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Let there be clades: phylogenetics of *Mimosa* series *Pachycarpae* and *Setosae* (Fabaceae) improves the infrageneric classification of the genus

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Full implementation of phylogenetic classifications remains pending, particularly to delimit infrageneric divisions of large genera. *Mimosa*, one of the largest genera of Fabaceae, includes five sections and 41 series, most of which are not monophyletic. Here we investigated the phylogenetic relationship among species of *Mimosa* series *Pachycarpae* and *Setosae*, two diverse series from the Brazilian Cerrado (savanna) Domain. We analysed morphological and molecular data for a wide taxonomic sample in a total-evidence approach. Our results show the non-monophyly of these series is more problematic than previously realized and extends to *M*. series *Piresianae*. Nonetheless, most taxa of *M*. series *Pachycarpae* and *Setosae* fall in a clade, which has an enlarged underground organ as one of its synapomorphies and an important functional trait underlying adaptation to fire in the Cerrado Domain. On the basis of these results, and after transferring some species to *M*. series *Piresianae*, we synonymize *M*. series *Setosae* under *M*. series *Pachycarpae*. These updates are a first step towards aligning the infrageneric classification of *Mimosa* with the tenets of phylogenetic systematics.

ADDITIONAL KEYWORDS: campos rupestres – dynamic homology – large genera – Leguminosae – taxonomy.

INTRODUCTION

Phylogenetic systematics has revolutionized taxonomic classifications. As we have moved towards naming only monophyletic groups, traditional classifications have had to be revised in all groups of organisms. These changes, however, are still far from complete. In angiosperms, as in other groups, updates to taxon limits generally proceeded from higher to lower levels of the taxonomic hierarchy, following increases in taxonomic and character sampling. Thus, even though many higher-level plant taxa are now robustly aligned with phylogeny (e.g. APG IV, 2016; LPWG, 2017), many less inclusive clades remain to be properly studied and aligned with clades. These groups were usually not extensively sampled in phylogenetic studies and include many genera, especially large genera.

According to Frodin (2004), 'big' plant genera are those with 500 or more species. Frodin's list of big genera included some Fabaceae, but not *Mimosa* L., now known to be the fifth largest genus in Fabaceae, with > 550 species (Barneby, 1991; Luckow, 2005; Simon *et al.*, 2011). Most *Mimosa* species are native to Tropical America, whereas 31 are endemic to Madagascar and a few occur in Asia and continental Africa (Villiers, 2002; Luckow, 2005; Simon *et al.*, 2011). Besides having a large number of species, the genus is also morphologically variable, a feature that supported the recognition of five sections, 41 series, 39 subseries and many infraspecific taxa (Barneby, 1991). Even though *Mimosa* is monophyletic (Bessega, Hopp & Fortunato, 2008; Simon *et al.*, 2011), initial

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phylogenetic trees showed that four of the five sections and at least 17 of the series are not monophyletic (Bessega *et al.*, 2008; Simon *et al.*, 2011).

Mimosa series *Pachycarpae* Benth. and *Setosae* Barneby embody these problems. These two together form a strongly supported monophyletic group (clade 'O' of Simon *et al.*, 2011, here referred to as the MPS clade), but lack of resolution in this clade precluded any assessment of whether these two series are really intermingled or sister to each other. This problem is aggravated by low taxon sampling, with only 26 out of 91 taxa (not accounting for autonyms, 31 are infraspecific) included in previous phylogenetic analyses (Bessega *et al.*, 2008; Simon *et al.*, 2011). Nonetheless, the phylogenetic affinity between *Mimosa* series *Pachycarpae* (hereafter referred to as *Pachycarpae*) and *M.* series *Setosae* (hereafter referred to as *Setosae*) is not unexpected. The original circumscription of *Pachycarpae* (Bentham, 1842, 1846, 1875, 1876) included taxa from both series, and the two are morphologically similar (Barneby, 1991). In fact, *Setosae* was described to accommodate species with a craspedium (a fruit that breaks up in one-seeded fragments, leaving behind intact margins; Spjut, 1994), rather than the distinctive unjointed craspedium of *Pachycarpae*, in which the valves break away intact from the margins (Barneby, 1991) (Fig. 1).

Besides having different fruit morphologies, *Pachycarpae* and *Setosae* encompass great variation in vegetative and reproductive features and growth forms, including virgate shrubs, humifuse subshrubs, rosette-like shrubs, shrubs and treelets (Barneby, 1991) (Fig. 2). Other prominent features of many species in these two series are the massive underground organs (despite the lack of anatomical studies, these are usually referred as xylopodia or lignotubers and are common in geoxyles, and also during early development of some shrubs and treelets), persistent stipules, thickened bark and congested leaves (Fig. 2). All of these traits are thought to be adaptations to the fire regimes prevalent across the Brazilian Cerrado Domain (Simon *et al.*, 2009; Simon & Pennington, 2012), to which most species of these two series are endemic (Barneby, 1991; Dutra *et al.*, 2020). Despite this large trait diversity, only a few morphological characters have been optimized onto phylogenetic trees (Bessega *et al.*, 2008; Simon *et al.*, 2011), none of which vary within the series. Thus, it remains to be seen if the MPS clade and its internal phylogenetic structure are supported by any morphological synapomorphies.

Considering that accurate classifications and knowledge about morphological evolution rely on densely sampled and well resolved phylogenetics trees, our goals are three-fold. First, we investigate whether increments in taxon and character sampling resolve phylogenetic relationships between *Pachycarpae* and *Setosae*. Second, we test for the presence of morphological synapomorphies for the clade (or clades) comprising members of both these series. Finally, we re-evaluate the classification of *Pachycarpae* and *Setosae* in light of our findings.

MATERIAL AND METHODS

Sampling and rooting

To achieve our goals, we assembled and analysed molecular and morphological data in a total-evidence approach. To build our molecular dataset, we used previously published sequences for the group

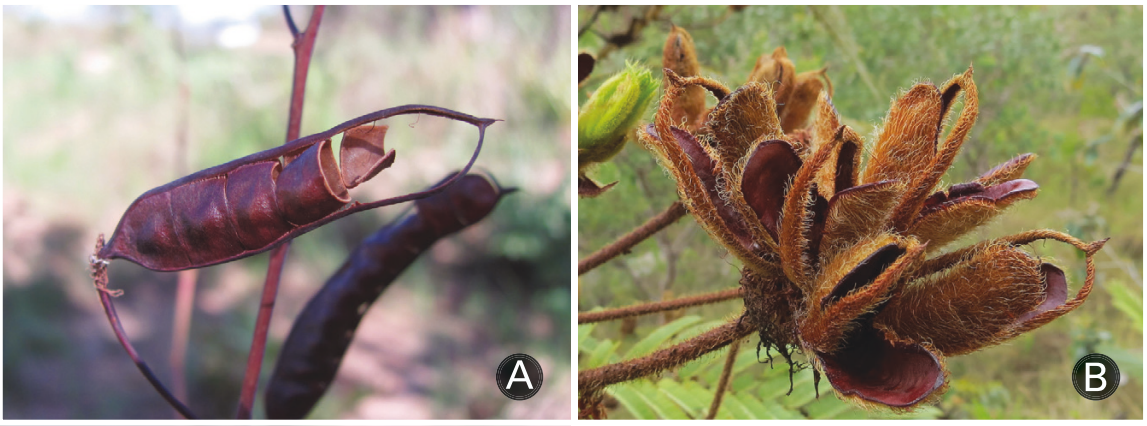

Figure 1. Fruit morphologies distinguishing *Mimosa* series *Pachycarpae* and *Setosae*. A, Fruit of *M. caliciadenia*, the common form of craspedium in the genus, showing the valves breaking into one-seeded segments or articles to leave a persistent replum. B, The unjointed craspedium of *M. longepedunculata*, a fruit type mostly restricted to *M.* series *Pachycarpae* in which the valves remain intact as they split from the pod margin.

