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On the Way to Speciation: Shedding Light on the Karstic Phylogeography of the Microendemic Cave Beetle *Aphaenops cerberus* in the Pyrenees

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Abstract

The highly modified morphology and ecological features of cave-dwelling organisms are a strong obstacle to dispersion. Hence, they represent ideal models for the study of historical biogeography at both large and fine timescales. Here, we study the phylogeography of *Aphaenops cerberus*, an endemic hypogean ground beetle with a fragmented distribution in the French Northern Pyrenees. We extracted 75 exemplars of 17 populations of *A. cerberus* and sequenced one mitochondrial and one nuclear marker to assess the geographic structuration as well as the recent biogeographic history of this species. We used Bayesian Inference and Maximum Likelihood to reconstruct the relationships among most of the extant populations of this species across its distributional range. We inferred divergence time estimates using carabid substitution rates and reconstructed haplotype networks to investigate the recent biogeographic history of this lineage. We recover a strong geographic structuration of the populations across the mountain range. The strong impact of geology on the structure of the populations is evidenced although geological continuity does not systematically lead to continual gene flow. The origin of the species is dated from the Early Pleistocene and the dispersal predates the main Last Glacial Maximum. Our results indicate broad similitudes between islands and karsts, which make cave organisms an excellent model for the study of evolution mechanisms.

Subject areas: Molecular systematics and phylogenetics, Population structure and phylogeography
Key words: Bayesian-relaxed clock, Carabidae, France, Trechini, haplotype network, Pleistocene, phylogeography.

Cave fauna constitutes a unique model to study speciation processes as most organisms confined to underground ecosystems present small distributional ranges and reduced dispersal abilities due to their morphological and physiological adaptations (Racovitza 1907; Vandel 1964; Juan et al. 2010). All cave adapted animals share well-known morphological adaptations such as the loss of eyes and pigmentation, lengthening of appendages, or apterism in insects often combined with elongation of life cycle (K strategy)

(Vandel 1964; MacArthur and Wilson 1967; Barr 1968). Such specialization may lead to a strong geographical structuration of populations confined to karstic areas, with high endemism, and very small ranges in cave animals (Jeannel 1926; Culver and Sket 2000). Subterranean environments of karstic areas therefore represent island-like systems, with multiple potentially isolated populations in reduced isolated areas (Culver 1970; Barr and Holsinger 1985; Caccone 1985; Juberthie 1989). Although limited in present day conditions, dispersal is not excluded for cave adapted fauna, and the timing and processes allowing species to extend their range are keystones to understand the setting-up of this peculiar biodiversity (Krekeler 1959; Rizzo et al. 2013). Moreover, hypogean species are not restricted to caves and numerous "cave" species can be sampled in the Superficial Hypogean Compartment ("Milieu souterrain superficiel", MSS, Juberthie and Bouillon 1983; Giachino and Vailati 2010). Pyrenees are an ideal area for studying the population history of cave animals, as it hosts hundreds of cave endemic invertebrate species, most of them belonging to lineages of hypogean restricted species in different group of insects, especially beetles (Coleoptera, Jeannel 1926). Beetles form the most diverse order of insects in subterranean ecosystems (Gibert and Deharveng 2002). Within the Pyrenees, as in the rest of the Palearctic area, the tribes Leptodirini (Leiodidae) and Trechini (Carabidae) represent the most diversified subterranean groups of beetles (Casale et al. 1998; Faille et al. 2010; Ribera et al. 2010). A few studies focused on population genetics of cave Trechini in North America, and concluded that geological features are strong barriers to gene flow for these species (Kane et al. 1992). In the Pyrenees, Leptodirini were the subject of detailed population studies, especially the genus *Speonomus* (Crouau-Roy 1987, 1989; Crouau-Roy and Bakalowicz 1993). These studies underlined the link between geology, paleogeographical events (especially quaternary glaciations), and population structures. However, these studies were based on allozyme data therefore preventing the estimation of a time frame for the diversification of populations.

Most of Pyrenean endemics are known from few caves only, some of them from a single locality. Here, we focused on a representative of the speciose genus *Aphaenops* Bonvouloir endemic to the Pyrenees. *Aphaenops* (*Cerbaphaenops*) *cerberus* (Dieck, 1869) is one of the most common and widespread species of the genus. It is known from numerous cavities of isolated karsts of the central Pyrenees between Ariege valley and Aspet area (Jeannel 1928; Coiffait 1958; Faille et al. 2007), and 5 subspecies were recognized on morphological basis, mainly differing in the shape of the pronotum and the head and the fore angles of elytra (Piochard de la Brûlerie 1872; Moravec et al. 2003). It is one of the few species occurring in more than 1 karstic unit, with a fragmented distribution of about 50 × 20 km in the central Pyrenees.

In this study, we aim to: (i) investigate the phylogeographical structure of *A. cerberus* across its distributional range, and (ii) identify the timing and the role of environmental barriers in the establishment of genetic structuration within this species.

