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Redescription of *Orculella aragonica* (Westerlund 1897), an Iberian species different from *O. bulgarica* (Hesse 1915) (Gastropoda: Pulmonata: Orculidae)

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Abstract. *Orculella bulgarica* (Hesse 1915) has been recorded from Bulgaria and western Asia, including Turkey, but has also been reported from Spain by several authors. Most studies on this species have been on subfossil shells. Recent findings of living populations in Turkey and Spain have allowed us to report on the reproductive system morphology and mtDNA sequences of this taxon. Despite the apparent lack of conchological differences between specimens from these two geographical areas, this new information revealed the presence of two species. Review of the literature pertaining to Iberian orculids, led us to conclude that the examined population in Spain species must be assigned to *Orculella aragonica* (Westerlund 1897), and all previous reports of *O. bulgarica* from the Iberian Peninsula should be ascribed to the former. This species is redescribed and diagnosed herein, highlighting differences between it and *O. bulgarica*. Some notes about its conservation status and biogeographic origin are also provided.

Key words: *Orculella aragonica*, *Orculella bulgarica*, distribution, Turkey, Spain, taxonomy.

Introduction

Orculella bulgarica was described by Hesse (1915) from "Gebedsche bei Varna" (Bulgaria). Subsequent authors extended its distributional range to two distant areas, namely (1) Bulgaria and Western Asia (Armenia, Azerbaijan, Russian Federation, Turkey) (Hausdorf 1996) and (2) Spain (Garrido *et al*. 2005, Robles & Martinez-Ortí 2009), with no records within this broad distributional hiatus. The studies were based on empty shells (fossil or "subfossil"), except those collected in Bulgaria by Urbański in 1960 (Gittenberger 1983). The genital anatomy remained unknown because of the lack of living material. As a result, Hausdorf (1996) questioned the generic position of *O. bulgarica*, because *Orculella* and *Schileykula* could only be distinguished on the basis of the genital structure, namely (mainly) the presence (*Orculella*) or absence (*Schileykula*) of a penial appendix (see also Páll-Gergely 2011).

The Iberian *Orculella* has been previously assigned to *Pupa dolium* Draparnaud 1801, *Pupa dolium* var. *plagiostoma,* Sandberger 1875, *Pupa dolium* var. nova Westerlund (in Calderon 1897), *Orcula dolium* forma *aragonica* Westerlund 1897 and *Orcula plagiostoma* (Sandberger) (in Brunnacker & Ložek 1969). The known localities were compiled by Gittenberger (1983) Gómez-Moliner (1988), Garrido et al. (2005) and Robles & Martinez-Ortí (2009).

Gittenberger (1983) compared Iberian *Orculella* to *O. bulgarica* from Bulgaria, concluding that they were conspecific on the basis of conchological characters. The shells from both areas show a protoconch microsculpture of irregular spiral ridges and the presence of a palatal lamella in body whorl of juveniles, but lacking in adults. More recently, Hausdorf (1996) distinguished *O. bulgarica lamellata* (living in southeastern Turkey) from *O. b. bulgarica* on the basis of well-developed lamella in adult shells.

Garrido et al. (2005) discovered four extant populations of the Iberian *Orculella* in Granada Province (southern Iberian Peninsula). They described the periostracum of live-collected juvenile shells (thin, oblique ribs in the shape of platelets which are lost in the adult shells), as well as the reproductive anatomy. The latter confirmed the generic position of the species within the genus *Orculella*.

Based on records spanning several million years in age (Plio-Pleistocene to Recent), Robles & Martinez-Ortí (2009) concluded that the species was formerly widely distributed along the eastern half of the Iberian Peninsula, but its geographical

range was significantly reduced recently. Gittenberger (1983) and Preece (1991) published detailed interpretations of this distributional contraction. At the present time, three of the four living populations recently discovered by Garrido et al. (2005) in Andalusia have become extinct, but seven new populations have been discovered after intense searching in the surrounding areas (Arrébola & Garrido 2008, Arrébola in prep.).

We have also found a small living population of *O. bulgarica bulgarica* in northeastern Turkey. This allowed us to compare specimens from the two distant geographic locations through analysis of shell and reproductive system morphology, and DNA sequences in order to clarify the systematic position of the Spanish populations.

