

1 Regular Article

2 Article title: An ant genus-group (*Prenolepis*) illuminates the biogeography and drivers of insect
3 diversification in the Indo-Pacific

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29 Abstract:

30 The Malay Archipelago and the tropical South Pacific (hereafter the Indo-Pacific region) are
31 considered biodiversity hotspots, yet a general understanding of the origins and diversification of
32 species-rich groups in the region remains elusive. We aimed to test hypotheses for the
33 evolutionary processes driving insect species diversity in the Indo-Pacific using a higher-level
34 and comprehensive phylogenetic hypothesis for an ant clade consisting of seven genera. We
35 estimated divergence times and reconstructed the biogeographical history of ant species in the
36 *Prenolepis* genus-group (Formicidae: Formicinae: Lasiini). We used a fossil-calibrated
37 phylogeny to infer ancestral geographical ranges utilizing a biogeographic model that includes
38 founder-event speciation. Ancestral state reconstructions of the ants' ecological preferences, and
39 diversification rates were estimated for selected Indo-Pacific clades. Overall, we report that
40 faunal interchange between Asia and Australia has occurred since at least 20–25 Ma, and early

41 dispersal to the Fijian Basin happened during the early and mid-Miocene (*ca.* 10–20 Ma).
42 Differences in diversification rates across Indo-Pacific clades may be related to ecological
43 preference breadth, which in turn may have facilitated geographical range expansions. Ancient
44 dispersal routes suggested by our results agree with the palaeogeography of the region. For this
45 particular group of ants, the rapid orogenesis in New Guinea and possibly subsequent ecological
46 shifts may have promoted their rapid diversification and widespread distribution across the Indo-
47 Pacific.

48 Keywords: biogeography; diversification rate; ecological shift; New Guinea; Pacific islands;
49 taxon cycle

50 **1. Introduction**

51 The islands of the Pacific Ocean constitute a relatively small amount of land area, but they
52 harbour an extraordinary number of endemic taxa (Keppel et al., 2009). Given the complexity of
53 their biogeographical history, multiple scenarios for the origin of the region's vast diversity have
54 been proposed (Gillespie et al., 2008; Keppel et al., 2009; Lohman et al., 2011; Stoddart, 1992),
55 but evaluations of them incorporating robust phylogenetic inferences, probabilistic biogeography
56 analyses, and ecological data have been scarce, especially as applied to species-rich groups (but
57 see, Gressitt, 1984; de Boer & Duffels, 1996; Sharma & Giribet, 2012; Clouse et al., 2015;
58 Economo et al., 2015b). In this study, we aim to understand the evolution of the ants within the
59 *Prenolepis* genus-group (Formicinae: Lasiini) (LaPolla et al., 2010a; Ward et al., 2016) as a
60 model for studying historical biogeography in the Malay Archipelago and the tropical South
61 Pacific (hereafter the TSP; Fig. 1). These ants are geographically widespread, and their lineages
62 exhibit a broad array of ecological preferences. They are a ubiquitous component of tropical

63 forests, they occupy all types of terrestrial habitats in the region, and some species have become
64 invasive.

65 Although various palaeogeographical reconstructions have been proposed for the region
66 (Baldwin et al., 2012; Hall, 2013; Lohman et al., 2011), most models agree that there was a
67 significant rise of subaerial (above water) land and orogenic activity within the past 5–10 Ma.
68 However, it is unclear whether there was ever a chain of volcanic activity and possible
69 archipelago connecting the Sunda Shelf in Southeast (SE) Asia and the Sahul Shelf, which
70 includes Australia and New Guinea (Hall, 2013; Lohman et al., 2011). If such a gateway did not
71 exist, then dispersal of many non-marine taxa between Asia and Australia across Wallace's Line
72 and the Wallacea region (the islands between Borneo and New Guinea) would have been limited
73 during most of the Miocene (i.e., 10–25 Ma); however dated molecular phylogenies of several
74 vertebrate groups strongly suggest faunal interchange across Wallacea throughout the Miocene
75 epoch (Cibois et al., 2014; Georges et al., 2014; Jönsson et al., 2011; Mitchell et al., 2014).
76 Molecular chronograms of insect taxa, nonetheless, largely agree with a later biotic exchange
77 scenario, around the late Miocene and Pliocene (i.e., <15 Ma; Balke et al., 2007; Condamine et
78 al., 2013, 2015; Müller et al., 2013; Tänzler et al., 2014; but see, Economo et al., 2015b), but
79 even among arthropods there are unexplained dispersal patterns (Clouse and Giribet, 2007). Not
80 only is the existence of such a Miocene land bridge unclear, but so is its possible extent, location,
81 and duration.

82 The island of New Guinea is a major centre of biotic endemism in the region. It is believed that
83 arthropod diversification there has occurred since the early Miocene (*ca.* 20–25 Ma; de Boer &
84 Duffels, 1996; Sharma & Giribet, 2012; Müller et al., 2013), accelerating during the past 5–10
85 Ma, concurrent with the orogeny of its Central Range (Toussaint et al., 2014). Although the

86 tectonic history of New Guinea is complex (Baldwin et al., 2012), broadly speaking there are two
87 competing hypotheses for the emergence of its land: 1) an early Miocene islands group along the
88 northern edge, so-called the proto-Papuan archipelago (Hall, 2002; Jønsson et al., 2011; Lohman
89 et al., 2011), and alternatively, 2) an Oligocene Papuan peninsular orogeny (*ca.* 30–35 Ma)
90 restricted to southeastern New Guinea (van Ufford and Cloos, 2005). These two models make
91 contrasting predictions about the first colonization events of New Guinea as either taking place
92 on the northern or southeastern sides of the island.

93 In the case of oceanic islands, it was hypothesized that most of the extant TSP archipelagos
94 emerged during the late Miocene and Pliocene (*ca.* 5–10 Ma; Gillespie et al., 2008; Gillespie &
95 Clague, 2009), but volcanic activity has been reconstructed since at least the Oligocene (*ca.* 30
96 Ma) (Neall and Trewick, 2008). Although palaeogeographic reconstructions depict ancient
97 Melanesian volcanic arcs extending from New Guinea into the Fijian Basin (Hall, 2002),
98 subaerial land may have been ephemeral (Hall, 2013). One hypothesis, the Vitiaz Arc Model,
99 suggests a relatively continuous archipelago extending throughout proto-Papua, the Solomon
100 Islands, Vanuatu and Fiji (Ewart, 1988; Rodda, 1994). Biogeographically, this scenario predicts
101 early stepping-stone dispersals to the southwest Pacific, which may have occurred until the
102 disruption of the island arc at about 10 Ma (Hall, 2002). Some of these earlier colonizers might
103 never have faced extinction because certain islands in Vanuatu and Fiji remained subaerial since
104 the early Miocene (*ca.* 25 Ma; Sarnat & Economo, 2012). In fact, time-calibrated insect
105 phylogenies strongly support the idea of a mid-Miocene colonization of Fiji from either New
106 Guinea or SE Asia (Balke et al., 2007; Economo et al., 2015a; Lucky and Sarnat, 2010; Sarnat
107 and Moreau, 2011).

108 Apart from the geological processes that might have facilitated the colonization of Melanesia,
109 including New Guinea, and the TSP, species diversification and distribution across islands may
110 have been driven locally by ecological processes, as suggested by recent advances in Island
111 Biogeography theory (Whittaker et al., 2008). The expansion/contraction phases, or the taxon
112 cycle hypothesis, is a classic, influential model that attempts to explain population differentiation
113 and geographic ranges over time in an ecological framework (Ricklefs and Cox, 1972; Wilson,
114 1961, 1959). In the expansion stage of the cycle, species colonize nearby archipelagos
115 (geographical expansion) facilitated by shifts in habitat preference towards marginal habitats
116 such as island coasts (ecological expansion). The following contraction stages involve
117 phenotypic and genetic differentiation of populations across islands, extinction of small
118 unspecialized populations, and the origin of single-island endemics. Although biogeographic
119 data and phylogenetic evidence have partially given support to the taxon cycles (Economato et al.,
120 2015b; Economato and Sarnat, 2012; Jönsson et al., 2014; Ricklefs and Bermingham, 2002), the
121 complexity of the hypothesis has hindered its comprehensive evaluation. For instance, other
122 factors have been proposed to explain species' expansion and contraction phases, such as recent
123 climatic fluctuations, character-displacement, and disparate dispersal abilities across lineages
124 (e.g., Pregill & Olson, 1981; Liebherr & Hajek, 1990; Losos, 1992).

125 Phylogenetically, the taxon cycle hypothesis predicts the repeated evolution of widespread
126 lineages exhibiting ecological shifts (Ricklefs and Cox, 1972). Lineages in the expansion phase
127 could give rise to daughter lineages adapted to marginal habitats, thus, the expansion phase may
128 also be represented by entire clades having wider ecological preferences. However, a potential
129 link between the expansion phase and phylogenetic diversification of Melanesian insects remains
130 to be tested (but see, Sarnat & Moreau, 2011; Economato & Sarnat, 2012; Economato et al., 2015b).

131 If expansion phases and phylogenetic diversification are indeed correlated, we would expect
132 increased diversification rates within certain clades due to expanded ecological opportunities.

133 In this study, we test four pairs of competing hypotheses that collectively inform our
134 understanding of diversity dynamics in the Indo-Pacific region:

135 1. ASIA-AUSTRALIA DISPERSAL IN THE MIOCENE

136 (1a) Dispersal between SE Asia and Australia was mostly post-late Miocene due to a lack of
137 subaerial land before 5–10 Ma.

138 (1b) Older colonization events of terrestrial lineages between these two areas were possible since
139 at least the early Miocene, either through overwater dispersal or via significant emergent land not
140 accounted for by the current palaeogeographic reconstructions.

141 2. COLONIZATION OF NEW GUINEA

142 (2a) The first colonization of—and diversification in—New Guinea was in a proto-Papuan
143 archipelago on the northern edge of the island.

144 (2b) The first emergence, colonization, and diversification in New Guinea occurred in the
145 present-day Papuan peninsula on the southeastern part of the island.

146 3. COLONIZATION OF EAST MELANESIA

147 (3a) Ancient colonization of east Melanesia (Vanuatu and Fiji) was facilitated by the
148 hypothesized ancient island chain associated with the Vitiaz arc, which may have lasted until
149 about 10 Ma.

150 (3b) Colonization of Vanuatu and Fiji occurred during the Pliocene (< 5 Ma) as a consequence of
151 substantial emergence of land above sea.

152 4. RANGE EXPANSION, SHIFTS IN ECOLOGICAL PREFERENCE, AND RADIATION

153 (4a) Lineages go through concordant shifts in ecological preference and geographical range
154 expansion, which may result in increased phylogenetic diversification rates.

155 (4b) Range expansions are not linked to niche shifts, and diversification remains constant over
156 time, thus, the extant species diversity in the region is time-dependent (i.e., older clades contain
157 more species diversity).

158 To test these hypotheses, we reconstruct a dated molecular phylogeny of seven ant genera in the
159 *Prenolepis* genus-group, calibrated using fossil data and secondary constraint, and infer its
160 ancestral geographical ranges, habitat preferences, and diversification rates on species-rich Indo-
161 Pacific clades.

162 2. Material and methods

163 2.1. Sample and dataset acquisition

164 The *Prenolepis* genus-group is monophyletic (Blaimer et al., 2015; LaPolla et al., 2010a) and has
165 recently been classified within the tribe Lasiini based on phylogenomic data (Ward et al., 2016).

166 The group has a worldwide distribution, occurring mostly in tropical and subtropical regions. In
167 the Malay Archipelago, TSP, and Australia, the focal study area, the group consists of 35
168 described species (AntWeb, 2016). We collected ants throughout the study region over a 10 year
169 period to obtain the most geographically and taxonomically extensive sampling of species as
170 possible, including undescribed and cryptic diversity. We sampled *ca.* 470 specimens that

171 represent 73 putative species distributed across our focal study area (Fig. 1; Table A.1 in
172 Appendix A). For most of these specimens (91%; see Table A.1), we sequenced the barcoding
173 region (659 bp) of the mitochondrial COI gene. Furthermore, for the phylogenetic analyses, we
174 expanded the molecular dataset for selected specimens to include at least one specimen per
175 species. This expanded aligned dataset consists of 3389 bp from six protein-coding genes: one
176 mitochondrial (COI) and five nuclear gene markers (CAD, EF-1 α F1, EF-1 α F2, LWR, and
177 wingless). We retrieved sequences from GenBank of outgroup taxa (tribe Lasiini: *Cladomyrma*,
178 *Lasius*, *Myrmecocystus*) (Ward et al., 2016) and all available sequences for 63 taxa within the
179 *Prenolepis* genus-group distributed around the world. All voucher specimens were deposited in
180 the Melanesian Ant Collection, Biology Centre of the Czech Academy of Sciences, with selected
181 duplicates deposited in the MCZ (Museum of Comparative Zoology, Cambridge, MA, USA).

182 Species determination was primarily based on morphology, whereas molecular divergences were
183 used to evaluate putative species boundaries and to detect cryptic species with substantial genetic
184 variation. We used the program bPTP (Zhang et al., 2013) to delimit species based on branch
185 length information. The multi-species coalescent model could not be utilized because of
186 computational limitations, convergence and mixing problems when analyzing our dataset
187 consisting of multiple genera (analyses not shown) (Yang and Rannala, 2010). However, the
188 species assignments by bPTP and programs based on the multi-species coalescent, such as
189 BP&P, may be similar in some cases (Toussaint et al., 2015). As input for the program bPTP, we
190 used a maximum likelihood tree with branch lengths representing number of substitutions as
191 inferred by RAxML v8.0 (Stamatakis, 2014). We used a multi-locus dataset, with 164 ingroup
192 specimens having unique COI haplotypes and at least one nuclear marker sequenced. The
193 molecular species delimitation analysis was conducted through the bPTP web server

194 (<http://species.h-its.org/ptp/>), having the following settings: 500,000 MCMC generations;
195 thinning by a factor of 100; and 25% burnin.

196 **2.2. Phylogenetic analyses**

197 Ingroup members of our molecular dataset consisted of *Prenolepis* genus-group specimens from
198 our focal study area and other regions in the world, and included in total 176 specimens from 124
199 species, having at least 4 out of 6 gene fragments sequenced (level of matrix completeness *ca.*
200 90%). Based on Bayes factors calculated by the stepping-stone sampling approach in MrBayes
201 v3.2.2 (Ronquist et al., 2012), we used the best-fit partitioning strategy consisting of by-gene and
202 by-coding positions in all phylogenetic analyses (21 partitions; Table A.2 in Appendix A). Other
203 less-fit partitioning schemes that were analyzed include: a) all sequences concatenated (1
204 partition), b) mitochondrial and nuclear sequences (2 partitions), c) each gene marker (7
205 partitions), and d) nucleotide sites grouped in bins with comparable relative evolutionary rates as
206 estimated by the program TIGER (Cummins and McInerney, 2011) (3 partitions), after verifying
207 congruence among single-gene tree topologies (Fig. A.4 in Appendix A).

208 Maximum likelihood analysis was conducted using RAxML v8.0 as implemented in CIPRES
209 (Miller et al., 2010). We performed "rapid bootstrapping" with 1000 iterations to assess the level
210 of node support. Bayesian inferences were conducted using MrBayes v3.2.2, with two
211 independent runs, each for 50 million generations with sampling every 5000 generations. We
212 applied the mixed option for nucleotide substitution (Huelsenbeck et al., 2004) to each partition
213 and a conservative burnin of 25%. We verified that the final average standard deviation split
214 frequencies were lower than 0.05, PSRF values were approaching unity, and log-likelihoods
215 reached a stationary distribution.

