

1 **Original Article**

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3 RRH: Biogeography of the relict butterfly *Elkalyce cogina*

4 LRH: G. Talavera *et al.*

5

6 **One note samba: the biogeographical history of the relict Brazilian butterfly**

7 ***Elkalyce cogina***

8

9 Gerard Talavera^{1,2,3*}, Lucas A. Kaminski^{1,4}, André V. L. Freitas⁴ and Roger Vila¹

10

11 ¹*Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra), 08003 Barcelona,*

12 *Spain, ²Department of Organismic and Evolutionary Biology and Museum of*

13 *Comparative Zoology, Harvard University, Cambridge, MA 02138, USA, ³Faculty of*

14 *Biology and Soil Science, St Petersburg State University, 199034 St Petersburg,*

15 *Russia, ⁴Departamento de Biologia Animal, Instituto de Biologia, Universidade*

16 *Estadual de Campinas, CP 6109, 13083-970, Campinas, SP, Brazil*

17

18 *Correspondence: Gerard Talavera, Institut de Biologia Evolutiva (CSIC-

19 Universitat Pompeu Fabra), Passeig Marítim de la Barceloneta 37, 08003

20 Barcelona, Spain

21 E-mail: gerard.talavera@csic.es

22

23

24 **ABSTRACT**

25 **Aim** Biogeographically puzzling taxa represent an opportunity to understand the
26 processes that have shaped current species distributions. The systematic
27 placement and biogeographical history of *Elkalyce cogina*, a small lycaenid
28 butterfly endemic to Brazil and neighbouring Argentina, are long-standing
29 puzzles. We use molecular tools and novel biogeographical and life-history data
30 to clarify the taxonomy and distribution of this butterfly.

31 **Location** South America, with emphasis on the Atlantic Rain Forest and Cerrado
32 biomes (Brazil and Argentina).

33 **Methods** We gathered a dataset of 71 Polyommatini (Lycaenidae) samples,
34 including representatives of all described subtribes and/or sections. Among
35 these, we contributed new sequences for *E. cogina* and four additional relevant
36 taxa in the target subtribes Everina, Lycaenopsina and Polyommata. We
37 inferred a molecular phylogeny based on three mitochondrial genes and four
38 nuclear markers to assess the systematic position and time of divergence of
39 *E. cogina*. Ancestral geographical ranges were estimated with the R package
40 BIOGEOBEARS. To investigate heterogeneity in clade diversification rates, we
41 used Bayesian analysis of macroevolutionary mixtures (BAMM).

42 **Results** Our results confirm the hypothesis that *E. cogina* belongs to the subtribe
43 Everina and not Lycaenopsina, but unexpectedly recovered it as the sister group
44 to the rest of Everina, with an estimated divergence time of approximately
45 10 Ma. Ancestral geographical range reconstruction points to an old colonization
46 from Asia, the centre of diversity for the Everina, to the New World. Whereas the
47 Neotropical Polyommata lineage diversified to produce almost 100 species in
48 multiple genera, the *E. cogina* lineage did not diversify at all. Such lack of

49 diversification is unique among the seven Everina/Polyommata lineages that
50 colonized the New World. We also show that the larvae of *E. cogina* feed on
51 Fabaceae, supporting the identification of this host-plant family as the ancestral
52 state for the whole group.

53 **Main conclusions** The age and biogeographical reconstruction of the *Elkalyce*
54 lineage are similar to those of the Neotropical lineage of Polyommata and
55 suggest that both travelled via the route proposed by Vladimir Nabokov (Asia–
56 Beringia–North America–South America). This coincidence suggests that the
57 climatic conditions at *ca.* 10 Ma favoured dispersal from Asia to the Neotropics
58 and that later events may have erased traces of these butterfly lineages in North
59 America.

60

61 **Keywords**

62 **Beringia; biogeographical disjunction; colonization; dispersal;**
63 **diversification; *Elkalyce cogina*; Fabaceae; Lepidoptera; New World; relict**

64

65

66 *"This is just a little samba,*

67 *Built upon a single note*

68 *Other notes are bound to follow,*

69 *But the root is still that note..."*

70

71 "Samba de Uma Nota Só" ("One-Note Samba") is a song composed by Antonio

72 Carlos Jobim and Newton Mendonça.

73

74 INTRODUCTION

75 Intriguing disjunct distributions that apparently formed after the break-up of
76 Gondwana are found within many butterfly families as the result of a complex
77 biogeographical history of colonization and extinction events (de Jong & van
78 Achterberg, 2007; Peña *et al.*, 2010). The Lycaenidae also include notable
79 disjunct distributions that are generally only attributable to long-distance
80 dispersal events, including examples in the Spalgini, Lycaenini, Heliphorini and
81 Polyommatini (de Jong & van Achterberg, 2007; Vila *et al.*, 2011). The study of
82 the affinities and origins of these taxa is crucial to understanding the processes
83 that led to the observed patterns of species distribution and diversification
84 worldwide.

85 With over 1200 species distributed worldwide, the tribe Polyommatini is,
86 one of the two richest lycaenid clades, alongside the Neotropical/Holarctic-
87 distributed Eumaeini (Eliot, 1973). These two tribes represent the only
88 Lycaenidae groups with important South American radiations, but their current
89 biogeographical patterns are strikingly different: whereas most Eumaeini
90 diversity occurs in the Neotropics, the Polyommatini are most species-rich in the
91 Holarctic region.

92 Within the Polyommatini, a complex scenario of independent New World
93 colonization events was hypothesized by Nabokov (1944, 1945) in the 1940s
94 and recently corroborated by Vila *et al.* (2011), who showed that at least five
95 colonization events from the Old World to the New World occurred between *ca.*
96 11 Ma and 1 Ma, apparently always through Beringia (the region surrounding
97 the Bering strait). This scenario was postulated and evaluated for the richest
98 subtribe, Polyommata, but other subtribes, like Leptotina, Everina,

99 Glaucospsychina and Brephidiina, also have representatives both in the Old
100 World and the New World.

101 Such complex biogeographical histories can only be unravelled by means of a
102 reliable and abundant fossil record or inferred from well-founded phylogenies.
103 For butterflies, although the fossil record is extremely poor (de Jong, 2007),
104 knowledge of their phylogenetic relationships is improving, resulting in novel
105 insights into long-standing biogeographical questions. Following the seminal
106 taxonomic revision by Eliot (1973), the higher-level systematics of Polyommatini
107 have recently been improved using molecular phylogenies (Wiemers *et al.*, 2010;
108 Vila *et al.*, 2011; Talavera *et al.*, 2013). Some enigmatic taxa remain dubiously
109 placed at the tribal and subtribal levels, however, as is the case for the
110 Neotropical species *Elkalyce cogina* (Schaus, 1902), which is restricted to Brazil
111 and Misiones province in northern Argentina. The species was originally
112 described within the genus *Lycaena* by Schaus (1902), but because no close
113 relatives were attributable based on morphology, the monotypic genus *Elkalyce*
114 Bálint & Johnson, 1955 was erected, thought to be sister to *Oreolyce* and so
115 belonging to the *Lycaenopsis* section (equivalent to the subtribe Lycaenopsina)
116 within Polyommatinae (Bálint & Johnson, 1996). More recently, Robbins &
117 Duarte (2006) suggested that *Elkalyce cogina* could be related to *Tongeia* in the
118 *Everes* section (subtribe Everina), also within Polyommatinae, supporting an
119 earlier unpublished hypothesis of Heinz Ebert and John H. Eliot (see comments
120 in Brown, 1993; Robbins & Duarte, 2006). In both hypotheses, the putative sister
121 groups of *E. cogina* are exclusively distributed in Asia, and therefore this taxon
122 seems to represent a South American endemic lycaenid whose closest relatives
123 occur in the Old World.

124 In this study, we present a detailed molecular phylogeny of Polyommattini
125 including *E. cogina* and thus provide evidence about its systematic placement
126 within the tribe. By inferring divergence times and ancestral geographical range,
127 we estimate the time of colonization of the New World by this lineage, and we
128 explore how diversification patterns within the group can elucidate the unique
129 biogeographical and evolutionary history of *E. cogina*.

130

131 **MATERIALS AND METHODS**

132 **Distribution and life history data**

133 Specimens of *E. cogina* were studied and collected in the field at three localities
134 in São Paulo state (SP), south-eastern Brazil: (1) Serra do Japi, Jundiaí (23°13' S,
135 46°58' W, 900–1200 m), (2) Grota Funda, Atibaia (23°10' S, 46°31' W, 1050–
136 1100 m), and (3) Alto do Capivari, Campos do Jordão (22°44' S, 45°31' W, 1600–
137 1800 m). Locality data for *E. cogina* were compiled from the list presented in
138 Robbins & Duarte (2006); additional data were also obtained from specimens
139 recently collected by the authors and deposited in the Zoological Collection of
140 Campinas State University, and from the personal communication of Ezequiel
141 Núñez-Bustos (Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia',
142 Buenos Aires, Argentina). A map of the updated distribution of *E. cogina* is
143 presented in Fig. 1.