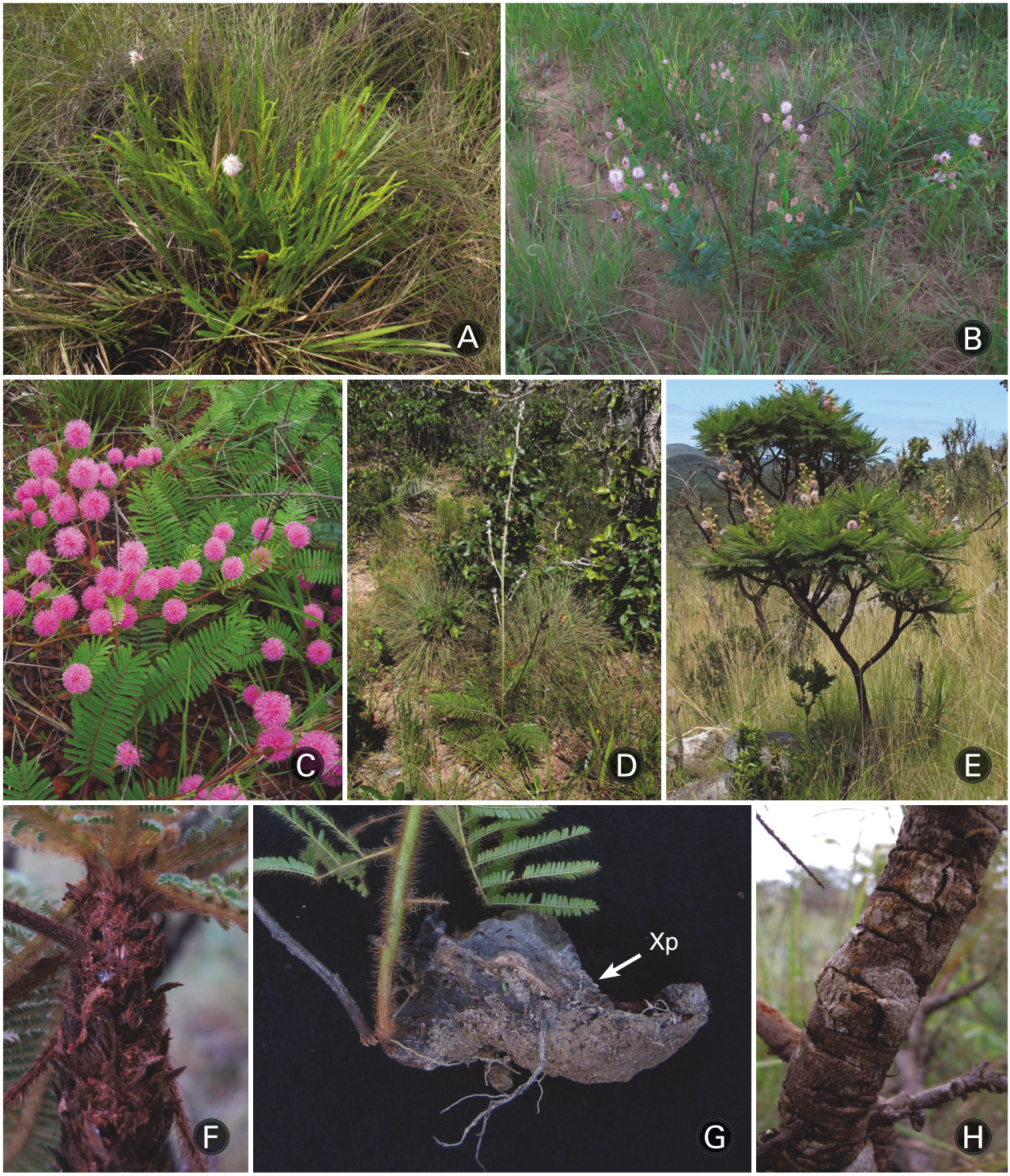

Figure 2. Morphological diversity of *Mimosa* series *Pachycarpae* and *Setosae*. A–E, Variation in plant habit. A, Rosette-like shrub of *M. speciosissima*. B, Shrub with branches fasciculate at the base, *M. foliolosa* var. *foederalis*. C, Humifuse subshrub, *M. prorepens*. D, Wand-like shrub, *M. eriorrhachis*. E, Treelet with leaves congested towards the apex of the shoots, *M. regina*. F–H, Fire adaptations in species of the Brazilian Cerrado Domain. F, Persistent stipules, *M. manidea*. G, Geoxyle with an enlarged underground organ (Xp), *M. diminuta*. H, Thick bark on the trunk, *M. claussenii* var. *prorsiseta*. Images also show different types of synflorescences. A, B and D, Axillary racemes nested within the foliage and C–E, racemes organized in terminal exserted synflorescences.

(Simon *et al.*, 2009; LPWG, 2017; Vasconcelos *et al.*, 2020) or extracted DNA from leaf tissue samples collected during multiple field expeditions throughout the geographical range of the MPS clade. Leaf samples were dried and stored in the CEN herbarium silica-dried leaf collection (acronyms according to Thiers, 2021) with some duplicates at RB. In total, we included data for 154 samples, of which 78 belong to *Pachycarpae* (60 taxa, 75% of the series), 18 to *Setosae* (13 taxa, 86% of the series). In addition, we included 55 other *Mimosa* spp. and one each of *Anadenanthera* Speg., *Stryphnodendron* Mart. and *Piptadenia* Benth. See the Supporting Information and the data availability statement for a detailed list of taxa, GenBank numbers and vouchers. Following previous phylogenetic studies for mimosoid legumes (Jobson & Luckow, 2007; Simon *et al.*, 2009, 2016), we rooted phylogenetic trees on *Anadenanthera colubrina* (Vell.) Brenan.

Molecular data

Our molecular dataset includes three plastid loci (*trnD-T* intergenic spacer, *trnL-F* intron and intergenic spacer and part of the *matK* gene) and one nuclear region (the nuclear ribosomal internal transcribed spacer region, ITS, including the ITS1 and ITS2 spacers and the intervening 5.8S subunit). Total DNA was isolated from silica-dried leaf samples using a modified version of the Cetyl trimethylammonium bromide protocol (Doyle & Doyle, 1987) or the DNeasy Plant Mini Kit (Qiagen, Crawley, UK). The *trnD-T* fragment was amplified using primers *trnD2* (Simon *et al.*, 2011), *trnTGGU* (Shaw *et al.*, 2005) and the internal primers *trnEUUC* and *trnYGUA* (Shaw *et al.*, 2005). The *trnL-F* region was amplified with primers *c* and *f* of Taberlet *et al.* (1991). For *matK*, we amplified the region between primers *1100F* (Wojciechowski, Lavin & Sanderson, 2004) and *trnK2R* (Lavin *et al.*, 2000). Finally, following Simon *et al.* (2016), ITS was amplified using a nested polymerase chain reaction (PCR) approach. The first reaction used primers ITS 5p and ITS 8p (Möller & Cronk, 1997), followed by a second PCR with primers ITS1 and ITS4 (White *et al.*, 1990). See Table A1 and Appendix for additional information on primer sequences and detailed amplification protocols.

We purified all PCR products using polyethylene glycol or shrimp alkaline phosphatase and exonuclease 1 (ExoSAP; USB Corp., Cleveland, OH, USA) and used Big Dye 3.1 Terminator (Applied Biosystems, Foster City, CA, USA) to sequence samples on an ABI 3730XL DNA sequencer (Applied Biosystems) at the Laboratório de Genética Vegetal, Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brazil. Alternatively, we sent PCR products for purification and sequencing at Macrogen Inc. Plastid regions were sequenced with the same primers used for PCRs, and for *trnD-T* all four primers were used to avoid problems sequencing through mononucleotide repeats (poly A/T). For ITS we sequenced with the same primers as the second PCR. ITS chromatograms included sites with multiple base calls, probably due to sequencing of paralogous copies, as reported for other mimosoids (Hughes, Bailey & Harris, 2002; Souza *et al.*, 2013). To minimize potential biases during phylogenetic analyses, we coded these rare instances as polymorphic. Sequenced strands for each region were assembled using Geneious v.6.1 (https://www.geneious.com/). All molecular alignments are available as Supporting Information.

Morphological data

We scored 75 characters from herbarium specimens held at CEN, K, NY, SPF and UB, and from field observations (see the Appendix and Supporting Information for details on character statements and sampling). When necessary, we also scored data from selected specimens held at the following herbaria: A, ALCB, B, BHCB, BM, CESJ, DIAM, ESA, F, G, HB, HBG, HRCB, HTO, HUEFS, HUFU, IAN, IBGE, LE, M, MG, MO, OUPR, P, PAMG, R, RB, RFA, S, SP, UEC, US, VIC and W. In a few cases, where features were absent on specimens, data were complemented by information in the literature (e.g. Barneby, 1991; Simon, Hughes & Harris, 2010; Borges, Simon & Pirani, 2014). We studied only fully developed structures from mature plants with the aid of a 10-63× magnification microscope. As phenotypic information was compiled by taxon, not individuals, morphological data are identical for different accessions of the same taxon.

Character statements follow Sereno (2007), who treated the presence or absence of particular traits as one type of character (neomorphic) and variations of this same trait as different characters (transformational). This approach is particularly beneficial when one structure varies in different aspects. For example, filiform setae, if present (character 6), vary in base shape (character 7), presence of a calcar (character 8), fusion to each other (character 9), ornamentation (character 10) and colour (character 11). We only deviated from Sereno's approach when a trait varied in a single attribute (e.g. character 19). We also chose to treat features related to habit as multiple characters. For example, the rosette-like shrub habit comprises three characters: presence of leaves congested towards the apex of the shoot (character 24: state 1) in a shrub (2: 0) with a thick and corky bark (16: 1). The morphological matrix is available in the Supporting Information and in MorphoBank (http://morphobank.org/permalink/?P4233).

Pre-alignment of DNA fragments

We performed a preliminary alignment for each molecular fragment with the Muscle plugin in Geneious v.6.1. Resulting alignments were then partitioned to avoid direct optimization of sequences (DO; Wheeler, 1996; Wheeler, 2003a) of coding regions and to improve computational performance during dynamic homology analyses, and had ends trimmed to avoid problems with missing data (Giribet, 2001).

Phylogenetic analyses

Phylogenetic analyses were performed in a total-evidence (molecules plus morphology) and dynamic homology approach using parsimony as the optimality criterion. This approach is grounded on particularities of our dataset and goals. First, dynamic homology (Wheeler, 2001) is an appropriate method to analyse sequences of variable length, i.e. that result in alignment gaps, such as the ones used here. Second, the lack of accurate models of trait evolution make parsimony a sensible criterion for phylogenetic analysis of morphological data (Goloboff, Torres Galvis & Arias Becerra, 2018). This is reinforced by the lack of topological discordance between parsimony and model-based analyses, as seen here (Supporting Information), for *Mimosa* as a whole (Simon *et al.*, 2011), and in many other empirical examples (Rindal & Brower, 2011; Brower, 2018). Finally, as we do not aim to estimate other evolutionary aspects, such as divergence time, our method is an appropriate and epistemologically coherent approach to infer phylogenetic relationships (see Pinto-da-Rocha *et al.*, 2014, and references therein).

