

1 **Evolution in caves: Darwin's "wrecks of ancient life" in the**  
2 **molecular era**

3

4 Carlos Juan<sup>1</sup>, Michelle T. Guzik<sup>2</sup>, Damià Jaume<sup>3</sup> and Steven J.B. Cooper<sup>2,4</sup>

5

6 <sup>1</sup> Departament de Biologia, Universitat de les Illes Balears, 07122 Palma de Mallorca,  
7 (Balearic Islands) Spain.

8 <sup>2</sup> Australian Centre for Evolutionary Biology and Biodiversity and School of Earth and  
9 Environmental Science, The University of Adelaide SA 5005, Australia.

10 <sup>3</sup>IMEDEA (CSIC-UIB), Mediterranean Institute for Advanced Studies, c/ Miquel  
11 Marquès 21, 07190 Esporles (Balearic Islands), Spain.

12 <sup>4</sup>Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, South  
13 Australia 5000, Australia.

14

15 Corresponding author: Carlos Juan, Departament de Biologia, Universitat de les Illes  
16 Balears, 07122 Palma de Mallorca, (Balearic Islands) Spain. Fax: +34971173184, e-  
17 mail: cjuan@uib.es

18

19 **Key words:** Subterranean, cave animals, phylogeography, cryptic species, speciation,  
20 vicariance and dispersal

21

22 **Running title:** Evolution in caves

23

24 **Abstract**

25 Cave animals have historically attracted the attention of evolutionary biologists due to  
26 their bizarre "regressive" characters and convergent evolution. However, understanding  
27 of their biogeographic and evolutionary history, including mechanisms of speciation,  
28 has remained elusive. In the last decade, molecular data have been obtained for  
29 subterranean taxa and their surface relatives, which have allowed some of the classical  
30 debates on the evolution of cave fauna to be revisited. Here we review some of the  
31 major studies, focusing on the contribution of phylogeography in the following areas:  
32 biogeographic history and the relative roles of dispersal and vicariance, colonisation  
33 history, cryptic species diversity and modes of speciation of cave animals. We further  
34 consider the limitations of current research and prospects for the future.

35 Phylogeographic studies have confirmed that cave species are often cryptic, with highly  
36 restricted distributions, but have also shown that their divergence and potentially  
37 speciation may occur despite the presence of gene flow from surface populations.  
38 Significantly, phylogeographic studies have provided evidence for speciation and  
39 adaptive evolution within the confines of cave environments, questioning the  
40 assumption that cave species evolved directly from surface ancestors. Recent technical  
41 developments involving "next generation" DNA sequencing and theoretical  
42 developments in coalescent and population modelling are likely to revolutionise the  
43 field further, particularly in the study of speciation and the genetic basis of adaptation  
44 and convergent evolution within subterranean habitats. In summary, phylogeographic  
45 studies have provided an unprecedented insight into the evolution of these unique fauna  
46 and the future of the field should be inspiring and data rich.

## 47 **Introduction**

48 Darwin (1859) identified cave animals, which often show peculiar convergent  
49 “regressive” traits, such as absence of eyes and lack of body pigment, as “*wrecks of*  
50 *ancient life*”. Indeed, the singularity of subterranean fauna has stimulated the  
51 imagination of several generations of biologists since the discovery of the first cave  
52 species, the salamander *Proteus anguinus* Laurenti, 1768 (Barr 1967; Poulson & White  
53 1969; Culver 1970). Cave ecosystems have been considered natural ecological and  
54 evolutionary laboratories due to the relative simplicity of their communities and the  
55 temporal and spatial isolation of their biota (Poulson & White 1969). Thus, cave  
56 animals can help in our understanding of evolutionary processes and historical factors  
57 related to biogeography and speciation in a similar manner to that of isolated oceanic  
58 island biotas (Cooper *et al.* 2007; Page *et al.* 2008). More than 7000 aquatic  
59 subterranean species (stygbionts) are known (Gibert & Deharveng 2002), whereas  
60 estimates of the number of terrestrial cave taxa (troglonits) point to a minimum of  
61 21000 worldwide (Juberthie & Decu 1994). These figures are likely to be an  
62 underestimation since caves and other subterranean environments are poorly studied and  
63 these habitats in many regions of the world still remain to be explored (Gibert &  
64 Deharveng 2002).

65       Historically, many hypotheses concerning the evolution of cave animals have  
66 proven to be controversial and some are still a matter of debate. These include the  
67 climate relict vs. ecological/parapatric modes of speciation by adaptive shift (Howarth  
68 1973; Holsinger 2000; Rivera *et al.* 2002; Wessel *et al.* 2007), the role played by  
69 dispersal versus vicariance to explain subterranean biogeographic patterns (see Porter  
70 2007 for a review), and selective (Yamamoto & Jeffery 2000; Jeffery 2005; Romero &  
71 Green 2005) vs. neutral (Kosswig 1960; Culver & Wilkens 2000; Leys *et al.* 2005)

72 hypotheses to explain regressive evolution (see Jeffery 2009 and Wilkens 2010 for  
73 recent reviews in cave fish). Finally, the irreversibility of ecomorphological adaptations  
74 to the subterranean (hypogean) habitat, and the possibility of recolonization of surface  
75 (epigean) habitats by hypogean lineages have been recently debated as well  
76 (Humphreys 2000; Volschenk & Prendini 2008; Prendini *et al.* 2010; Collin & Miglietta  
77 2008).

78         In the last decade, a wealth of molecular data has been gathered from both  
79 terrestrial and aquatic subterranean taxa (see Table 1 for a list of publications). These  
80 data have allowed the testing of hypotheses on biogeography and modes of subterranean  
81 evolution within explicit phylogenetic frameworks, distinguishing the role played by  
82 ecological adaptations and by historical factors in the evolution of cave faunas (Arnedo  
83 *et al.* 2007; Juan & Emerson 2010). Here we review the major contributions that  
84 molecular data have made in the field of subterranean biology, describing recent case  
85 studies from the literature. We also assess the limitations of current studies and discuss  
86 future prospects and directions in this emerging area. We focus on studies in the fields  
87 of biogeography/phylogeography and speciation, complementing a recent review of the  
88 neutral vs. selection hypotheses of regressive evolution (Wilkens 2010).

89

## 90 **The biogeographic history of subterranean animals: the role of dispersal and** 91 **vicariance**

92 There has been a long and recurrent debate on the relative roles played by vicariance  
93 and dispersal in the biogeography of subterranean taxa (see Culver *et al.* 2009 for a  
94 historical review). Traditionally, the extremely localised distributions of many  
95 subterranean taxa have been explained by their highly reduced dispersal capabilities due  
96 to intrinsic (low vagility, high specialization, small size) and extrinsic (habitat

97 fragmentation) factors (Crouau-Roy 1989; Holsinger 1991; Gibert *et al.* 1994). The  
98 relative contribution of dispersal and vicariance varies among geographical regions and  
99 between terrestrial and aquatic subterranean taxa, the latter usually showing broader  
100 ranges due to the higher connectivity between subterranean aquifers compared to karstic  
101 terrestrial habitats (Porter 2007, Culver *et al.* 2009).

102         A combination of dispersal and vicariance events are suggested to have  
103 influenced the biogeographic history of peri-Thyrranian stenasellid isopods (Ketmaier *et*  
104 *al.* 2003). The detachment of the Corsico-Sardinian microplate from the Pyrenees  
105 explains an old divergence in these isopods (between *Stenasellus racovitzai* and the  
106 mainland *S. virei*), while dispersal accounts for the unexpected low genetic divergence  
107 found between mitochondrial cytochrome oxidase subunit 1 (*cox1*) sequences of *S.*  
108 *racovitzai* lineages of Tuscany and Corsica (with an estimated divergence time of 2 to  
109 0.5 Mya, thus long after than the separation of the island from the Italian Peninsula).  
110 These results are based on a calibration of the molecular clock using two different  
111 geological events; the split of Corsica–Sardinia from the Pyrenees 29 million years ago  
112 (Mya), and the separation between Sardinia and Corsica (9 Mya).

113         The extreme disjunct global distributions displayed by many stygobiont  
114 crustaceans are thought to result from plate tectonic vicariant events that subdivided  
115 widely distributed ancestral populations once living along the margins of late Mesozoic  
116 seas (the full Tethyan track hypothesis, see Fig. 1, Stock 1993; Jaume & Christenson  
117 2001). To date, this hypothesis has not been well explored using molecular methods.  
118 However Page *et al.* (2008) recently used mitochondrial and nuclear sequences to study  
119 the endemic Western Australian subterranean shrimp genus *Stygiocaris* (Atyidae) and  
120 its relationship with other Australian and North Atlantic relatives. These authors  
121 conclude that the closest relative to the West Australian *Stygiocaris* is the Mexican cave

122 genus *Typhlatya*. Despite the presumed dispersal capacity of these shrimps during their  
123 larval stages, molecular data suggested that the two sister groups derived from an  
124 ancient vicariant event, namely the cessation of migration in populations of a coastal  
125 marine ancestor about 19 Mya by the closure of the Tethys Seaway following tectonic  
126 plate movements. Future molecular analyses should confirm if the extreme disjunct  
127 distributions observed in other anchialine (caves flooded by a mixture of marine water  
128 and terrestrial fresh groundwater lacking surface connections to the sea) groups of  
129 presumed Tethyan distribution, such as remipedes and some ostracod, copepod and  
130 thermosbaenacean crustaceans, have parallel genetic divergences dating back to the  
131 Tethys sea. However, it can be anticipated that a combination of mitochondrial and  
132 conserved nuclear DNA sequences in conjunction with different calibration points based  
133 on geological datings will be needed to robustly test this hypothesis.

134 Vicariance followed by secondary contact also seem to have operated over a  
135 more recent time-scale in *Stygiocaris*, with evidence provided for an east-west  
136 separation of the two currently recognized species, with the occurrence of a zone of  
137 sympatry in the northern portion of the Cape Range peninsula in Western Australia.  
138 This pattern has been related to isolation by the orogenic upraising of the peninsula  
139 followed by limited secondary contact during low sea-level stands (Page *et al.* 2008).  
140 One case demonstrating the occurrence of limited dispersal and coexistence of closely  
141 related subterranean taxa was recently revealed by Santos (2006) and Craft *et al.* (2008),  
142 who found low levels of gene flow and a high level of genetic differentiation in  
143 populations of the endemic Hawaiian anchialine shrimp *Halocaridina rubra* (Atyidae)  
144 on and between islands of the archipelago. Nevertheless, cases are known among  
145 anchialine stygobionts with a broad distribution where population structuring is  
146 virtually non-existent, suggesting the occurrence of panmixia mediated by marine

147 dispersal. This is the case, for example, in the endemic Hawaiian alpheid shrimp  
148 *Metabetaeus lohena* (Russ *et al.* 2010), with populations on O'Ahu, Maui and Hawaii;  
149 or in the neritilid gastropod *Neritilia granosa*, with populations set 200 km apart in the  
150 Philippines (Kano & Kase 2004).