216 2.3. Molecular dating of phylogenies

217 Divergence dates were obtained under the uncorrelated relaxed-clock model as implemented in
218 BEAST v2.3.1 (Bouckaert et al., 2014), using a reduced dataset comprising one specimen per
219 species. The level of completeness of this molecular dataset is nearly 85%. We calibrated the
220 phylogeny using three extinct *Prenolepis*-group species with affinities to three extant genera
221 (*Nylanderia*, *Prenolepis*, and *Pseudolasius*) found in Baltic amber (34–42 Ma) and two extinct
222 species within the extant genera *Nylanderia* and *Zatania* from Dominican amber (15–20 Ma)
223 (LaPolla et al., 2012; LaPolla and Dlussky, 2010) (see Appendix A for further details on fossil
224 taxa phylogenetic placement). Because the Baltic fossils cannot be confidently assigned to any
225 extant infrageneric group or clade, we conservatively constrained the stem ages of *Nylanderia*,
226 *Prenolepis*, and *Pseudolasius* using an exponential distribution with mean of 5.0 and offset to
227 34.0. The Dominican fossils, instead, closely resemble extant clades and are likely members of
228 crown groups (LaPolla et al., 2012; LaPolla and Dlussky, 2010). Therefore we constrained the
229 minimum crown ages of *Zatania* and the North American *Nylanderia parvula* clade following an
230 exponential distribution with mean of 3.0 and offset to 15.0. In addition, we also constrained the
231 stem of the genus *Lasius* (outgroup) based on fossil data from Baltic amber deposits, following
232 the same calibration parameters as above. A secondary calibration point was used to constrain
233 the root of the tree (i.e., the tribe Lasiini), by setting a broad normal distribution to the root
234 height with mean of 77.4 Ma (5% quantiles to 66.5 Ma and 88.3 Ma), following Blaimer et al.,
235 (2015).

236 We selected the Birth-Death Model as the tree prior (Stadler, 2009), and substitution models
237 were unlinked across partitions. The best substitution models for each gene partition were
238 calculated using jModelTest 0.1.1 (Posada, 2008) under the Bayesian Information Criterion:

239 GTR+ Γ model for CAD; HKY+ Γ model for EF-1 α F1; SYM+ Γ model for EF-1 α F2; GTR+ Γ
240 model for COI; GTR+ Γ model for LWR; and SYM+ Γ model for wingless. We ran the analyses
241 using four independent runs for 100 million generations each, sampling every 5000 generations.
242 We checked that ESS values were higher than 200 before combining and summarizing the
243 estimated trees (Figs 2 & 3). An additional analysis using the Yule tree model is presented in
244 Fig. A.5 in Appendix A.

245 **2.4. Biogeographical inferences**

246 We estimated ancestral geographic ranges to test the competing pairs of Hypotheses 1–3 on the
247 origin of New Guinean, Melanesian and TSP ant diversity (i.e., first faunal interchange between
248 SE Asia and Australia, and colonization events to New Guinea, Vanuatu, and Fiji). Although the
249 New World and the Afrotropics were considerably undersampled, it is probable based on
250 morphological similarities (Kallal and LaPolla, 2012; John S. LaPolla et al., 2011; LaPolla et al.,
251 2010a, 2010b) and global framework phylogenies (Blaimer et al., 2015; LaPolla et al., 2010a)
252 that such missing taxa would cluster along the well-defined, geographically correspondent clades
253 (Figs 2 & 3; see Appendix A for further accounts on morphology and distribution of missing
254 taxa). Geographical distributions of species were taken from the literature and public databases
255 (e.g., Clouse, 2007; Economo & Sarnat, 2012; Antweb, 2016), as well as from our field records
256 (<http://www.newguineants.org>).

257 Ancestral geographic ranges were estimated following the Dispersal-Extinction-Cladogenesis
258 model (Ree et al., 2005) and including a founder-event speciation parameter (DEC+J; Matzke,
259 2014). We conducted our biogeographical analyses by finely subdividing the focal region based
260 on biogeographical characteristics of Pacific fauna (Fig. 1; Keppel et al., 2009), resulting in a

261 total of nine global geographical areas. The palaeogeographic models of the Indo-Pacific
262 (Gillespie and Clague, 2009; Hall, 2013, 2002) were incorporated as differential rates of
263 dispersal (range expansion) across a time-stratified phylogeny at 25, 15, and 5 Ma. Relative
264 dispersal rates between two areas were arbitrarily set from 1.0 to 10^{-4} as an attempt to represent
265 the extent of geographic barriers such as sea straits and mountain ranges (see Appendix A for
266 further details). The analyses were performed on a likelihood framework using the R package
267 BioGeoBEARS (Matzke, 2013). Further analyses using other biogeographical models, including
268 the DIVA-LIKE and BAYAREA-LIKE, are described in Appendix A but in all cases the DEC+J
269 model outperformed other biogeographical models (Akaike weight = 0.998, Table 1).

270 **2.5. Reconstruction of ancestral habitat preferences and diversification rates**

271 We reconstructed ancestral ecological preferences of Indo-Pacific clades to evaluate any
272 association between niche shift and geographical range expansion (competing pair of Hypotheses
273 4a–b). We compiled habitat preferences for each species from the literature (e.g., Clouse, 2007;
274 Economo & Sarnat, 2012; Antweb, 2016) and our field records (<http://www.newguineants.org>).
275 Locality information for each collected specimen, including habitat type and GPS coordinates,
276 are presented in Appendix A, Table A.1, and each species' distributional information can be
277 found in Table A.4 and in the New Guinea Ants database (<http://www.newguineants.org>). Three
278 categories were set for the "habitat" character: 1) undisturbed primary rainforest, 2) disturbed
279 forest and savanna, 3) highly-disturbed areas including gardens, docks, coastal margins and
280 urban areas; and "elevation" was categorized as: 1) lowlands, 2) lower montane, and 3) montane.
281 The breaks between the three elevation categories were at 800 m and 1600 m on New Guinea,
282 and at 250 m and 500 m on TSP archipelagos, based on major distributional turnover (pers. obs.;
283 Sarnat & Economo, 2012). Character matrices were used to reconstruct and trace ancestral states

284 over the phylogeny, under the Fitch Parsimony (unordered) model and the Mk1 Likelihood
285 model, in Mesquite 3.10 (Maddison and Maddison, 2016).

286 To test for expanding geographic ranges and ecological opportunity driving diversification
287 (Hypothesis 4a), we estimated the diversification rates of four Indo-Pacific clades, two within
288 *Paraparatrechina* and two within *Nylanderia*. We used the branching times of these Indo-Pacific
289 lineages to evaluate the best-fitting diversification model using the R package DDD (Etienne et
290 al., 2012). We carried out the three following analyses: 1) constant pure-birth diversification
291 (Yule), 2) constant birth-death diversification (BD), and 3) diversity-dependence birth-death
292 diversification, with speciation linearly declining with diversity (DD). Akaike weights were used
293 to estimate the relative probability of each diversification model fitting our dataset.
294 Diversification rates were compared across clades to evaluate the alternative scenarios of
295 increased rates followed by diversity-dependent speciation, potentially linked to ecological shifts
296 (Hypothesis 4a) vs. constant phylogenetic diversification (Hypothesis 4b).

297 **3. Results**

298 **3.1. Species delimitation and phylogenetic relationships**

299 The estimated number of species recovered by bPTP ranged between 80–105, with a mean of 92
300 species (Fig. A.1). Most of the species boundaries were moderately to highly supported
301 (Bayesian supporting values > 0.8), and only nine species were not in agreement with our
302 morphologically-based sorting. Five of these conflicting lineages, however, represented
303 described species whose morphological identifications were based on available taxonomic keys
304 and comparisons with museum specimens. In cases where there was no sufficient molecular
305 evidence or conflicting resolutions of molecular species boundaries, we followed our

306 morphologically-based species delimitations (see Fig. A.1 for a visual inspection of molecular
307 divergences within these conflicting lineages). Nonetheless, the species diversity estimated in
308 this study should be taken as preliminary, and a thorough taxonomic investigation and the usage
309 of multi-species coalescent on smaller taxonomic groups are strongly demanded, whereas other
310 fast-evolving molecular markers (e.g., STRs) along with better population sampling may be
311 beneficial in finding any further cryptic species not detected here.

312 The monophyly of each genus and putative species with more than two individuals sampled was
313 strongly supported in every phylogenetic analysis (Fig. 2 and Figs A.2–A.4). All Melanesian
314 (which includes New Guinea), Australian, and TSP lineages clustered together in monophyletic
315 groups and closely related to SE Asian lineages. New World and African species were recovered
316 in separate clades, and apparently these geographical regions have not directly contributed to the
317 extant Melanesian ant fauna. We identified seven highly-supported Melanesian and TSP clades
318 (Figs 2 & 3; posterior probabilities [PP] ≥ 0.95 , bootstraps $\geq 90\%$): two clades within
319 *Parapatrechina* and *Pseudolasius*, and three clades within *Nylanderia*.

320 **3.2. Divergence times**

321 The crown age of the *Prenolepis* genus-group was estimated at 46.0 Ma (95% HPD 41.2–51.8
322 Ma). Ingroup divergence times and tree topologies were similar and consistently recovered
323 across independent analyses using distinct tree priors (Birth-Death and Yule) (Fig. A.5).
324 Moreover, our estimated timing of origin and diversification of the *Prenolepis* genus-group is in
325 line with Blaimer et al. (2015), who found that the group originated and began its diversification
326 by the late Paleocene and Eocene (i.e., about 45–60 Ma). The extant Melanesian lineages arrived
327 to the region and diversified as early as 23.5 Ma (95% HPD 19.4–27.9 Ma) (i.e., the crown age

328 of clade 2 within *Paraparatrechina*), but it is likely that the actual dispersal event may have been
329 earlier but probably not before 34.1 Ma (95% HPD 28.9–39.6 Ma), which represents the stem
330 age of clade 2 in our phylogeny.

331 **3.3. Ancestral state reconstructions and diversification rates**

332 A continental origin from Asia was reconstructed for all the Melanesian *Prenolepis* genus-group
333 lineages (Fig. 3). Geographic range expansions occurred during two main instances: at the early
334 Miocene (*ca.* 20–25 Ma) from SE Asia to New Guinea, and during the late Miocene and Plio-
335 Pleistocene (*ca.* 3–10 Ma), again from SE Asia to Melanesia, but also from the latter region
336 towards the remaining of Indo-Pacific islands (Fig. 4). The DEC+J biogeographical model fits
337 our molecular phylogeny better than others, such as BAYAREA-LIKE or DIVA-LIKE (Table 1).

338 Ancestral state reconstructions suggested that undisturbed (primary) rainforest was the most
339 likely habitat preferred by the common ancestor of three main Indo-Pacific clades (within
340 *Paraparatrechina* and *Nylanderia*). However, highly-disturbed and open-environments were
341 recovered as the most-likely ancestral state in clade 7 (within *Nylanderia*) with about 0.67
342 marginal probability, and within clade 2 (*Paraparatrechina*) with about 0.5 marginal probability.
343 On the other hand, the origin and evolution of clade 5 (within *Nylanderia*) is likely linked to
344 lower montane habitats on New Guinea (marginal probability of 0.97). At about 10 Ma, there
345 was a shift in habitat preference from lowland to montane environments in clade 2 with marginal
346 probabilities of more than 0.6. The colonization of montane areas in archipelagos of the TSP
347 occurred recently and simultaneously with the shift to disturbed environments within clades 2
348 and 7 (< 5.0 Ma).

349 The evolution of the smaller clades 1 and 5 (within *Paraparatrechina* and *Nylanderia*,
350 respectively) might be explained by two equally-probable diversification models, the constant-
351 rate or the diversity-dependence scenarios. The best-fit diversification model for the larger clades
352 2 and 7, however, was the diversity-dependence model (Akaike weights > 0.99). Estimated
353 diversification rates for the small clades varied between 0.06 and 0.17, whereas the
354 diversification rates for clade 2 was 0.26, for the *P. oceanica*–*P. minutula* subclade within clade
355 2 was 0.5, and for clade 7 was 0.61 (Table 2).

356 **4. Discussion**

357 Ants in the *Prenolepis* genus-group likely colonized New Guinea from continental SE Asia in
358 the early Miocene *ca.* 25 Ma. Dispersal to eastern Melanesia (Fiji and Vanuatu) is as old as 10–
359 20 Ma, suggesting that a subaerial island chain associated with the Vitiaz Arc may have
360 facilitated the colonization of these archipelagos during the Miocene. Substantially larger
361 diversification rates in the young and diverse clade 7 (within *Nylanderia*), and to a lesser extent
362 in clade 2 (within *Paraparatrechina*), may have been linked to expansions in both ecological
363 preference and geographical range. Therefore, it may be possible that ecological processes have
364 significantly shaped the ant diversity and distribution in Melanesia, as partially suggested by the
365 taxon cycle hypothesis.

366 **4.1. The timing of faunal interchange between SE Asia and Melanesia**

367 Our results support the hypothesis that faunal interchange via Wallacea occurred as early as the
368 Miocene (*ca.* 20–25 Ma; Hypothesis 1b), rather than after the late Miocene (Hypothesis 1a).
369 Ancestral range inferences showed that Melanesian (including New Guinea) and TSP lineages
370 very likely had a continental SE Asian origin, which agrees with the hypothesized centre of

371 origin and diversity of the *Prenolepis* genus-group in continental tropical Asia (Blaimer et al.,
372 2015; John S LaPolla et al., 2011; LaPolla et al., 2010a). Substantial subaerial land before 5–10
373 Ma may have arisen in Wallacea as scattered volcanic islands (Hall, 2012), which might have
374 facilitated overwater dispersal of terrestrial arthropods in a stepping-stone fashion. But
375 stratigraphic evidence in favour of emergent land in the region at the early Miocene is scarce
376 (Baldwin et al., 2012; Hall, 2013; Lohman et al., 2011; Stelbrink et al., 2012; van Ufford and
377 Cloos, 2005). However, biogeographical patterns and time-calibrated dispersal events suggest
378 that significant faunal interchange might have occurred already by 25 Ma (Cibois et al., 2014;
379 Georges et al., 2014; Jönsson et al., 2011; Mitchell et al., 2014), whereas it is likely that early
380 Miocene plant dispersal across Wallacea might have been enhanced by animals (Crayn et al.,
381 2015).

382 The hypothesis that this exchange was due to the presence of significant amounts of land is
383 strengthened by the independent dispersal events across unrelated ant clades at approximately the
384 same period of time (i.e., 15–25 Ma; Fig. 3), regardless of life history traits. For instance, ant
385 nesting preferences across these clades vary from rotten logs, leaf litter, to soil, and even some
386 *Pseudolasius* appear to live completely underground (LaPolla, 2004). Our results provide further
387 evidence that faunal interchange between Asia and Australia might be in fact an old
388 palaeozoographic event, aided by a significant increase of land above water, in both vertebrate
389 and invertebrate lineages, including other ants (Economo et al., 2015b).

390 **4.2. Colonization and diversification in New Guinea**

391 Biogeographical reconstructions of the older colonists of New Guinea supported Hypothesis 2a
392 that the most likely region of arrival covered the Central Range and northern New Guinea (Fig.

