



A new species of blind Trechinae from the Pyrenees of Huesca, and its position within *Aphaenops* (*sensu stricto*) (Coleoptera: Carabidae: Trechini)

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Abstract

A new trechine species *Aphaenops parvulus* **sp. n.** (Carabidae, Trechini) is described from Esjamundo cave in the Pyrenees of Huesca, Spain. The new species belongs to the subgenus *Aphaenops* (*sensu stricto*), but differs from its closest congeners by the small size—it is the smallest species of the group—and characters of the aedeagus. Molecular data based on fragments of a mitochondrial (COI) and a nuclear (LSU) genes recognised *Aphaenops parvulus* **sp. n.** as a sister taxon to *A. eskualduna* Coiffait.

Aphaenops eskualduna is reported from Spain with precision for the first time.

Key words: Carabidae, Trechini, *Aphaenops parvulus* **sp. n.**, subterranean environment, Pyrenees, Spain, molecular phylogeny

Introduction

The 41 species included in the genus *Aphaenops** Bonvouloir 1862 are all Pyrenean endemics highly adapted to troglobitic life. The genus was shown to be polyphyletic by Faille *et al.* (2010), composed of two independent lineages of cave beetles, the first occupying the west of the Pyrenean chain (subgenera *Aphaenops* *s. str.*, *Geophaenops* Cabidoche 1965, *Cephalophaenops* Coiffait 1962, in part, and *Arachnaphaenops* Jeanne 1967, in part), and the second inhabiting the central-eastern Pyrenees (*Cerbapphaenops* Coiffait 1962 plus some morphologically differentiated species) (Faille 2006; Faille *et al.* 2010). Mainly diversified in the French slope of the chain, the western radiation is extended in France between the area of Bagnères-de-Bigorre (Hautes-Pyrénées) and the Arbailles massif (Pyrénées Atlantiques); in Spain ten species belonging to this clade are known between Navarra (area of Zubiri) and Ribagorçana valley. During biospeleological expeditions in the area of Villanúa (Western Pyrenees, Huesca, Spain), we discovered an unexpected species of Trechini of the *Aphaenops* (*sensu stricto*) group.

* On the use of the graphy *Aphaenops* instead of *Aphoenops*, please see Faille *et al.* 2010

Methods and phylogenetic analyses

Specimens were collected alive by hand in the cave and preserved in absolute ethanol in the field or collected by mean of pitfall traps containing propylene glycol, known to preserve mitochondrial and nuclear DNA (Rubink *et al.* 2003; López & Oromí 2010) (Table 1). Extractions of single specimens were non-destructive,

TABLE 1. Sequenced specimens, with localities, collectors, codes and sequence accession numbers (unpublished sequences in bold).

