without statistical support of any of the nodes.

All three before mentioned clades within *Gomphrena* diversified at higher elevations in the Andes (Fig. 7). The mostly Andean clade diverged around 8 Ma (4.0–13.6 95% HPD) from lowland ancestors whereas the crown group has an age of just 4.3 Ma (1.8–10.3 HPD). The *G. meyeniana* clade (stem group age 7.8 Ma; 2.9–13.5 HPD) and the *G. radiata–G. tomentosa* clade (stem group age 8.8 Ma; 3.5–15.8 HPD) must have colonized the rising Andes independently. Considering that the central Andes had only about half of its modern elevation by the late Miocene (Gregory-Wodzicki 2000; Garzione & al. 2008; Jiménez & al. 2009) followed by a continuous uplift in the last 10 Ma to reach the modern elevation of the central Andean plateau of ~4 km further exceeded by the Eastern and Western Cordilleras, the dates estimated for the origin of the Andean clades of *Gomphrena* fit well to the geological history. Compared to the radiation of the Andean clade of *Lupinus L.* (*Fabaceae*) with a crown group age of about 1.5 Ma (Hughes & Eastwood 2006) the Andean diversification within *Gomphrena* started somewhat earlier (crown group ages of 2.6 Ma [0.7–7.1 and 0.2–5.0 95% HPD, respectively] for the Andean subclades A and B, and 2 Ma for the *G. meyeniana* clade). The youngest crown group within *Gomphrena* (*G. meyeniana* and allies) exclusively grows above 3000 m (up to 4700 m; Borsch & al. 2015b) whereas members of...
Andean subclades A and B are often range-restricted in dry inner Andean valleys or at elevations >3000 m. This underscores the importance of valley systems with dry climates that originated as a consequence of the Andean uplift (Strecker & al. 2007) in particular in the Eastern Cordillera (Bolivia and northwestern Argentina) and probably fuelled reproductive isolation as well as adaptation to dry habitats. However, better resolved species trees and a more thorough understanding of species limits and their exact geographical distribution will be needed to further illuminate the diversification of Gomphrena in the Andes (e.g. testing if the morphologically variable and widespread *G. perennis* is in fact a single species). Nevertheless, *Gomphrena* is an interesting model to study plant diversification with the uplift of the Andes as it contains at least three independent ascents onto the Andes.

The *Philoxerus*+Australian *Gomphrena* clade comprises all sampled Australian species as well as *Lithophila* and *Philoxerus*, the latter two of which are plants adapted to coastal environments with fleshy leaves (Fig. 4). The species of *Lithophila* and *Philoxerus* appear morphologically similar but *Lithophila* differs in the reduced number of two stamens (*Eliasson 1988, Fig. 6*). The filaments of *Lithophila* and *Philoxerus* are united only basally, forming a cup, and lateral filament appendages are absent. To the contrary, the Australian species have morphologically variable filaments, some with lateral filament appendages (Townsend 1993). Some Australian species differ from the neotropical species by longer stigmas (Palmer 1998), but this variation will be analysed in more detail in a future study. Nevertheless, all the species of the *Philoxerus*+Australian *Gomphrena* clade share the main morphological characteristics of the core *C*, *Gomphrena* clade (Fig. 5, 6) so that it is proposed here to include the two genera into *Gomphrena*.

**An Australian lineage derived recently from South American ancestors**

The crown group of the Australian clade is of very recent origin (3.9 Ma, 1.3–8.6 95% HPD) as is the more inclusive clade comprising *Philoxerus* and *Lithophila* (crown group age of 5.7 Ma [2.0–12.4 95%], Fig. 7 and Appendix S10). Therefore, a Gondwana vicariance hypothesis (Upchurch 2008; Barker & al. 2007a) for the origin of the Australian *Gomphrena* clade can be clearly rejected. Our results further show that this Australian clade is very deeply nested among South American and Caribbean ancestors, underscoring that long distance dispersal is the only plausible explanation for the origin of the Australian *Gomphrena* clade.

In Fig. 8 we present an overview on the global distribution of the core *C*, *Gomphrena* clade (pale yellow signature) as well as *Philoxerus* (dot map for each of the three currently accepted species). *Gomphrena* is not native in Africa (Townsend 1985) and therefore is not recorded for this continent although our two samples of *G. celosioides* are of African origin (Fig. 2, 3). The possible first branch of the *Philoxerus*+Australian *Gomphrena* clade is *Lithophila* (Fig. 2, 3), which is endemic to all Caribbean islands (Acevedo-Rodríguez & Strong 2012; not illustrated), where it grows in limestone crevices at the coast. *Gomphrena* and *Philoxerus* do not occur in the southernmost part of South America, nor on Tasmania or New Zealand, indicating that a trans-Tasmanian dispersal route is very unlikely for *Gomphrena* and relatives. Sanmartín & al. (2007) inferred this as a frequent pattern, apparently facilitated by westward directed circumpolar currents, but more in temperate plant groups.