393 3), rather than the southern part of the island including the Papuan peninsula (Hypothesis 2b),
394 which in the case of the *Prenolepis* genus-group was colonized later, after the mid-Miocene (<
395 15 Ma). Predaceous diving beetles from New Guinea have a similar pattern, though a younger
396 timing of colonization, from a probable origin in the Central Range followed by dispersal
397 towards the Papuan peninsula during the last 5 Ma (Toussaint et al., 2014). Although other
398 studies found that southern Papuan lineages may be older than northern taxa (Oliver et al., 2013),
399 these did not estimate time-calibrated species divergences thus a young origin, in the mid-
400 Miocene or later, of these southern Papuan lineages cannot be ruled out. In this study, the model
401 describing a proto-Papuan archipelago along the northern edge of New Guinea in the early
402 Miocene is favoured over an old Papuan peninsular origin of extant New Guinean species
403 diversity.

404 Older endemics that currently occur in montane habitats, such as the New Guinean clade 5 and
405 certain lineages within clade 2, probably originated at about 15 Ma, in agreement with the major
406 orogeny of the Central Range. Further sampling of montane ants along the Central Range is
407 necessary to infer the mechanisms driving ant montane speciation in the region. For instance,
408 ecological processes causing altitudinal segregation have been proposed as drivers of montane
409 diversification in birds (Diamond, 1973) and ants (Economo and Sarnat, 2012; Sarnat and
410 Economo, 2012). On the other hand, a large proportion of cladogenetic events in New Guinea
411 occurred within northern or southern lowland areas, and not across them as expected under a
412 "lowland vicariant" scenario driven by the Central Range. Ecological constraints and/or
413 geographical barriers within the northern and southern lowland areas may exist and explain the
414 observed pattern in the *Prenolepis* genus-group, such as strong habitat associations and riverine
415 barriers (Janda et al., 2016). Additionally, we found that on average each locality harbours 2–3

416 highly divergent species per genus. This may suggest that competitive exclusion of closely
417 related species, with perhaps similar ecological niches, might be an important mechanism of ant
418 community structure in New Guinean lowlands; a pattern described for other low-elevation ant
419 communities (Machac et al., 2011).

420 **4.3. Colonization of eastern Melanesia**

421 The earliest insect colonization of eastern Melanesia (Vanuatu and Fiji) was estimated at about
422 9–17 Ma (Balke et al., 2007; Liebherr, 2005; Lucky and Sarnat, 2010; Sarnat and Moreau, 2011),
423 consistent with the hypothesis that colonization was facilitated by emergent land as part of the
424 subaerial Vitiaz Arc as island chain (Hypothesis 3a). In the *Prenolepis* genus-group, dispersal
425 has occurred earlier than the recent re-exposure of land in eastern Melanesia during the Pliocene
426 (Hypothesis 3b), whereas early Miocene land has likely remained above water and harboured
427 older colonizers to the present. Our phylogenetic reconstructions indicate that New Guinea was
428 the likely source of ant colonists in Vanuatu and Fiji, with estimated ages congruent with
429 previous ant chronograms (*ca.* 10–16 Ma; Lucky & Sarnat, 2010; Sarnat & Moreau, 2011).
430 Dispersal to these archipelagos apparently ceased until the Plio-Pleistocene, when younger
431 colonization events occurred at about 5 Ma, consistent with the recent re-exposure of subaerial
432 land in the TSP. This apparent absence of dispersal during the late Miocene and early Pliocene
433 may be expected given the disruption of the arc at about 10 Ma, when the Ontong-Java Plateau
434 collided with the Vitiaz trench (Ewart, 1988; Hall, 2002). However, further dated phylogenies of
435 eastern Melanesian insects are needed to rule out other potential explanations, such as local
436 extinction events that erased traces of ant dispersal between 5 and 10 Ma.

437 Range expansions to Micronesia and Polynesia have happened since 5 Ma, in line with the
438 significant exposure of land in these regions during the late Miocene and Pliocene. Colonization
439 of Palau, the richest in species diversity among Micronesian and Polynesian archipelagos (six
440 *Prenolepis* genus-group taxa), occurred since 5 Ma from two sources: New Guinea and the
441 Philippines. No local ant radiation has been inferred, instead, the faunal assemblage in Palau
442 might be the result of a long history of colonization events by unrelated lineages (Rundell, 2008).
443 Dispersal to the Caroline Islands and to eastern Polynesia occurred geologically recently (< 4Ma)
444 by species with wide geographic ranges. These time-calibrated dispersal events significantly
445 post-date the geological origins of Western Pacific archipelagos inferred since at least the mid-
446 Miocene *ca.* 15 Ma (Rehman et al., 2013). Submergence of land and complete eradication of
447 Miocene fauna by the Miocene/Pliocene transition might explain the apparent "delay" in
448 colonization observed in our phylogeny. On the other hand, two invasive species, the longhorn
449 crazy ant (*Paratrechina longicornis*) and the robust crazy ant (*Nylanderia bourbonica*) most
450 likely spread throughout the Pacific due to commerce. In those cases, closely related COI
451 haplotypes were recovered from distant localities, and no apparent genetic structure across
452 islands was evident (in contrast with, for instance, *Nylanderia vaga*, which is similar in age and
453 distribution; Fig. A.3).

454 **4.4. The connection between niche shifts and diversification**

455 One of the main predictions of the taxon cycle hypothesis is that range expansions may be
456 facilitated by shifts in habitat preference, eventually resulting in ecological release whereby
457 relaxed selective pressures caused by ecological opportunity may promote phenotypic variability
458 and speciation. Species adapted to lowland marginal habitats, such as the coasts of islands, might
459 be more prone to colonize other archipelagos by overwater dispersal (Hypothesis 4a). Although

460 an explicit relationship between expansion/contraction phases and shifts in diversification rates
461 has not been formulated, it may be possible that expanding lineages diversify due to ecological
462 innovation through the key adaptation to marginal habitats. Using ancestral character state
463 reconstruction, we found that ant habitat preferences for highly disturbed and open environments
464 may be linked to geographical expansion across archipelagos (e.g., within clade 2 and in clade
465 7). Moreover, adaptation to disturbed environments might also explain one of the origins of ant
466 invasiveness, as some of the tramp species are closely related and derived from common
467 ancestors already adapted to marginal habitats (e.g., *Nylanderia bourbonica*, *N. vaga*, and *N.*
468 *obscura*, all within clade 7). In addition, alternative reproductive strategies are known to increase
469 the invasive potential of some ant species, such as *Paratrechina longicornis* (Pearcy et al., 2011),
470 by producing queens clonally (thelytoky) and avoiding the negative effects associated with small
471 founding populations (Rabeling and Kronauer, 2013).

472 Although diversification rates and any shift in evolutionary dynamics across clades need to be
473 studied in the light of a global, well-sampled phylogeny, we propose that speciation in the Indo-
474 Pacific may have not remained constant across clades. Older colonization events to the Indo-
475 Pacific do not necessarily explain the disparate species-richness observed across clades
476 (Hypothesis 4b), but it seems that ecological processes may enhance diversification and broaden
477 geographical distribution of the ants. The main diversification of extant *Prenolepis* genus-group
478 lineages in the Indo-Pacific has occurred on New Guinea since the past 15 Ma, but younger
479 clades (clades 2 and 7) hold decoupled diversification dynamics compared to older clades (clades
480 1 and 5).

481 We showed that diversification scenarios facilitated by ecological innovations (shifts in habitat
482 preference) in the Indo-Pacific region cannot be ruled out (Hypothesis 4a). Ecological

483 opportunity in a macroevolutionary perspective might be related to an initial burst in speciation
484 rates followed by diversity-dependent deceleration of diversification rates (Etienne and
485 Haegeman, 2012; Rabosky, 2010). On the contrary, under a time-dependent diversification
486 model (Yule or Birth-Death) speciation is constant, and the extant diversity across clades is
487 primarily explained by the age of lineage's origin (Hypothesis 4b). The diversification rates of
488 the clade 7 (and partially within clade 2) have accelerated compared to other Indo-Pacific taxa
489 (Table 2). This coincides with the reconstructed shift in ecological preference towards disturbed
490 habitats (Fig. 3), which may have increased the “ecological limits” determining the clades’
491 species richness (Rabosky, 2009). Accordingly, shifts in habitat preference (ecological release)
492 may not only favour geographical range expansion, as proposed by the taxon cycle hypothesis,
493 but may also trigger species diversification of terrestrial invertebrates in Melanesia. In this line,
494 recent developments of island biogeography models also predict that speciation rates would peak
495 before islands reach maximal topographic complexity, as empty niche space will provide more
496 opportunities for radiation (Whittaker et al., 2008). However, a more rigorous approach to study
497 macroevolutionary dynamics, including the study of ant ecology (e.g., worker abundances,
498 dispersal abilities) and mathematical models that consider species abundances (Rosindell and
499 Phillimore, 2011) are needed before attempting a formal test of the taxon cycle in the light of
500 molecular phylogenies..

501 **5. Conclusions**

502 We found evidence of old insect colonization events from SE Asia to New Guinea, dating to at
503 least the early Miocene (*ca.* 25 Ma). A broad, faunistic and floristic interchange between Asia
504 and Australia may have happened earlier than previously thought geographically possible.
505 Moreover, early insect radiations on New Guinea have apparently occurred along the northern

506 edge of the island, contrasting with the hypothesis of an older, subaerial Papuan peninsula.
507 Colonization of eastern Melanesia (i.e., Vanuatu and Fiji) may be as old as 10–20 Ma, in
508 agreement with the hypothesized ancient island chain Vitiaz Arc connecting the proto-Papuan
509 archipelago to the Fiji Basin. In addition, a link between ecological shifts, geographic range
510 expansion, and phylogenetic diversification of insular arthropods cannot be ruled out, and such
511 shifts may have promoted geographical range expansion and triggered diversification of the
512 Indo-Pacific ant fauna. Further studies of other New Guinean and Melanesian insect lineages
513 would clarify if speciation in the region is primarily driven by ecological factors, and if so,
514 whether that is characterized by the fragmentation of geographic ranges or adaptation to new,
515 empty niches.

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532 **Supplementary material**

533 Supplementary data (Appendix A) associated with this article can be found in the online version.

534 **Data accessibility**

535 We deposited the data in external repositories. DNA sequences: GenBank accessions KP231876-
536 KP232901. Phylogenetic data, including alignments and dated trees: TreeBASE accession
537 numbers 17090 and 18335.

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774

775

777 **Table 1**

778 Statistical comparison across eight biogeographical models as implemented in BioGeoBEARS.
 779 LnL: log-likelihood of the model; numparams: number of parameters; *d*: rate of anagenetic range
 780 expansion; *e*: rate of anagenetic range contraction; *j*: founder-event parameter weight. The
 781 relative probability of each model was estimated using Akaike weights based on the AICc values
 782 estimated in BioGeoBEARS. The model DEC+J stratified model (in asterisks) outperformed
 783 other biogeographical models.

	LnL	numparams	d	e	j	AICc	Akaike weights
DEC+J **	-298.42	3	0.026	0.003	0.246	602.84	0.99845
DIVA-LIKE+J	-304.89	3	0.029	0.002	0.252	615.79	0.00154
BAYAREA-LIKE+J	-311.57	3	0.026	0.004	0.241	629.14	0.00000
DEC	-328.26	2	0.041	0.009	-	660.51	0.00000
DIVA-LIKE	-333.74	2	0.046	0.007	-	671.49	0.00000
BAYAREA-LIKE	-364.74	2	0.044	0.046	-	733.48	0.00000

784

786 **Table 2**

787 Phylogenetic diversification analyses on selected Indo-Pacific clades using DDD and the median
 788 divergences times in the chronogram inferred by BEAST. Estimated parameters: number of
 789 parameters in each model (nP), net diversification rate (r) = speciation minus extinction rates,
 790 clade-level carrying capacity (K) in diversity-dependence model, maximum likelihood of the
 791 model (LogLik), Akaike Information Criterion (AIC), Akaike weights (A.W.) for each
 792 diversification scheme. The most probable model fitting the dataset is shown in asterisks (**).
 793 Yule: pure-birth diversification, (BD) constant birth-death diversification, (DD) diversity-
 794 dependent diversification.

<i>Clade 1 (P. opaca clade)</i>	nP	r	K	LogLik	AIC	A.W.
Yule	1	0.072	-	-23.304	48.609	0.530
BD	2	0.072	-	-23.305	50.609	0.195
DD	2	0.128	20	-22.961	49.922	0.275
<i>Clade 2 (P. oceanica–P. pallida clade)</i>						
Yule	1	0.084	-	-83.608	169.216	0.003
BD	2	0.084	-	-83.608	171.216	0.001
DD**	2	0.259	66	-76.812	157.623	0.996
<i>Clade 2, (P.oceanica–P. minutula subclade)</i>						
Yule	1	0.108	-	-51.925	105.851	0.000
BD	2	0.108	-	-51.925	107.851	0.000
DD**	2	0.502	38	-42.681	89.363	1.000
<i>Clade 5 (N. nuggeti clade)</i>						
Yule	1	0.063	-	-24.200	50.399	0.395
BD	2	0.063	-	-24.200	52.400	0.145
DD	2	0.170	15	-23.048	50.097	0.460
<i>Clade 7 (N. obscura clade)</i>						
Yule	1	0.157	-	-59.661	121.322	0.000
BD	2	0.157	-	-59.661	123.322	0.000
DD**	2	0.613	35	-50.165	104.330	1.000

795

797 **Figure legends**

798 **Fig. 1.** Map of the Indo-Pacific region. Red stars represent this study's sampling localities.
799 Locality information including GPS coordinates, elevation, and brief habitat descriptions are
800 presented in Table A.1 in Appendix A.