151 Marine transgressions/regressions have been proposed to generate endemic  
152 inland freshwater species derived from a common marine ancestor (Notenboom 1991;  
153 Holsinger 1994, 2000). The origin of many stygobiontic taxa (stygofauna) whose  
154 distribution falls within areas formerly covered by ancient seas have been explained by  
155 the stranding of populations of a marine ancestor in inland groundwaters followed by  
156 divergence and speciation as the sea retreats (Zakšek *et al.* 2007). However, some cases  
157 of direct surface freshwater derivation in presumed lineages of marine origin have been  
158 revealed by molecular analyses. Mitochondrial and nuclear phylogenies have shown  
159 that three different lineages (France-Iberian Peninsula, Dinaric-Caucasian and Western  
160 Caucasus clades) of the South European atyid cave shrimp *Troglocaris* are related to  
161 three different surface ancestors. A freshwater common ancestor of Caucasian and  
162 Dinaric cave shrimps was dated at 6–11 Myr, contemporary with the brackish and  
163 freshwater phase of the Paratethys (Zakšek *et al.* 2007). The same holds for two  
164 subterranean atyid genera from northern Australia (*Parisia*, *Pycnisia*), closely related to  
165 surface freshwater species of *Caridina* of the same region (Page *et al.* 2007, 2008).

166 In a comparative phylogeographic analysis, Trontelj *et al.* (2009) used  
167 mitochondrial and nuclear gene sequences from six freshwater subterranean species  
168 showing apparently broad ranges on the European mainland: *Niphargus virei* and *N.*  
169 *rhenorhodanensis* (Amphipoda, Niphargidae), *Troglocaris anophthalmus*, *T.*  
170 *hercegovinensis* and *Spelaecaris pretneri* (Decapoda, Atyidae) and *Proteus anguinus*  
171 (Amphibia, Caudata, Proteidae). They uncovered substantial levels of cryptic diversity

172 within each of these species (see the section on cryptic species below), with most of the  
173 identified lineages having narrow distributions. These findings lend support to the  
174 hypothesis that stygofauna generally have very narrow distributions due to  
175 hydrographical isolation and their low dispersal abilities.

176         Several molecular studies have been carried out on the recently discovered and  
177 largely unknown subterranean stygofauna of the isolated terrestrial limestone aquifers  
178 (called calcretes) of inland Western Australia. This faunistic assemblage includes  
179 Dytiscidae (diving-beetles) (Cooper *et al.* 2002; Leys *et al.* 2003; Leys & Watts 2008),  
180 Amphipoda (Cooper *et al.* 2007; Finston *et al.* 2007), Isopoda (Cooper *et al.* 2008;  
181 Finston *et al.* 2009) and Bathynellacea crustaceans (Guzik *et al.* 2008). The molecular  
182 phylogenies obtained from sixty species of diving beetles belonging to the tribes  
183 Bidessini and Hydroporini (Coleoptera: Dytiscidae), together with relaxed molecular  
184 clock calibrations, showed that individual calcrete aquifers in the Yilgarn region of  
185 Western Australia contain distantly related lineages derived from surface ancestors that  
186 independently colonised the calcretes between 9-4 Mya (Leys *et al.* 2003). This pattern  
187 of relict lineages restricted to individual calcretes has been corroborated using *coxI*  
188 mitochondrial sequence data (with some uncertainties still remaining due to lack of  
189 known surface ancestors among other factors) from subterranean amphipods  
190 (Paramelitidae and Chiltoniidae; Cooper *et al.* 2007), oniscoidean isopods (*Haloniscus*,  
191 Cooper *et al.* 2008) and bathynellid syncarids (Guzik *et al.* 2008). Isolation of calcrete  
192 populations was deduced to have occurred at least since the Pliocene, coinciding with  
193 the occurrence of a major aridity phase that led to the intermittent drying up of surface  
194 waters (Byrne *et al.* 2008). Other recent molecular studies of subterranean amphipods  
195 and isopods carried out in the Pilbara region of Western Australia (Finston *et al.* 2007;  
196 2009) show again that diversification among major lineages is consistent with the onset



197 of arid conditions during the Pliocene (data deduced from *cox1* mitochondrial  
198 phylogenies), a result consistent also with the estimated dates for central Western  
199 Australia (Yilgarn region). However, contrasting colonisation patterns are deduced in  
200 the two groups: the phylogeny is compatible with a single common ancestor in the  
201 amphipod diversification, while multiple invasions explain the divergent lineages found  
202 in *Pygolabis* isopods (Finston *et al.* 2009).

203         The study of genetic variation in insects, in particular beetles, was initiated in the  
204 1970's by pioneering surveys using allozymic markers (e. g. Carmody *et al.* 1972; Laing  
205 *et al.* 1976; Cockley *et al.* 1977; Caccone & Sbordoni 1987; Crouau-Roy 1989).  
206 Molecular DNA phylogenies have also been obtained in the last few years for many  
207 terrestrial cave animals including beetles, isopods, crickets, pseudoscorpions, spiders,  
208 and salamanders (see references in Table 1). Recent studies on terrestrial cave beetles  
209 suggest that the present distributions of some taxa tend to reflect those of ancestral  
210 surface ancestors and, presumably, could be caused by ancient vicariant events  
211 involving the establishment of barriers to dispersal in the subterranean domain (Faille *et*  
212 *al.* 2010; Ribera *et al.* 2010, see section on speciation within subterranean habitats  
213 below and Fig. 2).

214

### 215 **Colonisation history of subterranean animals: insights from phylogeography**

216 Understanding the evolutionary processes that lead to morphological specialization, and  
217 the genetics of speciation in cave animals require simple case-study models where  
218 recently evolved cave populations coexist with their presumed surface ancestors, as the  
219 early stages of the evolution of ancient subterranean taxa are difficult to reconstruct  
220 (Strecker *et al.* 2003). Phylogeographic frameworks and population genetic analyses can  
221 provide the basis for discriminating whether cave forms derive from a single

222 colonisation event followed by subterranean dispersal, or from multiple independent  
223 invasions of a fragmented subterranean habitat. This further allows discerning whether  
224 troglobiotic features (troglomorphies or ecomorphological adaptations to the hypogean  
225 habitat) have a single origin or appear by convergence, and can also be informative on  
226 the dynamics of gene flow between surface and/or cave populations.

227         One of the best model study systems that comprise closely related surface and  
228 cave populations is the neotropical fish *Astyanax fasciatus* (syn. *A. mexicanus* Filippi).  
229 This species is broadly distributed in Mexican surface waters, and includes many  
230 depigmented and blind-fish populations in karstic caves on the northeast of the country  
231 (Awise & Selander 1972; Mitchell 1977; Wilkens 1988; Espinasa & Borowsky 2001;  
232 Dowling *et al.* 2002; Strecker *et al.* 2003; 2004). The apparent independent evolution of  
233 cave populations and their co-existence and hybridisation with nearby surface  
234 populations have made it an ideal model system to explore the genetic basis of  
235 regressive and adaptive evolution of cave animals (see Wilkens 2010 and references  
236 therein). Mitochondrial cytochrome b (*cob*) data clearly show that several of the present  
237 cave populations of this fish originated from an ancestral genetic stock different from  
238 the current surface populations, and that a minimum of two independent invasions have  
239 occurred. The pattern is consistent with the extinction of surface populations, leaving  
240 behind relict underground populations due to Pleistocene climatic changes with  
241 subsequent recolonisation and expansion of surface populations from the south  
242 (Dowling *et al.* 2002; Strecker *et al.* 2003; 2004). Microsatellite data also suggest that  
243 the occurrence of repeated population bottlenecks in cave populations and of  
244 introgressive hybridization between surface and underground populations are a common  
245 feature in *Astyanax* (Strecker *et al.* 2003).

246         The Dinaric karst region of the Balkan Peninsula in Europe, host to the world's

247 richest subterranean fauna, also offers considerable potential for phylogeographic and  
248 population genetic analyses of related surface and cave populations (Sket *et al.* 2004).  
249 Although the composition of this fauna is known fairly well, molecular analyses have  
250 only recently been undertaken (e.g. Verovnik *et al.* 2004; Zakšek *et al.* 2009) and are  
251 offering unique insights into the evolutionary histories of cave species and how they  
252 have been influenced by the complex hydrographic history of the region. Verovnik *et al.*  
253 (2004) used *cox1* mitochondrial sequences from surface and troglobiontic populations  
254 of the crustacean isopod *Asellus aquaticus* to uncover its genetic variation and cave-  
255 surface relationships. In this study, samples grouped by hydrographic connection rather  
256 than by habitat, and at least three different colonisations of the caves were deduced to  
257 have occurred, probably after the ancestral surface populations were already  
258 differentiated by vicariance in this extremely fragmented karst habitat (Verovnik *et al.*  
259 2004).

260         The tropical snail genus *Georissa* (Hydrocenidae) in western Sabah, Malaysian  
261 Borneo, has a differentiated cave morph, which seems to be a direct derivative of the  
262 surface *G. saulae* (Schilthuizen *et al.* 2005). Connections between surface and  
263 subterranean populations have also been examined in the crayfish *Cambarus tenebrosus*  
264 (*rrnL* 16S rRNA mitochondrial gene) from the Cumberland Plateau of the southern  
265 Appalachians (Finlay *et al.* 2006). Phylogenetic and nested clade analyses demonstrated  
266 a lack of correspondence between habitat and phylogeny and no evidence for a recent  
267 invasion of the caves (Finlay *et al.* 2006).

268         *Palmorchestia hypogaea*, one of the few known terrestrial troglobiontic  
269 amphipods, occurs in lava tubes on the island of La Palma in the volcanic Canary  
270 archipelago. On the same island, a related surface form dwells in the leaf litter of the  
271 laurel forest (Stock & Martin 1988; Stock 1990). Villacorta *et al.* (2008) studied

272 populations of the surface and cave forms across the island using *cox1* and *cox2*  
273 mitochondrial sequences, revealing strong phylogeographic structuring and evidence for  
274 multiple invasions of the lava tubes. In cave and surface populations of the freshwater  
275 amphipod *Gammarus minus*, a similar pattern of spatial structuring, low genetic  
276 variation within caves or springs, and relatively high divergence among sites with  
277 limited gene flow have been reported in the Appalachian Mountains (Carlini *et al.*  
278 2009). As in the *Palmorchestia* study, population clustering is better explained by  
279 hydrological or geographic factors rather than by the type of habitat (i.e whether cave or  
280 surface), and there is some evidence for the past occurrence of repeated population  
281 bottlenecks in cave populations (Carlini *et al.* 2009).

282

### 283 **Assessing cryptic species diversity in cave environments using molecular data**

284 Recently, phylogeographic analyses have uncovered considerable levels of cryptic  
285 species diversity within subterranean ecosystems. "Cryptic species" usually refer to two  
286 or more taxa classified as a single species based on morphological similarity  
287 (Pfenninger & Schwenk 2007). These taxa can be a by-product of poor taxonomy  
288 (therefore "crypticism" often disappears when adequate morphological features are  
289 considered) or be independent evolutionary lineages showing morphological stasis.  
290 Convergent evolution resulting from exposure to similar selection pressures, or lack  
291 thereof, within the lightless subterranean environment, can produce genetically  
292 divergent but cryptic morphological lineages (e.g. Culver *et al.* 1995; Arntzen & Sket  
293 1997; Verovnik *et al.* 2003; Fišer *et al.* 2006; Hedin & Thomas 2010). In addition,  
294 convergence of form and adaptive modifications such as loss of traits (Porter &  
295 Crandall 2003) or their gain (Jones *et al.* 1992), can render evolutionary relationships  
296 elusive since the shared morphology may not reflect shared phylogenetic history (Wiens

297 *et al.* 2003). Cryptic species have been described for surface crustaceans (e.g. King &  
298 Hanner 1998; Witt & Herbert 2000; Penton *et al.* 2004) and this is also an emerging  
299 issue for subterranean crustaceans (Finston *et al.* 2007; Buhay & Crandall 2009; Zakšek  
300 *et al.* 2009) and salamanders (Wiens *et al.* 2003).