A striking result of this investigation is to find the Australian *Gomphrena* clade nested among species that inhabit coastal habitats. Their adaptations such as fleshy leaves, resistance to high concentrations of salt and adventitious roots allowing vegetative reproduction from broken off stems (Cordazzo 2007; Cordazzo & Seeliger 2003, T. Borsch pers. obs.) have apparently led to marine dispersal. *Philoxerus (= Blutaparon)* has reached the west coast of tropical Africa (see Fig. 8) but also the islands of Galapagos. *Blutaparon rigidum* is reported as a Galapagos endemic, which has some similarities to *Lithophila* in floral morphology (*Eliasson 1990*) but is a morphologically distinct plant with upright habit. However, it is only known from two historical collections and now extinct because it was covered by the eruption of the volcano on the island of Santiago (*Eliasson 1971*). But Galapagos harbours at least two other species of *Lithophila* (*L. radiata* Standl., *L. subcaposa* Hook. f.). And interestingly, other lineages of *Amaranthaceae* also reached Galapagos, so species of *Alternanthera*, which have a derived position among neotropical ancestors within this monophyletic genus (*Sánchez-del Pino & al. 2012*).

Based on the above, we hypothesize that *Philoxerus (= Blutaparon)* or *Lithophila*-like plants adapted to coastal environments were the ancestors of the Australian *Gomphrena*. They were dispersed to Australia across the Pacific Ocean through marine currents (Fig 8, based on Kasang 2018) such as the North and South Equatorial current, which then leads over into the East Australian current and which were present since about 6 Ma. Interestingly, the dispersal of the ancestor of *Philoxerus wrightii* could be explained with the Kuroshio current (Fig. 8). Grehan (2001) reported Galapagos-Central-America-Caribbean tracks were also reported in other organisms such as isopods (*Nesophilosa, Troglophiloscia*), snakes (*Antilophis*) or beetles (*Ablechrus*) and even Galapagos-Australia tracks in angiosperms (*Nicotiana*), termites (*Insitermes*) and beetles (*Pitinus*). The latter dispersals may have occurred in the late Miocene, which roughly corresponds to the stem age of the *Philoxerus*+Australian *Gomphrena* clade dated 10 Ma (5.7–16.9 95% HPD). A detailed phylogeographic analysis as well as the inclusion of all available specimens of this clade into a well-resolved dated phylogeny will certainly further illuminate this scenario in the future.
C₄ evolution in Gomphrena and allies

Within the subfamily Gomphrenoideae the C₄ photosynthetic pathway was reported from a large number of species of Gomphrena, as well as from several members of Alternanthera and the genera Blutaparon, Froelichia, Guilleminea, Lithopila and Tidestromia (Sage & al. 2007). Our results show that the C₄ species of Gomphrena (Fig. 2, 3, 7) all belong to a single clade. The members of this core Gomphrena clade are characterized by metaterticulate pollen where the tectum is reduced to a distal band (Borsch 1998; Fig. 7). On the other hand, most C₃ species of Gomphrena (e.g. G. elegans, G. mandonii) have pollen with closed tecta similar to Pfaffia. They are resolved in a distant lineage together with the C₃ genera Hebanthe and Pfaffia in both plastid and nrITS trees (Fig. 2, 3, 7 and Appendix S10). The only exception are the two C₃ species G. mollis and G. rupestris that belong to the core Gomphrena clade in which they share the morphology of pollen with reduced tecta (Ortuño & Borsch, unpubl. data).

Ancestral character state reconstruction using the chloroplast trees (Fig. 7 for matK-trnK; MCMC model; the same results are found with the 27-taxon set, not shown) indicates that C₄ photosynthesis arose in the common ancestor of the core Gomphrena clade plus Froelichia but reversed back to C₃ in the clade of G. mollis and G. rupestris. Analyses under a ML model in BayesTraits slightly favour a reversal in the common ancestor of the core C₄ Gomphrena clade plus the G. mollis–G. rupestris clade and a regain of the C₄ syndrome by the ancestor of a core C₄ Gomphrena clade (Appendix S11). However, this would be a much more complicated scenario that therefore seems less probable. To the contrary, the ITS trees favour a single origin of C₄ photosynthesis in a common ancestor of the core C₄ Gomphrena clade plus the Gomphrena prostrata–Guilleminea clade (Appendix S12). The about ten species of the core C₄ Gomphrena clade, for which leaf anatomy has been investigated so far (Carolin 1978; Estelita-Teixeira & Handro 1984; Fank-de-Carvalho & al. 2015), show thick-walled parenchymatous bundle-sheath, and in most cases dimorphic chloroplasts corresponding to an NADP-ME subtype. Similar anatomy was also found in Froelichia (Carolin 1978) and Guilleminea (Filippa & Espinar 1993). This was called “Gomphrena type” (Carolin 1978; Kadereit & al. 2003), and considered to be present in all C₄ Gomphrenoideae. Gomphrena prostrata has thickened and lignified bundle sheath cell walls (Estelita-Teixeira & Handro 1984), but this may be rather seen as an adaptation to xeric environments like white sand habitats. Leaf anatomical characters seem therefore not informative indicating a sole in the whole subfamily, contrary to the strong differentiation of leaf anatomy in Chenopodiaceae.
(Kadereit & al. 2003). It is nevertheless remarkable that a potential C3 reversal coincides with a possibly reticulate origin of the respective reversed C3 lineage (G. mollis–G. rupestris clade). An alternative explanation would be a hybridization of a C3 ancestor with an early branch of the Gomphrena C4 lineage as a maternal parent, so we actually see a captured C2-type chloroplast. Experimental hybrids of C3 and C4 species in Atriplex and Flaveria (see Kadereit & al. 2017 for review) rather showed differential segregation of different characters of photosynthesis types, leading to C2-like offspring. Further biochemical and physiological analysis of G. mollis and G. rupestris would be needed to see if they differ from other C4 Gomphrenoideae. Nevertheless, the δ13C values of G. mollis and G. rupestris (Sage & al. 2007) are not different from those C4 species.