801

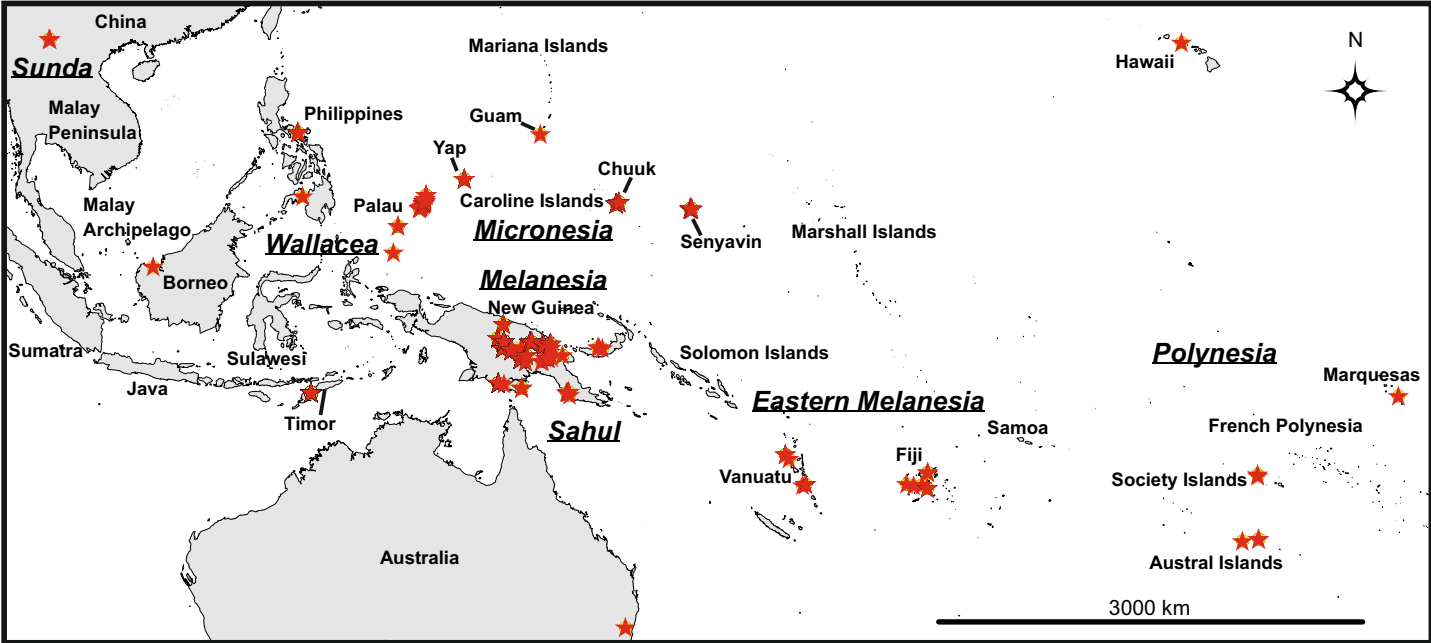
802 **Fig. 2.** Time-calibrated phylogeny of the *Prenolepis* genus-group and outgroup taxa (in grey) as
803 inferred in BEAST. Six fossil-based calibration points were used as minimum ages for crown
804 (*Zatania*, *Nylanderia parvula* group) or stem groups (*Lasius*, *Prenolepis*, *Pseudolasius*,
805 *Nylanderia*), based on morphological affinities or apomorphies sharing with close taxa (LaPolla
806 and Dlussky, 2010). A secondary calibration from (Blaimer et al., 2015) was used for the root of
807 the tree (tribe Lasiini). Posterior probabilities of each node are depicted as coloured asterisks
808 (black for > 0.95; red for 0.75–0.94; values < 0.75 are not shown). 95% credibility intervals for
809 divergence times are displayed over each node in the tree. The seven main Indo-Pacific clades
810 within the *Prenolepis* genus-group are numbered in the phylogeny. Scale bar in Ma. Inset,
811 specimen photographs in frontal and lateral views. Red scale bar in each photograph represents 1
812 mm. A: *Paraparatrechina opaca* (MJ15399, New Guinea); B: *Paraparatrechina* sp. VANU002
813 (CR120619-01-02, Vanuatu); C: *Pseudolasius* sp. PSEU002 (MJ9384, New Guinea); D:
814 *Pseudolasius* sp. PSEU005 (MJ16068, New Guinea); E: *Nylanderia nuggeti* (MJ6887, New
815 Guinea); F: *Nylanderia vaga* (MJ16124, Fiji).

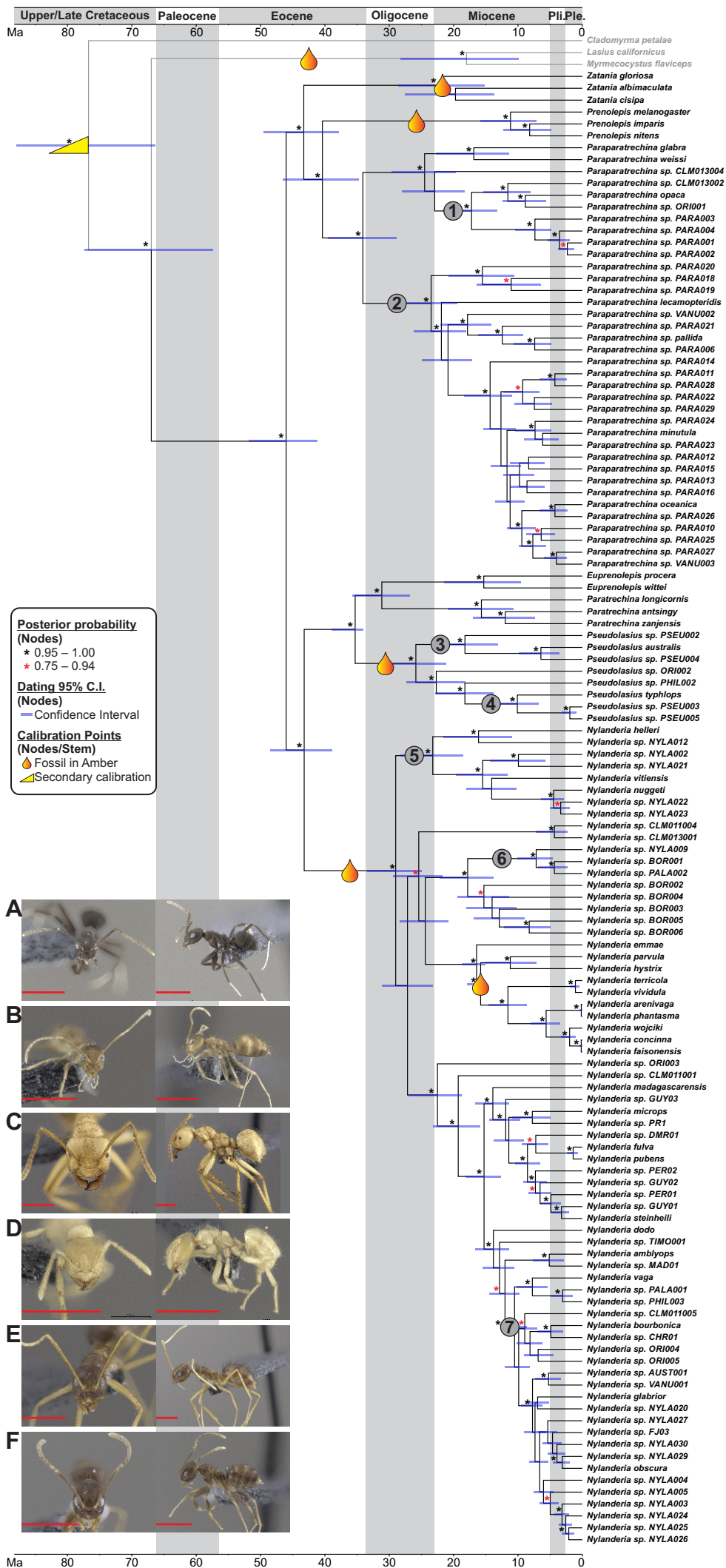
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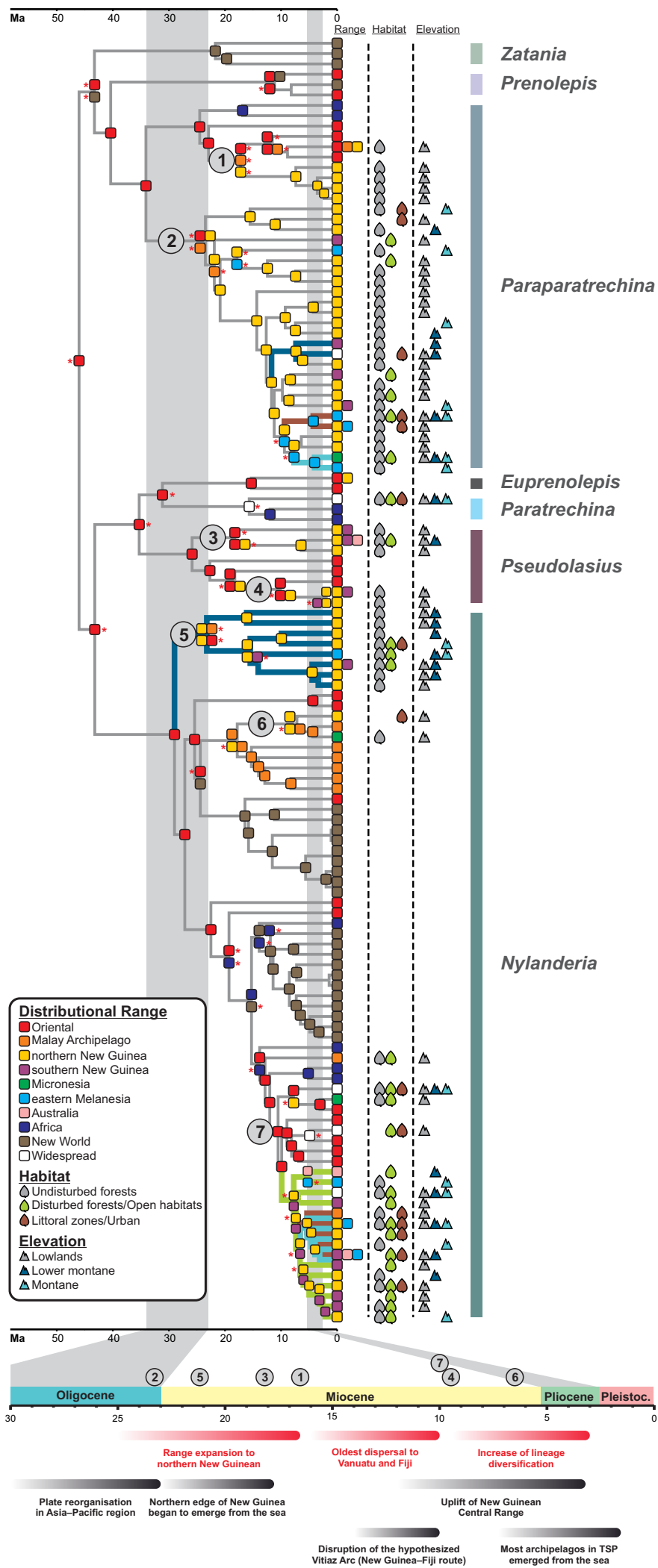
817 **Fig. 3.** Time-calibrated phylogeny of the Indo-Pacific *Prenolepis* shown in Fig. 2. Squares over
818 nodes and terminals in the phylogeny represent inferred and extant geographical ranges,
819 respectively. Coloured symbols depicts the recorded habitat preference (leave shape) and
820 elevation of each Indo-Pacific species (mountain shape). One or two most probable ancestral
821 geographical ranges estimated in BioGeoBEARS under the DEC+J model are depicted over each
822 node; red asterisks represent ranges with probabilities between 0.1 and 0.5, whereas ranges with
823 probabilities < 0.1 are not considered. Ancestral state reconstruction of habitat and elevation
824 preferences using Mesquite on each Indo-Pacific clade, are displayed as coloured branches. The
825 median ages of clades 1–7 (gray circles), main biogeographical events (red bars), and main
826 geological events (black bars), are each depicted below the phylogeny. Scale bar in Ma.

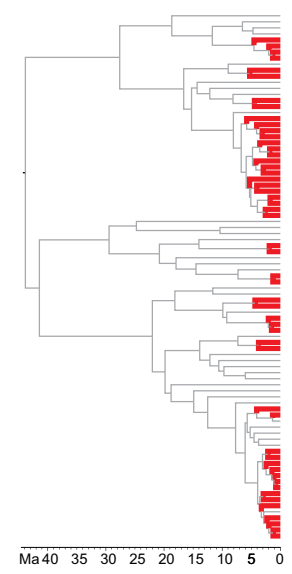
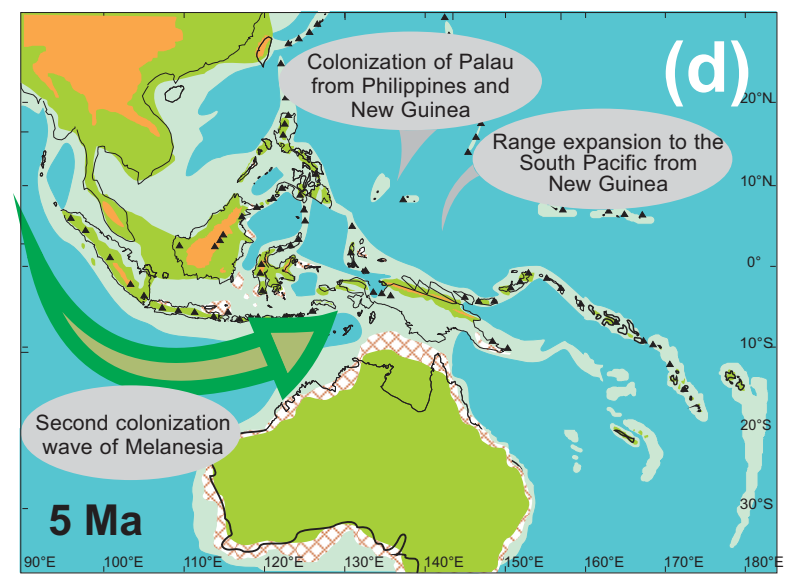
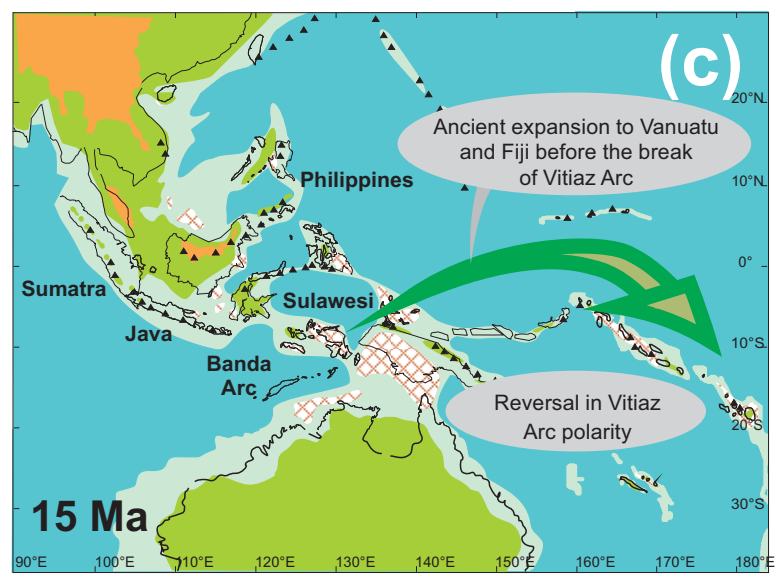
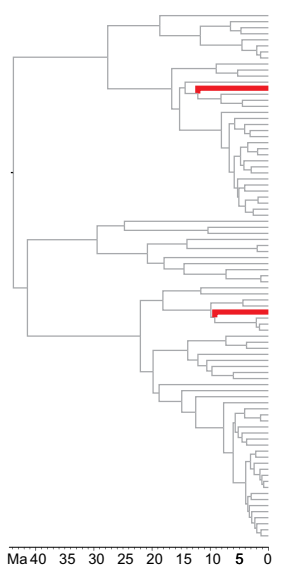
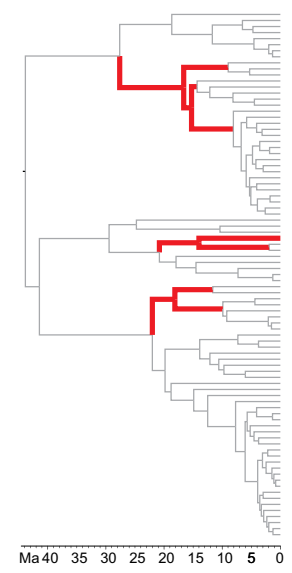
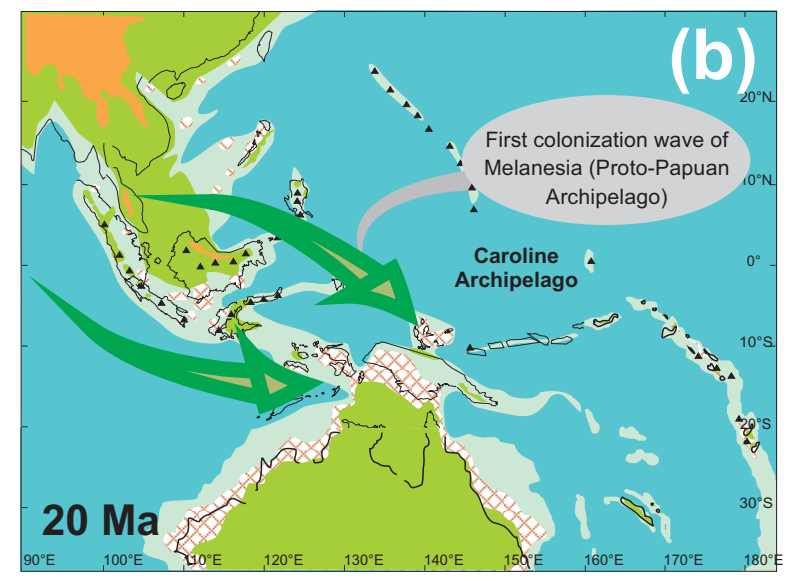
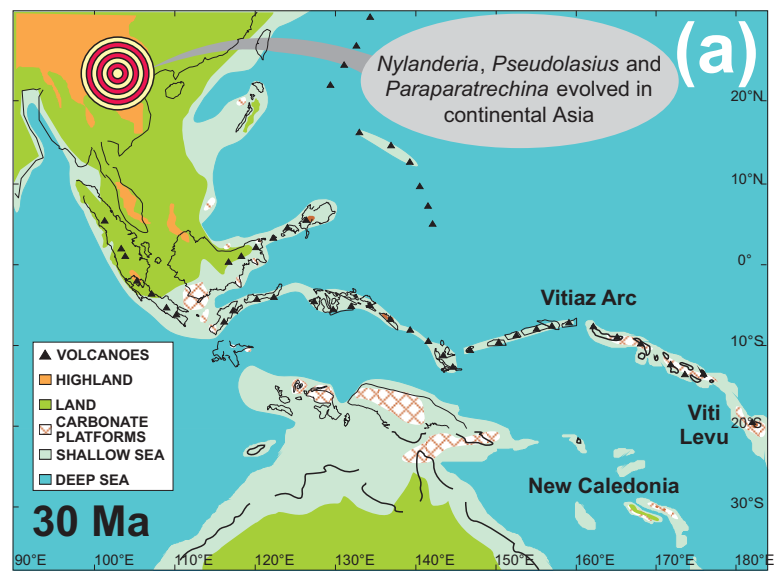
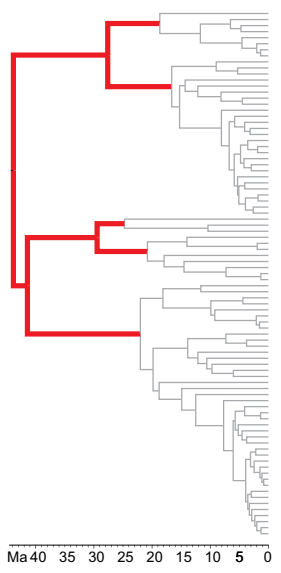
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828 **Fig. 4.** Palaeogeographic maps and evolutionary history of the *Prenolepis* genus-group in the
829 Indo-Pacific. Panels a–d depict the four time slices used in biogeographical analyses. The legend
830 in panel a describes the distribution of land and sea for all maps, whereas major dispersal events
831 are shown as green arrows. Maps were adapted from Hall (2013).