We estimated trees using POY v.5.1.1 (Wheeler *et al.*, 2015). Before running analyses, POY clears any gaps of the pre-aligned molecular matrices to allow DO. We treated the morphological matrix as a set of static characters. Thus, although included in tree inference, they were not subjected to DO.

The search strategy followed Pinto-da-Rocha *et al.* (2014) and used successive time-constrained rounds of DO search to generate a set of unique candidate trees, which were subsequently refined using the iterative pass optimization (IP) (Wheeler, 2003b). DO analyses were run with an indel opening cost set to zero, whereas indel extensions, transversions and transitions had equal weights (0:1:1:1). We set the number of DO rounds to 30 and the search time to 12 hours partitioned in three slots of four hours by gradually increasing number of searches and individual search times until tree cost stopped improving. We then multiplied the number of rounds by three and search time by two to maximize exploration of tree space.

The full set of most-parsimonious trees found during IP were used to generate a strict consensus tree. Based on that consensus tree, we generated static character matrices in the form of implied alignments (IA; Wheeler, 2003a). These character matrices and the consensus tree were used to calculate branch lengths and to estimate bootstrap support. We performed bootstrap (1000 replications) with TNT (Goloboff *et al.*, 2008).

To understand if incongruences between nuclear and plastid data recorded for other mimosoids (e.g. Hughes *et al.*, 2002, Souza *et al.*, 2013) also occur in *Mimosa*, we analysed ITS and plastid data independently using the methods described previously (see the Supporting Information). Aiming to provide an empirical justification to our methods, we also reanalysed the IA generated during IP using static methods and parsimony and posterior probability as optimality criteria. We concatenated datasets and coded indels with the 'simple index coding' (Simmons & Ochoterena, 2000) using 2matrix (Salinas & Little, 2014). Static parsimony analyses were performed with PAUP* v.4 (Swofford, 2003) and included two rounds of heuristic search, each with 1000 replicates of random taxon addition and tree bisection-reconnection branch swap, saving 15 trees per replicate. The second search round was performed using the trees saved during the first round. Branch support was estimated using 10 000 iterations of bootstrap resampling using the same parameters mentioned before. We conducted static Bayesian analyses with Mr Bayes, v.3.2 (Ronquist *et al.*, 2012), using the CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010). Following Simon *et al.* (2016), who analysed the same DNA regions for a closely related group, we performed two runs of four chains using a GTR + I + G model for molecular partitions and equal rates for the morphological partition for 10 million generations, sampling trees each 1000th generation. Sampled trees and branch posterior probabilities were summarized on a 50% majority rule tree after discarding 25% of samples as burn-in.

Optimization of morphological character states

Inferred morphological character state changes were optimized on the consensus tree with YBYRÁ (Machado, 2015). YBYRÁ categorizes character state changes that have different optimizations of equal cost as 'ambiguous', whereas those changes with a single placement are treated as 'unambiguous'. Unambiguous transformations are further subdivided into unique and non-homoplastic (the synapomorphy occurs only once in the tree and is shared by all terminals of the

clade); unique and homoplastic (the synapomorphy occurs only once in the tree, but it is transformed within the clade); and non-unique and homoplastic (the synapomorphy is not exclusive to the clade and neither shared by all of its members).

RESULTS

Phylogenetic analyses

DO analysis of the combined molecular and morphological datasets generated a pool of 109 unique trees with length ranging from 5270 to 5276 steps. After IP, this set of trees was reduced to 17 trees of length 5240. The strict consensus of these trees shows that species of *Pachycarpae* and *Setosae* are closely related to species belonging to series of *M*. section *Habbasia* DC. (Fig. 3, node XO). Within this group, *Pachycarpae* and most species of *Setosae* together form a monophyletic group, here referred to as the MPS clade. The remaining species of *Setosae* are placed in another clade (PIR hereafter) with members of *M*. series *Piresianae* Barneby, which is here shown to be polyphyletic. Although static analyses differ regarding placement of the PIR clade (Supporting Information), dynamic homology analysis place it as sister to a group containing species from five series [*M*. series *Leiocarpae* Benth., *Neptunioideae* Barneby, *Bipinnatae* DC., *Rojasianae* Barneby and *Glandulosae* (Benth.) Barneby] and this larger group, with *M. pigra* L., is sister to a clade containing the MPS clade plus *M. corynadenia* Britton & Rose. Most of these relationships have strong bootstrap support.

The MPS clade includes all sampled species of *Pachycarpae* and most of those belonging to *Setosae*, including the type species of both series (Fig. 4). Relationships in the MPS clade vary in the degree of resolution and have overall low bootstrap support (Fig. 4). The polytomy at the base of the clade includes five lineages with different degrees of resolution. Four of these lineages are internally resolved, although with low bootstrap support, and show species of *Setosae* intermingled with taxa of *Pachycarpae* (Fig. 4). Species belonging to these four lineages span the whole distribution of both series. The fifth lineage subtends a massive polytomy with weak support (here referred to as the coreMPS clade), which includes a large number of species of *Pachycarpae*, most of which are restricted to the campos rupestres of the Brazilian Central Plateau.

Despite uncertainties due to low bootstrap support and resolution, the tree also shows that most species with infraspecific taxa are not monophyletic (Fig. 4). Although varieties (or subspecies) of some taxa cluster together (e.g. *M. ulei* Taub.), many others do not (e.g. *M. albolanata* Taub., *M. claussenii* Benth., *M. densa* Benth. and *M. foliolosa* Benth.) (Fig. 4). At the same time, a few varieties of these non-monophyletic species form clades [e.g. *M. albolanata* var. *brasiliana* Barneby and *M. albolanata* var. *paucipinna* (Benth.) Barneby; *M. foliolosa* var. *paranani* Barneby and *M. foliolosa* var. *brevibractea* Barneby]. Finally, although in a few species (*M. adenotricha* Benth., *M. antrorsa* Benth., *M. diminuta* Marc.F.Simon & C.E.Hughes and *M. pseudofoliosa* Barneby) multiple accessions do not clade together, most multiple accessions of the same species do tend to group together in strongly supported monophyletic groups, as seen for *M. acroconica* Barneby, *M. aguapeia* Barneby, *M. heringeri* Barneby, *M. paludosa* Benth., *M. prorepens* Barneby and *M. speciosissima* Taub.

The analyses of the combined dataset with other methods and of plastid and nuclear datasets individually in general agreed with the results presented before (see Supporting Information). Differences were observed in the placement of the PIR clade, which appeared as sister to the MPS clade plus *Mimosa corynadenia* in both Bayesian and static parsimony analyses, and on DO of ITS. Individual analysis of plastid data placed *M. corynadenia* as sister to a larger clade including taxa belonging to *M*. sections *Habbasia* and *Batocaulon* DC. These differences are probably associated with the effects of DO, which is a more adequate method to analyse sequences of different length, such as the ones in our plastid data. Thus, we optimized morphological characters, discussed our results and made (conservative) taxonomic changes on the basis of the consensus tree inferred using POY.

Morphological synapomorphies

All morphological character state transformations were inferred to be homoplastic (Fig. 5), and mostly unique. Five state changes are non-unique (characters:states 1:1, 37:3, 32:2/3, 34:0 and 22:2/4) and three have ambiguous placements (72:inapplicable state, 26:7 and 4:3).

The MPS clade is supported by two synapomorphies: (1) the presence of an enlarged underground organ (1:1), which is subsequently lost (a non-unique change) in particular lineages in the clade (e.g. in the group formed by *M. caliciadenia* Barneby, *M. maguirei* Barneby and *M. acroconica*); and (2) the absence of a projection between pinnae pairs (32:0), which changes to other states (a non-unique transformation) in the clade [e.g. the presence of a laminar projection in *M. neonitens* L.M.Borges and *M. granitica* (Barneby) L.M.Borges]. It is also important to note that the unjointed craspedium (60:0) is also homoplastic, hypothesized to have evolved independently in three subclades (Fig. 5). Five synapomorphies, dichotomic branching (4:2), incurved (22:1) filiform setae with a bulbous base (7:1), absent paraphyllidia (36:0) and