Sage & al. (2007) mapped the taxonomic distribution of C4 photosynthesis using a matK-trnK tree of the Amaranthaceae under parsimony and depicted three C4 clades in subfamily Gomphrenoideae: (1) a clade of Froelichia–Guilleminia–Blataparón–Gomphrena (species with distal tectal bands in their pollen grains), (2) a sublineage of Alternanthera, and (3) Tidestromia. The plesiomorphic condition in the monophyletic genus Alternanthera was later confirmed to be C3 in a much more detailed study by Sánchez-del Pino & al. (2012) whereas C3 was considered to be derived within their clade B3. This clade is represented in our dataset D by A. microphylla and A. pungens, which is congruently inferred as nested within Alternanthera in our analyses (Fig. 7, Appendices S11, S12). However, the position of C4 Tidestromia is inconsistently shown as sister to Pedersenia (with ITS, Appendix S12), in a grade with Alternanthera and Pedersenia as successive sisters (with matK-trnK, Fig. 7) or as sister to Alternanthera (Sánchez-del Pino & al. 2009). This makes a precise dating of the age of Tidestromia as a C4 lineage difficult, but otherwise does not change the picture of three independent C4 origins in the Gomphrenoideae (Sage & al. 2007). Nevertheless, the early C4 evolution of the Gomphrena clad is more complex as evidenced by the improves sampling in this investigation. Amaranthaceae s.str. like the chenopod lineages (Kadereit & al. 2012) therefore exhibits multiple gains and losses of C4.

Recently, Bena & al. (2017) studied macroclimatic niche limits and C4 evolution in Gomphrenoideae. The authors compiled available plastid sequences from GenBank (mostly from Müller & Borsch 2005; Borsch & al. 2012; Sage & al. 2007; Sánchez-del Pino & al. 2009, 2012) and added new trnL-F sequences from ten species. However, they did not obtain any significant statistical support on nodes within in the gomphrenoids (sensu Sánchez-del Pino & al. 2009) which may be explained by the patchy matrices, and also they did not sample relevant taxa like Gomphrena mollis or G. rupestris, thus limiting conclusions on the origin of C4 in Gomphrenoideae. Nevertheless, the general conclusion of Bena & al. (2017) that C4 Gomphrenoideae, unlike C2 grasses, shifted their niches into regions with colder winter climates is in line with the results presented here.

The age of the crown group of the speciose core Gomphrena C4 clade plus Froelichia is inferred as 18 Ma (10.2–28.4 95% HPD; the stem diverged from C4 Xerosiphon at 20.6 Ma [11.8–32.9 95% HPD]), and thus is slightly younger than the earliest inferred gain of C4 in the crown group of Salsoideae/Camphorosmoideae (Kadereit & al. 2012). The stem of the C4 clade within Alternanthera originated at 18.5 Ma (7.8–33.7 95% HPD) and the C4 genus Tidestromia diverged at 27.7 Ma (19.3–38.9 95% HPD). Tidestromia is very different morphologically to the other extant genera and C4 photosynthesis might have evolved at some point along the stem later (we have only included one of the 14 species here; and morphologically intermediate ancestors might now be extinct). Thus, we can therefore assume that C4 originated in Gomphrena and other Gomphrenoideae in roughly the same time interval than in Chenopodiaceae s.str. subsequently to the drop of CO2 concentrations at Eocene-Oligocene boundary (Christin & al. 2011; Kadereit & al. 2012). Nevertheless, the core Gomphrena clade stands out by being a C4 clade that diversified at least twice into high elevation Andean environments. Species such as G. umbellata (3800–4400 m a.s.l.) and G. meyeniana (2600–4000 m a.s.l.) and allies probably constitute the highest populations of any C4 plant, thereby being competitive in sub-arid as well as humid Puna vegetation.

