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1 Strapline: Original article

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3 **Historical biogeography and phylogeny of *Typhlatya* cave shrimp**

4 **(Decapoda: Atyidae) based on mitochondrial and nuclear data**

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19 **Running head:** Zoogeography of *Typhlatya* cave shrimp

20 **ABSTRACT**

21 **Aim** Our aim was to produce a dated phylogeny of *Typhlatya*, a stygobiont shrimp genus
22 with an extremely disjunct localized distribution across the Mediterranean, Central Atlantic
23 and Eastern Pacific. Using phylogenetic analyses, we examine the role of dispersal and
24 plate tectonics in determining its distribution.

25

26 **Location** Western Mediterranean, Ascension Island, Bermuda, Bahamas, Yucatán,
27 Caribbean, Galápagos, Western Australia.

28

29 **Methods** Thirteen of the 17 species of *Typhlatya* were analysed, using *Stygiocaris*,
30 *Halocaridina* and *Antecaridina* as outgroups. Fragments of three mitochondrial and three
31 nuclear genes were combined into a data set of 2449 mitochondrial bp and 1374 nuclear
32 bp.

33

34 **Results** Phylogenetic trees clearly showed *Typhlatya* to be paraphyletic, with the
35 Galápagos species clustering with *Antecaridina*. Only the phylogenetic position of
36 *T. monae* (Hispaniola and Puerto Rico) showed some uncertainty, appearing as the sister
37 group to the Australian genus *Stygiocaris* on the most likely topology. We estimated an
38 average age of 45 Myr (30.6–61.1 Myr) for the most recent common ancestor of *Typhlatya*
39 + *Stygiocaris* + *Antecaridina* + *Halocaridina*. All *Typhlatya* (except *T. galapagensis*) +
40 *Stygiocaris* derived from a node dated to 35.7 Ma (25.7–47.0 Ma), whereas the ancestor of
41 all *Typhlatya* species (excluding *T. monae* and *T. galapagensis*) lived 30.7 Ma (21.9–
42 40.4 Ma).

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44 **Main conclusions** *Typhlatya* is paraphyletic and apparently absent from the eastern
45 Pacific, with *T. galapagensis* clustering with *Antecaridina*. The remaining *Typhlatya*
46 species form a robust monophyletic group with *Stygiocaris*, and both molecular and
47 morphological evidence support the recognition of three sublineages: (1) *Typhlatya* s. str.,
48 Atlantic–Mediterranean, embracing all *Typhlatya* species minus *T. monae*; (2) *Stygiocaris*,
49 limited to north-western Australia; and (3) *T. monae* (Caribbean), for which a new genus
50 could be erected. No congruence was found between temporal and geographical
51 projections of cladogenetic events within *Typhlatya/Stygiocaris* and the major plate
52 tectonic events underlying Tethyan history.

53

54

55 **Keywords**

56 ***COI*, *cyt b*, histone *H3A*, molecular clock, stygofauna, Tethyan relicts, *16S* rRNA, *18S***
57 **rRNA, *28S* rRNA.**

58

60 **INTRODUCTION**

61 Many aquatic subterranean crustaceans (stygbionts) exhibit broad transoceanic disjunct
62 distributions throughout tropical and subtropical latitudes, so that different congeneric
63 species may be isolated on continents or islands half the world apart (Stock, 1993). This
64 pattern is repeated in a diverse set of taxonomic groups including the remipedes,
65 thermosbaenaceans, amphipods, isopods, decapods, copepods and ostracods, and it has
66 been explained by the fragmentation of the continuous ranges of their ancestors by a series
67 of shared isolation events (Stock, 1993; Wagner, 1994). These so-called ‘Tethyan’
68 distribution patterns are best explained in terms of the vicariant isolation of the ancestral
69 lineages coincident with the fragmentation in the late Mesozoic and Tertiary of the Tethys
70 Sea, a predominantly shallow-water circumtropical ocean that existed from the Middle
71 Jurassic until 20 million years ago (Ma) (Sterrer, 1973; Stock, 1993). The progressive
72 breakup of this east–west palaeo-seaway with the collision of continental landmasses and
73 the formation of broad, deep oceanic basins could have resulted in the allopatric
74 diversification of the ancestors of present species, which subsequently became stranded in
75 inland aquifers. It follows, therefore, that genera displaying such distributions should have
76 an age that at least precedes the establishment of deep-water conditions in the north-central
77 Atlantic Ocean. They must also have persisted since then in a state of morphological stasis,
78 or alternatively, converged morphologically under the shared selection pressures posed by
79 the subterranean habitat (Barr & Holsinger, 1985; Hart & Manning, 1986).

80 The major drawback to the hypothesized Tethyan origin for these taxa and their
81 vicariance by plate tectonics is their frequent occurrence on relatively young oceanic
82 islands that have never been connected to continental shelves. Hart *et al.* (1985) proposed
83 that representatives of these lineages on Atlantic islands might be survivors from the time

84 when the Atlantic Ocean was very narrow, and that the forerunners of these islands were in
85 contact with or close to both shores of the ocean. Deep-sea dispersal along the crevicular
86 medium associated with the circumglobal system of spreading zones also represents a
87 feasible alternative explanation of the presence of some of these taxa on geologically
88 young oceanic islands (Boxshall, 1989).

89 *Typhlatya* Creaser, 1936 is a stygobiont genus of atyid shrimp with a punctuated
90 distribution throughout coastal continental and insular ground-waters of the Mediterranean,
91 north central Atlantic and east Pacific (Fig. 1). This taxon, which has never been reported
92 in open marine habitats but with most of the species inhabiting anchialine waters, has an
93 uncertain biogeographical history (see, for example, Croizat *et al.*, 1974; Monod, 1975;
94 Rosen, 1975; Buden & Felder, 1977; Iliffe *et al.*, 1983; Hart *et al.*, 1985; Manning *et al.*,
95 1986; Stock, 1993; Sanz & Platvoet, 1995, for proposals based on vicariance; and Chace &
96 Hobbs, 1969; Monod & Cals, 1970; Chace & Manning, 1972; Peck, 1974; Iliffe, 1986;
97 Stock, 1986; Banarescu, 1990, for alternative dispersalist explanations). The broad
98 distribution of *Typhlatya* has been described elsewhere as the result of Tethys
99 fragmentation (Buden & Felder, 1977; Stock, 1993). However, the ability of some
100 members of the (typically freshwater) family Atyidae to undertake part of their life cycle in
101 the marine environment (diadromy) and the presence of members of *Typhlatya* and other
102 closely related genera on young oceanic islands also support explanations based on marine
103 dispersal (Smith & Williams, 1981; Russ *et al.*, 2010). Recently, divergent
104 phylogeographical patterns among anchialine shrimp have been related to differences in
105 the duration of their respective planktonic larval (dispersive) phases (Santos, 2006; Craft *et*
106 *al.*, 2008; Russ *et al.*, 2010).

107 Several molecular phylogenetic analyses have dealt with *Typhlatya* species, but none
108 has addressed the phylogeny and biogeography of the genus as a whole. Thus, Hunter *et al.*
109 (2008) investigated the phylogeography of three species from Yucatán, the Caicos Islands
110 and Bermuda. Zakšek *et al.* (2007) analysed the molecular phylogeny of the stygobiont
111 genus *Troglocaris* Dormitzer, 1853, using species of *Typhlatya* from Spain and Yucatán as
112 outgroups. Page *et al.* (2008) found that *T. pearsei* (Yucatán) – the only *Typhlatya* included
113 in their analysis – was recovered as the closest relative of the endemic Western Australian
114 subterranean genus *Stygiocaris*, and suggested that they may have descended from a
115 common ancestor that lived in the coastal marine habitat of the ancient Tethys Sea, and
116 were subsequently separated by tectonic plate movements. Five *Typhlatya* species were
117 also included in a recent molecular phylogeny of the family Atyidae (von Rintelen *et al.*,
118 2012). Using a relaxed molecular clock and different calibration priors, these authors
119 estimated an age range from early Cretaceous to Palaeogene for what they defined
120 informally as the ‘*Typhlatya* group’ (*Antecaridina*, *Halocaridina*, *Halocaridinides*,
121 *Stygiocaris* and *Typhlatya*).

122 Here, we present the first genetic survey undertaken to reconstruct the phylogeny of
123 *Typhlatya*, based on 4 kb of nuclear and mitochondrial sequences and a geographically
124 representative sample. Our aim was to use phylogenetic analyses to test the roles of
125 dispersal and of plate tectonics in generating the distribution of *Typhlatya*. With
126 transoceanic dispersal, we would expect disparate estimates for divergence times,
127 inconsistent with those of Tethys fragmentation. In contrast, large divergences, preceding
128 the establishment of deep-water conditions in the Atlantic Ocean, would be anticipated if
129 the distribution pattern of *Typhlatya* was better explained by ancient vicariance.

