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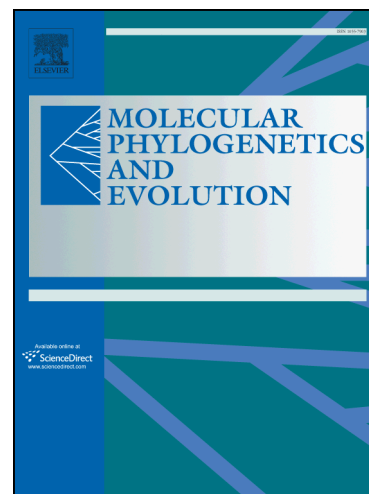
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A molecular phylogeny reveals the Cuban enigmatic genus *Behaimia* as a new piece in the Brongniartieae puzzle of papilionoid legumes

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ABSTRACT

The papilionoid legume tribe Brongniartieae comprises a collection of 13 genera with disparate morphologies that were previously positioned in at least three remotely related tribes. The Brongniartieae displays a wide geographical disjunction between Australia and the New World and previous phylogenetic studies had provided conflicting results about the relationships between the American and Australian genera. We carry out phylogenetic analyses of (1) a plastid *matK* dataset extensively sampled across legumes to solve the enigmatic relationship of the Cuban-endemic monospecific genus *Behaimia*; and (2) multilocus datasets with focus on all genera ever referred to Brongniartieae. These analyses resulted in a well-resolved and strongly-supported phylogenetic tree of the Brongniartieae. The monophyly of all American genera of Brongniartieae are strongly supported. The doubtful position of the Australian genus *Plagiocarpus* is resolved within a clade comprising all Australian genera. *Behaimia* has been traditionally classified in tribe Millettieae, but our new molecular data and re-assessment of morphological traits have unexpectedly resolved the genus within the early-branching papilionoid tribe Brongniartieae. Characters including the pinnately multifoliolate (vs. unifoliolate) leaves, a sessile (vs. stipitate) ovary, and an indehiscent or late dehiscent one-seeded pod distinguish *Behaimia* from its closer relatives, the South American genera *Cyclolobium* and *Limadendron*.


KEY WORDS: *Brongniartia*, Genistoids s.l. clade, Papilionoideae, *Plagiocarpus.*

1. INTRODUCTION

Increased molecular sampling in papilionoid legume phylogeny has dramatically changed our understanding of the evolution and taxonomic classification in this species rich lineage of economically and ecologically important legumes (family Leguminosae). The prevailing traditional hypotheses of generic relationships assumed that the papilionate flower would be a signature of the most "derived" groups, whereas the more caesalpinioid and mimosoid-like floral organizations, involving undifferentiated petals and free stamens, marked mostly the "primitive" tribes within the subfamily Papilionoideae (Polhill, 1981a). Hence, such an evolutionary perspective has largely influenced the way in which the modern subfamily, tribe, and even genus-level classification of legumes was built (LPWG, 2013, 2017). In the past 15 years, molecular phylogenetic studies have underpinned dramatic taxonomic changes after revealing unexpected relationships, suggesting that floral architecture is relatively labile in the early-branching papilionoid lineages (e.g. Pennington et al., 2001; Wojciechowski et al., 2004; Cardoso et al., 2012a, 2013a, 2015; Ramos et al., 2016).

The recent examples of broad phylogenetic re-alignments that were unexpectedly revealed in the early-branching genera of Papilionoideae (Cardoso et al., 2012a, 2012b, 2012c, 2013a, 2017; Ramos et al., 2016; Castellanos et al., in press) are not an exception across legumes. For example, dramatic shuffling in the placement of genera has also been necessary within the species-rich canavanine-accumulating clade (Wojciechowski et al., 2004; Silva et al., 2012; Sirichamorn et al., 2012, 2014; Queiroz et al., 2015). There are, however, few cases of generic interchange between lineages below (less inclusive) or above the (more inclusive) the node defining the canavanine-accumulating clade. This contrasts with the striking example of the changing historical circumscription of the tribe Brongniartieae (Queiroz et al., 2010).

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The Brongniartieae was defined by Arroyo (1981) to include only the amphitropical American genera *Brongniartia* Kunth and *Harpalyce* Moç. & Sessé ex DC., mostly for sharing a strongly bilabiate calyx and an embryo with a straight radicle, with the latter trait then considered as "underlining the ancient nature of the assemblage" (Arroyo, 1981: 387). However, these traits are also found in the so-called "Templetonia group" of the Australian tribe Bossiaeeae, leading both Polhill (1981c) and Arroyo (1981) to recognize the possible phyletic connection between the two groups with "their taxonomic separation rest[ing] on the grounds of convenience" (Arroyo 1981: 387). So it was not a surprise that phylogenetic studies found convincing evidence that the "Templetonia group" is more closely related to the American Brongniartieae than to the Australian "Bossiaea group" of the tribe Bossiaeeae (Crisp and Weston, 1987; Crisp et al., 2000; Kajita et al., 2001; Thompson et al., 2001; Wojciechowski et al., 2004; Cardoso et al., 2012a, 2013a). Crisp and Weston (1987) then formally transferred the genera *Hovea* R.Br. ex W.T. Ait., *Lamprolobium* Benth., *Plagiocarpus* Benth., and *Templetonia* R. Br. ex Ait. to the tribe Brongniartieae.

The American genera *Cyclolobium* Benth. and *Poecilanthe* Benth., currently placed in Brongniartieae, also present tortuous taxonomic histories. They were once considered as members of the subtribe "Pterocarpeae" of the tribe Dalbergieae (Bentham, 1865) or of the tribe Millettieae (as Tephrosieae; Geesink, 1981). *Poecilanthe* was later transferred to Robinieae (Geesink, 1984) but this was not supported by phylogenetic analyses of morphological (Lavin, 1987) or molecular data (Kajita et al., 2001; Wojciechowski et al., 2004), the latter studies suggesting a closer phylogenetic relationship with *Brongniartia*, *Harpalyce*, and *Hovea* of Brongniartieae. The inclusion of both *Cyclolobium* and *Poecilanthe* in the Brongniartieae was unequivocally demonstrated by Hu et al. (2000, 2002) and later confirmed by more

comprehensively-sampled phylogenetic analyses (Wojciechowski et al., 2004; Queiroz et al., 2010; Cardoso et al., 2012a, 2013a; Meireles et al., 2014). The latest addition to the Brongniartieae was the description of *Tabaroa* L.P. Queiroz, G.P. Lewis & M.F. Wojc., a newly discovered genus of one tree species from the Brazilian seasonally dry woodland or Caatinga vegetation (Queiroz et al., 2010). Together with this new taxon, the reinstatement of *Amphiodon* Huber, the segregation of the new genus *Limadendron* Meireles & A.M.G.Azevedo from *Poecilanthe* (Meireles and Azevedo-Tozzi, 2014; Meireles et al., 2014), and of *Cristonia* J.H. Ross and *Thinicola* J.H. Ross from *Templetonia* (Ross, 2001), the Brongniartieae now comprises 13 genera and c. 153 species (Ross and Crisp, 2005; Queiroz et al., 2010). It is worth emphasizing that these genera were once placed in at least three tribes of Polhill's Papilionoideae classification (1981a), namely the Brongniartieae from the early-branching Genistoid s.l. clade, and the Bossiaeeae and Millettieae from the canavanine-accumulating clade, thus demonstrating the morphological disparity and puzzling nature of the tribe. More recently, the African monospecific genus *Haplormosia* Harms was demonstrated as sister to the American-Australian tribe Brongniartieae (Cardoso et al., 2017). This genus has long been classified in the tribe Sophoreae and could represent another phylogenetically disparate member of the Brongniartieae, yet morphologically unusual in the tribe because of its free stamens.

Here we further refine the phylogeny and taxonomy of the Brongniartieae by fitting yet another unexpected piece in the puzzle that is this tribe. We report that the enigmatic Cuban-endemic, monospecific genus *Behaimia* Griseb., formerly of the tribe Millettieae (Geesink, 1984; Lewis, 1988; Schrire, 2005), is nested within the early-branching tribe Brongniartieae of the Genistoid clade. *Behaimia* was never formally

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associated with Brongniartieae before and it is among the genera of Leguminosae

without representation in any previously published molecular analysis (LPWG, 2013).

*Behaimia* is represented in herbaria by rather old and poorly-preserved

specimens but was recently collected by one of us (BMT) in Seasonally Dry Tropical

Forests over limestone deposits in south-central Cuba. The phylogeny of the

Brongniartieae is revisited to investigate the relationships between the American and

Australian genera and to confirm the position of *Behaimia* based upon molecular

phylogenetic analyses of (1) a broad sampling of the plastid *matK/trnK* region of

papilionoid legumes, and (2) a multilocus dataset.

## 2. MATERIAL AND METHODS

### 2.1. Taxon sampling and molecular data

Two sets of phylogenetic analyses were carried out in order to test the

phylogenetic position of *Behaimia* and investigate relationships within Brongniartieae.

