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SHORT COMMUNICATION

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First record of *Pisidium subtruncatum* Malm, 1855 (Bivalvia, Sphaeriidae) in an African cave

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Abstract

Studies on the bivalve family Sphaeriidae in North Africa are very limited at the surface water level, but even more for caves. During an expedition in 2019 to the Ait M'hamed cave (Oum Er Rabia Basin), six specimens of the genus *Pisidium* were collected. Morphometric and genetic analyses showed that these individuals belong to the species *Pisidium subtruncatum* Malm, 1855. This work is the first step towards future exploration of cave Sphaeriidae in North Africa.

Keywords

Molluscs, Subterranean, Invertebrates, Biospeleology, Ait M'hamed, Morocco

Introduction

Pisidium is a genus of freshwater bivalves belonging to the family Sphaeriidae that includes the smallest bivalves on Earth. Despite their small size, *Pisidium* species can be used for bioindication studies (Horsák 2001) and the usefulness of these species as markers of metal and organic pollution has been proved repeatedly (e.g. Ingram et

al. 1953; Wurtz 1955; Anderson 1977; Gadzała-Kopciuch et al. 2004; Alhejoj et al. 2017). The group is cosmopolitan and occurs in temporary and permanent aquatic environments. Along with Dreissenidae, Sphaeriidae is the only family of bivalves inhabiting subterranean habitats (Culver 2012; Prié 2019). Their occurrence in caves has been reported by a number -albeit few- of authors from different localities (e.g. *Pisidium hallae* Kuiper, 1983, *Sphaerium tasmanicum* Tenison Woods, 1876 from Australia (Kuiper 1983; Korniushin 2000), *Pisidium zoctanum* Poli, 1876 and *Pisidium crimeana* Stadnichenko, 1980 from Ukraine (Vargovitsh and Anistratenko 2016; Vinarski and Kantor 2016), *Pisidium casertanum* Poli, 1791 and *Pisidium personatum* Malm, 1855 from Scotland (Knight and Wood 2000; Knight 2018) and *Pisidium ljovushkini* Starobogatov, 1962, *P. cavatica* Zhadin, 1952 and *P. subterranea* Zhadin, 1932 from Caucasus (Vinarski and Kantor 2016)). In North Africa, studies on the freshwater clams of caves are lacking. In fact, in Morocco, even fewer studies are limited to the distribution of *Pisidium* species were seven species are reported (Kuiper 1972) and where extreme environments such as caves are not prospected. The aim of this paper is to report for the first time the occurrence of a Sphaeriidae species in a Moroccan cave.

Material and methods

In May 2019, we prospected the Ait M'hamed cave. This cave is located in Oum Er Rabia basin at 1693 m of altitude (31°52'48"N, 06°27'02"W). The cave is dug at the bottom of a cliff in the calcareous of Bajocian – Bathonian period with horizontal stratification (Doat et al. 2005). The water flowing inside the cave is drained from a spring since it is permanent water even during dry season and expeditors reported the continuity of flowing tributaries even after more than 1500 m from the cave entrance (Doat et al. 2005). The entrance to the cave is wide, about 5 m large and 2.50 m high (Fig. 1A). Physical-chemical parameters of the water were measured at two points, the cave entrance and the waterfall (Table 1) using a multiparameter tool (HI98194 portable probe).

The sampling was carried out with a sieve of 200 μm of diameter in muddy sediments and lead to the collecting of 6 specimens belonging to the genus *Pisidium* (Fig. 1B, C). The maximum distance explored of the cave is 4000 m, however, only 3052 m were topographically mapped (Fig. 2). The specimens were collected at two points: one at 100 m and the second at 500 m from the entrance. Specimens collected were placed in 80% ethanol for morphological and genetic analysis. No permit for sampling was required.

In the laboratory, the identification of the specimens was based on morphological characters following the descriptions of Adam (1960) and Killeen et al. (2004) using a stereomicroscope (Leica Microsystems CH 9435 Loupe). On the basis of the scaled images of the shells obtained with the stereomicroscope, we used TpsDig v. 2.31 (Rohlf 2005) to produce the following shell measurements for a better morphological diagnosis: **L** (shell length), **H** (shell height), **LP** and **LA** (length of posterior and anterior parts respectively), **LL** (length of ligament), **LE** (umbo length), **LH** (hinge length)

Figure 1. Study area Ait M'hamed cave **a** The cave entrance **b** the sampling and **c** the inside of the cave (Moutaouakil 2019).