144

145 **Taxon sampling and molecular data**

146 A large collection of 75 Polyommattini samples was used, including at least one
147 representative of each described subtribe or section *sensu* Eliot (1973). Most of
148 the dataset was gathered from our previous studies (Vila *et al.*, 2011; Talavera *et*

149 *al.*, 2013), from which multilocus sequences were available. An additional
150 specimen of *Elkalyce cogina* was collected from Petrópolis (Brazil) and taxon
151 sampling for the target subtribes Everina, Lycaenopsina and Polyommantina was
152 strengthened with additional taxa. (See Table S1 in Appendix S1 for a list of
153 specimens used in this study.) The samples (bodies in ethanol and wings in
154 glassine envelopes) are stored in the DNA and Tissues Collection of the Museum
155 of Comparative Zoology (Harvard University, Cambridge, MA, USA) and of the
156 Institut de Biologia Evolutiva (Barcelona, Spain). Genomic DNA was extracted
157 from a leg or from a piece of the abdomen using the DNeasy Tissue Kit (Qiagen,
158 Valencia, CA, USA) following the manufacturer's protocols. Fragments from three
159 mitochondrial genes - cytochrome oxidase I (*COI*), leucine transfer RNA (*leu-*
160 *tRNA*) and cytochrome oxidase II (*COII*) - and from four nuclear markers -
161 elongation factor-1 alpha (*EF-1 α*), 28S ribosome unit (*28S*), histone H3 (*H3*) and
162 wingless (*Wg*) - were amplified by polymerase chain reaction and sequenced as
163 described in Talavera *et al.* (2013). (GenBank codes for the set of sequences used
164 and obtained specifically for this study are listed in Table S2 of Appendix S1.)

165

166 **Alignment, phylogenetic inference and dating**

167 A molecular matrix was generated for each independent marker (the
168 mitochondrial markers were treated as a single evolutionary unit). All sequences
169 were edited and aligned, together with those obtained in Vila *et al.* (2011), using
170 GENEIOUS 6.1.5 (Biomatters, Auckland, New Zealand; available at:
171 <http://www.geneious.com/>). Ambiguously aligned regions and regions of the
172 matrix lacking more than 50% of data were removed using GBLOCKS 0.96 under a
173 relaxed criterion with the following parameters: *b2*, 50% + 1 of the sequences;

174 *b3*, 3; *b4*, 5; *b5*, all (Castresana, 2000; Talavera & Castresana, 2007). The final
175 combined alignment consisted of 4850 bp, comprising 2172 bp of *COI* + *leu-tRNA*
176 + *COII*, 1172 bp of *EF-1 α* , 811 bp of *28S*, 370 bp of *Wg*, and 328 bp of *H3* and is
177 available in TreeBASE at <http://www.treebase.org/> (study ID 17438).

178 Maximum likelihood (ML) and Bayesian inference (BI) were employed to
179 estimate evolutionary relationships. ML was run using PH γ ML 3.0 (Guindon *et al.*,
180 2010), and BI was employed to simultaneously infer phylogenetic relationships
181 and divergence times with the software BEAST 1.8.0 (Drummond *et al.*, 2012).
182 JMODELTEST 2.1.4 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012) was executed to
183 select the best-fitting DNA substitution models according to the Akaike
184 information criterion (AIC). For ML, the model GTR+I+G was selected for a single
185 concatenated alignment. For BI, the data were partitioned into six markers,
186 considering *COI* + *leu-tRNA* + *COII* a single evolutionary unit in the mitochondrial
187 genome. As a result, the HKY+I+G model was used for *H3*, the TN+I+G model for
188 *Wg* and a GTR+I+G model for *EF-1 α* , *28S* and the mitochondrial fragment. The
189 gamma distribution was estimated automatically from the data using six rate
190 categories. Because of the absence of reliable primary calibration points (fossils)
191 for the Lycaenidae studied, normally distributed Time to the Most Recent
192 Common Ancestor (TMRCA) priors based on published age estimates were used
193 as secondary calibrations on six well-supported nodes (posterior probability \geq
194 0.95) to estimate divergence times (Fig. 2). The 95% HPD distribution of these
195 TMRCA priors included the maximum and minimum ages inferred in Vila *et al.*
196 (2011) for the selected nodes. These inferences were based on the application of
197 a wide range of substitution rates described for mitochondrial regions in
198 invertebrates. For *COI*, a slow substitution rate of 6.5×10^{-9} and a fast rate of 9.5

199 $\times 10^{-9}$ (Quek *et al.*, 2004) were used, and for *COI + leu-tRNA + COII*, a substitution
200 rate of 11.5×10^{-9} substitutions site⁻¹ yr⁻¹ (Brower, 1994) was applied. The
201 resulting 95% HPD intervals were 3.26–6.06 Ma for node 1, 2.97–5.61 Ma for
202 node 2, 3.43–6.35 Ma for node 3, 6.76–12.2 Ma for node 4, 4.78–9.32 Ma for node
203 5, and 9.95–17.2 Ma for node 6. An uncorrelated relaxed clock (Drummond *et al.*,
204 2006) and a constant population size under a coalescent model were established
205 as priors. Two independent chains were run for 50 million generations each,
206 sampling values every 1000 steps. A conservative burn-in of 500,000
207 generations was applied for each run after checking Markov chain Monte Carlo
208 (MCMC) convergence through graphically monitoring likelihood values in TRACER
209 1.5 (available at: <http://beast.bio.ed.ac.uk/Tracer>). Independent runs were
210 combined in LOGCOMBINER 1.8.0, as implemented in BEAST, and all parameters
211 were analysed using TRACER 1.5 to determine whether they had also reached
212 stationarity. Tree topologies were assessed using TREEANNOTATOR 1.8.0 in the
213 BEAST package to generate a maximum-clade-credibility tree of all sampled trees
214 with median node heights. FIGTREE 1.4.0 (available at: <http://beast.bio.ed.ac.uk/FigTree>)
215 was used to visualize the consensus tree along with node ages,
216 age deviations and node posterior probabilities.

217

218 **Diversification rates and ancestral geographical range inference**

219 To investigate possible diversification-rate heterogeneity affecting the
220 Polyommata + Everina clade, and therefore the role of *Elkalyce cogina*, we used
221 Bayesian analysis of macroevolutionary mixtures (BAMM; Rabosky *et al.*, 2013,
222 2014a). The software was run on the BEAST maximum-credibility tree and non-
223 random missing data were accounted for by assigning the proportion of the

224 species sampled per genus. Four MCMC chains were run for 10 million
225 generations with a burn-in of 10%. Convergence of chains was checked in CODA
226 (Plummer *et al.*, 2006) and BAMM results were analysed and visualized with the
227 package BAMMTOOLS (Rabosky *et al.*, 2014b) in R 3.1.1 (R Core Team, 2014).

228 A lineages-through-time (LTT) plot was calculated for the Polyommatina +
229 Everina clade and contrasted with the diversification patterns of a dataset of
230 simulated trees. The R package TREESIM (Stadler, 2011) was used to simulate 100
231 trees by fixing the extant number of species per genus and the time since the
232 most recent common ancestor (function 'sim.bd.taxa.age'). The speciation and
233 extinction rates used in the simulations ($\lambda = 0.80$, $\mu = 0.40$) were calculated
234 through the extended version of the birth–death models (function 'bd.ext') in the
235 APE package (Paradis *et al.*, 2004), which allows phylogenetic and taxonomic data
236 for a given clade to be combined. Species richness for the genera was assigned
237 according to Talavera *et al.* (2013).

238 Ancestral geographical ranges were estimated in order to infer the most
239 likely biogeographical origin for the Everina subtribe and so know the directions
240 of the intercontinental dispersals that resulted in the current distributions. The
241 likelihoods of the two hypotheses were assessed with the R package
242 BIOGEOBEARS 0.2.1 (Matzke, 2013a,b) (Table S3 in Appendix S1). For all
243 analyses, 10 large-scale biogeographical regions and a dispersal multiplier
244 matrix were coded as in Vila *et al.* (2011). In order to avoid sampling effects,
245 terminals were considered placeholders for the genera they represented and the
246 distribution range of the genus was used (Table S4 in Appendix S1). A test was
247 performed for a dispersal–extinction–cladogenesis (DEC) model (Ree & Smith,
248 2008), a maximum-likelihood version of the dispersal–vicariance model

249 (DIVALIKE) (Ronquist, 1997) and a Bayesian biogeographical inference
250 (BAYAREALIKE) (Landis *et al.*, 2013). The three models were also tested when
251 allowing for founder-effect speciation (j) (Matzke, 2014). The resulting models
252 recovered the common ancestor of Everina and Polyommatina in several regions
253 simultaneously, including both the Old World and the New World, a scenario that
254 is biologically implausible. AIC scores and weights were used to detect the best-
255 performing model (BAYAREALIKE), which was subsequently used to
256 independently test each of the six favoured areas as a potential origin by fixing
257 the root.