A broad-level analysis of the Papilionoideae phylogeny based on the protein-coding

plastid *matK* gene of 975 accessions, including 787 accessions of Papilionoideae and a

broad outgroup of 183 accessions of other Leguminosae subfamilies (Cercidoideae: 6;

Duparquetioideae: 1; Dialioideae: 11; Detarioideae: 77; Caesalpinioideae: 86), and

seven other Fabales, was designed to investigate the position of *Behaimia* within the

papilionoid legumes, especially with respect to the tribes in which it was previously

classified. To accomplish this aim, all major lineages of the papilionoids (Cardoso et al.,

2012a, 2013a) were included, mostly from previously published *matK* sequences (e.g.,

Hu et al., 2000; Lavin et al., 2001, 2003; McMahon and Hufford, 2004; Wojciechowski

et al., 2004; Queiroz et al., 2010, 2015; Queiroz and Lavin, 2011; Delgado-Salinas et

al., 2011; Cardoso et al. 2012a, 2013a, 2015; Meireles et al., 2014; Snak et al., 2016).

Additionally, 35 new *matK* sequences of Brongniartieae were added to the dataset

(Table 1) covering all genera ever referred to the tribe (Ross and Crisp, 2005; Queiroz et

al., 2010).

The second set of analyses involved a multilocus phylogenetic approach to

investigate the generic relationships within the Brongniartieae. Individual and combined

phylogenetic analyses of plastid (*matK/trnK* and *trnL* intron) and nuclear rDNA

(ITS/5.8S) sequences were performed from a total sampling of 193 accessions of 49

species of the Brongniartieae. The outgroups for the second dataset were selected from

the Leptolobieae clade, following the results of the broad *matK* analyses of the

papilionoid legumes (see also Cardoso et al., 2012a, 2013a) and included the African

monospecific genus *Haplormosia*, recently demonstrated as the putative sister group of

the tribe Brongniartieae (Cardoso et al., 2017). Most molecular data came from a large

sampling of available sequences in GenBank (http://www.ncbi.nlm.nih.gov/genbank/)

as previously published in our phylogenetic studies of the Brongniartieae (Queiroz et

al., 2010; Cardoso et al., 2012a, 2017) and complemented with 14 new sequences of

ITS/5.8S, 35 of *matK/trnK* and 26 of *trnL* intron regions.

To avoid a large amount of missing data in concatenated analyses, we organized

our matrices by species instead of by accession (Table 1), except for some accessions of

the genera *Plagiocarpus* and *Brongniartia* for which there were conflicting results in

previous phylogenetic studies. ITS1 and *matK* accessions of the Australian

*Plagiocarpus axillaris* Benth. were reported in quite disparate positions in previous

works: the ITS1 accession embedded in the American genus *Brongniartia* (Thompson

et al., 2001), whereas the *matK* accession appeared in a clade together with the

Australian genera *Hovea*, *Lamprolobium*, and *Templetonia* (Queiroz et al., 2010).

Coincidently these two accessions were prepared from the same specimen (*M. Barrit 1406*, later renamed as *Plagiocarpus arcuatus* I.Thomps. by Ian Thompson, MEL) but we kept them separate in our analyses to test their probable position by sequencing a second accession of *P. axillaris* and another species of *Plagiocarpus*. Furthermore, the monophyly of *Brongniartia* was considered suspicious because *matK* accessions of the Bolivian *B. ulbrichiana* Harms did not group with a clade containing the Mesoamerican species of the genus (Queiroz et al., 2010), a result conflicting with our current individual analyses of ITS/5.8S and *trnL* intron regions. We kept the *matK/trnK* accession (from Queiroz et al., 2010) separate from a specimen newly sequenced for all regions to test the probable position of *B. ulbrichiana* with respect to other species of the genus.

## 2.2. DNA amplification and sequencing

DNA isolations from silica-gel dried leaf material for polymerase chain reaction (PCR) amplifications were performed mostly with Qiagen Kits (Qiagen, Santa Clarita, California) or with a modified 2× CTAB protocol (Doyle & Doyle, 1987). PCR amplifications were prepared in two ways. The first prepared in a final volume of 10 µL containing 6 µL of TopTaq Master Mix Kit (Qiagen GmbH, Hilden, Germany), 0.2 mM of each primer, 2 µL of TBT-PAR [trehalose, bovine serum albumin (BSA) and polysorbate-20] (Samarakoon et al., 2013), 1 µL of ×10 DNA template and adjusted with de-ionized water. The second protocol used 10 µL containing 1 µL of 10× PCR buffer, 0.5 µL of $MgCl_2$ (2.5 mM), 0.2 µL of dNTPs (10 mM), 2 µL TBT, 0.15 mM of each primer, 0.1 µL of *Taq* polymerase (Fenix Life Science, Madrid, Spain), 1 µL of total DNA or 2 µL of ×10 DNA template and adjusted with de-ionized water. We added 0.2 µL of DMSO for PCR of the ITS/5.8S region.

To amplify the *matK/trnK* region we used the forward primers trnK685F, matK4La, and matK1100L, and the reverse primers matK1932Ra, matK4LR, and trnK2R* (Wojciechowski et al., 2004). Reaction conditions for the *matK/trnK* region consisted of 4 min denaturing step at 94°C, followed by 40 cycles of 1 min at 94°C (denaturation), 30 s at 51–55°C (annealing), 1 min at 72°C (extension), and further extension for 7 min at 72°C. For the *trnL* intron amplification we used the two universal primers C (forward) and D (reverse) (Taberlet et al., 1991), and PCR conditions consisting of 3 min denaturing step at 94°C, 40 cycles of 1 min at 94°C (denaturation), 30 s at 50°C (annealing), 1 min at 72°C (extension), and further extension for 10 min at 72°C. For the nuclear ribosomal ITS/5.8S region we used the amplification primers 17SE and 26SE (Sun et al., 1994) and the sequencing primers ITS 92 (Desfeux and Lejeune, 1996) and ITS 04 (White et al., 1990). The ITS/5.8S region was amplified using a run program with a 3 min denaturing step at 94°C, followed by 28–30 cycles of 1 min at 94°C (denaturation); 1 min at 50–52°C (annealing); 2:30 min at 72°C (extension) and further extension for 7 min at 72°C.

PCR products were purified and sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems/Life Technologies Corporation, Carlsbad, California, USA). The products of sequencing were analyzed in the sequencer 3130 XL Genetic Analyzer (Applied Biosystems) at the Laboratório de Sistemática Molecular de Plantas (LAMOL) of the Universidade Estadual de Feira de Santana, Bahia, Brazil.

*2.3. Alignment and phylogenetic analyses*

The original chromatogram files were assembled into final sequences using the Geneious platform (Drummond et al., 2012). Sequences were automatically aligned in MUSCLE using default settings (Edgar, 2004) and then manually adjusted in SeaView

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v.4 (Gouy et al., 2010), using the similarity criterion described by Kelchner (2000). For the multilocus phylogenetic analyses focusing on the Brongniartieae, we prepared individual (single locus) datasets as well as datasets of combined partitions of plastid (*matK/trnK* + *trnL*) and nuclear and plastid (ITS/5.8S + *matK/trnK* + *trnL*) regions.

Searches for the most parsimonious trees were carried out in PAUP* 4.0b10 (Swofford, 2002) using heuristic methods with 1000 random taxon-additions and tree bisection-reconnection (TBR) branch swapping, saving 15 trees per replicate. Trees saved in this first round were used as starting trees in a second search using the same parameters, but saving a maximum of 10,000 trees. All character state transformations were weighted equally and unordered (Fitch, 1971). Nonparametric bootstrap resampling (BS; Felsenstein, 1985) was implemented to estimate clade support, which was assessed through 2000 BS replicates, each analyzed using the heuristic search parameters mentioned above and with 15 trees retained per replicate.

The combinability of the individual plastid and nuclear DNA markers and putative incongruence in the resulting tree topologies were assessed by comparing clade support (Wiens, 1998). Because Bayesian posterior probability values are often biased high (Alfaro et al., 2003; Erixon et al., 2003; Suzuki et al., 2002), we used the more conservative parsimony bootstrap supports to identify clade conflict between the DNA partitions. Incongruent clades with bootstrap supports >80% were taken as evidence for not combining data sets. Based on this criterion, we did not find any evidence of strong conflict between the individual data partitions; hence they were used in different sampling strategies for combined phylogenetic analyses. We have avoided the parsimony-based partition homogeneity test (incongruence length difference test; Farris et al., 1994), because it has been proven to produce misleading results (Dolphin et al., 2000; Yoder et al., 2001; Barker and Lutzoni, 2002).

The best-fit substitution model was selected for each dataset using the Akaike information criterion implemented in MrModelTest v. 2.2 (Nylander, 2004). GTR+$\Gamma$ was selected for plastid partitions, GTR+I+$\Gamma$ for ITS1 and ITS2 regions and the simpler model K80+ $\Gamma$ for the 5.8S region of the ITS (Table 2). The Bayesian analyses were carried out using MrBayes v.3.2.6 (Ronquist et al., 2012). Two separate runs of a Metropolis-coupled Markov Chain Monte Carlo (MCMC) permutation of parameters were each initiated with a random tree and eight simultaneous chains set at default temperatures (Ronquist and Huelsenbeck, 2003). Markov chains were run for $7\times10^{7}$ generations for the broad papilionoid legume *matK* dataset, and for $10^{7}$ generations for the second set of analyses, sampling every 1,000[th] generation. Convergence of runs was tested by inspecting whether the standard deviation of split frequencies of the runs was <0.01 and by using the effective sample sizes (ESS) >200 as calculated with Tracer v.1.5 (Rambaut and Drummond, 2007). We then used MrBayes command "sumt" to summarize 75% of the trees sampled from post burn-in generations into a 50% majority rule consensus tree that included posterior probabilities (PP) as branch support estimates.