Table 1. Measurements of physical and chemical parameters at two localities in the cave system (see Fig. 2, May 2019).

	H (%)	T(°C)	T(°C) of water	Dissolved oxygen. (mg/l)	Conductivity (µS/com)	pH	Nitrites (g/mol)	Phosphate ion (g/mol)	Ammonium (g/mol)
Cave entrance	28	20.7	20	5.32	421	7.2	0.08	0.11	0.03
Waterfall	28	19.1	21.6	4.65	432	7.09	0.071	0.06	0.05

and **HH** (hinge height). The mean shape of the shells was obtained on the basis of semi landmark coordinates plotted with TpsRelw v. 1.70 (Rohlf 2003) (Fig. 4).

Soft bodies were extracted for genetic analysis in order to confirm morphological identification. DNA isolation followed a CTAB protocol (Wilke et al. 2006). Amplification of mitochondrial gene fragments which are regularly used in sphaeriid barcoding and phylogenetics was unsuccessful. Therefore, Polymerase Chain Reaction after 9 cycles running for 1,5 h was performed with thermocycler Eppendorf Mastercycler using the nuclear gene H3 and primers of Colgan et al. (2000). Sequencing was carried out on an ABI 3730 at LGC Genomics, Berlin, Germany. Resulting sequences were checked in the NCBI database using nucleotide BLAST (BLASTn suite: megablast) returning highly similar sequences stored in the NCBI GenBank database (Zhang et al. 2000). The top five BLAST hits (sorted by max score; default) for each individual are shown in Table 3.

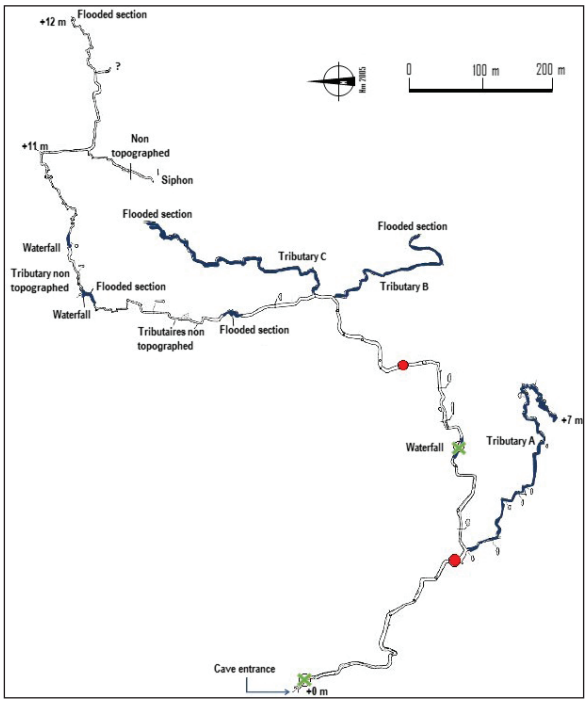

Figure 2. Cave topography. Red points: Sampling localities (green crosses included), green crosses: *P. subtruncatum* occurence.