Supplementary data

An ant genus-group (*Prenolepis*) illuminates the biogeography and drivers of insect diversification in the Indo-Pacific

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Appendix A: Detailed methods procedures and further analyses carried out in support of our analyses in the main text.

1. Further information on taxon sampling

Field work was conducted during 2002–2012, and specimens were collected following standard methods for sampling of ants in rainforest and other habitats. At majority of New Guinea sites, samples were collected by hand, baiting, and by leaf litter extraction (e.g., Agosti *et al.*, 2000) in a system of 20 m × 20 m plots, with 1 to 12 plots surveyed at each site. The sampling effort for each plot included nine samples of 1 m² leaf litter, extracted for 48–72 hours; 20 tuna bait traps on forest floor and 20 baits on understory vegetation exposed for at least one hour; and at least four person-hours of direct (hand) collecting. The total area at each site across which the surveyed plots were distributed ranged from 20–25 km². At some sites (Baitabag, Ohu, Madang Lagoon, Weam, Port Moresby), we increased population sampling by targeted collecting of *Prenolepis* genus-group species outside the surveys plots. In sites where the plot-based sampling was not possible, the ants were collected by direct hand-collecting and/or baiting. The habitats where specimens were collected encompass lowland and montane localities, primary rainforests, savanna, coastal environments, and urban areas (see Appendix S1 for locality information of each specimen and Table A.4 for an overview of habitat preference by species). Ants were stored in 70–100% ethanol and their morphology was investigated under an Olympus SZ50 microscope. Species identifications based on morphology were conducted by ant taxonomists with expertise in the *Prenolepis* genus-group.

2. Laboratory protocols and DNA sequences manipulation

Genomic DNA was extracted from whole ant specimens using the Geneaid Genomic Tissue DNA kit (Taiwan). DNA amplifications were carried out using 2 µl of isolated DNA, 1x of PPP Master Mix (Top-Bio, Prague), 0.4 µM of each forward and reverse primers, and completed with distilled water to 25 µl. PCR protocols consisted on 95°C for 5 min, 35 cycles of: 94°C for 30 sec, 50/55°C for 50 sec and 72°C for 90 sec, finalizing the program with 72°C for 5 min. Annealing temperature of primers varied from 50°C in the case of the mitochondrial COI and the nuclear genes wingless and LWR, to 55°C for the nuclear genes CAD, EF-1αF1 and EF-1αF2. Primer sequences are listed:

Gene region and primer sequences	Described in
Cytochrome c oxidase subunit I (COI) LCO: 5' <u>TAATACGACTCACTATAGGGGGTCAACAAATCATAAAGATATTGG</u> 3' HCO: 5' <u>ATTAACCCTCACTAAAGTAAACTTCAGGGTGACCAAAAAATCA</u> 3'	Folmer <i>et al.</i> (1994)
Carbonyl-phosphate synthase II (CAD). Done in two pieces Region 1, CD892: 5' <u>TAATACGACTCACTATAGGGGGYACCGRCGTTGYTAYATGAC</u> 3' Region 1, CD1491: 5' <u>ATTAACCCTCACTAAAGGCCGCARTTNAGRRCRGTYTGYCC</u> 3' Region 2, CD1423: 5' <u>TAATACGACTCACTATAGGGAGGTRATACRATCGGARAGRCCDGA</u> 3' Region 2, CD1910: 5' <u>ATTAACCCTCACTAAAGCCGAGRGGRTCRACRRTTYTCCATRTRTRCAYAC</u> 3'	Ward <i>et al.</i> (2010)
Elongation factor 1-alpha F1 paralog (EF-1αF1) F1-1424: 5' <u>TAATACGACTCACTATAGGGGCGCCKGCGGCTCTACCACCGAGG</u> 3' F1-1829: 5' <u>ATTAACCCTCACTAAAGGGAAGGCCTCGACGCACATMGG</u> 3'	Schultz & Brady (2008)
Elongation factor 1-alpha F2 paralog (EF-1αF2) F2-557: 5' <u>TAATACGACTCACTATAGGGGAACGTGAACGTGGTATYACSAT</u> 3' F2-1118: 5' <u>ATTAACCCTCACTAAAGTTACCTGAAGGGGAAGACGRAG</u> 3'	Schultz & Brady (2008) Brady <i>et al.</i> (2006)
Long wavelength rhodopsin (LWR) LW143: 5' <u>TAATACGACTCACTATAGGGGACAAAGTKCCACCRGARATGCT</u> 3' LW639: 5' <u>ATTAACCCTCACTAAAGYTTACCGRTTCCATCCRAACA</u> 3'	Ward & Downie (2005)
wingless WG578: 5' <u>TAATACGACTCACTATAGGGTGCACNGTGAARACYTGCTGGATGCG</u> 3' WG1032: 5' <u>ATTAACCCTCACTAAAGACYTCGCAGCACCARTGGAA</u> 3'	Ward & Downie (2005) Brady <i>et al.</i> (2006)

Successful PCRs were sent to Macrogen (South Korea) for standard sequencing in both directions using the universal primers T7promoter and T3 (underlined sequences listed above). We removed from the DNA alignment three intronic regions, two in CAD and one in LWR, and kept only the exonic regions for phylogenetic analyses. The dataset alignment was carried out using the Clustal W algorithm (Thompson *et al.*, 1994) (ClustalW cost matrix; Gap open cost: 16; Gap extend cost: 8) as implemented in the program Geneious R7. All DNA sequences are publicly available in GenBank and mitochondrial COI gene sequences in BOLD (ASPNA project). The aligned datasets and final trees were deposited in TreeBASE (submission IDs 17090 and 18335).

3. Phylogenetic analyses and divergence times estimation

3.1. Fossil taxa

The described fossil record for the *Prenolepis* genus-group includes five extinct species, most of them resembling extant members on the group (LaPolla *et al.*, 2012; LaPolla and Dlussky, 2010).

1: *Nylanderia vetula* † workers resemble species within the North American clade in *Nylanderia* (LaPolla *et al.*, 2010a), as regard of their lack of erect setae on scapes and legs, mandibular features, and the rounded dorsal face of the propodeum. This fossil was found in Dominican amber and it was dated to the early Miocene (15–20 Ma; LaPolla *et al.*, 2013). We enforced a minimum age of the

Nylanderia parvula clade in our phylogeny by assigning an exponential distribution with Median 17.1 Ma, and 5% and 95% Quantiles to 15.2 Ma and 24.0 Ma, respectively.

2: *Zatania electra* † was found in Dominican amber. A number of morphological characters including head, propodeum and mesosoma features in workers and male, confidently places this extinct species within crown *Zatania* (LaPolla et al., 2012). We therefore constrained the minimum age of the genus using an identical exponential distribution as for the *Nylanderia parvula* clade.

The remaining four extinct species were found in Baltic amber with an estimated age of 34–42 Ma (Dlussky and Rasnitsyn, 2010; LaPolla et al., 2013a).

3: *Nylanderia pygmaea* † is confidently described as the oldest definitive species of the genus *Nylanderia*, based on several apomorphic features observed in workers, males, and gynes (LaPolla et al., 2010a). This extinct species is the only representative of the genus known from Europe, and it cannot be confidently placed within any extant *Nylanderia* subclade. We therefore constrained the minimum stem age of the genus using an exponential distribution with Median 38.9 Ma, and 5% and 95% Quantiles to 34.4 Ma and 55.0 Ma, respectively.

4: *Prenolepis henschei* † is known from workers and reproductives, and may be related to extant species such as *Prenolepis imparis* (Nearctic) and *Prenolepis nitens* (Palearctic). However, a key feature among extant species, the thin and setose parameres, is remarkably different from the extinct taxon (LaPolla et al., 2010a). We constrained the stem age of *Prenolepis* using an exponential distribution with Median 37.5 Ma, and 5% and 95% Quantiles to 34.3 Ma and 49.0 Ma, respectively.

5: *Pseudolasius boreus* † is known only from workers, and its relation to extant *Pseudolasius* is not clear as in other *Prenolepis* genus-group extinct species. However, it likely belongs to the genus *Pseudolasius* and not to the closely related and morphologically similar genus *Euprenolepis*, given the high tooth count in workers of *Pseudolasius* ants (LaPolla et al., 2010a). We assigned a minimum stem age of *Pseudolasius* using an identical exponential distribution as for the genus *Prenolepis*.

Another extinct species that may belong to the *Prenolepis* genus-group is *Protrechina carpenteri* † from Arkansas amber. However, it is known only from a single specimen, which is deteriorated and the amber has become clouded leaving the specimen difficult to study (LaPolla et al., 2010a). Thus we did not include this fossil in our time-calibration analyses.

4. Biogeographical analyses

4.1. Taxonomic and geographical considerations of missing taxa

New World and Afrotropical *Prenolepis* genus-group taxa may form each monophyletic groups that have probably originated from Asian/Indo-Pacific lineages and that have further diversified locally (LaPolla et al., 2010a). Further taxonomic and distributional evidence is shortly described:

- The genus *Parapatrechina* has not been encountered in the New World, thus it is entirely confined to the Palearctic. Its centre of species diversity is SE Asia and the Indo-Pacific (LaPolla et al., 2010b). Only thirteen species are known from the Afrotropics and Malagasy regions and in the light of male genitalia these taxa appear to be allied. In our phylogeny, *P. glabra* (widespread across Madagascar) and *P. weissii* (widespread throughout Central Africa) are in fact closely related and sister to a large clade of SE Asian and Indo-Pacific taxa.

- The genus *Nylanderia* is widespread across tropical and subtropical regions around the world. The known species richness of the group is about 110 extant species, most of them distributed in Asia and the Indo-Pacific (AntWeb, 2016). Two monographic revisions within the Nearctic (Kallal and LaPolla, 2012) and the Afrotropics (LaPolla et al., 2011) have found only fourteen endemic species on each region, which may be largely the result of local radiation rather than several immigration events based on morphological features. We recovered two clades containing New World *Nylanderia* taxa: a Nearctic clade and a Neotropical clade. Little is known about the taxonomy of Neotropical *Nylanderia*, but all species from such region, including described and undescribed species, so far cluster within a monophyletic group sister to a Malagasy *Nylanderia*. However, an Asian origin of these taxa cannot be discarded, as a large number of continental Asian species remain to be added in the phylogeny.

- The genus *Zatania* (only five species) is restricted to the Caribbean and Central American regions, but may have originated by dispersal from Asia.

- The genus *Prenolepis* (about 20 species) is mainly distributed in SE Asia, whereas one species is found in the Nearctic region (likely a single dispersal event from Asia) and another species is endemic to southern Europe.

- The region of origin of the genus *Paratrechina* has been hypothesised to be SE Asia (LaPolla et al., 2013b), with further dispersal to and radiation in Africa (LaPolla and Fisher, 2014).

- The genera *Euprenolepis* and *Pseudolasius* are restricted to SE Asia and the Indo-Pacific.

4.2. Historical biogeography inferences

The focal study area was finely subdivided into seven areas, whereas two larger areas were considered as New World and Afrotropics and Malagasy:

- Oriental and Palearctic area with ant faunal assemblages of mostly Asian origin. It includes continental SE Asia and the Malay Peninsula in the study area.
- Malay Archipelago area defined as the intermediate zone between SE Asia and Sahul (Australia and New Guinea). It includes Wallacea (Sulawesi, the lesser Sundas, and the Moluccas), Borneo, and the Philippines.
- Northern New Guinea area covers the northern part of the island, including the Central Range.
- Southern New Guinea area delimits the region south of the Central Range, including the Papuan peninsula.
- Australia area covers tropical and subtropical regions of the mainland continent.
- Micronesia area defined as the cluster of oceanic islands in Palau, Caroline Islands, and Marianas Islands. Although Micronesia is a vast area and some archipelagos, such as Palau, are geographically closer to the Malay Archipelago, recent molecular phylogenies suggested that ant dispersal across such areas is relatively rare (Janda *et al.*, 2014b; Clouse *et al.*, 2015).
- Eastern Melanesia area encompasses the islands of the Vanuatu archipelago and the Fijian archipelago.

Polynesia, including Marquesas Islands, Austral Islands and Society Islands, harbours only a tiny proportion of *Prenolepis* genus-group species diversity, in all cases, as a result of recent dispersal by widespread taxa or introduction by man. Species that have reached Polynesia includes *Nylanderia bourbonica* (including Hawaii), *Nylanderia vaga*, and *Paratrechina longicornis* (Ramage, 2014; Wilson and Taylor, 1967).