Maximum Likelihood (ML) analyses were performed both for the broad *matK* dataset and for the second set of analyses using RAxML v.8 (Stamatakis, 2014), using GTRCAT as the evolutionary model and estimating gamma distribution and invariant sites during the run. Clade supports were assessed through 1000 BS replications.

All Bayesian and ML analyses were run in the Cyberinfrastructure for Phylogenetic Research (Cipres Science Gateway; Miller et al., 2010). All MP strict consensus trees, ML trees, and Bayesian 50% majority-rule consensus trees were visualized and partially edited in FigTree v.1.4 (Rambaut, 2012).

3. RESULTS

Both Bayesian and ML analyses of the broad *matK* dataset of 975 accessions recovered the genus *Behaimia* within a highly supported monophyletic Brongniartieae (PP = 1; BS = 100), where it appears as sister to the South American genus *Cyclolobium* (PP = 1; BS = 89). The Bayesian and the ML (LnL = -93503.79853) trees show the Brongniartieae as sister to the African genus *Haplormosia* and this *Haplormosia*-Brongniartieae clade appeared sister to the Leptolobieae with moderate to high support (PP = 0.99; BS = 78), and they collectively are sister to the core Genistoid clade (PP = 0.98; BS = 70; Figs. 1, S1).

Most phylogenetic analyses of individual and combined datasets focusing on Brongniartieae strongly support *Behaimia* as sister to *Cyclolobium* and this *Behaimia*-*Cyclolobium* clade as sister to the recently described Amazonian genus *Limadendron* (a segregate of the genus *Poecilanthe*) (Figs. 2, S2; Table 1). The exception was the analysis of the individual *trnL* intron dataset in which *Behaimia* appeared in a large unresolved polytomy (Fig. S2-C). The American taxa appeared as a basal paraphyletic grade comprising four subsequently diverging but strongly supported clades: (1) the genus *Poecilanthe*; (2) a clade comprised of the genera *Amphiodon*, *Tabaroa*, and *Harpalyce*; (3) a clade including *Limadendron*, *Behaimia*, and *Cyclolobium*; and (4) the genus *Brongniartia* with the ITS/5.8S accession of *Plagiocarpus axillaris* nested in it. The Australian genera comprise a clade sister to *Brongniartia* although this Australian clade received higher support only in the Bayesian analysis of the ITS/5.8S-*matK/trnK*-*trnL*, plastid (*matK/trnK*-*trnL*), and individual *matK/trnK* datasets (Figs. 2, S2).

The proportion of missing data in the combined datasets ranged between 18.2% and 19.7%, but this had no effect either in the resolution of the backbone of the tree or in supporting the (*Limadendron* (*Behaimia*, *Cyclolobium*)) clade (Table 2).

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4. DISCUSSION

*4.1. The Brongniartieae within the Genistoids s.l.*

All previous phylogenetic studies have shown the Brongniartieae to be one of the main subclades within the Genistoids s.l., the latter a species-rich early-branching papilionoid lineage including 94 genera and over 2,400 species, chiefly distributed in the Southern Hemisphere (Crisp et al., 2000; Cardoso et al., 2013a). The current circumscription of the Genistoids s.l. represents a broad expansion of the initial definition of the clade (Crisp et al., 2000; Wojciechowski et al., 2004). This resulted from much denser sampling that incorporated genera not previously sampled for molecular data, and by resolving deep polytomies, involving lineages such as the Ormosieae clade, which has been found as sister to the remaining Genistoids s.l., and namely the yet unresolved lineages of Brongniartieae, Leptolobieae and the core Genistoid clade (Cardoso et al., 2012a, 2013a). However, none of the previous studies clearly resolved the position of the Brongniartieae with respect to the other lineages of the Genistoids s.l. (Pennington et al., 2001; Wojciechowski et al., 2004; Cardoso et al., 2012a, 2013a). Our ML and Bayesian analyses of the broad *matK* dataset of papilionoid legumes included a denser sampling in the Brongniartieae (63 accessions in 37 species of 13 genera sampled here vs. 18 accessions in 16 species of 10 genera in Cardoso et al., 2013a). For the first time, Brongniartieae was recovered as sister to the Leptolobieae clade with moderate to high support (Figs. 1, S1).

Resolution of the Brongniartieae-Leptolobieae-core Genistoids polytomy is critical to a better understanding of floral morphology and chemical evolution in this spectacular radiation of early-branching papilionoids. Although sharing the more

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advanced papilionate flowers with a bilabiate calyx, joined stamens, and dimorphic

anthers with most genera in the core Genistoids (Boatwright et al., 2008), the

Brongniartieae shares with the Leptolobieae the presence of minute colleter-like glands

in the axils of leaflet pulvinules or within the stipules, bracts or bracteoles (Cardoso et

al., 2012c). This find implies that similar floral organization in Brongniartieae and core

Genistoids should have resulted from independent evolution from a weakly papilionate

flower involving free stamens and incompletely differentiated petals like those found in

Leptolobieae and in the early-branching lineages of the Genistoids s.l. clade (Cardoso et

al., 2012c, 2013a), demonstrating once again the labile nature of flower architecture in

the early-branching lineages of the papilionoid legumes (Cardoso et al., 2013b). Also, it

would imply an ancient divergence between the New World lineages of Brongniartieae

and Leptolobieae with respect to the predominantly African-Eurasian core Genistoids

(Lavin et al., 2005).

*4.2. Monophyly and relationships in the Australian clade of Brongniartieae*

The backbone of the Brongniartieae phylogeny shows an Australian clade of

genera nested within an otherwise American grade, with the American genus

*Brongniartia* more closely related to the Australian clade than to the remaining New

World genera. This pattern was recovered by different studies and with different

sampling densities using the plastid genes *matK* (Queiroz et al., 2010; Cardoso et al.,

2012a, 2013a) and *rbcL* (Kajita et al., 2001). However, sampling of nuclear ITS/5.8S

sequences cast doubt on the monophyly of the Australian group because *Plagiocarpus*

*axillaris* appeared embedded in the American *Brongniartia* (Thompson et al., 2001), a

result that was taken as evidence that *Plagiocarpus* could be more closely related to

*Brongniartia* than to the remaining Australian genera (Thompson et al., 2001; Ross and

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Crisp, 2005). In our analyses, the *matK* accession of *P. axillaris* appeared nested in a clade together with a second accession of *P. axillaris* and *P. conduplicatus* I.Thomps., whereas the ITS1 accession of the same specimen (taken from Thompson et al., 2001) was nested within the genus *Brongniartia* (Figs. 2, S2-A, C). *Plagiocarpus conduplicatus* and the second accession of *P. axillaris* were sampled here for all analyzed markers (ITS/5.8S, *matK/trnK*, and *trnL*) and they appeared nested within the Australian clade together with *Cristonia*, *Lamprolobium*, and *Thinicola*. This result is similar to that obtained in a previous morphological parsimony analysis of the Brongniartieae (Thompson et al., 2001). In that study, *Plagiocarpus axillaris* was found in the same clade as *Cristonia* [as *Templetonia biloba* (Benth.) Polhill], *Lamprolobium*, and *Thinicola* [as *Templetonia incana* J.H.Ross], supported by the exclusive synapomorphy of glandular hairs in the leaf axils, thus being consistent with the position of the *matK* accession of *P. axillaris*.

Geographical patterns of the tribe Brongniartieae also seem to agree with the position of the *matK* accession of *P. axillaris*. The tribe is widely disjunct between the New World and Australia and it is more probable that the Australian group is the result of an unique dispersal event (which is supported if we take into consideration the position of the *matK* accession), rather than two independent long-distance dispersal events from the New World to Australia, as implied by the position of the ITS/5.8S accession of *P. axillaris*. Thus, results from our analyses, together with morphological and biogeographical evidence concur in that the phylogenetic position of *Plagiocarpus* is not nested in the American *Brongniartia* (Thompson et al., 2001; Ross and Crisp, 2005), but very likely closely related to *Cristonia*, *Lamprolobium*, and *Thinicola* within the Australian clade (Fig. 2).

Our results also add strong support for the segregation of *Cristonia* and *Thinicola* from *Templetonia* (Ross, 2001; Thompson, 2010b). Morphological parsimony analysis already indicated that *Templetonia* [*Cristonia*] *biloba* and *Templetonia* [*Thinicola*] *incana* are more related to the genera *Lamprolobium* and *Plagiocarpus* than to the remaining species of *Templetonia* (Crisp and Weston, 1987). A similar result was obtained in a phylogenetic study using the nuclear ITS1 region (Thompson et al. 2001; but see the discussion about *Plagiocarpus* above). Alkaloid profiles also support the hypothesis that *Cristonia* and *Thinicola* are more related to *Lamprolobium* and *Plagiocarpus* since all these genera accumulate bicyclic quinolizidine alkaloids and mainly $\alpha$-pyridone bases, but lack the ormosanine-type alkaloids that are typical of the other species of *Templetonia* and *Hovea* (Greinwald et al., 1995a, 1995b, 1995c, 1996a, 1996b).