Toward a phylogeny-based circumscription of Gomphrena

The genus Gomphrena has a complex taxonomic history. It was established by Linnaeus (1753) with G. globosa L. and G. flava L. [= Alternanthera flava (L.) Mears]. In later publications, Linnaeus added nine more species which now belong to four genera: Gomphrena, Philoxerus, Froelichia and Alternanthera. The type of the genus Gomphrena was chosen later as G. globosa with the typification effectively published by Hitchcock (Hitchcock & Green 1929). Gomphrena globosa is included in the molecular trees of Sánchez-del Pino & al. (2009) in a position that corresponds to the mostly Andean clade (Fig. 2, 3) and thus belongs to the core C4 Gomphrena clade.

Martius (1826) then treated 57 species of Gomphrena with detailed descriptions and also described the genera Pfaffia, Hebanthe and Serturnera. Endlicher (1837) reduced these genera to sections of Gomphrena. This extended generic concept was largely followed by Moquin-Tandon (1849), who in addition recognized the section Xerosiphon, and also reduced the genera Ninanga and Wadapus described by Rafinesque (1837) to sectional level within Gomphrena. The currently used generic circumscription goes back to Seubert (1875) who excluded the sections Hebanthe, Pfaffia and Serturnera from Gomphrena. This was followed by Schinz (1893), Holzhammer (1955; 1956) and Townsend (1993), and confirmed
by more recent investigations using molecular and morphological characters (Borsch & Pedersen 1997; Müller & Borsch 2005; Sánchez-del Pino & al. 2009). The resurrection of *Xerosiphon* as an own genus contrary to its treatment as a section of *Gomphrena* since Seubert (1875) was proposed by Pedersen (1990), and is in line with results of this study as well as Müller & Borsch (2005) and Sánchez-del Pino & al. (2009). Siquera (1992) included *Pseudogomphrena* R. E. Fr. which has pollen and floral morphology similar to *Pfaffia* and therefore is probably distantly related to the *Gomphrena* clade (Eliasson 1988; Borsch 1998). The same applies to some other species currently included in *Gomphrena* with *Pfaffia*-type pollen (here represented by *G. mandonii*; see Fig. 1–3).

Generic concepts should be based on phylogenetic insights, rendering genera monophyletic, but there should also be morphological characters that allow to recognize genera. This investigation provides robust evidence from nuclear and plastid genomic compartments that genera. This investigation provides robust evidence from nuclear and plastid genomic compartments that *G. mandonii* and also morphological characters allow to recognize genera. This investigation provides robust evidence from nuclear and plastid genomic compartments that *G. mandonii* and also morphological characters allow to recognize *G. mandonii* and also *G. prostrata* and *G. tomentosa* (see previous paragraphs) further underscore that the species of these genera should in fact be classified within *Gomphrena*.

The situation in *Guilleminea* is different. The species of *Guilleminea* could represent a convergent origin of a procumbent habit in relation to *Gossypianthus* and the *Gomphrena radiata–G. tomentosa* clade. Whereas *Guilleminea* stands out by a perigynous flowers (Fig. 6, character 13; Eliasson 1988), its phylogenetic position and origin is not yet clear. This unique floral morphology historically always led authors to maintain *Guilleminea* at generic rank. *Guilleminea* could therefore (together with *G. prostrata*) be viewed as the earliest diverging branch in an even more widely circumscribed genus *Gomphrena* or maintained as an own genus. The latter would imply to find morphological characters that would support an inclusion of *G. prostrata* into *Guilleminea* or a generic status of *G. prostrata*. Our case of *Gomphrena* also exemplifies the stepwise progress in recognizing natural entities that can only be followed by a stepwise adjustment of classification systems to better reflect the natural history of the group (Borsch & al. 2015a). Based on the results of this investigation which consistently reveal the core *Gomphrena* clade as a distinct entity we propose to include *Blutaparon*, *Gossypianthus* and *Lithophila* into *Gomphrena* whereas any change of classification appears to be premature for *Guilleminea*.

The relationships of the Australian species of *Gomphrena* were debated since the early days of botanical systematics, even affecting the use of *Philoxerus* R. Br. and *Blutaparon* Raf. as generic names. Robert Brown (1810) created an own genus *Philoxerus* to accommodate *P. conicus* and *P. diffusus*. Standley (1917) lectotypified the genus name with *P. conicus*, which is a species of sandy soils close to coasts in Australia. This was in line with Hooker (1880) who had defined *Philoxerus* as a genus of coastal plants from the Americas, Africa and Australia rather than using discrete morphological characters to support his genus concept. When Mears (1982 a, b) proposed to use the name *Blutaparon* for the American (and African) taxa, he neither had solid morphological nor phylogenetic evidence for the distinctness of these taxa that could have supported his view. Consequently, Hernández-Ledesma & al. (2015) pointed out that *Blutaparon* should be treated as a synonym of *Philoxerus*. The phylogenetic results now presented in this paper solve this long-standing debate. Our phylogenetic data also show that the only partially fused androecium used by Robert Brown to discriminate species of *Philoxerus* from the then known New World species of *Gomphrena* with a complete staminal tube and lateral stamen appendages, represents a character state that is homoplastic within the *Gomphrena* clade (Fig. 6).