sp	locality	collector	code	LSU	cox1
<i>Aphaenops Bonvouloir, 1862</i>					
<i>Aphaenops Bonvouloir, 1862 (sensu stricto)</i>					
<i>Aphaenops leschenaulti</i> Bonvouloir, 1861	Grotte de Castelmouly - Bagnères-de-Bigorre (France-65)	C. Bourdeau, P. Déliot, A. Faille	MNHN-AF1	GQ293593	GQ293629
<i>Aphaenops catalonicus</i> Escolà & Canció, 1983	Cova des Toscllosses - Bonansa (Spain-Huesca)	C. Bourdeau, P. Déliot, J. Fresneda	MNHN-AF2	HM1921082	GQ293674
<i>Aphaenops toubensi</i> Jeannel, 1953	Salle de la Verna - Sainte-Engrâce (France-64)	C. Bourdeau, P. Déliot, A. Faille	MNHN-AF3	HM1921083	GQ293660
<i>Aphaenops abodiensis</i> Dupré, 1988	Villanueva de Aezkoa - Sierra de Abodi - P70 (Spain-Navarra)	C. Bourdeau, A. Faille, E. Quéinnec	MNHN-AF4 MNHN-	GQ293555	GQ293627
<i>Aphaenops bessoni</i> Cabidoche, 1961	Gouffre du Col d'Aran 3 - Bielle (France-64)	C. Bourdeau, E. Ollivier	AF122	GQ293554	HM1921076
<i>Aphaenops cabidochei</i> Coiffait, 1959	Salle de la Verna - Sainte-Engrâce (France-64)	C. Bourdeau, P. Déliot, A. Faille	MNHN-AF5	GQ293556	GQ293667
<i>Aphaenops ochsi</i> Gaudin, 1925	Grotte d'Ayssaguer - Larrau (France-64)	C. Bourdeau, P. Déliot, A. Faille	MNHN-AF7	HM1921084	GQ293666
<i>Aphaenops jeanneli</i> (Abeille de Perrin, 1905)	Aven d'Istaurdy - Aussurucq (France-64)	C. Bourdeau, P. Déliot, A. Faille	MNHN-AF11	GQ293594	GQ293661
<i>Aphaenops orionis</i> Fagniez, 1913	Gouffre EL71 - Château-Pignon (France-64)	C. Bourdeau, P. Déliot, A. Faille	MNHN-AF9	GQ293592	HM1921077
<i>Aphaenops alberti</i> Jeannel, 1939	Aven prox. Istaurdy - Aussurucq (France-64)	C. Bourdeau	MNHN-AF12	GQ293595	GQ293662
<i>Aphaenops parvulus</i> sp.n.	Cueva d'Esjamundo - Villanua (Spain-Huesca)	C. Bourdeau, A. Faille, J. Fresneda	MZB-AF133	HM1921085	HM1921078
<i>Cephalaphaenops</i> Coiffait, 1962					
<i>Aphaenops eskualduna</i> Coiffait, 1959	Salle de la Verna - Sainte-Engrâce (France-64)	C. Bourdeau	MNHN- AF132	HM1921086	HM1921079
<i>Geaphaenops Cabidoche, 1966</i>					
<i>Aphaenops rhadamanthus</i> (Linder, 1860)	Aven de Nabails - Arthez d'Asson (France-64)	C. Bourdeau, P. Déliot, A. Faille	MNHN-AF13	HM1921087	HM1921080
<i>Aphaenops ludovici</i> Colas & Gaudin, 1935	Grotte d'Ambielle - Arette (France-64)	C. Bourdeau, P. Déliot, A. Faille	MNHN-AF15 MNHN-	GQ293550	GQ293676
<i>Aphaenops quefféleci</i> Cabidoche, 1965	Gouffre Achacama - Urutchurdoky (France-64)	C. Bourdeau	AF134	HM1921088	HM1921081
<i>Cerbaphaenops</i> Coiffait, 1962					
<i>Aphaenops cerberus</i> (Dieck, 1869)	Grotte du Sendé - Moulis (France-09)	P. Déliot, A. Faille	MNHN-AF30	GQ293589	GQ293646

using a standard phenol-chloroform method or the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). After extraction, specimens were mounted on card. When necessary, the genitalia were extracted and stored in water-soluble dimethyl hydantoin formaldehyde resin (DMHF) on cards, and pinned beneath the specimen from which they had been removed. Vouchers and DNA samples are kept in the collections of the Museu de Zoologia of Barcelona (MZB), the Museum National d'Histoire Naturelle of Paris (MNHN), the Institut of Evolutionary Biology in Barcelona (IBE) and author's collections. We included 15 species of *Aphaenops* (*sensu stricto*) plus *Geaphaenops*, and the tree was rooted in a species belonging to the eastern radiation of Pyrenean troglotic Trechinae, *A. (Cerbaphaenops) cerberus* Dieck which is unambiguously outside the *Aphaenops* (*sensu stricto*) group (Faille *et al.* 2010).

