

INTEGRATING DNA AND MORPHOLOGICAL TAXONOMY TO DESCRIBE A NEW SPECIES OF THE FAMILY BATHYNELLIDAE (CRUSTACEA, SYNCARIDA) FROM SPAIN

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ABSTRACT

A. I. Camacho, B. A. Dorda & I. Rey. 2013. Integrated DNA and morphological taxonomy to describe a new species of the Family Bathynellidae (Crustacea, Syncarida) from Spain. *Graellsia*, 69(2): 179-200.

A new species of Bathynellidae Grobben, 1905 is described from Spain. *Vejdovskybathynella vasconica* **sp. nov.** displays an exclusive feature within the genus: eight-segmented antenna. Besides, the new species has a unique combination of morphological characters, including medial seta on exopod of antenna, antennule length similar to antenna, three-segmented mandibular palp without sexual dimorphism, four segments on endopod of thoracopod I to VII, three spines on the sympod of uropod, two claws on the endopod of uropod, first spine longer than the rest on the furcal rami, exopod smaller than endopod on female thoracopod VIII, a long frontal projection and medium size outer protuberance on penial region of male thoracopod VIII, and a medium size frontal crest with a small "spur" on basipod of male thoracopod VIII.

Partial sequences from mitochondrial gene cytochrome oxidase I (COI) and 18S ribosomal RNA (rRNA) gene have been obtained from specimens of the type locality of the new species. The analyses of molecular data demonstrate the presence of two highly divergent genetic units within the Bathynellidae, corresponding to two morphologically well differentiated genera.

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Keywords: Syncarida; Bathynellacea; Bathynellidae; Spain; groundwater fauna; new species; morphological data; mtDNA; COI; nuclearDNA; 18S.

RESUMEN

A. I. Camacho, B. A. Dorda & I. Rey. 2013. Integrando ADN y taxonomía morfológica para describir una nueva especie de la familia Bathynellidae (Crustacea, Syncarida) de España. *Graellsia*, 69(2): 179-200 (en inglés).

Se describe una nueva especie de la familia Bathynellidae Grobben, 1905 de España. *Vejdovskybathynella vasconica* **sp. nov.** presenta un carácter único dentro del género, tener la antena de 8 segmentos. Además la nueva especie exhibe una única combinación de caracteres morfológicos que incluye: seda mediana presente en el exopodio de la antena, antenu-

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la y antena de igual longitud, palpo mandibular de tres segmentos y sin dimorfismo sexual, cuatro segmentos en el endopodio de todas las patas, tres espinas en el simpodio del urópodo, dos uñas en el endopodio del urópodo, la primera espina de la furca más larga que las demás, toracópodo VIII hembra con exopodio más pequeño que el endopodio, toracópodo VIII macho con una larga prolongación frontal y una protuberancia externa de tamaño medio en la región peneana, así como una cresta frontal de tamaño medio con un pequeño espón en el basipodio.

Se han obtenido secuencias parciales del gen mitocondrial citocromo oxidasa I (COI) y del gen ribosomal 18S ARNr de especímenes de la localidad tipo de la nueva especie. El análisis de estos datos moleculares ha demostrado la presencia de dos unidades genéticas altamente divergentes entre ejemplares de la familia Bathynellidae correspondientes a dos géneros morfológicamente bien diferenciados. La información molecular obtenida en este trabajo complementa la descripción taxonómica tradicional, morfológica, apoyando la validez de la nueva especie perteneciente al género *Vejdovskybathynella*.

Palabras clave: Syncarida; Bathynellacea; Bathynellidae; Spain; fauna subterránea; nueva especie; morfología; ADNmt; COI; ADN nuclear; 18S.

Introduction

In traditional taxonomy a unique combination of morphological features is used to define a given taxon. Nevertheless, this approach does not seem to differentiate some species within certain groups such as the Bathyllenacea, an order of groundwater crustaceans where in some cases morphology is not fully satisfactory to distinguish between closely related species. Therefore, in order to conduct proper taxonomic and evolutionary studies in Bathynellacea it is highly recommended to obtain DNA sequences from different species to establish comparisons, combining a molecular and a morphological approach. This especially holds true for the Bathynellidae, where differences between species are particularly subtle and very difficult to find.

The Family Bathynellidae Grobben, 1905 is widespread in Europe (13 genera and 40 species) but is not well known in the Iberian Peninsula (Camacho, 2006, 2007; Camacho *et al.*, 2013) where only six species in three genera are known: *Hispanobathynella catalanensis* (Serban, Coineau et Delamare Deboutteville, 1971) (syn.: *Meridiobathynella catalanensis* Serban, Coineau et Delamare Deboutteville, 1971) from the Ter River Torroella de Montgrí in Catalonia, two species of the genus *Paradoxiclamourella* (Camacho *et al.*, 2013), and three species of the genus *Vejdovskybathynella* Serban and Leclerc, 1984 (Camacho, 2007, and Camacho *et al.*, 2013), all from caves in northern Spain. *Hispanobathynella* and *Paradoxiclamourella* belong to Tribe Gallobathynellini, subtribe Meridobathynellina Serban, Coineau et Delamare Deboutteville, 1971, and Gallobathynellina, res-

pectively; whereas *Vejdovskybathynella* belongs to Tribe Vejdovskybathynellini Serban, 1989. Some old records mention the presence of the genus *Bathynella* Vejdovsky, 1882 in Portugal, and even established subspecies for the material found (Noodt & Galhano, 1969). However, according to Serban (1977) these are probably representatives of the subfamily Gallobathynellinae and not the Bathynellinae, to which the genus *Bathynella* belongs.

In this paper we describe morphologically a new species of the genus *Vejdovskybathynella* (Bathynellidae) from the Iberian Peninsula. We obtained sequences of mitochondrial DNA (mtDNA) cytochrome c oxidase I (COI) and of nuclear DNA, 18S ribosomal RNA (rRNA) genes, from several specimens of the new species and of representatives of other taxa, some identified down to species level and others not, from the families Bathynellidae and Parabathynellidae. We have compared the divergent clades that can help to assign specimens to genera and to elucidate the phylogenetic relationships between taxa at both higher levels (family and genus) and at the species level.

Material and methods

TAXONOMIC SAMPLING/SPECIMEN COLLECTION

Specimens studied were found in 4 samples from Goikoetxe cave, Vizcaya (northern Spain). All samples were collected with a 0.1 mm mesh hand net (plankton type) from water in small gours and pools in the epikarstic zone. The specimens used in the morphological study were fixed in 4% buffered

formalin and stored in ethanol (70%). Specimens used for the molecular study were directly frozen at -20°C, in 400 ml of distilled water.

The morphological and molecular descriptions are based on the type series.

MORPHOLOGICAL STUDY

A total of 23 specimens were used in the morphological study: 16 females and seven males collected in 29 April 2011. They constitute the type series of the new species described herein.

A complete dissection of all anatomical parts of several specimens was done and, together with whole specimens, kept as permanent slides (special metal slides, glycerine gelatine stained with Methylene Blue as mounting medium). Anatomical observations were performed using an oil immersion lens (100X) with a Zeiss interference microscope provided with a drawing tube. The material is deposited in the Museo Nacional de Ciencias Naturales, Madrid (MNCN).

We follow the terminology proposed by Serban (1972 and following papers) to name Md and male Th VIII. Serban worked in depth on the family Bathynellidae (Serban 1989a, b, 1992; Serban & Leclerc, 1984; Serban *et al.*, 1971), revised and described many genera, and we think that his terminology is the most accurate and intuitive.

SPECIMEN COLLECTION FOR DNA EXTRACTION

Twenty eight specimens were used for DNA extraction, but successful DNA extraction was only possible from six, and are part of the type series.

This type material, the DNA extracted from the six specimens, is deposited in the Tissues and DNA Collection of the Museo Nacional de Ciencias Naturales, Madrid (MNCN). Voucher numbers are shown in Table 1.

In order to examine the phylogenetic relationships between bathynellids, we used partial DNA sequences of the mtDNA gene *COI* (508 bp) and the nuclear 18S rRNA (997 bp). The small subunit (SSU) 18S rRNA gene is one of the most frequently used genes in phylogenetic studies and an important marker for random target PCR in environmental biodiversity screening (Chenuil, 2006) and in general, rRNA gene sequences are easy to access due to their highly conserved flanking regions allowing the use of universal primers (Hillis & Dixon, 1991). *COI* sequences were obtained from two individuals, and 18S rRNA sequences were obtained from six specimens.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Extraction was carried out with Chelex following Walsh *et al.* (1991). Fresh specimens were cut in two in distilled water, and were placed in the wall of a 1.5 ml microcentrifuge tube with a sterile needle. Each tube contained 100 ml Chelex 100 (Bio-Rad; 5% in distilled water) and 400 ml of distilled water. The specimens were incubated overnight at 56°C, followed by 10 minutes at 100°C and centrifuged at 16,000g for 10 minutes.

A 510 base pair (bp) region of the *COI* gene was amplified with the primers C1-J-1718 (5'-GGAG-GATTTGGAAATTGATTAGTTC-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAA AATCA-3') (Folmer *et al.*, 1994; Simon *et al.*, 1994). A 997 base pair (bp) fragment of the 18S rRNA region was amplified in two fragments, using the primers 1F (5'-TACCTGGTTGATCCT-GCCAG TAG-3') and 3R (5'-AGGCTCCCTCTC-CGGAATCGAAC-3') and 3F (5'-GTTCGATTCC GGAGAGGGA-3') (Giribet *et al.*, 1996) and bi (5'-GAGTCTCGTTCGTTATCGGA-3') (Whiting *et al.*, 2002). Three ml of the DNA solution were used as a template. Other components of the 25 ml PCR reaction were: 1x of the corresponding buffer (75 mM Tris HCl, pH 9.0; 50 mM KCl and 20 mM (NH₄)₂SO₄), 2 mM MgCl₂, 10 mM dNTPs mix, 0.1 mM of both primers, 0.02% BSA, and 0.125 units AmpliTaq Gold® DNA Polymerase (Applied Biosystems). Six ml of PCR products were electrophoresed through a 1.5% agarose gel and visualized with Ethidium Bromide under ultraviolet light. PCR products were purified by treatment with ExoSAP-IT (USB Amersham, Buckinghamshire, UK) in a 5:1 amplicon: enzyme ratio and incubated at 37 °C for 45 min, followed by 15 min at 80 °C to inactivate the enzyme. The purified PCR product was then used to sequence in both directions using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems Inc., Foster City, USA) in a 10 µL volume, containing 15-20 ng of purified product and 3 pmol of primer. To verify that the sequences obtained came from a bathynellacean, they were compared with sequences from GenBank using Blast (Altschul *et al.*, 1997). The alignment of all bathynellacean *COI* and 18S gene sequences generated in our lab was performed and edited manually using MEGA 4.0 (Tamura *et al.*, 2007). Fine adjustments were made by eye, as the *COI* does not present any gaps. All sequences were submitted to GenBank (see Table 1 for collection

Table 1.- Localities of the studied samples of Bathynellacea, voucher numbers of the Tissues and DNA Collection of the Museo Nacional de Ciencias Naturales, CSIC (Spain) and GenBank accession numbers of specimens. * specimens used in phylogenetic reconstruction (Fig. 4A).