**Taxonomic treatment**

In this section we provide a taxonomic backbone for the species formerly classified under the segregate genera *Gossypianthus*, *Lithophila* and *Philoxerus*, including a full synonymy and type information. We also extend the synonymy of the genus *Gomphrena* to reflect the inclusion of the segregate genera as proposed here. Where relevant, provisions of the *International Code of Nomenclature for algae, fungi, and plants* (Turland & al. 2018) are cited (e.g. “Art. 52.1”).


\[ \equiv \]

* Lithophila Sw., Prodr.: 1, 14. 1788, syn. nov. – Type: *Lithophila muscoides* Sw.

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* Gossypianthus* Hook. in Icon. Pl. 3: t. 251. 1840, syn. nov. – Type (designated by Swart, ING card 09817. 1959): *Gossypianthus rigidiflorus* Hook.
= Chnoanthus Phil. in Anales Univ. Chile 21: 405. 1862. – Type: Chnoanthus mendozaeus Phil.
= Wadapus Raf., Fl. Tellur. 3: 77. 1837, nom. inval., nom. nud. (Art. 38.1(a)).


Notes — The name Gomphrena lanarophylla Span. already exists and is a synonym of the accepted name G. canescens R. Br., an Australian species (Palmer 1998). Therefore, a new name had to be found. It is inspired by the habit and appearance of this species that indeed has some superficial similarity with species of Paronychia Mill. (Caryophyllaceae). Because the epithet “paronychoiodes” is also part of the accepted name of a widespread species of Alternanthera, which may appear in the same habitat, we maintained the word particle “lanar” (Greek, woolly) to make the new species name unique and at the same time connect to the meaning of the old epithet “lanarophylla”.

Although Henrickson (1987) cited a “holotype” at P, there may be no holotype because Poiret merely mentioned a specimen that he had seen in “herb. Desfont.” Because there are three specimens at P, all labelled as “isotype” by Mears, Henrickson’s citation of “holotype” is interpreted as a first-step lectotypification (Art. 9.17). Here we designate the second-step, in order to narrow down to a single specimen.


Notes — The name Lithophila muscoides Sw. already exists and is a synonym of the accepted name G. canescens R. Br., an Australian species (Palmer 1998). Therefore, a new name had to be found. It is inspired by the habit and appearance of this species that indeed has some superficial similarity with species of Paronychia Mill. (Caryophyllaceae). Because the epithet “paronychoiodes” is also part of the accepted name of a widespread species of Alternanthera, which may appear in the same habitat, we maintained the word particle “lanar” (Greek, woolly) to make the new species name unique and at the same time connect to the meaning of the old epithet “lanarophylla”.

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Gomphrena portulacoides (A. St.-Hil.) T. Ortuño & Borsch, comb. nov. = Philoxerus portulacoides A. St.-Hil., Voy. Distr. Diam. 2: 436. 1833 = Iresine portulacaoides (A. St.-Hil.) Moq. in Candolle, Prodr. 13(2): 341. 1849 = Blutaparon portulacoides (A. St.-Hil.) Mears in Taxon 31: 115. 1982. – Holotype: Brazil, Province...


Note — The sheet is annotated as the holotype by Mears (Nov 1980), although there are three Tweedie specimens in the Hooker herbarium with different barcodes mounted on the same sheet. They are from the Isla Gorriti (34°57'12.2"S, 54°58'19.2"W), probably originating from three different gatherings as the locality information given on the respective labels is different.


= Alteranthera acaulis Andersson, Galapagos Veg.: 164. 1854. – Holotype: Ecuador, Galapagos, Chatham Island, Anderson s.n. (S-R-219!); isotype: BR [0000005235810]).

Note — There is further historical material of A. acaulis collected by N. J. Andersson from Galapagos under different numbers: no 64 in P [P00622604] and no. 67 in C [C100054391], LD [LD1567052], M [M-0241868!] and MEL [MEL2458701].


= Blutaparon breviflorum Raf., New Fl. 4: 45. 1838. – Type: United States, Florida, W. Baldwin [not located, see Mears 1982].

Note — Iresine surinamensis was synonymized with Blutaparon vermiculare var. vermiculare by Mears (1982). Moquin Tandon (1849) cited “A. Kappele!” without any collecting number. The P material is wrongly annotated by Mears (1975 in sched.) as Lithophila muscoides. The P specimen is weak material and the protologue also mentions the 3-veined tepals but no description of the androecium.


≡ *Gomphrena albiflora* Moq. in Candolle, Prodr. 13(2): 392. 1849. – Holotype: Venezuela, Maracaybo, Plee s.n. (P [P00622647!]).

Note — Compared to other individuals of *Gomphrena vermiculare*, the plants called *G. albiflora* deviate by longer tepals and very narrow, linear cauline leaves. The species, in addition to *Blutaparon vermiculare*, was accepted by Medina & al. (2008), who illustrated plants from the Caribbean coast of Venezuela that are clearly halophytes with succulent leaves. It will therefore be important to include specimens corresponding to this entity into molecular phylogeographic analyses of *G. vermiculare* and relatives.


