

1 Title: Evolution of andrenine bees reveals a long and complex history of faunal interchanges
2 between the Americas during the Mesozoic and Cenozoic

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15 ABSTRACT

16 Bees are presumed to have arisen in the early to mid-Cretaceous coincident with the
17 fragmentation of the southern continents and concurrently with the early diversification of the
18 flowering plants. Among the main groups of bees, Andreninae sensu lato comprise about
19 3000 species widely distributed with greatest and disjunct diversity in arid areas of North
20 America, South America, and the Palearctic region. Here, we present the first comprehensive
21 dated phylogeny and historical biogeographic analysis for andrenine bees, including
22 representatives of all currently recognized tribes. Our analyses rely on a dataset of 106 taxa
23 and 7952 aligned nucleotide positions from one mitochondrial and six nuclear loci.
24 Andreninae is strongly supported as a monophyletic group and the recovered phylogeny
25 corroborates the commonly recognized clades for the group. Thus, we propose a revised tribal

26 classification that is congruent with our phylogenetic results. The time-calibrated phylogeny
27 and ancestral range reconstructions of Andreninae reveal a fascinating evolutionary history
28 with Gondwana patterns that are unlike those observed in other subfamilies of bees.
29 Andreninae arose in South America during the Late Cretaceous around 90 Million years ago
30 (Ma) and the origin of tribes occurred through a relatively long time-window from this age to
31 the Miocene. The early evolution of the main lineages took place in South America until the
32 beginning of Paleocene with North American fauna origin from it and Palearctic from North
33 America as results of multiple lineage interchanges between these areas by long-distance
34 dispersal or hopping through landmass chains. Overall, our analyses provide strong evidence
35 of amphitropical distributional pattern currently observed in Andreninae in the American
36 continent as result at least three periods of possible land connections between the two
37 American landmasses, much prior to the Panama Isthmus closure. The andrenine lineages
38 reached the Palearctic region through four dispersal events from North America during the
39 Eocene, late Oligocene and early Miocene, most probably via the Thulean Bridge. The few
40 lineages with Afrotropical distribution likely originated from a Palearctic ancestral in the
41 Miocene around 10 Ma when these regions were contiguous, and the Sahara Desert was
42 mostly vegetated making feasible the passage by several organisms. Incursions of andrenine
43 bees to North America and then onto the Old World are chronological congruent with distinct
44 periods when open-vegetation habitats were available for trans-continental dispersal and at
45 the times when aridification and temperature decline offered favorable circumstances for bee
46 diversification.

47

48 *Keywords:* Amphitropical distribution, Andrenidae, diversity, biogeography, macroevolution,
49 phylogeny.

50

51 **1. Introduction**

52 Bees constitute a well-established clade of pollen-feeding Hymenoptera presumed to
53 have arisen during the Early to mid-Cretaceous approximately 113-132 Ma (million years
54 ago) (Cardinal and Danforth, 2013). Their origin coincides with the end of the Gondwana
55 breakup and the early diversification of eudicots, the major group of flowering plants
56 (Cardinal and Danforth, 2013). Currently presenting a worldwide distribution and about
57 20,000 described species (Ascher and Pickering, 2020), bees have been hypothesized to have
58 originated in the Southern Hemisphere, more specifically, in the xeric interior of Gondwana
59 (Michener, 1979; Litman et al., 2011; Hedtke et al., 2013). Over the last 20 years, much
60 progress has been made on our understanding of the origin and evolution of bees mainly due
61 to the use of molecular data and fossil information combined with statistical phylogenetic
62 methods to reconstruct bee phylogenies at different taxonomic levels (see Danforth et al.,
63 2013, for a review). Among the main groups of bees, the Andreninae remain the most poorly
64 understood lineage in terms of phylogenetic relationships of its supraspecific taxa and
65 biogeographic history (Danforth et al., 2013).

66 In the present work we focus on the phylogenetic and biogeographic history of
67 andrenine bees. This group provides an excellent system for exploring historical
68 biogeographic patterns of biotic interchanges that took place during the Cenozoic mainly
69 between the North and South America regions. Andreninae constitute a well-established clade
70 of bees with about 3,000 species that represent over 15% of all bee diversity (Ascher and
71 Pickering, 2020) and diverged from the clade (Halictinae (Stenotritinae + Colletinae)), around
72 90 Ma in the late Cretaceous (Cardinal and Danforth, 2013; Sann et al., 2018). The subfamily
73 is widely distributed on all continents, except Australia and tropical Asia, and are
74 exceptionally diverse in the temperate and xeric parts of the Western Hemisphere (Michener,
75 2007). Among the 61 known genera of andrenine, the New World fauna encompasses around

76 45 genera, with 31 of them found only in South America. Outside the Americas, Andreninae
77 have a smaller genus-level diversity, with 14 genera reported for the Palearctic region and 5 in
78 the Afrotropics.

79 Andreninae comprise typically slender medium-sized (6-10 mm long), black with some
80 yellow marks, and sparsely haired bees, but also robust and hairy bees such as Oxaeini
81 (Michener, 2007). It has been consistently recovered as a monophyletic group (Ascher, 2004;
82 Danforth et al., 2004, 2006a, 2006b; Cardinal and Danforth, 2013; Danforth et al., 2013;
83 Hedtke et al., 2013). The most distinctive morphological character is the subantennal area
84 defined by two subantennal sutures, with the presence of facial fovea in females and some
85 males, and the pointed glossa being also useful features in their recognition (Michener, 2007).
86 The subfamily is remarkable for the high proportion of pollen specialists (oligolectic) species,
87 each visiting a few species within the same plant family, such as Asteraceae, Cactaceae,
88 Fabaceae, Malvaceae, Melastomataceae, Onagraceae, Oxalidaceae, Passifloraceae,
89 Solanaceae, and Turneraceae (Michener, 2007). As far as we know, all andrenine species are
90 solitary, however, several groups also show communal nesting biology (such as *Andrena*,
91 *Oxaea*, Perditini, *Panurgus*, and some Protandrenini) (Michener, 2007). The nests are
92 excavated in the soil with one or a short series of cells at the end of each lateral burrow
93 radiating from the main burrow. Many species are uni- or bivoltine (i.e. producing one or two
94 generations per year), especially in seasonally dry areas, but many common polylectic species
95 are multivoltine. The cleptoparasitic associations of several species of nomadine bees with
96 andrenines are an interesting and complex system to be investigated (Rozen, 1989). In this
97 sense, resolution of the phylogenetic relationship among Andreninae lineages is an important
98 step for understanding the evolution of cleptoparasitism in bees (Rozen, 1992), as well as the
99 evolutionary history of bee-plant associations (Larkin et al., 2008; Danforth et al., 2013).

100 The classification of Andreninae has a complex history with variable number of
101 recognized internal taxa. For many years the Andreninae was treated as a slightly less-
102 inclusive group, excluding Oxaeini (Ascher et al., 2006; Engel, 2015). Members of this tribe
103 differs considerably from other Andreninae, what lead to its placement as a separate higher
104 taxon based on adult and larval morphology (e.g. Michener, 1944; Rozen, 1964, 1965, 1993;
105 Hurd and Linsley, 1976; Alexander and Michener, 1995). Ruz (1986, 1991) was the first to
106 investigate phylogenetic relationships among genera of the panurgine line, followed by Patiny
107 (1999), who analyzed the relationship among the Old-World members of panurgine based on
108 adult morphological characters proposing six tribes: Panurgini, “Camptopoeumini”,
109 Panurginini, Mermiglossini, Melitturgini, and “Paramelittergini”. The forms included in this
110 arrangement were classified into two tribes (Panurgini and Melitturgini) by Michener (2000,
111 2007), a proposal that was largely followed by subsequent authors. Later, Ascher (2004)
112 analyzed the whole Andreninae using morphological (adult and larvae) plus EF-1 α (F2 copy)
113 sequences, only partially published (in Rozen, 2003) in order to establish the phylogenetic
114 relationship of the enigmatic Nolanomelissini.

115 Our understanding of the phylogenetic relationships among andrenine bees remains
116 fragmented due to limited taxon sampling mainly from South America – the richest region in
117 diversity of supra specific taxa. Additionally, a global biogeographic analysis based on a well-
118 resolved fossil calibrated phylogeny of the subfamily has not yet been attempted thus far.
119 Thus, the primary goal of the work is to provide a solid phylogenetic framework with special
120 emphasis on the New World, as an important step to establish a stable classification of the
121 Andreninae and to the understanding of their evolution through the Cenozoic. This is the first
122 time the monotypic tribe Protomelitturgini has been included in a molecular phylogenetic
123 work. The current study provides information to understand the ancient diversification that
124 took place in South America and the complex biogeographic scenario and evolutionary

125 factors that have produced the biotic interchange between the warm areas of the Americas
126 during the Mesozoic and Cenozoic.