Material and methods

Samples

Samples from different localities in southern Spain (see Material Examined and Table 1 for descriptions) and one locality in Turkey were used for morphological studies of the shell and reproductive system. The Spanish locality habitats were described by Garrido et al. (2005). The Turkish locality was a wet, marshy bank of a stream, where specimens were crawling on the ground or on decaying plants among dense vegetation. The specimens are deposited in the collections of the Zoology Department of Seville University (Seville), Natural History Museum of Valencia and in the private collection of the last author (Mosonmagyaróvár, Hungary). Specimens of *O. aragonica* (6) and *O. bulgarica* (2) were preserved in 96% ethanol and used for molecular studies (Table 1). Five Spanish (P1, P5, P6) and wo Turkish specimes were dissected.

Abbreviations used in the text:

H: shell height

NHMW: Naturhistorisches Musem, Wien (Vienna, Austria)

DNA isolation

DNA was isolated from the foot of each snail using the Qiagen DNeasy Blood & Tissue Kit according to the manufacturer's instructions.

DNA Amplification and Sequencing

Polymerase chain reaction (PCR) was used to amplify two mitochondrial gene fragments, cytochrome oxidase subunit I (COI) and 16S rRNA (16S) using universal primers (Folmer et al. 1994, Palumbi et al. 1994, respectively).

The PCR amplifications were conducted in 23 µl reactions containing 2 µl diluted DNA, 2 µl dNTP (2.5 mM), 1.25 µl $MgCl_2$ (50 mM), 2.5 µl NH_4 buffer 10X, 0.5 µl of each primer (0.25 mM), 0.25 µl Taq Polymerase (5 U/µl) and 1 µl of BSA (10mg/ml). We used a Biorad iCycler thermal cycler with the following cycling conditions: an initial denaturation step at 96 ºC for 1 min; 35 cycles of denaturing at 94 ºC for 30 s, annealing at 51 ºC for COI or 56 ºC for 16S for 30 s and extending at 72 ºC for 1 min; and a final extending step at 72 ºC for 10 min. Reactions were held at 4 ºC.

PCR products were run in 1.5% agarose gels stained with GelRed, to verify the amplifications. Amplicons were sequenced using the BigDye Terminator Kit v1.1 (Applied Biosystems) in an ABI PRISM Model 3100 Avant Genetic Analyzer. The sequences were accessioned into GenBank (Table 1).

DNA sequences Analysis

Sequences were aligned using CLUSTALX v1.81 (Thompson et al. 1997) and then manually adjusted to minimize mismatches. The COI and 16SrRNA sequences were concatenated into a single dataset for phylogenetic analyses. *Pupilla muscorum* (Linnaeus 1758) was used as outgroup (sequences available in GenBank with accession numbers GQ921664.1 for COI and GQ921551.1 for 16SrRNA).

Phylogenetic reconstructions using PAUP 4.0b3 (Swofford 2002) were performed by a distance method using the neighbour-joining algorithm (NJ) (Saitou & Nei 1987). The HKY (Hasegawa et al. 1985) model was selected as the best-fit model of nucleotide substitution for the molecular data set by the Akaike information criteria approach using Modeltest 3.6 (Posada & Crandall 1998). The robustness of the trees was assessed by bootstrap resampling (1000 replications for NJ analysis; Felsenstein 1985).

We also performed a Bayesian analysis (BA) using MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001). The Markov Chain Monte Carlo (MCMC) search was performed using four chains for 2.5 million generations and using the most suitable model determined by Modeltest. Bayesian posterior probabilities were picked from the 50% majority rule consensus of trees sampled every 100 generations (the first 2000 trees were discarded as "burn in").

Uncorrected pairwise p-distances were calculated with PAUP v. 4.0b10 for the COI, 16S and concatenated dataset (length 635, 486 and 1121 nucleotides, respectively). These distances were interpreted as percentages.

Number of mtDNA haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π) and number of nucleotide differences (k) were calculated for the combined dataset using DnaSP 4.10.4 software (Rozas et al. 2003).

Results - Systematical part

Family Orculidae Pilsbry 1918

Genus *Orculella* Steenberg 1925

***Orculella aragonica* (Westerlund 1897)**

Material Examined (P= populations of *O. aragonica*) (total/dissected/DNA analyzed specimens): P1 (393/03/00) Rambla de la Viña spring, 940 m asl, 30SVG8029. P3 (12/00/01): 1400 m south from Barrio los Parrales (Lopera) (Potrera spring), 940 m asl, 30SVG7829. P5 (08/01/00): 325 m S-SE from Cortijo la Carrasca, 1417 m asl,

Table 1. Species abbreviations, geographical coordinates (UTM grid references) and Genbank accession numbers.