Three biogeographic models as implemented in BioGeoBEARS (Matzke, 2013a, 2013b) were assessed. First, we used the Dispersal-Extinction-Cladogenesis model (DEC; Ree *et al.*, 2005; Ree & Smith, 2008) allowing range expansion (dispersal) and contraction (extinction) as anagenetic processes (i.e., along branches). DEC also implements cladogenetic processes (i.e., at nodes) such as vicariance and the so-called *sympatric speciation* event *sensu* Matzke (2013c). Second, we used the BAYAREA-LIKE model (Landis *et al.*, 2013) which differs from the DEC model by disallowing vicariance and the so-called *subset sympatric speciation* event. The rationale of using these two models is to evaluate (1) the impact of vicariance (allowed in DEC but not in BAYAREA-LIKE) in

biogeographic reconstruction, like the emergence of New Guinean Central Range, and (2) the importance of anagenetic processes such as dispersal, since BAYAREA-LIKE is a simpler model accounting for fewer cladogenetic processes than DEC (Matzke, 2013a, 2013b). Third, the DIVA-LIKE model adds an extra vicariance parameter, a process that may inform on the effect of large biogeographic barriers such as the Central Range in New Guinea. Statistical comparison of these models using Akaike weights are presented in Table 1 (Main Text). The stratified DEC+J (Fig. A.6) was favoured over the remaining models.

In the stratified analyses, we sliced the chronogram into four temporal spans at 25, 15 and 5 Ma, each with different geological configurations. We arbitrarily assigned distinct dispersal rates between two areas at each time slice (Table A.3). Dispersal rates were constrained as an attempt to recreate palaeogeographical models:

- Rates were set to 1.0 when two areas are temporarily joint (e.g., northern and southern New Guinea);
- When two areas are separated by sea strait or geographic barrier, dispersal was set to either 0.5 or 0.1 depending on the extent of the separation;
- Long-distance dispersal involving three areas was set to 0.01 (e.g., SE Asia and Australia);
- Other dispersal types across areas were drastically reduced to a rate of 10^{-4} .
- Range expansion to areas that were under water during specific time slices (e.g., oceanic islands) were disallowed.

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Additional tables and figures

Table A.1. Voucher information, including sampling locality, habitat preference, and Genbank accession numbers for each gene marker and BOLD accession numbers (ASPNA project) for the COI gene marker. Outgroup taxa and described species that were not sampled in our phylogeny are presented in the first and last rows of the table, respectively.

Table A.2. Stepping-stone analyses to determine the best-fitting partitioning strategy. ESS: Effective Sample Size; BF-ss: Bayes Factor; SSML: Stepping-Stone Marginal Likelihood. The codon partitioning strategy outperformed other strategies.

Partition strategy	N. of partitions	ESS	Harmonic mean	SSML	BF-ss [A]	BF-ss [B]	BF-ss [C]	BF-ss [D]	BF-ss [E]
A (All)	1	4857.67	-45575.8	-47051.77	-				
B (By Genome)	2	5558.82	-43714.9	-45298.93	1752.84	-			
C (By Gene)	7	4615.77	-43616.8	-45276.41	1775.36	22.52	-		
D (By Bin)	3	5617.15	-42812.3	-44411.47	2640.3	887.46	864.94	-	
E (By Codon)*	21	1589.76	-42133.6	-44012.32	3039.45	1286.61	1264.09	399.15	-

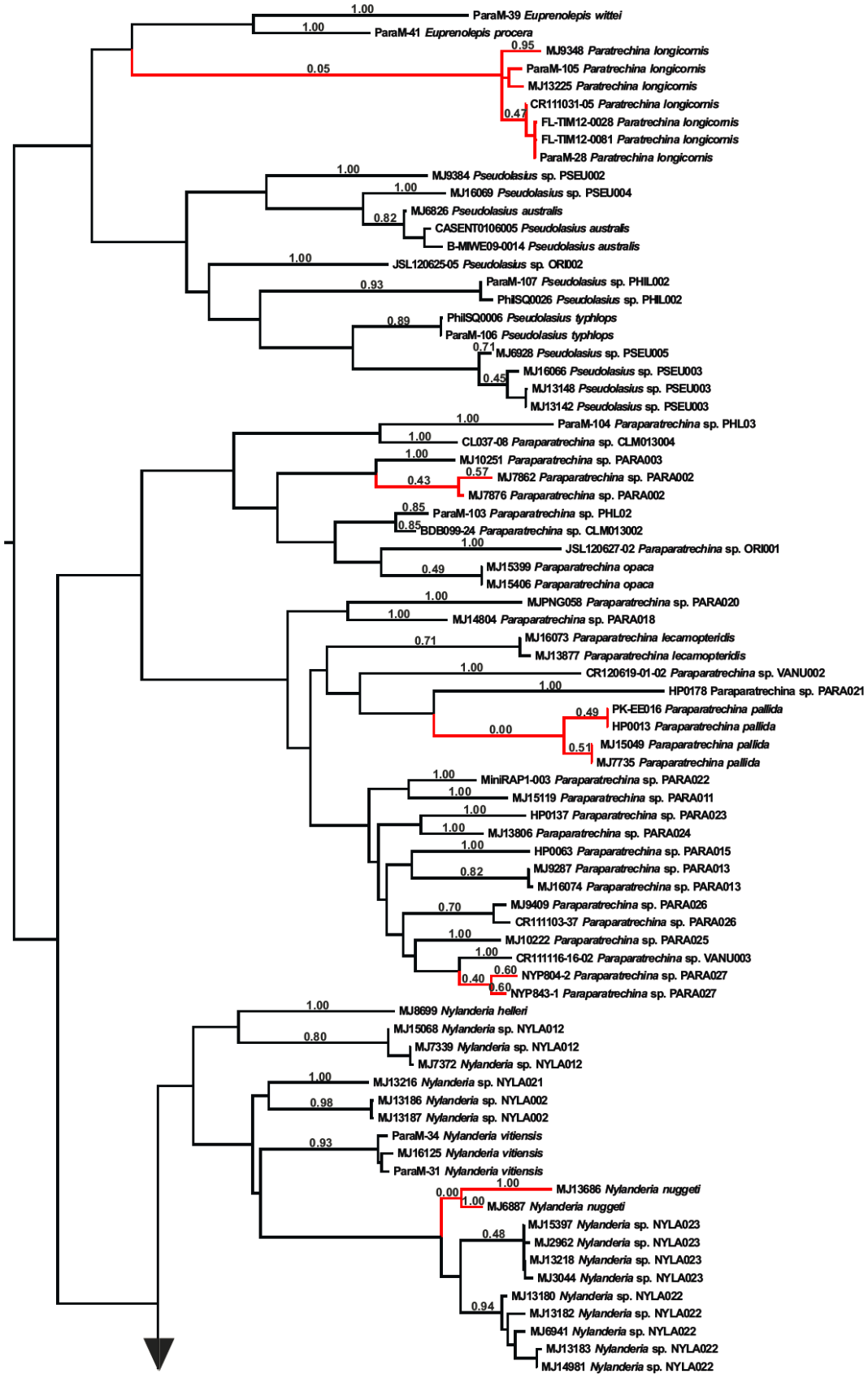
Table A.3. Manual dispersal multipliers (upper right) and areas allowed (lower left) settings used in the stratified biogeographical analyses in BioGeoBEARS. The areas were defined as 1) Oriental-Palaearctic (Or), 2) Malay Archipelago (MA), 3) northern New Guinea (NGn), 4) southern New Guinea (NGs), 5) Australia (Au), 6) Micronesia (Mi), 7) eastern Melanesia (eMe), 8) New World (NW), and 9) Afrotropics (Af).

Time Period: 5–15 Ma									
	Or	MA	NGn	NGs	Au	Mi	eMe	NW	Af
Or	1	0.5	0.1	0.1	0.01	0.01	0.001	0.01	0.5
MA	1	1	0.5	0.5	0.1	0.5	0.1	0.0001	0.001
NGn	1	1	1	0.5	0.5	0.5	0.5	0.0001	0.001
NGs	1	1	1	1	0.5	0.5	0.5	0.0001	0.001
Au	1	1	1	1	1	0.1	0.5	0.001	0.001
Mi	1	1	1	1	1	1	0.5	0.001	0.0001
eMe	1	1	1	1	1	1	1	0.001	0.0001
NW	1	1	1	1	1	1	1	1	0.001
Af	1	1	1	1	1	1	1	1	1
Time Period: 15–25 Ma									
	Or	MA	NGn	NGs	Au	Mi	eMe	NW	Af
Or	1	0.5	0.01	0.01	0.001	0.000001	0.0001	0.1	0.5
MA	1	1	0.1	0.1	0.01	0.000001	0.01	0.01	0.01
NGn	1	1	1	0.1	0.1	0.000001	0.1	0.00001	0.0001
NGs	1	1	1	1	0.1	0.000001	0.1	0.00001	0.0001
Au	1	1	1	1	1	0.000001	0.1	0.0001	0.0001
Mi	0	0	0	0	0	0	0.000001	0.000001	0.000001
eMe	1	1	1	1	1	0	1	0.0001	0.00001
NW	1	1	1	1	1	0	1	1	0.001
Af	1	1	1	1	1	0	1	1	1
Time Period: 25–47 Ma									
	Or	MA	NGn	NGs	Au	Mi	eMe	NW	Af
Or	1	0.5	0.1	0.01	0.0001	0.000001	0.001	0.1	0.5
MA	1	1	0.5	0.01	0.001	0.000001	0.01	0.01	0.01
NGn	1	1	1	0.1	0.1	0.000001	0.5	0.01	0.01
NGs	1	1	1	1	0.5	0.000001	0.1	0.001	0.001
Au	1	1	1	1	1	0.000001	0.1	0.01	0.001
Mi	0	0	0	0	0	0	0.000001	0.000001	0.000001
eMe	1	1	1	1	1	0	1	0.0001	0.00001
NW	1	1	1	1	1	0	1	1	0.001
Af	1	1	1	1	1	0	1	1	1
Time Period: > 47 Ma									
	Or	MA	NGn	NGs	Au	Mi	eMe	NW	Af
Or	1	0.1	0.000001	0.000001	0.00001	0.000001	0.000001	0.5	0.1
MA	1	1	0.000001	0.000001	0.00001	0.000001	0.000001	0.1	0.01
NGn	0	0	0	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001
NGs	0	0	0	0	0.000001	0.000001	0.000001	0.000001	0.000001
Au	1	1	0	0	1	0.000001	0.000001	0.1	0.001
Mi	0	0	0	0	0	0	0.000001	0.000001	0.000001
eMe	0	0	0	0	0	0	0	0.000001	0.000001
NW	1	1	0	0	1	0	0	1	0.001
Af	1	1	0	0	1	0	0	1	1

Table A.4. Geographical ranges, habitat preference, and elevational range for each species in Melanesia and the tropical South Pacific. In geographical range: As=Sundaland, including Philippines; Wa=Wallacea; NG=New Guinea (*n*: northern, *s*: southern); Au=Australia; Mi=Micronesia, including Palau and the Federated States of Micronesia; Va=Vanuatu; Fi=Fiji; and Po=Polynesia. In vegetation, UF=undisturbed rainforest; DF=disturbed forest; SP=savanna and pastures; CS=coastal scrubs; and UG=urban and gardens. In elevation, lw=lowlands; lm=lower montane; and mo=montane. The habitat preference recorded only in other islands/archipelagos but not in New Guinea are depicted as "Is". The number of examined colonies from our own field sampling are shown in the last column.

Voucher codes and species IDs	Geographical Range	Vegetation	Elevation	Field records
BDB086_11_Nylanderia_bourbonica	As-Wa-Mi-Po	DF-SP-CS-UG	lw	17
BDB096_01_Nylanderia_CLM011001	As	-	-	-
BDB096_04_Nylanderia_CLM011005	As	-	-	-
BDB099_24_Parapatrechina_CLM013002	As	-	-	-
BOR12_130D_Nylanderia_BOR003	As	-	-	-
BOR12_140_Nylanderia_BOR005	As	-	-	-
BOR12_204B_Nylanderia_BOR004	As	-	-	-
BOR12_204C_Nylanderia_BOR006	As	-	-	-
BOR12_244_Nylanderia_BOR001	As	-	-	-
BOR12_271_Nylanderia_BOR002	As	-	-	-
CASENTO200454_Parapatrechina_PARA029	NG(s)	UF	lm	1
CL018_19_Nylanderia_CLM013001	As	-	-	-
CL037_08_Parapatrechina_CLM013004	As	-	-	-
CR111103_37_Parapatrechina_PARA026	NG(n)-Va	UF(Va)-CS-UG(NG)	lw	2
CR111116_16_02_Parapatrechina_VANU003	Va	UF	mo	1
CR120619_01_02_Parapatrechina_VANU002	Va	UF	mo	1
CR120625_48_Nylanderia_VANU001	Va	UF	mo	1
FL_TIM12_0038_Nylanderia_TIMO001	Wa	UF-DF	lw	10
FL_TIM12_0050_Nylanderia_vaga	Wa-NG(n-s)-Mi-Va-Fi-Po	UF-DF-CS-UG	lw(NG)-lm-mo(ls)	67
FL_TIM12_0076A_Nylanderia_NYLA027	Wa	UF-UG	lw	3
HP0013_Parapatrechina_pallida	NG(n-s)	UF	lw	15
HP0063_Parapatrechina_PARA015	NG(n)	UF	lw	2
HP0112_Parapatrechina_PARA004	NG(n)	UF	lw	1
HP0137_Parapatrechina_PARA023	NG(n)	UF	lw	2
HP0178_Parapatrechina_PARA021	NG(n)	DF	lw	4
JCM_0062_Nylanderia_PALA002	Mi	UF	lw	1
JCM_0136D_Parapatrechina_minutula	NG(n)-Au-Mi	UF(NG&ls)-CS(NG)	lw(NG&ls)-lm(ls)	3
JCM_0283A_Nylanderia_PALA001	Mi	UF-DF	lw	11
JSL120625_05_Pseudolasius_ORI002	As	-	-	-
JSL120626_01_Nylanderia_ORI003	As	-	-	-
JSL120627_02_Parapatrechina_ORI001	As	-	-	-
JSL120627_03_Nylanderia_ORI004	As	-	-	-
JSL120629_04_Nylanderia_ORI005	As	-	-	-
MiniRAP1_003_Parapatrechina_PARA022	NG(s)	UF	mo	3
MJ10222_Parapatrechina_PARA025	NG(s)	UF	lw	1
MJ10251_Parapatrechina_PARA003	NG(s)	UF	lw	3
MJ13142_Pseudolasius_PSEU003	NG(n-s)	UF	lw	9
MJ13190_Nylanderia_NYLA002	NG(s)	UF	lm	7
MJ13213_Nylanderia_NYLA030	NG(n)	UF-DF-UG	mo	5
MJ13216_Nylanderia_NYLA021	NG(n)	UF-DF-UG	mo	4
MJ13220_Nylanderia_NYLA009	NG(n)	CS	lw	5
MJ13225_Paratrechina_longicornis	All	All	All	29
MJ13236_Nylanderia_NYLA020	NG(s)	SP	lw	2
MJ13237_Nylanderia_NYLA004	NG(s)	SP	lw	3
MJ13240_Nylanderia_glabrior	NG(n-s)-Au-Mi-Fi-Po	UF-SP	lw(NG)-lm-mo(ls)	14
MJ13243_Nylanderia_NYLA024	NG(s)	SP	lw	3
MJ13806_Parapatrechina_PARA024	NG(s)	UF	lm	1
MJ13855_Parapatrechina_PARA001	NG(n)	UF	lw	6
MJ13877_Parapatrechina_lectamopteridis	NG(s)	SP	lw	12
MJ13888_Parapatrechina_PARA012	NG(s)	SP	lw	3
MJ13913_Nylanderia_NYLA025	NG(s)	UF-SP	lw	2
MJ14804_Parapatrechina_PARA018	NG(n)	CS	lw	6
MJ14851_Nylanderia_NYLA003	NG(n)	ALL	lw	90
MJ15022_Parapatrechina_PARA028	NG(s)	UF	lw	1
MJ15119_Parapatrechina_PARA011	NG(s)	UF	lw	1
MJ15399_Parapatrechina_opaca	NG(n)	UF	lw	4
MJ15403_Nylanderia_NYLA005	NG(s)	UF	lm	10
MJ16065_Nylanderia_NYLA022	NG(s)	UF	lw-lm	20
MJ16069_Pseudolasius_PSEU004	NG(n)	UF	lw	7
MJ16074_Parapatrechina_PARA013	NG(s)	UF-SP	lw	6
MJ16121_Parapatrechina_oceanica	Fi	UF-DF-SP-UG	lw-lm-mo	2
MJ16123_Nylanderia_FJ03	NG(n)-Fi	UF-DF(Fi)-CS-UG(NG)	lw(NG)-lm-mo(ls)	3
MJ16125_Nylanderia_vitiensis	Fi	UF-DF	lm-mo	2
MJ18281_Parapatrechina_PARA019	NG(n)	UF	lm	1
MJ18414_Parapatrechina_PARA014	NG(n-s)	UF	lw	3
MJ3044_Nylanderia_NYLA023	NG(n)	UF	lw	15
MJ6826_Pseudolasius_australis	NG(n-s)-Au	UF-DF	lw-lm	22
MJ6887_Nylanderia_nuggeti	NG(n-s)	UF-DF	lw-lm	3
MJ6928_Pseudolasius_PSEU005	NG(n)	UF	lw	2
MJ6933_Nylanderia_NYLA026	NG(s)	UF-DF-SP	mo	3
MJ7339_Nylanderia_NYLA012	NG(s)	UF	lw-lm	7
MJ7781_Parapatrechina_PARA010	NG(s)	UF	lw	2
MJ7862_Parapatrechina_PARA002	NG(n)	UF	lw	2
MJ8699_Nylanderia_helleri	NG(n-s)	UF	lw-lm	9
MJ8839_Parapatrechina_PARA006	NG(s)	UF	lw	4
MJ9198_Nylanderia_obscura	NG(s)-Au-Va	DF-SP-UG	lw-lm	16
MJ9384_Pseudolasius_PSEU002	NG(n-s)	UF	lw	6
MJPNG058_Parapatrechina_PARA020	NG(n-s)	UF-UG	mo	5
MJPNG069_Parapatrechina_PARA016	NG(s)	UF	mo	6
MJPNG081_Nylanderia_NYLA029	NG(n)	UF	mo	1
NYP843_1_Parapatrechina_PARA027	Mi	UF-DF	lw-lm-mo	4
ParaM_39_Euprenolepis_wittei	As	-	-	-
ParaM_41_Euprenolepis_procera	As-NG(n)	-	-	-
PHILSQ0006_Pseudolasius_typhlops	As	-	-	-
PHILSQ0015_Nylanderia_Phil003	As	-	-	-
PHILSQ0026_Pseudolasius_Phil002	As	-	-	-
PL_03_K010_Nylanderia_AUST001	Au	DF-SP	lm	1