Materials and Methods

Taxon Sampling and Molecular Biology

We collected 75 specimens of *A. cerberus* (Carabidae, Trechini), covering the entire distributional range of the species (Figure 1; Table 1) as well as one specimen of the close *Cerbaphaenops* species *A. crypticola* as outgroup (Faille et al. 2010). Total genomic DNA of new specimens was extracted from complete specimen kept in 96% ethanol using the DNeasy kit (Qiagen, Hilden, Germany). We used standard PCR protocols (Faille et al. 2014; Toussaint et al. 2014) to amplify and sequence the 2 following gene fragments: cytochrome oxidase subunit 1 (COI, 776 bp) and carbamoyl phosphate synthetase 2 (CAD, 675 bp).

We used the following couples of primers: cox1: ron/Tom (Simon et al. 1994; Ribera et al. 2010); CAD: CD439F/CD688R (Wild and Maddison, 2008). We sequenced CAD only for 1 specimen per population as preliminary tests shown no nucleotide polymorphism within populations. DNA sequences were corrected with Sequencher 4.9, aligned using Muscle (Edgar 2004) and the reading frames checked under Mesquite 3.01 (http://mesquiteproject.org). The different datasets used to infer phylogenetic relationships were generated under Geneious R6 (Biomatters, http://www.geneious.com/).

Molecular Phylogenetics and Population Structure

We used Bayesian Inference (BI) and maximum likelihood (ML) to reconstruct phylogenetic relationships using a concatenated dataset.

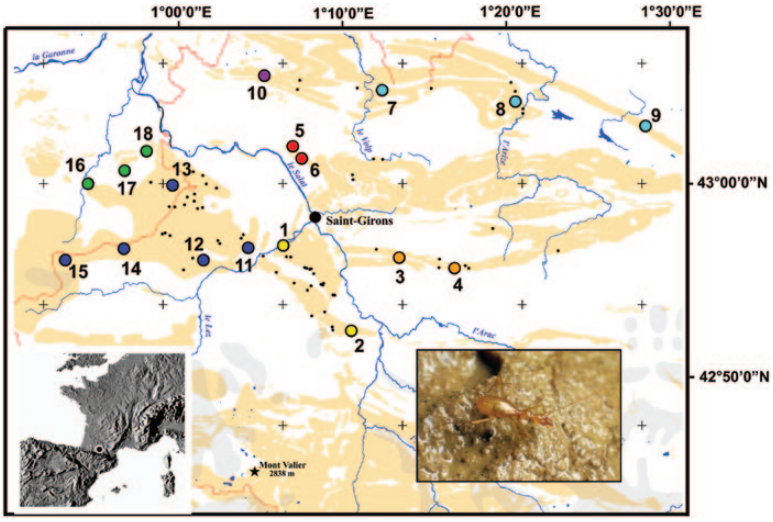

Figure 1. Distribution of the sampled sites of *A. cerberus*. Orange (gray) shaded: karstic areas. Yellow dots (1, 2): *A. cerberus cerberus*, Orange and red (3–6): *A. cerberus obtusus*, light-blue and purple (7–10): *A. cerberus inaequalis*, dark-blue and green (11–18): *A. cerberus bruneti*. Picture: C. Vanderbergh.

Table 1. Summary of sequenced exemplars and haplotype diversity. The locality numbers refer to Figure1.

Subspecies	Locality	Loc number	Number of samples	Haplotype diversity
A. cerberus cerberus (Dieck 1869)	Grotte du Sendé—Moulis (France-09)	1	5	4
A. cerberus cerberus (Dieck 1869)	Grotte d'Ardet—Rogalle (France-09)	2	6	2
A. cerberus obtusus (Jeannel 1926)	Grotte du Ker—Riverenert (France-09)	3	2	1
A. cerberus obtusus (Jeannel 1926)	Grotte de Rougé—Riverenert (France-09)	4	1	1
A. cerberus obtusus (Jeannel 1926)	Grotte de la Touasse—Gajan (France-09)	5	10	3
A. cerberus obtusus (Jeannel 1926)	Grotte de Sainte-Croix—Gajan (France-09)	6	1	1
A. cerberus inaequalis Abeille de Perrin, 1872	Grotte de la Quère—Mérigon (France-09)	7	4	2
A. cerberus inaequalis Abeille de Perrin, 1872	Grotte de Peyrounard—Mas d'Azil (France-09)	8	7	4
A. cerberus inaequalis Abeille de Perrin, 1872	Grotte des Cloutets—Aigues-Juntes (France-09)	9	6	1
A. cerberus inaequalis Abeille de Perrin, 1872	Grotte de Tourtouse—Tourtouse (France-09)	10	1	1
A. cerberus bruneti (Jeannel 1926)	Grotte de Liqué—Moulis (France-09)	11	9	4
A. cerberus bruneti (Jeannel 1926)	Grotte de Sainte-Catherine—Balaguères (France-09)	12	6	3
A. cerberus bruneti (Jeannel 1926)	Grotte de l'Estelas—Cazavet (France-09)	13	7	7
A. cerberus bruneti (Jeannel 1926)	Gouffre du Papillon—Buzan (France-09)	14	1	1
A. cerberus bruneti (Jeannel 1926)	Grotte de la Petite Marie—Galey (France-09)	15	1	1
A. cerberus bruneti (Jeannel 1926)	Grotte de la Marbrière—Montastruc-de-Salies (France-31)	16	6	5
A. cerberus bruneti (Jeannel 1926)	Grotte de Noustens—Urau (France-31)	17	1	1
A. cerberus bruneti (Jeannel 1926)	Grotte du Mont-de-Chac—Urau (France-31)	18	1	1