127

128 **2. Material and methods**

129 *2.1. Taxon and molecular sampling*

130 The taxon sampling comprises 91 species of the subfamily Andreninae sensu lato,
131 representing all currently recognized suprageneric taxa and the main geographical
132 occurrences within the subfamily. We follow the classification of Melo and Gonçalves (2005)
133 for the main lineages of bees, treated as subfamilies of a single family (Apidae sensu lato),
134 and Moure et al. (2012) for American taxa of Andreninae; in the case of Old-World taxa, we
135 use the subtribes of Ascher and Engel (2017) at tribal level, as originally proposed by Patiny
136 (1999). According to the classification adopted here, Andreninae is subdivided into the
137 following extant tribes: Andrenini, Euherbstiini, Calliopsini, Neffapini, Melitturgini,
138 Mermiglossini, Nolanomelissini, Oxaeini, Panurgini, Panurginini, Perditini, Protandrenini,
139 and Protomelitturgini. The higher-level bee phylogenies indicate a large clade uniting all the
140 short-tongued subfamilies (except Melittinae) (Danforth et al., 2006a, 2006b; Cardinal and
141 Danforth, 2013; Danforth et al., 2013; Hedtke et al., 2013), thus we choose fifteen terminals
142 to represent the subfamilies from this clade: Colletinae (7 spp.), Halictinae (6 spp.) and
143 Stenotritinae (2 spp.). Attention to the choice of species was taken in order to more accurately
144 place fossil calibration points and to maximize morphological diversity and geographic
145 distribution for the ancestral area reconstruction. In total, 106 taxa were included in the
146 analyses, as described in Supplementary Tables S1-S2 along with taxonomy, collection site,
147 voucher information, and GenBank accession numbers (GenBank accession numbers will be
148 indicated upon this manuscript acceptance).

149 Molecular sequence data were generated for this study fulfilling a huge gap of South
150 American andrenine bees in GenBank. Most newly sequenced specimens were field-collected
151 by us or obtained through donation or loans from other researchers and institutions (see
152 Acknowledgments). In addition, sequence data were obtained from the databases of Genbank
153 and BOLD (Barcode of Life Data System) mostly from Ascher et al. (2001), Ascher (2003,
154 2004), Danforth et al. (2006a, 2006b), Larkin et al. (2006), Almeida and Danforth (2009),
155 Cardinal and Danforth (2013), and Packer and Ruz (2017). Total DNA was mostly isolated
156 from bees preserved in EtOH, but also from pinned museum specimens collected up to twelve
157 years before processing. Muscular tissue was taken from thorax or legs and the DNA was
158 extracted using the Qiagen DNeasy blood and tissue extraction kit, following the
159 manufacturer's protocol. In the case of museum species, the whole body was maintained in an
160 insect relaxing chamber for 24-48 hours prior to extraction following the protocol of
161 Evangelista et al. (2017) for museum specimens, using the same extraction kit. Voucher
162 specimens of the newly generated sequences are housed in the DZUP – Entomological
163 Collection “Jesus Santiago Moure” at Federal University of Paraná, Brazil, or at their home
164 institutions (Supplementary Tables S1-S2).

165 The selected regions comprise four nuclear protein-coding genes: elongation factor-1 F2
166 copy (EF-1a), long-wavelength rhodopsin (opsin), RNA polymerase (poly) and wingless
167 (wg); two nuclear ribosomal RNA loci: large subunit 28S rRNA (28S) and 18S rRNA (18S);
168 and one mitochondrial protein-coding gene: cytochrome c oxidase subunit I (COI). These
169 genes have provided robust results for several phylogenetic studies of bees at distinct levels of
170 relationships. Newly generated sequences refer only to EF-1a (650 base pairs (bp)), wingless
171 (500 bp), and 28S rRNA (700 bp). Primer information and PCR conditions can be found in
172 electronic supplementary material (Table S3). PCR success was examined by gel
173 electrophoresis on 1% agarose gel and PCR products were purified and sequenced in forward

174 and reverse directions by Macrogen Inc. (South Korea). Sequence assembling was generated
175 with the Staden Package (Staden et al., 2000). Chromatogram quality evaluation and
176 corrections were done in BioEdit v5.0.9 (Hall, 1999).

177 All genes were separately aligned in MAFFT v. 7 (Katoh et al., 2019) with default
178 parameters: gap opening penalty = 1.53; offset value = 0. The alignment of the protein coding
179 genes EF-1a and opsin relied on the LINS-i strategy, which is recommended for sequences
180 with multiple conserved domains and long gaps. The alignment of the 18S rRNA, wingless
181 and COI relied on the G-INS-i strategy, recommended for sequences with a global homology.
182 The ribosomal 28S rRNA was aligned based on its secondary structure using the Q-INS-i
183 algorithm. Minor adjustments were made by eye in Geneious, and we made sure that the
184 introns/exon boundaries of EF-1a and opsin were maintained. Sequences from the seven gene
185 fragments were concatenated in Sequence Matrix v. 1.7.8 (Vaidya et al., 2010). The final
186 number of aligned nucleotides used in the analyses was 7952 base pairs in length (EF-1a:
187 1946 bp; opsin: 1494 bp; wg: 512 bp; poly: 842 pb; 28S rRNA: 1714 bp; 18S rRNA: 786 bp;
188 COI: 658 bp).

189

190 *2.2. Phylogenetic analysis and divergence time estimation*

191 All genes were concatenated into a single matrix subdivided as follows: introns and
192 exons of the protein coding regions EF-1a and opsin, poly, wg, COI, 28S rRNA, and 18S
193 rRNA totalizing 16-character sets. The search for the best partitioning scheme and models of
194 DNA substitution was performed in PartitionFinder v. 2 (Lanfear et al., 2017) based on the
195 Bayesian Information Criterion and the algorithm greedy (see Supplementary Table S4 for
196 specific information of data partition and their best fit models). The phylogenetic tree
197 searches were carried out using the concatenated matrix with nine data partitions under
198 maximum likelihood and Bayesian inferences. Maximum likelihood analysis was performed

199 in RaxML (Stamatakis, 2006) in the CIPRES server (Miller et al., 2011). Simultaneous
200 bootstrap analyses with 1000 replicates were conducted to evaluate node support. Bayesian
201 phylogenetic tree searches were conducted in MrBayes v. 3.2. (Ronquist et al., 2012) in
202 CIPRES. The Markov chain Monte Carlo (MCMC) was run for 20 million generations
203 sampled every 1000th generation. The stationarity of all parameters and convergence of both
204 runs were accessed in Tracer v. 1.7.1 (Rambaut et al., 2018). A 25% burn-in was applied and
205 a 50% majority rule consensus was computed with the remaining trees in TreeAnnotator (part
206 of BEAST 2.0 package, Bouckaert et al., 2014). Resulting ML and Bayesian trees were
207 visualized and edited in FigTree v. 1.4.4 (Rambaut, 2016).