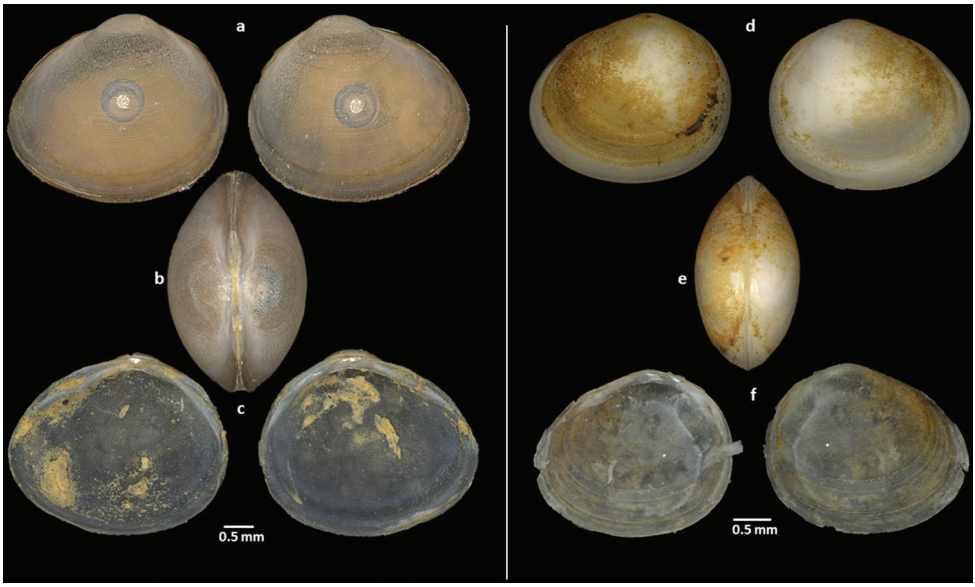

Figure 3. Two specimens of *P. subtruncatum* from Ait M'hamed cave **a, d** external view of the shell of the left and right sides of both specimens **b, e** dorsal view of both specimens **c, f** internal view of left and right valves of both specimens.

Table 2. Measurements of internal shell features.

	N	L	H	LA	LP	LE	LL	LH	HH
Mean ± SD	4	2.96 ± 0.81	2.28 ± 0.53	1.8 ± 0.64	1.16 ± 0.31	0.81 ± 0.24	0.48 ± 0.09	1.44 ± 0.38	0.15 ± 0.05

Table 3. List of the first five significant BLAST hits (NCBI GenBank accessed on 15/06/2019).

Description	Max score	E value	Percent identity	Accession
Pisidium subtruncatum isolate 17469 histone 3 (H3) gene, partial cds	599	3e-167	99.39%	KU376244.1
Pisidium atkinsonianum isolate 6024 histone 3 (H3) gene, partial cds	595	3e-166	99.39%	KU376227.1
Pisidium viridarium isolate 15834 histone 3 (H3) gene, partial cds	590	2e-164	99.09%	KU376246.1
Pisidium personatum isolate 17456 histone 3 (H3) gene, partial cds	590	2e-164	98.78%	KU376241.1
Pisidium casertanum isolate 17462 histone 3 (H3) gene, partial cds	586	2e-163	98.78%	KU376228.1

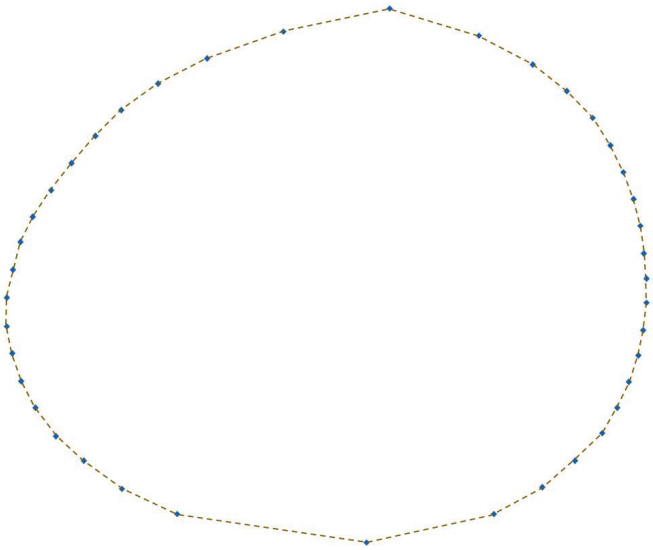

Figure 4. Mean overall shell outline shape of the four adult specimens of *P. subtruncatum*. The mean shape was generated from semilandmarks coordinates of the right valves using the tpsRelw.

Results and discussion

Morphometric results of the four specimens collected showed that they have a length ranging between 3.49 and 1.91 mm and height between 2.93 and 1.62 mm. The shell is silky with slight striations and the umbo is narrow and located posteriorly. The shape of the shell is sub-angulated, the most extreme point of the anterior part is located lower than the middle of the shell height (Figs 3, 4). The anterior part is clearly longer than the posterior part (see measurements on Table 2). The hinge is thicker, more or less wide. The ligament pit is long. The left valve with two long cardinal teeth, the lower (C_2) and the uppermost (C_4) parallelly located, C_4 overlaps C_2 at anterior end, C_3 is long and slightly curved (Fig. 5). All individuals found in the present work are exactly similar