130

131 **MATERIALS AND METHODS**

132 **Material examined**

133 The 17 species of *Typhlatya* currently recognized are found on eastern Pacific and mid-
134 Atlantic islands, the coasts of the Caribbean, the Antillean Arch, the Bahamas and the
135 western Mediterranean (Fig. 1, Table 1); thirteen were included in the analysis. A single
136 population of each species was analysed, except for *T. galapagensis*, *T. consobrina*,
137 *T. miravetensis* and *T. monae* (see Table 2).

138 The two species of *Stygiocaris* Holthuis, 1960, plus *Halocaridina rubra* and
139 *Antecaridina lauensis*, were included in the data set because of their demonstrated
140 relationship to *Typhlatya* (Monod & Cals, 1970; Page *et al.*, 2008). Other analyses using
141 only 16S rRNA [*rrnL*], 28S rRNA [*LSU*] and histone *H3A* sequences were performed in
142 conjunction with GenBank sequences from the closest relatives of the *Typhlatya* /
143 *Stygiocaris* cluster (von Rintelen *et al.*, 2012).

144

145 **Sequences and alignments**

146 Genomic DNA was isolated from whole specimens using the DNeasy Tissue Kit (Qiagen,
147 Hilden, Germany). Polymerase chain reaction (PCR) was used to amplify fragments of the
148 mitochondrial cytochrome *c* oxidase subunit I (*COI*; two non-overlapping fragments),
149 cytochrome *b* (*cyt b*), and *rrnL* genes using the primers shown in Table 3. Fragments of
150 three other nuclear genes were also amplified: histone *H3A*, *SSU*, and 28S rRNA (*LSU*)
151 (Table 3). The combined data set consisted of 3823 bp (2449 bp of the mitochondrial and
152 1374 bp of the nuclear genome).

153 PCR was performed in a reaction containing (1×) NH₄ buffer, 3.5–5.0 mM MgCl₂,
154 0.2 mM of each dNTP, 0.2–0.4 μM each primer, 0.5 U of *Taq* DNA polymerase and 1–5 μL

155 of DNA template, in a final volume of 25 μ L. The amplification conditions consisted of
156 one cycle of 94 °C for 2 min and 35 cycles of 94 °C for 30 s, 47–55 °C for 30 s, and 72 °C
157 for 1 min, followed by a final extension step at 72 °C for 10 min. The amplified fragments
158 were sequenced in both directions using the ABI Prism BigDye Reaction Kit v. 2.0 and an
159 ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Nucleotide
160 sequences were aligned using MAFFT 4.0 software, taking into account the RNA secondary
161 structure of ribosomal genes (Kato *et al.*, 2005).

162

163 **Phylogenetic analyses**

164 The program JMODELTEST (Posada, 2008) was used to select the best evolutionary model
165 for each partition, according to the Bayesian information criterion (BIC). The best model
166 was HKY+ Γ , except for the *SSU+LSU* partition, for which GTR+ Γ was the best.

167 Incongruence length difference (ILD) tests (Farris *et al.*, 1995) were performed with PAUP*
168 4.0b10 (Swofford, 2002) to check for incongruence among genes. We implemented
169 different evolutionary models, data partitioning strategies, tree construction methods and
170 clock estimation methods to assess their effect on tree topologies, branch lengths, and
171 evolutionary rates (Phillips, 2009). We explored five different partitioning strategies: (1)
172 seven partitions: considering first, second and third codon positions of mtDNA as three
173 different partitions, plus *rrnL*, histone *H3A*, *SSU* and *LSU* as individual partitions; (2) six
174 partitions: as above, but combining the first and second mtDNA positions into a single
175 partition; (3) five partitions: as in (2), but with the nuclear ribosomal genes merged into a
176 single partition; (4) each gene as an independent partition (six partitions); and (5) the
177 mitochondrial and nuclear sequences treated as two different partitions. The competing
178 partition strategies were compared using Bayes factors (Brown & Lemmon, 2007).

179 Marginal likelihoods and harmonic means were estimated using TRACER 1.4 (Rambaut &
180 Drummond, 2007). The best partition scheme among the five tested was option 3.

181 Bayesian phylogenetic analyses were conducted in the parallel version of MRBAYES
182 3.1.2 (Huelsenbeck & Ronquist, 2001). In each Bayesian search, two independent runs
183 were performed, starting with the default prior values, random trees, and three heated and
184 one cold Markov chains, which ran for two million generations, sampled at intervals of
185 1000 generations. All parameters were unlinked and rate models were allowed to vary
186 freely over partitions. The convergence of all parameters of the two independent runs was
187 assessed in MRBAYES 3.1.2 and TRACER 1.4, obtaining effective sample sizes > 200
188 (Rambaut & Drummond, 2007). After the 10% burn-in samples, the remaining trees from
189 the two independent runs were combined into a single majority consensus topology, and
190 the frequencies of the nodes in the majority rule tree were taken as the posterior
191 probabilities (Huelsenbeck & Ronquist, 2001).

192 Maximum likelihood (ML) analyses using the partition schemes described above were
193 performed using RAXML 7.0.4 (Stamatakis *et al.*, 2005). Bootstrap support values were
194 estimated using the fast bootstrapping method, with 500 replicates.

195

196 **Molecular clock analyses**

197 We estimated node ages using BEAST 1.6.0 (Drummond & Rambaut, 2007), enforcing a
198 relaxed molecular clock with an uncorrelated lognormal distribution and a Yule speciation
199 model. For tree calibration, we used the known age ranges of three major events affecting
200 the diversification of particular lineages as flat priors: (1) the isolation of the populations of
201 *T. galapagensis* from Santa Cruz and Isabela islands in the Galápagos, which cannot be
202 older than the age of the Cocos Ridge and associated seamounts. These now-submerged

203 structures probably formed when the oceanic crust moved over the Galápagos hotspot, and
204 it is probable that an archipelago has existed continuously above the current Galápagos
205 area for the past 14.5 Myr (see Werner *et al.*, 1999, and references therein), so the interval
206 5–14 Ma has been proposed for the separation of the two populations; (2) the isolation of
207 the ancestor of *Stygiocaris lancifera* and *S. styliifera* after the emergence of the Cape Range
208 anticline in north-western Australia (7–10 Ma; Page *et al.*, 2008; see above); and (3) the
209 occlusion of the Havana–Matanzas Channel in Cuba at 5–6 Ma (Iturralde-Vinent *et al.*,
210 1996), which could have triggered the isolation of the ancestors of the sister species
211 *T. consobrina* and *T. taina*.

212 We assumed three independent substitution rates, implemented as three clocks: a rate
213 for the mitochondrial protein-coding genes (*COI*, *cyt b*), another for *rrnL*, and the third for
214 the nuclear data set (histone *H3A*, *SSU* and *LSU*). BEAST analyses were run for fifty million
215 generations, sampling every 1000 generations. The outputs were analysed with TRACER 1.4
216 and TREEANNOTATOR 1.6.0 (Drummond & Rambaut, 2007), after the first five million
217 generations had been discarded.

218

219 **RESULTS**

220 Data regarding the species, populations, collection sites, and corresponding EMBL
221 accession numbers of the DNA sequences used in this study are shown in Table 2. Note
222 that it was not possible to recover the entire sequences of some gene fragments for some
223 populations.

224

225 **Intraspecific divergences**

226 *Typhlatya galapagensis* from Santa Cruz and Isabela islands showed a considerably higher
227 pairwise *COI* genetic distance (8%) than those found among populations of *T. monae* or
228 *T. miravetensis*, or between *T. garciai* and *T. kakuki* (see Appendix S1 in Supporting
229 Information). This suggests that these two island populations are differentiated at the
230 species level.

231 The three different *T. monae* populations from Hispaniola, located at opposite corners
232 of the Dominican Republic, and the population from Bosque Guánica in Puerto Rico
233 showed very low genetic divergences (< 0.5% for *COI*). Moreover, a comparison of
234 *T. monae* from Playa Frontón (Samaná Peninsula, northern Dominican Republic) and
235 *T. utilaensis* from the single locality known thus far showed that two of the four diagnostic
236 morphological characters considered for the latter species (Alvarez *et al.*, 2005) are similar
237 in both taxa. Unfortunately, the single *T. utilaensis* specimen available proved to be useless
238 for molecular analysis. Sequencing further samples could confirm the conspecific status of
239 these two taxa in the future.

240 *Typhlatya garciai* from Providenciales (Caicos Islands) and *T. kakuki* from Acklins
241 Island (Bahamas; see Table 2) showed identical histone H3A sequences and low
242 divergences for *COI* and *rrnL* (< 0.6%). We consider here that *T. kakuki* is only a
243 population of *T. garciai* that has a completely regressed cornea.

244 Three populations of *T. miravetensis*, separated by up to 40 km in eastern Spain,
245 showed a divergence in the mitochondrial markers of 1–3%, but their nuclear sequences
246 were identical. The two Cuban populations of *T. consobrina* included here showed
247 significant divergences in *rrnL* (2.3%), *cyt b* (6.1%) and *COI* (4.7%).