Tabla 1.- Localidades de las muestras estudiadas de Bathynellacea, número de la colección de Tejidos y ADN del MNCN y número de acceso a GenBank de cada uno de los ejemplares estudiados. *ejemplares usados en el análisis filogenético (Fig. 4A).

Order/Family/Species	Locality	N	W	Z	Specimens voucher MNCN/ADN	GenBank accession number COI	GenBank accession number 18S
BATHYNELLACEA CHAPPUIS, 1915							
Parabathynellidae Noodt, 1965							
<i>Iberobathynella burgalensis</i> Camacho, 2005	Ojo Guareña cave (OG53), Comejo (Burgos)	43.03188	3.65821	724	29520	HQ659859	KC469529
<i>Iberobathynella parasturiensis</i> Camacho et Serban, 1998							
<i>Iberobathynella imuniensis</i> Camacho, 1987	Torca de Treslajorá (CO209), Peñarrubia (Cantabria)	43.26725	4.59085	1132	29566	KC469544	KC469530
<i>Iberobathynella celiana</i> Camacho, 2003	Torca Morteros cave, Imunia (Burgos)	43.14786	3.59539	1280	29166	HQ659850	KC469528
<i>Hexabathynella sevillensis</i> Camacho, 2005	Viar stream, Castiblanco (Sevilla)	37.71374	5.87772	60	29452	HQ659862	KC469527
<i>Paraiberobathynella cf. maghrebenensis</i> (Boutin et Coineau, 1987)	Santiago el Grande Cave, Carmona, (Sevilla)	38.03013	5.90429	358	29545	HQ659869	KC469526
<i>Paraiberobathynella cf. fagei</i> (Delamare et Angelier, 1950)	Well, route Nador to Berkane, Maghreb (Morocco)	34.95238	2.59795	276	29935	KC469542	KC469532
	Sima de la Higuera, Pliego (Murcia)	37.97836	1.50170	496	29667	KC469541	KC469533
Bathynellidae Grobben, 1904							
<i>Vejdovskybathynella edelweissi</i> Camacho, 2007	Ojo Guareña Cave (OG01), Comejo (Burgos)	43.03188	3.65821	724	29415	HQ596664	KC469512
<i>Vejdovskybathynella edelweissi</i>							
<i>Vejdovskybathynella carolori</i> Camacho, 2007	Sima Huesos Cave, Comejo (Burgos)	43.03231	3.63881	705	29440	HQ596571	KC469513
<i>Vejdovskybathynella vasconica</i> sp. nov. 1*	Gándara cave, Soba (Cantabria)	43.19015	3.58604	740	29877	KC469538	KC469525
<i>Vejdovskybathynella vasconica</i> sp. nov. 2*	Goikoetxe cave, Busturia (Vizcaya)	43.36026	2.70195	149	29646	KC469535	KC469521
<i>Vejdovskybathynella vasconica</i> sp. nov.	Goikoetxe cave, Busturia (Vizcaya)	43.36026	2.70195	149	29889	KC469536	KC469516
<i>Vejdovskybathynella vasconica</i> sp. nov.	Goikoetxe cave, Busturia (Vizcaya)	43.36026	2.70195	149	29623	KC469518	KC469518
<i>Vejdovskybathynella vasconica</i> sp. nov.	Goikoetxe cave, Busturia (Vizcaya)	43.36026	2.70195	149	29635	KC469519	KC469519
<i>Vejdovskybathynella vasconica</i> sp. nov.	Goikoetxe cave, Busturia (Vizcaya)	43.36026	2.70195	149	29638	KC469520	KC469520
<i>Paradoxiciamoussella fideli</i> Camacho et al., 2013	Pozo del Agua cave (CO69), Peñamellera Baja (Asturias)	43.28383	4.52318	374	29746	JX121253	KC469524
<i>Paradoxiciamoussella fideli</i>							
<i>Paradoxiciamoussella cf. fideli</i>	Fuente del Camero (CO220) Herrierias (Cantabria)	43.28680	4.47784	156	29735	JX121252	KC469523
Bathynellidae indeterminate	Torca de Treslajorá (CO209), Peñarrubia (Cantabria)	43.26725	4.59085	1132	29594	JX121249	JX121235
Bathynellidae indeterminate	Imunia cave, Imunia (Burgos)	43.14632	3.59660	1280	29921	KC469534	KC469515
Bathynellidae indeterminate	Redonda Cave, Comejo (Burgos)	43.03236	3.62914	668	29523	HQ596573	KC469515
Bathynellidae indeterminate	La Nava cave, Lamasón (Cantabria)	43.25928	4.50022	492	29914	KC469537	KC469514
Bathynellidae indeterminate	Ojo Guareña cave (Erizos river), (Burgos)	43.03188	3.65821	724	29487	HQ596572	KC469514
Bathynellidae indeterminate	Río Chico cave, Soba (Cantabria)	43.28383	4.52318	575	29728	JX121244	KC469522
Bathynellidae sp1	Australia					JN817409	
ANASPIDACEA CALMAN, 1904							
Anaspididae Thomson, 1893							
<i>Anaspides tasmaniae</i> (Thomson, 1893)	Mount Wellington, Tasmania						DO310660

Table 2.– COI genetic distances among and within species groups of the Spanish and Australian Bathynellidae.

Tabla 2.– Distancia genética encontrada en el gen COI entre y dentro de grupos de especies de la familia Bathynellidae de España y Australia.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 Bathynellidae Erizos	0														
2 Bathynellidae Chico	2.56	0													
3 <i>V. edelweiss</i> 1OG01	14.57	14.96	0												
4 <i>V. edelweiss</i> Huesos	14.76	14.76	1.57	0											
5 Bathynellidae Imunia	14.57	14.96	13.39	12.99	0										
6 Bathynellidae Redonda	16.93	16.93	15.16	14.37	6.10	0									
7 <i>V. vasconica</i> sp. nov.															
Goiko	12.99	13.39	14.17	14.17	12.80	14.76	0								
8 <i>V. vasconica</i> sp. nov.															
Goiko	13.19	13.58	14.76	14.76	13.39	15.35	0.59	0							
9 Bathynellidae Nava	15.75	15.55	16.73	16.73	16.34	17.72	16.14	16.34	0						
10 <i>P. cf. fideli</i> CO209	16.54	16.54	17.91	18.11	16.14	18.31	16.73	16.73	10.63	0					
11 <i>P. fideli</i> CO69	17.13	17.72	18.31	17.91	15.35	17.91	15.75	15.95	11.22	7.30	0				
12 <i>P. fideli</i> Carnero	16.73	17.32	17.91	17.52	14.96	17.52	15.75	15.95	10.83	6.89	0.39	0			
13 <i>V. caroloi</i> Gándara	17.52	17.72	19.68	18.90	17.72	18.31	16.53	16.73	16.92	19.45	17.91	17.52	0		
14 Bathynellidae sp1 Australia	21.85	21.85	21.65	20.87	17.72	19.68	18.70	19.29	19.29	19.68	19.29	19.29	21.46	0	
15 <i>I. imuniensis</i> Morteros	25.20	25.20	22.64	22.05	22.05	23.43	22.64	23.03	23.23	22.64	23.03	23.03	23.43	22.83	0

voucher numbers of each specimen and the GenBank Accession Number).

PHYLOGENETIC AND DNA SEQUENCE ANALYSIS

Phylogenetic reconstruction based on COI and 18S rRNA sequence data involved Bayesian Inference (BI), maximum likelihood (ML) and maximum parsimony (MP) approaches, using separate data sets. To examine relationships between species of Bathynellidae, we analysed the mtDNA COI sequences obtained by us from several species of *Vejdovskybathynella* Serban et Leclerc, 1984 (*V. edelweiss* Camacho, 2007 and *V. caroloi* Camacho, 2007), *Paradoxiclamoussella* Camacho, Dorda et Rey, 2013 (*P. fideli* Camacho, Dorda et Rey, 2013 and *P. cf. fideli*) and other undetermined genera from Spain. The Parabathynellid *Iberobathynella imuniensis* from Spain was chosen as out-group (GenBank accession number HQ659850). To examine relationships between families and genera, we used 18S rRNA sequences obtained by us. Genera of Parabathynellidae included *Iberobathynella* Schminke, 1973, *Paraiberobathynella* Camacho et Serban, 1998 and *Hexabathynella* Schminke, 1972, all from Spain. In the case of the Bathynellidae, we used the genera *Vejdovskybathynella*, *Paradoxiclamoussella*, plus other not yet determined material from Spain (voucher and

GenBank accession numbers shown in Table 1). The Anaspidid Syncarida *Anaspides tasmaniae* Thomson, 1893 was chosen as out-group (GenBank accession number DQ310660). Pairwise comparisons of observed proportional sequence divergence (p-distance) (Tables 2 and 3) and corrected sequence divergence (Kimura-2-parameter model), were determined using PAUP*4.0b10 (Swofford, 2002). To test for possible saturation of nucleotide substitutions (only with COI sequences), we plotted p-distance (y) versus corrected estimates of proportional sequence divergence (x) for first, second and third codon positions, as well as for transitions and transversions separately (not shown). We initially explored the dataset using distance analyses (neighbour joining, NJ) with PAUP*4.0b10. Phylogenetic analyses were conducted using Maximum Likelihood (ML; Felsenstein, 1981, 1985) and Bayesian inference (BI) (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). All characters were equally weighted. Modeltest 3.7 (Posada & Crandall, 1998) were used to identify the model of sequence evolution that best fit the data, based on Akaike information criteria (AIC), for use in the phylogenetic (ML) and distance analyses (NJ). The general time-reversible model of evolution (GTR) with gamma parameter and a proportion of invariable positions

Table 3.— 18S genetic distances among and within species groups of the Spanish Bathynellacea.