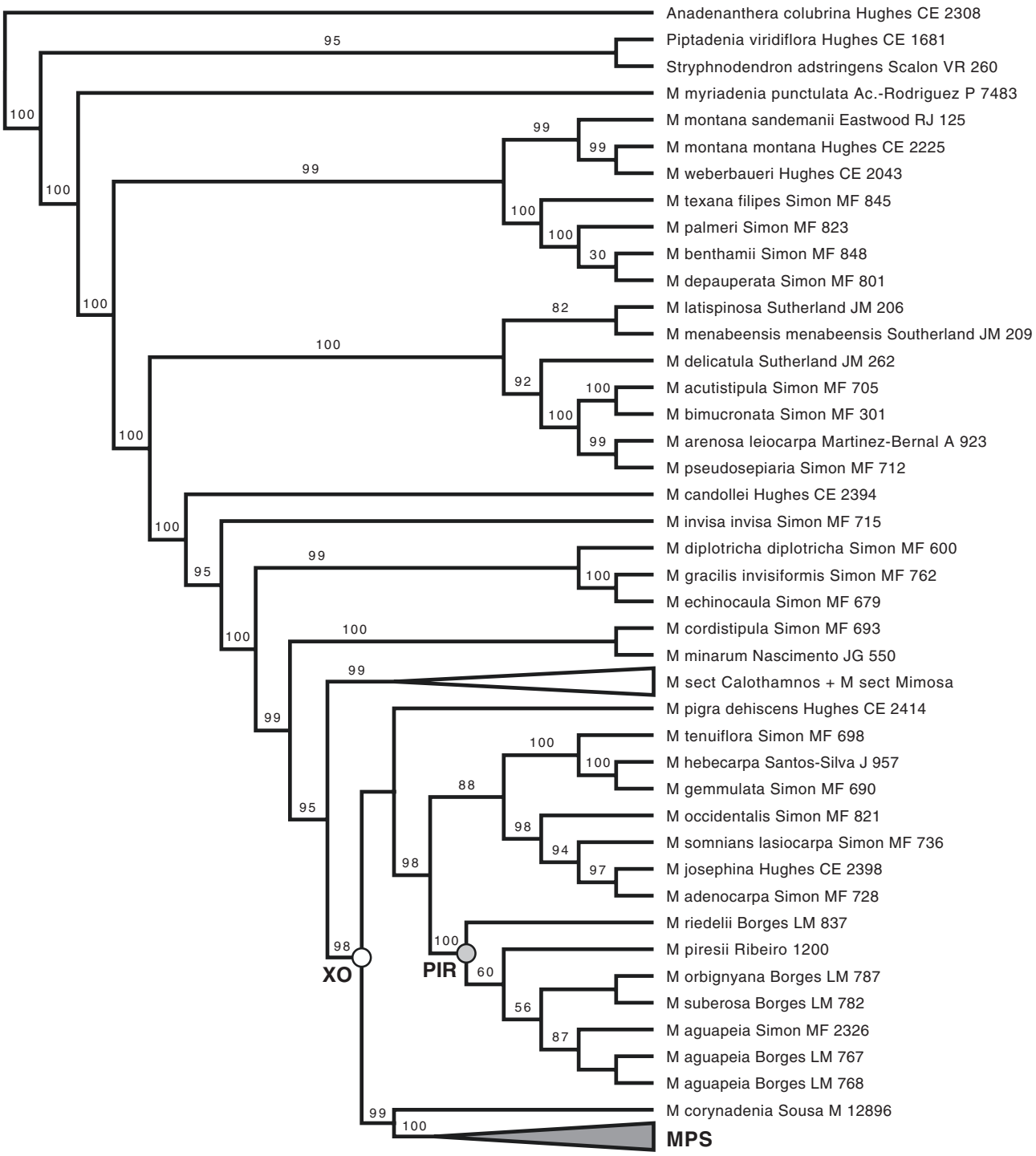

Figure 3. Phylogenetic tree for *Mimosa*. Strict consensus of 17 most-parsimonious trees of length 5240 steps found during the iterative pass optimization showing the relationship of *Mimosa* series *Pachycarpae* and *Setosae* to the remainder of the genus. Numbers above nodes are bootstrap values > 50%. See Figure 4 for details on the relationships in the MPS.

presence of glandular setae on the calyx rim (54:1), support the coreMPS clade, which forms a large polytomy. Inside this group, most characters show high levels of homoplasy.

Moving out of the MPS, the placement of a small subset of species of *Setosae* in a clade with species of *M.* series *Piresianae* is supported by six unique and one non-unique transformations: lack of interpinnal

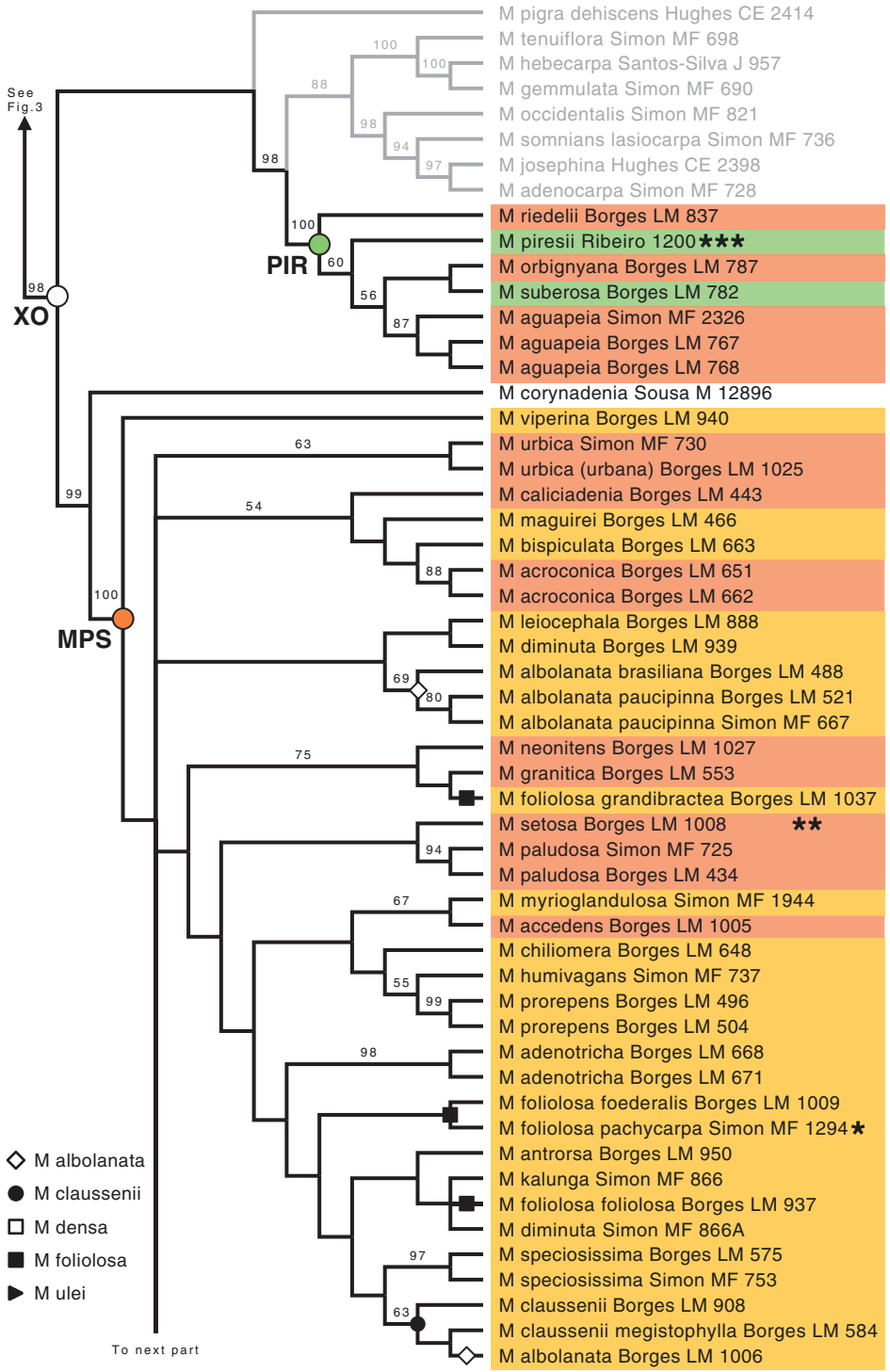

Figure 4. Phylogenetic tree of the MPS clade. Strict consensus of 17 most-parsimonious trees of length 5240 steps found during the iterative pass optimization. The tree is cropped at node XO of Figure 3. Species names in orange belong to *Mimosa* series *Pachycarpae*, in red to *M.* series *Setosae* and in green to *M.* series *Piresianae*. *type of *Mimosa* series *Pachycarpae*. **type of *Mimosa* series *Setosae*. ***type of *Mimosa* series *Piresianae*. Numbers above nodes are bootstrap values > 50%. Symbols indicate the placement of infraspecific taxa belonging to five polymorphic species.