248

249 **Phylogenetic analyses**

250 A preliminary Bayesian analysis including our species data set plus a selection of the taxa
251 considered by von Rintelen *et al.* (2012), and using the same three gene markers as those
252 authors, showed that the cluster *Antecaridina–Halocaridina–T. galapagensis* is the
253 monophyletic sister group of the remaining *Typhlatya/Stygiocaris* species (Fig. 2). ILD
254 tests of our complete data set indicated that the six partitions were not incongruent (P
255 > 0.13). Total evidence derived from the Bayesian and maximum likelihood phylogenetic
256 trees corroborated the paraphyly of the genus *Typhlatya* because the Galápagos species
257 clustered with *A. lauensis* with a high posterior probability (PP = 1.0; Fig. 3). The
258 Australian genus *Stygiocaris* clustered as nested to *Typhlatya*, as suggested by Page *et al.*
259 (2008). The only tree node showing weak support involved *T. monae*, which appeared as
260 the sister group to *Stygiocaris* (PP = 0.93; 56% bootstrap support in the ML analysis).
261 However, this species appeared basal to the rest of *Typhlatya + Stygiocaris* (PP = 0.87) in
262 an analysis that included additional outgroup species and a reduced (three genes) data set
263 (Fig. 2). Shimodaira–Hasegawa tests revealed no significant differences between the two
264 alternative topologies.

265 Based on the most probable topology and molecular rates, and using the three
266 palaeogeographical events as calibration points, a relaxed molecular clock estimated an age
267 of 30.6–61.1 Myr for the most recent common ancestor (MRCA) of *Typhlatya +*
268 *Stygiocaris + Antecaridina + Halocaridina* (Figs 4 & 5, Appendix S2). This analysis also
269 estimated an age of 5.0–7.7 Myr for the ancestor of the divergent populations of
270 *T. galapagensis* from Santa Cruz and Isabela (node ‘f’ in Fig. 4). The age of the MRCA of
271 all *Typhlatya* species (minus *T. galapagensis*) + *Stygiocaris* (node ‘b’ in Fig. 4) was 25.7–
272 47.0 Myr, whereas the ancestor of all *Typhlatya* species (minus *T. monae* and
273 *T. galapagensis*) (node ‘e’ in Fig. 4) lived 21.9–40.4 Ma.

274

275 **DISCUSSION**

276 Molecular dating using the node age priors separately or in combination produced
277 compatible age estimates, particularly for the most recent nodes (< 20 Myr; see Fig. 5 and
278 Appendix S2). We found a marked inconsistency between the divergence time estimates in
279 our phylogeny and one of the major palaeogeographical events in Tethys history – the
280 establishment of deep water between the two shores of the north-central Atlantic Ocean at
281 about 110 Ma (Sclater *et al.*, 1977; Jones *et al.*, 1995). Our estimates date the separation
282 between the western Atlantic / Caribbean (minus *T. monae*) and the Mediterranean lineage
283 of *Typhlatya* at 21.9–40.4 Ma (see Fig. 4), which is much later than the disruption of the
284 shallow-water connections between the two shores of the Atlantic. Thus, the distribution of
285 *Typhlatya* / *Stygiocaris* cannot be explained solely by the vicariant isolation that
286 accompanied the fragmentation of Tethys Sea. We suggest that this disjunct amphi-Atlantic
287 distribution could be the result of the extinction of species from central and eastern Atlantic
288 archipelagos, and that new *Typhlatya* species might even await discovery in the
289 Macaronesian islands. There is compelling geological evidence for the presence of
290 drowned archipelagos and seamounts in the central East Atlantic Ocean from at least
291 60 Ma (Geldmacher *et al.*, 2001, 2005; Fernández-Palacios *et al.*, 2011). These Palaeo-
292 Macaronesian islands were located much closer to the western Mediterranean than they
293 would be today and were affected by the east-to-west warm circumequatorial marine
294 Tethys Sea current (Fernández-Palacios *et al.*, 2011). The existence of these vanished
295 archipelagos supports the potential presence of *Typhlatya* in the area and also the relatively
296 recent divergence of the Mediterranean and western Atlantic *Typhlatya* lineages (21.6–
297 44.4 Ma; see Fig. 4).

298 The sister relationship found between *Typhlatya* and the Australian genus *Stygiocaris*
299 (with the caveat that corroboration is needed from additional molecular evidence) is hardly
300 compatible with their presumed vicariant divergence due to the occlusion of the connection
301 between the Mediterranean and the Indian Ocean (Page *et al.*, 2008). The time frame
302 established for the collision of the Arabian Plate with Anatolia (16–20 Ma; Meulenkamp &
303 Sissingh, 2003) not only post-dates our age estimate for the divergence of *Typhlatya* s. str.
304 and *Stygiocaris* (25.7–47.0 Ma; Fig. 4), but also (and more relevantly) the divergence of
305 the sister taxa *T. monae* and *Stygiocaris* (22.0–42.3 Ma; Fig. 4).

306

307 **Paraphyly of *Typhlatya***

308 Monod & Cals (1970) assigned a series of juvenile blind atyids from Santa Cruz and
309 Isabela in the Galápagos to *Typhlatya*, although they noted some morphological similarities
310 to *Antecaridina*. In our phylogenetic analyses, *Typhlatya galapagensis* is placed as the
311 sister taxon to *Antecaridina lauensis* with strong statistical support, rendering the genus
312 *Typhlatya* paraphyletic in its current conception. Our study also suggests a possible
313 species-level differentiation between the populations of *T. galapagensis* from the islands
314 Santa Cruz and Isabela. *COI* genetic distance between these two populations (8%)
315 approaches the minimum of 10% found in our study to distinguish different *Typhlatya*
316 species (Appendix S1), but is considerably higher than the maximum interpopulational
317 distances we identified for *T. monae*, *T. garciai / kakuki* and *T. miravetensis*.

318

319 ***Stygiocaris* and *Typhlatya monae***

320 *Stygiocaris* and *T. monae* are morphologically peculiar, even though they cluster with the
321 rest of the *Typhlatya* species in a robust monophyletic group (Fig. 3). The position of

322 *T. monae* is not fully established on the tree, but occurs either as the sister taxon to the rest
323 of *Typhlatya* or as the sister taxon to *Stygiocaris* (as shown in Figs 2 & 3, respectively). In
324 any event, our analysis confirms the long-independent evolution of these three sublineages
325 (i.e., *Typhlatya* s. str., *Stygiocaris* and *T. monae*; see Fig. 3).

326 *Stygiocaris* is a stygobiont genus endemic to north-western Australia, composed of
327 three species, only two of which have been formally described (Holthuis, 1960; Page *et al.*,
328 2008). Page *et al.* (2008) have already noted the sister relationship between *Stygiocaris* and
329 *Typhlatya* based on molecular evidence. Using a combined nuclear and mitochondrial
330 sequence data set, they found that the Mexican *T. pearsei* was the sister taxon of
331 *Stygiocaris*, rather than any surface or cave atyids from Australia or the Indo-Pacific
332 region.

333 *Typhlatya monae*, with an apparently broad distribution in the Caribbean, is unique in
334 displaying a uniramous pereopod (the fifth), whereas the other *Typhlatya* species display a
335 well developed exopod on all pereopods. The introduction of a new genus to
336 accommodate *T. monae* on the basis of this feature should be considered, and would give
337 taxonomic relevance to the broad molecular divergence of the *Typhlatya* s. str., *Stygiocaris*
338 and *T. monae* lineages.

339 Zakšek *et al.* (2007) and Sket & Zakšek (2009) have, however, recently challenged the
340 relevance of features such as the presence of certain spines on the anterior margin of the
341 cephalothorax or the absence of exopods on the pereopods in distinguishing atyid genera.
342 These authors, based on molecular markers, have shown that a presumed *Typhlatya* from
343 the Balkans is actually a modified *Troglocaris*, which displays smooth anterior margins on
344 its cephalothorax and uniramous pereopods. Our own data for *Antecaridina* lend support
345 to this hypothesis, because *T. galapagensis* (which lacks spines on the anterior margin of

346 the cephalothorax) occurs in our phylogram as a sister group to *A. lauensis*, a taxon
347 displaying both suborbital and pterygostomial spines. Our own observations of the shape of
348 the sternal process in *Typhlatya* s. str. also indicate that it is identical to *Stygiocaris*,
349 supporting the congeneric status of the two taxa.

350

351 **Dispersal, population structure and divergence of *Typhlatya* species**

352 The distribution patterns of the Atyidae are dependent on life-history traits, such as their
353 dispersal capacities and species-specific tolerance to local conditions (Page & Hughes,
354 2007). *Typhlatya* species are usually very localized, limited in most instances to a single
355 island or narrow portion of coast. However, several *Typhlatya* species display relatively
356 broad distributions that, in some instances, include territories separated by stretches of sea.
357 Thus, *T. monae* is known from Mona Island, Puerto Rico and Hispaniola (Greater Antilles),
358 the more distant Barbuda (Lesser Antilles), and Curaçao and San Andrés islands, the last
359 two at opposite sides of the Caribbean (see Table 1). Our own data for three different
360 populations from Hispaniola and one from Puerto Rico suggest the occurrence of panmixia
361 (Appendix S1).