Tabla 3.— Distancia genética en el gen 18S entre y dentro de grupos de especies españolas de la familia Bathynellidae.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 Bathynellidae Goiko	0															
2 Bathynellidae Redonda	1.94	0														
3 Bathynellidae Chico	1.63	0.31	0													
4 <i>V. caroloi</i> Gándara	3.48	2.15	2.05	0												
5 <i>V. edelweiss</i> OG01	1.94	0.41	0.31	2.15	0											
6 <i>V. edelweiss</i> Huesos	1.94	0.41	0.31	2.15	0	0										
7 <i>P. fideli</i> CO69	6.31	7.13	7.03	7.97	6.93	6.93	0									
8 <i>P. cf. fideli</i> CO209	6.12	7.04	6.95	7.77	6.84	6.84	1.41	0								
9 <i>I. burgalensis</i> OG53	8.85	8.24	8.36	9.72	8.13	8.13	11.63	11.55	0							
10 <i>I. parasturiensis</i> CO209	8.32	7.70	7.81	9.08	7.60	7.60	11.51	11.43	1.24	0						
11 <i>I. imuniensis</i> Morteros	8.52	8.12	8.02	9.49	8.01	8.01	12.02	11.95	1.34	0.93	0					
12 <i>I. celiána</i> Sevilla	8.35	7.94	7.85	9.33	8.04	8.04	11.76	11.69	2.17	1.55	1.55	0				
13 <i>H. sevillaensis</i> Sevilla	9.68	8.66	8.78	9.51	8.76	8.76	11.83	11.64	6.94	6.20	6.51	6.12	0			
14 <i>Pi. cf. maghrebenis</i>	8.84	8.44	8.34	9.92	8.43	8.43	12.13	12.06	1.34	1.55	1.24	1.97	6.93	0		
15 <i>Pi. cf. fagei</i> Higuera	8.96	8.02	7.93	9.51	8.02	8.02	11.62	11.54	0.83	1.13	0.82	1.55	6.52	0.41	0	
16 <i>Anaspides tasmaniae</i>	12.98	12.38	12.50	13.67	12.27	12.27	14.55	14.98	11.95	11.51	11.61	11.45	12.16	11.94	11.51	0

(GTR+G+I) was selected as the best fit and was used for ML (Yang, 1994; Gu *et al.*, 1995; Swofford *et al.*, 1996) and BI analyses. ML analyses with empirical base frequencies were performed using Garli (Zwickl, 2006; Zwickl & Balhoff, 2006). We used nonparametric bootstrapping (500 pseudoreplicates) to assess the stability of internal branches in the resulting topologies (Felsenstein, 1985; Felsenstein & Kishino, 1993). BI analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) assuming six discrete gamma categories. Bayesian analyses were initiated with random starting trees and run for 1,000,000 generations sampled every 100 generations. The convergence occurred during the first million generations, the likelihood values converged to relative stability after approximately 100,000 generations; subsequently we conservatively discarded all samples obtained during the first hundred thousand (10%) generations as burn-in. Robustness of the observed clades was assessed with Bayesian posterior probabilities.

Systematic account

According to Serban (2000), it was assumed that the largest part of France and the Iberian Peninsula

is populated by representatives of the subfamily Gallobathynellinae Serban *et al.*, 1971. This subfamily consists of four tribes (the differences are shown in Table 4), two subtribes, 11 genera, and 25 species including the new species described herein (Camacho, 2007; Camacho *et al.*, 2013). The majority of the species occur in France, two are known from Italy, one from Switzerland, one from Germany, and eight, including the new species, from Spain.

The new species belongs to the Tribe Vejdovskybathynellini Serban, 1989 (see Table 4) based on the number of main teeth on the Mandible (5), the three-segmented mandibular palp, the four segmented endopod of the thoracopods I to VII, an antennule similar in size to antenna, basipod of the male thoracopod VIII vertical and with frontal crest with frontal spur and penial region with a lobe with outer protuberance, a developed frontal projection and one outer lobe of similar length. This Tribe has a single genus, *Vejdovskybathynella* Serban et Leclerc, 1984, to which the new species belongs. Table 5 summarizes the main features of the different genera from subtribes Gallobathynellina and Meridiobathynellina, as well as the genus *Vejdovskybathynella*, clearly identifying the differences between them.

Table 4.— Differences amongst the tribes of the Subfamily Gallobathynellinae Serban, Coineau et Delamare Debutteville, 1971: Gallobathynellini Serban, Coineau et Delamare Debutteville, 1971; Pseudobathynellini Serban, 1989; Sardobathynellini Serban, 1992 and Vejdovskybathynellini Serban, 1989. (Modified from Serban 1989). Terminology of the Thoracopod VIII male translated from the terminology used by Serban: région pénienne= penial region; lobe= lobe (lb); prolongement rostral= frontal projection (fr. pj.); lobule interne= inner lobe (I.lb); lobule externe= outer lobe (O.lb); proéminence externe= outer protuberance (O. prt.).

Tabla 4.— Diferencias entre las tribus de la subfamilia Gallobathynellinae Serban, Coineau et Delamare Debutteville, 1971: Gallobathynellini Serban, Coineau et Delamare Debutteville, 1971; Pseudobathynellini Serban, 1989; Sardobathynellini Serban, 1992 y Vejdovskybathynellini Serban, 1989. (Modificado de Serban 1989). La terminología usada para el Th VIII macho está traducida de la terminología empleada por Serban: région pénienne= región peneana; lobe= lóbulo (lb); prolongement rostral= proyección frontal (fr. pj.); lobule interne= lóbulo interno (I.lb); lobule externe= lóbulo externo (O.lb); proéminence externe= prominencia externa (O. prt.).

	Gallobathynellini	Pseudobathynellini	Sardobathynellini	Vejdovskybathynellini
A.I: number of segments	6/7	7	7	7
A.II: endopod	6-7-8	8	7	7/8
Medial seta of exopod	present/absent	present	present	present
A.I/A.II	A.I=A.II/A.I>A.II/A.I<A.II	A.I>A.II	A.I>A.II	A.I=A.II
Md: n° of principal teeth	5-6	7 teeth	7 teeth	5-6 teeth
Segments on palp	1-3	3	3	3
Sexual dimorfism on palp	not	yes	yes	yes/not
Th. 1 to 7: endopod	4/4/4/4/4/4/4 4/3/3/3/3/4/4 4/3/3/3/3/3/3/3 3/3/3/3/3/4/4	4/4/4/4/4/4/4	4/4/4/4/4/3/3	4/4/4/4/4/4/4
Th 8 male: type	<i>Gallobathynella</i>	<i>Pseudobathynella</i>	<i>Pseudobathynella</i>	<i>Vejdovskybathynella</i>
Penial region	Lb+F.pj+O.lb/or not	Lb+I.Lb+small O.lb	I.Lb+small O.lb	lobe+F. prj+ big O.lb
frontal projection (F. prj)	present	present	absent	present
outer protuberance (O.prt)	on Bsp	on Bsp/on coxopod	included on coxopod	included on coxopod
Basipod (Bsp)	inclined 35°	inclined 30°	inclined 30°	vertical
Th 8 female: coxal seta	long	short	short	long/absent
epipod	present (big)/absent	present (big)	present (big)	present (big)
endopod/exopod size	endopod<exopod	endopod=exopod	endopod=exopod	endopod<=>/exopod
Uropod: sympod	3-4 spines	3-4 spines	3 spines	3-4 spines
Endopod	2-3 claws	2-3 claws	4 claws	2-3 claws
Furca: spines	similar/different	different	similar	similar/different

Tribe *Vejdovskybathynellini* Serban, 1989

DIAGNOSIS (translated from Serban 1989a, 1989b and modified)

A. I and A. II ordinary. Md.: mandibular palp with three segments with the setae longer in the males than in the females or not. Th. I–VII: endopod with four segments in all the pairs. Male Th. VIII: penial region with one lobe that shows an elongated frontal projection and an outer lobe, vertical basipod with a very prominent frontal crest (Fr. crt.) and with a distal region curved on the external side, forming a spur (“eperon”) (S. fr. crt.); endopod a single segment, elongated and shorter than the exopod. Female Th. VIII ordinary, coxal seta elongated or absent (see Table 4).

Genus *Vejdovskybathynella* Serban et Leclerc, 1984

DIAGNOSIS (modified from Serban and Leclerc 1984 and Camacho, 2007)

A. I: with seven segments. Md.: mandibular palp with or without sexual dimorphism. Male Th. VIII: penial region with frontal projection and a large outer lobe; the basipod has a vertical position and a prominent frontal crest (Fr. crt.). Female Th. VIII: with an exopod simplified. Uropod: sympod with three or four spines and endopod with two or three spines. Furcal rami: first or second spine the longest (see Table 5).

TYPE SPECIES. *Vejdovskybathynella balazuci* Serban et Leclerc, 1984.

Table 5.- Differences amongst the two subtribes of the Tribe Gallobathynellini and their genera: Gallobathynellina Serban, Coineau et Delamare Deboutteville, 1971 (*Gallobathynella* Serban, Coineau et Delamare Deboutteville, 1971), *Clamousella* Serban, Coineau et Delamare Deboutteville, 1972, *Clamousella* cf. and *Vandelibathynella* Serban, Coineau et Delamare Deboutteville, 1971), and Meridiobathynellina Serban, 1989 (*Meridiobathynella* Serban, Coineau et Delamare Deboutteville, 1971, *Hispanobathynella* Serban, 1977 and *Parameridiobathynella* Serban et Leclerc, 1984). Abbreviations: endp.= endopod; exp.= exopod; Fr.projct.= frontal projection; i.Lb.= inner lobe; Lb.= penial lobe; M= medium size; O.lb.= outer lobe; O.prt.= outer protuberance; S= small; XL= extra large size.

Tabla 5.- Diferencias entre las dos subtribus de la Tribu Gallobathynellini y sus generos: Gallobathynellina Serban, Coineau et Delamare Deboutteville, 1971 (*Gallobathynella* Serban, Coineau et Delamare Deboutteville, 1971), *Clamousella* Serban, Coineau et Delamare Deboutteville, 1972, *Clamousella* cf. y *Vandelibathynella* Serban, Coineau et Delamare Deboutteville, 1971), and Meridiobathynellina Serban, 1989 (*Meridiobathynella* Serban, Coineau et Delamare Deboutteville, 1971, *Hispanobathynella* Serban, 1977 and *Parameridiobathynella* Serban et Leclerc, 1984). Abreviaturas endp.= endopodio; exp.= exopodio; Fr.projct.= proyección frontal; i.Lb.= lóbulo interno; Lb.= lóbulo peneano; M= mediano; O.lb.= lóbulo externo; O.prt.= protuberancia externa; S= pequeño; XL= muy grande.