We amplified fragments of two genes, one mitochondrial (675bp of the 5' end of cytochrome c oxidase subunit 1, *cox1*) and one nuclear (ca 895 bp of the large ribosomal unit, LSU) (see Table 2 for primers used, and Faille *et al.* (2010) for general PCR conditions). Sequences were assembled and edited with Bioedit v. 7.00 (Hall, 1999) or Sequencher 4.6 (Gene Codes, Inc., Ann Arbor, MI). New sequences have been deposited in GenBank with Acc. Nos. HM921076–HM921088 (Table 1). Part of the sequences for 12 of the species were taken from Faille *et al.* (2010) (Table 1).

We aligned the sequences using the MAFFT online v.6 with the Q-INS-i algorithm and default parameters (Katoh & Toh 2008). Maximum likelihood analyses were conducted on a combined data matrix with RaxML online (Stamatakis, 2006; Stamatakis *et al.*, 2008), using two partitions corresponding to the two genes and with a GTR+G approximation as the evolutionary model. We used the default values for other parameters of the search (Stamatakis *et al.* 2008). RAXML estimates node support with a fast bootstrapping algorithm, and at the same time performs a maximum likelihood search that gives an optimal tree (Stamatakis *et al.* 2008).

TABLE 2. Primers used in the study.

marker	primer	sequence	Ref
Cox1	RON	GGATCACCTGATATAGCATTCCC	Simon <i>et al.</i> (1994)
	TOM1	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	Ribera <i>et al.</i> (2010)
28S-LSU	D3	5' GCATAGTTCACCATCTTTC 3'	Ober (2002)
	D1	5' GGGAGGAAAAGAACTAAC 3'	Ober (2002)

***Aphaenops (Aphaenops) parvulus* Faille, Bourdeau & Fresneda sp. n.**
(Figs. 1, 3)

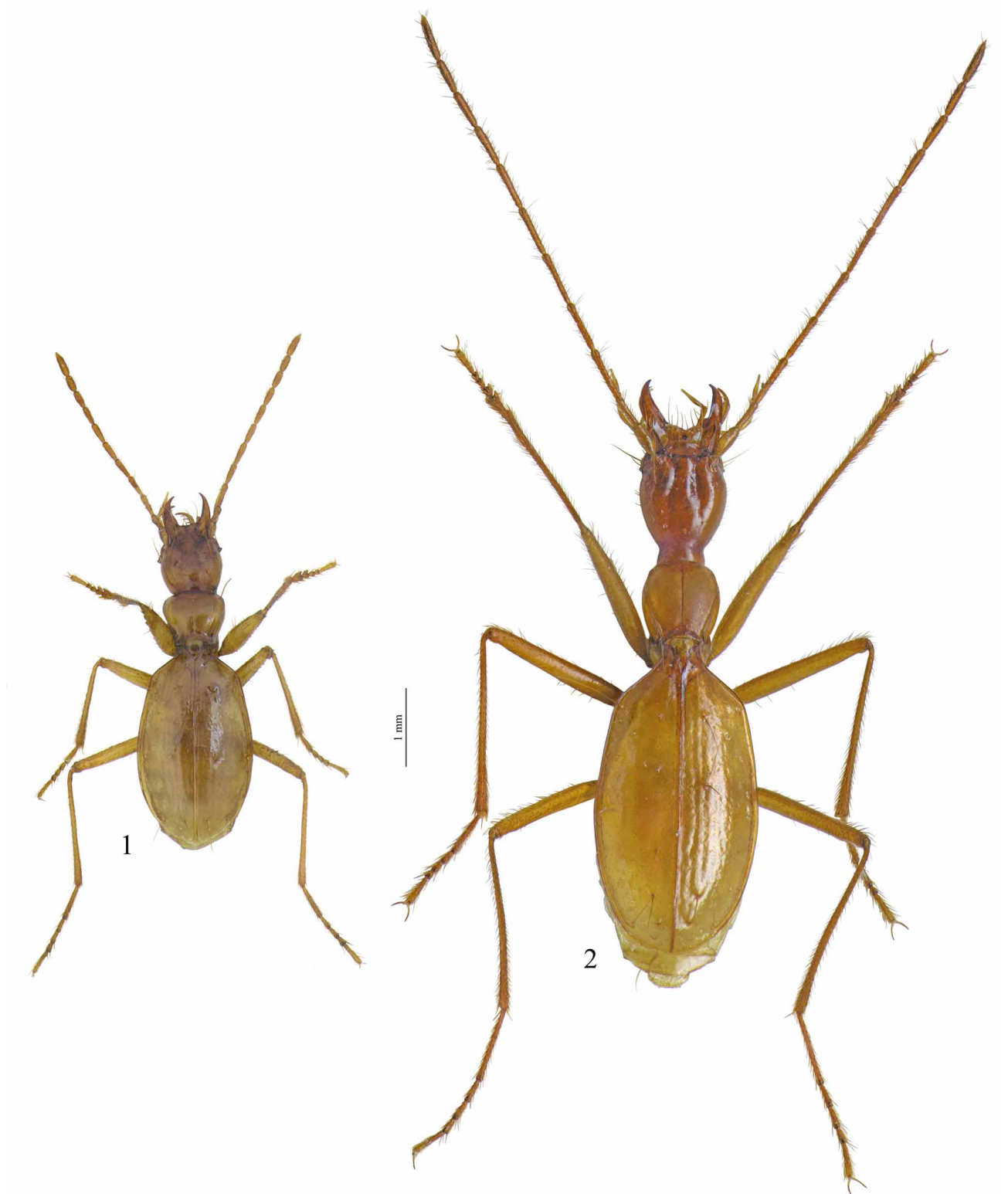
Diagnosis. *Aphaenops parvulus* sp. n. shows the characteristics of the genus, namely, depigmentation, ocular furrows shortened, not complete, striae almost erased, elytra glabrous with only some long setae in the border of elytra and on the disk, chitinous membrane of the endophallus staying on the right side, not symmetrically placed. It is easily distinguishable from geographically related species of the genus because of its small size.