301 Molecular data provide new insights into the levels of species diversity in cave  
302 systems. Historically, allozyme genetic divergence data permitted the discrimination of  
303 geographically distant cave populations and the possible occurrence of cryptic species  
304 (Cesaroni *et al.* 1981; Sbordoni *et al.* 1981; Kane *et al.* 1992; Allegrucci *et al.* 1997).  
305 Subsequent advances in sequencing technology have led to the use of DNA sequence  
306 data for the identification of cryptic species (e.g. King & Hanner 1998; Witt & Herbert  
307 2000; Penton *et al.* 2004), particularly in subterranean crustaceans (Proudlove & Wood  
308 2003; Finston *et al.* 2007; Buhay & Crandall 2009; Zakšek *et al.* 2009) and salamanders  
309 (Wiens *et al.* 2003). The recent development of DNA barcoding thresholds for  
310 delineating species (16% divergence in *cox1*), genera and families based on crustacean  
311 sequence data has provided a new benchmark for species recognition (Lefébure *et al.*  
312 2006). While the of these thresholds, coupled with phylogeographical analyses, have  
313 been useful in revealing the possible presence of cryptic species of subterranean animals  
314 (Trontelj *et al.* 2009), their generalized application poses several problems. First,  
315 recently evolved species cannot be discriminated, with several studies demonstrating the  
316 occurrence of lower levels of genetic divergence between morphologically distinct  
317 species (Leys *et al.* 2003; Guzik *et al.* 2008; Trontelj *et al.* 2009). Second, systematic  
318 categories such as genus and family are morphologically defined entities that do not  
319 necessarily reflect any clear time-scale of evolution, particularly when different animal  
320 groups are compared. Finally, the high levels of saturation in DNA sequences at these  
321 higher taxonomic levels can pose additional problems. We conclude therefore, that the

322 use of molecular thresholds may have a place in delineating species taxonomic research,  
323 however, resolution above the species level is likely to be questionable.

324

### 325 **Modes of speciation in subterranean animals**

326 Two alternative models are proposed to explain speciation in cave faunas: the “climatic-  
327 relict” and the “adaptive-shift” hypotheses (Howarth 1973; Holsinger 2000; Rivera *et al.*  
328 2002). The first model was proposed for continental temperate ecosystems (Holsinger  
329 1988, 2000; Peck & Finston 1993) where invasion of the cave environment by surface  
330 species is coupled with an initial phase of gene flow between surface and cave  
331 populations. This phase is followed by speciation in strict allopatry (between cave and  
332 surface populations) after extinction of the epigeal populations due to climatic  
333 fluctuations (e.g. glaciations or aridity). However, during the 1970s a diverse cave fauna  
334 in the tropics was discovered along with some of the extant closely-related surface  
335 relatives, leading to the proposal of the “adaptive shift” hypothesis (Rouch &  
336 Danielopol 1987; Howarth 1987; Desutter-Grandcolas & Grandcolas 1996). This model  
337 assumes the occurrence of active colonisation of the subterranean environment  
338 accompanied by adaptive differentiation of surface and cave populations, reduction of  
339 gene flow and, eventually, parapatric speciation. A parapatric “adaptive-shift” model is  
340 reliant upon divergent natural selection, while the allopatric model requires an  
341 accumulation of neutral or “regressive” mutations, but may or may not involve adaptive  
342 shifts in relation to reproductive isolation. It is therefore the presence/absence of gene  
343 flow during divergence (and not selection) that is the key factor that allows  
344 discrimination between these two models (Niemiller *et al.* 2008). An additional question  
345 is whether reproductive isolation can be reached without the involvement of an  
346 allopatric phase to complete the speciation process.

347 Molecular phylogenies could, in theory, help to test these two hypotheses as they  
348 allow reconstruction of sister relationships between cave and surface taxa (Rivera *et al.*  
349 2002). In particular, phylogenetic contrasts coupled with the analysis of present  
350 geographical distributions (viz. surface and cave populations with allopatric  
351 distributions; relict subterranean species; or parapatric distributions of both surface and  
352 cave populations) can potentially distinguish between the above-mentioned hypotheses  
353 (Rivera *et al.* 2002; Leys *et al.* 2003). Additional tests have been proposed that correlate  
354 divergence times and branching patterns with geological/palaeo-climatic data (Leys *et*  
355 *al.* 2003). However, phylogenetic studies have some limitations, particularly when the  
356 divergence of cave-lineages are very old and their related surface relatives became  
357 extinct, or have departed from the region after the advent of environmental changes. In  
358 addition, molecular clock estimates are subject to considerable errors, as few reliable  
359 rate calibrations exist for cave animals, whereas information is very limited on whether  
360 rates of molecular evolution increase or decrease when animals live underground.  
361 Finally, single or a few loci only, are often used to reconstruct phylogenetic  
362 relationships, with gene tree divergence times not necessarily equating to species  
363 divergence times.

364 In the subterranean diving beetles of the arid zone of Western Australia, the  
365 transition to the subterranean environment, especially in sympatric sister-species pairs,  
366 was coincident in time with a period of aridity that commenced approximately 10-5  
367 Mya. This finding suggested that climate change (and thus a “climatic-relict” allopatric  
368 speciation model) could be the main factor inducing speciation (Leys *et al.* 2003). On  
369 the contrary, in Hawaiian terrestrial isopods studied using *cox1* sequences, Rivera *et al.*  
370 (2002) showed that the sister-relationship and parapatric distribution of cave and surface  
371 species of the genus *Littorophiloscia* could be explained by the occurrence of an

372 adaptive shift from a marine supralittoral to a subterranean terrestrial life-style.

373         Two recent studies, the first focused on tropical land snails (Schilthuizen *et al.*  
374 2005) and the other on North American salamanders (Niemiller *et al.* 2008), suggest the  
375 occurrence of speciation scenarios of “divergence with gene flow” (Rice & Horstert  
376 1993). An unnamed cave population of the land snail *Georissa* from Sabah (see above)  
377 showed a sister-relationship to the surface *G. saulae* based on *rrnL* mitochondrial  
378 sequences and shell characters. The occurrence of a morphologically intermediate  
379 population in the twilight zone of the cave showing both surface and cave haplotypes  
380 suggests the occurrence of gene flow between the surface and cave populations  
381 (Schilthuizen *et al.* 2005). Cases of subterranean taxa from volcanic caves with extant  
382 surface relatives living near the cave entrance are also reported from the Galapagos and  
383 Canarian archipelagoes (see Fig. 3 for a series of Canary Islands representative cave  
384 arthropods). Molecular phylogenies have revealed that many of them involve cases of  
385 sister-species relationship (Peck & Finston 1993; Arnedo *et al.* 2007; Contreras-Díaz *et*  
386 *al.* 2007; Villacorta *et al.* 2008). A parapatric mode of speciation with ecological shift  
387 via resource segregation has been suggested to be applicable in these cases.

388         In the Tennessee cave salamander species-complex of the genus *Gyrinophilus*  
389 (Plethodontidae), Niemiller *et al.* (2008) used mitochondrial (*rrnS* and *cob*) and nuclear  
390 (RAG-1) gene genealogies to show that the cave-dwelling populations have recently  
391 diverged from the surface species *G. porphyriticus* via local adaptation and ecological  
392 speciation. Cave salamanders have specific traits absent in spring salamanders such as  
393 reduced eyes, expanded lateral systems, permanent aquatic life cycle and  
394 pedomorphosis, the latter likely promoting premating isolation (Niemiller *et al.* 2008).  
395 Coalescent analyses using the program IM (Hey & Nielsen 2004) found significant  
396 evidence of gene flow from the surface *G. porphyriticus* into the cave *G. p. palleucus*



397 and *G. gulolineatus* populations, and from the subterranean *G. p. necturoides* to the  
398 surface *G. porphyriticus*, but failed to detect it among cave populations. This suggests a  
399 scenario of speciation by divergence with gene flow, a model that has been suggested in  
400 other non-subterranean systems (Rice & Hostert 1993; Coyne & Orr 2004).

401

#### 402 **Speciation within subterranean habitats**

403 Prior to recent molecular genetic studies, it had generally been viewed that subterranean  
404 species had reduced phenotypic and genetic diversity due to population bottlenecks  
405 resulting from their isolation in cave habitats and adaptation to a stable environment, a  
406 hypothesis attributed to White & Poulson (1968). This hypothesis suggested that there  
407 would be a limited capacity for subterranean species to undergo adaptive evolution and  
408 formation of new species. More recently, however, the occurrence of substantial  
409 intraspecific (population level) genetic diversity has been observed within cave species  
410 (Stepien *et al.* 2001; Buhay & Crandall 2005; Finlay *et al.* 2006; Lejeusne &  
411 Chevaldonné 2006; Guzik *et al.* 2009). These findings support the alternative view  
412 (Barr 1968) that after a reduction in diversity following the initial founder events during  
413 cave colonisation, genetic diversity can increase considerably as a subterranean species  
414 expands both its range and population size following colonisation of new uninhabited  
415 subterranean areas.

416       These above studies suggest that sufficient genetic variation can build up within  
417 subterranean species to allow for adaptive evolution and speciation. However, to date  
418 few studies have examined post-colonisation speciation processes within the  
419 subterranean habitat. One recent exception consists of a series of studies dealing with  
420 sympatric diving beetles from calcrete aquifers in Western Australia (Cooper *et al.*  
421 2002; Leys *et al.* 2003; Leys & Watts 2008; Guzik *et al.* 2009). These calcretes contain

422 at least 12-13 cases of sister-species pairs or triplets of diving-beetle species, where  
423 sympatric species fall into non-overlapping size classes. These findings suggest the  
424 presence of niche partitioning of beetle species within the calcrete and the potential they  
425 evolved by sympatric speciation (Cooper *et al.* 2002; Leys *et al.* 2003; Leys & Watts  
426 2008). However, several alternative modes of speciation also may explain this  
427 phylogenetic pattern. First, it is possible that sympatric sister species evolved following  
428 repeated colonisation events from the same ancestral species at different time periods, a  
429 process of allopatric divergence. Second, speciation may have occurred within the  
430 aquifer by allopatric divergence at a fine spatial scale (referred to as micro-allopatry),  
431 possibly through partitioning of the calcrete following water level fluctuations, or by a  
432 combination of allopatric/parapatric divergence. In a recent study Guzik *et al.* (2009)  
433 used fine-scale comprehensive sampling of a 3.5 km<sup>2</sup> grid of bore holes in a single  
434 aquifer in Western Australia and examined the mitochondrial gene genealogies of three  
435 sympatric sister species of diving beetles. These taxa comprise non-overlapping size  
436 classes and form a reciprocally monophyletic clade with respect to other subterranean  
437 and surface species of the genus *Paroster*, suggesting that they evolved within the  
438 calcrete from a single common ancestor (Watts & Humphreys 2006; Leys & Watts  
439 2008). The phylogeographic and population analyses undertaken suggested the  
440 occurrence of patchy genetic structures (at least in two of the three species) and that  
441 demographic histories had involved fluctuations in population size. Isolation by distance  
442 in the three species was deduced to have occurred as well. These results, added to the  
443 lack of detailed information on niche specialization for the triplet of species, suggest  
444 that an allopatric speciation model cannot be ruled out for these species. The dynamics  
445 of the environment, however, suggests that micro-allopatry is unlikely to persist for  
446 long periods of time, and that speciation might have occurred by a combination of

447 micro-allopatric and parapatric divergence (Guzik *et al.* 2009).