208 Divergence times were estimated in a Bayesian framework using BEAST 2 (Bouckaert
209 et al., 2014) by employing the same partition scheme and models of phylogenetic analyses.
210 Four bee fossils were used to calibrate the Andreninae tree, two belonging to this subfamily
211 and the others to Halictinae and Colletinae. Compression fossils attributed to *Andrena* from
212 the Eocene of Florissant, Colorado (Priabonian Age: 37.2–33.9 Ma) were used to calibrate the
213 node of extant *Andrena* species. There are four species from Florissant deposit attributed to
214 *Andrena*: *A. grandipes* Cockerell, 1911; *A. hypolitha* Cockerell, 1908; *A. percontusa*
215 Cockerell, 1914 and *A. sepulta* Cockerell, 1906. Since the descriptions by Cockerell, their
216 identity has not been much investigated, but all have been undoubtedly attributed to the
217 megadiverse genus *Andrena*. For this point, a lognormal distribution prior was applied with
218 offset = 34, stdev = 0.623 and Mean = 1.8, corresponding to a minimum bound of 34 Ma,
219 median value of 40 and 95% quantile of 50. *Heterosarus eickworti* Rozen, 1996 from the
220 Miocene Dominican amber (20.43–13.65 Ma) was used to calibrate the node uniting the two
221 extant species included in our study. The crown group of *Heterosarus* was given a lognormal
222 distribution with offset 14, stdev = 0.432 and M = 1.8, corresponding to a minimum bound of
223 14 Ma, median value of 20, and 95% quantile of 26.31. Two other species from Dominican

224 amber, *Chilicola (Hylaeosoma) gracilis* Michener and Poinar, 1996 and *Chilicola*
225 *(Hylaeosoma) electrodominicana* Engel, 1999, were attributed to the crown group of
226 *Chilicola* (Colletinae, Xeromelissini), following the same prior used in *Heterosarus*.
227 *Electrolictus antiquus* Engel, 2001 from the Eocene Baltic amber was used to calibrate the
228 stem group of Halictini (in Halictinae) given a lognormal distribution prior with the same
229 parameter as the *Andrena* fossils, since the Baltic amber has also a Priabonian age. All
230 geological ages attributed to the fossils mentioned here were retrieved from Fossilworks
231 (Behrensmeyer and Turner, 2013).

232 A normal prior distribution was attributed to the crown age of Andreninae+(Colletinae,
233 Halictinae) based on previous broader age estimates for Hymenoptera (Aculeata): from
234 Branstetter et al. (2018) (89.3 Ma, 95% HPD: 76.66-100.17 Ma) and Peters et al. (2017) (99,
235 95% HPD 82-118). An average value between these two estimates was calculated and the
236 calibration point was given a normal distribution, offset 94, sigma 2.5 and mean 1 (5%
237 quantile 90.1, 95% 99.9). The tribes Protandrenini and Mermiglossini were constrained to be
238 monophyletic based on the Maximum Likelihood and Bayesian analyses, which consistently
239 recovered these clades. Supplementary Figure S1 depicts all points of fossil calibration,
240 secondary calibration and monophyletic constraints. Substitution and clock models were
241 unlinked among partitions, but trees were linked. A Yule speciation model was used as tree
242 prior. Markov chain Monte Carlo (MCMC) searches were conducted for 100×10^6 generations
243 sampled every 10,000 generations with the first 25% discarded as burn-in. Convergence and
244 stationarity of the runs were accessed in Tracer v 1.7.1 (Rambaut et al., 2018) using the ESS
245 scores. The final tree was created in TreeAnnotator (both part of BEAST 2 package).
246 Resulting maximum clade credibility tree was visualized and edited in FigTree v. 1.4.4
247 (Rambaut, 2016). All trees with associated data matrix will be deposited in TreeBASE upon
248 manuscript acceptance.

249

250 *2.3. Ancestral range estimation*

251 We were interested in illuminating the biogeographic history of Andreninae and
252 identifying their most probable ancestral range and the events of dispersal between the New
253 World and the Old World and between North and South America. For achieving this purpose,
254 we conducted an ancestral range estimation analysis using five biogeographically relevant
255 regions representing the total distribution of Andreninae and outgroups: A. South America, B.
256 North America, C. Palearctic, D. Afrotropical and E. Australian. The Palearctic is here used in
257 broad sense, including some extralimital elements whose distributions extend to the Oriental
258 region. Geographical occurrences that could be assumed to be secondary were considered
259 biogeographically uninformative. For example, the species-rich genus *Andrena* was
260 considered here as occurring only in North America and in the Palearctic region, in spite of
261 known geographic records in the Neotropics, Afrotropics and Oriental region, since its
262 presence in these latter regions has been attributed to younger clades (Pisanty et al., 2021).
263 Biogeographic coding for *Melitturga* considered the known distribution of the whole genus,
264 even though we only sampled palearctic species.

265 Ancestral ranges of Andreninae were estimated using R package BioGeoBEARS
266 (Matzke, 2013, 2014) comparing different maximum likelihood versions of three classic
267 biogeographic methods: DEC (Dispersal-Extinction-Cladogenesis: Ree and Smith, 2008),
268 BayArea (Bayesian Inference of Historical Biogeography for Discrete Areas: Landis et al.,
269 2013) namely BAYAREALIKE, and DIVA (Dispersal-Vicariance Analysis: Ronquist, 1997)
270 namely DIVALIKE. The maximum clade credibility tree derived from the BEAST analysis
271 was used as input and each terminal taxon was coded for presence/absence in the five
272 geographic areas (Supplementary Table S5). We applied a dispersal multipliers matrix to give
273 different probabilities for different events of dispersal between continents. Dispersals

274 between, for example, South America and Africa, which is very implausible for Andreninae
275 bees after Gondwana breakup, were given a 0 probability; while reasonable events, for
276 example well-documented long-distance dispersals between South and North America, before
277 the closure of the Panama Isthmus, were given a 1 probability. We assessed the overall fitness
278 of the models with likelihood-ratio tests and AIC values.

279

280 **3. Results**

281 *3.1. Phylogenetic Relationships and Divergence Time Estimation*

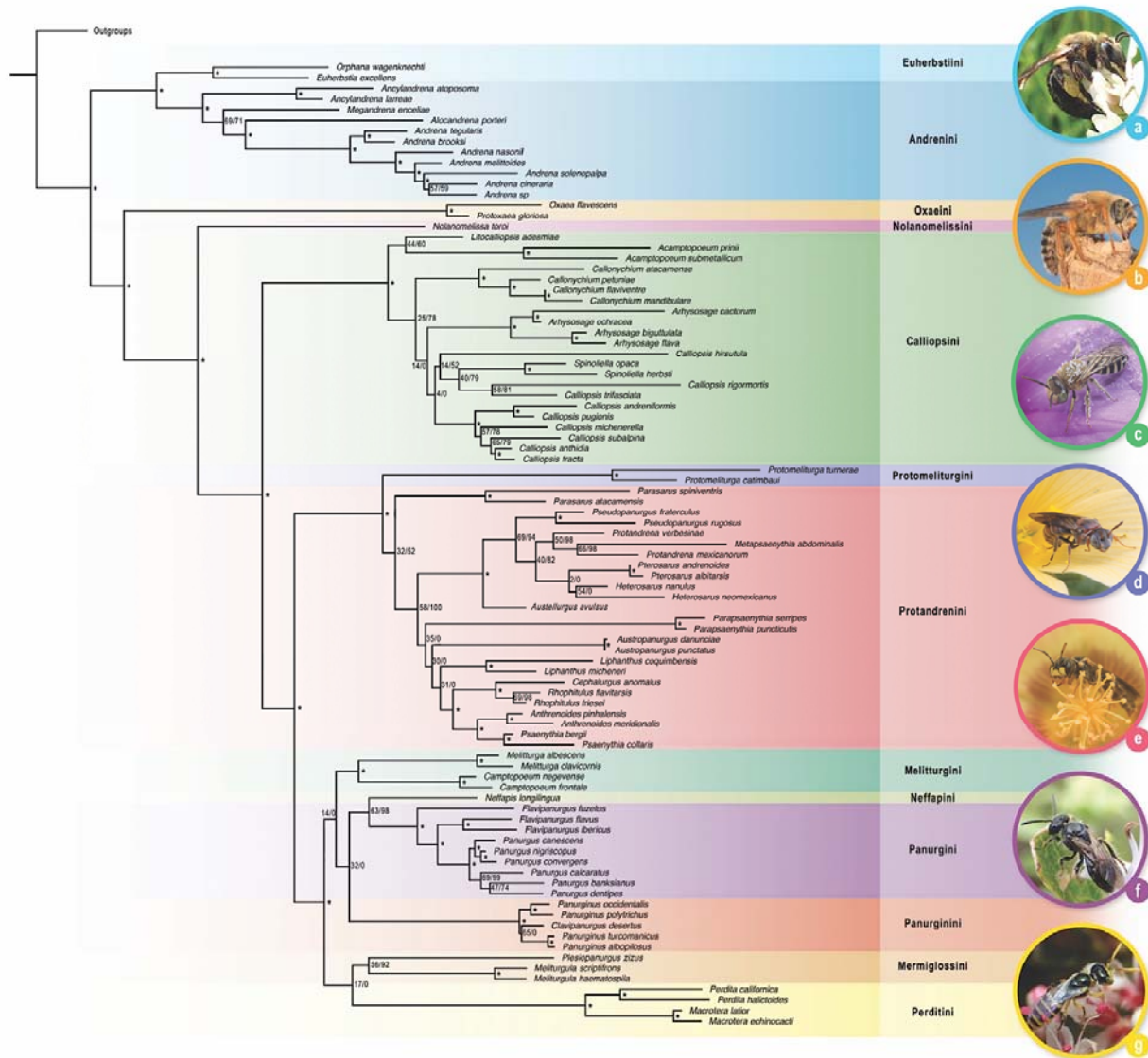
282 We assembled the largest molecular dataset for andrenine bees up to date: our final
283 matrix contains 7952 nucleotides and 106 taxa, comprising approximately 70% of the
284 currently recognized genera in Andreninae *sensu lato*. The monophyly of most tribes and
285 many of their relationships are well-supported based on bootstrap proportions and Bayesian
286 posterior probabilities (i.e. node supports higher than $\geq 70\%$ ML bootstrap and $\geq 98\%$
287 Bayesian posterior probability). Figure 1 and Supplementary Figure S2 depict the phylogeny
288 of Andreninae recovered by ML and Bayesian inference, respectively. Figure 2 and
289 Supplementary Figure S3 show the fossil-calibrated tree and ages for main nodes and related
290 95% highest posterior probability intervals are listed in Table 1.