Type locality. Spain, Huesca, Villanúa, Cueva de Esjamundo (= Cueva Nueva), 980 m, UTM (WGS 84): 30 T 701860 4728657.

Type series. Holotype (Fig. 1): ♂ (Museu de Zoologia, Barcelona), [label data: voucher number MZB-AF133] Cueva de Esjamundo, Villanúa, (Spain, Huesca), 8.VIII.2009, leg. C. Bourdeau, A. Faille & J. Fresneda. **Paratypes** (Muséum National d'Histoire Naturelle, Paris, and authors' collections): 1 ♂ (MNHN), remains of 2 ♂ (elytra, abdomen with genitalia), remains of 1 ♀ (head, pronotum, elytra, abdomen with genitalia) and 11 exemplars (elytra, 4 exemplars with pronotum and head, sex unknown): same locality, data and collectors than the holotype. Remains of 1 ♂ (elytra, abdomen with genitalia) and 1 ♀ (head, pronotum, elytra, abdomen with genitalia), 6.VI.2009, same locality and collectors. Remains of 7 exemplars (elytra and pronotum, 2 exemplars with head, sex unknown), 27.IX.2009, same locality and collectors.

Description of the holotype. *Habitus and size.* Length from labrum to apex of elytra: 5 mm. Anophthalmous and depigmented; stocky, robust, with cordate pronotum (Fig. 1). microsculpture fine but distinct, consisting of isodiametric meshes.

Chetotaxy. Surface of elytra glabrous with the exception of a periscutellar seta, two setae on the disk, four humeral setae, four setae along lateral margin and two preapical setae. Marginal setae of pronotum present, the basal ones located before the first third of the length. Ventral pubescence limited to one seta on each half sternite.



FIGURES 1–2. Habitus. 1—holotype of *Aphaenops parvulus* sp. n.; 2—*A. eskualduna* Coiffait.

Head. Glabrous; round; mandibles robust with a tooth at internal edge of the right mandible (width 1 mm). Penultimate segment of maxillary palpus distinctly pubescent.