258

259 **RESULTS**

260 **Distribution and life history**

261 Published geographical distribution data and field observations show that
262 *E. cogina* is associated with montane habitats, usually above 800 m (with the
263 exception of the southernmost records in Argentina) (Fig. 1). The species has
264 usually been observed associated with open habitats, especially partly flooded
265 montane swamps (as noted by Robbins & Duarte, 2006), where other
266 polyommattines such as *Leptotes cassius*, *Hemiargus hanno* and *Zizula cyna* are
267 also common (although these three species have much broader geographical and
268 elevational distributions). Adults are generally only found in particular localities
269 where populations exist, sometimes displaying notable densities. For example,
270 we observed about 20 individuals in a small swamp area of *ca.* 100 m² in Serra
271 do Japi (Jundiaí, SP), and more than 50 individuals in a 30-m sector of a trail
272 through a swamp area in Campos do Jordão (SP). Adults of *E. cogina* are present
273 all year round, although they are more common from January to April. They have

274 a low, fluttering flight, usually 10–50 cm above ground, usually flying inside the
275 vegetation or just above it. Adults were observed feeding from flowers of small
276 Asteraceae, Fabaceae and Lamiaceae that were growing in the marshy areas. In
277 Serra do Japi, one larva has been reared on inflorescences of *Desmodium*
278 *uncinatum* (Fabaceae), but no oviposition behaviour has ever been observed.
279 This represents the first host plant record for *E. cogina*. Because no ants were
280 recorded tending larvae at any of the studied sites, a low degree of
281 myrmecophily or a non-myrmecophilous condition is assumed.

282

283 **Phylogenetic systematics**

284 ML and BI recovered phylogenetic trees displaying generally concordant
285 topologies that are very similar to those previously published for Polyommagini
286 (Vila *et al.*, 2011; Talavera *et al.*, 2013) for the supported nodes (Fig. 2, Fig. S1 in
287 Appendix S1). *Elkalyce cogina* was recovered basally in the Everina subtribe with
288 strong support (posterior probability, 1.0; bootstrap support, 100), and is thus
289 most closely related to the Old World genera *Tongeia*, *Talica* and *Cupido*. The
290 inferred divergence times also generally matched previously published
291 probability distributions, and the estimated divergence between *E. cogina* and
292 the common ancestor of Everina was 10.17 Ma (95% highest posterior density
293 interval, HPD, 7.69–12.86 Ma).

294

295 **Diversification**

296 Net diversification rates, as estimated by the BAMM model, accelerated noticeably
297 during the Palaeartic Polyommatina radiation (Fig. 3a). The 95% HPD sampled
298 by BAMM comprised the ten most probable distinct shift configurations. The

299 single best shift configuration in BAMM inferred one increasing rate shift for the
300 most diverse Palaeartic Polyommata clade, with a probability of 0.52 (Fig. 3b).
301 The second-best configuration ($f = 0.14$) suggested an additional minor second
302 shift in the form of a diversification decrease for the *E. cogina* lineage (Fig. 3c).
303 The diversification-through-time curve showed that diversification rates peaked
304 between 7 and 4 Ma (Fig. 3d). This strong signal can be attributed to the shift
305 detected for Palaeartic Polyommata; the peak disappeared when
306 diversification rates in this clade were not considered (Fig. 3e). The LTT plot
307 showed an overall accumulation of lineages increasing fairly smoothly (Fig. 3f).
308 The curve displayed minor fluctuations in the cladogenesis through time that
309 might result from clade diversification heterogeneity, but generally fitted within
310 the margins of the simulated trees under a constant-rate model (Fig. 3f). No
311 strong shifts in diversification rate, either increases or decreases, were detected
312 in the lineages colonizing the New World, and only *Elkalyce* displayed a
313 marginally significant rate decrease. Indeed, *Elkalyce* was recovered as the oldest
314 lineage with a single extant taxon in the Everina + Polyommata tree (Fig. 3g).

315

316 **Ancestral geographical range**

317 The BAYAREALIKE model (LnL, -137.28; AIC, 278.4) was by far the best among
318 all models tested with BIOGEOBEARS, and the addition of founder-event
319 speciation (j) parameters improved the results (LnL, -134.71; AIC, 275.4) (Table
320 S3 in Appendix S1). Among the six potential ancestral regions for the root of the
321 tree, the Oriental region was strongly favoured (LnL, -142.96; AIC, 291.92),
322 followed by the East Palaeartic region (LnL, -144.30; AIC, 294.60) (Table S3 in
323 Appendix S1). Thus, a dispersal event from Asia to the New World is recovered

324 as the most likely hypothesis, although the exact route is uncertain.

325

326 **DISCUSSION**

327 **The systematic position of *Elkalyce cogina***

328 Our results contradict the hypothesis of Bálint & Johnson (1996) that *Elkalyce*
329 *cogina* belongs to the subtribe Lycaenopsina (*Lycaenopsis* section). Rather, the
330 present data and analyses support the close relationship to the Everina (*Everes*
331 section) proposed by Robbins & Duarte (2006) and confirm the ideas of H. Ebert,
332 as mentioned by Brown (1993). Talavera *et al.* (2013) suggest 15 Myr as a
333 minimum age for subtribes in Polyommataini. Accordingly, the monotypic genus
334 *Elkalyce* (10.17 Myr; HPD, 7.69–12.86 Myr) may be included within Everina.
335 (Note that Everina is the correct name for this subtribe, rather than Cupidina as
336 used by Talavera *et al.*, 2013.) Concerning the position within the subtribe, our
337 phylogeny shows *Elkalyce* to be the sister group to all the other Everina sampled
338 (Fig. 2), and thus this taxon is not ‘sister to, or congeneric with, *Tongeia*’ as
339 suggested by Eliot (1988, unpublished letters to Robbins) as described in
340 Robbins & Duarte (2006). Interestingly, Robbins & Duarte (2006) expressed
341 doubts regarding the homology of the male genital ‘false alulae’ because of slight
342 differences in their position, which led them to be cautious about synonymizing
343 *Elkalyce* with *Tongeia*. Even though monotypic genera are sometimes considered
344 of dubious classificatory value (Farris, 1976), the phylogenetic position and age
345 (cf. discussion of generic ages in Talavera *et al.*, 2013) of *Elkalyce* support the
346 acceptance of this genus.

347

348 Historical biogeography and diversification

349 Ancestral geographical range reconstruction indicates the most likely scenario to
350 consist of the common ancestor of Everina + Polyommata originating in the Old
351 World – probably in the Oriental region or in the Eastern Palaearctic – followed
352 by dispersal to the New World by the *Elkalyce* lineage (Table S3 in Appendix S1).
353 The centre of diversity for Everina is clearly Southeast Asia, as is the case for
354 several other lycaenid groups. Indeed, all the genera except *Elkalyce* occur there,
355 some of them (*Bothrinia*, *Shijimia* and *Talicada*) exclusively. The remarkable
356 disjunction between *Elkalyce* and the other Everina taxa does not, however,
357 represent the only example of a New World lycaenid whose closest relatives are
358 in the Old World. Other notorious biogeographical disjunctions exist, including
359 those of two species of *Brephidium*, apparently related to South African taxa in
360 the same genus and in *Oraidium*, and *Zizula cyna* – the only other species of
361 *Zizula* occurring in Africa, Southeast Asia and Australia (Robbins & Duarte, 2006;
362 de Jong & van Achterberg, 2007). Although debate about a Gondwanan vicariant
363 or dispersal origin has been generated for other higher-level butterfly taxa (e.g.
364 de Jong, 2003; Hall *et al.*, 2004; Braby *et al.*, 2005; Kodandaramaiah & Wahlberg,
365 2007; Peña *et al.*, 2010), most cases in Lycaenidae are arguably too young to
366 consider a Gondwanan vicariant-origin hypothesis.

367 Vila *et al.* (2011) proposed that at least five colonization events from the Old
368 World to the New World through Beringia occurred within the Polyommata,
369 the sister subtribe to Everina. The ancestor of *Elkalyce* might represent another
370 case that occurred approximately 10 Ma, at a similar time to the ancestor of the
371 current Neotropical Polyommata (*ca.* 10.7 Ma) and the ancestor of the *Icaricia*
372 + *Plebulina* clade (*ca.* 9.3 Ma). During this period, climatic conditions were

373 warmer than at present and a land-bridge existed in Beringia, which
374 undoubtedly facilitated this dispersal route for presumably warm-adapted
375 butterflies. The discovery that *E. cogina* feeds on Fabaceae, as was presumably
376 also the case for the ancestors of the Neotropical Polyommata and of the
377 *Icaricia* + *Plebulina* clade (Vila *et al.*, 2011), helps explain their parallel
378 biogeographical histories. In addition, this result strengthens the hypothesis that
379 Fabaceae was the ancestral host plant for both Polyommata and Everina
380 subtribes (see Fig. S7 in Vila *et al.*, 2011). Within the Everina, the genus *Cupido*
381 includes two apparent sister species in the Nearctic – *Cupido comyntas* and
382 *Cupido amyntula* – that are estimated to have colonized the New World less than
383 2 Ma, the estimated time of divergence between *C. comyntas* and the Palaearctic
384 *Cupido alcetas* (Fig. 2). Overall, at least seven Old World-to-New World
385 colonization events have occurred within the Polyommata + Everina clade (Fig.
386 4, Table 1).