Sample	U.T.M._1km	GenBank accession numbers COI / 16S
*1-O.bulgarica**		JQ765693 / JQ765685
*2-O.bulgarica**		JQ765694 / JQ765686
3-O.aragonica P6a	30SVG6818	JQ765695 / JQ765687
4-O.aragonica P9	30SVG6523	JQ765700 / JQ765692
5-O.aragonica P6b	30SVG6818	JQ765696 / JQ765688
6-O.aragonica P8	30SVG6020	JQ765699 / JQ765691
7-O.aragonica P3	30SVG7829	JQ765697 / JQ765689
8-O.aragonica P7	30SWG2354	JQ765698 / JQ765690

**O. bulgarica* specimens: Turkey, on the borders of vil. Erzurum and vil. Erzincan, stream near Tercan tüneli (tunnel), 39°50'25.36"N, 40°34'0.56"E, leg. Páll-Gergely, B., 05.07.2010.

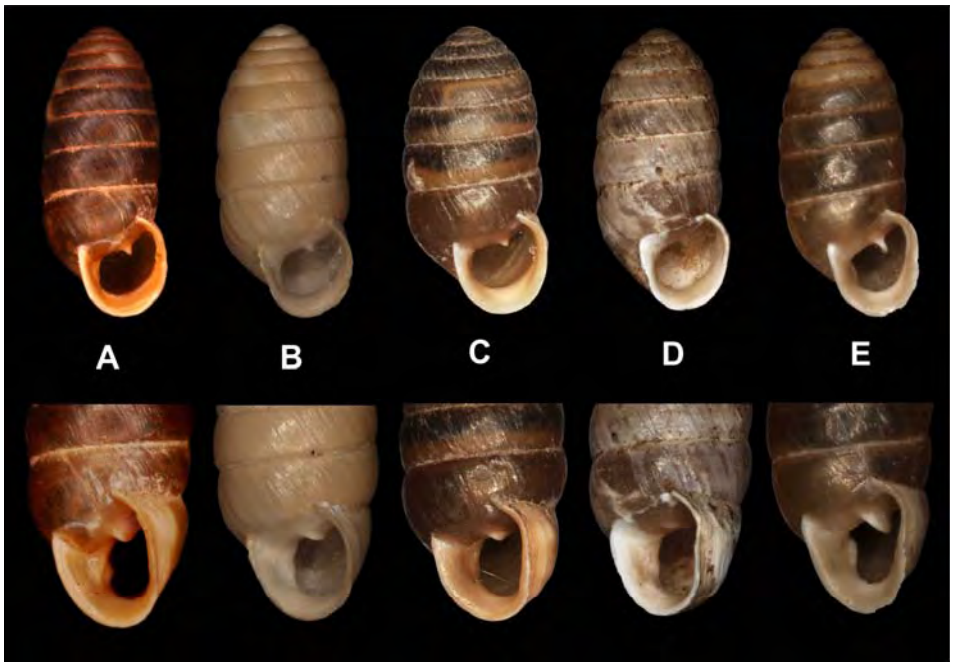

Figure 1. Shells of *Orculella* species. **A**: *Orculella aragonica* (Westerlund 1897) 325 m S-SE from Cortijo la Carrasca, 1417 m asl, 30SWG0938, H= 7,94 mm **B**: *Orculella aragonica*, labelled as *Orcula dolium plagiostoma*, Spain, Prov. Granada, Galva, Coll. Klemm, leg. Falkner 1968, NHMW 60920, H= 6.5 mm; **C**: *Orculella bulgarica bulgarica* (Hesse 1915) Turkey, on the border of vil. Erzurum and vil. Erzincan, Tercan tüneli, stream near the tunnel, 39°50'25.36"N, 40°34'0.56"E, leg. Páll-Gergely, B., 5.7.2010, NHMW ALSV6606, H=6.6 mm; **D**: *Orculella bulgarica bulgarica* (Hesse 1915), Turkey, Gaybi, 15km SW Eregli, Coll. Klemm, leg. Ressl 10.6.1965, det. B. Hausdorf 1989, NHMW 55500. H= 6.8 mm; **E**: *Orculella bulgarica lamellata* Hausdorf 1996, paratype, Turkey, Yesilyurt, Coll. Klemm, leg. Ressl 2.6.1965, det. B. Hausdorf 1992, NHMW 55531, H= 7.7 mm.