291 All analyses recovered the monophyly of Andreninae *sensu lato* with high support. The
292 divergence time estimation indicates that Andreninae diverged from the clade Halictinae
293 (Colletinae, Stenotritinae) in the Late Cretaceous and evolved rapidly soon afterwards,
294 considering the age of 96 (91–101) Ma for the crown group. Most andrenine tribes were
295 recovered as monophyletic with maximum support, except for tribes Melitturgini (99 BPP,
296 72% BS) and Panurgini (100 BPP, 88% BS) and the not well supported Protandrenini and
297 Mermiglossini. Furthermore, the position of all tribes agrees between ML and BI, expect for
298 Panurginini and Mermiglossini.

299 The basalmost divergence within Andreninae involves two major clades, both
300 containing widespread species-rich subclades. The first split corresponds to a clade formed by
301 the mainly Holarctic species-rich tribe Andrenini and an endemic Chilean group, the
302 Euherbstiini originated around 73 (59–86) Ma, in the Late Cretaceous. The monophyly of
303 Andrenini is recovered encompassing the monospecific genus *Alocandrena*, endemic to Peru,
304 as sister group of the speciose *Andrena*, a genus widely distributed in the Holarctic region.
305 The second major clade in Andreninae contains a series of tribes, involving both New- and
306 Old-World elements. The base of this clade contains a grade with two New World tribes, the
307 Oxaeini and the Nolanomelissini, which have diverged from the remaining tribes quite early
308 in the Late Cretaceous. Oxaeini is an ancient lineage, having diverged in the Campanian
309 period (~85 Ma), but whose crown is estimated to be much younger. Nolanomelissini, a
310 monospecific group from Chile, is very distinctive and diverged from the panurgine line (see
311 below) around 77 (67–87) Ma.

312 The remaining tribes of this second major clade form a group treated as the Panurginae
313 in Michener's classification and that we refer here as the panurgine line. This large
314 assemblage contains Calliopsini, splitting at the base as sister group of the large clade formed
315 by Protandrenini, Protomeliturcini, Perditini, Melitturgini, Mermiglossini, Neffapini,
316 Panurginini and Panurgini. The divergence between Calliopsini and the other tribes of the
317 panurgine line is estimated to have occurred at 69 (60–79) Ma, while the crown age of
318 Calliopsini is 20 million years younger (Fig. 2 and Table 1). The relictual tribe
319 Protomeliturcini, endemic to the dry areas of northeastern Brazil, is recovered with strong
320 support as sister clade of Protandrenini. The monophyly of Protandrenini is confirmed,
321 although with low support, excluding the monotypic genus *Neffapis* which proved to be
322 phylogenetically related to the Panurgini. Within Protandrenini, the North American genera
323 *Heterosarus*, *Protandrena*, *Pseudopanurgus* and *Pterosarus* were shown to form a clade that

324 diverged from de South American genera in the early Oligocene, around 33 (26–41) Ma (Fig.



325 2).

326 FIGURE 1. Phylogenetic relationships among 91 species of Andreninae bees resulting
 327 from a Maximum Likelihood analysis of concatenated data set of the following seven gene
 328 loci: elongation factor-1a F2 copy, long wavelength rhodopsin, wingless, RNA polymerase II,
 329 28S rRNA, 18S rRNA, and cytochrome c oxidase subunit I. Complete tree of Maximum
 330 likelihood and Bayesian analysis are shown as Figure S1 and S2, respectively. Maximum
 331 likelihood bootstrap support (BS) and Bayesian posterior probability (BPP) values are shown
 332 at nodes. Clade support $\geq 98\%$ BPP and $\geq 70\%$ BS is indicated by asterisk on subtending node.

333 (a) *Andrena vicina*, female. (b) *Oxaea* sp., male. (c) *Acamptopoeum prinii*, male. (d)
334 *Protomeliturga catimbaui*, female. (e) *Cephalurgus anomalus*, male. (f) *Panurginus* sp.,
335 female. (g) *Perdita rhois*, male. Photo credits: Tom Barnes (a, f, g); Julio Pupim and Adriana
336 Tiba (b, c, e); Clemens Schindwein (d).

337

338 The remaining tribes of the panurgine line form a large clade distributed in the
339 Afrotropical (Mermiglossini), Nearctic (Perditini and Panurginini), and Palearctic (Panurgini,
340 Panurginini and Melitturgini) regions, except for Neffapini, a monotypic tribe known only
341 from northern Chile. Phylogenetic relationships among these tribes remain somewhat
342 uncertain, varying to some degree depending on the method used to analyze the data,
343 probably due to the short length of the involved branches (Fig. 1 and Supplementary Fig. S1).
344 The sister-group relationship between Neffapini and Panurgini is recovered in all analyses
345 (79% BS, 100 BPP) and their crown age estimates correspond to about 44 (28–50) Ma.

346 Our analyses recovered the monophyly of all non-monotypic genera, except *Calliopsis*
347 (*Calliopsini*) and *Heterosarus* (*Protandrenini*). This last one was placed in a trichotomy with
348 *Pterosarus* by the Bayesian analysis, but monophyletic in the ML results. *Calliopsis* is
349 paraphyletic, with the species endemic to South America forming a clade distantly related to
350 those from the Nearctic region. Most of the genera in Andreninae (~65%) had their origin in
351 the Miocene (Fig. 2).

352

353 TABLE 1. Divergence time estimates for crown and stem groups of most relevant clades
354 of Andreninae bees. Values in parentheses refers to 95% Highest Posterior Density intervals.
355 Ages in million years (My).

Clade	Crown age	Stem age
Andreninae	91 (82–99)	96 (91–101)
Andrenini + Eueherbstiini	72 (59–86)	91 (82–99)
Andrenini	61 (51–74)	72 (59–86)
<i>Alocandrena</i> + <i>Andrena</i>	50 (40–58)	55 (45–65)
Eueherbstiini	35 (11–61)	72 (59–86)

Oxaeini	11 (2–23)	84 (75–94)
Nolanomelissini	-	76 (67–86)
Calliopsini	48 (37–59)	69 (59–79)
<i>Calliopsis</i> (North America)	20 (12–31)	42 (32–53)
Protomeliturgini + Protandrenini	53 (43–62)	63 (53–72)
Protomeliturgini	20 (8–34)	53 (43–62)
Protandrenini	48 (39–57)	53 (43–62)
Protandrenini (North America)	28 (22–34)	33 (25–41)
Holarctic-Afrotropical clade*	56 (46–65)	63 (53–72)
Melitturgini	39 (26–51)	50 (38–58)
Mermiglossini	34 (21–47)	42 (32–52)
Neffapini	-	43 (28–50)
Neffapini + Panurgini	43 (28–50)	50 (38–58)
Panurgini	37 (19–40)	43 (28–50)
Panurginini	14 (7–21)	48 (38–59)
Perditini	23 (14–32)	42 (32–52)