448 An additional case study suggesting the occurrence of intra-cave speciation  
449 involves sympatric troglobiont spider species of the genus *Dysdera* in the Canary  
450 Islands. These species often show segregation by body size into groups of large, mid-  
451 sized and small species coupled with cheliceral modifications, suggesting specialisation  
452 to different prey species (Arnedo *et al.* 2007). The case of the sister-species pair *D.*  
453 *hernandezii* and *D. esquivelei* in Tenerife is particularly relevant, since they are very  
454 similar both morphologically and genetically (*cox1* and *rrnL* sequences) with the  
455 exception of their cheliceral structure. These data suggest that they possibly evolved in  
456 sympatry by divergent selection of prey-capture strategies (Arnedo *et al.* 2007).

457 The recent use of molecular data has raised the possibility of occurrence of  
458 extensive allopatric speciation within the underground habitat, resulting from  
459 subterranean radiations (Faille *et al.* 2010; Ribera *et al.* 2010). Several beetle groups,  
460 Leiodidae (suborder Polyphaga) and Trechinae (suborder Adephaga), appear to  
461 represent large monophyletic lineages comprising mostly cave species. Faille *et al.*  
462 (2010) have used a combination of mitochondrial (*cox1*, *cob*, *rrnL*, *trnL*, *nad1*) and  
463 nuclear (SSU, LSU) sequences to study the phylogeography and speciation of Trechinae  
464 in the Pyrenees. The robust phylogeny obtained for this group demonstrated monophyly  
465 for all cave taxa in this mountain range, to the exclusion of all surface and all  
466 subterranean species from other geographical areas (Cantabrian and Iberian mountains,  
467 Alps). Within the Pyrenees, eastern and western splits were evident, with contrasting  
468 evolutionary patterns in each clade apparently related to differences in limestone  
469 structure (Fig. 2). In a similar study on Mediterranean Leptodirini (Coleoptera,  
470 Leiodidae), Ribera *et al.* (2010) revealed the presence of several monophyletic cave-  
471 dwelling lineages in the mountain ranges of the Iberian peninsula. These authors

472 proposed an ancient origin for the main Western Mediterranean lineages of Leptodirini,  
473 which would have derived from Early-Mid Oligocene (34-25 Mya) ancestors already  
474 adapted to subterranean life. Their findings contradict the traditional view that  
475 subterranean lineages invariably derive from multiple colonisations by surface  
476 ancestors.

477

#### 478 **Prospects for future research**

479 While phylogeographic and population genetic analyses have greatly contributed to our  
480 understanding of the evolutionary and biogeographic history of cave animals, many  
481 questions still remain, particularly in the areas of speciation and adaptive and regressive  
482 evolution. Recent theoretical developments in speciation modelling (Gavrilets & Vose  
483 2005; 2007; Gavrilets *et al.* 2007) and massive parallel sequencing techniques (next  
484 generation sequencing, Tautz *et al.* 2010) offer enormous potential in the study of these  
485 topics. Similarly, the development of more complex coalescence models (e.g. IMA, Hey  
486 2010) using multiple genetic markers to study dispersal and isolation processes, will  
487 allow a more rigorous assessment of the colonisation and population genetic history of  
488 subterranean animals.

489 Gavrilets *et al.* (2007) have shown that if several factors co-occur, such as few  
490 loci controlling local adaptation, intermediate selection and carrying capacity, and if  
491 there is a strong effect of non-random mating control loci, parapatric or sympatric  
492 (defined as a population where mating is random with respect to the birthplace of the  
493 mating partners, Gavrilets 2003, 2004) speciation may be possible. In cave ecosystems,  
494 the occurrence of strong selection has been demonstrated for several traits conferring an  
495 advantage in the subterranean environment, and these can be rapidly fixed in small  
496 populations (see Wilkens 2010 for a review in cave fish). However, recurrent gene flow

497 from surface to cave populations may break co-adapted gene complexes unless there is  
498 strong assortative mating, probably via the evolution of behavioural changes in  
499 reproductive traits. So-called “magic traits” (Gavrilets 2004), which are traits under  
500 diversifying selection that can simultaneously result in reproductive isolation, provide a  
501 basis for speciation to proceed to completion in the presence of gene flow, but their  
502 existence in cave animals is not well documented. Paedomorphosis in cave salamanders,  
503 where subterranean species retain gills allowing them to live permanently under water,  
504 may represent an example of a magic trait, as it also restricts the location of mating,  
505 which can occur outside the water in surface salamanders (Niemiller *et al.* 2008). In  
506 order to develop realistic individual-based models for the study of processes of  
507 colonisation of subterranean habitats and of subsequent speciation with gene flow in  
508 cave animals, good estimates of dispersal, levels of trait selection, number of loci  
509 involved, etc. are necessary from an appropriate model system. The latter should  
510 comprise cave and surface species preferably displaying parapatric distributions  
511 (Gavrilets *et al.* 2007).

512 Advances in parallel sequencing technology are starting to change the way we  
513 think about many disciplines in molecular biology and evolution (Tautz *et al.* 2010).  
514 Several exciting new approaches using large-scale genome sequencing can be envisaged  
515 in subterranean organisms. Some, but not all are: (i) scanning adaptation and  
516 convergence evolution at the genome level, and identifying the genetic basis of these  
517 processes, using suitable phylogenetic contrasts of cave and surface species, (ii) the  
518 assessment of gene-flow across the genome among surface and subterranean taxa to test  
519 the adaptive shift hypothesis, (iii) studying the genetics of phenotypic differences in  
520 cave and surface related forms through transcriptional profiling, (iv) the development of  
521 tools for rapid biodiversity assessment and detection of cryptic species based on

522 environmental sampling methodologies, and (v) using an across-the-genome multi-gene  
523 approach to obtain more reliable molecular datings and estimates of how long it takes  
524 for a particular subterranean species to evolve.

525 For all of these advances to be successful there is a need for study systems in  
526 which both cave and surface populations are of relatively recent origin and where the  
527 key biological, geographical and phylogenetic contexts are well established. Case  
528 studies lacking any evidence of the occurrence of allopatric phases would be  
529 particularly useful to study the likelihood of ecological speciation through the  
530 methodologies above mentioned, and this is expected to be more likely to occur among  
531 cave troglo- or stygobionts from the tropics and their relatives.

532

### 533 **Acknowledgements**

534 We are very grateful to Bill Humphreys, Brent Emerson and Pedro Oromí for their  
535 constructive comments on an early version of the manuscript. The valuable comments  
536 and discussions on the paper of three anonymous reviewers and the suggestions of  
537 Louis Bertnachez greatly improved the manuscript. Tom Iliffe, Sergio Montagud,  
538 Ignacio Ribera and Pedro Oromí kindly provided photographs for figures. Financial  
539 support has been received from the Ministerio de Ciencia e Innovación, Spain (projects  
540 CGL2006-01365 to CJ and CGL2009-08256 to DJ, co-financed with EU FEDER  
541 funds). CJ visits to the University of Adelaide (Australia) have been funded by the UIB,  
542 and to the University of East Anglia (United Kingdom) and Institute of Zoology,  
543 Beijing (China) by MEC (PR2009-0231). MTG and SJBC received support for their  
544 research from the Australian Research Council (DP0663675 and LP0669062).

545

546

547 **References**

- 548 Allegrucci G, Minasi MG, Sbordoni V (1997) Patterns of gene flow and genetic  
549 structure in cave-dwelling crickets of the Tuscan endemic, *Dolichopoda*  
550 *schiazzii* (Orthoptera, Rhaphidophoridae). *Heredity*, **78**, 665-673.
- 551 Allegrucci G, Todisco V, Sbordoni V (2005) Molecular phylogeography of  
552 *Dolichopoda* cave crickets (Orthoptera, Rhaphidophoridae): a scenario suggested  
553 by mitochondrial DNA. *Molecular Phylogenetics and Evolution*, **37**, 153-164.
- 554 Arnedo M, Oromí P, Murría C *et al.* (2007) The dark side of an island radiation:  
555 systematics and evolution of troglotic spiders of the genus *Dysdera* Latreille  
556 (Araneae: Dysderidae) in the Canary Islands. *Invertebrate Systematics*, **21**, 623-  
557 660.
- 558 Arntzen JW, Sket B (1997) Morphometric analysis of black and white European cave  
559 salamanders, *Proteus anguinus*. *Journal of Zoology*, **241**, 699-707.
- 560 Avise JC, Selander RK (1972) Evolutionary genetics of cave-dwelling fishes of the  
561 genus *Astyanax*. *Evolution*, **26**, 1-19.
- 562 Balke M, Watts CHS, Cooper SJB, *et al.* (2004) A highly modified stygobitic diving  
563 beetle of the genus *Copelatus* (Coleoptera, Dytiscidae): Taxonomy and cladistic  
564 analysis based on mtDNA sequences. *Systematic Entomology*, **29**, 59-67.
- 565 Baratti M, Kheziba MY, Messana G (2004) Microevolutionary processes in the  
566 stygobitic genus *Typhlocirolana* (Isopoda Flabellifera Cirolanidae) as inferred  
567 by partial 12S and 16S rDNA sequences. *Journal of Zoological Systematics and*  
568 *Evolutionary Research*, **42**, 27-32.
- 569 Barr TC (1967) Observations on the Ecology of Caves. *The American Naturalist*, **101**,  
570 475-491.

- 571 Barr TC (1968) Cave ecology and the evolution of troglobites. In: *Evolutionary Biology*  
572 (eds. Dobzhansky T, Hecht MK, Steere WC), pp. 35-102. Appleton-Century-  
573 Crofts, New York.
- 574 Bichain JM, Gaubert P, Samadi S, Boisselier-Dubayle MC (2007) A gleam in the dark:  
575 Phylogenetic species delimitation in the confusing spring-snail genus *Bythinella*  
576 Moquin-Tandon, 1856 (Gastropoda: Rissoidae: Amnicolidae). *Molecular*  
577 *Phylogenetics and Evolution*, **45**, 927-941.
- 578 Bonett RM, Chippindale PT (2004) Speciation, phylogeography and evolution of life  
579 history and morphology in plethodontid salamanders of the *Eurycea multiplicata*  
580 complex. *Molecular Ecology*, **13**, 1189-1203.
- 581 Borowsky RL, Mertz L (2001) Genetic differentiation among populations of the cave  
582 fish *Schistura oedipus* (Cypriniformes: Balitoridae). *Environmental Biology of*  
583 *Fishes*, **62**, 225-231.
- 584 Borowsky RL, Vidthayanon C (2001) Nucleotide diversity in populations of balitorid  
585 cave fishes from Thailand. *Molecular Ecology*, **10**, 2799-2805.
- 586 Bradford T, Adams M, Humphreys WF, Austin AD, Cooper SJB (2010) DNA  
587 barcoding of stygofauna uncovers cryptic amphipod diversity in a calcrete aquifer  
588 in Western Australia's arid zone. *Molecular Ecology Resources*, **10**, 41-50.
- 589 Buhay J, Crandall K (2005) Subterranean phylogeography of freshwater crayfishes  
590 shows extensive gene flow and surprisingly large population sizes. *Molecular*  
591 *Ecology*, **14**, 4259-4273.
- 592 Buhay JE, Moni G, Mann N, Crandall KA (2007) Molecular taxonomy in the dark:  
593 Evolutionary history, phylogeography, and diversity of cave crayfish in the  
594 subgenus *Aviticambarus*, genus *Cambarus*. *Molecular Phylogenetics and*  
595 *Evolution*, **42**, 435-448.