362 *Typhlatya garciai / kakuki* is known from north-eastern and north-western Cuba,
363 Providenciales (Caicos Islands) and Acklins Island (Bahamas; see Table 1). Our own
364 observations of the latter two populations, separated by a deep-water sea arm of 173 km,
365 indicated very low molecular divergence, which could be explained either by continuous
366 gene flow through dispersal over sea or, more likely given the separation of the
367 populations, by recent colonization and subsequent isolation.

368 The high dispersal potential of *Typhlatya* across subterranean waters has already been
369 pointed out by Hunter *et al.* (2008) on the Yucatán Peninsula, where haplotypes of

370 *T. mitchelli* are shared between populations separated by up to 235 km. However, we found
371 significant isolation among the populations of *T. consobrina* (sampled from two locations
372 in Cuba about 330 km apart) and *T. miravetensis* (three populations separated by up to
373 40 km in eastern Spain) (Appendix S1).

374

375 **Trans-oceanic dispersal of *Typhlatya***

376 Two closely related species, *Typhlatya iliffei* (Bermuda) and *T. rogersi* (Ascension), are
377 found on mid-oceanic islands in the Atlantic. Bermuda is the cap of a mid-plate rise in a
378 sector of the north-western Atlantic with no other seamounts or ridges that could have
379 harboured members of *Typhlatya* in the past. The pillow lavas that formed the original
380 Bermuda shield volcano are no older than 47–40 Myr, and at 40–36 Ma, the Bermuda
381 platform had already risen to sea level (Vogt & Jung, 2007). Ascension, located about
382 7000 km to the southeast of Bermuda, occurs 90 km west of the Mid-Atlantic Ridge on 7-
383 Myr-old oceanic lithosphere. Its oldest subaerial lava flows have been dated recently at
384 2.5 Ma (Minshull *et al.*, 2010).

385 Iliffe *et al.* (1983) proposed that *T. iliffei* and *T. rogersi* represent an ancient atyid
386 stock that survived on submerged and emergent seamounts along or associated with the
387 Mid-Atlantic Ridge. Alternatively, Hart *et al.* (1985) and Manning *et al.* (1986) proposed
388 that the ancestral form of *Typhlatya* was a deep-sea benthic organism that originally
389 entered the cave environment directly from deep water via cracks and fissures on
390 submerged seamount slopes during the Mesozoic. Opposing this view is the alleged
391 primary freshwater condition of the family Atyidae, which already included limnic
392 representatives by the middle Cretaceous (Rabadà y Vives, 1993), and the fact that
393 *Typhlatya* has never been recorded in open marine habitats. Hunter *et al.* (2008) favoured

394 an alternative scenario, where mid-Atlantic species reached their present distributions by
395 transoceanic dispersal of a shallow-water ancestor. These researchers identified a sister
396 relationship between *T. iliffei* from Bermuda and *T. garciai* from Providenciales (Caicos
397 Islands) based on molecular evidence, and suggested that the taxon from Bermuda might
398 have derived from a Bahamian ancestor dispersed via the Gulf Stream.

399 The most common recent ancestor of the Bahamas, Ascension and the Bermudian taxa
400 lived 18.6–33.9 Ma (see Fig. 4), which is compatible with the colonization of Bermuda by
401 overseas dispersal of a Bahamian ancestor, as proposed by Hunter *et al.* (2008) (the age of
402 Bermuda is 40–36 Myr; see above). Our estimate for the divergence of the Bermuda–
403 Ascension lineages (1.9–5.3 Ma; see Fig. 4) is also compatible with the age of Ascension
404 (2.5 Myr; see above). Although both islands are separated by a huge expanse of ocean and
405 the prevailing equatorial currents would make the derivation of one from the other by long-
406 distance over-sea dispersal untenable, the most likely explanation for their origin is that
407 they derived from a diadromous Bahamian lineage which colonized Bermuda first and
408 subsequently colonized Ascension Island.

409

410 **CONCLUSIONS**

411 In this study, we examined the molecular phylogeny of *Typhlatya* shrimp using nuclear and
412 mitochondrial gene sequences and a relaxed molecular clock. These stygobiont atyids show
413 an extremely disjunct distribution, which has been suggested to derive from plate-tectonic
414 vicariance. Our results confirm the paraphyly of *Typhlatya*, because *T. galapagensis* from
415 the Galápagos Islands is the sister taxon to *Antecaridina*. Furthermore, the Greater
416 Antillean *T. monae* probably represents an independent sister lineage to the Australian
417 genus *Stygiocaris*. We have analysed the relaxed molecular clock for *Typhlatya* using three

418 different calibration points based on three independent palaeogeographical events. We
419 show that in *Typhlatya* / *Stygiocaris*, the ages of the corresponding subclades postdate the
420 establishment of deep water between the north-central Atlantic Ocean shores. In addition,
421 the divergence of the *T. monae* lineage from the rest of the genus *Typhlatya* preceded the
422 cladogenesis of *Typhlatya* s. str. into a Mediterranean and a Caribbean/Mid-Atlantic clade.
423 Therefore, our results are inconsistent with a simple explanation of the origin of the group
424 based on plate-tectonic vicariance.

425

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448

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661

662 **SUPPORTING INFORMATION**

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

664

665 **Appendix S1** Percentage uncorrected pairwise genetic distances among the *COI* sequences.

666 **Appendix S2** Mean ages of the tree nodes in Fig. 4 and molecular evolutionary rates,
667 calculated using different calibration points.

668

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673

674

675 **BIOSKETCH**

676 **Alejandro Botello** is a carcinologist who shares with the other authors a vivid interest on
677 the evolution and biogeography of anchialine crustaceans. His research is mainly focused on
678 the cave decapod fauna of the Yucatán Peninsula.

679

680 Author contributions: A.B., C.J. and D.J. conceived the ideas; A.B., F.A., T.I. and D.J.
681 collected the data; A.B., J.P. and C.J. analysed the data; and A.B., C.J. and D.J. led the writing.

682

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684

685 **Table 1** *Typhlatya* diversity and distribution. Asterisks denote species retaining pigmented
686 eyespots.

687

Species	Distribution	References
<i>T. arfae</i> Jaume & Bréhier, 2005	France	Jaume & Bréhier (2005)
<i>T. campechae</i> Hobbs & Hobbs, 1976	Yucatán Peninsula (Mexico)	Hobbs & Hobbs (1976)
<i>T. consobrina</i> Botosaneanu & Holthuis, 1970	Cuba	Botosaneanu & Holthuis (1970)
<i>T. dzilamensis</i> Alvarez, Iliffe & Villalobos, 2005	Yucatán Peninsula (Mexico)	Alvarez <i>et al.</i> (2005)
<i>T. elenae</i> Juarrero, 1994	Cuba	Juarrero (1994)
<i>T. galapagensis</i> Monod & Cals, 1970	Santa Cruz and Isabela Islands (Galápagos)	Monod & Cals (1970)
<i>T. garciadebrasi</i> Juarrero & Ortiz, 2000	Cuba	Juarrero & Ortiz (2000)
<i>T. garciai</i> Chace, 1942	Cuba; Caicos Islands	Botosaneanu & Holthuis (1970); Buden & Felder (1977); Chace (1942); Holthuis (1977)
* <i>T. iliffei</i> Hart & Manning, 1981	Bermuda	Hart & Manning (1981)
* <i>T. kakuki</i> Alvarez, Iliffe & Villalobos, 2005	Acklins (Bahamas)	Alvarez <i>et al.</i> (2005)
<i>T. miravetensis</i> Sanz & Platvoet, 1995	Spain	Sanz & Platvoet (1995)
<i>T. mitchelli</i> Hobbs & Hobbs, 1976	Yucatán Peninsula (Mexico)	Hobbs & Hobbs (1976)
* <i>T. monae</i> Chace, 1954	Puerto Rico; Dominican Republic; Mona Island (Puerto Rico); Barbuda (Lesser Antilles); Curaçao (Netherlands Antilles); San Andrés Island (Colombia)	Chace (1954; 1975); Debrot (2003); Sket (1988)
<i>T. pearsei</i> Creaser, 1936	Yucatán Peninsula (Mexico)	Cárdenas (1950); Creaser (1936; 1938); Hobbs & Hobbs (1976)
* <i>T. rogersi</i> Chace & Manning, 1972	Ascension Island	Chace & Manning (1972)
<i>T. taina</i> Estrada & Gómez, 1987	Cuba	Estrada & Gómez (1987)
* <i>T. utilaensis</i> Alvarez, Iliffe & Villalobos, 2005	Utila Island (Honduras)	Alvarez <i>et al.</i> (2005)
<i>Typhlatya</i> sp.	Belize	T. Iliffe, pers. obs.
<i>Typhlatya</i> sp.	Aruba (Netherlands Antilles)	L. Botosaneanu, Zoölogisch Museum, Amsterdam. pers, comm.
<i>Typhlatya</i> sp.	Bonaire (Netherlands Antilles)	L. Botosaneanu, Zoölogisch Museum, Amsterdam. pers, comm.

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689**Table 2** Collection sites and EMBL accession numbers of *Antecaridina*, *Halocaridina*, *Stygiocaris* and *Typhlatya* species included in this
690analysis. Accession numbers in bold correspond to sequences obtained from GenBank (Ivey and Santos 2007; Page *et al.*, 2008).