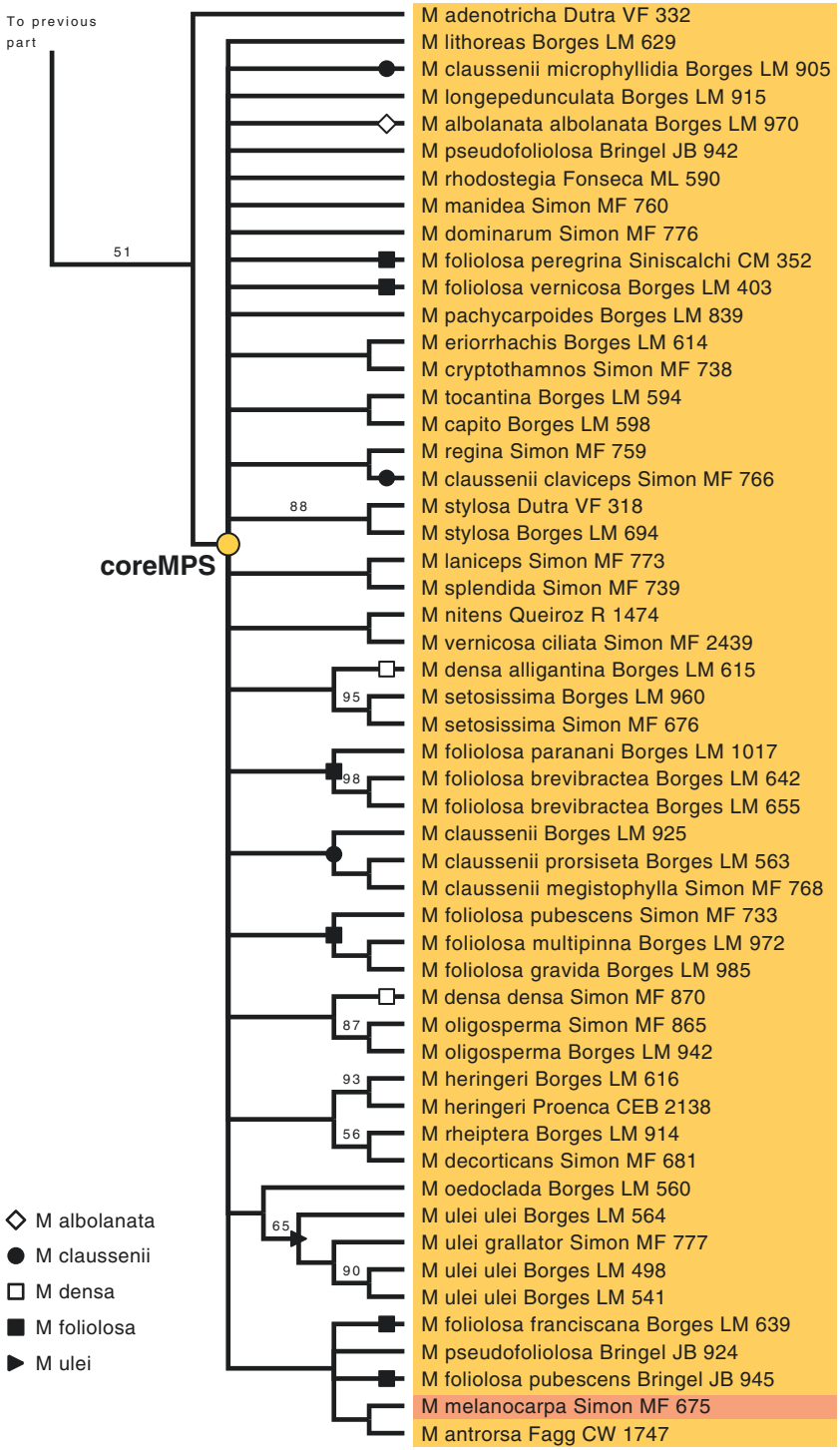

Figure 4. Continued.

projections (32:0), absence of glandular setae on rachides (35:0) and leaflet margins (41:0), absence of filiform setae on rachillas (37:0), ovate leaflets (39:3), leafless inflorescences (43:0) and presence of filiform setae on pedicels (49:1).

DISCUSSION

PHYLOGENETIC RELATIONSHIPS

Our results confirm, here with wider taxon sampling and stronger support, the close phylogenetic affinity

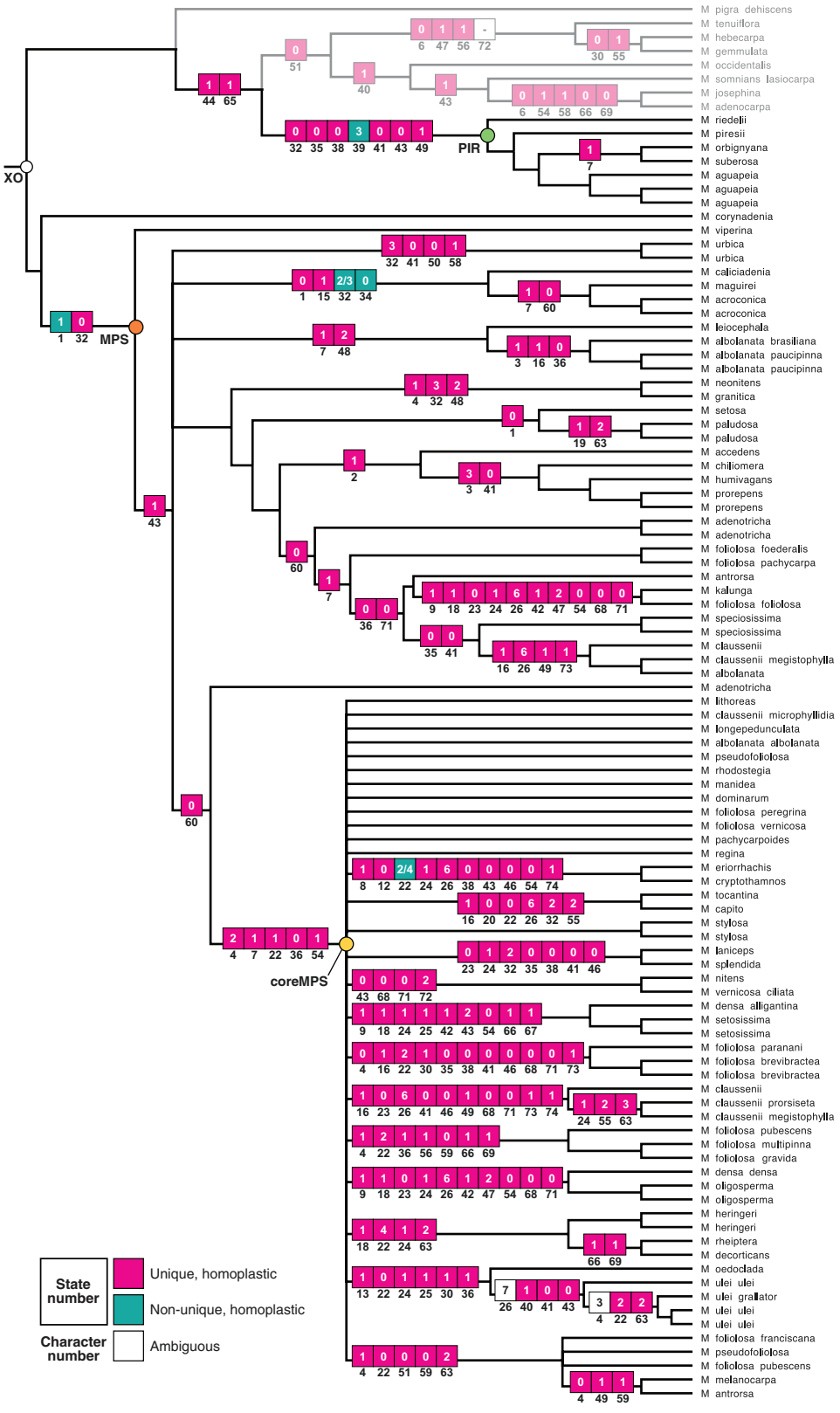

Figure 5. Morphological character state changes optimized onto the strict consensus of trees found during iterative pass optimization cropped at node XO of Figure 3. Autapomorphies are not shown. Character and character state definitions are given in the Appendix.

between *Pachycarpae* and *Setosae* (Bessega *et al.*, 2008; Simon *et al.*, 2011). Most species of these two series are placed in the MPS clade, which we show to be robustly supported (100% BS) and to include two morphological synapomorphies (see below). Another result in line with previous analyses is the placement of *M. corynadenia* as sister to the MPS clade. Although *M. corynadenia* belongs to *M.* series *Glandulosae*, this relationship is not surprising, given the morphological similarities between *M. corynadenia* and *M. caliciadenia,* a member of *Setosae* (Barneby, 1991: 350). Nonetheless, the placement of *M. corynadenia* does not reflect the overall pattern of strong geographical structure across the phylogenetic tree for *Mimosa* as a whole (Simon *et al.*, 2011). *Mimosa corynadenia* is widespread in other Central and South American countries and the only Brazilian collection comes from Amazonia (Barneby, 1991), distant from the distribution of species of *Pachycarpae*. Also, *M. adenocarpa* Benth., the only other member of *M.* series *Glandulosae* included in phylogenetic analyses, is not closely related to the MPS (Fig. 3; see also Simon *et al.*, 2011). Thus, we suggest that evolutionary or taxonomic conclusions regarding the relationship between *Pachycarpae* and *M. corynadenia* should wait for further sampling of this still poorly known species and of other members of *M.* series *Glandulosae*.

A close relationship between *Pachycarpae* and *Setosae* found here confirms previous phylogenetic analyses (Bessega *et al.*, 2008; Simon *et al.*, 2011) and was already evident in taxonomic treatments of these groups. For example, *M. setosa*, which was described on the basis of flowering material only, was originally placed in *Pachycarpae* (Bentham, 1842). Similarly, Barneby described *M. acroconica* based on specimens without fruits as belonging to *Setosae* (Barneby, 1991), whereas the extremely similar *M. bispiculata* Barneby was placed in *Pachycarpae* (Barneby, 1997). Although fruits of *M. bispiculata* are still unknown, *M. acroconica* is now known to have unjointed craspedia with papery valves, contradicting the original placement of the species in *Setosae*, which was established to minimize fuzzy delimitation of *Pachycarpae*.