- 596 Buhay JE, Crandall K (2009) Taxonomic revision of cave crayfish in the genus  
597 *Cambarus* subgenus *Aviticambarus* (Decapoda: Cambaridae) with descriptions of  
598 two new species, *C. speleocoopi* and *C. laconensis*, endemic to Alabama, USA.  
599 *Journal of Crustacean Biology*, **29**, 121-134.
- 600 Byrne M, Yeates DK, Joseph L *et al.* (2008) Birth of a biome: insights into the  
601 assembly and maintenance of the Australian arid zone biota. *Molecular Ecology*,  
602 **17**, 4398-4417.
- 603 Caccone S, Sbordoni V (1987) Molecular evolutionary divergence among North  
604 American cave crickets. I. Allozyme variation. *Evolution*, **41**, 1198-1214.
- 605 Caccone A, Sbordoni V (2001) Molecular biogeography of cave life: a study using  
606 mitochondrial DNA from bathysciine beetles. *Evolution*, **55**, 122-130.
- 607 Carlini D, Manning J, Sullivan P, *et al.* (2009) Molecular genetic variation and  
608 population structure in morphologically differentiated cave and surface populations  
609 of the freshwater amphipod *Gammarus minus*. *Molecular Ecology*, **18**, 1932-1945.
- 610 Carmody GR, Murphy G, Peck SB (1972) Preliminary studies on electrophoretic  
611 variation in cavernicolous *Ptomaphagus* beetles (Coleoptera, Leiodidae,  
612 Catopinae). *Annales de Spéléologie*, **27**, 399-404.
- 613 Cesaroni D, Allegrucci G, Caccone A *et al.* (1981) Genetic variability and divergence  
614 between populations and species of *Nesticus* cave spiders. *Genetica*, **56**, 81-92.
- 615 Cockley DE, Gooch J, Weston DP (1977) Genetic diversity in cave dwelling crickets.  
616 *Evolution*, **31**, 313-318.
- 617 Colli L, Pagalianti A, Berti R *et al.* (2009) Molecular phylogeny of the blind cavefish  
618 *Phreatichthys andruzzii* and *Garra barreimiae* within the family Cyprinidae.  
619 *Environmental Biology of Fishes*, **84**, 95-107.

- 620 Collin R, Miglietta MP (2008) Reversing opinions on Dollo's Law. *Trends in Ecology*  
621 *and Evolution*, **23**, 602-609.
- 622 Contreras-Díaz HG, Moya O, Oromí P, Juan C (2007) Evolution and diversification of  
623 the forest and hypogean ground-beetle genus *Trechus* in the Canary Islands.  
624 *Molecular Phylogenetics and Evolution*, **42**, 687-699.
- 625 Cooper SJB, Hinze S, Leys R, Watts CHS, Humphreys WF (2002) Islands under the  
626 desert: molecular systematics and evolutionary origins of stygobitic water beetles  
627 (Coleoptera:Dytiscidae) from central Western Australia. *Invertebrate Systematics*,  
628 **16**, 589-598.
- 629 Cooper S, Bradbury J, Saint K *et al.* (2007) Subterranean archipelago in the Australian  
630 arid zone: mitochondrial DNA phylogeography of amphipods from central Western  
631 Australia. *Molecular Ecology*, **16**, 1533-44.
- 632 Cooper S, Saint K, Taiti S *et al.* (2008) Subterranean archipelago: mitochondrial DNA  
633 phylogeography of stygobitic isopods (Oniscidea: *Haloniscus*) from the Yilgarn  
634 region of Western Australia. *Invertebrate Systematics*, **22**, 195-203.
- 635 Coyne JA, Orr HA (2004) *Speciation*. Sinauer Associates, Sunderland, Massachusetts.
- 636 Craft JD, Russ AD, Yamamoto MN *et al.* (2008) Islands under islands: The  
637 phylogeography and evolution of *Halocaridina rubra* Holthuis, 1963 (Crustacean:  
638 Decapoda: Atyidae) in the Hawaiain archipelago. *Limnology and Oceanography*,  
639 **53**, 675-689.
- 640 Crouau-Roy B (1989) Population studies on an endemic troglobitic beetle: geographical  
641 patterns of genetic variation, gene flow and genetic structure compared with  
642 morphometric data. *Genetics*, **121**, 571-582.
- 643 Culver DC (1970) Analysis of simple cave communities: niche separation and species  
644 parking. *Ecology*, **51**, 949-958.

- 645 Culver DC, Wilkens H (2000) Critical review of the relevant theories of the evolution of  
646 subterranean animals. In: *Ecosystems of the World. Subterranean Ecosystems* (eds  
647 Wilkens H, Culver DC, Humphreys WF), pp. 389–407. Elsevier, Amsterdam.  
648 London, 1, 496–499.
- 649 Culver DC, Kane TC, Fong DW (1995) *Adaptation and natural selection in caves: The  
650 evolution of Gammarus minus* Harvard University Press, Cambridge,  
651 Massachusetts.
- 652 Culver D, Pipan T, Schneider K (2009) Vicariance, dispersal and scale in the aquatic  
653 subterranean fauna of karst regions. *Freshwater Biology*, **54**, 918-929.
- 654 Darwin C (1859) *On the Origin of Species by Means of Natural Selection, or the  
655 Preservation of Favoured Races in the Struggle of Life*. London: John Murray;  
656 1859.
- 657 Desutter-Grandcolas L, Grandcolas P (1996) The evolution toward troglobitic life: a  
658 phylogenetic reappraisal of climatic relict and local habitat shift hypotheses.  
659 *Memoirs of Biospeology* **23**, 57-63.
- 660 Dowling T, Martasian D, Jeffery W (2002) Evidence for multiple genetic forms with  
661 similar eyeless phenotypes in the blind cavefish, *Astyanax mexicanus*. *Molecular  
662 Biology and Evolution*, **19**, 446-455.
- 663 Espinasa L, Borowsky RB (2001) Origins and relationship of cave populations of the  
664 blind Mexican tetra, *Astyanax fasciatus*, in the Sierra De El Abra. *Environmental  
665 Biology of Fishes*, **62**, 233-237.
- 666 Faille A, Ribera I, Deharveng L *et al.* (2010) A molecular phylogeny shows the single  
667 origin of the Pyrenean subterranean Trechini ground beetles (Coleoptera:  
668 Carabidae). *Molecular Phylogenetics and Evolution*, **54**, 97-106.
- 669 Finlay J, Buhay J, Crandall K (2006) Surface to subsurface freshwater connections:

- 670 phylogeographic and habitat analyses of *Cambarus tenebrosus*, a facultative cave-  
671 dwelling crayfish. *Animal Conservation*, **9**, 375-387.
- 672 Finston T, Johnson M, Humphreys W, *et al.* (2007) Cryptic speciation in two  
673 widespread subterranean amphipod genera reflects historical drainage patterns in an  
674 ancient landscape. *Molecular Ecology*, **16**, 355-365.
- 675 Finston T, Francis C, Johnson M (2009) Biogeography of the stygobitic isopod  
676 *Pygolabis* (Malacostraca: Tainisopidae) in the Pilbara, Western Australia: evidence  
677 for multiple colonisations of the groundwater. *Molecular Phylogenetics and*  
678 *Evolution*, **52**, 448-60.
- 679 Fišer C, Trontelj P, Sket B (2006) Phylogenetic analysis of the *Niphargus orcinus*  
680 species-aggregate (Crustacea: Amphipoda: Niphargidae) with description of new  
681 taxa. *Journal of Natural History*, **40**, 2265-2315.
- 682 Fresneda J, Bourdeau C, Faille A (2009) *Baronniesia deliotti* gen. n. sp. n., a new  
683 subterranean Leptodirini from the French Pyrenees (Coleoptera: Leiodidae:  
684 Cholevinae). *Zootaxa*, **1993**, 1-16.
- 685 Gavrilets S (2003) Models of speciation: what have we learned in 40 years? *Evolution*,  
686 **57**, 2197-2215.
- 687 Gavrilets S (2004) *Fitness Landscapes and the Origin of Species*. Princeton University  
688 Press, Princeton, New Jersey.
- 689 Gavrilets S, Vose A (2005) Dynamic patterns of adaptive radiation. *Proceedings of the*  
690 *National Academy of Sciences USA*, **102**, 18040-18045.
- 691 Gavrilets S, Vose A (2007) Case studies and mathematical models of ecological  
692 speciation. 2. Palms on an oceanic island. *Molecular Ecology*, **16**, 2910-2921.
- 693 Gavrilets S, Vose A, Barluenga M, Salzburger W, Meyer A (2007) Case studies and  
694 mathematical models of ecological speciation. 1. Cichlids in a crater lake.

- 695 *Molecular Ecology*, **16**, 2893-2909.
- 696 Gibert J, Danielopol DL, Stanford JA (1994) *Groundwater Ecology*. Academic Press,  
697 New York.
- 698 Gibert J, Deharveng L (2002) Subterranean ecosystems: a truncated functional  
699 biodiversity. *BioScience*, **52**, 473-481.
- 700 Gorički S, Trontelj P (2006) Structure and evolution of the mitochondrial control region  
701 and flanking sequences in the European cave salamander *Proteus anguinus*. *Gene*,  
702 **387**, 31-41.
- 703 Guzik MT, Abrams KM, Cooper SJB *et al.* (2008) Phylogeography of the ancient  
704 Parabathynellidae (Crustacea: Bathynellacea) from the Yilgarn region of Western  
705 Australia. *Invertebrate Systematics*, **22**, 205-216.
- 706 Guzik MT, Cooper SJB, Humphreys WF, Austin AD (2009) Fine-scale comparative  
707 phylogeography of a sympatric sister species triplet of subterranean diving beetles  
708 from a single calcrete aquifer in Western Australia. *Molecular Ecology*, **18**, 3683-  
709 3698.
- 710 Hedin M and Thomas SM (2010) Molecular systematics of eastern North American  
711 Phalangodidae (Arachnida: Opiliones: Laniatores), demonstrating convergent  
712 morphological evolution in caves. *Molecular Phylogenetics and Evolution*, **54**,  
713 107-121.
- 714 Hey J (2010) Isolation with migration models for more than two populations. *Molecular*  
715 *Biology and Evolution*, **27**, 905-920.
- 716 Hey J, Nielsen R (2004) Multilocus methods for estimating population sizes, migration  
717 rates and divergence time, with applications to the divergence of *Drosophila*  
718 *pseudoobscura* and *D. persimilis*. *Genetics*, **167**, 747-760.