≡ *Philoxerus surinamensis* Moq. ex Moq. in Candolle, Prodr. 13(2): 339. 1849, nom. inval., pro syn. (Art. 36.1(b)).

≡ *Philoxerus maracaybensis* Klotzsch ex Benth. & Hook. f., Gen. Pl. 3: 40. 1880, nom. inval., nom. nud. (Art. 38.1(a)).

Note — Suessenguth annotated the specimen *Moritz 1016* in BM (BM000839401!) from Venezuela, Maracaybo, as “*Philoxerus maracaybensis* Suess.”, which was considered an isotype by Mears (in sched.). However, this name was never published by Suessenguth.


≡ *Philoxerus wrightii* Hook. f. in Bentham & Hooker, Gen. Pl. 3: 40. 1880, nom. inval., nom. nud. (Art. 38.1(a)).


Notes — Turner (2016) pointed out that the name ascribed to Hooker (1880) in *Genera plantarum* was not validly published because the only and insufficient descriptive terms given are “species parvula”. A full description was later provided by Maximowicz (1886), who also validated the name. Apart from the revision by Mears (1982), the species was accepted in *Flora of Japan* (Ohwi 1965) and *Flora of China* (Bao & al. 2003). In GBIF (https://doi.org/10.15468/39omei – accessed 6 Apr 2020) there are 46 records spanning the Ōsumi-Shōtō Islands, Ryukyu Islands, Nansei-Shōtō, Okinawa-Islands to the south of Taiwan. The species does not occur in Australia (the three specimens in L are incorrectly attributed).

Acknowledgements

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Appendix 1. Specimens used in phylogenetic analysis and reconstruction of ancestral character states including voucher information and EMBL/GenBank accession numbers for sequences. All sequences that were published in previous papers are cited with a superscript number corresponding to the respective source immediately after the accession number: 1 from Müller & Borsch 2005; 2 from Sánchez-del Pino & al. 2009; 3 from McCauley & Ballard 2007; 4 from Borsch & al. 2011; 5 from Sage & al. 2007; 6 from Hammer & al. 2015; 7 from Di Vincenzo & al. 2018; 8 from Borsch & al. 2018.

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- **Code**: Accession number
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- **Taxon name**: Scientific name
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- **Voucher**: Voucher information

This table provides a list of taxa with their respective accessions, identification numbers, scientific names, field/garden origins, and voucher information.
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Appendix 2. List of morphological characters and their states

Habit and vegetative morphology (Characters 1–6)

1. Life cycle. Character 1
Annual herbs in *Gomphrena* and allies complete their life cycle in a single year and survive the dry season as seeds. Perennial herbs, subshrubs, shrubs, lianas or vines live for many years.
States: annual (0), perennial (1)

2. Type of root. Character 2
Annual herbs have a slender primary root with short lateral roots (such “simple” roots exist for example in *Gomphrena haenkeana* and *G. radiata*). Perennial herbs have taproots, with a strongly developed main root. Such taproots are often thickened as in *Guilleminea densa* (Henrickson 1987), *G. meyeniana* and *G. tomentosa*. Adventitious roots appear from the nodes of usually decumbent stems, different to the primary root system. They are present in the perennial herbs *Philoxerus portulacoides* and *P. vermicularis*.
States: simple root (0); taproot (1), taproot and adventitious roots (2)

3. Basal leaves. Character 3
Basal leaves clustered into a rosette growing at a reduced main axis are present in species of *Guilleminea*, *Gossypianthus* (Henrickson 1987) and also *Gomphrena meyeniana*. Pedersen (1990) described this character as “scapose flowering stem”. The basal leaves are bigger than the cauline leaves but otherwise similar.
States: absent (0), dense rosette in prostrate or decumbent plants (1), rosette with few leaves in erect plants (2)

4. Orientation of growth of stems. Character 4
Several modes of growth are present in the plants. They are procumbent, when the stems grow spreading on the ground for their lifetime; decumbent when stems initially spread on the ground but later grow upward. Upright stems are called “erect”. In the case of *G. meyeniana* the main axes appear to be condensed (coded as erect) and synflorescences are at the tip of decumbent to ascending branches.
States: procumbent (0), decumbent (1), erect (2)

5. Vegetative branching system. Character 5
Species of *Gomphrena* and allies have different branching systems. In some of them erect stems are unbranched, although a plant can develop several stems from a rootstock. Unbranched stems are only present in perennial plants. But many annual and perennial species have stems branched from near the base.
States: only with unbranched principal stems (0), with secondary and tertiary stems (1)

6. Determinate vs. indeterminate growth of stems. Character 6
The term “determinate growth” is used in the sense to characterize the main axes as stem formed by an apical meristem. This meristem may abort after some period of functioning or it may transform into a specialized structure such as a flower or inflorescence, impeding its further extension capacity (Barthélémy & Caraglio 2007). Here “determinate” growth is understood to be present in those species of *Gomphrena* in which all stems (principal but also secondary or tertiary) terminate in inflorescences, such as in *G. flaccida*, *G. haenkeana* and *G. meyeniana*. In contrast, indefinite extension refers to an axis on which an apical meristem indefinitely maintains its growth potential (Barthélémy & Caraglio 2007). This is found in species with procumbent stems such as *G. tomentosa* or *Guilleminea densa*.
States: indeterminate (0), determinate (1)