The partitions and corresponding optimal models of substitution were searched under PartitionFinder 1.1.1 (Lanfear et al. 2012) using the "*greedy*" algorithm, either the "*mrbayes*" of "*raxml*" set of models and the Akaike Information Criterion corrected (AICc) to compare the fit of the different models. The BI analyses were performed using MrBayes 3.2.2 (Ronquist et al. 2012). Instead of selecting the substitution models a priori based on the results of PartitionFinder, we used the different partitions recovered but used reversible-jump MCMC to explore the entire space of substitution models (Huelsenbeck et al. 2004). Two simultaneous and independent runs consisting of 8 Metropolis-coupled Markov chain Monte Carlo (MCMC, 1 cold and 7 incrementally heated) running 10 million generations were used, with a tree sampling every 1000 generations to calculate posterior probabilities (PP). In order to investigate the convergence of the runs we investigated the split frequencies and effective sample size (ESS) of all the parameters, and plotted the log-likelihood of the samples against the number of generations in Tracer 1.5 (http://BEAST.bio.ed.ac.uk/Tracer). A value of ESS > 200 was acknowledged as a good indicator of convergence. All trees that predated the time needed to reach a log-likelihood plateau were discarded as burn-in. The remaining samples were used to generate a 50% majority rule consensus tree. ML analyses were conducted with the best partitioning scheme selected in PartitionFinder 1.1.1 (Lanfear et al. 2012) using RAxML (Stamatakis 2006). We performed 1000 bootstrap replicates (BS) to investigate the level of support at each node. A calculated PP ≥ 0.95 or a BS ≥ 70 was considered to indicate strong support for a given clade (Hillis and Bull 1993; Erixon et al. 2003). We also constructed a haplotype network of the CO1 dataset under SplitsTree4 (Huson and Bryant 2006) using the NeighborNet algorithm.

Divergence Time Estimate

Divergence times were inferred with BEAST 1.8.0 (Drummond et al. 2012). The partitions and models of nucleotide substitution were selected under PartitionFinder 1.1.1 (Lanfear et al. 2012) using the "*greedy*" algorithm, the "*beast*" set of models and the AICc. We tested the hypothesis of molecular clock for the concatenated dataset (CO1 and CAD gene fragments) using Paup* (Swofford 2003). To do so, we used a fixed topology and estimated the likelihood of this same tree with or without enforcing a molecular clock. We then performed a Likelihood Ratio Test (LRT) to compare the likelihood scores of both analyses. The hypothesis of molecular clock was significantly rejected ($P < 0.001$), and therefore we used a Bayesian relaxed clock allowing rate variation among lineages as implemented in BEAST. In order to calibrate the tree, we used the 2 different CO1 substitution rates calculated by Andúar et al. (2012) for the genus *Carabus*, in order to acknowledge the overlap of the fragment we sequenced with the 2 fragments for which rates were calculated by these authors. These rates were previously used for cave beetles and were found to be consistent with biogeographical calibrations used to obtain absolute divergence times (Faille et al. 2014). We used a uniform prior encompassing the following credibility intervals calculated for the 2 different CO1 fragments: rate 1 (0.0081–0.0145) and rate 2 (0.01–0.0198). The runs were conducted under a Coalescent: Constant Size Tree prior. We run 30 million generations sampled every 2000 generations. The convergence of the runs was investigated using ESS, a conservative burn-in of 25% applied after checking the log-likelihood curves and the different runs merged using LogCombiner 1.8.0 (Drummond et al. 2012). The maximum credibility tree, median ages, and their 95% highest posterior density (HPD) were generated afterwards under TreeAnnotator 1.8.0 (Drummond et al. 2012).

Data Archiving

In fulfillment of data archiving guidelines (Baker 2013), haplotypes identified in this study were submitted to EMBL Nucleotide Sequence Database (Accession numbers available in Supplementary Table 1).

Results

Phylogenetic Relationships and Phylogeography

We found a strong geographic structuration of *A. cerberus* populations. Two large clades CI and CII were inferred with strong support (Figure 2). The relationships between the different populations were extremely well-supported across the topology except between the 2 subspecies *A. cerberus obtusus* and *A. cerberus cerberus*.