387 It is generally understood that older lineages have had more time to
388 accumulate species (McPeck & Brown, 2007) and an exponential correlation
389 between time and number of species is expected if we assume a constant
390 diversification rate. The number of species versus time of colonization
391 approximates an exponential curve across the New World lineages, with the
392 exception of *Elkalyce* (Fig. 4). There is a particularly strong contrast between the
393 successful Neotropical Polyommata and the monotypic *Elkalyce* which, despite
394 having similar ages, have resulted in *ca.* 91 and 1 species in the Neotropics,
395 respectively. We cannot be certain that no undescribed species exist within
396 *Elkalyce*; because there is no apparent bias in the intensity of research carried
397 out in the different lineages of South American polyommata, however, it

398 seems unlikely that the documented contrast in richness is an artefact. This
399 pattern shows that *Elkalyce* has been much diversified much less than most
400 extant New World lineages. Nevertheless, our BMM results show that the
401 decrease in the diversification rate of the *Elkalyce* lineage is only marginally
402 significant. This could be due to the generally low species richness of *Everina* and
403 of the early divergent branches in Polyommata. Moreover, other examples of
404 old singletons exist in the Polyommata, including the Neotropical
405 *Pseudochrysops bornoi* [9.89 (7.97–11.88) Myr old]. *Pseudochrysops* is only found
406 on Caribbean islands, and islands are well known for harbouring relict lineages.
407 *Elkalyce cogina* is one of the few polyommata that occur in eastern South
408 America. Regardless of any factor that explains the low diversity in *Elkalyce*, the
409 Neotropical Polyommata, which are most diverse in western South America,
410 may exhibit high species richness due to habitat heterogeneity and Andean
411 orogenesis, as found for other Neotropical butterflies (see Wahlberg & Freitas,
412 2007; Elias *et al.*, 2009).

413 A significant increase of the diversification rates in Polyommata was
414 detected by BMM for the hyperdiverse Palearctic genera. This clade is generally
415 associated with mountainous habitats and arid conditions, similar to the
416 Polyommata in South America, which also display high relative species
417 numbers. Polyommata and *Everina* are both typical of open vegetation, with
418 very few species occurring in forested habitats. This could in part explain why
419 lineages such as *Elkalyce* or *Pseudochrysops* have not diversified: both are
420 endemics of areas with a low diversity of open habitats, which could prevent
421 diversification by vicariance in these two lineages. Therefore, even if the relict
422 distribution of *Elkalyce* suggests extinction in the intervening areas after

423 colonization and dispersal through the New World, the limited diversification
424 observed for this lineage might also be a product of a low speciation rate.
425 Although *Elkalyce* maintained the apparently ancestral host-plant family
426 (Fabaceae), New World Polyommatina genera including *Pseudolucia*, *Icaricia*,
427 *Plebejus* and *Agriades* displayed host-plant shifts to new families of plants, which
428 is a well-known driver of butterfly diversification (Nylin & Wahlberg, 2008;
429 Fordyce, 2010; Nylin *et al.*, 2014).

430 Larval association with ants (myrmecophily) is another factor that might
431 increase diversification in Lycaenidae (Pellissier *et al.*, 2012). This may be
432 important when comparing the diverse and generally myrmecophilous
433 Polyommatina with the Everina (*Cupido* section in Fiedler, 1991; Benyamini,
434 1995), but it is not particularly relevant in explaining the uniqueness of *Elkalyce*,
435 larvae apparently display a low degree of myrmecophily, similar to that observed
436 in the other Everina taxa.

437

438 **CONCLUSIONS**

439 We provide the first molecular assessment of the taxon *Elkalyce cogina*, a species
440 of Polyommatini, endemic to Brazil and neighbouring Argentina, of unclear
441 systematic placement and biogeographical history. Our results show that
442 *E. cogina* belongs to the Everina subtribe, which agrees with one of the published
443 hypotheses based on morphology. The supported position of *E. cogina* as sister
444 to the other Everina taxa studied and the estimated divergence time of about
445 10 Ma are, however, unexpected. We observed the larvae of *E. cogina* feeding on
446 *Desmodium uncinatum* (Fabaceae), which suggests that the common ancestor of
447 both the sister subtribes Everina and Polyommatina fed on Fabaceae. A

448 biogeographical history parallel to that of the Neotropical Polyommata (Asia–
449 Beringia–North America–South America) is proposed. The lack of diversification
450 in *Elkalyce* is unique among lineages of New World colonists.

451

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468

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613 *ITS2* sequences (Lepidoptera: Lycaenidae). *European Journal of Entomology*,
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615

616 SUPPORTING INFORMATION

617 Additional Supporting Information may be found in the online version of this
618 article:

619 **Appendix S1** Supporting tables (Tables S1–S4) and figure (Fig. S1).

620

621 BIOSKETCH

622 Members of the research team are actively engaged in the study of the

623 biodiversity, biogeography and evolution of Lepidoptera, with specific interest in
624 unravelling the historical and present-day factors responsible for current species
625 distributions.

626

627 Author contributions: G.T. and R.V. conceived the study; R.V., L.A.K. and A.V.L.F.
628 collected the data; R.V. obtained molecular data and G.T. performed phylogenetic
629 and diversification analyses. G.T. led the writing. All authors contributed in the
630 form of discussions and suggestions, and approved the final manuscript.

631

632 Editor: Michelle Gaither

633 **TABLES**

634 **Table 1** Inferred timing of colonization (mean \pm SD) and approximate number of
 635 species and genera for New World lineages of Polyommatinae. Values for non-
 636 inferred nodes were extracted from Vila *et al.* (2011). See also Fig. 4.

Ancestor	Date of colonization (Ma)	Approximate number of species	Number of genera
Neotropical Polyommatina	11.74 (9.74–13.86)	91	9
<i>Elkalyce</i>	10.17 (7.69–12.86)	1	1
<i>Icaricia-Plebulina</i>	8.49 (6.94–10.04)	8	2
<i>Plebejus</i>	2.40 (1.50–4.70)	4	1
<i>Cupido</i>	1.65 (1.02–2.45)	2	1
<i>Agriades glandon</i> group	1.10 (0.15–2.40)	3	1
<i>Agriades optilete</i>	1.00 (0.49–2.00)	1	1

637

638 **FIGURE LEGENDS**

639

640 **Figure 1** Recorded distribution of *Elkalyce cogina* (black dots) in South America.

641 Locality data include records compiled by Robbins & Duarte (2006), new

642 collections and museum data.

643

644 **Figure 2** Bayesian chronogram for Polyommatini based on seven genes: *COI*, *leu-*645 *tRNA*, *COII*, *EF-1 α* , *Wg*, *28S* and *H3* (4850 bp). Thick lines indicate supported646 relationships (posterior probabilities ≥ 0.95); node bars show uncertainty in

647 estimated divergence times. The six well-supported nodes (posterior probability

648 ≥ 0.95) used for clock calibration are marked with clock symbols. Age priors for649 these nodes were based on Vila *et al.* (2011). Branches in blue indicate

650 Polyommatina and Everina lineages that have colonized the New World. The two

651 Polyommatini subtribes involved in taxonomic hypotheses for *Elkalyce cogina*

652 (Bálint & Johnson, 1996; Robbins & Duarte, 2006) and Polyommatina are

653 indicated with coloured boxes. Upperside and underside photographs of the

654 monotypic species *Elkalyce cogina* are shown (female specimen Ex. Oberthür655 Coll. British Museum 1927-3, with label indicating: *Brésil, Nova Friburgo, P.*656 *Germain, Février 1884*. Photos: Zsolt Bálint).

657

658 **Figure 3** Diversification for the Polyommatina + Everina clade. (a) BAMM phylorate

659 plot showing average net diversification rates along each branch of the

660 Polyommatina + Everina clade. Warmer colours denote faster diversification

661 rates (in lineages per Myr). Number of species per genera is indicated in

662 parentheses. (b,c) The two shift configurations with the highest posterior

663 probability. Circles denote locations of rate shifts (red, rate increase; blue, rate
664 decrease) and are proportional to the overall marginal probability of a shift on
665 the branch. Trajectories of diversification rate through time (RTT) for (d) the
666 whole Polyommata + Everina clade and (e) after excluding the hyperdiverse
667 Palaearctic Polyommata subclade. Colour density shading illustrates the
668 relative probability of a rate at any point in time. (f) Lineages-through-time
669 (LTT) plot for the Polyommata + Everina clade (red) and LTT plots for 100
670 simulated trees (black). (g) Species richness of each terminal lineage
671 (representing genera) against their estimated ages (Myr). The red dot
672 corresponds to *Elkalyce cogina*.

673

674 **Figure 4** Representative specimen photographs (dorsal view) and maps showing
675 the current distribution of the seven lineages (a–g) of Polyommatae that
676 colonized the New World. (h) Correlation between number of species and
677 estimated time since colonization of New World (Ma). The monotypic genus
678 *Elkalyce* (in red) can be considered an outlier breaking up any correlation.
679

SUPPORTING INFORMATION

One note samba:

the biogeographical history of the relict Brazilian butterfly *Elkalyce cogina*

Gerard Talavera, Lucas A. Kaminski, André V. L. Freitas and Roger Vila

Table S1 Samples used in this study: taxon name, specimen label, sample accession number at the Museum of Comparative Zoology (Cambridge, MA, USA), or at the Institute of Evolutionary Biology (Barcelona, Spain) in the case of *Maurus vogelii*, and sample collection locality.