Inflorescence morphology (Characters 7–10)

7. Shape of paracladia. Character 7
The flower unit in *Gomphrenoideae* is solitary with flowers subtended by one bract and two bracteoles. Considering that *Amaranthaceae* have a cymous inflorescence architecture, the partial florescences are reduced to a solitary flower in *Gomphrenoideae* (Borsch & Pedersen 1987; Acosta & al. 2009). These paracladia have been described as globose heads, subglobose or short to elongate capitate or glomerate spikes by some authors (Standley 1917; Eliasson 1988; Henrickson 1987; Borsch 2001). This includes lateral (co-florescences) and terminal (main florescences).
States: globose to subglobose with flowers densely adjacent (0), elongate to cylindric with flowers not densely aggregated (1)

8. Arrangement of paracladia (synflorescence structure). Character 8
*Gomphrena* and other *Gomphenoideae* exhibit very complex synflorescence structures, part of which were described by Borsch & Pedersen (1987) for the thyrsoid structures in *Hebanthe* and by Acosta & al. (2009) in their wider analysis of the *Amaranthaceae*. This character includes the arrangement of the paracladia (= partial florescences) and therefore, it defines the different structures of the branching system that is constituted by the axes bearing paracladia.
States: paracladia solitary and terminal on the main vegetative axes (0); paracladia solitary on main axes as well as on lateral branches (1); paracladia on the axes of one of the opposite cauline leaves, and in terminal parts of
major branches of the plant on elongated or very reduced axes appearing in one of the leaf axils (2); paracladia arranged on strongly condensed axes in a way that multiple paracladia appear in a terminal whorl-like structure with a terminal partial florescence surrounded by usually 5 (3–6) paracladia without visible axes (3); paracladium with a regular asymmetric arrangement arising from the axillary bud of a large cauline leaf and a very condensed branching structure with multiple branching orders and smaller paracladia arising from axillary buds of smaller cauline leaves that usually appear only on one side of the stem (4); paracladia arranged in a complex thyrsoid structure (a terminal paracladium as main florescence and lateral paracladia are co-florescences, with up to two orders of branching (5)

9. Apical leaves subtending paracladia. Character 9
In the case of synflorescences with condensed axes bearing partial florescences (paracladia: character 8 state 3), there may be specialized cauline leaves that subtend the paracladia. For these leaves that are morphologically deviant in shape and size but also often in indumentum and texture from the cauline leaves we propose the term “apical leaves subtending paracladia”. In the case of the pseudanthium in Gomphrena, the number of subtending apical leaves is reduced to one per paracladium (= partial florescence). Alternatively, leaves subtending partial florescences can be completely absent, so that partial florescences appear pedunculate.

States: absent (0), two oppositely arranged and un-specialized, similar to cauline leaves (1), broadened, (4–)5(–6) and stellately arranged in a whorl (2), 5–10, irregularly arranged, similar in texture to cauline leaves but very distinct through their narrower shape (3)

10. Cauline leaves supporting branches in synflorescence. Character 10
The paracladia may appear in a complex branching system (synflorescence) where the respective cauline leaf organs are not just reduced in size but considerably modified and differ from the other normal photosynthetic cauline leaves also in texture and anatomy (Acosta & al. 2009). With the presence of such scales, an inflorescence appears leafless.

States: cauline-like (0), membranous scales (1)

Photosynthesis (Character 11)

11. Photosynthesis of C₃ and C₄ type. Character 11
The photosynthesis type is here understood as a complex character syndrome corresponding to either C₃ with un-specialized anatomy or C₄ with visible specialized Kranz anatomy. The complex character syndrome also includes characteristic δ¹³C values for C₃ and C₄ (following the broad assessment of Amaranthaceae by Sage & al. 2007).

States: C₃ photosynthesis (0), C₄ photosynthesis (1)

Floral morphology (Characters 12–13)

The morphology of the inner two sepals differ in shape, size and texture. The character is here defined as complex trait (composite coding; see Torres-Montúfar & al. 2018) with three states as further analysis will require detailed microscopic work that goes beyond the scope of this study.

States: all five tepals equal in shape and texture (0), inner two tepals distinctly smaller than the outer three, strongly compressed and carinate in fruit (1), inner two tepals smaller than the outer two but equal in texture (2)

13. Perigynous vs. hypogynous flowers. Character 13
In Gomphrena and most related genera flowers are hypogynous with sepals and androecium tube attached to the axis below the ovary. This feature is different in the species of Guilleminiae where the androecium is adnate to the perianth and the flowers are perigynous (Mears 1967; Eliasson 1988).