691

Species	Collection site	EMBL accession numbers						
		<i>COI 5'</i>	<i>COI 3'</i>	<i>cyt b</i>	<i>rrnL (16S)</i>	Histone <i>H3A</i>	<i>SSU (18S)</i>	<i>LSU (28S)</i>
<i>Antecaridina lauensis</i>	North East Point, Christmas Island (SE Indian Ocean)	HE80089 8	HE80091 9	N/A	EU12385 1	HE80096 5	HE80101 6	N/A
<i>Halocaridina rubra</i>	Kohala District (Hawaii Is.; Hawaii): anchialine pool PT (Santos, 2006)	DQ9174 32	DQ9174 32	DQ9174 32	FN99536 8	HE80096 4	HE80101 5	HE801036
<i>Stygiocaris lancifera</i>	Cape Range (W Australia): Tulki well	HE80090 1	HE80092 2	HE80094 8	EU12382 7	HE80096 8	HE80101 9	HE801039
<i>Stygiocaris stylifera</i>	Cape Range (W Australia): Kuddamurra well (Palms)	N/A	HE80092 3	N/A	EU12383 6	HE80096 9	HE80102 0	HE801040
<i>Typhlatya arfeae</i>	Salses (Perpignan; France): Font Estramar	HE80090 6	HE80092 9	HE80095 4	HE80100 0	HE80097 5	HE80102 5	HE801045
<i>Typhlatya consobrina</i>	Bolondrón (Matanzas; Cuba): Cueva Chicharrones	HE80091 0	HE80093 3	HE80095 6	HE80100 4	HE80097 9	HE80102 8	HE801048
	El Veral (Guanahacabibes Peninsula; W Cuba): Cueva del Agua	HE80091 5	HE80094 0	HE80096 2	HE80101 1	HE80098 6	HE80103 4	N/A
<i>Typhlatya dzilamensis</i>	Dzilam de Bravo (Yucatán; Mexico): Cenote Cervera	N/A	HE80092 6	HE80095 1	HE80099 7	HE80097 2	N/A	N/A
<i>Typhlatya galapagensis</i>	Sta. Cruz Is. (Galápagos)	HE80089 9	HE80092 0	HE80094 6	HE80099 1	HE80096 6	HE80101 7	HE801037
	Isabela Is. (Galápagos)	HE80090 0	HE80092 1	HE80094 7	HE80099 2	HE80096 7	HE80101 8	HE801038
<i>Typhlatya garciai</i>	Providenciales (Caicos)	HE80090 9	HE80093 2	HE80095 5	HE80100 3	HE80097 8	N/A	N/A
<i>Typhlatya iliffei</i>	Bermuda: Tucker's Town Cave	HE80090 4	HE80092 7	HE80095 2	HE80099 8	HE80097 3	HE80102 3	HE801043
<i>Typhlatya kakuki</i>	Salinas Point (Acklins Is.; Bahamas): Shrimp Hole	N/A	HE80094 1	N/A	HE80101 3	HE80098 8	N/A	N/A
<i>Typhlatya miravetensis</i>	Pla de Cabanes (Castellón; Spain): Ullal de la Rambla de Miravet	HE80090 5	HE80092 8	HE80095 3	HE80099 9	HE80097 4	HE80102 4	HE801044
	Well at Peñíscola (Castellón; Spain)	HE80091 6	N/A	HE80096 3	HE80101 2	HE80098 7	HE80103 5	N/A
	Well at Alcalá de Xivert (Castellón; Spain)	HE80091 7	N/A	N/A	N/A	N/A	N/A	N/A

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<i>Typhlatya mitchelli</i>	Hoctún (Yucatán; Mexico): Cenote de Hoctún	HE80090 2	HE80092 4	HE80094 9	HE80099 5	HE80097 0	HE80102 1	HE801041
<i>Typhlatya monae</i>	Well at Juan Dolio (Dominican Rep.)	HE80090 7	HE80093 0	N/A	HE80100 1	HE80097 6	HE80102 6	HE801046
	Jaragua NP (Oviedo; Pedernales; Dominican Rep.): Pozimán Cadena	N/A	HE80093 5	N/A	HE80100 6	HE80098 1	N/A	HE801050
	Cave at Bosque Guánica (SW Puerto Rico)	HE80091 2	HE80093 6	HE80095 8	HE80100 7	HE80098 2	HE80103 0	N/A
	Cave at Bosque Guánica (SW Puerto Rico)	HE80091 3	HE80093 7	HE80095 9	HE80100 8	HE80098 3	HE80103 1	N/A
	Cave at Bosque Guánica (SW Puerto Rico)	HE80091 4	HE80093 8	HE80096 0	HE80100 9	HE80098 4	HE80103 2	N/A
	Cave at Bosque Guánica (SW Puerto Rico)	N/A	HE80093 9	HE80096 1	HE80101 0	HE80098 5	HE80103 3	N/A
	Samaná Peninsula (Dominican Rep.): well at Playa del Frontón	N/A	HE80094 2	N/A	HE80101 4	N/A	N/A	N/A
	Well at Juan Dolio (Dominican Rep.)	N/A	HE80094 3	N/A	N/A	N/A	N/A	N/A
	Well at Juan Dolio (Dominican Rep.)	N/A	HE80094 4	N/A	N/A	N/A	N/A	N/A
<i>Typhlatya pearsei</i>	Sacalum (Yucatán; Mexico): Cenote Nohchen	HE80090 3	HE80092 5	HE80095 0	HE80099 6	HE80097 1	HE80102 2	HE801042
<i>Typhlatya rogersi</i>	Anchialine pool at Ascension Is.	N/A	HE80093 1	N/A	HE80100 2	HE80097 7	HE80102 7	HE801047
<i>Typhlatya taina</i>	Puerto Escondido (Sta. Cruz del Norte; La Habana; Cuba): Cueva de la India	HE80090 8	HE80093 4	HE80095 7	HE80100 5	HE80098 0	HE80102 9	HE801049

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694**Table 3** Primers used to amplify different mitochondrial and nuclear fragments.
695

Amplified fragment	Primer	Primer sequence (5'-3')	Reference	
Mitochondrial				
<i>COI</i>	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer <i>et al.</i> (1994)	
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer <i>et al.</i> (1994)	
	NANCY	CCYGGTAAAAATAAAAATATAAATCTC	Simon <i>et al.</i> (1994)	
	Pat	TCCAATGCACTAATCTGCCATATTA	Simon <i>et al.</i> (1994)	
	Jerry	CAACATTTATTTGATTTTTTGG	Simon <i>et al.</i> (1994)	
	COIF1	AAAAAAGAAAACMTTYGGYACNYTAGG	This study	
	COIR1	TTNARDCCTARGAARTGYTGRGG	This study	
	F12	GCCTTCCCCCGGATRAAYAYAT	This study	
	R27	CGGTCGGTCAGCAGYATNGTRATNGC	This study	
	<i>cyt b</i>	CB1	TAIGTACTACCATGAGGACAAATATC	Barraclough <i>et al.</i> (1999)
		CB4	AAAAGAAARTATCATTGAGGTTGAAT	Barraclough <i>et al.</i> (1999)
	<i>rrnL (16S)</i>	M14	CGCCTCTTTATCAAAAACAT	Xiong & Kocher (1991)
M74		CTCCGGTTTGAACTCAGATCA	Xiong & Kocher (1991)	
Nuclear				
Histone <i>H3A</i>	H3aF	ATGGCTCGTACCAAGCAGACVGC	Colgan <i>et al.</i> (1998)	
	H3Ar	ATATCCTTRGGCATRATRGTGAC	Colgan <i>et al.</i> (1998)	
<i>SSU (18S rRNA)</i>	18S3'	CACCTACGGAAACCTTGTTACGAC	Shull <i>et al.</i> (2001)	
	18S2.0	ATGGTTGCAAAGCTGAAAC	Shull <i>et al.</i> (2001)	
<i>LSU (28S rRNA)</i>	Ver28Sf	CAAGTACCGTGAGGGAAAGTT	Lefébure <i>et al.</i> (2006)	
	Ver28S2	GTTACCATCTTTCGGGTC	Lefébure <i>et al.</i> (2006)	

697
698 **Figure captions**
699

700 **Figure 1** Global distributions of *Typhlatya* and *Stygiocaris*. The inset shows the
701 distribution of *Typhlatya* in the Caribbean region. See Table 1 for the precise
702 distribution of each taxon. *Typhlatya mitchelli* and *T. pearsei* are broadly distributed
703 throughout the northern Yucatán Peninsula, and are shown schematically.