Subtribe/ Section	Taxon	Sample code	Locality
Polyommata	<i>Afarsia morgiana</i> (Kirby, 1871)	VL-02-X393	Iran, Kerman, Kuh-e-Lalazar Mts
	<i>Agriades podarce</i> (Felder & Felder, 1865)	AS-92-Z130	USA, California, Leek Spring
	<i>Alpherakya sarta</i> (Alphéraky, 1881)	VL-02-X098	China, Xinjiang, Kuqa
	<i>Aricia agestis</i> (Denis & Schiffermüller, 1775)	NK-00-P712	Kazakhstan, Kayandy
	<i>Chilades lajus</i> (Stoll, 1780)	DL-99-T242	Thailand, Prachuap Khiri Khan Province, Ampuh Thap Sakae
	<i>Cyaniris semiargus</i> (Rottemburg, 1775)	AD-00-P206	Russia, Low Volga, Volgograd region, Kamytsinsky
	<i>Cyclargus ammon</i> (Lucas, 1857)	JE-01-C283	USA, Florida, Big Pine Key
	<i>Echinargus isola</i> (Edwards, 1871)	AS-92-Z185	USA, California, Alpine, Carson River
	<i>Eldoradina cyanea</i> (Balleto, 1993)	RV-05-M735	Peru, Lima, Oyón
	<i>Eumedonia eumedon</i> (Esper, 1780)	AD-03-B062	Russia, Altai, Aktash
	<i>Freyeria trochylus</i> (Freyer, 1844)	VL-01-L462	Turkey, Artvin, Kiliçkaya
	<i>Glabroculus cyane</i> (Eversmann, 1837)	VL-02-X159	Kazakhstan, Karaganda region, Aktchatau
	<i>Hemiargus hanno</i> (Stoll, 1790)	MH-01-I001	Puerto Rico, Culebra Island, Flamenco Beach
	<i>Icaricia icarioides</i> (Boisduval, 1852)	AS-92-Z065	USA, California, Nevada, Donner Pass
	<i>Itylos titicaca</i> (Weymer, 1890)	MFB-00-N206	Chile, P.N. Lanca, Las Cuevas
	<i>Kretania eurypilus</i> (Freyer, 1851)	VL-01-L152	Turkey, Gümüşhane Prov., 35 km SW Gümüşhane, Dilekyolu
	<i>Luthrodes pandava</i> (Horsfield, 1829)	MWT-93-A009	Malaysia, Kepong
	<i>Lysandra bellargus</i> (Rottemburg, 1775)	AD-00-P129	Armenia, Transcaucasus, Amberd Valley, Aragatz Mt.
	<i>Maurus vogelii</i> (Oberthür, 1920)	RVcoll.09-X164	Morocco, Khenifra, S. Timahdite, Col du Zad
	<i>Nabokovia cuzquenha</i> Bálint & Lamas, 1997	RV-03-V234	Peru, Cuzco, Pisac
<i>Neolysandra coelestina</i> (Eversmann, 1843)	AD-00-P092	Armenia, Gegadyr, Gegamsky Mountains	

Subtribe/ Section	Taxon	Sample code	Locality
	<i>Pamiria chrysopsis</i> (Grum-Grshimaïlo, 1888)	VL-05-Z998	Tajikistan, East Pamir, Sarykolski Range, Dunkeldyk Lake
Polyommata	<i>Paralycaeus inconspicua</i> (Draudt, 1921)	RV-03-V188	Peru, Arequipa, Cañón del Colca
	<i>Patricius lucifer</i> (Staudinger, 1866)	VL-05-Z995	Russia, Altai, Chikhacheva Range, Sailugem Mountain
	<i>Plebejidea loewii</i> (Zeller, 1847)	AD-00-P266	Armenia, Gnishyk, Aiodzor Mts.
	<i>Plebejus idas</i> (Linnaeus, 1761)	NK-00-P165	Russia, St Petersburg, Luga
	<i>Plebulina emigdionis</i> (Grinnell, 1905)	CCN-05-I856	USA, California, Kern, W. Onyx
	<i>Polyommatus amandus</i> (Schneider, 1792)	AD-00-P053	Russia, Volgograd region, Kamysinsky
	<i>Pseudochrysops bornoi</i> (Comstock & Huntington, 1943)	MAC-04-Z114	Dominican Republic, Punta Cana
	<i>Pseudolucia chilensis</i> (Blanchard, 1852)	MFB-00-N227	Chile, Farellones
	<i>Rimisia miris</i> (Staudinger, 1881)	NK-00-P575	Kazakhstan, Altai, Oktyabrsk
	<i>Rueckbeilia fergana</i> (Staudinger, 1881)	NK-00-P777	Kazakhstan, Shymkent Reg., Karatau Mountains, Turpan Pass
Everina	<i>Cupido alcetas</i> Hoffmannsegg, 1804	MAT-99-Q954	Spain, Catalonia, Montseny
	<i>Cupido comyntas</i> (Godart, 1824)	AS-92-Z312	USA, California, Davis
	<i>Cupido minimus</i> (Fuessly, 1775)	AD-00-P540	Russia, Tula, Tatinki
	<i>Elkalyce cogina</i> (Schaus, 1902)	TQ-05-M179	Petrópolis, Rio de Janeiro, Brazil
	<i>Talicauda nyseus</i> (Guerin, 1843)	JXM-99-T709	India, Karala, Trivandrum
	<i>Tongeia bisudu</i> Zhdanko & Yakovlev, 2001	VL-02-X094	China, Xinjiang, Kuqa
	<i>Tongeia fischeri</i> (Eversmann, 1843)	NK-00-P594	Kazakhstan, Altai, Oktyabrsk
Cacyreus	<i>Cacyreus marshalli</i> (Butler, 1897)	AH-95-Y685	Republic of South Africa, Capetown, Pinelands
Lampides	<i>Lampides boeticus</i> (Linnaeus, 1767)	MWT-93-E012	Malaysia, Poring Hot Spring
Actizera	<i>Actizera lucida</i> (Trimen, 1883)	AP-98-W773	Republic of South Africa, Kwazulu Natal, Hillcrest
Azanus	<i>Azanus mirza</i> (Plötz, 1880)	TL-96-W903	Ghana, Kibi, Atewa
Brephidium	<i>Oraidium barberae</i> (Trimen, 1868)	AAM-98-V076	Republic of South Africa, Springbok
Castalius	<i>Castalius rosimon</i> (Fabricius, 1775)	MWT-93-B024	Malaysia, Selangor
Catochrysops	<i>Catochrysops panormus</i> (C. Felder, 1860)	KD-93-C044	Australia, Queensland, Pinalba
Danis	<i>Psychonotis caelius</i> (C. & R. Felder, 1860)	KD-93-C021	Australia, Queensland, Nathan
Eicochrysops	<i>Eicochrysops hippocrates</i> (Fabricius, 1793)	TL-97-W513	Cameroon, Korup N.P.
Euchrysops	<i>Euchrysops cnejus</i> (Fabricius 1798)	KD-93-C045	Australia, Queensland, Pinalba
	<i>Lepidochrysops dukei</i> Cottrell, 1965	AH-98-Y715	Republic of South Africa, Eastern Swartberg, Blesberg Mountain
Famegana	<i>Famegana alsulus</i> (Herrich-Schäffer, 1869)	KD-95-Z506	Australia, Queensland, Wulguru
Glaucopygma	<i>Euphilotes enoptes</i> (Mattoni, 1955)	AS-92-Z024	USA, California, Nevada, Donner Pass

Subtribe/ Section	Taxon	Sample code	Locality
	<i>Glaucopsyche lygdamus</i> (Doubleday, 1841)	AS-02-Z131	USA, California, Leek Spring
	<i>Maculinea arion</i> (Linnaeus, 1758)	RV-03-N585	Spain, Barcelona, Gombren
<i>Jamides</i>	<i>Jamides alecto</i> (Felder, 1860)	MWT-93-A070	Malaysia, Kepong
<i>Leptotina</i>	<i>Leptotes marina</i> (Reakirt, 1868)	AS-92-Z272	USA, California, Santa Barbara
	<i>Leptotes trigemmatius</i> (Butler, 1881)	RV-03-V095	Chile, Coquimbo, Alcohuas
<i>Lycaenopsis</i>	<i>Acytolepis puspa</i> (Horsfield, 1828)	MWT-93-D010	Malaysia, Sabah, Kokol
	<i>Celastrina echo</i> (Edwards, 1864)	AS-92-Z186	USA, California, Alpine, Carson River
	<i>Lycaenopsis haraldus</i> (Fabricius, 1787)	MWT-93-B038	Malaysia, Pelindung
	<i>Megisba malaya</i> (Horsfield, 1828)	MWT-93-D008	Malaysia, Sabah, Kokol
<i>Nacaduba</i>	<i>Nacaduba angusta</i> (Druce, 1873)	MWT-93-B058	Malaysia, Kepong
<i>Petrelaea</i>	<i>Pseudonacaduba aethiops</i> (Mabille, 1877)	TL-97-W507	Cameroon, Korup N.P.
<i>Phylaria</i>	<i>Phylaria cyara</i> (Hewitson 1876)	TL-96-W917	Ghana, Kibi, Atewa
<i>Theclinesthes</i>	<i>Theclinesthes miskini</i> (Lucas, 1889)	KD-93-Q030	Australia, Queensland, Mount Gammie
<i>Una</i>	<i>Una usta</i> (Distant, 1886)	DL-02-P705	Thailand, Chiang Mai, Doi Suthep-Pui N.P.
<i>Upolampes</i>	<i>Caleta elna</i> (Hewitson 1876)	MWT-93-D027	Malaysia, Sabah, Kokol
<i>Uranothauma</i>	<i>Uranothauma falkensteini</i> (Dewitz, 1879)	TL-96-W908	Ghana, Kibi, Atewa
<i>Zintha</i>	<i>Zintha hintza</i> (Trimen, 1864)	AH-00-T289	Republic of South Africa, Gautony, Helepoort
<i>Zizeeria</i>	<i>Zizeeria karsandra</i> (Moore, 1865)	KD-94-Q064	Australia, Queensland, Townsville, Hermit Park
<i>Zizula</i>	<i>Zizula hylax</i> (Fabricius, 1775)	KD-94-R033	Australia, Queensland, Inglewood

Table S2 GenBank codes for the Polyommata specimens used in the current study. Codes in bold indicate new sequences obtained.