30SWG0938. P6 (14/01/02): spring on Barranco de las Ramillas, 1431 m asl, 30SVG6818. P7 (21/00/01): 400 m NW from Cortijo del Olivar, 730 m asl, 30SWG2354. P8 (18/00/01): Collado de Puerto Blanco, 1400 m asl, 30SVG6020. P9 (13/00/01): La Torre spring, 1364 m asl; 30SVG6523.

Shell (Fig. 1A): Conical-oval in shape, dark brown in colour and somewhat translucent. Protoconch with 1 ½ to 1 ¾ whorls and microsculpture of fine, irregular, spiral ridges on the surface, more accentuated at the periphery, but absent in the teleoconch. 7 ¼ to 9 ¼ slowly and regularly growing, slightly convex whorls with shallow suture. Last whorl slightly raised near the peristome and larger than the previous one. Shell surface partly covered with dense, fine, transverse ribs. The umbilicus is

elongated, narrow and shallow. Aperture more or less rounded, somewhat taller than wide. Peristome interrupted, thickened, without (or with a very weak) parietal callosity and slightly reflected, especially in the umbilical area. Palatal wall slightly concave with a crenulated edge laterally. Adults with a short and small parietal lamella originating some distance from the edge, internally extended ½ whorl. The deep columellar lamellae are not always visible in frontal view. Juveniles with both thin columellar and parietal lamella in the aperture, a rounded and deep umbilicus and sculpture made up of thin, oblique ribs in the shape of platelets which are lost in the adult stages (see Garrido et al. 2005).

Measurements: H=7.24 mm, SD= 0.38 mm (P5, n= 57). H=6.73 mm SD= 0.37 (P1, n= 393).

Genital structure (Fig. 2B): Retractor muscle of right ommatophore running between penis and vagina. Vagina short and thick, the penis relatively short, generally the proximal portion thicker, but in some specimens the entire penis is cylindrical (of same width). The penial appendix relatively long, the first portion thicker, the second gradually tapering to the end. The "penial caecum" is not a true "caecum", but forms a loop (only visible at high magnification) with weak fibres on proximal portion (near distal end of penis). Retractor muscle attached to distal end of penis, near base of the "penial caecum". Epiphallus slightly (narrower) than penis, narrowest at proximal portion and slowly tapering towards vas deferens. Bursa copulatrix long and the bursa itself is not conspicuously thickened.

Differential diagnosis: *O. aragonica* has a loop-like "penial caecum" whereas *O. bulgarica* has a true penial caecum and the penis and the epiphallus is shorter in *O. bulgarica* than in *O. aragonica* (see Fig. 2 and Table 2).

Habitat: General data obtained from localities where or adjacent to where living specimens of *O. aragonica* were collected suggested that this species has a narrow thermoclimatic tolerance, being associated to humid/sub-humid environments. The species prefers hygrophilous habitats such as small marshes; permanently moist and waterlogged soil habitats in permanent freshwater spring areas associated with limestone. This species would be restricted to the moistest habitats in semi-arid areas during the summer months (Garrido et al. 2005; Arrébola & Garrido 2008; Arrébola in prep.).

Geographic range: Fossil and subfossil shells of *O. aragonica* have been found in the eastern half of the Iberian Peninsula (Robles & Martinez-Ortí 2009), but living specimens are only known from Granada Province (Andalusia, southern Iberian Peninsula).

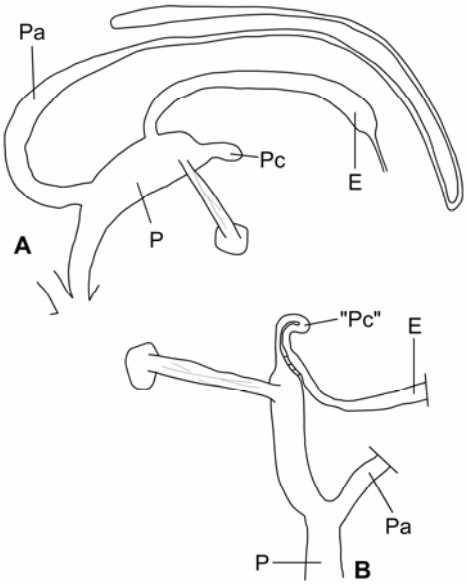

Figure 2. Male genital structures of *Orculella* species. A: *Orculella bulgarica* (Hesse 1915) (Turkey, on the border of Erzurum and Erzincan vilayets, bank of the Tercan stream close to the Tercan tüneli [tunnel]); B: *Orculella aragonica* (Westerlund 1897) (Spain, 325 m S-SE from Cortijo la Carrasca). Abbreviations: E: epiphallus; P: penis; Pa: penial appendix; Pc: penial caecum.