Antennae. Proportionally short. Length: 3.4 mm; densely pubescent from the second antennomere. Antennal formula: see Table 3.

TABLE 3. Antennal formula of the holotype of *Aphaenops parvulus* sp. n. (mm).

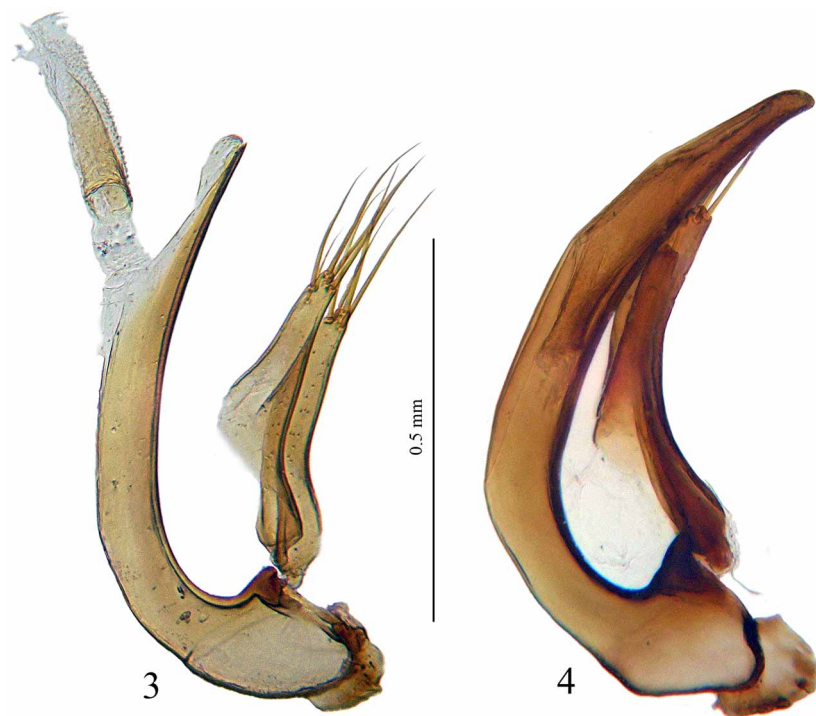
Article	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Length	0.275	0.25	0.35	0.3375	0.325	0.3125	0.3125	0.3	0.275	0.2625	0.4

Pronotum. Cordate, transverse and strongly narrowing towards base (length 0.8 mm x width 0.9 mm); sides very arcuate, maximum width in the anterior third, without any distinct puncture. Pronotal hind angles protruding, very acute.

Elytra. About one time and half longer than wide, glabrous, convex, with marked shoulders (length: 2.9 mm). Striation obsolete; isolated punctures distributed irregularly.

Legs. Proportionally short, pubescent, tibiae straight. Male protarsomeres 1–2 strongly dilated, first wider than apex of protibia; onychium longer than protarsomeres 2–4 combined.

Aedeagus (Fig. 3). Median lobe elongate, in lateral view curved, parameres with respectively four (left) and five (right) setae. Copulatory piece sclerotized, elongate, forming a gut.



FIGURES 3–4. Aedeagus in lateral view. 3—holotype of *Aphaenops parvulus* sp. n.; 4—*A. eskualduna* Coiffait.

Female diagnosis. Only the remains of two females are known with certainty. Head, pronotum and elytra with same characteristics as the holotype. Gonocoxites glabrous.

Etymology. Specific epithet referring to the small size of the new species.

Distribution and ecology. *Aphaenops (Aphaenops) parvulus* sp. n. is so far known from a cave only, the Cueva de Esjamundo located in the Pyrenean chain of Huesca, north of Jaca, Spain (Fig. 5).