Sample code	Taxon	COI + COII	EF-1 α	Wg	28S	H3
VL-02-X393	<i>Afarsia morgiana</i>	JX093487	JX093302	—	JX093228	JX093344
AS-92-Z130	<i>Agriades podarce</i>	GQ128943	GQ128632	GQ128839	GQ128447	GQ128731
VL-02-X098	<i>Alpherakya sarta</i>	JX093451	JX093311	JX093446	JX093224	JX093353
NK-00-P712	<i>Aricia agestis</i>	AY496801	AY496824	GQ128843	GQ128451	GQ128735
DL-99-T242	<i>Chilades lajus</i>	GQ128946	GQ128635	GQ128844	GQ128452	GQ128736
AD-00-P206	<i>Cyaniris semiargus</i>	JX093491	JX093301	JX093412	JX093235	JX093338
JE-01-C283	<i>Cyclargus ammon</i>	GQ128948	GQ128637	GQ128846	GQ128454	GQ128738
AS-92-Z185	<i>Echinargus isola</i>	DQ018914	DQ018914	DQ018885	GQ128456	GQ128740
RV-05-M735	<i>Eldoradina cyanea</i>	GQ128952	GQ128641	GQ128850	GQ128459	GQ128743
AD-03-B062	<i>Eumedonia eumedon</i>	GQ128953	GQ128642	GQ128851	GQ128460	GQ128744
VL-01-L462	<i>Freyeria trochylus</i>	GQ128955	GQ128644	GQ128853	GQ128462	GQ128746
VL-02-X159	<i>Glabroculus cyane</i>	JX093489	JX093283	JX093434	JX093221	JX093356
MH-01-I001	<i>Hemiargus hanno</i>	GQ128960	GQ128649	GQ128858	GQ128467	GQ128751
AS-92-Z065	<i>Icaricia icarioides</i>	GQ128963	GQ128652	GQ128861	GQ128470	GQ128754
MFB-00-N206	<i>Itylos titicaca</i>	GQ128970	GQ128659	GQ128867	GQ128477	GQ128760
VL-01-L152	<i>Kretania eurypilus</i>	JX093457	JX093298	JX093433	JX093218	JX093345
MWT-93-A009	<i>Luthrodes pandava</i>	GQ128951	GQ128640	GQ128849	GQ128458	GQ128742
AD-00-P129	<i>Lysandra bellargus</i>	JX093472	JX093299	JX093410	JX093225	JX093340
RVcoll.09-X164	<i>Maurus vogelii</i>	JX093485	JX093316	—	JX093230	JX093361
RV-03-V234	<i>Nabokovia cuzquenha</i>	GQ128985	GQ128674	GQ128883	GQ128493	GQ128776
AD-00-P092	<i>Neolysandra coelestina</i>	JX093490	JX093303	JX093417	JX093223	JX093337
VL-05-Z998	<i>Pamiria chrysopsis</i>	JX093469	JX093312	JX093447	—	JX093348
RV-03-V188	<i>Paralycaeus inconspicua</i>	GQ128987	GQ128676	GQ128885	GQ128495	GQ128778
VL-05-Z995	<i>Patricius lucifer</i>	JX093475	—	JX093443	—	JX093357
AD-00-P266	<i>Plebejidea loewii</i>	GQ128989	GQ128678	GQ128887	GQ128497	GQ128780
NK-00-P165	<i>Plebejus idas</i>	GQ128974	GQ128663	GQ128872	GQ128482	GQ128765
CCN-05-I856	<i>Plebulina emigdionis</i>	GQ128991	GQ128680	GQ128890	GQ128500	GQ128783
AD-00-P053	<i>Polyommatus amandus</i>	JX093452	JX093315	JX093418	JX093204	JX093321
MAC-04-Z114	<i>Pseudochrysops bornoi</i>	GQ128994	GQ128683	GQ128894	GQ128504	GQ128787
MFB-00-N227	<i>Pseudolucia chilensis</i>	GQ128999	GQ128688	GQ128899	GQ128509	GQ128792
NK-00-P575	<i>Rimisia miris</i>	JX093493	AY496848	JX093445	JX093213	JX093352
NK-00-P777	<i>Rueckbeilia fergana</i>	JX093477	AY496850	JX093448	JX093231	JX093358
MAT-99-Q954	<i>Cupido alcetas</i>	KP824778	KP824781	KP824791	KP824772	KP824787
AS-92-Z312	<i>Cupido comyntas</i>	GQ128954	GQ128643	GQ128852	GQ128461	GQ128745
AD-00-P540	<i>Cupido minimus</i>	GQ128947	GQ128636	GQ128845	GQ128453	GQ128737
TQ-05-M179	<i>Elkalyce cogina</i>	KP824777	KP824782	KP824794	KP824774	KP824785
JXM-99-T709	<i>Talicauda nyseus</i>	GQ129009	GQ128697	GQ128908	GQ128519	GQ128801

Sample code	Taxon	COI + COII	EF-1 α	Wg	28S	H3
VL-02-X094	<i>Tongeia bisudu</i>	KP824775	KP824780	KP824790	KP824773	KP824786
NK-00-P594	<i>Tongeia fischeri</i>	GQ129010	GQ128698	GQ128909	GQ128520	GQ128802
AH-95-Y685	<i>Cacyreus marshalli</i>	EU919304	GQ128703	GQ128914	GQ128526	GQ128808
MWT-93-E012	<i>Lampides boeticus</i>	GQ129023	GQ128712	GQ128924	GQ128537	GQ128819
AP-98-W773	<i>Actizera lucida</i>	GQ129013	GQ128701	GQ128911	GQ128523	GQ128805
TL-96-W903	<i>Azanus mirza</i>	GQ129014	GQ128702	GQ128913	GQ128525	GQ128807
AAM-98-V076	<i>Oraidium barberae</i>	GQ129029	GQ128718	GQ128930	GQ128544	GQ128826
MWT-93-B024	<i>Castalius rosimon</i>	GQ129016	GQ128705	GQ128916	GQ128528	GQ128810
KD-93-C044	<i>Catochrysops</i> <i>panormus</i>	GQ129017	GQ128706	GQ128917	GQ128529	GQ128811
KD-93-C021	<i>Psychonotis caelius</i>	GQ129033	GQ128723	GQ128935	GQ128549	GQ128831
TL-97-W513	<i>Eicochrysops</i> <i>hippocrates</i>	GQ129019	GQ128708	GQ128919	GQ128531	GQ128813
KD-93-C045	<i>Euchrysops cnejus</i>	GQ129020	GQ128709	GQ128920	GQ128532	GQ128814
AH-98-Y715	<i>Lepidochrysops</i> <i>dukei</i>	GQ129024	GQ128713	GQ128925	GQ128538	GQ128820
KD-95-Z506	<i>Famegana alsulus</i>	GQ129021	GQ128710	GQ128922	GQ128534	GQ128816
AS-92-Z024	<i>Euphilotes enoptes</i>	AY675410	AY675363	GQ128921	GQ128533	GQ128815
AS-02-Z131	<i>Glaucoopsyche</i> <i>lygdamus</i>	AY675411	AY675364	GQ128923	GQ128535	GQ128817
RV-03-N585	<i>Maculinea arion</i>	AY675422	AY675375	GQ128928	GQ128541	GQ128823
MWT-93-A070	<i>Jamides alecto</i>	GQ129022	GQ128711	AF233556	GQ128536	GQ128818
AS-92-Z272	<i>Leptotes marina</i>	GQ129025	GQ128714	GQ128926	GQ128539	GQ128821
RV-03-V095	<i>Leptotes</i> <i>trigemmatius</i>	JX093474	GQ128660	GQ128868	GQ128478	GQ128761
MWT-93-D010	<i>Acytolepis puspa</i>	KP824776	KP824783	KP824792	KP824770	KP824788
AS-92-Z186	<i>Celastrina echo</i>	GQ129018	GQ128707	GQ128918	GQ128530	GQ128812
MWT-93-B038	<i>Lycaenopsis</i> <i>haraldus</i>	GQ129026	GQ128715	GQ128927	GQ128540	GQ128822
MWT-93-D008	<i>Megisba malaya</i>	KP824779	KP824784	KP824793	KP824771	KP824789
MWT-93-B058	<i>Nacaduba angusta</i>	GQ129027	GQ128716	GQ128929	GQ128542	GQ128824
TL-97-W507	<i>Pseudonacaduba</i> <i>aethiops</i>	GQ129032	GQ128722	GQ128934	GQ128548	GQ128830
TL-96-W917	<i>Phylaria cyara</i>	EU919287	GQ128719	GQ128931	GQ128545	GQ128827
KD-93-Q030	<i>Theclinesthes</i> <i>miskini</i>	DQ456544	DQ456617	GQ128936	GQ128550	GQ128832
DL-02-P705	<i>Una usta</i>	GQ129034	GQ128724	GQ128937	GQ128551	GQ128833
MWT-93-D027	<i>Caleta elna</i>	GQ129015	GQ128704	GQ128915	GQ128527	GQ128809
TL-96-W908	<i>Uranothauma</i> <i>falkensteini</i>	EU919282	GQ128725	GQ128938	GQ128552	GQ128834
AH-00-T289	<i>Zintha hintza</i>	GQ129035	GQ128726	GQ128939	GQ128553	GQ128835
KD-94-Q064	<i>Zizeeria karsandra</i>	GQ129036	GQ128727	GQ128940	GQ128554	GQ128836
KD-94-R033	<i>Zizula hylax</i>	GQ129037	GQ128728	GQ128941	GQ128555	GQ128837