	Gallobathynellini					Vejdovskybathynellini				
	Gallobathynellina	Clamousella	Paradoxyclamousella	Vandelibathynella	Meridiobathynellina	Hispanobathynella	Parameridiobathynella	Vejdovskybathynella	Meridiobathynellina	Vejdovskybathynella
A.I.: number of segments	6/7	7	7	7	6	6	6	7	6	7
A.II: number of segments	7/8	8	8	8	6	6	8	7/8	8	7/8
medial seta of exopod	absent	absent	present	present	absent	absent	present	present/absent	AI>AII/ AI=AII	AI>AII/ AI=AII
A.I/A.II	AI>AII	AI=AII	AI slightly>AII	AI <AII	AI>AII	AI>AII	AI>AII	3	3	3
Md: palp number of segments	2	not	yes/not	not	not	not	not	yes/not	not	yes/not
palp sexual dimorphism	3/3/3/3/3/4/4	3/3/3/3/3/4/4	3/3/3/3/3/4/4	3/3/3/3/3/4/4	4/3/3/3/3/3/3/3	4/3/3/3/3/3/4/4	4/4/4/4/4/4/4	4/4/4/4/4/4/4	4/4/4/4/4/4/4	4/4/4/4/4/4/4
Th. 1 to 7: endopod (segts.)	long	long	medium	long	long	long	long	medium/absent	long	medium/absent
Th 8 female: coxal seta	present (large)	present (large)	present (large)	present (large)	absent	absent	present (large)	present (large)	present (large)	present (large)
epipod	exp.>endp.	exp.>endp.(big)	exp.>endp.(reduced)	absent	absent	absent	small exp.<endp.	exp.>/<=endp.	small exp.<endp.	exp.>/<=endp.
exopod/endopod	elongated	elongated	not very long	elongated	rounded	slightly elongated	elongated	elongated	elongated	elongated
penial region	Lb+O.prt (L)	Lb+O.prt (L)	Lb+O.prt, O.Lb,i.Lb	Lb+O.prt (S)	Lb+O.prt (L)	Lb+O.prt (XL)	Lb+O.prt (XL), O.Lb	Lb+O.prt (L)+ O.Lb (M)	Lb+O.prt (XL), O.Lb	Lb+O.prt (L)+ O.Lb (M)
outer lobe	absent	absent	present (M)	absent	absent	absent	present (S)	present (M)	present (M)	present (M)
frontal projection	present (M)	present (M)	present (L)	present (short)	present (L)	present (L)	present (L)	present (M)	present (L)	present (M)
basipod	inclined 35°	inclined 35°	inclined 35°	inclined 35°	inclined 35°	inclined 35°	inclined 35°	vertical	inclined 35°	vertical
Crest	present (S)	present (L)	absent	absent	absent	absent	present (M)	present (M/L) with spur	present (S)	present (M/L) with spur
endopod	well-developed	well-developed	small	absent/reduced	absent/reduced	absent	well-developed	well-developed	well-developed	well-developed
exopod	big	well-developed	big	big	well-developed	well-developed	big	big	big	big
Uropod: sympod	3-4 spines	4 spines	4 spines	3 spines	4 spines	4 spines	4 spines	3-4 spines	4 spines	3-4 spines
Endopod	2 claws	2 claws	2 claws	2 claws	3 claws	3 claws	3 claws	2-3 claws	3 claws	2-3 claws
Furca: second seta	similar/different	a few different	a few different	similar	similar	different	different	similar/different	different	similar/different
longest spine	none/second (M)	second (XL)	third (S)	first (S)	none	second (XL)	second (M)	first (S)/second (M/L)	second (M)	first (S)/second (M/L)

***Vejdovskybathynella vasconica* sp. nov.**

(Figs. 1-3)

MATERIAL EXAMINED

TYPE LOCALITY. Goikoetxe Cave, Busturia (Vizcaya, Spain); coordinates N 43.36703, W 2.70195 and Z: 3149 (WGS84); 29 April 2011 (69 specimens collected, six of them juveniles).

Type series comprises Holotype male of 0.79 mm (MNCN 20.04/9119), allotype female of 0.66 mm (MNCN 20.04/9120), 6 males and 15 females paratypes (MNCN 20.04/9121 to 9141) and 6 DNAtypes (MNCN/ADN: 29646; 29889; 29623; 29633; 29635 and 29638).

DESCRIPTION

Body. Total length of males 0.65-0.79 mm, of females 0.56-0.85 mm. Body elongated, segments slightly widening towards posterior end; each approximately nine times as long as wide. Head twice as long as wide. Pleotelson with one small smooth dorsal seta at each side. All drawings are of the holotype (male) except for Th VIII female, and one figure of Md that belongs to the allotype.

Antennule (Fig. 1A). As long as antenna, seven-segmented; length of first three segments slightly longer than the other four segments; fourth and sixth segment similar and smaller than seventh; fifth smallest; inner flagellum rectangular; setation as in Fig. 1A; segment six and seven with two terminal aesthetascs, all similar in size.

Antenna (Fig. 1B). Eight-segmented; as long as antennule; distal and fifth segment longer than rest, terminal longest; last four segments twice as long as the first four; ventromedial seta of exopod present, and two apical setae, one of them a bifurcated sensory seta; setal formula: $0/0/2+exp/2+0/1+0/0+0/2+2/4$.

Labrum (Fig. 1C). Almost triangular and smooth.

Paragnaths (Fig. 1D). Elongated and with a projection on the distal part; with fine setules around distal part.

Mandible (Figs. 1E, F). Palp with three segments, terminal segment with two equal and barbed claws. Masticatory part: pars molaris with five simple main teeth, *processus incisivus accessorius* with one tooth and incisor process (*pars incisiva*) with two teeth.

Maxillule (Fig. 1G). Proximal endite with four setae; distal endite with six teeth, four of which provided with denticles other two simple, seta-like; three plumose setae, of similar size, on the outer margin of endite.

Maxilla (Fig. 1H). Four-segmented; setal formula 6, 4, 6, 4.

Thoracopods I-VII (Figs. 2A, E and 3A, B). Th I (Fig. 2A) smaller than rest; Th II (Fig. 2B) to V (Fig. 2E) similar in size, Th VI (Fig. 3A) to VII (Fig. 3B) longer than rest. Th I without epipod; coxa with a long strong plumose seta; basipod with two barbed setae. Basipod of Th II and Th III with two smooth setae; one smooth seta on basipod of rest of thoracopods. Exopod one-segmented in all thoracopods, similar in size to the endopod in Th I to Th V, and half length of endopod in Ths VI and VII; exopods with five barbed setae, with groups of ctenidia at base of setae. Endopod with four segments in all thoracopods; all setae smooth except those on distal outer corner of segment two, that is plumose; groups of ctenidia at lateral internal edge of endopod in all segments. Thoracopod endopod setal formulae: Th I, $3+0/3+1/3+0/4$; Th II: $2+0/2+1/1+0/4$; Th III: $1+0/2+1/2+0/4$; Th IV: $0+0/1+1/1+0/4$; Th V: $0+0/0+1/1+0/3$; Th VI: $0+0/0+1/0+0/2(1)$; Th VII: $0+0/0+1/0+0/2(1)$.

Male thoracopod VIII (Fig. 3C, D). Outer lobe (O. lb.), similar to the frontal projection and not exceeding the basipod, both with transverse circular section; expanded outer protuberance (O. prt.); vertical rectangular basipod (Bsp.) without seta; frontal crest of basipod not very prominent and with a very small spur; exopod with six setae; endopod one-half the size of the exopod, with two small terminal setae of similar length.

Female thoracopod VIII (Fig. 3E). Coxa without setae; very large epipod reaching, beyond distal end of exopod; endopod small and slightly more slender than exopod, single segment with two apical smooth setae; exopod with two apical unequal smooth setae of different lengths.

First pleopod (Fig. 3F). Two-segmented, first segment with one seta; second segment with four setae, all smooth.

Uropod (Fig. 3G). Sympod 1.6 times longer than wide and 1.6 times longer than endopod, with three equal spines; endopod slightly longer than exopod, with two strong similar claws, terminally with two barbed setae (one of them very long) and with two plumose setae located dorsolaterally; exopod with two barbed terminal setae, of which external very long and two small barbed medial setae.

Pleotelson (Fig. 3H). With one short, smooth dorsal seta at either side near base of furca.

Furcal rami (Fig. 3H). Almost square, bearing five spines; dorsal spine a little shorter than second

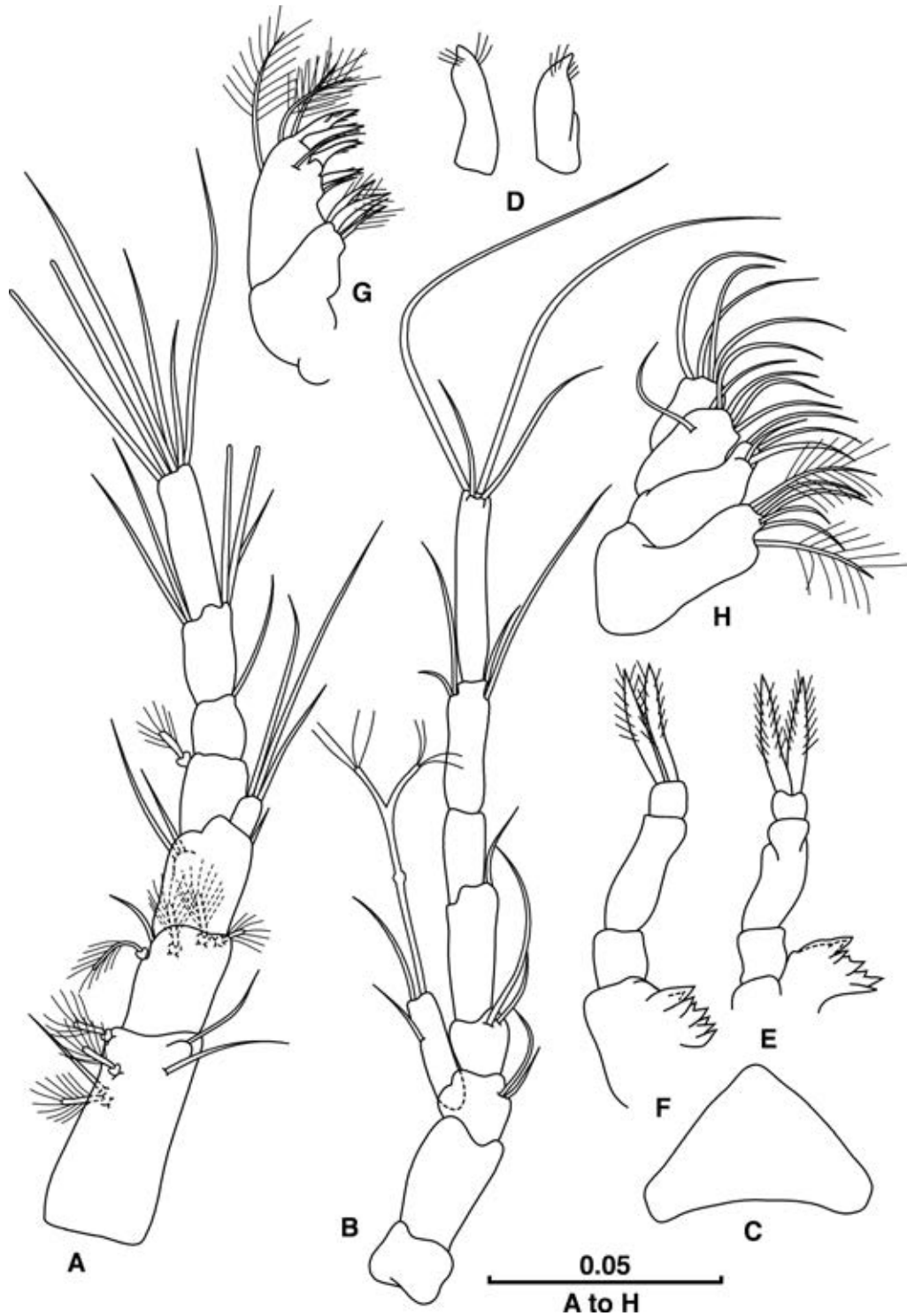


Fig. 1.— *Vejdovskybathynella vasconica* **sp. nov.**, male holotype. (A) Antennule (dorsal view); (B) Antenna (dorsal view); (C) Labrum; (D) Paragnaths; (E) Mandible; (F) female Mandible; (G) Maxillule; (H) Maxilla. Scale bar in mm.