- 719 Holsinger JR (1988) Troglobites: the evolution of cave-dwelling organisms. *American*  
720 *Scientist*, **76**, 146-153.
- 721 Holsinger JR (1991) What can vicariance biogeographic models tell us about the  
722 distributional history of subterranean amphipods? *Hydrobiologia*, **223**, 43-45.
- 723 Holsinger JR (1994) Pattern and process in the biogeography of subterranean  
724 amphipods. *Hydrobiologia*, **287**, 131-145.
- 725 Holsinger JR (2000) Ecological derivation, colonisation and speciation. In:  
726 *Subterranean Ecosystems* (eds Wilkens H, Culver D, Humphreys W), pp. 399–415.
- 727 Howarth FG (1973) The cavernicolous fauna of Hawaiian lava tubes. I. Introduction.  
728 *Pacific Insects*, **15**, 139–151.
- 729 Howarth FG (1987) Evolutionary ecology of aeolian and subterranean habitats in  
730 Hawaii. *Current Biology*, **18**, 295-396.
- 731 Humphreys WF (2000) Relictual faunas and their derivation. In: *Ecosystems of the*  
732 *World. Subterranean Ecosystems*. Vol. 30. (eds Wilkens H, Culver D and  
733 Humphreys W), pp. 417–432. Elsevier, Amsterdam.
- 734 Hunter RL, Webb MS, Iliffe TM, Alvarado Bremer JR (2008) Phylogeny and historical  
735 biogeography of the cave-adapted shrimp genus *Typhlatya* (Atyidae) in the  
736 Caribbean Sea and western Atlantic. *Journal of Biogeography*, **35**, 65-75.
- 737 Jaume D, Christenson K (2001) Amphi-Atlantic distribution of the  
738 subterranean amphipod family Metacrangonyctidae (Gammaridea). *Contributions to*  
739 *Zoology*, **70**, 99-125.
- 740 Jeffery WR (2005) Adaptive evolution of eye degeneration in the Mexican blind  
741 cavefish. *Journal of Heredity*, **96**, 185-196.
- 742 Jeffery WR (2009) Regressive evolution in *Astyanax* cavefish. *Annual Review of*  
743 *Genetics*. **43**, 25-47.

- 744 Jones R, Culver DC, Kane TC (1992) Are parallel morphologies of cave organisms the  
745 result of similar selection pressures? *Evolution*, **46**, 353-365.
- 746 Juan C, Emerson BC (2010) Evolution underground: shedding light on the  
747 diversification of subterranean insects. *Journal of Biology*, **9**, 17.
- 748 Juberthie C, Decu V eds. 1994. *Encyclopaedia Biospeologica*. Tome 1. 2. Moulis  
749 (France): Société de Biospéologie.
- 750 Kane TC, Culver DC, Jones RT (1992) Genetic structure of morphologically  
751 differentiated populations of the amphipod *Gammarus minus*. *Evolution*, **46**,  
752 272-278.
- 753 Kano Y, Kase T (2004) Genetic exchange between anchialine cave populations by  
754 means of larval dispersal: the case of a new gastropod species *Neritilia*  
755 *cavernicola*. *Zoologica Scripta*, **33**, 423-437.
- 756 Ketmaier V, Argano R, Caccone A (2003) Phylogeography and molecular rates of  
757 subterranean aquatic Stenasellid Isopods with a peri-Tyrrhenian distribution.  
758 *Molecular Ecology*, **12**, 547-555.
- 759 King JL, Hanner R (1998) Cryptic species in a “living fossil” lineage: taxonomic and  
760 phylogenetic relationships within the genus *Lepidurus* (Crustacea: Notostraca)  
761 in North America. *Molecular Phylogenetics and Evolution*, **10**, 23-36.
- 762 Kosswig C (1960) Darwin und die degenerative evolution. *Abhandl. U. Verh. Naturw.*  
763 *Verein Hamburg, N.F.*, **4**, 21-42.
- 764 Kupriyanova EK, Ten Tove HA, Sket B, Zakšek V, Trontelj P, Rouse GW (2009)  
765 Evolution of the unique freshwater cave-dwelling tube worm *Marifugia cavatica*  
766 (Annelida: Serpulidae). *Systematics and Biodiversity*, **7**, 389-401.
- 767 Laing C, Carmody RG, Peck SB (1976) Population genetics and evolutionary biology  
768 of the cave beetle *Ptomaphagus hirtus*. *Evolution*, **30**, 484-497.

- 769 Lefébure T, Douady C, Gouy M *et al.* (2006) Phylogeography of a subterranean  
770 amphipod reveals cryptic diversity and dynamic evolution in extreme  
771 environments. *Molecular Ecology*, **15**, 1797-1806.
- 772 Lefébure T, Douady C, Malard F, Gibert J (2007) Testing dispersal and cryptic diversity  
773 in a widely distributed groundwater amphipod (*Niphargus rhenorhodanensis*).  
774 *Molecular Phylogenetics and Evolution*, **42**, 676-686.
- 775 Lejeusne C, Chevaldonné P (2006) Brooding crustaceans in a highly fragmented  
776 habitat: the genetic structure of Mediterranean marine cave-dwelling mysid  
777 populations. *Molecular Ecology*, **15**, 4123-4140.
- 778 Leys R, Watts C, Cooper SJB, Humphreys WF (2003) Evolution of subterranean diving  
779 beetles (Coleoptera: Dytiscidae: Hydroporini, Bidessini) in the arid zone of  
780 Australia. *Evolution*, **57**, 2819-2834.
- 781 Leys R, Cooper SJB, Strecker U, Wilkens H (2005) Regressive evolution of an eye  
782 pigment gene in independently evolved eyeless subterranean diving beetles.  
783 *Biology Letters*, **1**, 496-499.
- 784 Leys R, Watts CHS (2008) Systematics and evolution of the Australian subterranean  
785 hydroporine diving beetles (Dytiscidae), with notes on *Carabhydrus*. *Invertebrate*  
786 *Systematics*, **22**, 217-225.
- 787 Li ZQ, Guo BC, Li JB, *et al.* (2008) Bayesian mixed models and divergence time  
788 estimation of Chinese cavefishes (Cyprinidae: *Sinocyclocheilus*). *Chinese Science*  
789 *Bulletin*, **53**, 2342-2352.
- 790 Mitchell RW, Russell WH, Elliot WR (1977) Mexican eyeless characin fishes, genus  
791 *Astyanax*: Environment, distribution, and evolution. *Special Publications Museum*  
792 *Texas Tech University*, **12**, 1-89.
- 793 Moulds T, Murphy N, Adams M *et al.* (2007) Phylogeography of cave pseudoscorpions



- 794 in southern Australia. *Journal of Biogeography*, **34**, 951-962.
- 795 Murphy NP, Adams M, Austin AD (2009) Independent colonisation and extensive  
796 cryptic speciation of freshwater amphipods in the isolated groundwater springs of  
797 Australia's Great Artesian Basin. *Molecular Ecology*, **18**, 109-122.
- 798 Niemiller M, Fitzpatrick B, Miller B (2008) Recent divergence with gene flow in  
799 Tennessee cave salamanders (Plethodontidae: *Gyrinophilus*) inferred from gene  
800 genealogies. *Molecular Ecology*, **17**, 2258-2275.
- 801 Notenboom J (1991) Marine regressions and the evolution of groundwater dwelling  
802 amphipods (Crustacea). *Journal of Biogeography*, **18**, 437-454.
- 803 Page T, von Rintelen K, Hughes JM (2007) Phylogenetic and biogeographic  
804 relationships of subterranean and surface genera of Australian Atyidae (Crustacea:  
805 Decapoda: Caridea) inferred with mitochondrial DNA. *Invertebrate Systematics*,  
806 **21**, 137-145.
- 807 Page, T, Humphreys W, Hughes J (2008) Shrimps down under: evolutionary  
808 relationships of subterranean crustaceans from Western Australia (Decapoda:  
809 Atyidae: *Stygiocaris*). *PLoS ONE*, **3**, e1618.
- 810 Panaram K, Borowsky R (2005) Gene flow and genetic variability in cave and surface  
811 populations of the Mexican tetra, *Astyanax mexicanus* (Teleostei, Characidae).  
812 *Copeia*, **2005**, 409-416.
- 813 Paquin P, Hedin MC (2004) The power and perils of 'molecular taxonomy' a case study  
814 of eyeless and endangered *Cicurina* (Araneae: Dictynidae) from Texas caves.  
815 *Molecular Ecology*, **13**, 3239-3255.
- 816 Peck SB, Finston TL (1993) Galapagos islands troglobites: the questions of tropical  
817 troglobites, parapatric distributions with eyed-sister-species, and their origin by  
818 parapatric speciation. *Memoires de Biospeliologie*, **20**, 19-37.

- 819 Penton EH, Hebert PD, Crease TJ (2004) Mitochondrial DNA variation in North  
820 American populations of *Daphnia obtusa*: continentalism or cryptic endemism?  
821 *Molecular Ecology*, **13**, 97-107.
- 822 Pfenninger M, Schwenk K (2007) Cryptic animal species are homogeneously  
823 distributed among taxa and biogeographical regions. *BMC Evolutionary Biology*,  
824 **7**, 121.
- 825 Porter M (2007) Subterranean biogeography: what have we learned from molecular  
826 techniques? *Journal of Cave and Karst Studies*, **69**, 179-186.
- 827 Porter ML, Crandall K (2003) Lost along the way: the significance of evolution in  
828 reverse. *Trends in Ecology and Evolution*, **18**, 541-547.
- 829 Poulson TL, White WB (1969) The cave environment. *Science*, **165**, 971-981.
- 830 Prendini L, Fancke OF, Vignoli V (2010) Troglomorphism, trichobothriotaxy and  
831 typhlochactid phylogeny (Scorpiones, Chactioidea): more evidence that  
832 troglobitism is not an evolutionary dead-end. *Cladistics*, **26**, 117-142.
- 833 Proudlove G, Wood PJ (2003) The blind leading the blind: cryptic subterranean species  
834 and DNA taxonomy. *Trends in Ecology and Evolution*, **18**, 272-273.
- 835 Ribera I, Fresneda J, Bucur R *et al.* (2010) Ancient origin of a Western Mediterranean  
836 radiation of subterranean beetles. *BMC Evolutionary Biology*, **10**, 29.
- 837 Rice WR, Hostert EE (1993) Laboratory experiments on speciation: what have we  
838 learned in 40 years? *Evolution*, **47**, 1637-1653.
- 839 Rivera MAJ, Howarth FG, Taiti S, Roderick GK (2002) Evolution in Hawaiian cave  
840 isopods (Oniscidea: Philosciidae): vicariant speciation or adaptive shifts?  
841 *Molecular Phylogenetics and Evolution*, **25**, 1-9.
- 842 Romero A, Green SM (2005) The end of regressive evolution: examining and  
843 interpreting the evidence from cave fishes. *Journal of Fish Biology*, **67**, 3-32.