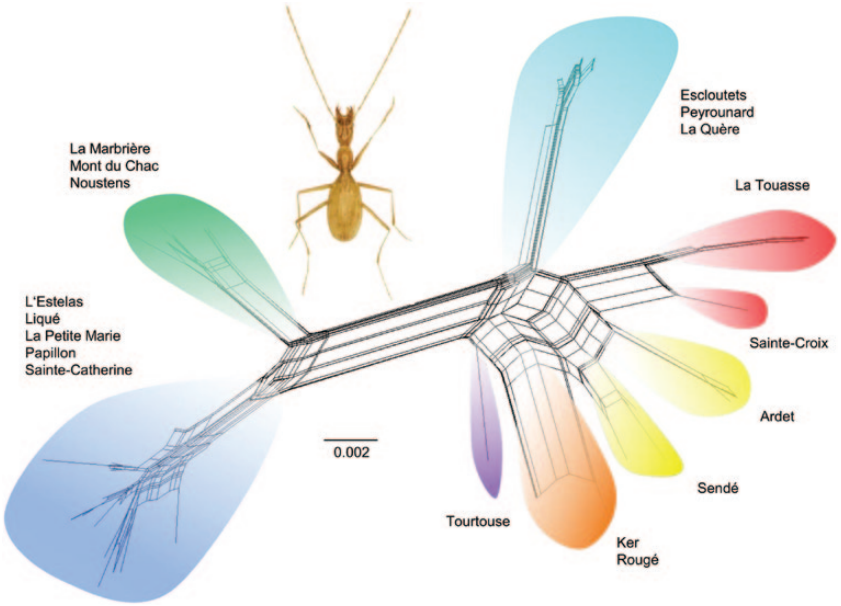

Figure 2. Haplotype network for the populations of *A. cerberus* obtained from SplitsTree using the NeighborNet algorithm and uncorrected p-distances derived from the CO1 alignment.

Within CI, group I corresponds to the subspecies *bruneti* Jeannel (Figures 2 and 3), isolated from the other populations by the Salat and Lez rivers (Figure 1). The isolation between the ssp. *bruneti* and the other populations of *A. cerberus* is corroborated by the morphological differences of this subspecies, especially an elongated pronotum and angulate humeri (Jeannel 1928). It was divided in 2 well-supported subclades, IA and IB. IA comprised 3 populations isolated in the periphery of the large karstic massif of L'Estelas, where are located all the other populations of the subclade IB. The subclade IB was also structured, although the support of the internal nodes was weak. All specimens of the population from the cave of L'Estelas (N°13 in Figure 2) were grouped together, whereas the other populations (N°11, 12, 14, 15, Figure 2) were intermixed. The main topology obtained was in accordance with the recognition of a distinct entity located on the western side of the Lez valley (Jeannel 1926, 1928).

The Clade II comprised all the remaining populations of *A. cerberus* located East of the Lez valley and North of the Salat valley (Figures 1 and 2). It was splitted into 2 subclades: IIA comprised all populations from the isolated Plantaurel massif (ssp. *inaequalis*) whereas IIB gathered the nominal subspecies together with the ssp *obtusus*. The locality isolated on the left side of the Volp valley (N°10, Figure 1) was sister to the other populations of the subclade IIA. The other localities were intermixed, but the 2 most distant localities were not intermixed, suggesting a low level of gene flow between these 3 populations. Nevertheless, further investigations are required given the general low support of the relationships between clades.

The analysis of the nuclear data alone indicated that the CAD fragment sequenced unambiguously supports the split between clades CI and CII (Figure 2). However, the signature of geographic structuration at a finer scale was not recovered compared to the mitochondrial data.

Divergence Time Estimates

Our results suggest a split between *A. cerberus* and the outgroup *A. crypticola* around 9 Ma with a large credibility interval (≈4–17 Ma when combining the credibility intervals of both analyses). Ages obtained with rate 2 were slightly younger than the ones inferred with the slower rate 1 (Figure 2). The split between the 2 main clades I and II is estimated from the Early Calabrian, ca 1.5–1.8 million years ago (Ma). The splits between the 4 main subclades IA and IB and IIA and IIB are dated from the end of the Calabrian (≈0.75–1.0 Ma), and the divergence between the different populations sampled is very recent, most likely during the last 500 thousand years (Kyr).

Discussion

Because of their low dispersal abilities, the present day distribution of cave organisms can be very informative regarding paleogeography and biogeographical histories (Faille et al. 2014). Although alternative modes of speciation have been suggested (see e.g. Wessel et al. 2013) allopatric speciation is considered prevailing in cave-dwelling lineages (Barr and Holsinger 1985; Faille et al. forthcoming; review in Juan et al. 2010). Here, we show that the dispersal of the population of *A. cerberus* occurred once fully adapted to subterranean environment but predates the main last glacial cycle. This is in agreement with previous studies showing that cave species can expand their range during favorable climatic windows (Rizzo et al. 2013). The divergence time estimates obtained are in accordance with previous studies on the evolution of pyrenean subterranean Trechini (Faille et al. 2010, 2011). Nevertheless the current fragmented distribution is genetically strongly structured (Figure 2). The hydrographic history of the area played a major role in the isolation of populations and likely drove their genetic structuration across the massif. As suggested for other hypogean Trechini of the Pyrenees (Faille et al. Forthcoming), Lez and Salat valleys are the 2 main obstacles to dispersal between populations in this geographic area (Figure 1).