Fig. A.1. Species delimitation analysis using bPTP based on a combined dataset consisting of 164 specimens with unique COI haplotypes and at least one gene marker sequenced. The input tree was the maximum likelihood phylogeny inferred by RAxML. bPTP calculated Bayesian supporting values (shown along branches) for each delimited species. We consider highly supported species boundaries to probabilities of more than 0.8. Conflicts between species delimitations based on morphology and bPTP are highlighted as red coloured branches, whereby bPTP suggested further split into two species for each lineage. Taxon label at each tip consists of each specimen voucher code and species name.



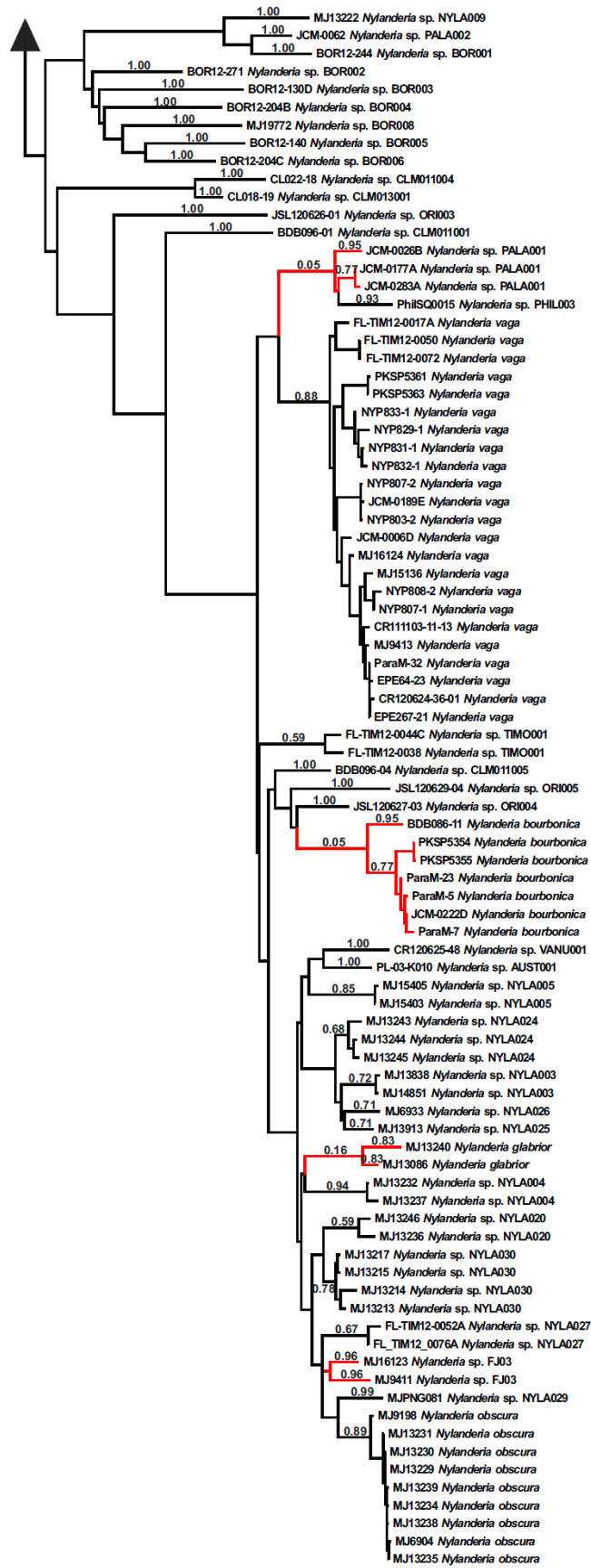


Fig. A.2. Consensus tree under the 50% majority rule of the multi-locus dataset using Bayesian inference as implemented in MrBayes. Branch lengths represent expected substitutions per site. Posterior probabilities from MrBayes and bootstrap support values from RAxML (1000 re-sampling events) are shown as coloured stars following the legend next to the tree. Taxon label at each tip consists of each specimen voucher code and species name. Major defined clades in the main manuscript are presented as numbered nodes on each major clade.

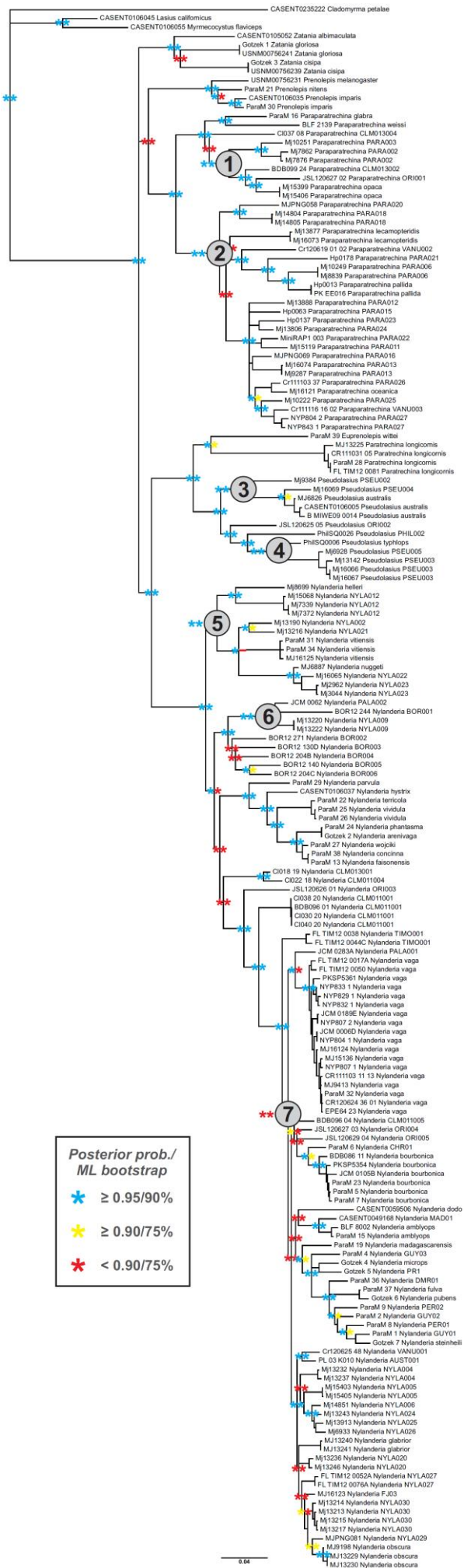
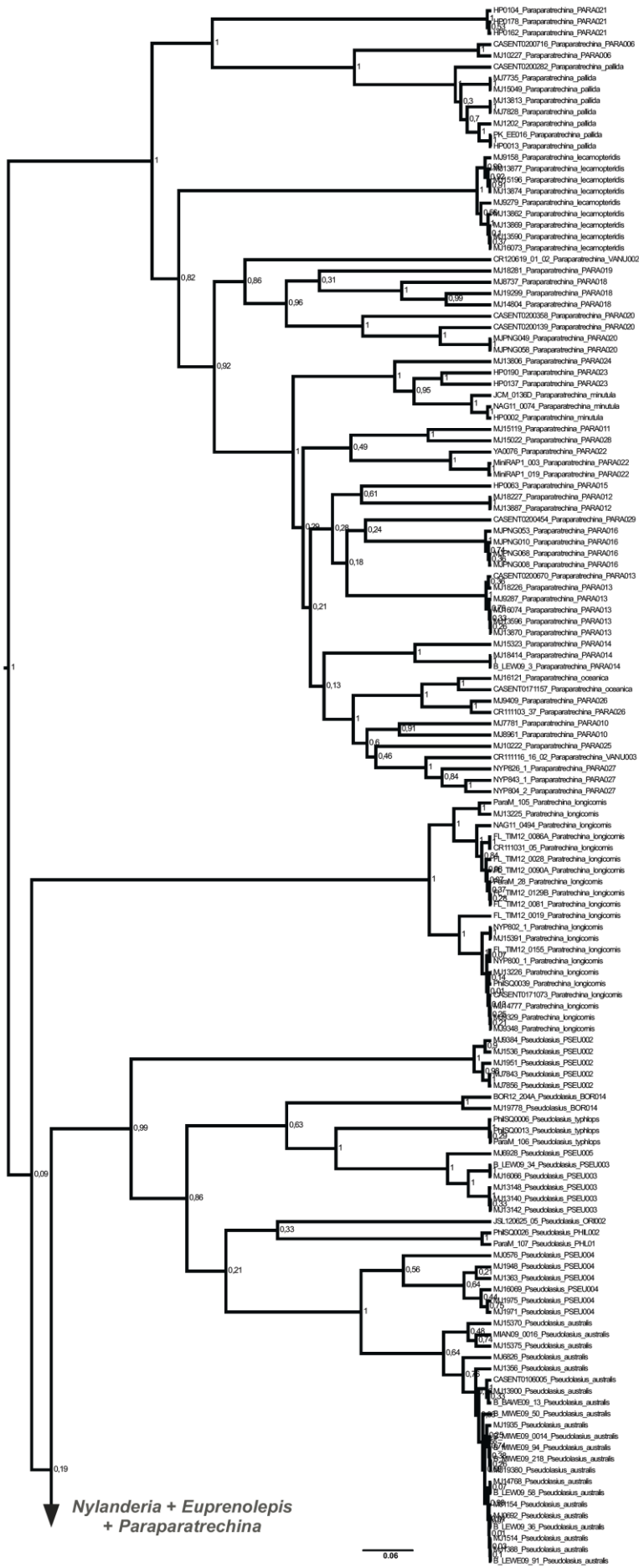


Fig. A.3. Gene tree inferred in BEAST based on a dataset consisting of unique COI haplotypes. Posterior probabilities are presented next to each node. Taxon label at each tip consists of each specimen voucher code and species name.