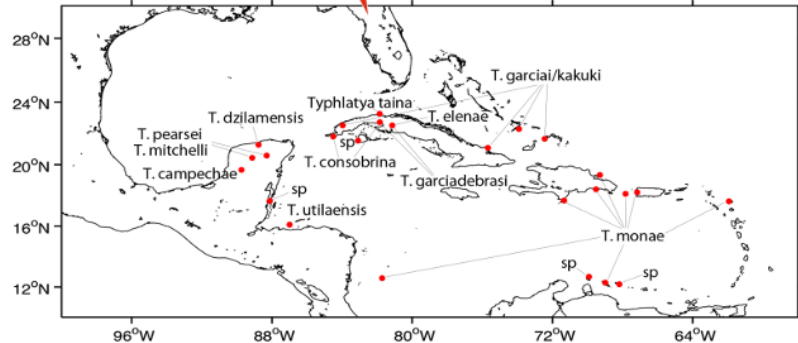
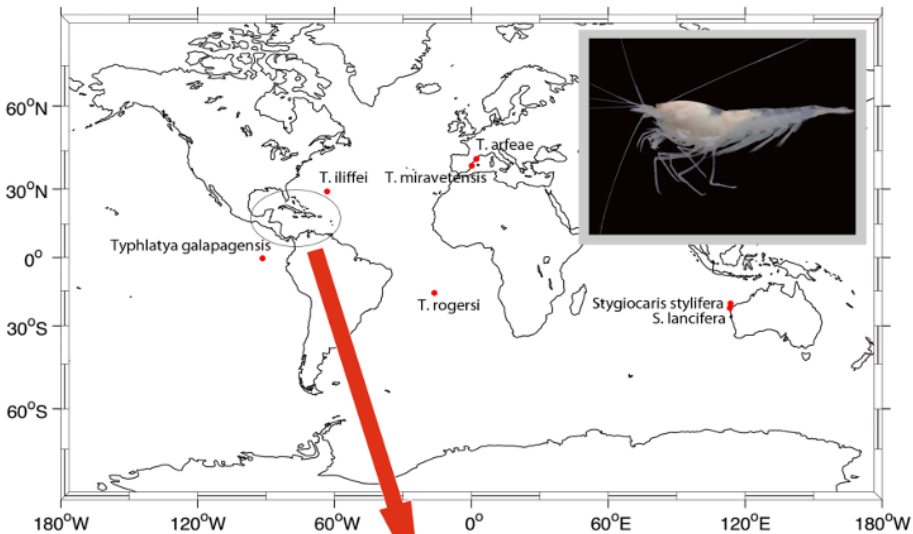
704 **Figure 2** Bayesian phylogram of *Typhlatya* / *Stygiocaris* and related genera based on
705 *rrnL*, *LSU* and histone *H3A* sequences. Numbers beside nodes show Bayesian posterior
706 probabilities.

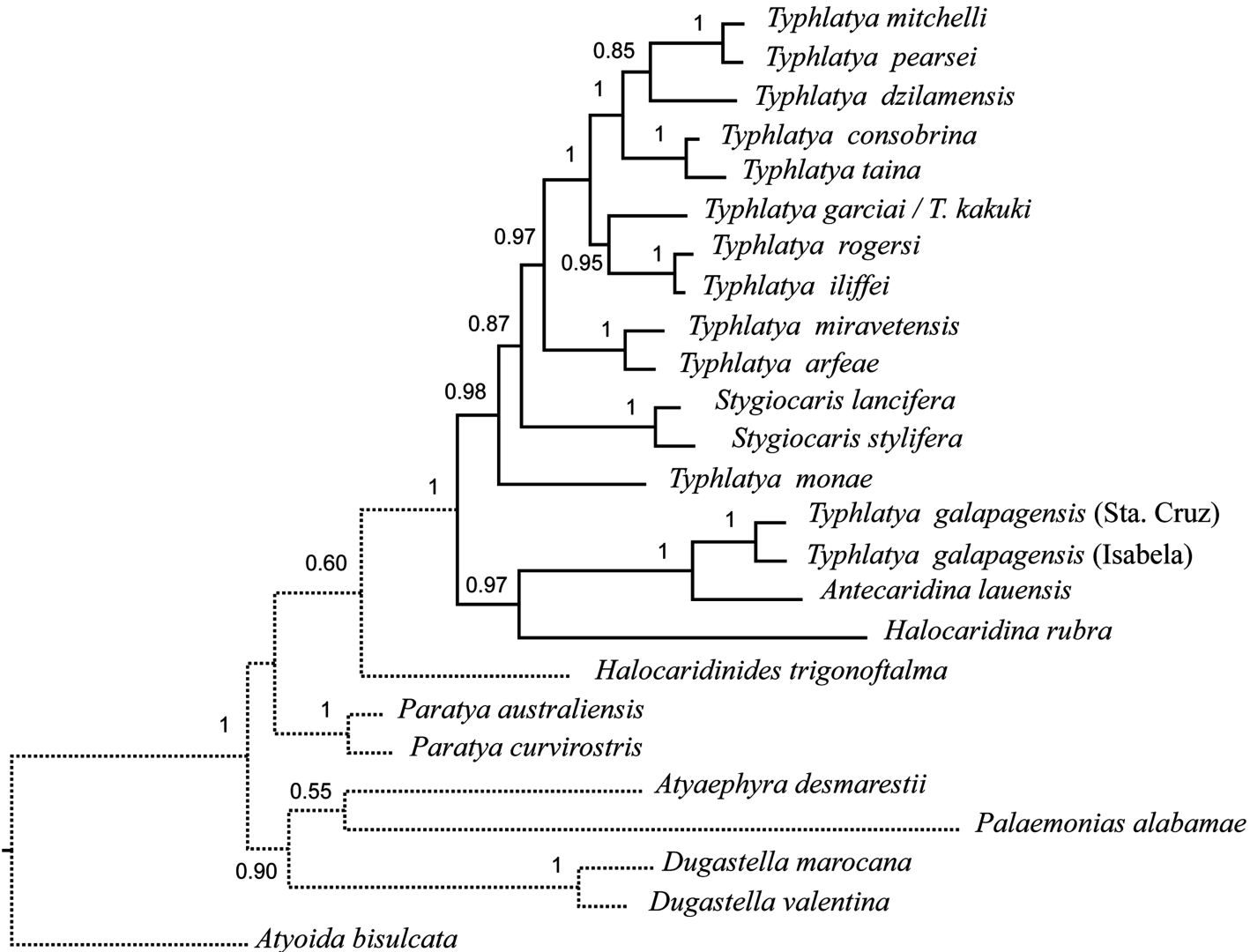
707 **Figure 3** Bayesian phylogram showing the relationships among the *Typhlatya* /
708 *Stygiocaris* species based on *rrnL*, *COI*, *cyt b*, *LSU*, *SSU* and histone *H3A* sequences,
709 with *Halocaridina rubra* and *Antecaridina lauensis* as the outgroups. The numbers
710 above the nodes show the Bayesian posterior probabilities, and those below the nodes
711 show the bootstrap support values estimated with maximum likelihood.

712 **Figure 4** Chronogram showing the estimated age ranges (Ma; 95% high posterior density
713 limits as confidence intervals) of the cladogenetic events within the *Typhlatya*–
714 *Stygiocaris*–*Antecaridina*–*Halocaridina* lineage. Asterisks indicate the nodes used as
715 calibration points (see text for details).

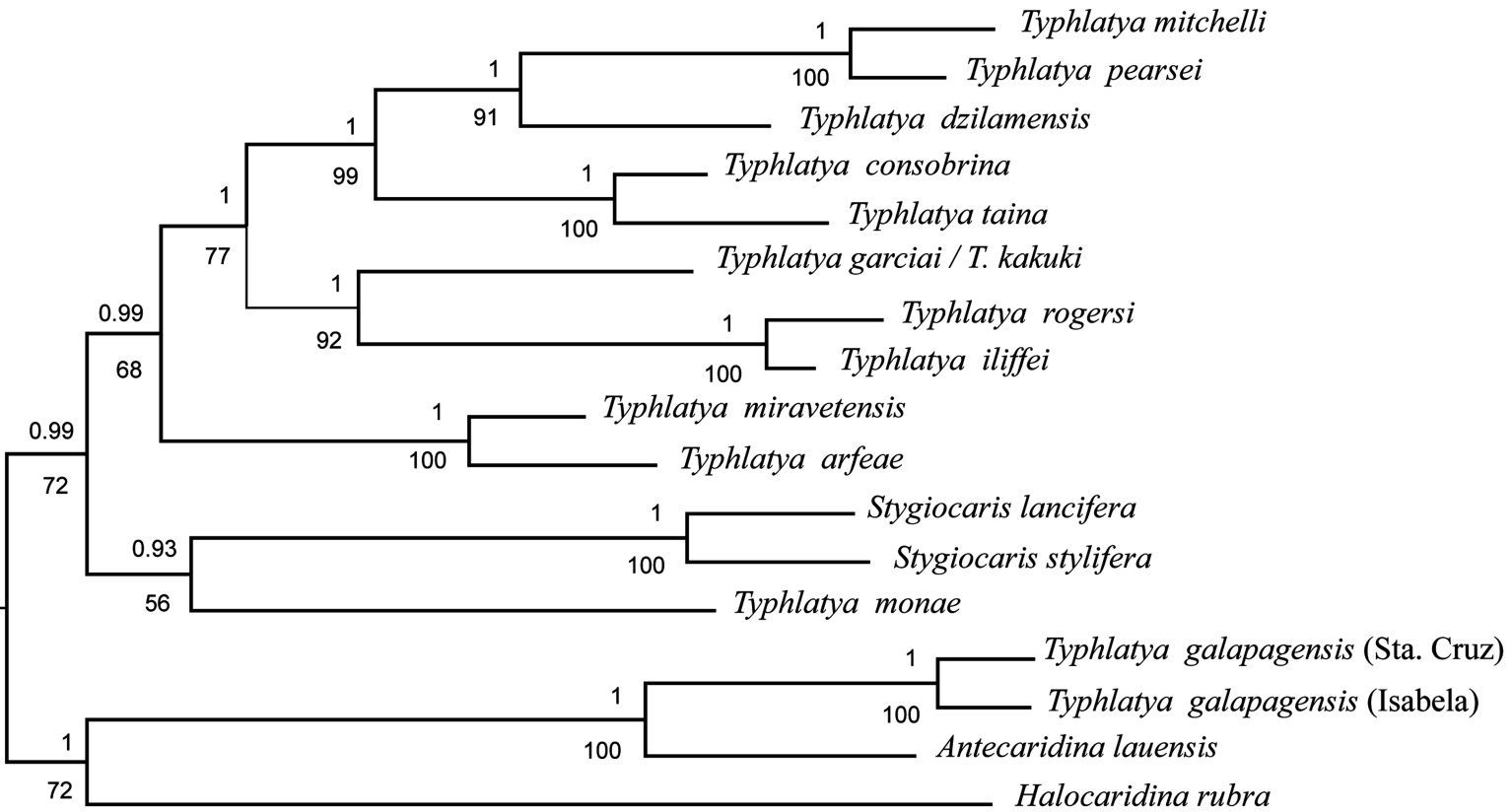
716 **Figure 5** Plot showing the mean age estimates (Myr) for the nodes shown in Fig. 4,
717 obtained with three different calibration points (see text for details). Black broken lines
718 show the 95% high posterior density limits for the node ages using the three-point
719 combined calibration.