Fig. 1.— *Vejdovskybathynella vasconica* **sp. nov.**, holotipo macho. (A) Anténula (vista dorsal); (B) Antena (vista dorsal); (C) Labro; (D) Paragnatos; (E) Mandíbula; (F) Mandíbula de la hembrae; (G) Maxilula; (H) Maxila. escala en mm.

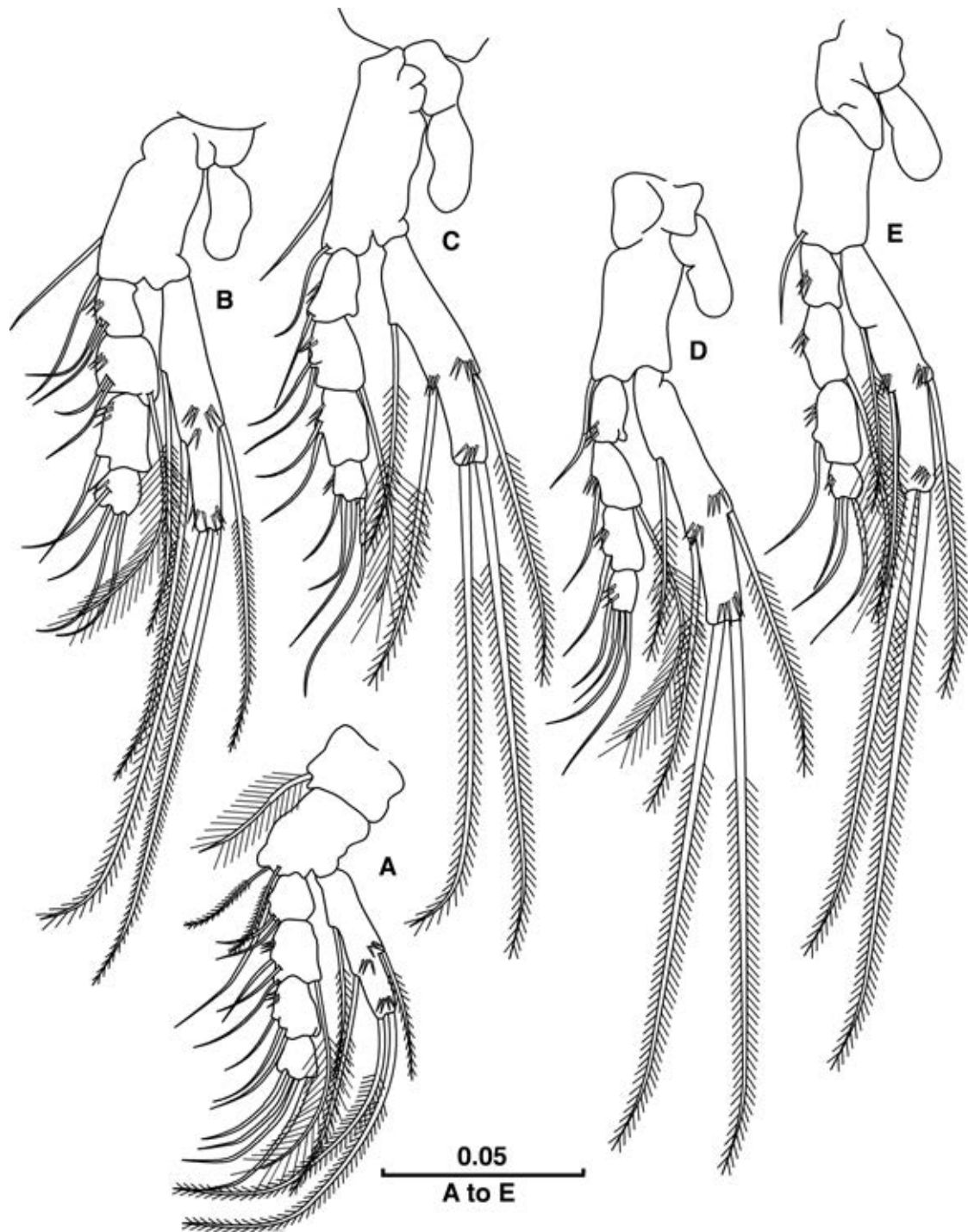


Fig. 2.— *Vejdovskybathynella vasconica* sp. nov., male holotype. (A) Thoracopod I; (B) thoracopod II; (C) thoracopod III; (D) thoracopod IV; (E) thoracopod V. Scale bar in mm.

Fig. 2.— *Vejdovskybathynella vasconica* sp. nov., holotipo macho. (A) Toracópodo I; (B) toracópodo II; (C) toracópodo III; (D) toracópodo IV; (E) toracópodo V. Escala en mm.

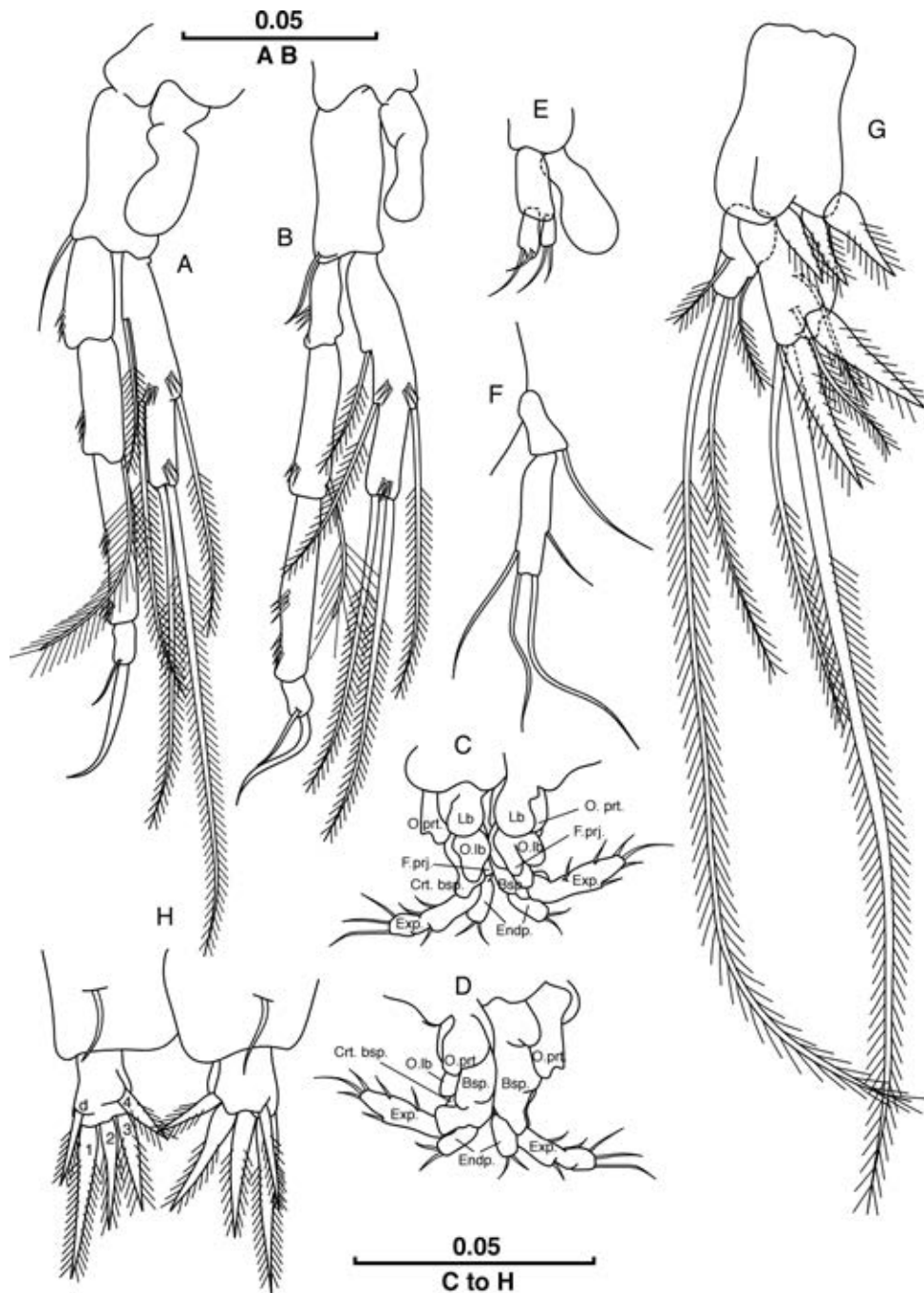


Fig 3.— *Vejdovskybathynella vasconica* sp. nov., male holotype. (A) thoracopod VI; (B) thoracopod VII; (C) thoracopod VIII (frontal view); (D) thoracopod VIII (ventral view); (E) female thoracopod VIII (frontal view); (F) pleopod (frontal view); (G) uropod (latero-external view); (H) furcal rami (dorsal view). Scale bar in mm. Abbreviations: Bsp, basipod; Crt. bsp., crest of basipod; d., dorsal spine; Endp, endopod; Exp, exopod; Fr. prj., frontal projection; O. prt., outer protuberance; O. Lb, outer lobe.

Fig 3.— *Vejdovskybathynella vasconica* sp. nov., holotipo macho. (A) toracópodo VI; (B) toracópodo VII; (C) toracópodo VIII (vista frontal); (D) toracópodo VIII (vista ventral); (E) toracópodo VIII de la hembra (vista frontal); (F) pleópodo (vista frontal); (G) urópodo (vista latero-externa); (H) ramas furcales (vista dorsal). Escala en mm. Abreviaturas: Bsp, basipodio; Crt. bsp., cresta del basipodio; d., espina dorsal; Endp, endopodio; Exp, exopodio; Fr. prj., proyección frontal; O. prt., protuberancia externa; O. Lb, lóbulo externo.