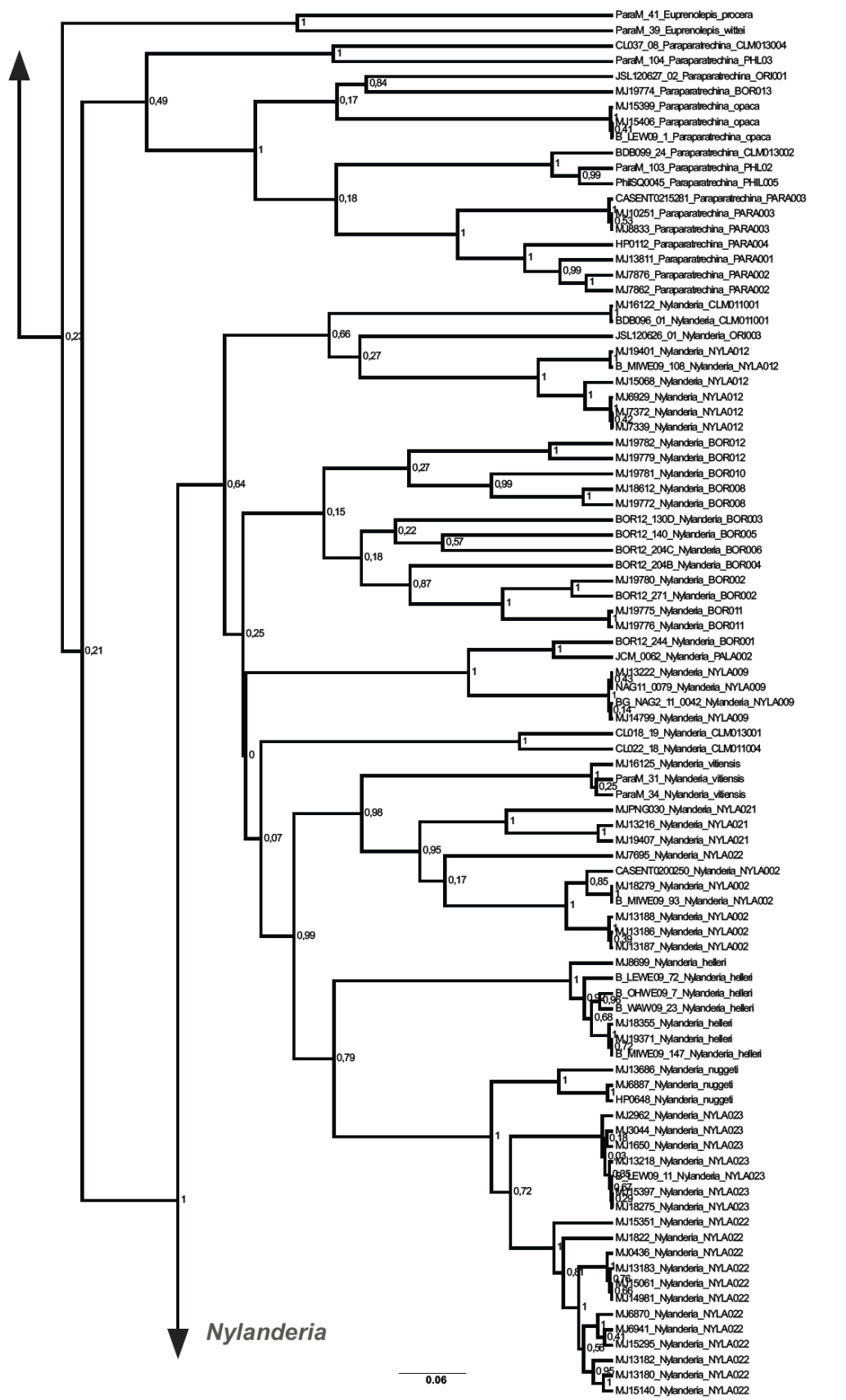
Paraparatrechina

Paratrechina

Pseudolasius

Nylanderia + *Euprenolepis*
+ *Paraparatrechina*

0.06

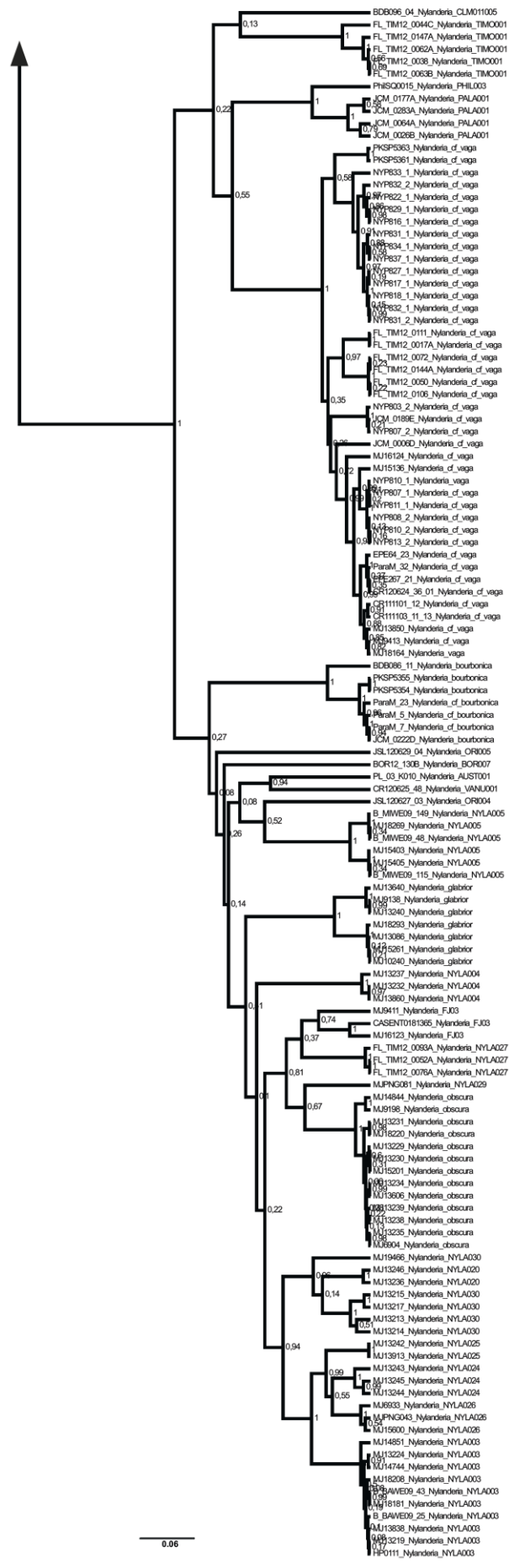


Parapatrechina

Nylanderia

Nylanderia

0.06



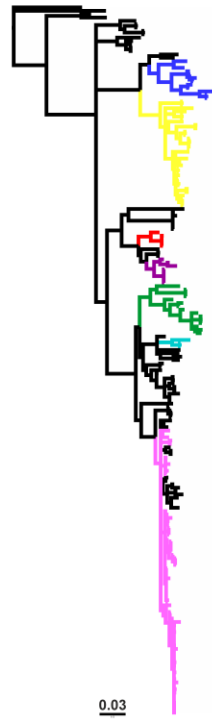
Nylanderia

Fig. A.4. Phylogenetic analyses of single gene markers in MrBayes. Branch lengths are represented in the same scale, thus, they are proportionate and comparable among trees. For single-gene analyses only, we included and excluded introns from the alignment in separate analyses to further investigate congruence among datasets. Branches are coloured for each genus following the legend next to the figure.

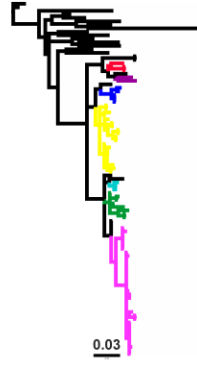
CAD gene, only exons



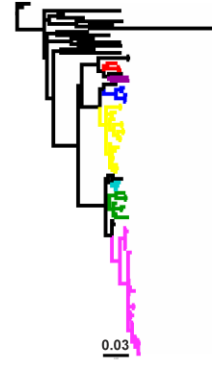
CAD gene, exons & introns



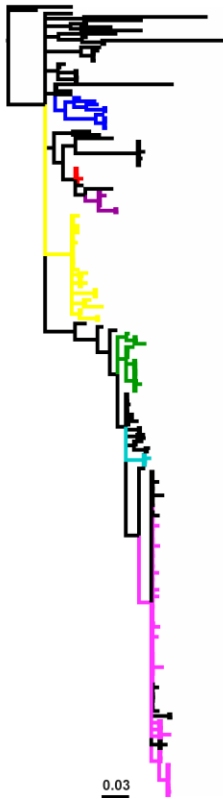
LWR gene, only exons



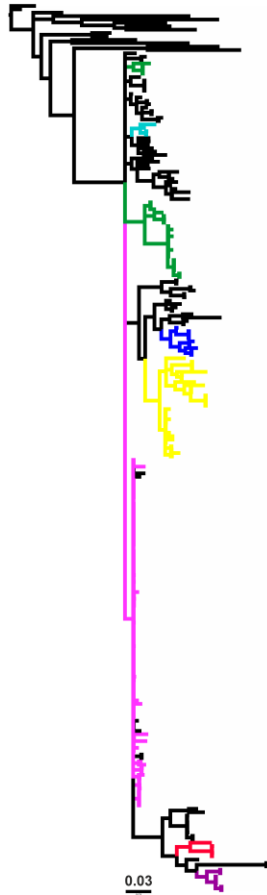
LWR gene, exons & introns



EF1 gene



EF2 gene



wingless gene

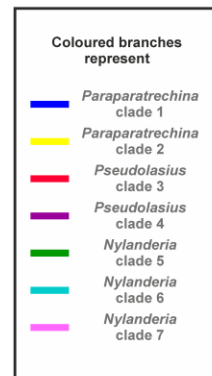
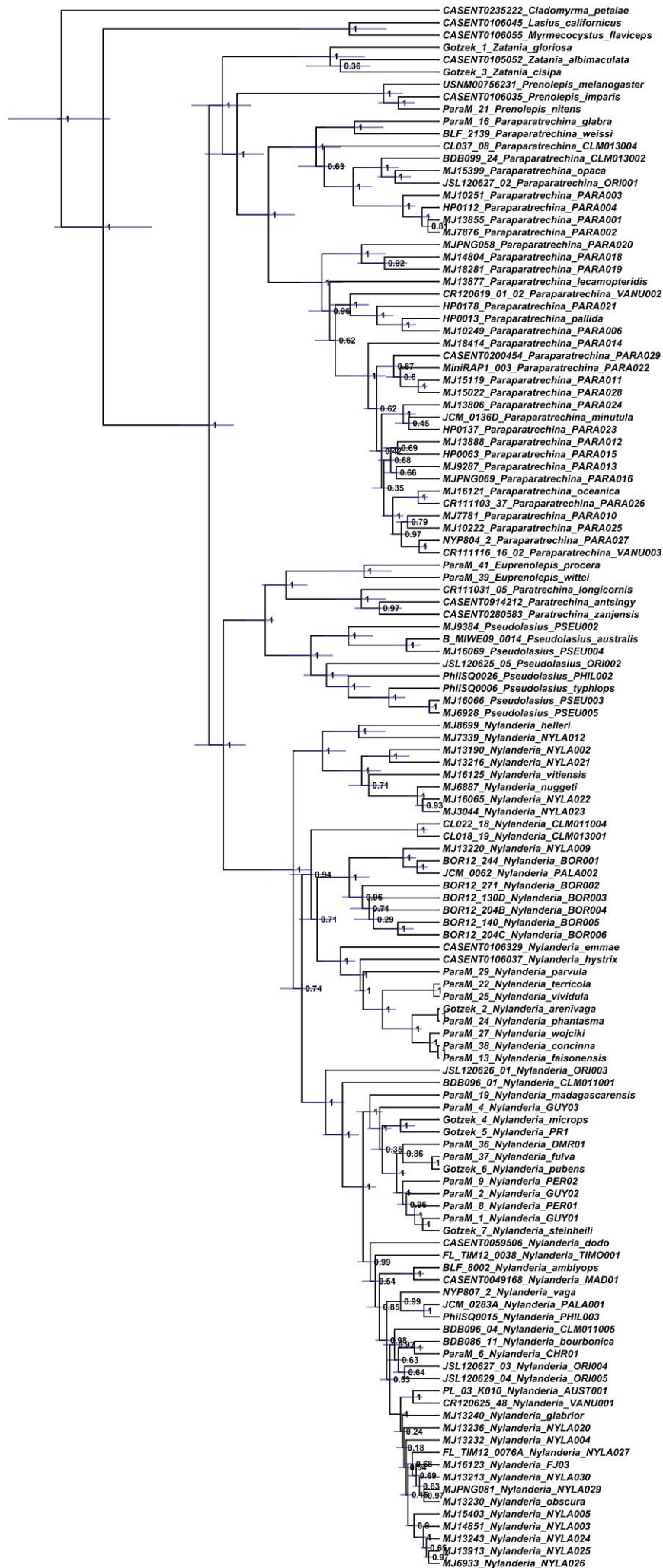


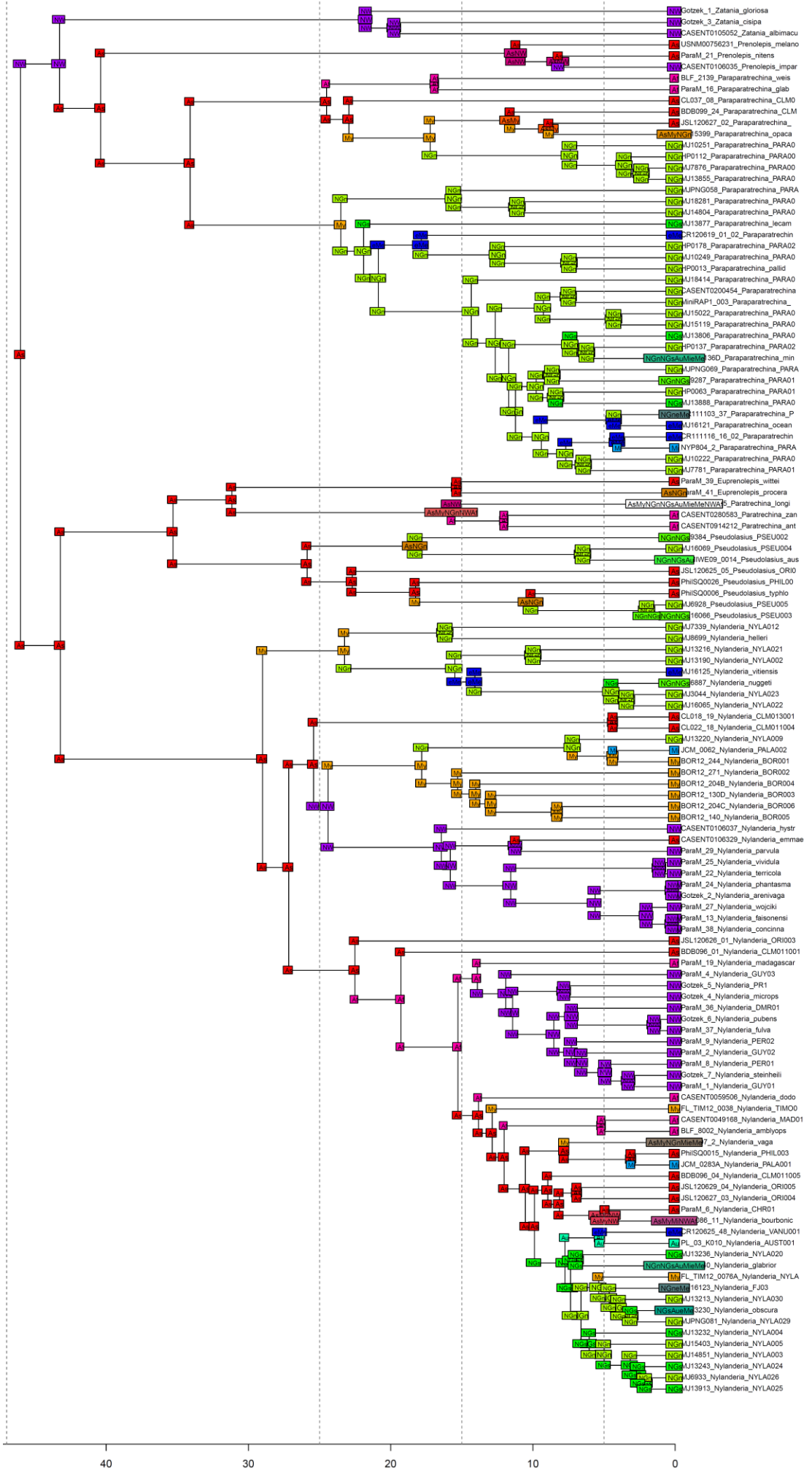
Fig. A.5. Divergence time estimation under the Yule tree prior in the program BEAST. The chronogram was summarized under the Maximum Clade Credibility approach and node information as the median estimate. 95% confidence interval displayed as bars on nodes. Posterior probabilities are labelled on each node. Time axis in million years ago.



-85 -80 -75 -70 -65 -60 -55 -50 -45 -40 -35 -30 -25 -20 -15 -10 -5

Fig. A.6. Output tree including pie charts from the biogeographical reconstruction analysis in BioGeoBEARS. The analyses was conducted under the preferred DEC+J model and the phylogeny was stratified in four time periods. The first tree shows on each node the most probable geographic range, while the second tree shows the pie chart probabilities for each node.

BioGeoBEARS DEC+J on Nylanderia stratified
ancstates: global optim, 9 areas max. d=0.0263; e=0.0026; j=0.2465; LnL=-298.42



40

30

20

10

0