- 844 Rouch R, Danielopol DL (1987) L'origine de la faune aquatique souterraine, entre le  
845 paradigme du refuge et le modèle de la colonisation active. *Stygologia*, **3**, 345-372.
- 846 Russ A, Santos SR, Muir C (2010) Genetic population structure of an anchialine  
847 shrimp, *Metabetaeus lohena* (Crustacea: Alpheidae), in the Hawaiian Islands.  
848 *Revista de Biología Tropical*, **58**, 159-170.
- 849 Santos SR (2006) Patterns of genetic connectivity among anchialine habitats: a case  
850 study of the endemic Hawaiian shrimp *Halocaridina rubra* on the island of Hawaii.  
851 *Molecular Ecology*, **15**, 2699-2718.
- 852 Sbordoni V, Allegrucci G, Caccone A *et al.* (1981) Genetic variability and divergence  
853 in cave populations of *Troglophilus cavicola* and *T. andreinii* (Orthoptera,  
854 Rhabdophoridae). *Evolution*, **35**, 3276-3233.
- 855 Schilthuizen M, Cabanban AS, Haase M (2005) Possible speciation with gene flow in  
856 tropical cave snails. *Journal of Zoological and Evolutionary Research*, **43**, 133-  
857 138.
- 858 Sket B, Paragamian K, Trontelj P (2004) A census of the obligate subterranean fauna of  
859 the Balkan Peninsula. In: *Balkan Biodiversity: Pattern and Process in the*  
860 *European Hotspot* (eds Griffiths HI, Kryštufek B, Reed JM), pp. 309-322. Kluwer  
861 Academic Publisher, Dordrecht.
- 862 Stepien CA, Morton B, Dabrowska KA *et al.* (2001) Genetic diversity and evolutionary  
863 relationships of the troglodytic 'living fossil' *Congeneria kusceri* (Bivalvia:  
864 Dreissenidae). *Molecular Ecology*, **10**, 1873-1879.
- 865 Stock JH (1990) A new forest-hopper (Amphipoda, Talitridae) from La Palma, Canary  
866 Islands. *Vieraea*, **18**, 91-98.
- 867 Stock JH (1993) Some remarkable distribution patterns in stygobion Amphipoda.  
868 *Journal of Natural History*, **27**, 807-819.

- 869 Stock JH, Martín JL (1988) A new cavehopper (Amphipoda: Talitridae) from lava tubes  
870 in La Palma, Canary Islands. *Journal of Natural History*, **22**, 1121-1133.
- 871 Strecker U, Bernatchez L, Wilkens H (2003) Genetic divergence between cave and  
872 surface populations of *Astyanax* in Mexico (Characidae, Teleostei). *Molecular*  
873 *Ecology*, **12**, 699-710.
- 874 Strecker U, Faundez V, Wilkens H (2004) Phylogeography of surface and cave  
875 (Teleostei) from Central and North America based on cytochrome sequence data.  
876 *Molecular Phylogenetics and Evolution*, **33**, 469-481.
- 877 Tautz D, Ellegren H, Weigel D (2010) Next generation molecular ecology. *Molecular*  
878 *Ecology*, **19**, 1-3.
- 879 Trontelj P, Douady C, Fiser C, *et al.* (2009) A molecular test for cryptic diversity in  
880 ground water: how large are the ranges of macro-stygobionts? *Freshwater Biology*,  
881 **54**, 727-744.
- 882 Venarsky MP, Anderson FE, Wilhem FM (2009) Population genetic study of the U.S.  
883 federally listed Illinois cave amphipod, *Gammarus acherondytes*. *Conservation*  
884 *Genetics*, **10**, 915-921.
- 885 Verovnik R, Sket B, Prevorcnik S, Trontelj P (2003) Random amplified polymorphic  
886 DNA diversity among surface and subterranean populations of *Asellus aquaticus*  
887 (Crustacea: Isopoda). *Genetica*, **119**, 155-165.
- 888 Verovnik R, Sket B, Trontelj P (2004) Phylogeography of subterranean and surface  
889 populations of water lice *Asellus aquaticus* (Crustacea: Isopoda). *Molecular*  
890 *Ecology*, **13**, 1519-1532.
- 891 Villacorta C, Jaume D, Oromí P, Juan C (2008) Under the volcano: phylogeography and  
892 evolution of the cave-dwelling *Palmorchestia hypogaea* (Amphipoda, Crustacea) at  
893 La Palma (Canary Islands). *BMC Biology*, **6**, 7.

- 894 Volschenk ES, Prendini L (2008) *Aops oncodactylus*, gen. et sp. nov., the first  
895 troglobitic urodacid (Urodacidae : Scorpiones), with a re-assessment of  
896 cavernicolous, troglobitic and troglomorphic scorpions. *Invertebrate*  
897 *Systematics*, **22**, 235-257.
- 898 Watts CHS, Humphreys WF (2006) Twenty-six new Dytiscidae (Coleoptera) of the  
899 genera *Limbodessus* Guignot and *Nirripiriti* Watts & Humphreys, from underground  
900 waters in Australia. *Transactions of the Royal Society of South Australia*, **1**, 123–
- 901 Wewalka G, Ribera I, Balke M (2007) The second known species of groundwater  
902 Hyphdrini, *Microdytes trontelji* sp.n. from Hainan, China based on morphology  
903 and DNA Sequence data (Coleoptera: Dytiscidae). *Koleopterologische Rundschau*  
904 **76**, 51-54.
- 905 Wiens JJ, Chippindale PT, Hillis DM (2003) When are phylogenetic analyses misled by  
906 convergence? A case study in Texas cave salamanders. *Systematic Biology* **52**, 501-  
907 514.
- 908 Wilkens H (1988) Evolution and genetics of epigeal and cave *Astyanax fasciatus*  
909 (Characidae, Pisces). *Evolutionary Biology*, **23**, 271-367.
- 910 Wilkens H (2010) Genes, modules and the evolution of cave fish. *Heredity*,  
911 doi:10.1038/hdy.2009.184
- 912 Wilcox TP, García de León FJ, Hendrickson DA, Hillis DM (2004) Convergence  
913 among cave catfishes: long-branch attraction and a Bayesian relative rates test.  
914 *Molecular Phylogenetics and Evolution*, **31**, 1101–1113.
- 915 Witt JDS, Herbert PDN (2000) Cryptic species diversity and evolution in the amphipod  
916 genus *Hyalella* within central glaciated North America: a molecular  
917 phylogenetic approach. *Canadian Journal of Fisheries and Aquatic Science*, **57**,  
918 687-698.

- 919 Yamamoto Y, Jeffery WR (2000) Central role for the lens in cave fish eye degeneration.  
920 *Science*, **289**, 631-633.
- 921 Zakšek V, Sket B, Trontelj P (2007) Phylogeny of the cave shrimp *Troglocaris*:  
922 Evidence of a young connection between Balkans and Caucasus. *Molecular*  
923 *Phylogenetics and Evolution*, **42**, 223-235.
- 924 Zakšek V, Sket B, Gottstein S, *et al.* (2009) The limits of cryptic diversity in  
925 groundwater: phylogeography of the cave shrimp *Troglocaris anophthalmus*  
926 (Crustacea: Decapoda: Atyidae). *Molecular Ecology*, **18**, 931-946.

For Review Only

927 **Figure 1.** Some examples of extreme disjunct distributions among subterranean  
928 crustaceans. These patterns are best explained by vicariance by plate tectonics of  
929 shallow-water marine ancestors once broadly distributed along the shores of late  
930 Mesozoic seas. Photographs of *Metacrangonyx* (Amphipoda) and *Tethysbaena*,  
931 (Thermosbaenacea) by Thomas Ilife and *Typhlatya* (Decapoda) by Sergio  
932 Montagud.

933 **Figure 2.** Schematic representation of simplified phylogenies, approximate  
934 geographical ranges and diversification times of cave beetles of tribes Leptodirini  
935 (Leiodidae) and Trechini (Carabidae) in the Pyrenees. A: *Aphaenops*, G: *Geaphaenops*,  
936 H: *Hydraphaenops*. Numbers in the Leptodirini phylogeny refer to monophyletic clades  
937 that include species from different genera with particular geographical distributions.  
938 Phylogenetic reconstructions were based on mitochondrial (*cox1*, *cob*, *rrnL*, *trnL* and  
939 *nad1*) and nuclear (SSU and LSU) markers. Trees were reconstructed using Bayesian  
940 analyses and estimation of divergence times performed by a Bayesian relaxed  
941 phylogenetic approach implemented in BEAST v1.4.7 calibrated by standard  
942 mitochondrial substitution rates (Trechini) and biogeographical dating (Leptodirini).  
943 Photos of the representative Pyrenean taxa *Aphaenops alberti* (Trechini) and  
944 *Baronniesia deliotti* (Leptodirini) from Faille *et al.* (2010) and Fresneda *et al.* (2009)  
945 respectively. Figure modified from Faille *et al.* (2010) and Ribera *et al.* (2010).

946 **Figure 3.** Canarian representative cave species: *Dysdera unguimmanis* (Araneae) (a),  
947 *Loboptera troglobia* (Blattaria) (b), *Collartida anophthalma* (Heteroptera) (c) and  
948 *Maiorerus randoi*. (Opiliones). Photographs obtained by Pedro Oromí.

**Table 1.** A list of the main studies on biogeographic and colonisation histories, cryptic species diversity and modes of speciation published in the last decade using DNA molecular markers in cave-dwelling fauna. Mitochondrial and nuclear markers are indicated for each study.

Taxonomic group	Markers		Geographical region	Approach	References
	Mitochondrial	Nuclear			
<b>Annelids</b>					
Polychaetes		SSU, LSU	Dinaric karst	Phylogeny	Kupriyanova <i>et al.</i> 2009
<b>Insects</b>					
Crickets	<i>cox1, rrnL</i>		W Mediterranean	Phylogeny, molecular dating	Allegrucci <i>et al.</i> 2005
Beetles	<i>cox1</i>		W Mediterranean	Phylogeny, phylogeography,	Caccone & Sbordoni 2001
	<i>cox1, rrnL, nad1</i>		W Australia	molecular dating, modes of	Cooper <i>et al.</i> 2002
	<i>cox1, rrnL, nad1</i>		W Australia	speciation	Leys <i>et al.</i> 2003
	<i>cox1, rrnL, nad1</i>		W Australia		Balke <i>et al.</i> 2004
	<i>cox1, cox2</i>	ITS-2	Macaronesia		Contreras-Díaz <i>et al.</i> 2007
	<i>cox1, rrnL</i>	SSU, Histone H3	China		Wewalka <i>et al.</i> 2007
	<i>cox1, rrnL, nad1</i>		NW Australia		Leys & Watts 2008
	<i>cox1, cob, rrnL, nad1</i>	SSU, LSU	W Mediterranean		Faille <i>et al.</i> 2010
	<i>cox1, cob, rrnL, nad1</i>	SSU, LSU	W Mediterranean		Ribera <i>et al.</i> 2010
	<i>cox1</i>		W Australia		Guzik <i>et al.</i> 2009
<b>Arachnids</b>					
Spiders	<i>cox1</i>		Texas, US	Species identification,	Paquin & Hedin 2004
	<i>cox1, rrnL</i>		Canary Is.	phylogeography, phylogeny	Arnedo <i>et al.</i> 2007
	<i>cox1</i>		SE United States		Hedin & Thomas 2010
Pseudoscorpions	<i>cox1</i>		S Australia	Phylogeography	Moulds <i>et al.</i> 2007
<b>Molluscs</b>					
Bivalves	<i>cox1, rrnL</i>		Dinaric karst	Genetic variability, phylogeny	Stepien <i>et al.</i> 2001
Gastropods	<i>cox1</i>		Philippines	Mode of speciation	Kano & Kase 2004
	<i>rrnL</i>		Borneo	Species delimitation	Schilthuizen <i>et al.</i> 2005
	<i>cox1</i>	ITS-1	France		Bichain <i>et al.</i> 2007
<b>Crustaceans</b>					
Amphipods	<i>cox1</i>	LSU	Central Europe	Phylogeography, speciation,	Lefébure <i>et al.</i> 2006
	<i>cox1, rrnL</i>	LSU	France	cryptic species, phylogeny	Lefébure <i>et al.</i> 2007
	<i>cox1</i>		NW Australia		Finston <i>et al.</i> 2007
	<i>cox1</i>		W Australia		Cooper <i>et al.</i> 2007
	<i>cox1, cox2</i>	Histone H3	La Palma (Canary Is.)		Villacorta <i>et al.</i> 2008