Pleistocene in the Pyrenees

The Pleistocene glaciations are known to have had a strong impact on the distribution and diversification of the present day biodiversity (Hewitt 1999, Galbreath and Cook 2004). The glaciations were

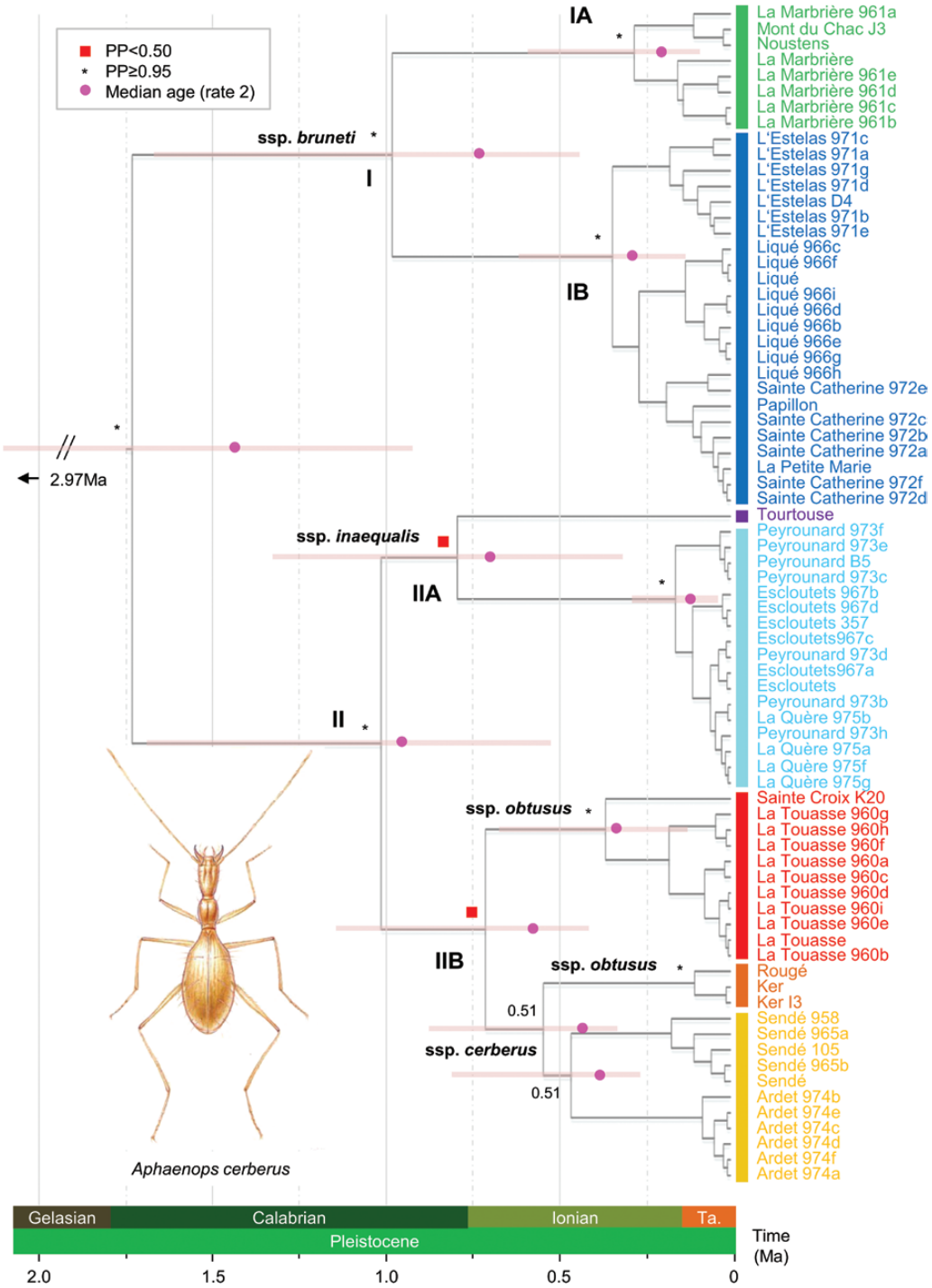

Figure 3. Divergence time estimates of *A. cerberus* population in the Pyrenees obtained from BEAST based on the concatenated dataset (CO1 and CAD) and different CO1 substitution rates. Calibrated tree resulting from the first set of calibration based on the first substitution rate highlighting the median age estimates for the most important nodes of the phylogeny. The median ages yielded by the second rate of substitution are shown with dots for each of these nodes. The different karstic areas are coded with the same color in the online version (see Figure 1). Posterior probabilities for each node are shown following the caption at the top left corner of the figure. Horizontal bars present the 95% HPD heights of the most important nodes.

very early considered as a main factor of isolation and speciation (Peyerimhoff 1906, Jeannel 1908, 1949; Vandel 1958; Barr 1968, Bellés 1986; Juberthie et al.1990; Crouau-Roy and Bakalowicz 1993; Foulquier et al. 2008). Some authors consider the glacial withdrawal as responsible for the isolation of troglobitic populations, by destruction of the epigean nivicolous populations (Peck 1973; Juberthie 1984; Hernando et al. 1999). Crouau-Roy and Bakalowicz (1993) considered that glaciations led to speciation by isolation of populations of troglobitic beetles (Pyrenean species of the genus *Speonomus*) and subsequent founder events after the recolonization of the karst. Jeannel was the first to study the distribution patterns of cave beetles (Pyrenean Bathysciinae and Trechinae) and compared

them to the maximal extension of the Quaternary glaciers (Jeannel 1908, 1919, 1948). These comparisons allowed him to postulate that the glaciations had a strong impact on the present day distribution. Our work show that the main split between the 2 population groups of *A. cerberus* (ssp *bruneti* vs. other populations, Figure 2) may have occurred during the Late Gelasian-Early Calabrian, a period with no significant change in environmental conditions (Cita et al. 2008). This event might be linked with the beginning of the first cycle of glaciation, the Donau glaciation (Penck and Brückner 1909). This first Pleistocene glaciation period is poorly documented in the Pyrenees (Calvet et al. 2011).