Table 2. Measurements (in mm) of some sections of the genitalia of *Orculella aragonica* (Westerlund 1897). (P5: 325 m S-SE from Cortijo la Carrasca; P6: spring on Barranco de las Ramillas) and *Orculella bulgarica* (Hesse 1915) (Turkey, on the border of Erzurum and Erzincan vilayets, bank of the Tercan stream close to the Tercan tüneli [tunnel]). Abbreviations: P1: penis until the junction with the penial appendix; P2: penis until the junction with the epiphallus; BC: bursa copulatrix. Other abbreviations correspond with Figure 3.

	P1	P2	E	Pc	Pa	BC
O. aragonica P5	1.5	2.4	3.5	0.5	7.7	6.2
O. aragonica P6	1.3	1.9	3.5	0.6	7.0	6.8
O. bulgarica	0.7	1.5	1.6	0.2	6.0	4.2

DNA sequence characteristics

The aligned COI dataset consisted of 635 bp and 31 (4.88%) characters were parsimony informative. The sequences had a nucleotide composition of: T (38.5%), C (14.7%), A (28.3%) and G (18.5%).

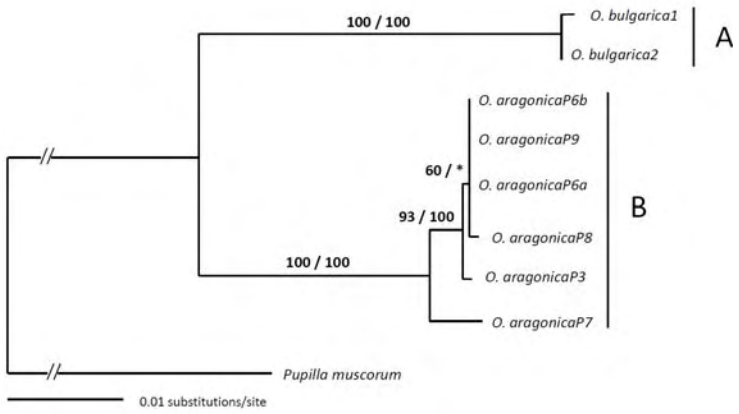

Figure 3. Neighbour-joining tree constructed following the HKY model of the combined dataset of the COI and 16S genes. NJ bootstrap values and Bayesian posterior probabilities are shown at each node (NJ/BA). The asterisk indicates a node where the BA topology was different.

The aligned 16SrRNA fragment consisted of 486 bp and 27 (5.56%) characters were parsimony informative. The nucleotide composition was: T (36.4%), C (14.1%), A (33.1%) and G (16.4%).

The combined length of the two aligned fragments was 1121 bp and 58 (5.17%) characters were parsimony informative. The sequences had a nucleotide composition of: T (37.8%), C (14.2%), A (30.4%) and G (17.6%).

Phylogenetic analyses

The two methods of phylogenetic inferences (NJ and BA) yielded almost the same tree topology. The NJ tree of the concatenated dataset is presented in Figure 3 indicating both NJ bootstrap and BA posterior probabilities.

The phylogenetic analyses showed two monophyletic lineages that were strongly supported. They are indicated as A (specimens from Turkey) and B (specimens from Spain). Pairwise sequences divergence based on uncorrected p-distance between these clades ranged from 5.27% to 5.45% in the concatenated dataset.

The clade A joined the two specimens from Turkey and this monophyly was supported by high bootstrap values and posterior probability (100%, 100% for NJ, BA). The haplotype diversity's value (Hd) was 1, because the two specimens differed from each other by 1 bp.