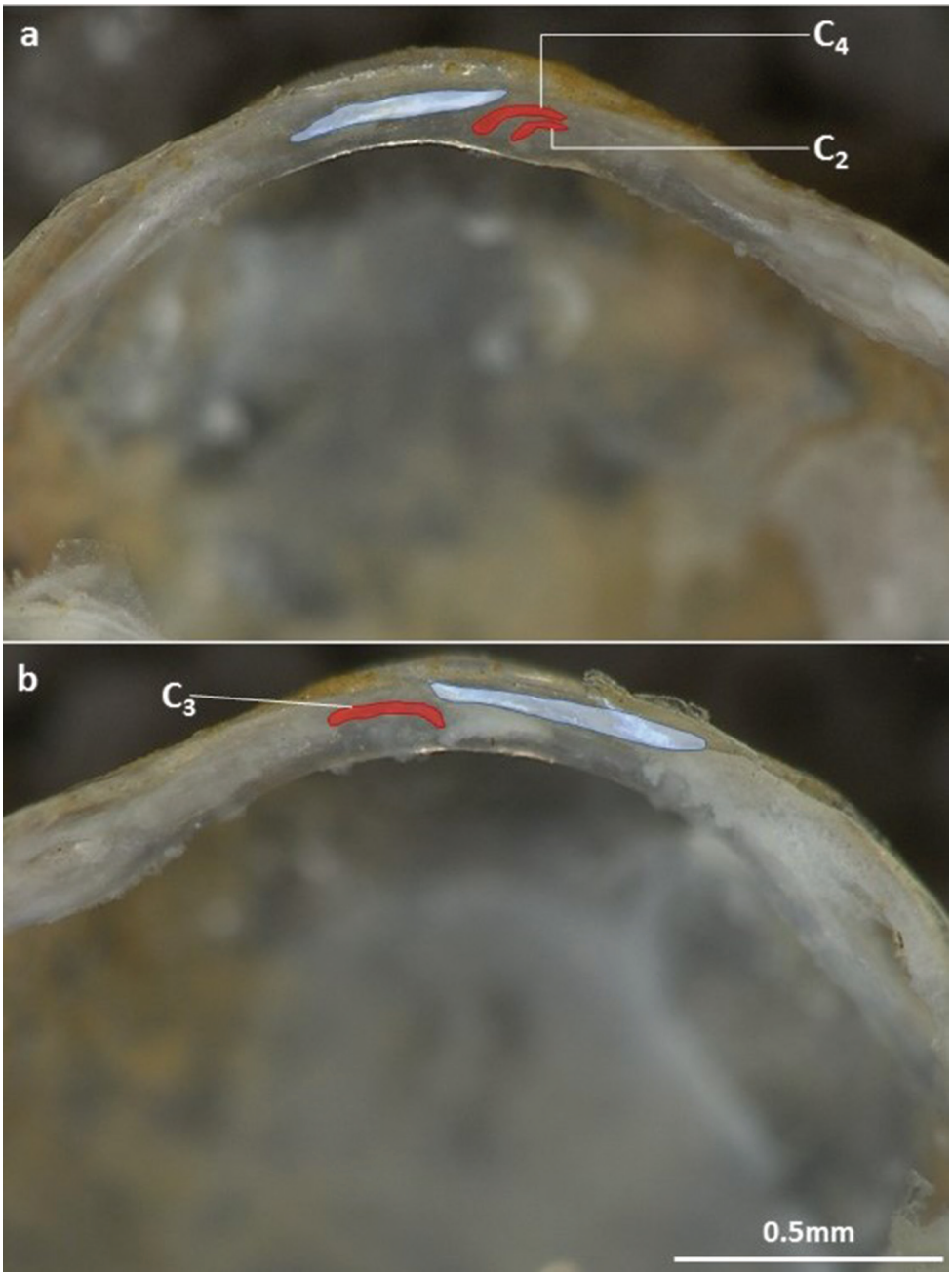

Figure 5. Position and shapes of cardinal teeth and ligament pits in left (**a**) and right valve (**b**). c: cardinal teeth.

to the description given by other authors (for a review see Adam 1960; Piechocki 1989; Killeen et al. 2004). Moreover, the identification was also confirmed by a specialist researcher who is familiar with *Pisidium* (M. Zettler Warnemünde 2019, in litt.).