The Esjamundo cave is a large cavity with high humidity, calcareous concretions and clay. The first part of the cave is paved with sandy soil. All the specimens of *Aphaenops parvulus* sp. n. were found in this dry and sandy part of the cave. None of the specimens or remains were observed in the most humid area of the cave. The fact is something exceptional within the genus *Aphaenops*, the species of which are usually highly hygrophilic. In spite of morphological affinities, the ecology of *A. parvulus* sp. n. is very different from that of

the species of the *Geaphaenops* group, which are endogean, living in the ground and found only occasionally in caves (Jeannel 1938; Cabidoche 1965).

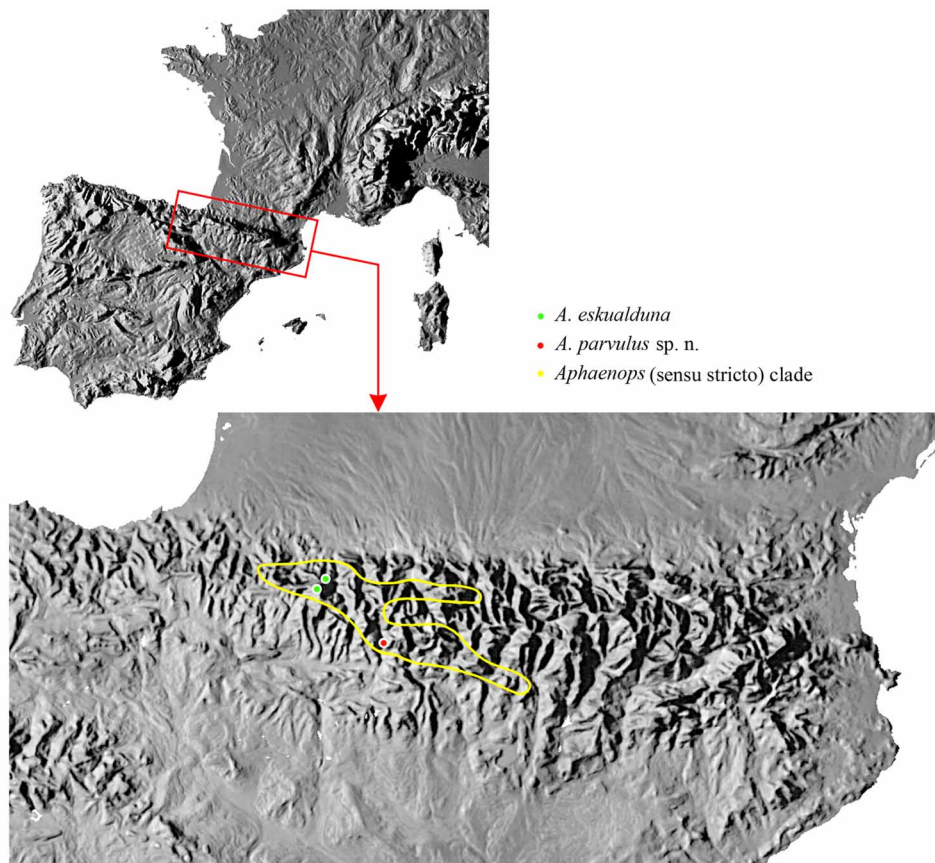


FIGURE 5. Map of Pyrenees showing the distribution of *Aphaenops parvulus* **sp. n.** (red circle), *A. eskualduna* Coiffait (green circles) and the general distribution of the *Aphaenops* (sensu stricto) clade (yellow line).

The reduced area where the new species was discovered is already known for its richness in hypogean endemic Coleoptera. The new species is sympatric with the Leptodirini *Bathysciola obermaieri* Bolívar 1918 (Leiodidae, Cholevinae), which is relatively abundant especially in the soil at the entrance and in the first part of the cave, as already noted by Español (1966). The region is inhabited also by several troglotic trechine species. *Geotrechus dumonti* Español 1977 is known only from a nearby cavity, Las Guixas, but was not observed in the Esjamundo cave. High altitude shafts of Peña Collarada, above Villanúa, are colonized by two species of blind Trechini, *Hydraphaenops penacollaradensis* Dupré 1991 and *Aphaenops valleti* Casale & Genest 1986.