Clade B grouped the six specimens collected from five sites in Granada Province (southern Spain). The number of haplotypes (H) in this clade was 4 and the haplotype diversity's value (Hd) 0.8. The nucleotide diversity's value (π) was 0.00328 and the number of nucleotide differences (k) 3.67. The specimen *O. aragonica P7* was the most different within clade B and the genetic distance between this sample and the others from Granada ranged from 0.80% to 0.89% in the concatenated dataset. Collection site of *O. aragonica P7* was 67 km away from the other four collecting sites.

Discussion

Taxonomy

Due to the remarkable conchological similarity between the Iberian *Orculella* specimens and *O. bulgarica* (Fig. 1), Gittenberger (1983) considered them to be conspecific. *Pupa/Orcula dolium* Draparnaud was most commonly employed by previous authors for the Iberian *Orculella* until Gittenberger (1983). However, *Orcula dolium* is an Alpine-Carpathian species (Kerney et al. 1983) absent from the Iberian Peninsula. *Pupa dolium* var. *plagiostoma* ('Braun' Sandberger 1875) (in Pilsbry 1922-1926, Gittenberger 1983) is a smaller form of *O. dolium* with one or no lamella on the columella (Fig. 1B), typically found in loess in Central Europe (Alsace to Austria, Hungary, Lombardia, Switzerland and France). Due to similar shell morphology (Gittenberger 1983) and habitats, Brunnacker and Ložek (1969) erroneously employed the name *Orcula plagiostoma* to refer to some shells of the Iberian *Orculella* found in the loess of Almería. Robles and Martinez-Ortí (2009) examined original material of *Pupa dolium* var. nova cited by Calderon (1897) from Alhama de Aragón (northeastern Iberian Peninsula) deposited

in the National Museum of Natural History (Madrid). These authors relegated this name to the synonymy of *O. bulgarica* because although Calderon (1897) mentioned that it was a new ("nova") variety of *O. dolium* he did not formally name it: "*Plica parietali immerse, brevi, tenui et plicis columellaribus profundissimis obsoletes distincta*" (defined by Westerlund 1897). However, Robles and Martinez-Ortí (2009) overlooked that in the same year Westerlund (1897: 69) described and named this taxon as a new "forma" of *O. dolium* as follows: "*aragonica n., t. tenuiss. striatula, lutescenti-cornea, ap. callo palatali angusto translucente, tuberculo ang. et lam. par. tenui, brevi v. brevissima, caeterum edentate* (Hisp., Aragonia)". This description distinguishes *Orcula dolium* from the Iberian *Orculella* (the only orculid species actually known in this area), which consequently must be named as *Orculella aragonica* (Westerlund 1897). Although Westerlund (1897) did not specify any locality for *aragonica* (only the geographic region "Aragonia") there is no doubt that the material on which the description was made originated from Alhama de Aragon and was collected by Calderon (1897). Consequently, this must be assigned the type locality. On the other hand, the specimens from the Calderon collection deposited in Natural Sciences Museum of Madrid (MNCN): MNCN-15.05/37017 (11 shells) and MNCN-s/n (col. Azpeitia nº 2481) (5 shells) may not have been seen by Westerlund and therefore can not be considered types.

Since the Iberian specimens are clearly different from *O. bulgarica* (Hess 1915) and the correct name for these populations has been shown to be *O. aragonica* we propose to remove *O. bulgarica* from the faunal list of the Iberian Peninsula and consider all previous records of it in this area referable to *Orculella aragonica* (Westerlund 1897).

Shell and reproductive system morphology

The finding of live specimens of *O. bulgarica* from Turkey made the conchological, anatomical and genetic comparison of the two species possible. According to our observations, the shell of *O. aragonica* is indistinguishable from *O bulgarica bulgarica* (Fig. 1), as it was stated by Gittenberger (1983). *Orculella bulgarica lamellata* Hausdorf 1996 (Fig. 1E) has a much stronger developed lamella than *O. aragonica* and *O. bulgarica bulgarica*. Some differences can be seen in the reproductive organs of *O. aragonica* and *O. bulgarica*. The latter species has a true penial caecum, whereas *O. aragonica* has a loop-like structure (see Fig. 2). Garrido et al. (2005) described the latter's reproductive anatomy, but failed to mention the loop-like "penial caecum". This is unique in *Orculella* and may be for the entire Orculidae. All other anatomically known orculids have true penial caeca. We consider this difference substantial and find it surprising that no conchological differences have been found.

The penis and the epiphallus are shorter in *O. bulgarica* than in *O. aragonica*. The measurements of some sections of the genitalia of the two species are compiled in Table 2.