Despite the broader DNA and morphological character sampling used here, we were still not able to resolve many relationships in the MPS clade (Fig. 4). Lack of resolution is especially prevalent across the coreMPS clade, hindering conclusions about relationships between different subclades. Nonetheless, two features are worth noting. First, the coreMPS, which is the largest polytomy in the MPS clade, includes most members of *Pachycarpae* and only one member of *Setosae* (*M. melanocarpa* Benth.). Species of the coreMPS occur chiefly in campos rupestres (rocky fields) and altitudinal cerrados of the Brazilian Central Plateau, a region well known for its high endemic species diversity, particularly in *Mimosa* (Simon & Proença, 2000). Second, our expanded taxonomic sampling removes any doubt about the polyphyly of *Setosae*. In contrast to previous studies that sampled three taxa (Simon *et al.*, 2011), the 12 species of *Setosae* sampled here are placed in different subclades, which in general also include taxa of *Pachycarpae*. More importantly, three species of *Setosae* are placed outside the MPS clade. These three species were already considered as morphologically distinct from core *Setosae* (Barneby, 1991) and one, *M. orbignyana* Barneby, was previously shown to fall outside of the MPS clade (Atahuachi *et al.*, 2016). Here we show that these outlying *Setosae* are related to *M.* series *Piresianae*, a group sampled for the first time by us.

Until now, phylogenetic placement of *M.* series *Piresianae*, which comprises five species, was uncertain (Simon *et al.*, 2011). When describing the series, Barneby postulated a possible relationship to *Pachycarpae*, but dismissed that idea on the basis of inflorescence arrangement and fruit morphology (Barneby, 1991: 486). We show here that *M.* series *Piresianae* is more closely related to *M.* series *Bipinnatae*, *Neptunioideae*, *Rojasianae*, *Auriculatae* Barneby and most *Leiocarpae* (clades L, M and N of Simon *et al.*, 2011). Although additional improvements in taxon sampling are needed in this part of the tree, the phylogenetic affinities of all these series, including *M.* series *Piresianae*, fits in large part with their geographical distribution centred in the western and northern limits of the Cerrado Domain. Nonetheless, it is clear from our results that *M.* series *Piresianae* should be broadened to include *M. aguapeia*, *M. orbignyana* and *M. riedelii* Benth.

Morphological synapomorphies and trait evolution

Placement of *Setosae* species together with *M.* series *Piresianae* is supported by seven morphological synapomorphies, including ovate leaflets (39:3), which are typical of these taxa, but rarely seen in other species of *Pachycarpae* and *Setosae*. Curiously, the joint occurrence of this particular leaflet shape and of ample leafless inflorescences (43:0), another synapomorphy for the PIR clade, was not previously used as evidence to place *M. aguapeia*, *M. riedelii* and *M. orbignyana* as members of *M.* series *Piresianae* (Barneby, 1991). At the same time, these three species were thought to be linked to *Pachycarpae*, in part due to the shared absence of interpinnal projections (Barneby, 1991), a feature we show to be homoplastic across the PIR and the MPS clades.

Lack of interpinnal projections and presence of an enlarged underground organ are the only two morphological synapomorphies of the MPS clade,

although both are homoplastic, show multiple subsequent reversals in the clade and characterize other unrelated lineages of *Mimosa*. The presence of interpinnal projections, either spicular or laminate, is even synapomorphic for a few small subclades, such as *M. caliciadenia*, *M. maguirei* + *M. acroconica* or *M. laniceps* Barneby + *M. splendida* Barneby. A similar situation is seen for the losses of an enlarged rootstock, particularly associated with subclades outside of coreMPS. However, there are many uncertainties regarding state changes of this character, particularly due to lack of resolution in this clade and because data on the underground organ of most treelet species are lacking. Although some of these treelets bear enlarged rootstocks during early developmental phases (e.g. *M. antrorsa*; L.M.B, pers. obs. in the field), some do not (e.g. *M. decorticans* Barneby; L.M.B, pers. obs. in the field). Moreover, unlike for many other Cerrado geoxyles (Appezzato-da-Glória & Estelita, 2000; Hayashi & Appezzato-da-Glória, 2007; Appezzato-da-Glória *et al.*, 2008; Melo-de-Pinna *et al*., 2022), detailed developmental studies are needed to confirm whether massive rootstocks in *Mimosa* are indeed xylopodia or other structures, such as lignotubers (Appezzato-da-Glória, 2015).

Despite uncertainties over the true nature of the enlarged rootstock in *Mimosa*, this organ was probably important during evolution and diversification of the MPS clade in the Cerrado (Simon *et al.*, 2009). Two prominent environmental stresses in the Cerrado, seasonal lack of water and fire, can be overcome by specialized underground organs, which store water and promote re-sprouting after damage to aerial parts (Rizzini & Heringer, 1962; Appezzato-da-Glória, 2015). Rapid re-sprouting from buds in these geoxylic systems may even be a competitive advantage over graminoids following fire, especially when fires occur frequently (Fidelis *et al.*, 2014). Functional synapomorphies that facilitate and promote establishment and diversification of a lineage in a new adaptative zone can be regarded as key innovations (Bond & Opell, 1998; Assis & Carvalho, 2010). Thus, as seen in *Chamaecrista* (L.) Moench series *Coriaceae* (Benth.) H.S.Irwin & Barneby, another species-rich Cerrado/campo rupestre endemic (Rando *et al.*, 2016), such underground organs may represent a key innovation for the MPS clade and a possible explanation for its increased rate of species diversification (Koenen *et al.*, 2013; Vasconcelos *et al.*, 2020).

The geoxyle key innovation may explain how *Pachycarpae* colonized and diversified in the fire-prone Cerrado, but it does not necessarily explain the wide morphological diversity of species of *Pachycarpae*, which is concentrated in the coreMPS clade. This clade is supported by five morphological synapomorphies, none of which appear to be of particular functional significance. At the same time, homoplasy is so high in the coreMPS that it probably would not lower significantly even after resolution improvements. If this holds true, it would indicate that the great morphological disparity observed among species of *Pachycarpae* may be linked to repeated developmental recombination, a processes in which phenotypic variation originates through changes in developmental pathways (West-Eberhard, 2005). Such changes are important evolutionary drivers (e.g. in the evolution of cichlid fish and leaf morphological diversity: Ichihashi *et al.*, 2014; Singh *et al.*, 2017). Furthermore, developmental evolution does not require genomic change and may be especially prevalent in heterogeneous environments (West-Eberhard, 2005), such as the highly patchy campos rupestres (Silveira *et al.*, 2016) where many *Pachycarpae* occur. Variations in a set of developmental switches could explain why species of *Pachycarpae* differ mainly in alternative combinations of a limited set of features. Moreover, phenotypic diversification may also arise through modification in the timing of developmental stages (heterochrony; Minelli, 2016). Heterochronic variation occurs in other mimosoid legumes (Grimes, 1992) and clearly also affected traits in *Pachycarpae*. For example, synflorescence architecture is in part related to development timing or complete suppression of leaves subtending each individual flowering shoot. Also, the loss of a single developmental step (splitting of valves) likely underpins the origin of the unjointed craspedium.

Until now, it was not possible to know whether variations in the presence of the unjointed craspedium across species of *Pachycarpae* was due to convergence or to reversals to the basic craspedium morphology. Here we show that the unjointed craspedium is not a synapomorphy for the MPS clade, and it was independently acquired in some of its subclades. Although changes from unjointed craspedium to an intermediate state occur (partially articulate valves; 60:1), complete reversals are rare and the presence of craspedia in species of *Pachycarpae* is mostly due to maintenance of a plesiomorphic state. This indicates that, even if variations in the thickness and splitting of the valve exist (Simon, Hughes & Harris, 2010), the unjointed craspedium, once gained, is evolutionarily stable. This particular fruit type with its apparently low seed dispersal capability has been considered to promote the high species endemism across the campos rupestres seen in coreMPS (Barneby, 1991). Indeed, micro-endemic species of *Pachycarpae* from that region tend to occur in small and dense populations (Barneby, 1991; Simon & Amaral, 2003). However, common widespread species with thick-walled unjointed craspedia (Simon & Hay, 2003) cast doubt on the universality of this hypothesis.

Understanding the functional, evolutionary and adaptive significance of morphology of *Pachycarpae* will require further investigations. Enhanced phylogenetic resolution is specially needed to allow for better estimation of homology/homoplasy levels, especially in coreMPS, which comprises the majority of morphological disparity in *Pachycarpae*. To that end, the Mimobaits gene set specifically developed for mimosoid legumes are promising (Koenen *et al.*, 2020). However, even with a much larger number of loci we expect high levels of gene tree conflict across the MPS clade given its apparently recent and rapid adaptive radiation (Koenen *et al.*, 2013; Vasconcelos *et al.*, 2020). This rapid diversification event is probably a function of the evolution of the Cerrado and campos rupestres flora (Simon *et al.*, 2009; Vasconcelos *et al.*, 2020; Rapini *et al.*, 2021) and intrinsic traits associated with the MPS clade, such as the presence of enlarged rootstocks. Although these and other questions regarding evolution of *Pachycarpae* remain open, the more precise definition of the MPS clade here provides a useful framework for future studies.

Taxonomic implications

Our analysis reinforces previous evidence that the current circumscription of *Mimosa* series *Pachycarpae* and *M. series Setosae* does not fit clade-based taxonomy. Thus, as suggested before (Bessega *et al.*, 2008), these two series are here fused. Given that the type taxon of each series (*M. foliolosa* var. *pachycarpa* (Benth.) Barneby and *M. setosa* Benth. var. *setosa*) are nested in the MPS (Fig. 4), we reinstate the initial circumscription of *M.* series *Pachycarpae* (Bentham, 1842, 1875, 1876), synonymizing *M.* series *Setosae* in it. In this context, species conservatively described in *Pachycarpae* (e.g. Borges *et al.*, 2014) were correctly placed. Fusion of *Pachycarpae* and *Setosae* requires segregation of all species from the latter that do not belong to the MPS clade, but are instead placed in the paraphyletic *M.* series *Piresianae* (*M. aguapeia*, *M. orbignyana* and *M. riedelii*). With this change, both *M.* series *Pachycarpae* and *M.* series *Piresianae* would be rendered monophyletic.