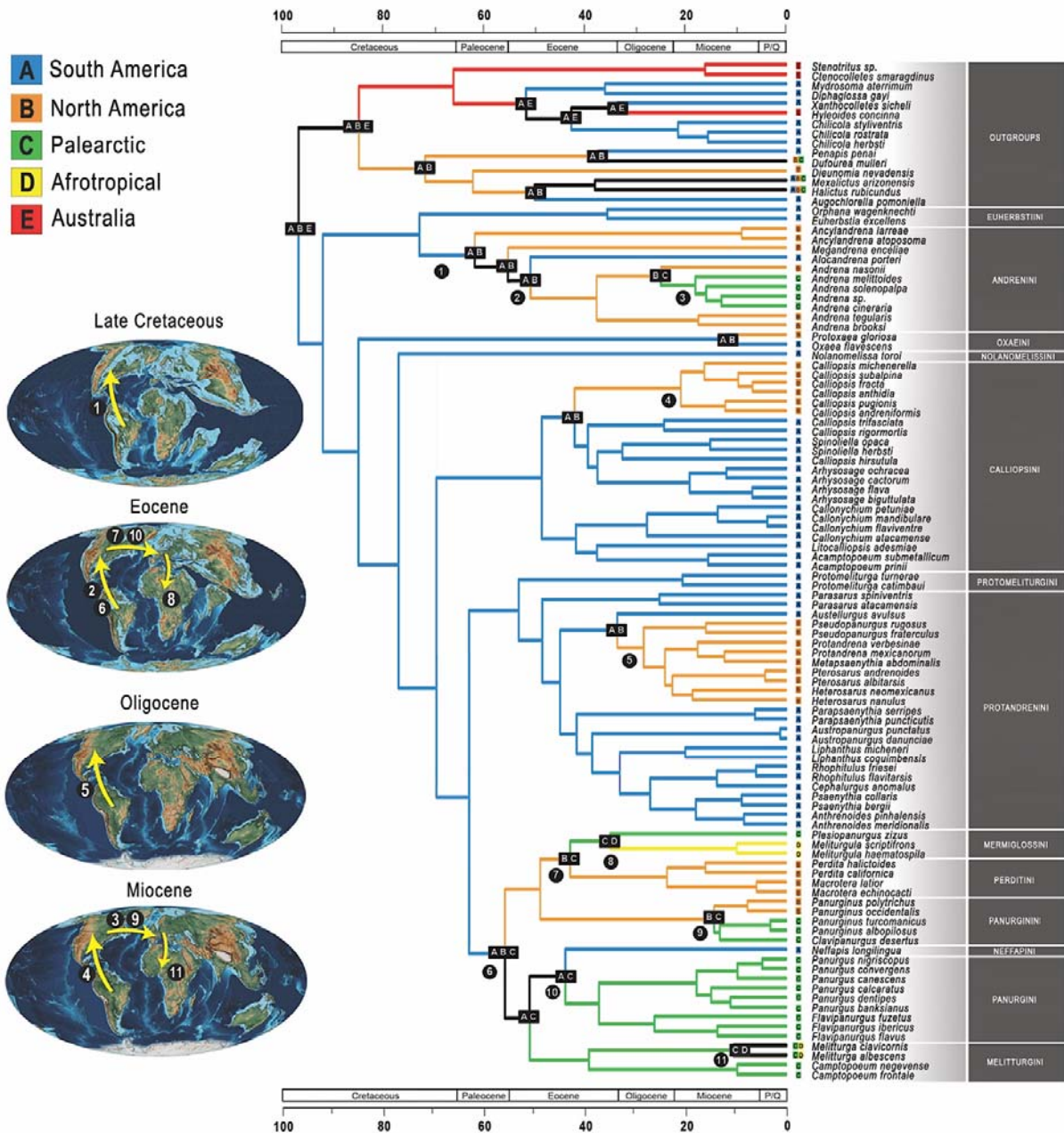
* It includes also the Chilean tribe Neffapini; the whole group could be referred as the supertribe Panurgidi Leach

356

357 *Ancestral Range Estimation*

358 The model DEC yielded the highest likelihood and best AICc scores for ancestral range
359 estimation for the Andreninae phylogeny. Statistical results for all three applied models are
360 given in Supplementary Table S6. Ancestral range estimates plotted in the Maximum Clade
361 Credibility tree are show in Figure 2, for the most important nodes, and in Supplementary
362 Figure S3 for all nodes. The reconstructed scenario shows unequivocally that the most recent
363 common ancestor (MRCA) of the subfamily Andreninae originated in South America in the
364 Late Cretaceous. The early evolution of its main lineages took place in this continent until the
365 end of the Cretaceous, when the first event of dispersal to North America occurred in the
366 Maastrichtian involving the lineage that gave rise to the tribe Andrenini. Other incursions
367 from South to North America and vice-versa involved different lineages in the Eocene within
368 Andrenini and the panurgine line, and during the Oligocene within Protandrenini and
369 Calliopsini, with the last two tribes retaining a largely South American distribution. This

370 indicates at least three periods of long-distance dispersal or possible land connections between
 371 the two continents, much prior to the closure of the Panama Isthmus.



372

373

374 **FIGURE 2.** Maximum likelihood ancestral range estimation in Andreninae, using the best
 375 model DEC (dispersal-extinction-cladogenesis model) implemented in BioGeoBEARS. The
 376 pie diagrams at nodes show the relative probability of the possible states (areas or
 377 combinations of areas). Color boxes show the distribution of each taxa within the clade. Black
 378 boxes represent nodes at which more than an ancestral area was recovered. Black circles

379 represent nodes at which lineage-interchange events may have occurred. Maps adapted from
380 Scotese (2014).

381

382

383 **4. Discussion**

384 *4.1. Phylogeny and Higher Classification of Andrenine Bees*

385 Andreninae is recovered as a well-supported monophyletic group, including the
386 Oxaeini, as previously proposed (Ascher, 2003, 2004; Danforth et al., 2006a, 2006b; Cardinal
387 and Danforth, 2013; Hedtke et al., 2013). One of the earliest-branching lineages of
388 Andreninae corresponds to the already previously recognized clade uniting the tribes
389 Andrenini and Euerbstiini (Pisanty et al., 2021). Our results show *Alocandrena*, a monotypic
390 genus from the Peruvian Andes, nested within Andrenini. Due to its unusual morphology in
391 relation to the other Andrenini, Michener (2000) placed *Alocandrena* in a separate higher
392 taxon, a classificatory decision corroborated neither by the present study nor by previous
393 molecular phylogenies (Pisanty et al., 2021). The phylogenetic relationships among the
394 genera of Andrenini remain controversial. We found *Ancylandrena* (*Megandrena*
395 (*Alocandrena*, *Andrena*)), while Pisanty et al. (2021) recovered *Andrena* as sister to
396 *Alocandrena* (*Ancylandrena*, *Megandrena*).

397 In the other basalmost clade of Andreninae, we have Oxaeini recovered as sister to the
398 remainder of this clade. This tribe is composed of 22 species of quite large and fast hover
399 flying bees restricted to warmer regions of the Western Hemisphere (Ascher et al., 2006;
400 Engel, 2015). The tribe is considerably different morphologically from the other Andreninae,
401 what lead to its placement as a separated family of the traditional classification (e.g. Rozen,
402 1964, 1965, 1993; Hurd and Linsley, 1976), mainly on the basis of several distinct features of
403 their mature larvae and adults (Hurd and Linsley, 1976; Michener, 2007; Engel, 2015).
404 However, broader studies using both larval and adult morphological characters, strongly

405 support the position found here with the group nested within Andreninae sensu lato
406 (Michener, 1944; Graf, 1966; Rozen, 1993, 1994; Alexander and Michener, 1995; Ascher,
407 2003, 2004; Danforth et al., 2006a, 2006b; Hedtke et al., 2013; Engel, 2015).

408 The enigmatic species *Nolanomelissa toroi*, known only from the southern border of the
409 Atacama Desert in Chile, appears as sister to remaining tribes of the panurgine line. This
410 species is oligolectic on pollen of *Nolana rostrata* (Solanaceae) (Rozen, 2003; Michener,
411 2007), and has a number of morphological apomorphies, but also shares characteristics with
412 different tribes of Andreninae making it difficult to assign to a tribe unequivocally (Rozen,
413 2003). However, our results agree with the placement of *Nolanomelissa* in its own tribe,
414 Nolanomelissini, as proposed by Rozen and Ascher (in Rozen, 2003). Calliopsini, here
415 represented by all of its genera (except *Xeranthrena*) and including South American species
416 of *Calliopsis* is recovered as monophyletic. Phylogenetic relationships based in adult
417 morphology have recovered a clade containing the genera *Callonychium*, *Spinoliella*,
418 *Xeranthrena* and *Arhysosage* excluding *Calliopsis*, *Acamptopoeum* and *Litocalliopsis* (Ruz,
419 1991; Roig-Alsina and Compagnucci, 2003; Gonzalez et al., 2017). Data on larval
420 morphology have also supported a close relationship between *Arhysosage*, *Callonychium* and
421 *Spinoliella* (Rozen, 2013; referred by him as the *Spinoliella* group). Our results, however,
422 indicate that *Calliopsis* sensu lato belongs to this group and *Spinoliella* makes the large genus
423 *Calliopsis* paraphyletic.