The remaining species of *Templetonia* are clustered in two highly supported clades, each corresponding to two morphologically distinct groups. *Templetonia egena* (F.Muell.) Benth. and *T. sulcata* (Meisn.) Benth.belong to a group of leafless species with the branches green, ridged or angular, and occasionally flattened like a cladode, whereas the *T. hookeri-T. retusa* clade comprises leafy species. The "leafless clade" is additionally distinguished from the "leafy clade" of *Templetonia* by having taxa with relatively small flowers, short pedicels, brown-chartaceous bracts and bracteoles, and few-seeded, non-partitioned pods (Thompson, 2010a) and could merit status as a distinct genus. However, phylogenetic relationships between the "leafy" and "leafless" clades and the clade comprising other Australian taxa were not resolved (Fig. 2), and sampling was rather sparse (two of seven species in the leafless clade and four out of six in the leafy clade), thus precluding any inference concerning alternative taxonomic solutions.

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*4.3. Relationships in the American grade of Brongniartieae*

Our multilocus analyses confirm that *Brongniartia* is phylogenetically more

related to the Australian clade (Thompsom et al., 2001; Queiroz et al., 2010; Cardoso et

al. 2012a, 2013a) than to the American Brongniartieae taxa. However, even without

considering the ITS/5.8S accession of *Plagiocarpus axillaris* (see discussion above), the

monophyly of the genus *Brongniartia* was considered doubtful because of the finding

that *Brongniartia ulbrichiana* did not cluster with other species of the genus in a

previous *matK* analyses (Queiroz et al., 2010). Our analyses of the individual ITS/5.8S

and *trnL* intron data sets and of the combined plastid and ITS/5.8S-*matK/trnK-trnL*

datasets recovered *Brongniartia* as monophyletic (excluding *P. axillaris*) with high

support (Figs. 2, S2). However, individual analyses of the *matK/trnK* region did not

recover *B. ulbrichiana* as sister to the remaining *Brongniartia* either in the broad

analysis of the papilionoid legumes or in the analysis of the Brongniartieae (Figs. 1, S1,

S2-B). Thus, the previous hypothesis of non-monophyly of *Brongniartia* (Queiroz et al.,

2010) seems to be an idiosyncrasy of the results of analysis of the *matK/trnK* region,

perhaps the result of a greater molecular divergence of *B. ulbrichiana* from the clade

that includes the remaining species of the genus, with some homoplasious base

substitutions. Thus, most of the evidence supports the monophyly of *Brongniartia* with

an amphitropical distribution pattern, where the Bolivian *B. ulbrichiana*, which is

diagnosed by all stamens joined in a tube, appears as sister to a larger Mesoamerican,

mostly Mexican clade comprising the species with a free vexillary stamen (but with

only nine out of c. 65 species sampled here; Dorado, 1999; Ross and Crisp, 2005). A

similar geographically structured phylogenetic pattern was also recovered in *Harpalyce*,

in which a Mesoamerican clade (section *Harpalyce*) and an Eastern Brazilian clade

(section *Brasilianae*; Arroyo, 1976) are reciprocally monophyletic (Fig. 2). The biogeographic history and associated diversification processes in Neotropical Brongniartieae will be further assessed in a more comprehensively-sampled analyses (São-Mateus, Cardoso, Queiroz et al., unpublished data). If such geographical and phylogenetic patterns in *Brongniartia* and *Harpalyce* found here are confirmed with broader sampling, it could shed light on the processes that drove the wide geographic disjunctions observed within the tribe.

The dry forest-inhabiting Eastern Brazilian genus *Tabaroa* was previously reported as sister to the genus *Harpalyce* by Queiroz et al. (2010). The rain forest Amazonian endemic genus *Amphiodon* was not sampled in that study, but all other phylogenetic studies that sampled these three genera recovered *Tabaroa* as sister to *Amphiodon*, nested within a (*Harpalyce* (*Tabaroa*, *Amphiodon*)) clade (Cardoso et al., 2012a, 2013a; Meireles et al., 2014). This relationship is also confirmed here using a more comprehensive sampling of taxa and genes (Figs. 1, 2, S2). The flowers of *Tabaroa* are superficially more similar to those of *Amphiodon* (Cardoso et al., 2012a), but differ by possessing a calyx with imbricate lobes (vs. valvate lobes in *Amphiodon*), all ten stamens joined in a tube with apiculate anthers (vs. the vexillary stamen free and anthers not apiculate) and a 2-ovulate ovary (vs. 7–9-ovulate). *Tabaroa* and *Harpalyce* share an explosive pollen display (Queiroz et al., 2010). While such data are unavailable for *Amphiodon*, flowers in herbarium sheets do not seem to possess this feature.

*4.4. Phylogenetic position of* Behaimia

All evidence derived from our analyses unequivocally indicate that *Behaimia* should be placed in tribe Brongniartieae, thus rejecting previous proposals that considered it close to *Lonchocarpus* Kunth and related genera, and then moved to

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different tribes where *Lonchocarpus* had been placed, such as Dalbergieae (Taubert, 1894), Lonchocarpeae (Hutchinson, 1964) or Millettieae (Geesink, 1984; Schrire, 2005). The putative relationship of *Behaimia* to *Lonchocarpus* was probably based on the two genera sharing indehiscent fruits, imparipinnate leaves with opposite leaflets, and papilionate flowers with stamens joined in a diadelphous androecium with the vexillary stamen free from the sheath made by the remaining nine stamens. Additionally, the androecium of *Behaimia* was described as possessing uniform anthers (Hutchinson, 1964), which reinforced its association with *Lonchocarpus*. However, dissection of flower buds of *B. cubensis* (*E. L. Ekman 9293*, K; *F. Rugel 342*, K) reveals that anthers are alternately short and dorsifixed, and, long and basifixed, a condition found in most genera of Brongniartieae (Queiroz et al., 2010).

The present inclusion of *Behaimia* in Brongniartieae is clearly justified also on morphological grounds. Except for the morphologically divergent genera *Brongniartia* and *Harpalyce*, most American genera of Brongniartieae have small flowers borne in short racemes, a calyx typically bilabiate with at least the two vexillary (adaxial) lobes united, and showing some degree of anther dimorphism (Queiroz et al., 2010). All of these features also characterize *Behaimia*. In fact, although *Behaimia* was not considered related with the Brongniartieae before, its possible relationship with *Poecilanthe* and *Cyclolobium* was previously suspected by Polhill (1981b: 235), who stated that there were some "small genera whose position remains equivocal" amongst them "*Poecilanthe*, *Cyclolobium* and *Behaimia* form a small complex referred to Tephrosieae" [= Millettieae]. After analyses of nuclear rDNA ITS/5.8S (Hu et al., 2002), plastid *matK* (Hu et al., 2000) and *rbcL* (Kajita et al., 2001) demonstrated that *Poecilanthe* and *Cyclolobium* should be moved to Brongniartieae, it is not surprising

that the morphologically similar *Behaimia* should also be found to belong within this tribe.

Within the Brongniartieae, *Behaimia* appears strongly supported as sister to *Cyclolobium* and the *Behaimia-Cyclolobium* clade as sister to the genus *Limadendron* (see Table 3 for a morphological comparison between the genera). These three genera are very similar in flower morphology as they possess relatively small flowers (8–15 mm long), with the two vexillary calyx lobes joined in an upper calyx lip, and the vexillary stamen free from the sheath made by the fusion of the filaments of the remaining nine stamens. However, *Behaimia* has yellow petals (J. León, Instituto de Ecologia y Sistemática, Havana, Cuba, personal communication) and a sessile ovary, while both *Cyclolobium* and *Limadendron* have dark red or reddish-purple petals and a stipitate ovary (Table 3).

Leaf morphology also distinguishes *Behaimia* from *Cyclolobium* and *Limadendron*. While *Behaimia* has imparipinnate leaves with 3 to 9 pairs of opposite leaflets and a single terminal leaflet and minute and caducous stipels, both *Cyclolobium* and *Limadendron* have unifoliolate leaves, which can have long and rigid stipels in *Cyclolobium* (Hoehne, 1941; Warwick and Pennington, 2002; Meireles and Azevedo-Tozzi, 2014). *Limadendron* sometimes exhibits imparipinnate leaves with 1 to 3 pairs plus a single terminal leaflet and small caducous stipels. Although this condition was described as very rare (Meireles and Azevedo-Tozzi, 2014), it shows some morphological similarities to the leaf traits of *Behaimia*.

*Behaimia* has elliptic-oblong, flat, one-seeded fruits, with a thin coriaceous, transversely reticulate-veined pericarp (Geesink, 1984; Lewis, 1988; Beyra Matos, 1998). They can be indehiscent or late dehiscent and are superficially very similar to those found in the less closely related genus *Tabaroa*. The more closely related genera

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*Cyclolobium* and *Limadendron* present different fruit characteristics. In *Cyclolobium*, the one-seeded, thin-valved fruit is distinguished by its raised and thin margins, which form a circling wing and a samaroid fruit (Hoehne, 1941; Warwick and Pennington, 2002) that is unique among the Brongniartieae (Queiroz et al., 2010). Fruits of *Limadendron* are elastically dehiscent with the woody valves becoming twisted during seed release (Meireles and Azevedo-Tozzi, 2014).