States: perigynous (0), hypogynous (1)

Androecium (Characters 14–21)
The members of Gomphrena and related genera have five functional stamens, with the exception of Lithopha, which has two stamens (Eliasson 1988). The filaments are united only basally into a cup or to higher degrees into an elongate tube, with an usually small free portion of filaments (Eliasson 1988; Sánchez-del Pino & al. 2019). In some taxa, stamen tube appendages develop from the tubular meristem and then alternate with filaments. The free portion of the androecium tube can be a simple extension of the filament or possess lateral appendages on each side. The characters and states describe these qualitative features of the androecium and also the proportions.

14. Length of androecial tube. Character 14
Due to gradual variation five size classes for the length of the tube are distinguished. Measurements are from the connection to the floral axis (proximal) to the distal tip of the filament where it is connected to the anther.

States: 0.6–4.2 mm (0), 4.3–5.9 mm (1), 6–7 mm (2), 8.1–10.2 mm (3), > 30 mm (4)

15. Fusion of androecial tube. Character 15
This character exhibits gradual variation and stands for the relation of the total size of the androecium as in character 14 to the free part measured from the deepest point between two stamens or between the stamen and the adjacent androecial tube appendage to the distal tip of the filament. Six ranks for the degree in percent of the free part are differentiated.
16. **Shape of androecial tube.** Character 16
States: broadly cup-shaped with distinctly spaced linear filaments (0), more or less broadly cup-shaped with V-shaped connection between gradually narrowing filaments (1), more or less broadly cup-shaped with U-shaped connection between more or less abruptly narrowing filaments (2), filaments broadly linear with narrowly U-shaped connection between filaments (3)

17. **Androecial tube appendages.** Character 17
The androecial tube appendages were called “pseudostaminodia” and transitions to stamen appendages have been described (Townsend 1993). Considering that either such pseudostaminodia or stamen appendages on both sides of the filament were found, both were considered to represent modifications of one character (Townsend 1993; Eliasson 1988). Here we define the androecial tube appendages as an own character that is not homologous to lateral filament appendages following Vrijdaghs & al. (2014) and Sánchez-del Pino & al. (2019).
States: absent (0), present (1)

18. **Shape of androecial tube appendage.** Character 18
States: broadly rounded to truncate (0), narrowly oblong and obtuse at apex (1), linear with cleft apex (2)

19. **Filament appendages.** Character 19
States: absent (0), present (1)

20. **Length of filament appendages.** Character 20
This is a quantitative character, for which four size classes are distinguished.
States: 0.1–0.2 mm (0), 0.3–0.5 mm (1), 0.6–1.0 mm (2), 1.1–1.5 mm (3)

21. **Shape of filament appendages.** Character 21
States: lanceolate-acuminate apex (0), lanceolate with rounded apex (1), falcate (2), narrowly acute (3)

**Appendices S1–S12**

The following Appendices are published electronically under Supplemental content online (see https://doi.org/10.3372/wi.50.50301).

**Appendix S1.** Sequence data of the *matK-trnK* dataset B-1. Includes the multiple sequence alignment with hotspots annotated, the sequence matrix used for tree inference, the list of indels and the indel matrix.

**Appendix S2.** Sequence data of the *matK-trnK* dataset B-2. Includes the multiple sequence alignment with hotspots annotated, the sequence matrix used for tree inference, the list of indels and the indel matrix.

**Appendix S3.** Sequence data of the ITS dataset B-2. Includes the multiple sequence alignment with hotspots annotated, the sequence matrix used for tree inference, the list of indels and the indel matrix.

**Appendix S4.** Sequence data of the *matK-trnK* dataset C. Includes the multiple sequence alignment with hotspots annotated and the sequence matrix used for tree inference.

**Appendix S5.** Sequence data of the *matK-trnK* dataset D-1. Includes the multiple sequence alignment with hotspots annotated and the sequence matrix used for tree inference.

**Appendix S6.** Sequence data of the ITS dataset D-2. Includes the multiple sequence alignment with hotspots annotated and the sequence matrix used for tree inference.

**Appendix S7.** Matrix of morphological characters.

**Appendix S8.** Ancestral states of morphological characters 7 and 10 in *Gomphrena* and allies reconstructed over the 27-taxon tree (dataset A).

**Appendix S9.** Ancestral states of morphological characters 15, 18, 20 and 21 in *Gomphrena* and allies reconstructed over the 27-taxon tree (dataset A).

**Appendix S10.** Complete tree of the BEAST analysis based on the extended dataset C depicting node ages with confidence intervals of the *Amaranthaceae–Chenopodiaceae*, supplemented by a tree showing all nodes numbered through and a supplementary table with the precise ages and HPDs of these nodes.

**Appendix S11.** Ancestral states of C₃ and C₄ photosynthesis (character 11) in the extended *matK-trnK* dataset (dataset D) as inferred using BayesTraits under an MCMC model and an ML model.

**Appendix S12.** Ancestral states of C₃ and C₄ photosynthesis (character 11) using the extended nrITS dataset (dataset D) as inferred using BayesTraits under an MCMC model and an ML model.