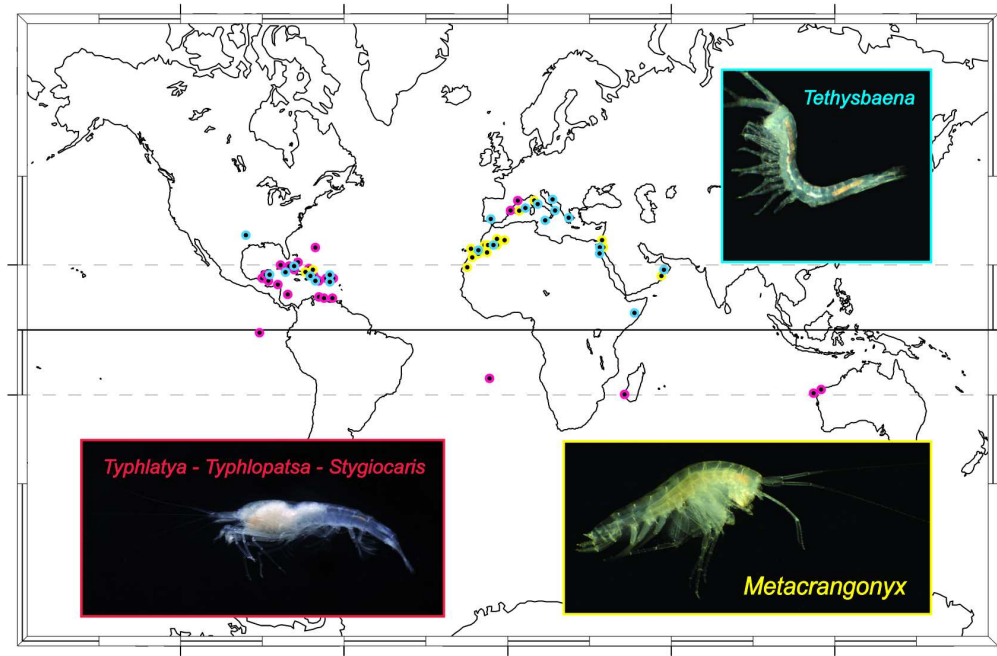


	<i>cox1</i>		Australia		Murphy <i>et al.</i> 2009
	<i>cox1</i>	ITS-1	Virginia, US		Carlini <i>et al.</i> 2009
	<i>cox1</i>		Illinois, US		Venarsky <i>et al.</i> 2009
	--	LSU	Europe		Trontelj <i>et al.</i> 2009
Isopods	<i>cox1</i>		W Australia		Bradford <i>et al.</i> 2010
	<i>cox1</i>		Hawaii Is.	Phylogeny, phylogeography, molecular dating, cryptic species, modes of speciation	Rivera <i>et al.</i> 2002
	<i>cox1</i>		W Mediterranean		Ketmaier <i>et al.</i> 2003
	--	RAPDs	NW Balkans		Verovnik <i>et al.</i> 2003
	<i>rrnS, rrnL</i>		W Mediterranean		Baratti <i>et al.</i> 2004
	<i>cox1</i>		NW Balkans		Verovnik <i>et al.</i> 2004
	<i>cox1</i>		W Australia		Cooper <i>et al.</i> 2008
<i>cox1</i>		W Australia	Finston <i>et al.</i> 2009		
Mysids	<i>cox1</i>		NW Mediterranean	Phylogeography and population genetics	Lejeune & Chevaldonné 2006
Decapods	<i>rrnL</i>		SE United States	Phylogeny, large-scale biogeography, phylogeography, molecular dating	Buhay & Crandall 2005
	<i>rrnL</i>		E United States		Finlay <i>et al.</i> 2006
	<i>cox1, rrnL</i>	LSU	France, W Balkans		Zakšek <i>et al.</i> 2007
	<i>cox1, rrnS, rrnL</i>	Histone H3, GAPDH	Tennessee, Alabama		Buhay <i>et al.</i> 2007
	<i>cox1</i>		Hawaiian Is.		Santos 2006
	<i>cox1, rrnL</i>		Australia & Indo-Pacific		Page <i>et al.</i> 2007
	<i>cox1, rrnL</i>	Histone H3	Australia & Indo-Pacific		Page <i>et al.</i> 2008
	<i>cox1, cob, rrnL</i>		Caribbean & W Atlantic		Hunter <i>et al.</i> 2008
	<i>cox1, rrnL</i>	ITS-2	W Balkans		Zakšek <i>et al.</i> 2009
	<i>cox1</i>		Hawaiian Is.		Russ <i>et al.</i> 2010
Bathynellaceans	<i>cox1</i>		W Australia	Phylogeny and phylogeography	Gukiz <i>et al.</i> 2008
<b>Vertebrates</b>					
Fish	<i>rrnL</i> , control region		Thailand	Phylogeography, gene flow analysis, phylogeny	Borowsky & Mertz 2001
	--	RAPDs	Thailand		Borowsky & Vidthayanon 2001
	<i>nad2</i>		Mexico		Dowling <i>et al.</i> 2002
	<i>cob</i>	Microsatellites	Mexico		Strecker <i>et al.</i> 2003
	<i>cob</i>		Mexico, Belize,		Strecker <i>et al.</i> 2004
	<i>cob, rrnL, rrnS</i>		Guatemala		Wilcox <i>et al.</i> 2004
	--	RAPDs, microsatellites	Mexico, Texas US		Panaram & Borowsky 2005
	<i>cob, nad4</i>		China		Li <i>et al.</i> 2008
	<i>cob</i>		SE Arabian Peninsula		Colli <i>et al.</i> 2009
Salamanders	<i>cob</i>	Allozymes	Texas, US	Phylogeography, gene flow	Wiens <i>et al.</i> 2003

<i>cob, nad4</i>		E North America	analysis, modes of speciation	Bonett & Chippindale 2004
<i>cob, rrnS</i> , control region		Balkans		Gorički & Trontelj 2006
<i>rrnS, cob</i>	RAG-1	Tennessee, US		Niemiller <i>et al.</i> 2008

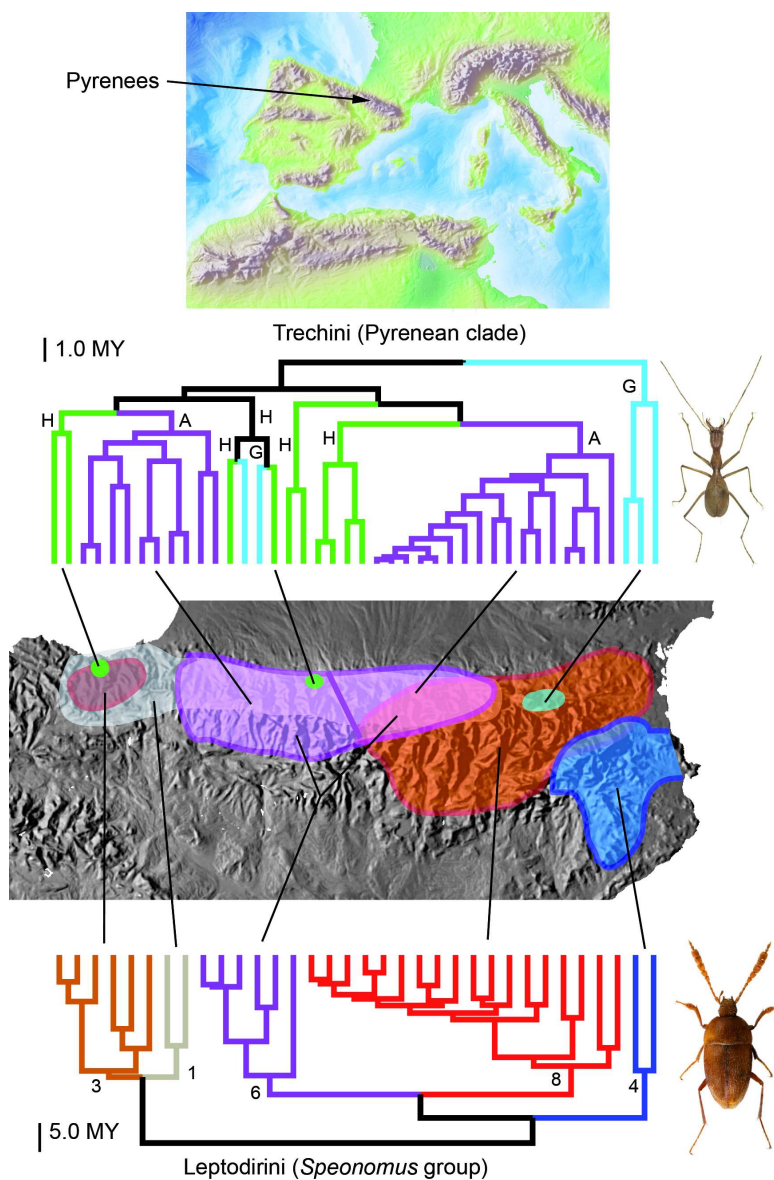
---

For Review Only



Some examples of extreme disjunct distributions among subterranean crustaceans. These patterns are best explained by vicariance by plate tectonics of shallow-water marine ancestors once broadly distributed along the shores of late Mesozoic seas. Photographs of *Metacrangonyx* (Amphipoda) and *Tethysbaena*, (Thermosbaenacea) by Thomas Ilife and *Typhlatya* (Decapoda) by Sergio Montagud.  
142x93mm (300 x 300 DPI)

www.Only



Schematic representation of simplified phylogenies, approximate geographical ranges and diversification times of cave beetles of tribes Leptodirini (Leiodidae) and Trechini (Carabidae) in the Pyrenees. A: *Aphaenops*, G: *Geaphaenops*, H: *Hydraphaenops*. Numbers in the Leptodirini phylogeny refer to monophyletic clades that include species from different genera with particular geographical distributions. Phylogenetic reconstructions were based on mitochondrial (*cox1*, *cob*, *rrnL*, *trnL* and *nad1*) and nuclear (SSU and LSU) markers. Trees were reconstructed using Bayesian analyses and estimation of divergence times performed by a Bayesian relaxed phylogenetic approach implemented in BEAST v1.4.7 calibrated by standard mitochondrial substitution rates (Trechini) and biogeographical dating (Leptodirini). Photos of the representative Pyrenean taxa *Aphaenops alberti* (Trechini) and *Baronniesia deliotti* (Leptodirini) from Faille et al. (2010) and Fresneda et al. (2009) respectively. Figure modified from Faille et al. (2010) and Ribera et al. (2010).

136x209mm (328 x 328 DPI)

For Review Only



Canarian representative cave species: *Dysdera unguimmanis* (Araneae) (a), *Lobotera troglobia* (Blattaria) (b), *Collartida anophthalma* (Heteroptera) (c) and *Maiorerus randoi*. (Opiliones).  
Photographs obtained by Pedro Oromí.  
249x122mm (600 x 600 DPI)

View Only