The geographic distributions of *Behaimia*, *Cyclolobium*, and *Limadendron* do not overlap and they exhibit distinct ecologies. *Behaimia* is known mostly from coastal seasonally dry forests and thickets in Cuba Occidental (Pinar del Rio, Artemisa, Havana, Mayabeque), Central (Matanzas, Cienfuegos), and Oriental (Las Tunas, Holguín, Santiago de Cuba, Guatánamo; Beyra Matos, 1998). The genus *Limadendron* includes two species from lowland rain forests in the Amazon (Meireles and Azevedo-Tozzi, 2014). The monospecific genus *Cyclolobium* is more widely distributed in seasonally dry forest patches from the southern border of the Amazon to eastern Bolivia (Santa Cruz), dry forest sites in Brazilian Cerrado vegetation, eastern Brazil (Bahia, Minas Gerais, and São Paulo) and eastern Paraguay (Warwick and Pennington, 2002; Meireles et al., 2014).

## 5. CONCLUSIONS AND PERSPECTIVES

The multilocus phylogenetic study reported here yielded a highly-resolved phylogeny of the Brongniartieae, with strong support for most clades. The phylogeny was especially resolved in the basal American grade in which all genera, as currently circumscribed, were strongly supported as monophyletic. By resolving the position of *Plagiocarpus axillaris* in the Australian clade, our study clearly refutes a previous hypothesis placing *Plagiocarpus* closer to the American genus *Brongniartia* than to the

remaining Australian genera, thus supporting a single clade containing all Australian genera. This finding implies that colonization of Australia by these taxa from the New World occurred just once.

Despite modest species numbers, tribe Brongniartieae displays great morphological diversity in leaf, flower and fruit traits, and the inclusion of *Behaimia*, and possibly of *Haplormosia*, brings more morphological diversity to the tribe. Nevertheless, there are a few cohesive characters that distinguish the tribe, mostly the presence of colleter-like glands in the axil of leaflet pulvinules or on the inner surface of stipules, bracts and bracteoles, an embryo with a straight radicle, and papilionate flowers in which the upper two lobes of the calyx are joined to some extent and the anthers are slightly to strongly dimorphic, alternately short and dorsifixed and long and basifixed. The resolution of the Brongniartieae-Leptolobieae-core Genistoids polytomy will be required to distinguish which, if any, of these characters are synapomorphies for Brongniartieae.

The Brongniartieae evince a striking morphological diversity, a wide ranging geography, and a marked variation in ecological preferences. Except for *Brongniartia* (c. 65 species), *Harpalyce* (c. 25), and *Hovea* (c. 37), the Brongniartieae is a collection of rather small genera, most comprising less than 10 species each, and nine out of the 14 genera have only one or two species. This low species richness in papilionate-flowered genera contrasts with the diversity patterns in the other Genistoid lineages in which genera with low species diversity have non-papilionate flowers (Cardoso et al. 2012a). Geographically, the tribe is marked by a wide intercontinental disjunction between the New World and Australia, but also by recurrent wide, within-continent disjunctions coincident with biome shifts among sister American genera (e.g., the eastern Brazilian dry forest genus *Tabaroa* sister to the Amazonian rain forest genus *Amphiodon*; the

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Cuban dry forest genus *Behaimia* sister to the Amazonian rain forest *Limadendron*) or within genera (e.g., the Mesoamerican dry forest *Harpalyce* sect. *Harpalyce* sister to the central-eastern Brazilian savanna *Harpalyce* sect. *Brasilianae*; *Brongniartia ulbrichiana* from Bolivian inter-Andean dry valleys sister to a xerophytic Mesoamerican *Brongniartia* clade). More densely sampled phylogenies, divergence time estimation, and biogeographical analyses are necessary to fully resolve those patterns and to shed light on the processes that generated them.

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FIGURE CAPTIONS

**Fig. 1.** Majority-rule (50%) consensus phylogram from Bayesian analysis of 787 *matK* accessions of Papilionoideae and a broad outgroup 183 accessions of other Leguminosae subfamilies (Cercidoideae: 6; Duparquetioideae: 1; Dialioideae: 11; Detarioideae: 77; Caesalpinioideae: 86), and seven other Fabales, showing the placement of *Behaimia* in the Brongniartieae. Groups in which the genera now included in tribe Brongniartieae were once classified (Polhill, 1981a), both above (Millettieae and Bossiaeeae) and below (Brongniartieae) the node defining the canvanine-accumulating clade, are highlighted by color boxes. The close-up shows phylogenetic relationships within the Brongniartieae depicted as a cladogram. Posterior probabilities (left) and Maximum Likelihood bootstrap support values (right) are shown only for the major clades discussed in the text. A complete version of the tree from Bayesian analysis with all PP support values > 0.5 is available in the Appendix S1.

**Fig. 2.** Majority-rule consensus tree from the Bayesian analysis of the combined nuclear (ITS/5.8S) and plastid (*matK/trnK + trnL*) datasets of the Brongniartieae. Numbers above branches are posterior probabilities (PP) and branches in bold have PP = 1. Numbers below branches are bootstrap support (BS) values from the Maximum Parsimony (left) and Maximum Likelihood (right) analyses. Asterisk indicates BS < 50%. The inset shows the corresponding phylogram to represent branch lengths.

**Appendix S1.** Majority-rule (50%) consensus tree from Bayesian analysis of 967 *matK* accessions of Leguminosae and seven other Fabales. The phylogenetic position of *Behaimia* and groups discussed in the text are highlighted by color boxes. Numbers at

the nodes are posterior probabilities > 0.5. Leguminosae subfamilies are presented according to LPWG (2017).