Some specimens of *Aphaenops parvulus* **sp. n.** were parasitized by the Ascomycete *Rhachomyces girardii* Lapesme & Tempère 1948, which was described on specimens collected on *Aphaenops ochsi* Gaudin, and known on *Aphaenops valleti* Casale & Genest, *A. loubensi* Jeannel and *A. alberti* Jeannel (Santamaria & Faille 2007). We observed this Laboulbeniale also on *Aphaenops bessoni* Cabidoche, but never on the members of the subgenus *Geaphaenops*.

Affinities. *A. (Aphaenops) parvulus* **sp. n.** is a representative of the western lineage of *Aphaenops* (sensu stricto) (in the sense of Faille *et al.*, 2010). It is the smallest species of this group and it shares common characters with some species of the subgenus *Geaphaenops*, especially *A. rebereti* Gaudin 1946 and *A. cissauguensis* Faille & Bourdeau 2008. In spite of an external “*Geaphaenops*-like” morphology, the head of the new species is glabrous, whereas it is pubescent in the *Geaphaenops* group. The elongate median lobe of the aedeagus suggests affinities of the new species with *A. eskualduna* Coiffait (Fig. 4). The latter (Fig. 2) was described from La Verna cave, Pierre Saint Martin massif, Sainte Engrâce, in the French western Pyrenees and was supposed to be close to *A. bucephalus* Dieck 1869 from Ariège and Haute-Garonne (Coiffait 1959). The study of troglotic Trechini at the Museu de Zoologia of Barcelona allowed us to identify a specimen of *A.*

eskualduna collected in the Spanish side of the Pyrenees: 1 ♂, Huesca, Ansó, Gamueta, Avenc G-171, 9.VII.1983, O. Escolà leg. (new record).

A. parvulus **sp. n.** shares the pubescent penultimate segment of maxillary palpus with *A. eskualduna* and all species of *Geaphaenops*, whereas that segment is glabrous in all other species of the *Aphaenops* (sensu stricto) clade. This character is also shared with *A. mentioni* Lagar, species of uncertain affinities from the central Spanish Pyrenees.