Table 6.— Differences amongst the six species known of the genus *Vejdovskybathynella* Serban & Leclerc, 1984. 1.- *Vejdovskybathynella balazuci* Serban & Leclerc, 1984 (France); 2.- *V. espattyensis* Serban & Leclerc, 1984 (France); 3.- *V. leclerci* Serban, 1989 (France); 4.- *V. edelweiss* (Spain); 5.- *V. caroloi* (Spain); 6.- *V. pascalis* (Spain) and 7.- *V. vasconica* sp. nov. (Spain). A.I= antennule; A.II= Antenna; Plp= pleopod; exp.= exopod; endp.= endopod; segs.= segments; P= present; A= absent

Tabla 6.— Diferencias entre las seis especies conocidas del género *Vejdovskybathynella* Serban & Leclerc, 1984. 1.- *Vejdovskybathynella balazuci* Serban & Leclerc, 1984 (Francia); 2.- *V. espattyensis* Serban & Leclerc, 1984 (Francia); 3.- *V. leclerci* Serban, 1989 (Francia); 4.- *V. edelweiss* (España); 5.- *V. caroloi* (España); 6.- *V. pascalis* (España) y 7.- *V. vasconica* sp. nov. (España). A.I= anténula; A.II= Antena; Plp= pleópodo; exp.= exopodito; endp.= endopodito; segs.= segmentos; P= presente; A= ausente.

	1	2	3	4	5	6	7
A. I: aesthetacs							
on segs. VI/VII	2/2	2/2	2/2	2/2	3/3	2/3	2/2
A.II: setal formula	0/1/2/1/0/4/4	0/1/2/1/0/4/4?	0/0/2/1/1/4/4	0/2/2/1/0/4/4	0/1/2/1/0/4/4	1/2/2/2/0/4/4	0/0/2/2/1/0/4/4
Exp.:medial seta	P	P?	A	P	A	A	P
Md: sexual dimorphism	Y			Y	Y	Y	N
Mx.II: setal formula	---	---	---	5/4/6/5	6/5/7/4	6/4/6/5	6/4/6/4
Th II-V	---	---	---	exp.<endp.	exp.<endp.	exp.>endp.	exp.=endp.
Th VI-VII	---	---	---	exp.<endp.	exp.<endp.	exp.<endp.	exp.<endp.
Th VIII female	exp.>endp.	exp.>endp.?	---	exp.=endp.	exp.>>endp.	exp.>>endp.	exp.<endp.
Th VIII male							
Outer protuberance				small	small	small	medium
Frontal projection	very large	large	very large	large	large	very large	large
Bsp.: frontal crest	P (large)	P (large)	A	P (large)	P (large)	P (large)	P (medium)
Endopod	large	large	---	large	small	very large	large
Exp.: length/width	4 times	3 times	---	2 times	2 times	3 times	4 times
Pleopod: setae	1+4	1+6	1+4	1+5	1+5	---	1+4
Uropod:							
Sympod: number spines	4	4	4	4	4	4	3
Spines	similar	similar?	similar	similar	distal long	basal small	similar
Endopod: claws	2	2?	2	3	3	2	2
Furca: ratio among							
second/first spine	4 times longer	4 times longer	2 times longer	2.5 times long.	4 times longer	2 times longer	2/3 longer
Dorsal/first spine	dorsal=first	dorsal=first	dorsal=first	dorsal>first	dorsal=first	dorsal>first	dorsal<first
Max. length of male	0.65	---	1.10	0.94	0.67	0.51	0.79
length of female	0.75	---	---	1.03	1.00	0.50	0.85

and third spine but thinner, a little longer than fourth spine, and 1.7 times shorter than the first, which is the longest, but not very long, attaining 2.5 times length of furca.

ETYMOLOGY. The species name, *vasconica*, is derived from the Basque Country, northeastern Spain, where the Goikoetxe cave is located and where the new species has been found. It is the first species of Bathynellacea described from the Basque Country.

REMARKS. The new species is medium-sized within the genus (see Table 6). Its antenna has eight segments, while the other six species of the genus display only seven-segmented antenna; the first segment lacks setae but the setal formula of the rest of

segments is similar to *V. edelweiss* (see Table 6) and the presence of a medial seta on the exopod occurs only in *V. balazuci* and in one of the Spanish species, *V. edelweiss*. *V. vasconica* sp. nov. is the only species of the genus that lacks sexual dimorphism on the male mandibular palp. The combination of setae on the segments of the endopod of Th. I and II is unique (see Table 7), as well as the combination of setae on the basipod of all thoracopods; within Spanish species only *V. edelweiss* has two setae as does *V. vasconica* sp. nov. on the basipod of Th. I to III, but *V. edelweiss* has also two setae on Th. IV (as in the French species) whereas *V. vasconica* sp. nov. has only one as the rest of Spanish species. The female Th VIII of *V. vasconica* sp. nov. has the exopod shorter than the endopod while *V. edelweiss* has

Table 7.— Setal formula of endopod of Thoracopods of species of *Vejdovskybathynella* Serban et Leclerc, 1984. Condition of *V. espattyensis* unresolved. Number of setae on basipod in brackets.

Tabla 7.— Fórmula setal del endopodio de los toracópodos de las diferentes especies del género *Vejdovskybathynella* Serban et Leclerc, 1984. Carácter desconocido en la especie *V. espattyensis*. Entre paréntesis el número de sedas del basipodio.

	<i>V. balazuci</i>	<i>V. leclerci</i>	<i>V. edelweiss</i>	<i>V. caroloi</i>	<i>V. pascalis</i>	<i>V. vasconica</i> sp. nov.
Th. I	(3) 3+0/1+1/2+0/4	(3) 3+0/1+1/2+0/4	(2) 3+0/2+1/2+0/4	(3) 2+0/2+1/2+0/4	(2) 2+0/2+1/2+0/4	(2) 3+0/3+1/3+0/4
Th. II	(2) 2+0/1+1/2+0/3	(2) 2+0/1+1/2+0/3	(2) 2+0/2+1/2+0/4	(2) 2+0/2+1/2+0/4	(2) 2+0/2+1/2+0/4	(2) 2+0/2+1/1+0/4
Th. III	(2) 1+0/0+1/2+0/3	(2) 2+0/1+1/2+0/3	(2) 2+0/2+1/2+0/4	(1) 2+0/2+1/2+0/3	(1) 1+0/2+1/2+0/4	(2) 1+0/2+1/2+0/4
Th. IV	(2) 1+0/0+1/2+0/3	(2) 2+0/1+1/2+0/3	(2) 1+0/1+1/1+0/4	(1) 1+0/1+1/1+0/3	(1) 1+0/1+1/1+0/4	(1) 0+0/1+1/1+0/4
Th. V	(1) 1+0/0+1/1+0/3	(1) 1+0/1+1/2+0/3	(1) 1+0/1+1/1+0/4	(1) 0+0/1+1/1+0/3	(1) 0+0/1+1/1+0/4	(1) 0+0/0+1/1+0/3
Th. VI	(1) 0+0/0+0/0+0/2	(1) 0+0/0+0/0+0/2	(1) 1+0/0+1/0+0/2	(1) 0+0/0+1/0+0/2	(1) 0+0/0+1/0+0/2	(1) 0+0/0+1/0+0/2
Th. VII	(1) 0+0/0+0/0+0/2	(1) 0+0/0+0/0+0/2	(1) 1+0/0+1/0+0/2	(1) 0+0/0+1/0+0/2	(1) 0+0/0+1/0+0/2	(1) 0+0/0+1/0+0/2

the exopod and endopod equal in size; all other species known in this genus have the exopod longer than the endopod. The size and proportion between the different lobes of the male Th. VIII is unique, with the exopod being four times as long as wide; in *V. edelweiss* and *V. caroloi* it is twice as long as wide; the frontal crest of the basipod is very small in the new species and small in *V. edelweiss*, whereas in the rest of species it is very well developed; the spur on the frontal crest is not well developed in the new species, but very developed in *V. edelweiss*; the endopod is large, as in the majority of species of this genus. In *V. vasconica* sp. nov. the second segment of the pleopod has four setae as in *V. balazuci* and *V. leclerci*. The new species is the only one with three spines on the sympod of the uropod, while the rest of the species have four; nevertheless, it has two claws on the endopod as *V. pascalis* and the three French species. Another unique feature of the new species is that the first spine of the furcal rami is the longest while in the rest of species it is the second which is longest; in addition, the dorsal spine is shorter than the first three spines, whereas in the rest of species they are of similar size, or with the first one somewhat longer.

Morphological discussion

There is no doubt that the new species belongs to the genus *Vejdovskybathynella*, described originally from the South of France, which includes three species from France, *V. balazuci* *V. leclerci*

and *V. espattyensis* Serban et Leclerc, 1984 and three from Spain, *V. edelweiss*, *V. caroloi* and *V. pascalis*.

Tables 6 and 7 show the similarities and differences between the seven species of the genus *Vejdovskybathynella*. In the original description of *V. espattyensis* Serban et Leclerc, 1984, only the differences with the type species *V. balazuci* Serban et Leclerc, 1984 were described, and no drawings of the majority of the body parts were provided. For this reason, there is not the same level of detail in the comparisons between species in our tables. For example, Mx. I and Mx. II are unknown in the French species.

The larger species are *V. leclerci* and *V. edelweiss*, and the smallest is *V. pascalis*; the new species is medium-sized. The three French species show more similarities between themselves than those seen between the Spanish species.

Only *V. caroloi* has three aesthetascs on segment 6 of antennule.; only *V. vasconica* sp. nov. has eight segments on antenna; only *V. pascalis* has setae on the first segment of A. II; the medial seta of the exopod of A. II is absent in *V. leclerci*, *V. caroloi* and *V. pascalis*.

The sexual dimorphism in the mandibular palp of the Md. is more evident in *V. edelweiss* and *V. caroloi* than in the other species where it is very subtle and it does not exist in the new species.

The setal formula of the endopod of Th. I-VII in the French species is mostly similar, with only slight differences in the number of setae of Th. III-V (Table 7). The Spanish species differ in number

Table 8.— Summary of genetic divergence (%) for COI between different level of taxa of populations of Bathynellidae from Northern of Spain. Outgroup used: *Iberobathynella imuniensis* of the Parabathynellidae family.

Tabla 8.— Resumen de la divergencia genética encontrada en el gen COI entre diferentes poblaciones de la familia Bathynellidae del Norte de España, pertenecientes a varios géneros y especies. Grupo externo usado: *Iberobathynella imuniensis* de la familia Parabathynellidae.

	Intraspecific	Intrageneric	Intergeneric	Intrafamily	Interfamily
Bathynellidae/Parabathynellidae					22.05-25.20
Bathynellidae Spain/Bathynellidae Australia				17.72-21.85	
<i>Vejdovskybathynella/Paradoxiclamousella</i>			15.75-19.45		
<i>Vejdovskybathynella/ Vejdovskybathynella</i>		14.17-19.68			
<i>Vejdovskybathynella edelweiss</i>	1.57				
<i>Paradoxiclamousella fideli</i>	0.39-7.30				
<i>Vejdovskybathynella vasconica</i> sp. nov.	0.59				

of setae on all the segments of the endopod of Th. I-VII, as well as in the number of setae on the basipod of the thoracopods (Table 7); only *V. edelweiss* has one seta on the first segment of the endopod of Th. VI and VII. The exopod of the thoracopods is smaller than the endopod in all thoracopods in *V. edelweiss* and *V. caroloi*, whereas it is larger or almost similar in Th. I-V in *V. pascalis* and *V. vasconica* sp. nov.