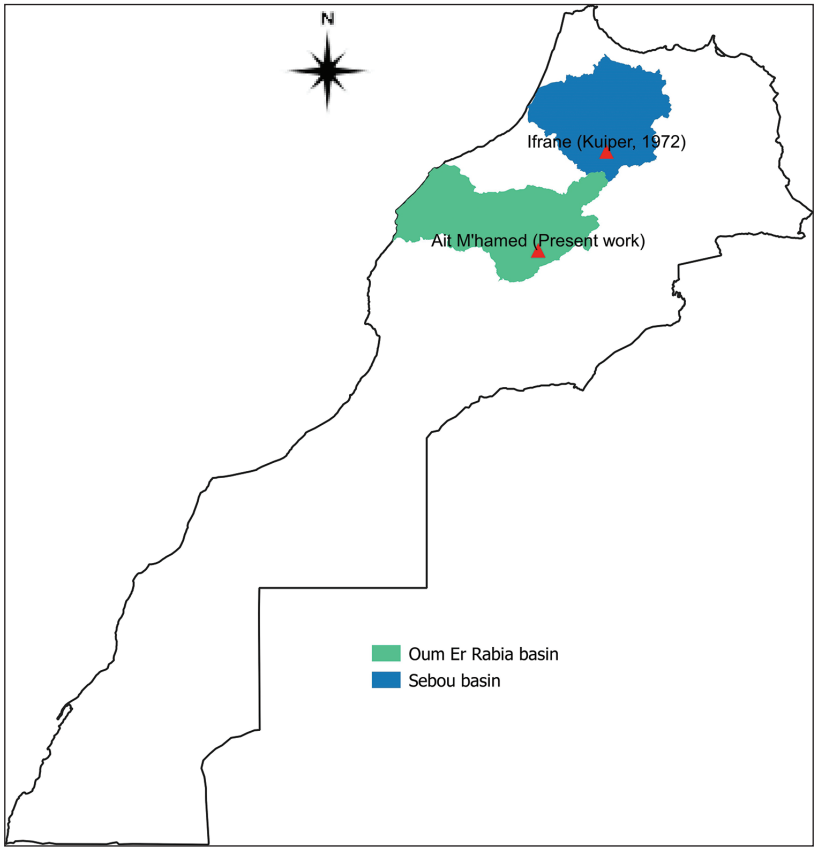

Figure 6. Map of occurrence localities of *Pisidium subtruncatum* from this paper and previous record (red triangles).

Genetic results did not contradict the identification of the species as *P. subtruncatum* and, as presented in the list of significant BLAST hits (Table 3), the five first sequences with the highest similarity with our sequences are *Pisidium subtruncatum*, *Pisidium atkinsonianum* Theobald, 1876, *Pisidium viridarium* Kuiper, 1956, *Pisidium personatum* and *Pisidium casertanum*, all from Nepal (Boessneck et al. 2016). With all uncertainty related to the conservative nature of the marker H3, these results (max score 599, see Table 3) support the morphological determination of the cave specimens as *P. subtruncatum*.

P. subtruncatum was already recorded in a river of the Sebou basin (Kuiper 1972) (Fig. 6), but no published studies cited the presence of this species in the Oum Er Rbia basin. The IUCN conservation status of this species in North Africa is considered as endangered because of its restricted area of occupancy and declining quality of habitat (García et al. 2010). The four individuals collected were from two localities and they inhabited a dark and muddy environment with no sign of anthropogenic influence. The water depth did not exceed 1 m and its overall quality is assessed as good (Table 1) (ONSSA 2018). The ecology of the genus *Pisidium* is resulting in surprising flexibility

as outlined by the current finding of a species living in the solid interstitial environment in Germany (Groh et al. 2020). *Pisidium subtruncatum* is an euryecious species with a palearctic distribution, inhabiting different kinds of habitats, its optimum conditions are met in small rivers with sandy-muddy substratum (Piechocki 1989), especially when being concentrated with macro-ions and organic matter (Bespalaya 2015). This agrees with our findings (e.g. high conductivity). The influence of darkness was not considered for the present note; however, it is known that all bivalves have light-sensitive cells (Cofransesco 2002) and the impact of light on bivalves growth had been proved by Medcof and Kerswill (1965).

Conclusion

In general, the Sphaeriidae family is neglected in North Africa and studies on this group of benthic organisms are very limited compared to other taxa. The originality of this work consists in the recording for the first time of a member of the Sphaeriidae family in an African cave and to our knowledge the first record of *P. subtruncatum* in a cave. Studies such as ours reported here should be expanded to other caves in Morocco (Fig. 6). This is important in order to enhance our faunal knowledge and to determine the actual conservation status of *Pisidium* species. Moreover, this need becomes urgent given the increasing human pressure including habitat loss and anthropogenic transformation of habitats of *Pisidium* species (e.g. rivers, lakes and springs) in a Mediterranean biodiversity hotspot region such as Morocco.

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