Table S3 Model testing output parameters for the BIOGEOBEARS analysis of the Polyommata + Everina clade. The two best models with unconstrained region at the root are highlighted in bold, but they result in biologically unrealistic scenarios. The best model with constrained region at root is shaded. OR, Oriental; EPa, East Palaearctic; WNa, West Nearctic; SSA, southern South America; ENa, East Nearctic; WPa, West Palaearctic.

Model	Constrained region at root	LnL	d.f.	<i>d</i>	<i>e</i>	<i>j</i>	AIC	ΔAIC
BAYAREALIKE+J	Unconstrained	-134.71	3	0.0214	0.0601	0.0205	275.40	0.00
BAYAREALIKE	Unconstrained	-137.28	2	0.0248	0.0897	0.0000	278.60	3.20
BAYAREALIKE+J	OR	-142.96	3	0.0272	0.0566	0.0170	291.92	16.52
BAYAREALIKE+J	EPa	-144.30	3	0.0273	0.0577	0.0187	294.60	19.20
BAYAREALIKE	EPa	-148.63	2	0.0312	0.0835	0.0000	301.26	25.86
BAYAREALIKE+J	WPa	-148.60	3	0.0293	0.0601	0.0253	303.20	27.80
BAYAREALIKE	SSA	-149.65	2	0.0318	0.0915	0.0000	303.30	27.90
BAYAREALIKE+J	SSA	-149.01	3	0.0294	0.0645	0.0243	304.02	28.62
BAYAREALIKE+J	WNa	-150.12	3	0.0302	0.0640	0.0271	306.24	30.84
BAYAREALIKE+J	ENa	-150.35	3	0.0290	0.0606	0.0307	306.70	31.30
BAYAREALIKE	WNa	-151.52	2	0.0335	0.0947	0.0000	307.04	31.64
BAYAREALIKE	WPa	-152.09	2	0.0335	0.0936	0.0000	308.18	32.78
BAYAREALIKE	ENa	-153.27	2	0.0339	0.1002	0.0000	310.54	35.14
DEC	Unconstrained	-159.49	2	0.0419	0.0159	0.0000	323.00	47.60
DEC+J	Unconstrained	-159.49	3	0.0419	0.0159	< 0.0001	325.00	49.60
DIVALIKE	Unconstrained	-162.64	2	0.0483	0.0135	0.0000	329.30	53.90
DIVALIKE+J	Unconstrained	-162.48	3	0.0474	0.0183	< 0.0001	331.00	55.60
BAYAREALIKE	OR	-176.49	2	0.0316	0.0819	0.0000	356.98	81.58

Table S4 Taxon name and biogeographic regions where it occurs for terminals used in the Ancestral area reconstruction with BIOGEOBEARS for the Polyommata + Everina clade. In order to avoid sampling effects, terminals were considered placeholders for the genera they represented and the distribution range of the genus was used. Af, Africa; Au, Australia; CA, Central America–Caribbean; ENa, East Nearctic; EPa, East Palaearctic; NSA, northern South America; Or, Oriental; SSA, southern South America; WNa, West Nearctic; WPa, West Palaearctic.

Terminal	Af	Au	CA	ENa	EPa	NSA	Or	SSA	WNa	WPa
<i>Elkalyce cogina</i>	0	0	0	0	0	0	0	1	0	0
<i>Cupido minimus</i>	0	0	0	0	1	0	0	0	0	1
<i>Cupido alcetas</i>	0	0	1	1	1	0	0	0	1	1
<i>Talicauda nyseus</i>	0	0	0	0	0	0	1	0	0	0
<i>Tongeia bisudu</i>	0	0	0	0	1	0	1	0	0	0
<i>Freyeria trochylus</i>	1	1	0	0	0	0	1	0	0	1
<i>Plebejus idas</i>	0	0	0	1	1	0	0	0	1	1
<i>Patricius lucifer</i>	0	0	0	0	1	0	0	0	0	0
<i>Pamiria chrysopsis</i>	0	0	0	0	1	0	0	0	0	0
<i>Neolysandra coelestina</i>	0	0	0	0	1	0	0	0	0	1
<i>Polyommatus amandus</i>	0	0	0	0	1	0	0	0	0	1
<i>Lysandra bellargus</i>	0	0	0	0	1	0	0	0	0	1
<i>Cyaniris semiargus</i>	0	0	0	0	1	0	0	0	0	1
<i>Rimisia miris</i>	0	0	0	0	1	0	0	0	0	0
<i>Agriades podarce</i>	0	0	0	1	1	0	0	0	1	1
<i>Eumedonia eumedon</i>	0	0	0	0	1	0	0	0	0	1
<i>Plebejidea loewii</i>	0	0	0	0	1	0	0	0	0	1
<i>Maurus vogelii</i>	0	0	0	0	0	0	0	0	0	1
<i>Aricia agestis</i>	0	0	0	0	1	0	0	0	0	1
<i>Glabroculus cyane</i>	0	0	0	0	1	0	0	0	0	0
<i>Alpherakya sarta</i>	0	0	0	0	1	0	0	0	0	0
<i>Kretania eurypilus</i>	0	0	0	0	1	0	0	0	0	1
<i>Afarsia morgiana</i>	0	0	0	0	1	0	0	0	0	0
<i>Rueckbeilia fergana</i>	0	0	0	0	1	0	0	0	0	0
<i>Icaricia icarioides</i>	0	0	0	0	0	0	0	0	1	0
<i>Plebulina emigdionis</i>	0	0	0	0	0	0	0	0	1	0
<i>Paralycaeus inconspicua</i>	0	0	0	0	0	0	0	1	0	0
<i>Itylos titicaca</i>	0	0	0	0	0	1	0	1	0	0
<i>Pseudochrysops bornoi</i>	0	0	1	0	0	0	0	0	0	0
<i>Pseudolucia chilensis</i>	0	0	0	0	0	0	0	1	0	0
<i>Nabokovia cuzquenha</i>	0	0	0	0	0	1	0	1	0	0
<i>Eldoradina cyanea</i>	0	0	0	0	0	0	0	1	0	0
<i>Hemiargus hanno</i>	0	0	1	1	0	1	0	1	1	0
<i>Cyclargus ammon</i>	0	0	1	1	0	0	0	0	0	0
<i>Echinargus isola</i>	0	0	1	0	0	0	0	0	1	0
<i>Edales pandava</i>	0	0	0	0	1	0	1	0	0	1
<i>Chilades lajus</i>	1	0	0	0	0	0	1	0	0	0