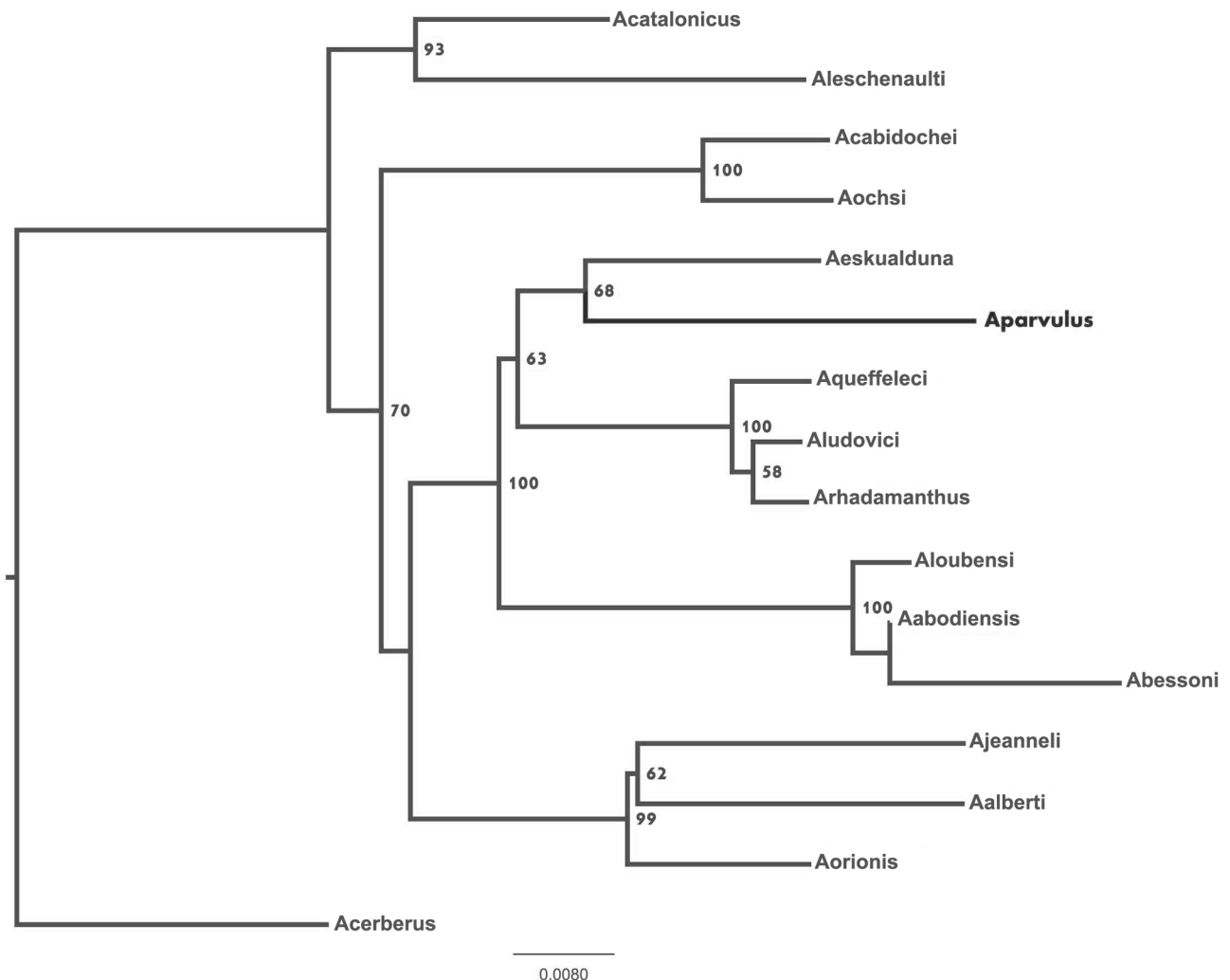


FIGURE 6. Phylogram obtained with RAxML and the combined mitochondrial and nuclear data. Above nodes, bootstrap support values when superior to 65. See Table 1 for the localities of the specimens.

Molecular phylogeny of the species of the *Aphaenops* (sensu stricto) group

The resulted topology (Fig. 6) is in accordance with the one published by Faille *et al.* (2010). The species newly added in the analyses were *A. queffeleci* Cabidoche, *A. eskualduna* Coiffait and *A. parvulus* **sp. n.** As suggested by its descriptor, *A. queffeleci* is very close to the *rhadamanthus* group, especially to *A. ludovici* Colas & Gaudin. *Aphaenops eskualduna* was said to belong to the *Cephalaphaenops* group and considered close to *A. bucephalus* (Coiffait, 1959). Our results suggest that this species should be considered as the sister species of *A. parvulus* **sp. n.** The clade *Geaphaenops* could be sister to these two species but the support is low. As previously said, due to the lack of some species it is not possible to unambiguously determine the status of *Geaphaenops*. *Aphaenops parvulus* **sp. n.**, *A. eskualduna*, the *Geaphaenops* species and the group of *A. loubensi* (*A. loubensi* Jeannel, *A. bessonni* Cabidoche and *A. abodiensis* Dupr) form a clade with very strong support.

A comprehensive phylogeny including more genes and species will be necessary to study in detail the radiation of the *Aphaenops* (sensu stricto) group in this area of the Pyrenees and to test the monophyly of *Geaphaenops*, gathering species on the base of external morphological characters (pubescence of the head, “anophthalmous” facies typical of endogean species).

The discovery of *Aphaenops parvulus* sp. n. and the geographical gap between the new species and *A. eskualduna* suggest that biospeleological explorations in the western Pyrenees of Aragon could still allow the discovery of new species of blind Trechini, what would much help to understand the diversification of the subterranean Pyrenean diversity of this group of ground beetles.

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