In the context of these updates, the definition of *Pachycarpae* once again becomes difficult due to extensive morphological variation (Bentham, 1842, 1875; Barneby, 1991), and the fact that the two morphological synapomorphies, enlarged rootstocks and lack of interpinnal projections are homoplastic. Thus, diagnoses of members of the MPS clade must be made with one-to-one comparisons with other groups of *M.* section *Habbasia* and some series of *M.* section *Batocaulon* (e.g. *M.* series *Glandulosae*; see Barneby, 1991). Nonetheless, taxa belonging to the MPS usually have an indumentum composed by simple trichomes, filiform and glandular setae, triangular stipules and infundibuliform flowers with setulose corola lobes. Interpinnal projections and aculei also occur, but these are usually restricted to species with craspedial fruits. Finally, species with these main features plus unjointed craspedial fruits can be readily placed in *Pachycarpae*, as other *Mimosa* spp. with this same fruit morphology differ in number of stamens, number of pinnae pairs and leaflet disposition (e.g. *M. brachycarpa* Benth. and members of *M.* section *Mimosa* and *M.* series *Stipellares* Benth.; see Barneby, 1991: 366).

We also show that the infraspecific classification applied to many species of *Pachycarpae* and *Setosae* (Barneby, 1991) may be unnatural. Improvements in resolution and a detailed assessment of potential gene paralogy, expected to occur in polyploid taxa, could change this view. However, although a few species of *Pachycarpae* are indeed polyploid (e.g. *M. paludosa*), most are not (Dahmer *et al.*, 2011; S. Marcal, pers. comm.). In this context, our results do not support the complex hierarchical arrangement of polymorphic species of the MPS clade, such as *M. albolanata*, *M. claussenii*, *M. densa* and *M. foliolosa* (Barneby, 1991). Species monophyly has been used as a criterion for species delimitation, including in *Mimosa* (e.g. Särkinen *et al.*, 2011), even though it is not necessary at this level of the biological hierarchy (Hennig, 1968; Crisp & Chandler, 1996; Rieppel, 2010). At the same time, it is expected that widespread species from the Cerrado Domain may not be monophyletic, particularly if speciation was allo- or parapatric (Pennington & Lavin, 2016). Nonetheless, extensive species paraphyly indicates that species hypotheses, or at least rank choice, were not strongly formulated, particularly in face of additional evidence, such as morphology. For example, the taxonomic update of *M. setosa* based on morphology and preliminary phylogenetic evidence (Borges, Simon & Pirani, 2017) agrees with our results. Revision of taxa limits, however, must be made with care, particularly for polymorphic species of the coreMPS polytomy, such as *M. claussenii* and *M. foliolosa*.

Taxonomy

Following our results, here we make the necessary taxonomic adjustments and list the species belonging to each series.

Mimosa series ***Pachycarpae*** (Benth.) Benth., Trans. Linn. Soc. London 30: 439. 1875. *Mimosa* § [unranked] *Pachycarpae* Benth., J. Bot. (Hooker) 4: 404. 1842. Type species: *M. pachycarpa* Benth. (1842: 406). Lectotype: Brazil. Minas Gerais: Vallo Fundo,

fr., *F. Sello s.n.* [syntype: F (fragment of a destroyed B specimen)] = *M. foliolosa* var. *pachyparpa* (Benth.) Barneby.

= *Mimosa* § [unranked] *Antrorsae* Benth., *J. Bot. (Hooker) 4: 403. 1842. Type species: M. antrorsa* Benth. (1842: 403). Lectotype (designated by Barneby 1991): Brazil. [Minas Gerais: ad Pedro Pereira (indicated later on Bentham, 1876)], *J.B.E. Pohl d. 1426* [=2891] (lectotype: K, isolectotypes: F, K, M, NY, US, W). *Mimosa adversa* Bentham (1875: 439), nom. subst. illeg. Syn. by Barneby (1991).

= *Mimosa* series *Setosae* Barneby, Mem. New. York Bot. Gard. 65: 350. 1991. Type species: *M. setosa* Benth. (1842: 404). Lectotype (designated by Barneby 1991: 355): Brazil. Goiás: ad Rio São Marcos [locality in Bentham (1876) (Barneby 1991)], December 1818, *J.B.E. Pohl 846* [=d. 1409] [lectotype: K (herb. Benth.); isotypes: F, K (herb. Hooker), NY, W]. **Syn. nov.**

Species list: 1. *Mimosa accedens* Barneby, 2. *M. acroconica* Barneby, 3. *M. adenotricha* Benth., 4. *M. albolanata* Taub., 5. *M. antrorsa* Benth., 6. *M. auriberbis* Barneby, 7. *M. bispiculata* Barneby, 8. *M. caliciadenia* Barneby, 9. *M. capito* Barneby, 10. *M. chiliomera* Barneby, 11. *M. claussenii*Benth., 12. *M. cryptothamnos* Barneby, 13. *M. decorticans* Barneby, 14. *M. densa* Benth., 15. *M. diminuta* Marc.F.Simon & C.E.Hughes, 16. *M. dominarum* Barneby, 17. *M. eriorrhachis* Barneby, 18. *M. foliolosa* Benth., 19. *M. gardneri* Benth., 20. *M. granitica* (Barneby) L.M.Borges, 21. *M. heringeri* Barneby, 22. *M. humivagans* Barneby, 23. *M. irwinii* Barneby, 24. *M. kalunga* Marc.F.Simon & C.E.Hughes, 25. *M. laniceps* Barneby, 26. *M. leiocephala* Benth., 27. *M. lithoreas* Barneby, 28. *M. longepedunculata* Taub., 29. *M. maguirei* Barneby, 30. *M. manidea* Barneby, 31. *M. melanocarpa* Benth., 32. *M. myrioglandulosa* V.F.Dutra & F.C.P.Garcia, 33. *M. neonitens* L.M.Borges, 34. *M. nitens* Benth., 35. *M. oedoclada*Barneby, 36. *M. oligosperma* Barneby, 37. *M. pachycarpoides* Malme, 38. *M. paludosa* Benth., 39. *M. perplicata* L.M.Borges, 40. *M. prorepens*Barneby, 41. *M. pseudofoliolosa* Barneby, 42. *M. pycnocoma* Benth., 43. *M. rava* Barneby, 44. *M. regina* Barneby, 45. *M. rheiptera* Barneby, 46. *M. rhodostegia* Barneby, 47. *M. rupigena* (Barneby) L.M.Borges, 48. *M. serpensetosa* L.M.Borges, 49. *M. setosa* Benth., 50. *M. setosissima* Taub., 51. *M. speciosissima* Taub., 52. *M. splendida* Barneby, 53. *M. struthionoptera* Barneby, 54. *M. stylosa* Barneby, 55. *M. tocantina* Taub., 56. *M. ulei* Taub., 57. *M. urbica* (Barneby) Marc.F.Simon, 58. *M. viperina* Marc.F.Simon & C.E.Hughes.

Mimosa series ***Piresianae*** Barneby, Mem. New. York Bot. Gard. 65: 486. Type species: *M. piresii* Barneby (1991: 487). Type: Brazil. Pará. Perto do Rio Xingú, 27 June 1978, *J. Murça Pires 16067* (NY; isotype: MG, RB, US).

Species list: 1. *Mimosa aguapeia* Barneby, 2. *M. dasilvae* A.S.L.Silva & Secco, 3. *M. kuhlmannii* Hoehne, 4. *M. orbignyana* Benth., 5. *M. piresii* Barneby, 6. *M. macropogon* Barneby, 7. *M. riedelii* Benth., 8. *M. suberosa* Atahuachi & C.E.Hughes.

CONCLUSIONS

With increased taxonomic and character sampling, we confirm that *Mimosa* series *Pachycarpae* and most species of *M. series Setosae* together form a clade, but are not individually monophyletic. Moreover, some species of *Setosae* are more closely related to *M.* series *Piresianae*. These relationships are supported by morphological synapomorphies, even though the typical fruit of *Pachycarpae*, an unjointed craspedium, is not one of them. Instead, one synapomorphy of the MPS clade is the presence of an enlarged underground organ. This organ, which stores water and promotes re-sprout of aerial parts, is a functional trait that may have had an adaptive role during evolution of the MPS clade in the fire-prone Cerrado.

Finally, our results indicate the need for taxonomic updates, both at infraspecific and infrageneric levels. First, the infraspecific subdivision of species of *Pachycarpae* appears to be largely unnatural, as many subspecies and/or varieties of the same species do not cluster together. This evidence supports current examination of the validity of infraspecific taxa in *Mimosa* (e.g. Borges *et al.*, 2017; Jordão, Morim & Baumgratz, 2018) and similar genera (e.g. *Chamaecrista*; Rando, Loeuille & Pirani, 2013). Second, to mirror phylogenetic structure, after transferring some species to *M.* series *Piresianae*, we merge *M.* series *Setosae* with *M.* series *Pachycarpae*. These new circumscriptions are a first step towards updating the infrageneric classification of the genus. If similar changes are consistently applied to other infrageneric groups, classification of *Mimosa* will be fully consistent with phylogenetic systematics and a more adequate source of taxonomic and evolutionary information.