**Appendix S2.** Majority-rule (50%) consensus trees from Bayesian analyses of individual datasets of nuclear ITS/5.8S (A), and plastid *matK/trnK* region (B) and *trnL* intron (C) and of a combined plastid (*matK/trnK* + *trnL*) dataset (D) of Brongniartieae. Numbers above the branches are posterior probabilities > 0.5.
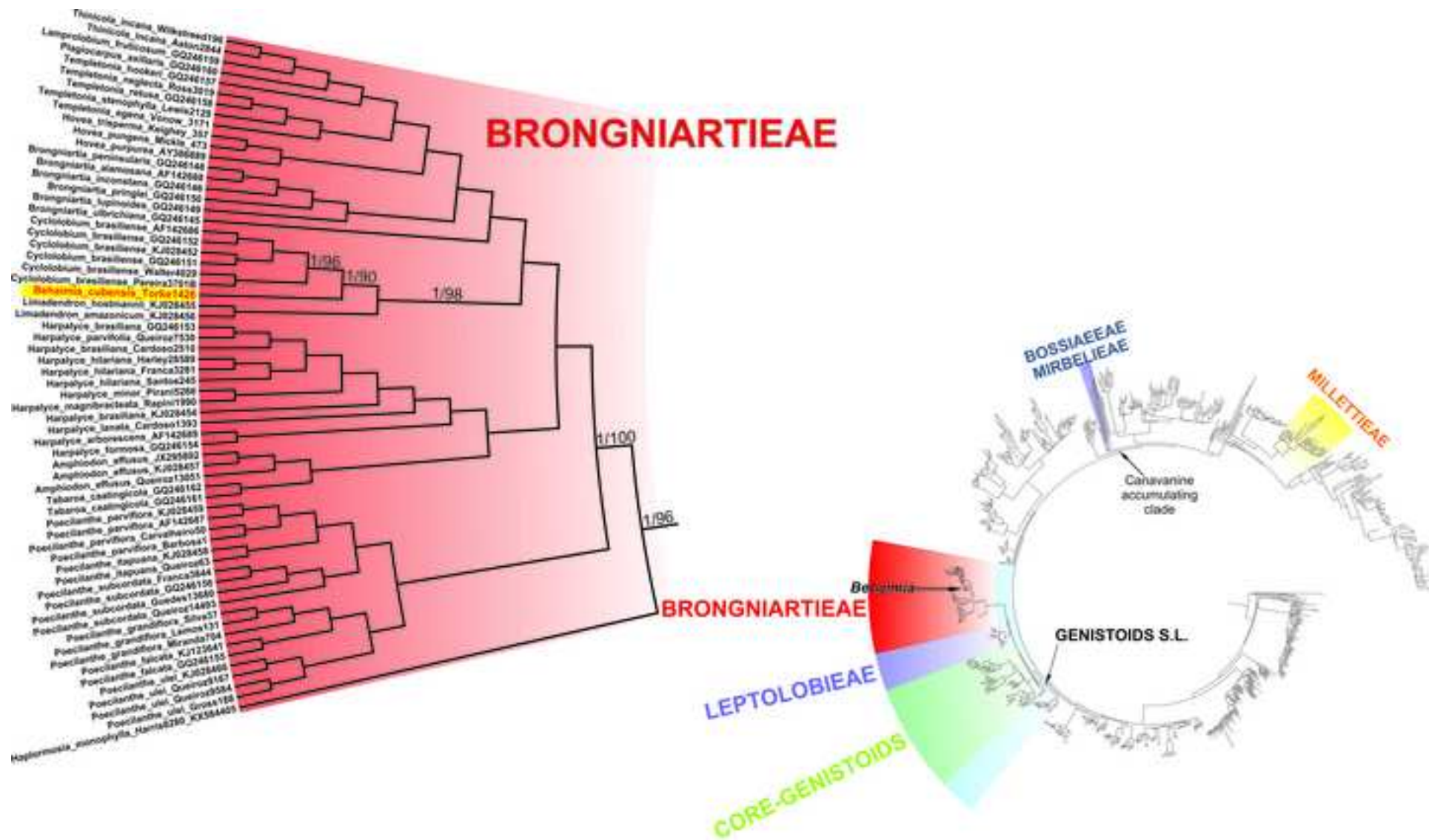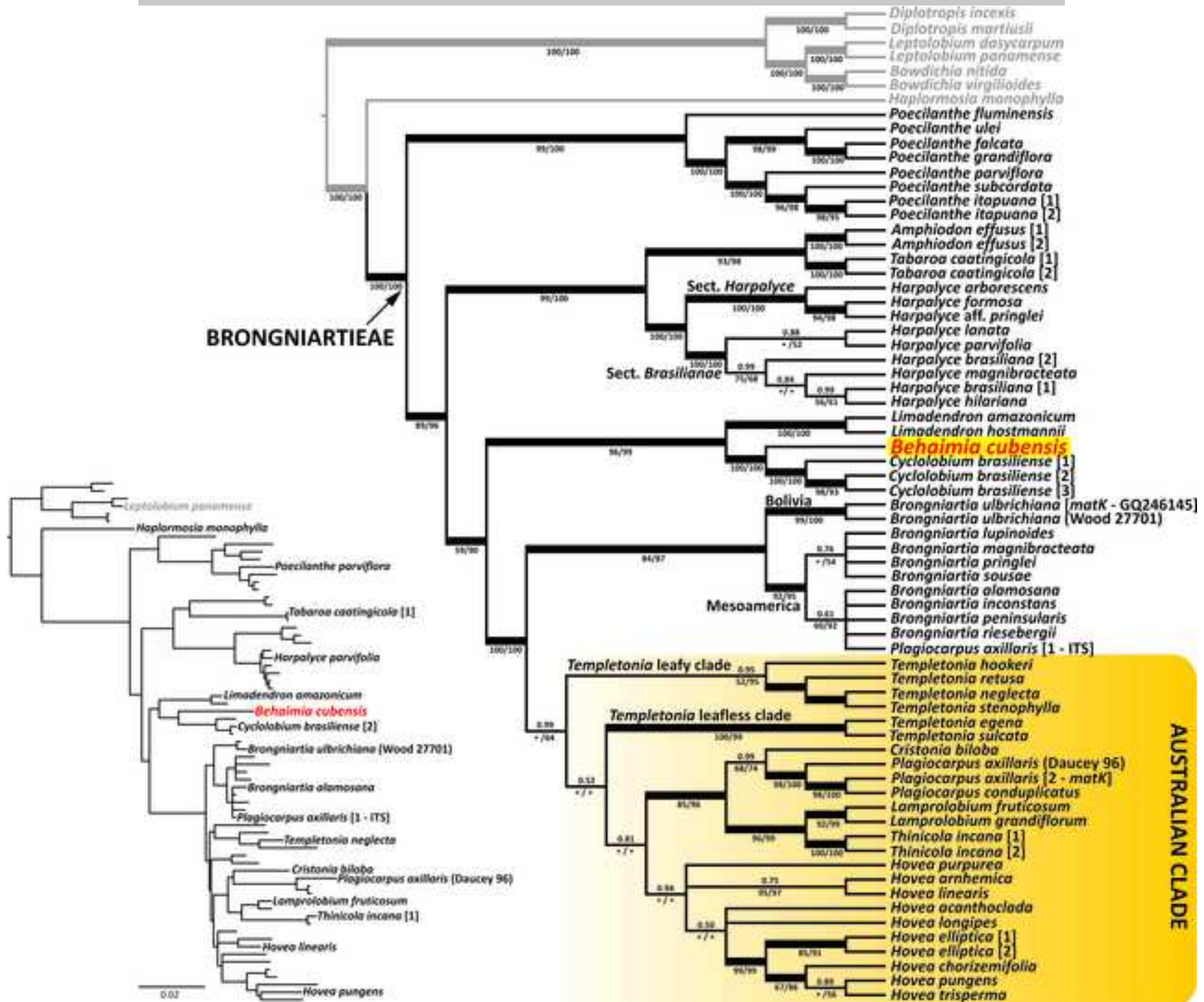
Table 1

**Table 1.** List of taxa and accessions used in the phylogenetic analyses of the Brongniartieae. Original sequences are presented with an asterisk at

the Genbank accession number. Species epithets between quotation marks are from undescribed species and those names are recorded in the

NCBI Genbank database.

[NOTE – accessions marked with GB are being submitted to NCBI genbank]

| Taxon | Voucher | ITS | *matK/trnK* | *trnL* intron |
|---|---|---|---|---|
| **Outgroups** | | | | |
| *Bowdichia nitida* Spruce ex Benth. | L.P. Queiroz 13073 (HUEFS) | JX124477 | – | – |
| | D.Cardoso 2916 (HUEFS) | – | JX124395 | JX124433 |
| *Bowdichia virgilioides* Kunth | D.Cardoso 1764 (HUEFS) | JX124476 | JX124393 | – |
| | R.T. Pennington 477 (UB) | – | – | AF309486 |
| *Diplotropis incexis* Rizzini & A. Mattos | D.Cardoso 2109 (HUEFS) | JX124486 | JX124401 | JX124440 |
| *Diplotropis martiusii* Benth. | L.P. Queiroz 13066 (HUEFS) | JX124484 | – | JX124438 |
| | Beck et al. 166 (US) | – | AY386938 | – |
| *Haplormosia monophylla* Harms | D.J.Harris 8280 (E) | KX584396 | KX584405 | KX584377 |

| | | | | |
|---|---|---|---|---|
| *Leptolobium dasycarpum* Vogel | D.Cardoso 2616 (HUEFS) | JX124496 | – | JX124450 |
| | F.Sacrpa s.n. (UEC) | – | JX124408 | – |
| *Leptolobium parvifolium* (Harms) Sch. Rodr. & A.M.G. Azevedo | R.Schutz-Rodrigues 1498 (UEC) | – | JX124411 | – |
| *Leptolobium panamense* (Benth.) Sch. Rodr. & A.M.G. Azevedo | C.Hughes 1308 (FHO) | JX124498 | AF142684 | AF208891 |
| **Brongniartieae** | | | | |
| *Amphiodon effusus* Huber (1) | H.C. Lima 7166 (HUEFS) | KX652250* | – | – |
| | L.P. Queiroz 13051 (RB) | – | KX652219* | KX584391 |
| *Amphiodon effusus* Huber (2) | J.E.Meireles 395 (RB) | KJ028464 | KJ028457 | – |
| | H.C.Lima 7166 (RB) | – | JX295892 | – |
| *Behaimia cubensis* Griseb. | B.M. Torke 1426 (NY) | KX652251* | KX652220* | KX652194* |
| *Brongniartia alamosana* Rydb. | Hu 1120 (DAV) | AF467022 | – | – |
| | Hu 1227 (DAV) | – | AF142688 | – |
| *Brongniartia inconstans* S. Watson | H. Hernandez 9407 (K) | GQ250075 | GQ246146 | – |
| *Brongniartia lupinoides* (Kunth) Standl. | R.A. Werling 363 (ASU) | – | GQ246149 | – |
| *Brongniartia magnibracteata* Schltdl. | M. Lavin 5795 (CANB) | AF287652 | – | – |
| *Brongniartia peninsularis* Rose | J. Rebman 4214 (ASU) | GQ250076 | GQ246148 | – |

| | | | | |
|---|---|---|---|---|
| *Brongniartia pringlei* Rydb. | J.C. Soto-Nunez 12205 (K) | AF204710 | – | – |
| | J.C. Soto-Nunez 11604 (K) | – | GQ246150 | – |
| *Brongniartia riesebergii* Dorado | O. Dorado 1775 (K) | AF204709 | – | – |
| *Brongniartia sousae* Dorado | M. Sousa 11942 (K) | KX652252* | KX652221* | KX652195* |
| *Brongniartia ulbrichiana* Harms | C. Hughes 2459 (K) | – | GQ246145 | – |
| | J. Wood 27701 (K) | KX652253* | GB | KX652196* |
| *Cristonia biloba* (Benth.) J.H. Ross | J.A. Chappill s.n. (MEL) | AF204703 | | |
| | R. Davis 12082B (PERTH) | – | GB | GB |
| *Cyclolobium brasiliense* Benth. (1) | J. Ratter 7431 (E) | AF287637 | – | – |
| | B.M.T. Walter 4029 (HUEFS) | – | KX652222* | KX652197* |
| *Cyclolobium brasiliense* Benth. (2) | J.E. Meireles 486 (RB) | KJ028461 | KJ028452 | – |
| | B.A.S. Pereira 3701-B (HUEFS) | | GB | KX584390 |
| *Cyclolobium brasiliense* Benth. (3) | J. Ratter 7431 (E) | AF467041 | – | AF309857 |
| | H.C. Lima s.n. (RB) | – | AF142686 | – |
| | B.B. Klitgaard 86 (K) | – | GQ246151 | – |
| | J.R. Wood 17607 (K) | – | GQ246152 | – |