In the female Th. VIII the exopod is larger than the endopod, except in *V. edelweiss* and *V. vasconica* sp. nov., where they are similar in size or shorter. Only the new species lacks setae on the coxopod.

The male Th. VIII of *V. edelweiss* and *V. vasconica* sp. nov. is more similar to *V. balazuci* and *V. espattyensis* than to the Spanish species, though they have a less-developed frontal projection and a more rectangular basipod than the French species. The male Th. VIII of *V. pascalis* and *V. caroloi* have a more square aspect.

The pleopod of the new Spanish species has four setae on the second segment like two of the French species, whereas the third French species has six setae and the other Spanish species have five.

V. vasconica sp. nov. is the only species of the genus with three spines on the sympod of the uropod. The new species and *V. pascalis* have two claws on the endopod of the uropod like the French species; the rest of Spanish species display three claws.

V. edelweiss and *V. pascalis* have dorsal spines on the furcal rami that are longer than the first

spine; in the case of the new species they are smaller and in the rest of the species they are all similar; in *V. vasconica* sp. nov. the first spine of the furcal rami is longest, whereas the second spine is longest in the rest of species of the genus and is very long in *V. balazuci*, *V. espattyensis*, and *V. caroloi* (four times longer than the first spine).

Molecular Results

NUCLEOTIDE ANALYSIS

The alignment of all Bathynellacean COI gene sequences (15 specimens in total) resulted in a consensus length of 508 bp, of which 58% were variable. The models of sequence evolution selected for the mtDNA were GTR + I + G. No stop codons or gaps were observed in any of the translated amino acid sequences suggesting that the genuine mtDNA COI gene was sequenced. The base frequencies were as follows: A= 0.29, C= 0.13, G= 0.14 and T= 0.44.

The alignment of all Bathynellacean 18S rRNA gene sequences (16 specimens) comprised 45% variable sites; base frequencies were similar in all cases, about 25% (24.9% A, 24.2% C and T and 26.7% G).

GENETIC DIVERGENCES

The genetic divergence of COI is relevant at the species level, with significant results found within the different morphospecies (Camacho *et al.*, 2011, 2012). The uncorrected sequence divergence esti-

Table 9.— Summary of 18S rRNA genetic divergence (%) between different families, genera, populations of Bathynellacea from Spain. Outgroup used: *Anaspides tasmaniae*.

Tabla 9.— Resumen de la divergencia genética (%) encontrada en el gen 18S ARNr entre diferentes familias, géneros y poblaciones de Bathynellacea de España. Grupo externo usado: *Anaspides tasmaniae*.

	Intrageneric	Intergeneric	Interfamily	Interorder
Anaspidacea/Bathynellacea				11-44-14.98
Anaspidacea/Bathynellidae				12.27-14.98
Anaspidacea/Parabathynellidae				11.45-12.16
<i>Bathynellidae/Parabathynellidae</i>			7.60-12.06	
<i>Hexabathynella/Paraiberobathynella</i>		6.52-6.93		
<i>Hexabathynella/Iberobathynella</i>		6.12-6.94		
<i>Paraiberobathynella/Iberobathynella</i>		0.83-1.97		
<i>Paraiberobathynella/Paraiberobathynella</i>	0.41			
<i>Iberobathynella/Iberobathynella</i>	0.93-2.17			
<i>Vejdovskybathynella/Paradoxicalamousella</i>		6.84-7.97		
<i>Vejdovskybathynella/Vejdovskybathynella</i>	0-2.15			
<i>Paradoxicalamousella/Paradoxicalamousella</i>	0-1.41			

mates between the specimens and the outgroup taxa are summarized in Table 2. The genetic divergence between the outgroup used, *Iberobathynella imuniensis* Camacho, 1987 (Parabathynellidae) and the Bathynellidae species is 22% to 25.2%. The average uncorrected sequence divergence estimates between families and genera taxa are summarized in Table 8. The sequence divergence for COI among genera was defined (*Vejdovskybathynella* and *Paradoxicalamousella*) as ranging between 15.75% to 19.45%. The divergence between species of *Vejdovskybathynella* was similar to the distance shown between *Vejdovskybathynella* and *Paradoxicalamousella*, 14.17% to 19.68%. Finally, *Vejdovskybathynella caroloi* showed more genetic distance with *V. edelweiss* (18.9% to 19.7%) than with *V. vasconica* sp. nov. (16.5% to 16.7%).

Within the same genus, the new species showed a genetic distance of 14.2 % to 16.7 with respect to other species of *Vejdovskybathynella*, the maximum being with *V. caroloi* and the minimum with *V. edelweiss* (Table 2). The divergence of the new species with different populations of *Paradoxicalamousella fideli* ranged from 15.7% to 16.7%, while the divergence with the only known COI sequence of an Australian species of the family Bathynellidae showed the highest genetic distance (from 18.7% to 19.3%). The lowest genetic distance found between species was with the assigned specimen from Imunia cave (12.8%). In

the case of the specimens from Río Chico cave and Erizo rivers in the Ojo Guareña cave, genetic distances ranged from 13% to 13.6%. Finally, the genetic distance between the two specimens studied of *V. vasconica* sp. nov. was only 0.6%.

The uncorrected sequence divergence estimates of 18S rRNA between all the specimens studied and the outgroup taxa are summarized in Table 3. The genetic divergence between the outgroup used, *Anaspides tasmaniae* (Thomson, 1893) and both families of the Bathynellacea ranges from 11.44% to 14.98%. The average uncorrected sequence divergence estimates between families and genera, and with the outgroup taxa are summarized in Table 9. *Anaspides* shows more genetic divergence with the Bathynellidae than with the Parabathynellidae. Genetic divergence between genera, in both families, are normally between 6% and 8%, with the exception of the distance shown between *Iberobathynella* and *Paraiberobathynella*, which is only about 2%, a value more typical of intrageneric genetic divergence. The lowest distance between both families occurred between *I. parasturiensis* and *V. edelweiss* (7.60%), and the highest between *Pi. cf. maghrebensis* and *P.cf. fideli* (12.06%).

In both families, the lowest distance between two species in the same genus occurred in the *Paraiberobathynella* (0.41%), and the highest between *Iberobathynella burgalensis* (from the North of Spain) and *I. celiana* (from southern Spain)

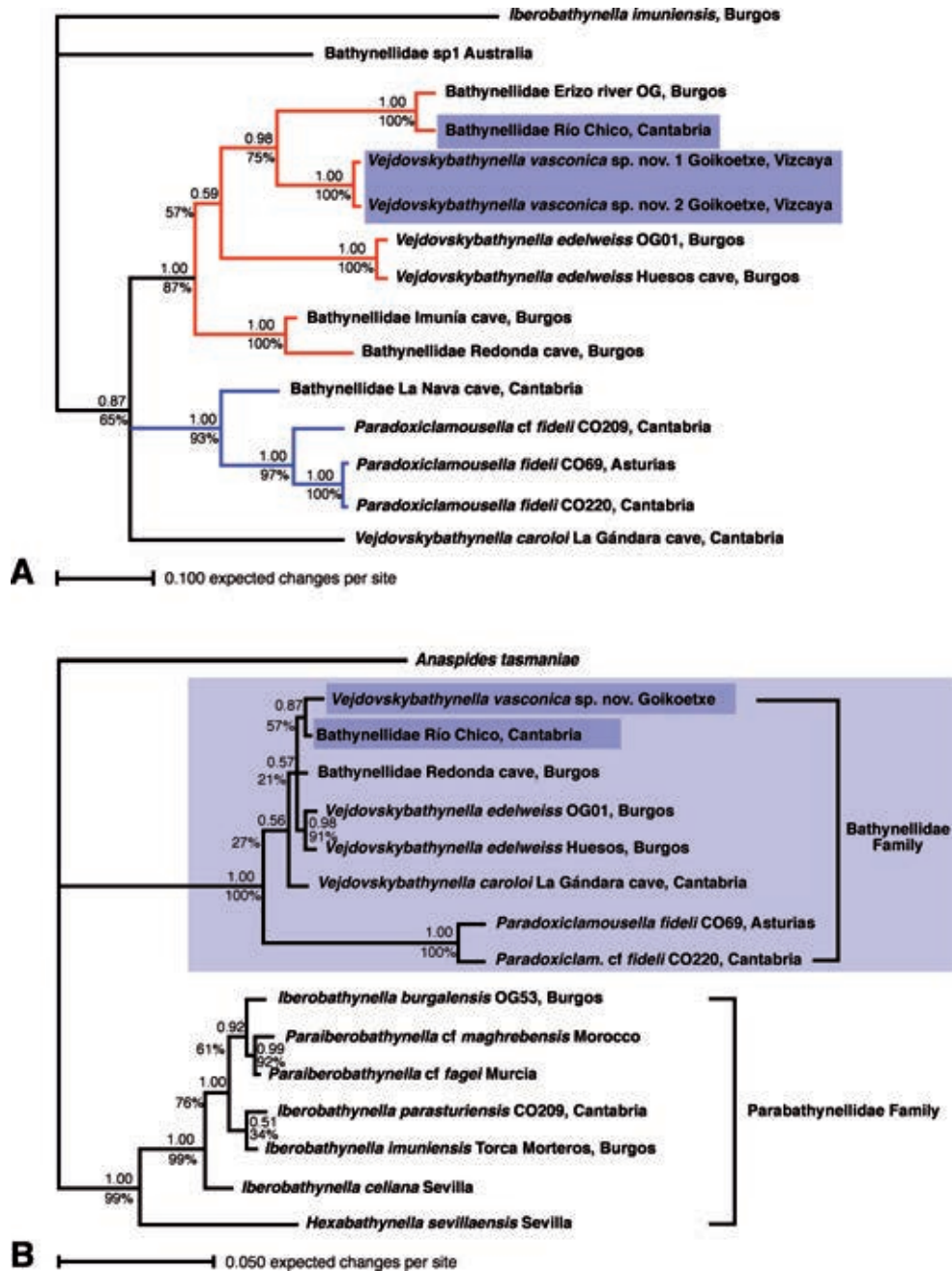


Fig. 4.– Phylogenetic reconstruction of the mitochondrial lineages (A) of Spanish Bathynellidae and the 18S rRNA data (B) of Spanish Bathynellacea. Posterior probability Bayesian consensus tree with re-estimated branch lengths using the GTR + I + G model of substitution. Bayesian posterior probabilities and credibility values are shown above on corresponding nodes, and Maximum likelihood (ML) below.

Fig. 4.– Reconstrucción filogenética de linajes mitocondriales (A) de la familia Bathynellidae de España y a partir de datos del 18S ARNr (B) de Bathynellacea de España. Árbol de consenso con probabilidad posterior Bayesiana, longitud de ramas reestimada usando el modelo de sustitución GTR + I + G. Los valores de probabilidad bayesiana se muestran encima de los nodos correspondientes, y la probabilidad máxima (ML) debajo.