The last glaciations (<100 ka) were considered as responsible for the geographic structuration of the populations in pyrenean cave beetles of the genus *Speonomus* (Crouau-Roy and Bakalowicz 1993). We could not detect any effect of the Würm glaciations occurring during the Upper Pleistocene (126000–11700 years), as all the significant splits are much older (Figure 3), but further investigation with faster markers would be required for a better understanding of the more recent dispersal events, including intrakarst gene flow.

In spite of geological continuity, the split of populations comprised in the clade IB into 2 clades suggests a probable lack of gene flow between the populations of L'Estelas cave (N°13, Figure 1) and the other populations located in the southern border of the massif. On the other side, the fact that some individuals from different caves of the massif (N°11, 12, 14, 15, Figure 1) share a common haplotype strongly suggests intrakarst gene flow, as observed for North American cave ground beetles or other hypogean invertebrates (Turanchik and Kane 1979; Verovnik et al. 2004).

Peripatric speciation (Mayr 1963) was invoked to explain the occurrence of a close but distinct species in a small karstic area west of the range of *A. cerberus* (*A. jauzioni*, Faille et al. 2007). The complete geographic isolation explain the 2 main splits IA and IB in the clade I (Figure 3). The populations of IA (16–18 in Figure 1) are completely isolated in the periphery of the massif of L'Estelas-Arbas where the other populations (11–15) of the clade occur (Figure 1).

The strongly geographically structured pattern observed for *A. cerberus* (Figures 2 and 3) plead for a general allopatric mode of speciation for cave animals, already considered as a major way of speciation in troglobites (Jeannel 1941; Barr 1968). Another interesting result is the higher genetic diversity in L'Estelas massif than in the other surrounding areas, suggesting a positive correlation between the size of the karstic unit and the genetic diversity. This hypothesis should be further investigated by comparing the patterns observed with other cave invertebrates and other fragmented areas. If confirmed, this would comfort what is observed in oceanic island settings where the size of the islands may directly be correlated with the number of species or haplotypes observed (MacArthur and Wilson 1967; Schoener 1976; Losos and Schluter 2000).

Conclusion

The diversification of the *A. cerberus* population originated in the Early Pleistocene, long time before the Last Glacial Maximum. The distribution pattern of the population is highly geographically structured. The dispersal is very limited, and the Lez valley is the main barrier isolating 2 subgroups of populations. The last glacial cycle (Jalut et al. 1992) did not impact the phylogeographic structure observed, suggesting a lower speciation rate than the one observed for other cave insects (Wessel et al. 2013). Our results reinforce the paradigm of subterranean organisms as ideal insular study systems to unveil macroevolutionary processes of lineage diversification and ecological adaptation.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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References

Andújar C, Serrano J, Gómez-Zurita J. 2012. Winding up the molecular clock in the genus *Carabus* (Coleoptera: Carabidae): assessment of methodological decisions on rate and node age estimation. *BMC Evol Biol*. 12:40.

Baker CS. 2013. Journal of heredity adopts joint data archiving policy. *J Hered*. 104:1.

Barr TC. 1968. Cave ecology and the evolution of troglobites. In: Dobzhansky T, Hecht MK, Steere WC, editors. *Evolutionary biology*. New York: Plenum Press. p. 35–102.

Barr TC, Holsinger JR. 1985. Speciation in cave faunas. *Annu Rev Ecol Syst*. 16:313–37.

Bellés X. 1986. Distribution of the iberian bathysciinae (Coleoptera: Catopidae). An explanation. 9e Congreso Internacional de Espeleologia. *Barcelona*. 2:134–137.

Caccone A. 1985. Gene flow in cave arthropods: a qualitative and quantitative approach. *Evolution*. 39:1223–1234.

Calvet M., Delmas M., Gunnell Y., Braucher R., Bourlès D. 2011. Recent advances in research on quaternary glaciations in the pyrenees. In: Ehlers J, Gibbard PL, Hughes PD, editors. *Quaternary glaciations – extent and chronology. developments in quaternary science*, vol. 15. Amsterdam: Elsevier. p. 127–139.

Casale A, Vigna Taglianti A, Juberthie C. 1998. Coleoptera Carabidae. In: Juberthie C, Decu V, editors. *Encyclopaedia Biospeologica, tome II*. Moulis: Société Internationale de Biospéologie. p. 1047–1081.

Cita MB, Capraro L, Ciaranfi N, Di Stefano E, Lirer F, Maiorano P, Marino M, Raffi I, Rio D, Sprovieri R, *et al*. 2008. The Calabrian stage redefined. *Episodes* 31:408–419.

Coiffait H. 1958. Note sur les Trechitae cavernicoles et endogés de la région Pyrénéenne. *Notes Biospeol*. 13:7–23.