| Taxon | Voucher | | | |
|---|---|---|---|---|
| *Harpalyce arborescens* A. Gray | J. Hu 1225 (DAV) | – | AF142689 | – |
| | P.T. Lezama 19187 (K) | KX652254* | – | KX652198* |
| *Harpalyce brasiliana* Benth. (1) | D. Cardoso 2510 (HUEFS) | KX652255* | KX652223* | KX584388 |
| *Harpalyce brasiliana* Benth. (2) | R.S. Rodrigues 1153 (RB) | KJ028470 | KJ028454 | – |
| | Fonsera et al. 885 (K) | – | GQ246153 | – |
| | W. São Mateus 333 (HUEFS) | – | – | KX652199* |
| *Harpalyce formosa* DC. | C. Hughes 2109 (K) | KX652256* | GQ246154 | – |
| | A. Campos 1778 (K) | – | – | KX652200* |
| *Harpalyce hilariana* Benth. | A.P. Fortuna-Perez 43 (RB) | KJ028469 | – | – |
| | R.M. Harley 28589 (HUEFS) | – | KX584414 | KX584386 |
| | * F. França 3281 (HUEFS) | | KX652224* | |
| | * H.G.P. Santos 245 (HUEFS) | | KX652225* | |
| *Harpalyce lanata* L.P. Queiroz | W. Ganev 2674 (K) | GQ250077 | – | – |
| | D. Cardoso 1393 (HUEFS) | – | KX584415 | KX584387 |
| *Harpalyce magnibracteata* São-Mateus , D.B.O.S.Cardoso & L.P.Queiroz | A. Rapini 1490 (HUEFS) | KX652257* | KX652226* | KX652201* |
| *Harpalyce minor* Benth. | J.R. Pirani 5266 (HUEFS) | | KX652227* | |

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|---|---|---|---|---|
| *Harpalyce parvifolia* H.S. Irwin & Arroyo | L.P. Queiroz 7530 (HUEFS) | KX652258* | KX584416 | KX652202* |
| *Harpalyce* aff. *pringlei* Rose | Torres 1825 (K) | GQ250078 | – | – |
| | Pringle 6827 (K) | – | KX652228* | – |
| *Hovea acanthoclada* F. Muell. | S. Kern 17468 (PERTH) | KX652259* | KX652229* | KX652203* |
| | M.G. Corrick 11008 (MEL) | AF204691 | – | – |
| *Hovea arnhemica* J.H.Ross | G. Wightman 444 (MEL) | AF204692 | – | – |
| *Hovea chorizemifolia* DC. | G.J. Keighery 448 (PERTH) | KX652260* | KX652230* | KX652204* |
| *Hovea elliptica* (Sm.) DC. (1) | M. Crisp 8924 (CANB) | AF287640 | – | – |
| | C.P. Dornan 357 (PERTH) | – | – | KX652205 |
| *Hovea elliptica* (Sm.) DC. (2) | C.W. Huggins s.n. (MEL) | AF204693 | – | – |
| | M. Crisp 8924 (CANB) | – | – | AF518123 |
| *Hovea linearis* (Sm.) R.Br. | I.R. Thompson 368 (MEL) | AF204694 | – | – |
| *Hovea longipes* Benth. | Clarkson 7343 (K) | GQ250079 | – | – |
| | P.I. Forster 16970 (MEL) | AF204695 | – | – |
| *Hovea pungens* Benth. | B. Archer s.n. (MEL) | AF204697 | – | – |
| | D.A. Mickle 473 (PERTH) | – | KX652231* | KX652206* |

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|---|---|---|---|---|
| *Hovea purpurea* Sweet | M.F. Wojciechowski 869 (ASU) | – | AY386889 | – |
| *Hovea trisperma* Benth. | M. Crisp 8925 (CANB) | AF287639 | – | – |
| | G.J. Keighery 1300 (PERTH) | – | KX652232* | KX652207* |
| | CHW 27 | AF204698 | – | – |
| *Lamprolobium fruticosum* Benth. | Clarkson 8827 (K) | GQ250080 | GQ246159 | – |
| *Lamprolobium grandiflorum* Everist & R.J.F.Hend. | J.R. Clarkson 9874 (MBA) | AF204699 | – | – |
| *Limadendron amazonicum* (Ducke) Meireles & A.M.G.Azevedo | J.E. Meireles 390 (RB) | KJ028465 | KJ028456 | – |
| *Limadendron hostmannii* (Benth.) Meireles & A.M.G.Azevedo | J.E. Meireles 462 (RB) | KJ028466 | KJ028455 | – |
| *Plagiocarpus axillaris* Benth. (1 ITS) | M. Barrit 1406 (MEL) | AF204700 | – | – |
| *Plagiocarpus axillaris* Benth. (2 *matK*) | M. Barrit 1406 (K) | – | GQ246160 | – |
| *Plagiocarpus conduplicatus* I.Thomps. | M.D. Barret 4013 (PERTH) | KX652261* | KX652233* | KX652208* |
| *Poecilanthe falcata* (Vell.) Heringer | B.B. Klitgaard 4 (K) | AF467492 | GQ246155 | – |
| | J.E. Meireles 398 (RB) | – | KJ123641 | – |
| | D. Cardoso 2218 (HUEFS) | – | – | KX652209* |
| *Poecilanthe grandiflora* Benth. | J.R. Lemos 131 (HUEFS) | KX652262* | KX584409 | KX584381 |
| | * E.B. Miranda 704 (HUEFS) | | KX652234* | |

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|---|---|---|---|---|
| | * P. Silva 37M (HUEFS) | | KX652235* | |
| *Poecilanthe itapuana* G.P.Lewis (1) | J.E. Meireles 433 (RB) | KJ028462 | KJ028458 | – |
| | W. São Mateus 322 (HUEFS) | – | – | KX652210* |
| *Poecilanthe itapuana* G.P.Lewis (2) | G.P. Lewis 2018 (K) | GQ250081 | – | – |
| | E.P. Queiroz 63 (HUEFS) | – | KX652236* | – |
| *Poecilanthe parviflora* Benth. | H.C. Lima 2816 (RB) | AF187089 | AF142687 | AF208897 |
| | J.E. Meireles 487 (RB) | – | KJ028459 | – |
| | * O. Barbosa 01 (HUEFS) | | KX652237* | |
| | * A.L. Carvalheiro 50 (HUEFS) | | KX652238* | GB |
| *Poecilanthe subcordata* Benth. | R.M. Harley 21205 (K) | GQ250082 | GQ246156 | – |
| | F. França 3844 (HUEFS) | – | KX652239* | – |
| | L.P. Queiroz 14493 (HUEFS) | – | KX652240* | KX584393 |
| | M.L.S. Guedes 13680 (HUEFS) | | KX652241* | |
| *Poecilanthe ulei* (Harms) Arroyo & Rudd | J.E. Meireles 422 (RB) | KJ028468 | KJ028460 | – |
| | L.P. Queiroz 9167 (HUEFS) | – | KX652242* | KX652211* |
| | * E. Gross 188 (HUEFS) | | KX652243* | |

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|---|---|---|---|---|
| | * L.P. Queiroz 9584 (HUEFS) | | KX652244* | |
| *Tabaroa caatingicola* L.P.Queiroz, G.P.Lewis & M.F.Wojc. (1) | L.P. Queiroz 7827 (HUEFS) | GQ250083 | GQ246161 | – |
| | D. Cardoso 3253 (HUEFS) | – | – | KX652212* |
| *Tabaroa caatingicola* L.P.Queiroz, G.P.Lewis & M.F.Wojc. (2) | B.L. Stannard 5143 (K) | GQ250084 | GQ246162 | – |
| *Templetonia egena* (F.Muell.) Benth. | Goldie s.n. | HM116997 | – | – |
| | H.P. Vonow 3171 (PERTH) | – | KX652245* | KX652213* |
| | I. Thompson 151 (MEL) | AF204708 | – | – |
| *Templetonia hookeri* (F.Muell.) Benth. | M.J.S. Sands 4961 (K) | GQ250085 | GQ246157 | – |
| | S. Legge 76 (PERTH) | – | – | KX652214* |
| | C.R. Dunlop 8554 (MEL) | AF204707 | – | – |
| *Templetonia neglecta* J.H. Ross | J.H. Ross 3021 (MEL) | AF204706 | – | – |
| | J.H. Ross 3019 (MEL) | – | KX652246* | – |
| | W.A. Thompson 2043 (MEL) | – | – | KX652215* |
| *Templetonia retusa* (Vent.) R.Br. | M. Crisp 8996 (CANB) | AF287636 | – | – |
| | G.P. Lewis 3833 (K) | – | GQ246158 | – |
| | T.J. Alford 203 (PERTH) | – | – | KX584380 |

| | | | | |
|---|---|---|---|---|
| | W.R. Archer 810951 (MEL) | AF204704 | – | – |
| *Templetonia stenophylla* (F.Muell.) J.M.Black | I.C. Clarke 2699 (MEL) | AF204705 | – | – |
| | G.P. Lewis 2129 (K) | – | KX652247* | KX652216* |
| *Templetonia sulcata* (Meisn.) Benth. | M. Crisp 9057b (CANB) | AF287635 | – | AF518122 |
| | M.S. Graham 2079 (PERTH) | – | KX652248* | – |
| *Thinicola incana* (J.H.Ross) J.H.Ross (1) | N. Gibson 4714 (PERTH) | KX652263* | – | – |
| | P. Wicksteed 196 (CANB) | – | KX652249* | KX652217* |
| *Thinicola incana* (J.H.Ross) J.H.Ross (2) | H.I. Aston 2844 (PERTH) | AF204702 | KX584408 | KX652218* |