720

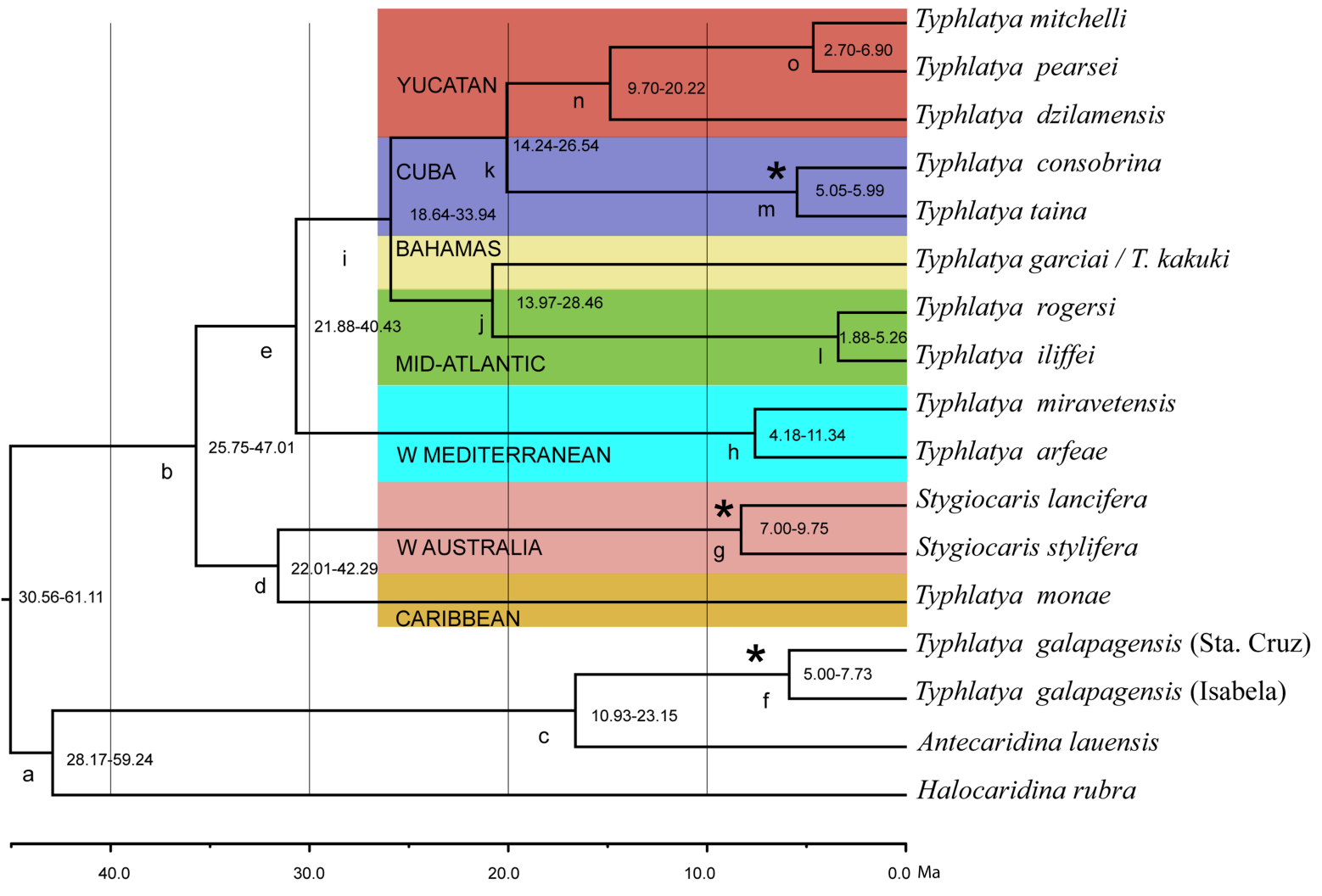




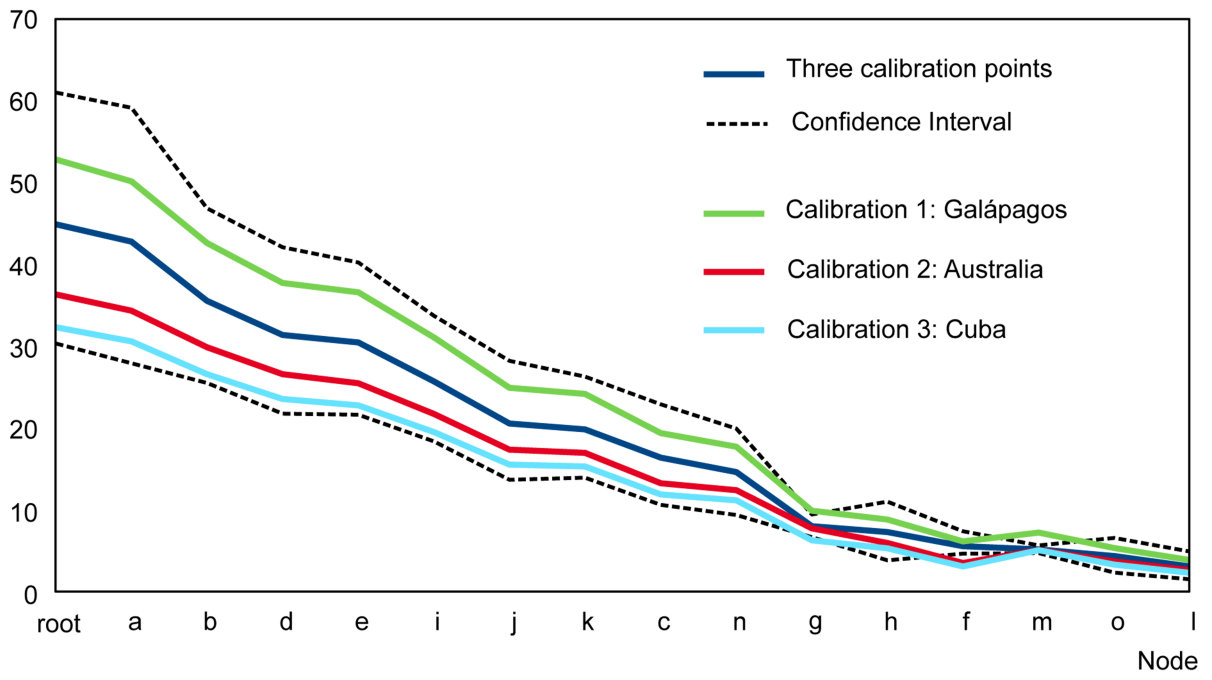
0.09 nt substitutions



0.07 nt substitutions



Node age (Myr)