424 Species of *Calliopsis* are classified in nine subgenera, three of them endemic to South
425 America and six to North America. Although Ruz (1991) evidenced the monophyly of
426 *Calliopsis* sensu lato, she highlighted the South American *C. (Liopoeum)* as one of the most
427 distinctive subgenera, with some species having the female metasomal terga with hair bands
428 and others with pigmented yellow bands. It seems clear that the genus *Calliopsis* still require
429 a more thorough phylogenetic investigation, with a broader taxonomic sampling to

430 understand the limits and definitions of the endemic South American subgenera in order to
431 define their taxonomic status. *Litocalliopsis* was corroborated as a distinct lineage sister to
432 *Acamptopoeum*, contradicting previous hypothesis proposing it as sister to *Calliopsis* (Roig-
433 Alsina and Compagnucci, 2003). In fact, these authors have also indicated several
434 morphological features shared between *Litocalliopsis* and *Acamptopoeum*.

435 Our molecular phylogeny provides a novel sister-group relationship between
436 Protomeliturgini and Protandrenini with maximum support. Previous hypotheses suggested
437 Protomeliturgini as close to Perditini and Calliopsini (Ruz, 1986, 1991). Protomeliturgini
438 contains a single genus with two described species distributed in semiarid areas of
439 northeastern Brazil and oligolectic in flowers of Turneraceae (Medeiros and Schlindwein,
440 2003). *Protomeliturga* is easily distinguished from other lineages by the very elongated first
441 two articles of the labial palpus, strongly curved 2nd abscissa of the vein M (basal vein), and
442 tergum 7 of male strongly curved forward, with a pair of apicolateral teeth (Schlindwein and
443 Moure, 2005; Michener, 2007).

444 Protandrenini is morphologically heterogenous, therefore not obviously monophyletic,
445 but was recovered by the present molecular dataset. Our results unequivocally exclude
446 *Neffapis* from Protandrenini. The placement of *Austellurgus avulsus* (Ramos & Melo, 2006)
447 as sister to the North American Protandrenini is here reinforced. Michener (2007) gave
448 *Protandrena* an overly broad scope, recognizing as subgenera the South American species of
449 *Austropanurgus* and *Parasarus* and North American species of *Heterosarus*, *Metapsaenythia*,
450 and *Pterosarus*. Yet, *Pseudopanurgus* was also previously recognized in a much broader
451 sense synonymizing the genera *Heterosarus*, *Pterosarus*, and *Xenopanurgus*, but based on an
452 incomplete sampling of those lineages. Our results do not provide support to these
453 propositions, showing *Protandrena* sensu Michener (2007) as polyphyletic with respect to
454 genera from South America and *Pseudopanurgus* sensu Ascher (2004) paraphyletic in

455 relation to *Protandrena*. We have also found out *Protandrena* sensu stricto paraphyletic to
456 *Metapsaenythia* as already indicated by both morphological and molecular data (Ascher,
457 2004; Michener, 2007).

458 Within the panurgine line, we also found a large clade formed by the tribes Neffapini,
459 Perditini, Mermiglossini, Melitturgini, Panurgini and Panurginini (see below for decision on
460 classification system). Relationships within this large and heterogeneous clade, containing
461 both New and Old World taxa, differed somewhat between the analyses, with the alternative
462 arrangements involving mainly Panurginini and Mermiglossini. The monospecific Neffapini,
463 the only South American element in this clade, came out as sister-group to the Old World
464 *Flavipanurgus* and *Panurgus* (Panurgini). *Neffapis*, endemic to Coquimban desert in Chile,
465 exhibits many unique characters such as the extremely long glossa and third labial palpus and
466 a minute maxillary palpus with only two palpomeres (Rozen and Ruz, 1995), which led to
467 difficulties in positioning it within existing tribes. Proposal of a separate tribe for *Neffapis* by
468 Ascher (in Engel, 2005) is corroborated here, although in a rather different phylogenetic
469 scenario.

470 Monophyly of Perditini and its two genera, *Perdita* and *Macrotera*, corroborates
471 morphological phylogenies (Danforth, 1996) and recognition of *Macrotera* as a valid genus
472 distinct from *Perdita*. This tribe is close to Palearctic and Afrotropical tribes (Patiny, 1999;
473 Ascher, 2003). The phylogenetic relationships of *Camptopoeum* with *Melitturga* and
474 *Melitturgula* with *Plesiopanurgus* corroborate the establishment of the tribes Melitturgini and
475 Mermiglossini, respectively. It is possible that this large clade may also include the intriguing
476 species *Simpanurgus phyllopodus* (Warncke, 1972), endemic to the Iberian Peninsula and
477 known only from males. Previous treatments indicated *Simpanurgus* as a subgenus of
478 *Panurgus* (Michener, 2007) or as *incertae sedis* in relation to the Old-World tribes (Ascher
479 and Engel, 2017). The inclusion of molecular data from this species, as well as a broader

480 sampling of Old-World elements, would improve our understanding of its position in the
481 phylogeny of Andreninae.

482 The classification system adopted here for this large clade of the panurgine line gives
483 tribal status to the main lineages. The systematic position of the genera within this large clade
484 varies in different papers and differs according to the suprageneric-level classification
485 adopted (Table 2). A comprehensive background about the distinct classification systems
486 adopted for these taxa is provided by Ascher and Engel (2017). Here we propose the
487 following taxonomic arrangements: Mermiglossini include *Plesiopanurgus* and *Meliturgula*,
488 represented here by a total of three species, and the non-represented genera *Flavomeliturgula*,
489 *Gasparinahla* and *Mermiglossa*; Melitturgini include *Camptopoeum*, *Melitturga* and the
490 genera not sampled here *Avpanurgus* and *Borgatomelissa*; Panurgini include *Panurgus* and
491 *Flavipanurgus*; Panurginini include only *Panurginus*; and Perditini comprise *Macrotera* and
492 *Perdita*. We disagree from previous classifications that give status of subtribe to these
493 lineages, as proposed by Ascher (2004), Engel (2005), and Ascher and Engel (2017), based
494 on the following reasons: (1) We found no evidence for a monophyletic Panurgini sensu lato;
495 Neffapini would have to be included to make it monophyletic; (2) Use of tribal level
496 preserves the status of well-known groups, as the Perditini; (3) There is a large amount of
497 heterogeneity within the clade, surpassing that found in other andrenine lineages, to assemble
498 them under a single tribe; (4) The first divergences within the clade, dated from the early
499 Eocene, have ages comparable to those of other lineages given tribal status within the
500 Andreninae. The classification system adopted here is summarized in Table 2.

501

502 4.2. Early evolution of the Andreninae

503 Our fossil calibrated tree and ancestral area estimation indicate that Andreninae arose in
504 South America during the Turonian, around 90 Ma in the Late Cretaceous, an age consistent

505 with previous estimates (Cardinal and Danforth, 2013; Sann et al., 2018). The biogeographic
506 history of Andreninae involved multiple northward transcontinental dispersal events, from
507 South to North America, with subsequent incursions to the Palearctic region and from there to
508 the Afrotropics, as a result of lineage interchanges between these landmasses. Our results,
509 therefore, suggest that the breakup of Gondwana seem to have had minor impact on the early
510 evolution of Andreninae.

511 Distribution, relationships, and divergence times among andrenine tribes suggest a
512 pattern of diversification mainly related to historical connections between North and South
513 America. The andrenine tribes originated during a relatively long time-window between the
514 Maastrichtian in the Late Cretaceous (crown age of Andrenini: 72 Ma) and the Miocene
515 (crown age of Oxaeini: 11 Ma). These exchanges between the land masses of the Western
516 Hemisphere gave rise to what is known as the amphitropical distributional pattern (see
517 Michener, 1979), which is exhibited by several lineages of Andreninae, as well as many other
518 New World bee groups.