**Table 2.** Summary of the molecular phylogenetic analyses with focus on the Brongniartieae, including the characteristics of the sequence data

and resulting trees for the different datasets analysed. The placement of *Behaimia* in Brongniartieae and its relationships with the genera

*Cyclolobium* ("Cyc") and *Limadendron* ("Lim") are indicated by support values in different analyses (posterior probability in Bayesian inference

/ maximum likelihood bootstrap / maximum parsimony bootstrap). "N" is the number of terminal taxa and "Incomplete Taxa" shows the

percentage of missing sequences in the dataset.

| Dataset | N | Length (bp) | Incomplete Taxa (%) | Best fit model | Parsimony informative characters | Tree length / CI / RI in MP analysis | *Behaimia* in Brongniartieae | (Beh, Cyc) | (Lim (Beh, Cyc)) |
|---|---|---|---|---|---|---|---|---|---|
| **ITS/5.8S** | 73 | 739 | – | GTR+I+Γ (ITS 1, 2), K80+Γ (5.8S) | 316 | 1174 / 57 / 78 | 1 / 100 / 96 | 1 / 98 / 86 | 0.53 / 36 / – |
| *matK* | 81 | 1971 | – | GTR+Γ | 445 | 1048 / 74 / 92 | 1 / 100 / 100 | 0.99 / 88 / 84 | 1 / 100 / 98 |
| *trnL* | 47 | 692 | – | GTR+Γ | 119 | 309 / 83 / 90 | 0.99 / 96 / 93 | – / – / – | * |
| *matK* + *trnL* | 62 | 2663 | 19.7 | – | 518 | 1327 / 75 / 88 | 1 / 100 / 100 | 0.98 / 86 / 88 | 1 / 100 / 98 |
| **ITS** + *matK* + *trnL* | 69 | 3402 | 18.2 | – | 826 | 2480 / 66 / 83 | 1 / 100 / 100 | 1 / 100 / 100 | 1 / 100 / 96 |

* The genus *Limadendron* was not included in the *trnL* intron dataset.

**Table 3.** Morphological comparison between *Behaimia* and the phylogenetically closely related genera *Cyclolobium* and *Limadendron* of the Brongniartieae.

| | *Behaimia* | *Cyclolobium* | *Limadendron* |
|---|---|---|---|
| **Habit** | Shrub or small tree | Shrub or small tree to 20m high | Small tree to 10–12 m |
| **Leaf** | Pinnate, 7–19-foliolate, leaflets opposite | Unifoliolate | Unifoliolate, very rarely to 3–7-foliolate and then leaflets opposite |
| **Stipels** | Early caudcous or absent | Persistent and rigid | Minute and caducous |
| **Inflorescence** | Short (3–4 cm long) racemes clustered in axillary, erect panicles | Short (1–9 cm long) axillary, erect racemes | Short (2–8 cm long) axillary or ramiflorous, pendant racemes |
| **Flower length** | c. 8 mm long | 5–13 mm long | 8–15 mm long |
| **Calyx** | Two vexillary (adaxial) lobes almost completely fused making an oblong lip | Two vexillary lobes slightly fused | Two vexillary lobes almost completely fused making an oblong lip |
| **Petal color** | Yellow | Dark red or purplish red | Dark red or purplish red |
| **Androecium** | Diadelphous, the vexillary stamen free | Diadelphous, the vexillary stamen free | Diadelphous, the vexillary stamen free |
| **Anthers** | Slightly dimorphic, shorter dorsifixed alternating with longer basifixed | Uniformly shaped but alternately dorsifixed and basifixed | Slightly dimorphic, shorter dorsifixed alternating with longer basifixed |
| **Ovary stipe** | Absent, ovary sessile | Present and slender | Present and slender |
| **Fruit** | Indehiscent or late dehiscent, elliptic or elliptic-oblong pod, with indistinct margins, and thick coriaceous, pubescent, transversely reticulate-veined valves | Indehiscent, ovate, obovate or orbicular samaroid fruit, with margins raised and thin wing-like, and thin and coriaceous, glabrous and smooth or with slightly raised reticulate veined valves. | Obovate, D-shaped [*L. amazonicum*] or oblong [*L. hostmannii*], elastically dehiscent pods, with indistinct margins and woody, glabrous, slightly or strongly transversely reticulate-veined valves. |
| **Seeds** | 1(2) | 1 | 1–2 [*L. amazonicum*] or 3–5 (–7) [*L. hostmannii*] |
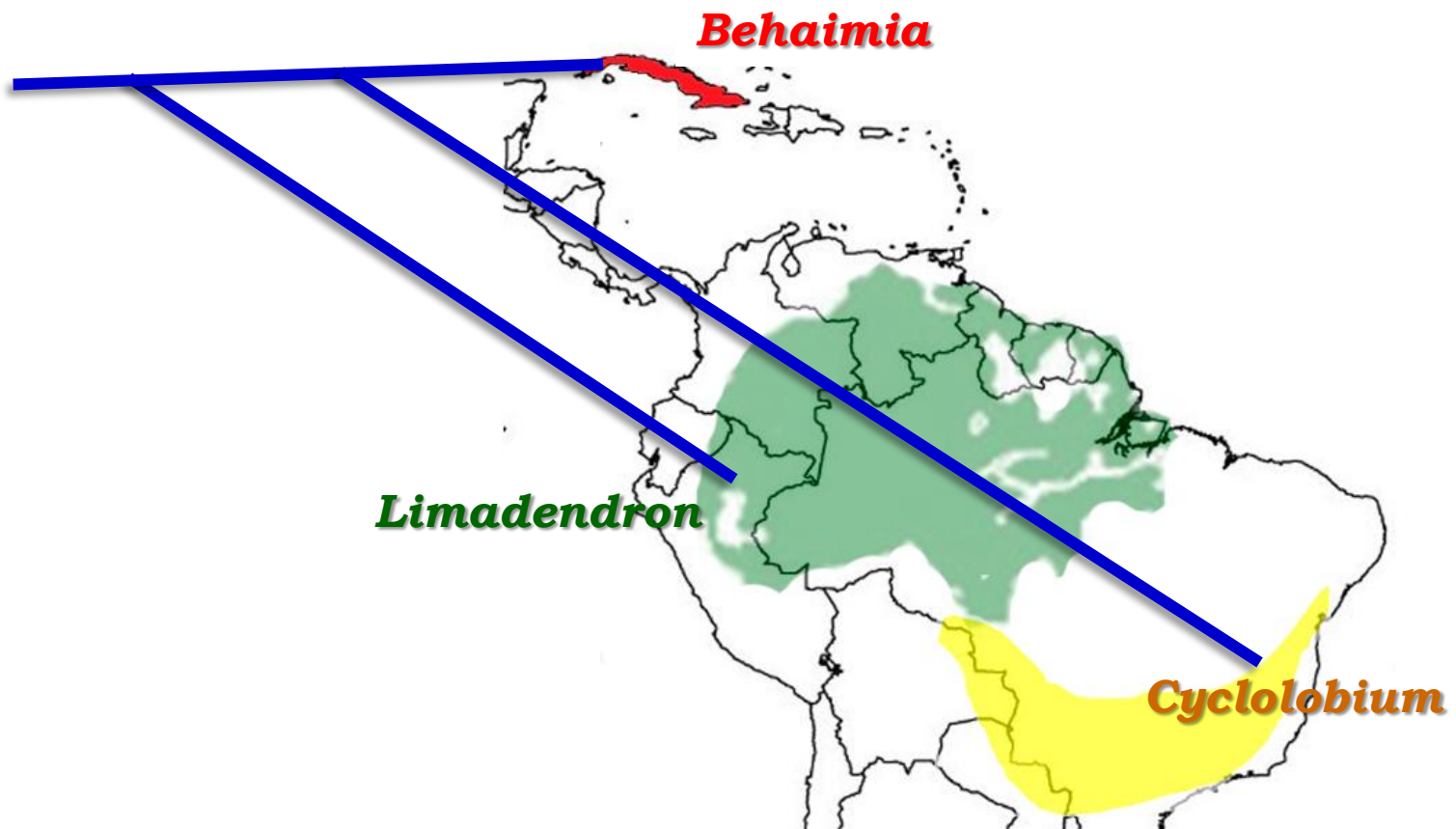
A molecular phylogeny unfolds the Cuban enigmatic genus *Behaimia* as a new piece in the Brongniartieae puzzle of papilionoid legumes

- The monotypic Cuban-endemic genus *Behaimia* is part of the tribe Brongniartieae

- The Australian genera of the Brongniartieae make a clade

- All American genera of Brongniartieae are monophyletic as currently circumscribed

- The American genus *Brongniartia* is sister to the Australian clade