Crouau-Roy B. 1987. Spéciation et structure génétique des populations chez les coléoptères *Speonomus*. *Mem Biospeol*. 14:1–315.

Crouau-Roy B. 1989. Population studies on an endemic troglobitic beetle: geographical patterns of genetic variation, gene flow and genetic structure compared with morphometric data. *Genetics*. 121:571–582.

Crouau-Roy B., Bakalowicz M. 1993. Genetic Structure in Cave Beetles and Past Environment Changes. *Biochem Syst Ecol*. 21:549–556.

Culver DC. 1970. Analysis of simple cave communities. I. Caves as islands. *Evolution*. 24:463–474.

Culver DC, Sket B. 2000. Hotspots of subterranean biodiversity in caves and wells. *J Cave Karst Stud* 62:11–17.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol*. 29:1969–1973.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. 32:1792–1797.

Erixon P, Svennblad B, Britton T, Oxelman B. 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Syst Biol*. 52:665–673.

Faille A, Déliot P, Queinnec E. 2007. A new cryptic species of *Aphaenops* (Coleoptera, Trechinae) from french Pyrenean cave: Congruence between morphometrical and geographical data confirm species isolation. *Ann Soc Entomol Fr (NS)* 43:363–370.

Faille A, Ribera I, Deharveng L, Bourdeau C, Garnery L, Quéinnec E, Deuve T. 2010. A molecular phylogeny shows the single origin of the Pyrenean subterranean Trechini ground beetles (Coleoptera: Carabidae). *Mol Phylogenet Evol*. 54:97–106.

Faille A, Casale A, Ribera I. 2011. Phylogenetic relationships of west Mediterranean troglobitic Trechini groundbeetles (Coleoptera: Carabidae). *Zool Scr*. 40:282–295.

Faille A, Andújar C, Fadrique F, Ribera I. 2014. Late Miocene origin of a Ibero-Maghrebian clade of ground beetles with multiple colonisations of the subterranean environment. *J Biogeogr*. 41:1979–1990.

Faille A, Bourdeau C, Bellés X, Fresneda J. Forthcoming. On the role of allopatric speciation in diversification of hypogean fauna, with description of four new species of *Geotrechus* from Eastern Pyrenees (Spain) (Coleoptera, Carabidae, Trechini). *Arthropod Syst Phylogeny*..

Foulquier A, Malard F, Lefébure T, Douady CJ, Gibert J. 2008. The imprint of Quaternary glaciers on the present-day distribution of the obligate groundwater amphipod *Niphargus virei* (Niphargidae). *J Biogeogr*. 35:552–564.

Galbreath KE, Cook JA. 2004. Genetic consequences of Pleistocene glaciations for the tundra vole (*Microtus oeconomus*) in Beringia. *Mol Ecol*. 13:135–148.

Giachino PM, Vailati D. 2010. The subterranean environment. Hypogean life, concepts and collecting techniques. *WBA Handbooks*, Vol. 3. Verona (Italy): World Biodiversity Association Onlus.

Gibert J., Deharveng L. 2002. Subterranean ecosystems: A truncated functional biodiversity. *Bioscience*. 52:473–481.

Hernando C, Ribera I, Vogler AP. 1999. Alpine and cave or endogean habitats as postglacial refugia: Examples from palearctic ground beetles, with comments on their possible origins (Coleoptera: Carabidae). *Coleopt Bull*. 53:31–39.

Hewitt GM. 1999. Post-glacial re-colonization of European biota. *Biol J Linn Soc*. 68:87–112.

Hillis, DM, Bull, JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst biol*. 42:182–192.

Huelsenbeck JP, Larget B, Alfaro ME. 2004. Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. *Mol Biol Evol*. 21:1123–1133.

Huson DH, Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol*. 23:254–267.

Jalut G, Monserrat Marti J, Fontugne M, Delibrias G, Vilaplana JM Julia R. 1992. Glacial to interglacial vegetation changes in the northern and southern Pyrénées: Deglaciation, vegetation cover and chronology. *Quat Sci Rev*. 11:449–480.

Jeannel R. 1908. Etudes sur le genre *Speonomus* Jeann. et sur sa distribution géographique. *L'Abeille*. 31:57–102.

Jeannel R. 1919. Sur la distribution géographique des coléoptères cavernicoles dans les Pyrénées. Boletin de la Asociación española para el Progreso de las Ciencias. *Bilbao*. 1–8.

Jeannel R. 1926. *Faune cavernicole de la France avec une étude des conditions d'existence dans le domaine souterrain*. Paris: Lechevalier.

Jeannel R. 1928. *Monographie des Trechinae. Morphologie comparée et distribution d'un groupe de Coléoptères*. Troisième Livraison: les Trechini cavernicoles. L'Abeille. 35:1–808.

Jeannel R. 1941. L'isolement, facteur de l'Evolution. *Rev Fr Entomol*. 8:101–110.

Jeannel R. 1948. Influence des glaciers quaternaires sur la répartition des *Aphaenops* dans les Pyrénées Occidentales. *Notes Biospeol*. 3:29–34.

Jeannel R. 1949. Influence du glaciaire sur la répartition de certains Coléoptères. *C R Soc Biogeogr*. 26:29–33.