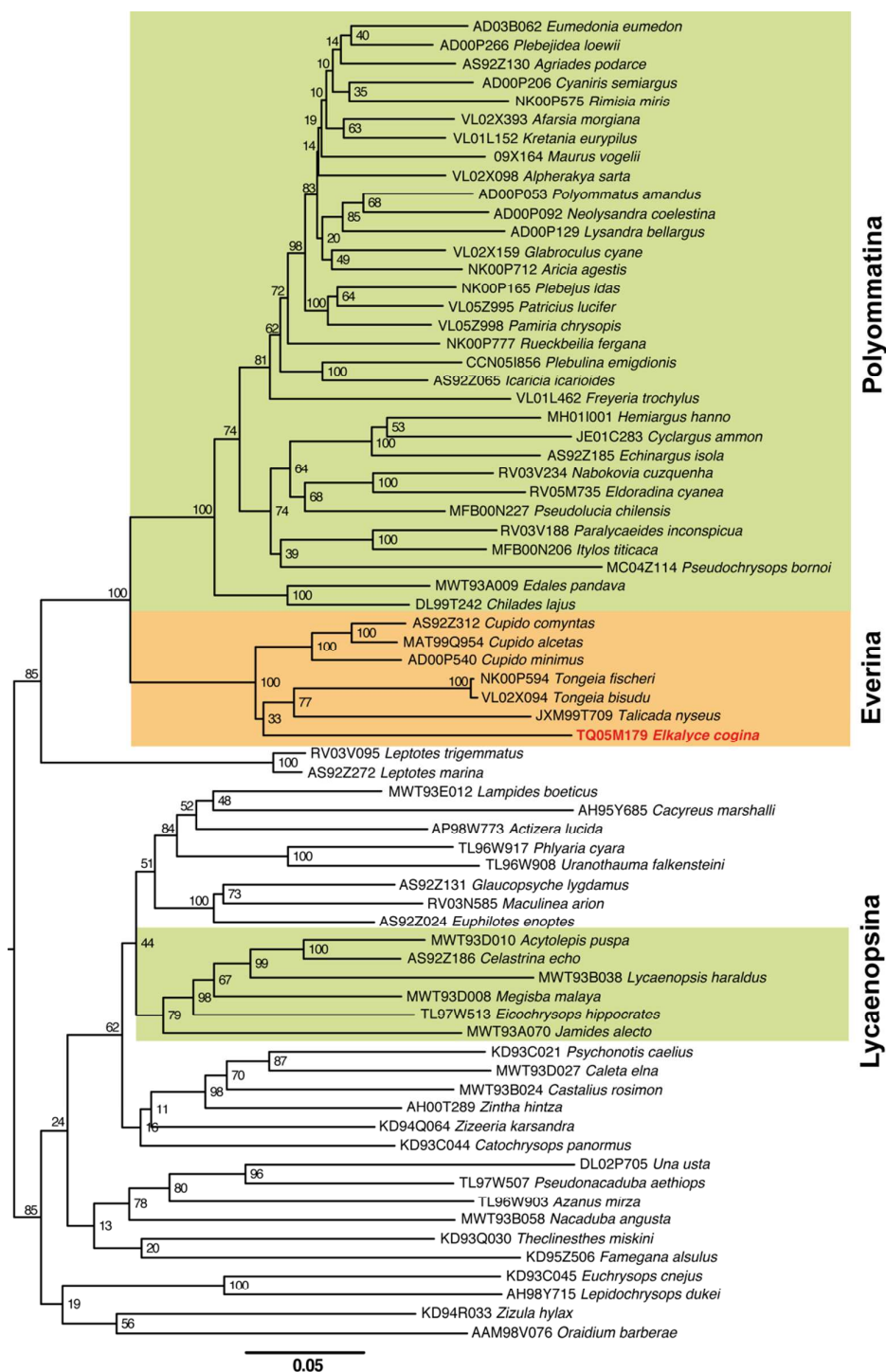
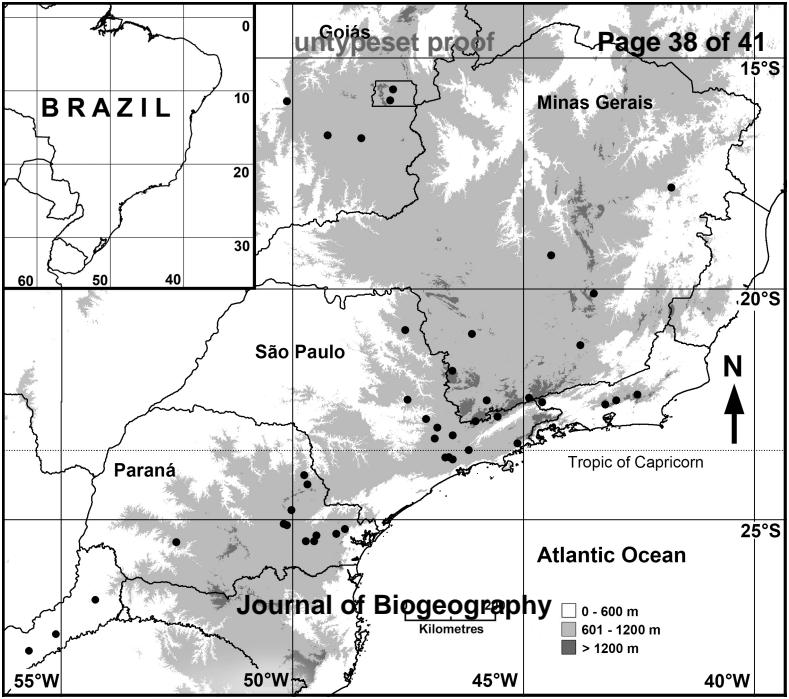


Figure S1 Maximum-likelihood phylogenetic tree for the Polyommataini inferred from three mitochondrial genes – cytochrome oxidase I (*COI*), leucine transfer RNA (*leu-tRNA*), and cytochrome oxidase II (*COII*) – and from four nuclear markers – elongation factor-1 alpha (*EF-1 α*), 28S ribosome unit (*28S*), histone H3 (*H3*) and wingless (*Wg*). Bootstrap support is shown at nodes. The scale bar represents substitutions per position.



BRAZIL

Goiás

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Minas Gerais

São Paulo

Paraná

Tropic of Capricorn

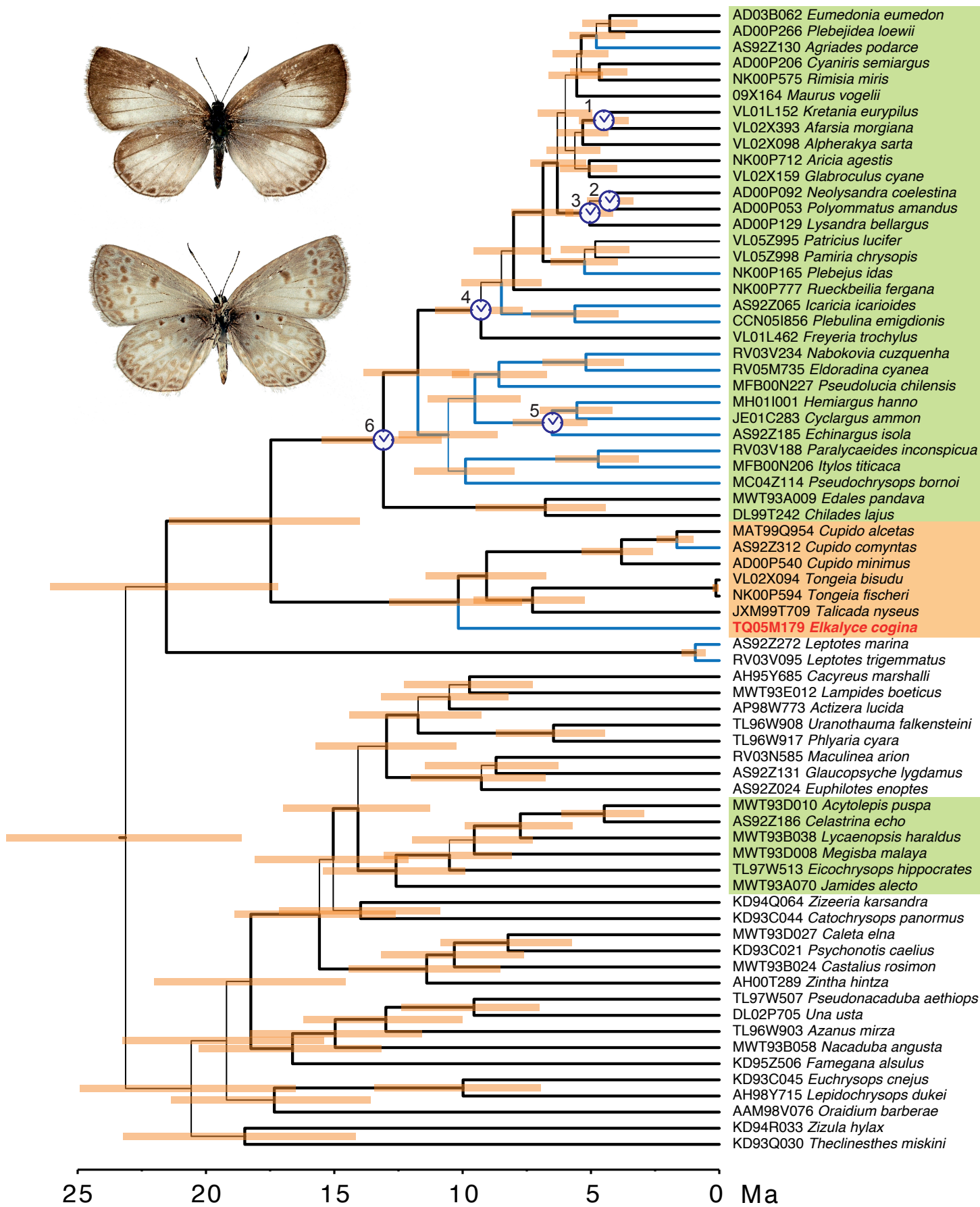
Atlantic Ocean

Journal of Biogeography

Kilometres

- 0 - 600 m
- 601 - 1200 m
- > 1200 m





Polyommattina

Everina

Lycaenopsina