519 Aside from land connections, dispersal of andrenine bees across Mesoamerica would
520 have required the availability of suitable habitats. Here we were able to reconstruct a more
521 specific biogeographic scenario showing the early differentiation of Andreninae taking place
522 under conditions similar to those prevalent today in the xeric regions of western South
523 America. Although a large diversity is currently distributed in open vegetation of the
524 American continent, arid and semiarid regions with Mediterranean climate played an
525 important role in the diversification of andrenine bees, especially the western portion of South
526 America (Chile and Argentina) and southwestern North America (Californian deserts and the
527 desertic regions of the southwestern United States and northern Mexico). The importance of
528 these areas to the evolution of Andreninae is supported by the occurrence of many endemic
529 taxa (Michener, 1979; Simpson and Neff, 1985; Turchetto-Zolet et al., 2013). Occurrence of

530 the ancient relictual lineages *Euherbstiini*, *Nolanomelissini* and *Neffapini* west of the Andes
531 in the southern Atacama and Coquimban deserts of Chile is a further evidence of these areas
532 as refugia for bees. The complex geological scenario of Andean orogeny, a long process
533 beginning in the Paleogene, has affected the climate and biodiversity in South America
534 (Hoorn et al., 2010), favoring a progressive formation of xeric habits along western portions
535 of South America (Armijo et al., 2015) and acting as a barrier to bee dispersal. This picture of
536 Andean formation is consistent with our data that shows that the first mountain chain may
537 have acted as isolation barrier for earlier andrenine lineages as *Euherbstiini*, *Nolanomelissa*,
538 *Neffapis*, and *Alocandrena*, and also as a strong barrier that prevented the spread of eastern
539 taxa such as the *Oxaeini*.

540

541 *4.3. Bee faunal interchanges between South and North America*

542 The evolutionary history of the Andreninae involves multiple faunal interchanges
543 between the South and North Americas in at least three different periods suggesting the
544 existence of land connections prior to the final closure of the Panama Isthmus in the Miocene.
545 The time range of the events estimated in our dating analyses is chronologically consistent
546 with different hypotheses of the geological history of connections between the two large
547 landmasses of the American continent from the Late Cretaceous onward. Contrary to
548 Michener's (1979) hypotheses, we found evidence that south-to-north dispersal of bees is
549 older than the reverse direction.

550 The early divergence between the tribes *Andrenini* and *Euherbstiini* in the Late
551 Cretaceous involved a geodispersal to North America (Fig. 2, Clade 1). Examples of similar
552 exchanges during this period have been reconstructed in other insect groups, such as riodinid
553 butterflies (Espeland et al., 2015), melanopline grasshoppers (Chintauan-Marquier et al.,
554 2011), and in bees of both eucerine and apine lines (see Martins and Melo, 2016). This

555 exchange event between the Americas, during the Campanian and the Maastrichtian, has been
 556 named as the First American Biotic Interchange (FABI), and has been originally proposed
 557 from patterns found in vertebrates (Goin et al., 2012).

558

559 TABLE 2. A comparison of suprageneric-level treatments for Andreninae classification.

Patiny (1999)	Ascher (2004)	Michener (2007)	Ascher and Engel (2017)	Present study
NT	Andrenini	Andreninae ¹	NT	Andrenini
NT	Euherbstiini	Andreninae	NT	Euherbstiini
NT	Oxaeinae	Oxaeinae	NT	Oxaeini
then undescribed	Nolanomelissini	Nolanomelissini	Nolanomelissini	Nolanomelissini
Calliopsini	Calliopsini	Calliopsini	Calliopsini	Calliopsini
Protandrenini	Protandrenini	Protandrenini	Protandrenini	Protandrenini
NT	Panurgini: Protomelitturgina	Protomelitturgini	Protomelitturgini	Protomelitturgini
NT	Panurgini: Neffapina	Protandrenini	Neffapini	Neffapini
Camptopoeumini* ² , Melitturgini*	Panurgini: Camptopoeumina* ² , Melitturgina*	Panurgini*, Melitturgini*	Panurgini: Camptopoeina*, Melitturgina*	Melitturgini
Mermiglossini*, Paramelitturgini* ³	Panurgini: Mermiglossina*, Melitturgulina*	Melitturgini	Panurgini: Mermiglossina*, Melitturgulina*	Mermiglossini
Panurgini	Panurgini: Panurgina	Panurgini	Panurgini: Panurgina	Panurgini
Panurginini	Panurgini: Panurginina	Panurgini	Panurgini: Panurginina	Panurginini
Perditini	Panurgini: Perditina	Perditini	Panurgini: Perditina	Perditini

560 NT: not treated. * In part. ¹*Alocandrena* excluded. ²*Nomen imperfectum* (Engel 2005).

561 ³Invalid name (Engel, 2001)

562

563 Distinct relative periods of isolation and contact of these landmasses from the
 564 Cretaceous to the Pleistocene, and its impact on the amphitropical fauna and flora diversity
 565 have long been a subject of discussion. In this way, the disjunct distribution in the American
 566 continent implies that Central America served as an important setting for diversification of
 567 such taxa in the past. Historical connections between North and South America involve a
 568 complex geodynamic of the Caribbean plate that modulated the relative isolation of such
 569 areas during distinct geological periods (Iturralde-Vinent and MacPhee, 1999; Ortiz-
 570 Jaureguizar and Pascual, 2007; Pindell and Kennan, 2009; Woodburne, 2010; Cody et al.,

571 2010; Farris et al., 2011; Giunta and Orioli, 2011; Coates and Stallard, 2013; Bacon et al.,
572 2013, 2015; Montes et al., 2012, 2015; Cione et al., 2015). Since there is no evidence for an
573 arid corridor through Central America or a full connection of the North and South landmasses
574 before the closure of the Panama Isthmus, geodispersal events should be invoked to explain
575 the diversification of the andrenine bees in the Americas, which perhaps have been facilitated
576 by transient arid conditions in the Central America terrains.

577 The sister relationships between the Peruvian genus *Alocandrena* and the Holarctic
578 *Andrena* represent another trans-Caribbean geodispersal event, but estimated here to have
579 occurred in the Eocene (~50 Ma) (Fig. 2, Clade 2). Pisanty et al. (2021) also recovered an
580 early Eocene age for the split between *Alocandrena* and their North American relatives and
581 explained this pattern by long distance wind dispersal through the Central American Seaway,
582 possibly aided by island hopping. Our results also indicate another ancient dispersal event
583 taking place at the beginning of the Eocene and involving the South American lineage that
584 gave rise to a large clade presently distributed in the Nearctic, Palearctic and Afrotropical
585 regions, but also containing a single South American genus, the Chilean *Neffapis*, nested
586 within it (Fig. 2, Clade 6). The distribution pattern exhibited by this clade implies
587 biogeographic scenarios invoking a direct dispersal from South America to the Palearctic
588 region. Such direct connections are unlike to have occurred during this timeframe and
589 considering the presence of some elements in the Nearctic region, as well the Chilean lineage,
590 we presuppose that this ancestral lineage also entered the Old World following a route
591 through North America. This South America–Palearctic disjunct scenario, however,
592 presupposes some extinctions in the Nearctic region since only a few lineages of this clade
593 have North American representatives. Inter-American exchanges during the same time period
594 in the early Eocene have previously been documented for the bee tribes Halictini and
595 Sphecodini (Danforth et al., 2004).

596 Additional inter-American exchanges occurring under younger ages have been also
597 recovered in our study. Within the tribes Calliopsini and Protandrenini, we found a repeated
598 biogeographic pattern with incursions to North America occurring between 42–28 Ma (late
599 Eocene to early Oligocene) and 33–20 Ma (early Oligocene to early Miocene), respectively
600 (Fig. 2, Clades 4 and 5). The tribe Oxaeini also exhibits an amphitropical distribution and
601 likely represents another dispersal event within this scenario (Fig. 2), but whose divergence
602 estimation needs further investigation with a more comprehensive taxon sampling.