Genetic background

Genetic distances within pulmonate gastropod species can present variable values. Maximal intraspecific 16SrRNA gene sequences above 10% have been reported several times: Watanabe & Chiba 2001: 14% for *Euadra quaesita*; Goodacre 2002: 10% for species of the genus *Partula*; Parmakelis et al. 2003: 21.4% for species of the genus *Mastus;* Teshima et al. 2003: 23% for *Ainohelix editha;* Pinceel et al. 2005: 21% for species of the genus *Arion*). Most estimates of divergence rates for stylommatophorans ranged from 5% to 14% for 16SrRNA (Chiba 1999, Hayashi & Chiba 2000, Thacker & Hadfield 2000, Van Riel et al. 2005). But among closely related species mean genetic distances can be very different. For example average COI genetic distances in the two sister species *Candidula unifasciata* and *C. rugosiuscula* was 12.5% (Pfenninger et al. 2003). In *Vertigo gouldii* COI sequence divergences ranged from 1.6% to 11.2% (Nekola et al. 2009).

In the present study genetic distances between the two monophyletic lineages were 4.88%-5.35% for COI, 5.38%-5.80% for 16S and 5.18%- 5.45% for the concatenated dataset. Although the two monophyletic groups were strongly supported, knowing the genetic variability that exists between gastropod species, we cannot determine if the obtained two clades belong to different species basing exclusively on genetic distances. But taking the anatomical and biogeographical criteria into account, we can conclude that *Orculella* populations from Turkey (and probably other neighbouring areas) and from the Iberian Peninsula (clades A and B of the phylogenetic analysis) are two different species.

Origin

The genus *Orculella* is composed of more than 25 species distributed in the Iberian Peninsula, Maghreb, Sicilia and the eastern Mediterranean region

(Balkan Region, Anatolia, Iran). Anatolia and Crete (12 and 8 species, respectively) have the highest diversity (Brandt 1956, Hausdorf 1988, 1996, Schütt & Şeşen 1998, Gittenberger & Hausdorf 2004, Robles & Martínez Orti 2009, Zilch 1960). This suggests that the center of origin for *Orculella* can be placed in the Aegean-Anatolia region, where the genus shows the greatest genetic diversity. The ancestor of *O. bulgarica* and *O. aragonica* may have lived in this region. The *Orculella* distributional expansion west to the Iberian Peninsula could have occurred across Europe. Nevertheless, there are no fossil records west of the Balkans and, thus, paleontological data cannot confirm this hypothesis (Mania 1995, Esu 1995). By contrast, the presence of *Orculella* in Libya, Sicilia and northern Africa (Brandt 1956, Gittenberger & Hausdorf 2004, Hausdorf 1988), suggests that the colonization of the Iberian Peninsula could have occurred via the southern margin of the Mediterranean Sea. One possibility is that this colonization occurred at the end of the Pliocene, when the Mediterranean Sea experienced a period of intense desiccation (Messinian Salinity Crisis, 5-6 My BP) (Hsü et al. 1977). The oldest fossils of *Orculella aragonica* are dated in the Pliocene-Pleistocene (Almenara-Casablanca-1 deposit, 1.8 MY, Robles & Martínez-Ortí 2009, Agustí et al. 2010). Molecular distances obtained in the present work (about 5%) also suggested a more recent separation between *O. aragonica* and *O. bulgarica*. Although there is no calibrated molecular clock rate available for *Orculella* or other closely related genera, land snails are suspected to have fast-evolving mitochondrial genome with an estimated divergence time of 2-5% sequence divergence per one million years (Pfenninger et al. 2003).

Whatever its route of dispersal, through Europe or from North-Africa, *O. aragonica* is now an endemic species of the eastern half of the Iberian Peninsula. The reduction of its range to the current status could have occurred sometime in the Holocene, when the track of the populations is lost in the southern half of the "Sistema Central" and Valencia.

Conservation Status

The conservation status of *O. aragonica* was assessed for the IUCN Species Red List (as *O. bulgarica*) by Arrébola and Páll-Gergely (2011) (see also Cuttelod et al. 2011). According to the methodology and criteria of the IUCN Red List Program, it was considered as a critically endangered species. Taking into account that *O. aragonica* is now restricted to a few, fragmented locations in southern Spain, this category is apt for this species.

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