Appendix S1 Percentage of uncorrected pairwise genetic distance among the 29 COI sequences of *Halocaridina*, *Antecaridina*, *Stygiocaris*, and *Typhlatya* species studied here. Both COI fragments were combined to estimate the distances. Bold numbers highlight intra-specific divergences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
1 <i>H. rubra</i>	-																														
2 <i>A. lauensis</i>	19.49	-																													
3 <i>T. galapag.</i> (Sta Cruz)	22.59	16.59	-																												
4 <i>T. galapag.</i> (Isabela)	22.09	17.14	8.01	-																											
5 <i>S. lancifera</i>	22.06	19.02	18.29	18.16	-																										
6 <i>S. stylifera</i>	19.50	19.16	19.94	18.59	14.78	-																									
7 <i>T. mitchelli</i>	22.72	22.32	20.27	19.16	18.98	20.65	-																								
8 <i>T. pearsei</i>	22.16	20.61	19.93	18.82	17.66	19.38	10.28	-																							
9 <i>T. dzilamensis</i>	20.36	19.80	21.70	20.74	19.81	19.45	17.42	16.32	-																						
10 <i>T. iliffei</i>	21.53	21.45	21.91	23.53	21.42	19.99	21.87	20.99	23.84	-																					
11 <i>T. arfeae</i>	18.89	19.14	21.23	18.70	18.60	17.37	19.82	18.63	18.22	20.58	-																				
12 <i>T. monae</i> (J. Dolio)-1	20.38	19.05	19.59	19.70	17.25	17.52	19.05	18.10	19.56	20.09	17.35	-																			
13 <i>T. monae</i> (J. Dolio)-9	20.60	18.11	18.86	19.33	17.17	17.40	20.21	17.80	19.71	21.07	16.52	0.00	-																		
14 <i>T. monae</i> (J. Dolio)-8	20.60	18.11	18.86	19.33	17.17	17.40	20.21	17.80	19.71	21.07	16.52	0.00	0.00	-																	
15 <i>T. monae</i> (Oviedo)-2	20.88	18.33	19.30	19.99	17.02	17.85	20.16	18.00	19.57	21.27	16.73	0.18	0.19	0.19	-																
16 <i>T. monae</i> (Samaná)-7	20.60	18.11	18.86	19.33	17.17	17.40	20.21	17.80	19.71	21.07	16.52	0.00	0.00	0.00	0.19	-															
17 <i>T. monae</i> (P. Rico)-3	20.25	19.02	19.68	19.69	17.13	18.32	19.83	18.13	19.54	20.42	17.72	0.00	0.00	0.00	0.22	0.00	-														
18 <i>T. monae</i> (P. Rico)-4	20.26	19.21	19.77	19.78	17.24	18.55	19.64	18.13	19.13	20.51	17.81	0.18	0.44	0.44	0.23	0.44	0.18	-													
19 <i>T. monae</i> (P. Rico)-5	20.15	19.15	19.90	19.85	17.29	18.54	19.90	18.32	19.15	20.78	18.11	0.19	0.43	0.43	0.23	0.43	0.19	0.00	-												
20 <i>T. monae</i> P. Rico 6	20.28	17.98	19.01	19.34	16.98	18.13	22.04	17.85	19.73	21.64	17.11	0.00	0.00	0.00	0.21	0.00	0.00	0.44	0.43	-											
21 <i>T. rogersi</i>	20.37	20.39	21.91	22.87	21.28	22.37	21.37	20.83	24.43	9.23	20.72	20.28	21.51	21.51	21.61	21.51	20.34	20.52	21.07	21.54	-										
22 <i>T. garciai</i>	18.03	19.14	21.11	20.75	21.35	22.02	21.54	20.58	17.47	20.67	18.91	20.14	20.00	20.00	20.37	20.00	19.76	19.77	19.74	19.25	22.66	-									
23 <i>T. kakuki</i>	16.89	16.99	19.29	20.06	19.41	21.17	21.92	20.04	17.92	18.22	17.50	19.21	19.42	19.42	19.65	19.42	19.25	19.28	19.30	19.47	21.96	0.42	-								
24 <i>T. consobrina</i> (Veral)	21.49	19.94	21.10	18.95	18.33	19.40	17.87	17.52	19.23	20.73	19.68	19.22	18.76	18.76	19.03	18.76	19.31	19.49	19.75	19.02	20.98	18.18	15.97	-							
25 <i>T. consobr.</i> (Bolondrón)	21.22	18.60	20.62	18.73	17.94	18.54	17.88	16.83	17.44	20.03	18.79	18.21	17.77	17.77	18.21	17.77	18.55	18.73	19.13	18.34	19.86	17.94	15.90	4.70	-						
26 <i>T. taina</i>	19.88	19.63	18.62	17.95	17.45	19.73	17.46	17.38	16.46	21.52	18.53	17.46	18.07	18.07	17.95	18.07	17.83	17.65	17.77	18.89	21.37	18.04	16.36	12.25	11.53	-					
27 <i>T. miravet.</i> (Cabanes)	19.43	20.15	20.39	18.45	18.75	17.49	18.11	16.87	18.55	19.81	10.08	16.00	14.58	14.58	15.16	14.58	16.34	16.44	16.55	15.28	20.23	17.52	15.78	17.49	17.16	17.07	-				
28 <i>T. miravet.</i> (Peñíscola)	18.84	21.69	20.38	19.04	18.03	n/a	18.31	17.69	n/a	20.32	10.41	18.12	n/a	n/a	n/a	n/a	18.15	18.14	18.86	n/a	20.14	20.25	n/a	18.88	18.15	17.68	3.24	-			
29 <i>T. miravet.</i> (Xivert)	19.14	21.79	22.10	20.13	17.00	n/a	18.64	17.95	n/a	20.66	11.31	17.70	n/a	n/a	n/a	n/a	17.74	17.73	18.38	n/a	20.13	19.38	n/a	19.50	17.70	16.77	3.13	1.20	-		

Appendix S2. Mean ages for the tree nodes of Figure 4 in Myr plus lower and higher confidence interval values estimated from 95% high posterior densities for three different calibration points and their combined analysis. Calibration 1: 5–14 Ma as the age interval of the MRCA of *Typhlatya galapagensis* from Santa Cruz and Isabela islands in the Galapagos (Werner *et al.*, 1999); calibration 2: age interval of the MRCA of *Stygiocaris lancifera* and *S. stylifera* explained by the emergence of the Cape Range anticline in NW Australia at 7–10 Ma (Page *et al.*, 2008) and calibration 3: 5–6 Ma as the age interval of the MRCA of *Typhlatya consobrina* and *T. taina* using the occlusion of the Havana-Matanzas Channel in Cuba (Iturralde-Vinent *et al.*, 1996). The last three rows show the inferred molecular rates per million years per lineage for different gene partitions ($\times 10^{-2}$).

Tree nodes	Calibration point 1			Calibration point 2			Calibration point 3			The three calibration points combined		
	mean	lower	upper	mean	lower	upper	mean	lower	upper	mean	lower	upper
root	52.97	25.28	90.16	36.53	20.52	55.71	32.54	18.09	48.51	45.08	30.56	61.11
a	50.30	24.23	86.47	34.57	18.37	53.29	30.84	16.38	46.44	42.96	28.17	59.24
b	42.80	19.66	73.60	30.12	17.90	44.55	26.82	15.96	39.06	35.75	25.75	47.01
c	19.66	9.20	33.74	13.57	7.22	21.11	12.18	6.53	18.51	16.66	10.93	23.15
d	37.92	16.50	65.55	26.85	15.35	39.60	23.82	13.70	35.15	31.62	22.01	42.29
e	36.78	16.93	63.24	25.74	14.96	38.10	23.04	13.87	33.29	30.72	21.88	40.43
f	6.48	5.00	9.95	3.85	1.66	6.35	3.42	1.51	5.57	5.91	5.00	7.73
g	10.22	3.74	18.86	8.08	7.00	9.65	6.63	3.46	10.21	8.33	7.00	9.75
h	9.14	3.66	16.58	6.31	3.02	10.24	5.63	2.71	9.00	7.63	4.18	11.34
i	31.30	14.28	54.19	22.01	13.21	32.81	19.78	12.05	28.22	25.95	18.64	33.94
j	25.16	10.77	44.02	17.63	9.86	26.69	15.84	9.23	23.34	20.84	13.97	28.46
k	24.43	10.93	42.74	17.27	9.87	25.56	15.60	9.71	22.25	20.12	14.24	26.54
l	4.21	1.45	7.88	2.98	1.38	4.85	2.67	1.30	4.24	3.45	1.88	5.26
m	7.55	2.52	14.15	5.46	2.72	8.64	5.42	5.00	5.92	5.52	5.05	5.99
n	17.99	7.57	31.52	12.73	6.92	19.31	11.48	6.68	16.83	14.91	9.70	20.22
o	5.69	2.15	10.41	4.06	2.02	6.50	3.66	1.85	5.68	4.70	2.70	6.90
Molecular evolutionary rates												
Mitochondrial protein coding genes (COI + Cyt b)	4.52	1.76	7.45	6.18	3.25	9.64	6.97	3.67	11.01	5.00	3.08	7.19
<i>rrnL</i> (16 rRNA)	0.87	0.34	1.45	1.19	0.63	1.83	1.31	0.73	1.99	0.96	0.63	1.33
Nuclear genes combined	0.15	0.07	0.24	0.21	0.12	0.31	0.24	0.14	0.35	0.17	0.12	0.22