603 The majority of the most recent and well-documented episodes of multitaxon
604 interchanges between North and South America occurred after formation of a permanent land
605 corridor in the Pliocene (~3 Ma). This biogeographic event – the Great American Biotic
606 Interchange (GABI) – resulted from the rise of the Isthmus of Panama and climatic changes
607 (mainly glaciations) that allowed many animal groups to cross the Panamanian connection
608 (Simpson and Neff, 1985; Cody et al., 2010; Goin et al., 2012; Wilson et al., 2014; Cione et
609 al., 2015). On the other hand, our results support that andrenine bees have migrated during
610 Oligocene and Miocene along Central America around times when patches of open habitats
611 might have been available. Similar to the Andreninae, species in the bee genera *Diadasia* and
612 *Centris* are also associated with arid regions and their evolutionary history endorses a
613 scenario with prevailing climatic conditions and plant formations adapted to dry conditions in
614 the connecting areas between the American landmasses (Wilson et al., 2014; Martins and
615 Melo, 2016). Hines (2008) also suggested that bumblebees may have arrived in South
616 America in the Miocene crossing Central America when favorable patches of temperate
617 habitats arose.

618 The GABI was initially characterized by the movement of land mammals between
619 North and South America which requires a contiguous area for overland migration (reviewed
620 by Goin et al., 2012, 2015). Several studies suggest that inter-American connections and

621 interchange of organisms, after Eocene age, could have happened earlier than the final closure
622 of the Isthmus of Panama. Cody et al. (2010) reveal the asynchrony between the evolutionary
623 history of several animal taxa related to GABI and plant migrations from South to North
624 America (at least 20 Ma earlier). As observed for plants, northward transcontinental dispersal
625 events around this same time window have been reconstructed here for the Calliopsini and the
626 Protandrenini, and have also been found in other bee groups [see Ramírez et al. (2010) for
627 early Miocene divergences within the orchid-bee genera *Euglossa* and *Eufriesea*, Almeida et
628 al. (2012) for the colletine *Caupolicana* and *Eulonchopria*, Wilson et al. (2014) for the
629 emporine genus *Diadasia* and Martins and Melo (2016) for the oil-bee genus *Centris*].

630 Geological favorable scenarios invoked to understand the biotic movements through the
631 American continent around Oligocene-Miocene include overwater long-distance dispersal
632 (Michener, 1979; Cody et al., 2010), volcanic island hopping (Iturralde-Vinent and MacPhee,
633 1999; Sturge et al., 2009) or through emergent peninsulas formed in Central America (Kirby
634 et al., 2008; Monte et al., 2012, 2015). Although there is still little evidence for an earlier age
635 for the closure of the Isthmus of Panama sensu stricto as highlighted by O’Dea et al. (2016),
636 the current biological evidence, including that brought in the present contribution, endorse a
637 pattern of early interchanges across Central America much before the closure of the Isthmus
638 of Panama has been completed.

639

640 4.4. Into the Palearctic and Afrotropical regions

641 The evolutionary history of the Andreninae occurring in the Old World is explained by
642 our data through four dispersal events from North America to the Palearctic region in two
643 distinct historical periods. Most ancient dispersal movements occurred in the Eocene and
644 involve the evolutionary history of Mermiglossini, which seems to have derived from a North
645 American ancestor common to Perditini, and also of the lineage that gave rise to the clade

646 containing Melitturgini, Panurgini and Neffapini. In both cases, the incursions to the
647 Palearctic region imply a North American MRCA. We also find evidence of a biotic
648 connection between North America and the Palearctic region during the late Oligocene (~24
649 Ma) and early Miocene (~14 Ma) indicated by diversification episodes within of the Holarctic
650 genera *Andrena* and *Panurginus* (Fig. 2, Clades 3 and 8, respectively). The presence of
651 Panurgini and Panurginini in Eurasia via North America was also early inferred by Michener
652 (1979) and Ruz (1986).

653 Evidence for these two dispersal pulses among the Northern Hemisphere landmasses
654 has been previously advocated by Praz and Packer (2014) for the long-tongued bee tribes
655 Ancylini and Eucerini. These authors reconstructed a first geodispersal event involving the
656 origin of the clade composed by the genera *Ancyla* and *Tarsalia* reaching the Old World from
657 the New World during the early Eocene, and a second distinct period of exchanges between
658 the Eastern and Western Hemispheres in the Miocene involving some eucerine genera.
659 Intercontinental dispersal events in the opposite direction, i.e. from the Palearctics to the
660 Nearctics, during Miocene age is also inferred to explain the evolutionary history of
661 bumblebees (Hines 2008) and honeybees (Engel et al., 2009).

662 Expansion of New World terrestrial biotic elements onto the Old World, through a
663 North American route, suggests the existence of Northern Hemisphere land bridges with
664 suitable environmental conditions in distinct ages, such as the Beringia, Thulean and De Geer
665 land bridges (Sanmartín et al., 2001; Brikiatis, 2014). The Bering route functioned in warm
666 periods connecting East Asia and North America. The De Geer Bridge chronologically
667 coincides with the Bering route but connected Eastern North America to Eurasia through the
668 Greenland to Fennoscandia (Sanmartín et al., 2001). The Thulean Bridge was also an
669 intercontinental land bridge that connected the Northern Hemisphere via Greenland but
670 became established well after the interruption of the De Geer route, offering a southerly

671 connection through Greenland, Iceland, Faroe Islands and Scotland. In addition to land
672 connections, the progressive aridification and temperature decline documented during the
673 middle Eocene to early Oligocene led to favorable circumstances for bee diversification. This
674 particular climatic condition that caused the displacement of tropical and subtropical forests
675 led also to further expansion of temperate savanna-like vegetation to as far north as the
676 Beringian and North Atlantic Land bridges in North America (Graham, 1999; Cardinal,
677 2018).

678 As similarly inferred by Praz and Packer (2014) for the tribe Ancylaini, the incursion of
679 andrenine bees into the Old World during the Eocene possibly occurred through the Thulean
680 route, taking into account that it implies warmer environmental conditions, due its more
681 southern position, and its connections to the western Palaearctics where the andrenine bees
682 are more diverse today. Additional examples of geodispersals related to the Thulean route in
683 the same period and invoking similar environmental conditions are known from other groups
684 of organisms (see Praz and Packer, 2014).

685 Afrotropical distribution of *Melitturga* (Melitturgini) and *Meliturgula* (Mermiglossini)
686 are estimated by our analyses as deriving from two distinct events involving Palearctic
687 ancestral groups. While the event involving the Melitturgini seems to have taken place during
688 the Tortonian age in the Miocene (around 10 Ma; 3-18 HPD 95%), that in the Mermiglossini
689 occurred at a much older age (Fig. 2, Clade 8). Indeed, the scenario within the Mermiglossini
690 is more complex and involves the Afrotropical genus *Mermiglossa*, not sampled here, known
691 from Namibia to central East Africa (Ascher and Engel, 2017). *Mermiglossa* is closely related
692 to *Plesiopanurgus* based on morphological data (Ruz, 1986; Patiny, 1999; Ascher, 2004;
693 Michener, 2007; Ascher and Engel, 2017) what indicates that its presence in the Afrotropics
694 derives from an ancient occupation of Palearctic arid areas in northern Africa and the Middle
695 East by the ancestral lineage of the Mermiglossini.

696 **5. Conclusions**

697 In the current study we present the first most comprehensive dated phylogeny to
698 understanding of the complex evolutionary history of Andreninae bees. We provide molecular
699 phylogenetic evidence that corroborate the monophyly of several tribes currently recognized
700 based on morphological evidence. The relationships within the large clade containing the
701 tribes Neffapini, Melitturgini, Mermiglossini, Perditini, Panurgini and Panurginini remain
702 poorly resolved and will require further investigation. The reconstructed evolutionary
703 scenario for the Andreninae sensu lato also provided new insights into the biogeographic
704 patterns and revealed repeated biotic interchanges between the major landmasses. The early
705 evolution of the main lineages took place in South America, during the Late Cretaceous, and
706 extended to the beginning of the Paleocene, with North American lineages originating from
707 South American relatives. Presence of the clade in the Palearctic region results from multiple
708 exchanges with North America by long-distance dispersal or hopping through landmass
709 chains. Our analyses provide strong support for the amphitropical pattern currently observed
710 in the American continent resulting from at least three periods of possible land connections
711 between the two American landmasses. The last incursion from South to North America is
712 reported in the Miocene, much prior to the well documented Panama Isthmus closure.

713

714 **CRedit authorship contribution statement**

715 Kelli Ramos: designed research, data analysis, writing - original draft. Aline Martins: data
716 analysis, writing - review & editing. Gabriel Melo: designed research, data analysis, writing -
717 review & editing.

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738 **Data availability**

739 Supplementary data are provided in Appendix A.

740 Phylogenetic matrix are provided in Appendix B.

741

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