Juan C, Guzik MT, Jaume D, Cooper SJ. 2010. Evolution in caves: Darwin's 'wrecks of ancient life' in the molecular era. *Mol Ecol*. 19:3865–3880.

Juberthie C. 1984. La colonisation du milieu souterrain; théories et modèles, relations avec la spéciation et l'évolution souterraine. *Mem Biospeol*. 11:65–102.

Juberthie C. 1989. Insularité et spéciation souterraine. *Mem Biospeol*. 16:3–14.

Juberthie C, Bouillon M. 1983. Présence des Aphaenops (Coléoptères Trechinae) dans le milieu souterrain superficiel des Pyrénées françaises. *Mem. Biospeol*. 10:91–98.

Juberthie C, Dupré E, Jalut G. 1990. Aphaenops rebereti: espèce « endogée » du sous-genre *Geaphaenops* présente dans le M.S.S. de la vallée glaciaire d'Ossau, date de 12000 B.P. *Mem Biospeol*. 17:181–190.

Kane TC, Barr TC Jr, Badaracca WJ. 1992. Cave beetle genetics: geology and gene flow. *Heredity*. 68:277–286.

Krekeler CH. 1959. Dispersal of cavernicolous beetles. *Syst Zool*. 8:119–130.

Lanfear R, Calcott B, Ho SY, Guindon S. 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol Biol Evol*. 29:1695–1701.

Losos JB, Schluter D. 2000. Analysis of an evolutionary species-area relationship. *Nature*. 408:847–850.

MacArthur RH, Wilson EO. 1967. *The Theory of Island Biogeography*. Princeton (NJ): Princeton University Press.

Mayr E. 1963. *Animal species and evolution*. Cambridge: Harvard University Press.

Moravec P, Uéno SI, Belousov IA. 2003. Carabidae: Trechinae: Trechini. In: Löbl I, Smetana A, editors. *Catalogue of palaearctic coleoptera*, Vol. 1. Stenstrup: Apollo Books. p. 288–346.

Peck SB. 1973. A systematic revision and the evolutionary biology of the *Ptomaphagus* (*Adelops*) beetles of North America (Coleoptera: Leiodidae: Catopinae), with emphasis on cave-inhabiting species. *Bull Mus Comp Zool*. 145:29–162.

Penck AFK, Brückner E. 1909. *Die Alpen im Eiszeitalter*. Leipzig: Chr. Herm Tauchnitz.

Peyerimhoff P. de. 1906. Considérations sur les origines de la faune souterraine. *Ann Soc Entomol Fr*. 223–233.

Piochard de La Brûlerie C. 1872. Etude des variations de l'*Anophthalmus cerberus* et remarques sur l'influence que peut avoir l'habitation des cavernes sur la variabilité des espèces. *Ann Soc Entomol Fr*. 461–472.

Racovitza EG. 1907. Essai sur les problèmes biospéologiques. *Arch Zool Exp Gen. 4ème série*. 6:371–488.

Ribera I, Fresneda J, Bucur R, Izquierdo A, Vogler AP, Salgado JM, Cieslak A. 2010. Ancient origin of a Western Mediterranean radiation of subterranean beetles. *BMC Evol Biol*. 10:29.

Rizzo V, Comas J, Fadrique F, Fresneda J, Ribera I. 2013. Early Pliocene range expansion of a clade of subterranean Pyrenean beetles. *J Biogeogr*. 40:1861–1873

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol*. 61:539–542.

Schoener TW. 1976. The species-area relation within archipelagos: models and evidence from island land birds. In: *Proceedings of the XVI international ornithological congress*, vol. 6. Canberra: Australian Academy of Science. p. 629–642.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann Entomol Soc Am*. 87:651–701.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*. 22:2688–2690.

Swofford DL. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sunderland (MA): Sinauer Associates.

Toussaint EF, Hall R, Monaghan MT, Sagata K, Ibalim S, Shaverdo HV, Vogler AP, Pons J, Balke M. 2014. The towering orogeny of New Guinea as a trigger for arthropod megadiversity. *Nat Commun*. 5:4001.

Turanchik EJ, Kane TC. 1979. Ecological genetics of the cave beetle *Neaphaenops tellkampfii* (Coleoptera: Carabidae). *Oecologia*. 44:63–67.

Vandel A. 1958. La répartition des cavernicoles et la Paléogéographie. *Actes du 2e Congrès International de Spéléologie, Bari (Italie)*. 2:31–43.

Vandel A. 1964. *Biospéologie. La Biologie des Animaux Cavernicoles*. Paris: Gauthier-Villars.

Verovnik R, Sket B, Trontelj P. 2004. Phylogeography of subterranean and surface populations of water lice Asellus aquaticus (Crustacea: Isopoda). *Mol Ecol*. 13:1519–1532.

Wessel A, Hoch H, Asche M, von Rintelen T, Stelbrink B, Heck V, Stone FD, Howarth FG. 2013. Founder effects initiated rapid species radiation in Hawaiian cave planthoppers. *Proc Natl Acad Sci USA*. 110:9391–9396.

Wild AL, Maddison DR. 2008. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. *Mol Phylogenet Evol*. 48:877–891.