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CONFLICT OF INTEREST STATEMENT

The research was conducted in the absence of any commercial, financial or personal relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY

The data underlying this article are available in the article and in its online supplementary material, as well as in GitHub at https://github.com/TaxEP/Pachycarpae_phylogeny. Morphological data is also available in MorphoBank at http://morphobank.org/permalink/?P4233.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

APPENDIX

List of morphological characters

1. enlarged underground organ: (0) absent (1) present
2. habit: (0) shrub (1) subshrub (2) treelet
3. stem, disposition: (0) erect (1) prostrate (2) deflexed (3) humifuse
4. branching system: (0) regular (1) fasciculate (2) dichotomic (3) wand-like
5. trichomes: (0) absent (1) present
6. filiform setae: (0) absent (1) present
7. filiform setae, base, shape: (0) tapering (1) bulbous
8. filiform setae, base, calcar: (0) absent (1) present
9. filiform setae, base, fusion: (0) absent (1) present
10. filiform setae, ornamentation: (0) absent (1) projections
11. filiform setae, colour: (0) ocraceous (1) whitish to grey (2) orange-red
12. glandular setae: (0) absent (1) present
13. glandular setae, shape: (0) clavate (1) capitate
14. glandular setae, stipe: (0) stipitate (1) sessile
15. prickles: (0) absent (1) present
16. branch, greyish cork: (0) absent (1) present
17. branch, peridermis, wax: (0) absent (1) present
18. branch, periderm, peeling: (0) absent (1) present
19. branch, prickles: (0) absent (1) patent (or slightly inclined) (2) antrorse (3) retrorse
20. branch, trichomes: (0) absent (1) present
21. branch, filiform setae: (0) absent (1) present
22. branch, filiform setae orientation: (0) patent (1) incurved (2) forwardly appressed (3) retrorse (4) antrorse (but not appressed) (5) inclined
23. branch, glandular setae: (0) absent (1) present (2) clavate (3) capitate
24. leaves, congestion: (0) absent (1) present
25. leaves, indumentum, distribution along primary axis: (0) homogeneously distributed (1) concentrated on pulvinoles
26. leaves, stipules, shape: (0) lanceolate/triangular (1) linear (2) ovate-lanceolate (3) ovate (4) broadly-ovate-acuminate (6) triangular (7) broadly triangular
27. leaves, stipules, base, fusion: (0) absent (1) present
28. leaves, stipules, dimorphism: (0) absent (1) present
29. leaves, dimorphic stipules shape: (0) lanceolate/triangular (1) linear (2) ovate-lanceolate (3) ovate (4) broadly-ovate-acuminate (6) triangular (7) broadly triangular
30. leaves, stipules, persitency: (0) caducous (1) persistent
31. leaves, petiole, stipels: (0) absent (1) present
32. leaves, rachis, interpinnal projection: (0) absent (1) present (2) spiculate (3) laminar (4) glandular
33. leaves, rachis, prickles: (0) absent (1) present
34. leaves, rachis, filiform setae: (0) absent (1) present
35. leaves, rachis, glandular setae: (0) absent (1) present
36. leaves, rachilla, paraphyllidia: (0) absent (1) present
37. leaves, rachilla, filiform setae: (0) absent (1) present
38. leaves, rachilla, glandular setae: (0) absent (1) present
39. leaves, leaflets, overall shape: (0) oblong (1) elliptic (2) oblong-falcate (3) ovate (4) lanceolate (5) linear
40. leaves, leaflets, secondadry veins promination in relation to primary veins: (0) not or less prominent (1) equally prominent
41. leaves, leaflets, margin, glandular setae: (0) absent (1) present
42. inflorescence, exhibition: (0) excerpt from foliage (1) nested in the foliage
43. inflorescence, associated leaf, development: (0) do not develop (1) partially at anthesis, fully when in fruit (2) fully or almost so at anthesis (3) partially at anthesis, with a diminute leaf when in fruit
44. inflorescence, secondary arrangement: (0) absent (1) frondose paniculate (2) bracteose paniculate
45. floral bract, filiform setae: (0) absent (1) present
46. floral bract, glandular setae: (0) absent (1) present
47. flower, calyx, shape: (0) cupulate (1) campanulate (2) shallowly cupulate (3) tubular
48. flower, calyx, lobes: (0) absent (1) fringed (2) shallowly triangular (3) ovate (4) triangular (5) present
49. flower, calyx, pedicel, filiform setae: (0) absent (1) present
50. flower, calyx, rim, filiform setae: (0) absent (1) present

51. flower, calyx, lobes, plane setae: (0) absent (1) present (3) ovate
52. flower, calyx, plane setae, fusion: (0) absent (1) present
53. flower, calyx, plane setae, location: (0) throughout rim (1) present just in half or less of rim
54. flower, calyx, rim, glandular setae: (0) absent (1) present
55. flower, corolla, shape: (0) infundibuliform (1) campanulate (2) narrowly infundibuliform
56. flower, corolla, lobes, trichomes: (0) absent (1) present
57. flower, corolla, lobes, filiform setae: (0) absent (1) present
58. flower, corolla, lobes, glandular setae: (0) absent (1) present
59. flower, corolla, lobes, indument coverage: (0) does not conceal surface (1) conceals surface
60. fruit, valves, segmentation: (0) integer (1) partially articulated (2) completely articulated
61. fruit, articles, time of separation relative to dehiscence of valves and liberation of seeds: (0) together (1) after
62. fruit, stipe, relative length to width: (0) less then 4 × (1) 5 × or more
63. fruit, shape: (0) oblong (1) rounded (2) narrowly oblong (3) elliptic (4) linear
64. fruit, apex, projection: (0) absent (1) present
65. fruit, margin, undulation: (0) absent (1) present
66. fruit, valves, trichomes: (0) absent (1) present
67. fruit, valves, filiform setae: (0) absent (1) present
68. fruit, valves, glandular setae: (0) absent (1) present
69. fruit, margin, trichomes: (0) absent (1) present
70. fruit, margin, filiform setae: (0) absent (1) present
71. fruit, margin, glandular setae: (0) absent (1) present
72. fruit, indumentum, setae orientation: (0) patent (1) incurved (2) forwardly appressed (3) retrose (4) antrorse (not appressed)
73. fruit, indumentum, concentric pattern of organization: (0) absent (1) present
74. fruit, valves, indument coverage: (0) does not conceal surface (1) conceals surface
75. fruit, valves, separation between exo- and endocarp: (0) absent (1) present

Additional information on primers and fragment amplification

We performed PCR of plastid fragments in 10 µL solutions containing 5 µL of Top Taq DNA polymerase (Qiagen, Crawley, UK), 3.7 µL ddH_2O, 0.15 µL of each primer (at 15 mM) and 1 µL of template DNA.

For amplification of ITS we performed two nested PCRs to maximize total DNA product and to avoid amplification of non-specific regions. Solutions of the first PCR reaction contained 0.25 µL of Taq DNA polymerase (Phoneutria, Belo Horizonte, Brazil), 1.5 µL of Buffer (10 × Platinum HF), 1.2 µL of dNTP mixture (2.5 mM), 1.2 µL of bovine serum albumin (BSA), 0.45 µL of each primer (5 µM), 0.45 µL of $MgCl_2$ (50 mM), 3.9 µL of betaine (5 M), 4.5 µL of ddH_2O, 1 µL of template DNA. Solution of the second reaction followed the composition of the first, but the DNA template was replaced by 1 µL of the first reaction product. Primer sequences are shown in Table A1. PCR conditions for *trnD-E* were 94 °C for 3 min; 30 cycles of 94 °C for 50 s, 55 °C for 1 min, 72 °C for 1.5 min and a final extension of 5 min at 72 °C; for *trnY-T* they were 80 °C for 10 min; 35 cycles of 94 °C for 1 min, 49 °C for 1 min, 65 °C for 5 min and a final extension of 4 min at 65 °C; for *trnL-F* were 95 °C for 2 min; 35 cycles of 94 °C for 1 min, 56 °C for 50 s, 72 °C for 2.5 min and a final extension of 5 min at 72 °C; for matK 94 °C for 5 min; 40 cycles of 94 °C for 50 s, 55 °C for 50 sec, 72 °C for 50 s and a final extension of 6 min at 72 °C; for ITS both reactions were set to 95 °C for 2 min; 30 cycles of 95 °C for 20 s, 50 °C for 30 s, 72 °C for 1.5 min and a final extension of 7 min at 72 °C.

Table A1. List of primer sequences used for amplification and sequencing of DNA fragments

Fragment	Primer	Sequence (5′–3′)
trnD-T	trnD2	GTG TAC AGC ATG CAT ATT CTT ACG
	trnE^uuc	AGG ACA TCT CTC TTT CAA GGA G
	trnY^gua	CCG AGC TGG ATT TGA ACC A
	trnT^ggu	CTA CCA CTG AGT TAA AAG GG
trnL-F	c	GAT TTT CAG TCC TCT GCT CTA C
	f	CG AAA TCG GTA GAC GCT ACG
matK	trnK2R	CCCGGAAC- TAGTCGGATG
	1100F	TTCAGTGGTACGGAGTCAAATG
ITS	ITS1	GTA GGT GAA CCT GCA GAA GGA
	ITS4	TCC TCC GCT TAT TGA TAT GC
	ITS5p	GGA AGG AGA AGT CGT AAC AAG
	ITS8p	CAC GCT TCT CCA GAC TAC A