(2.17%), followed by 2.15% between *Vejdovskybathynella edelweiss* and *V. caroloi*.

PHYLOGENETIC ANALYSES

The results of the phylogenetic analyses (ML, Bayesian) are summarized in Fig. 4A and B. The COI mtDNA sequence data analysis produced a tree (A) in which all samples of Bathynellidae are clearly separated from *Iberobathynella imuniensis* (Parabathynellidae) and Bathynellidae sp1 from Australia. There are two groups supported in ML and Bayesian analyses (> 100% Bayesian posterior probability and 93% and 87% bootstrap values). The phylogenetic reconstruction revealed that the major clades were clearly grouped in at least two different genera: *Paradoxiclamousella* and *Vejdovskybathynella*. The first robust group (93% bootstrap) is formed by *P. fideli* from CO69 cave, the population of CO209 cave and an unassigned Bathynellidae from La Nava cave (probably belonging to *Paradoxiclamousella* genus). The other clade (87% bootstrap) is formed by the specimens of *Vejdovskybathynella edelweiss* (Ojo Guareña and Huesos caves), the new species (*Vejdovskybathynella vasconica*) and two subgroups formed by unassigned Bathynellidae from Imunia and Redonda caves and Erizo river and Río Chico caves respectively (probably belonging to *Vejdovskybathynella*). The species from La Gándara cave, *Vejdovskybathynella caroloi*, appears as the only phylogenetic incongruence in the topologies observed in the gene tree for COI. The type locality of the new species is geographically farther from *Paradoxiclamousella* populations than *Vejdovskybathynella edelweiss* and *V. caroloi* populations (see Figure 5). *V. vasconica* sp. nov. shows enough genetic distance with respect to *V. edelweiss* (about 15%) and *V. caroloi* (15.75% to 16.73%) as to be separated into a different clade (see Table 2).

The 18S rRNA sequence data analysis produced a tree (B) in which there are two groups supported in ML and Bayesian analyses (> 100% Bayesian posterior probability and 99% and 100% bootstrap values). All samples of Bathynellidae are clearly separated from the Parabathynellidae. The phylogenetic reconstruction of the Parabathynellidae family revealed that the major clades clearly grouped, at least, two different genera: *Hexabathynella* and *Iberobathynella*. *Paraiberobathynella* appears nested within the group of *Iberobathynella* species,

and although the bootstrap value is not very high (61%), this indicates a small phylogenetic incongruence. The phylogenetic reconstruction of the Bathynellidae revealed that the first robust group (100% bootstrap) is formed by *P. fideli* from CO69 cave and the population of CO209 cave. The other group is formed by *Vejdovskybathynella* (including the new species described herein) and various unassigned specimens from several populations, but the support is somewhat weak (27%).

General discussion and conclusion

According to Serban (2000), none of the Iberian species belong to the genus *Bathynella* Vejdovsky, 1882, which ranges mainly to the northeast of France and never beyond the Pyrenees (Camacho, 2007). Current knowledge suggests that other representatives of the subfamily Gallobathynellinae Serban, Coineau et Delamare Deboutteville, 1971, populate most of France and the entire Iberian Peninsula. Here we describe a new species from northern Spain belonging to *Vejdovskybathynella*. This is the fourth species of this genus found in Spain, all of them being limited to the north of the Iberian Peninsula (Fig. 5).

The new species lives in groundwater of epikarstic gours and pools in a Basque cave and shows very subtle morphological differences with other *Vejdovskybathynella* species.

In this study we have complemented the morphological analysis with a molecular analysis to support the validity of the new species described. We present all the morphological characters needed to establish a new taxon, as required by classical taxonomy, but we also have added new characters (mtDNA and 18S rRNA sequences) obtained with molecular techniques.

The genetic divergences observed in partial COI mtDNA sequences of the new species, 14.17 to 16.73%, should be enough to discriminate between congeneric species. At the moment, we still lack clear taxon definitions, and the demarcation of species using genetic divergences based on a single mitochondrial sequence is still imprecise (Meier *et al.*, 2008). In a previous paper, we found values of divergence between 14 and 17% for different morphospecies of the genus *Vejdovskybathynella* (Camacho *et al.*, 2011, 2012), and values ranging between 15.8 and 23.6% between morphospecies

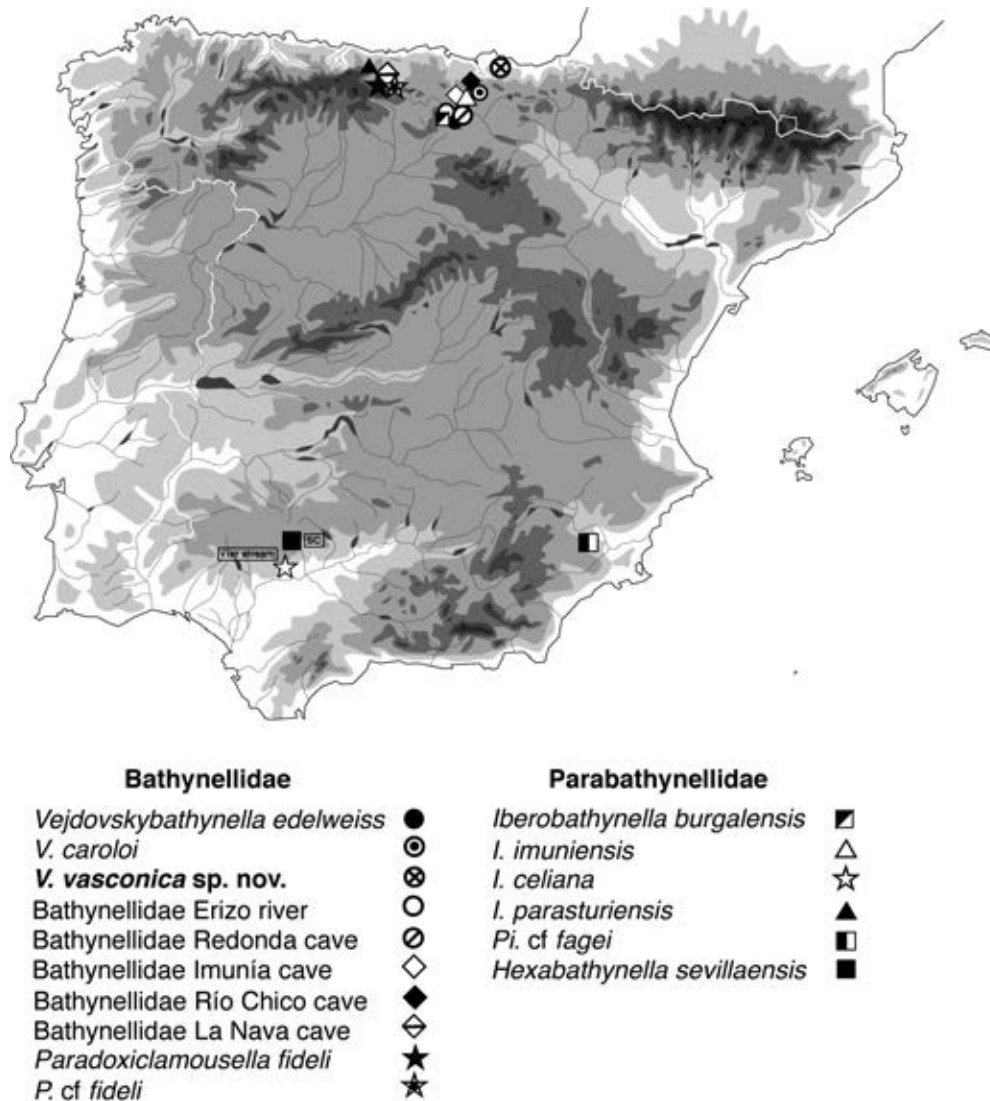


Fig. 5.– Distribution of Spanish Bathynellacean with COI and 18S rRNA sequences known.

Fig. 5.– Distribución de las especies españolas de Bathynellacea de las que se conocen secuencias de los genes COI y 18S ARNr.

of *Iberobathynella* Schminke, 1973 (Parabathynellidae) (Camacho *et al.*, 2012). Nevertheless, since genetic divergence thresholds can vary among organisms, currently it is not clear what level of divergence designates a significant difference within and among lineages. Within crustaceans, Lefébure *et al.* (2006) investigated the relationship between morphospecies and genetically diverse species and identified 16% genetic

divergence to be a consistent threshold for distinct species. Costa *et al.* (2007) suggested that a 17% divergence assessed different species between decapoda crustaceans. Abrams *et al.* (2012) found genetic divergences varying from 6.2% to 15.9% among 11 species of *Brevisomabathynella* Cho, Park et Ranga Reddy, 2006. The divergence between species in *Atopobathynella* Schminke, 1973 (Parabathynellidae) from Australia was much lar-

ger, ranging from 15.8% to 24.6%, . For the purpose of this study, the genetic divergences found and the robustness of the main clades assure us that our new species, *V. vasconica* sp. nov., is certainly valid and belongs to the genus *Vejdovskybathynella*. The results also show that the specimens from Erizo river and Río Chico cave probably belong to *Vejdovskybathynella*, and that the species of these two caves, is closer to *V. vasconica* sp. nov. than to *V. edelweiss*. In addition, the phylogenetic position established for some unassigned taxa provides a first indication of their relationship both to each other and to other species currently morphologically well identified and supported by their geographical distribution (see map of the Fig. 5).

Besides the COI sequences, partial sequences of 18S rRNA (about 1000 bp) have been obtained in this study. This is the first time this is done in Spanish Bathynellacea, so we do not have previous data to establish comparisons. Abrams *et al.* (2012) obtained partial sequences (about 700 bp) of several species belonging to three Australian genera of Parabathynellidae, and one more sequence of one unassigned specimen of Australian Bathynellidae. They found on average 18S rRNA sequence divergence among genera between 3.1% and 8.8%, and an average divergence between all Australian parabathynellid species of 4%. In our study, the genetic divergences observed in partial 18S rRNA sequences between the new species and other Spanish Bathynellidae ranged from 1.63 to 6.12% (see Table 3). We have not included the Genbank sequences of Australian species in our data-set since the combined number of base pairs was very small to offer good results. In the phylogenetic tree obtained using only our data several small incongruences within two major clades that correspond to both families were found. For example, *Iberobathynella burgalensis*, *I. parasturiensis* and *I. imuniensis* appear in the same group, but *Paraiberobathynella* appears nested within them. In addition, the group corresponding to *Vejdovskybathynella* is not very well supported, despite the geographical proximity of species (see Fig. 5).

It would be necessary to obtain longer sequences of these genes, as well as to consider more genes in order to expand the molecular studies. This should be done in parallel to further morphological analyses to outline the populations composed by specimens of mixed species, of different or the same genera. The use of both classical taxono-

mic tools and molecular techniques will generate more precise knowledge on the taxonomy of a group of groundwater invertebrates that is proving to be much more diverse than previously considered.

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