Split distribution, biogeography and morphological and genetic diversity of the Iberobathynellini Tribe in the family Parabathynellidae (Crustacea, Malacostraca, Bathynellacea)

Ana Isabel Camacho | ORCID: 0000-0003-0596-7678

Museo Nacional de Ciencias Naturales (CSIC), Dpto. Biodiversidad y Biología Evolutiva,
C/ José Gutiérrez Abascal 2, 28006 Madrid, Spain
mcnac22@mncn.csic.es

Paloma Mas-Peinado | ORCID: 0000-0002-9290-5805

Museo Nacional de Ciencias Naturales (CSIC), Dpto. Biodiversidad y Biología Evolutiva,
C/ José Gutiérrez Abascal 2, 28006 Madrid, Spain
Centro de Investigación en Biodiversidad y Cambio Global CIBC-UAM, Facultad de
Ciencias, Universidad Autónoma de Madrid, c/Darwin 2, 28049 Madrid, Spain
maspeina@gmail.com

E. Karen López-Estrada | ORCID: 0000-0001-5539-4826

Museo Nacional de Ciencias Naturales (CSIC), Dpto. Biodiversidad y Biología Evolutiva,
C/ José Gutiérrez Abascal 2, 28006 Madrid, Spain
lokaren21@gmail.com

Beatriz A. Dorda | ORCID: 0000-0002-2741-1841

Museo Nacional de Ciencias Naturales (CSIC), Dpto. Colecciones, Colección de Tejidos
y ADN, C/ José Gutiérrez Abascal 2, 28006 Madrid, Spain
balvarez@mncn.csic.es

Isabel Rey | ORCID: 0000-0002-2122-5124

Museo Nacional de Ciencias Naturales (CSIC), Dpto. Colecciones, Colección de Tejidos y ADN,
C/ José Gutiérrez Abascal 2, 28006 Madrid, Spain
mcnrf3g@mncn.csic.es

Abstract

The “*Iberobathynella* group”, or Iberobathynellini tribe, is a complex of six genera consisting of 33 nominal species and several cryptic species with an amphiatlantic distribution (in Europe, North Africa and North

America). A modern systematic revision of this group of subterranean crustaceans is presented here. A phylogenetic and biogeographic study using morphological and molecular data (mitochondrial COI and nuclear 18S) was carried out and allowed to a) re-evaluate the taxonomic status and validity of previously erected subtribes, genera and subgenera that show congruence in the data; b) assess whether the identified mitochondrial lineages represent cryptic species; c) provide a plausible phylogenetic hypothesis for the relationships within Iberobathynellini and with the other two genera of the family Parabathynellidae that inhabit North America and Europe (*Montanabathynella* and *Parabathynella*, respectively); d) propose a plausible temporal and historical framework (paleobiogeographic scenario) for the diversification and evolution of the Iberobathynellini tribe based on the current distribution of morphotypes and their estimated times of divergence. Our results show that in parabathynellids, molecular and morphological divergences are not always congruent. Subtribe and subgenus are invalid categories so they must be eliminated. *Paraiberobathynella* genus needs to be revisited. The molecular dating results support the early divergence of the Iberobathynellini Tribe (Upper Cretaceous, around 78 Mya) and the vicariance by plate tectonics as main factor to explain the *amphi*-Atlantic distribution shown by this ancient subterranean crustacean group. Since there are species morphologically very similar to *I. magna* and *I. imuniensis*, but genetically different, we can ensure the existence of at least three cryptic species. *Texanobathynella* is undoubtedly a valid genus distinct from *Iberobathynella*. *Montanabathynella* and *Parabathynella* are two well-differentiated genera closely related to the Iberobathynellini tribe.

Keywords

Parabathynellidae – Iberobathynellini tribe – stygofauna – taxonomic uncertainties – systematics – molecular tools – 18S – COI – palaeobiogeography

Introduction

The Iberobathynellini tribe Camacho & Serban, 1998 is one of the most diverse and best known groups within the family Parabathynellidae Noodt, 1965. It includes six genera distributed in Spain and Portugal within Europe and in North Africa and North America. *Iberobathynella* Schminke, 1973, the most speciose genus of the Tribe, with its 22 formally described species (and some cryptic ones), is the third largest genus in terms of number of species, after *Allobathynella* Morimoto & Miura, 1957 (with 25 species) and the cosmopolitan genus *Hexabathynella* Schminke, 1972 (with 23 species) (Camacho, 2019) inside to the Parabathynellidae family. *Iberobathynella* is widely distributed

throughout the Iberian Peninsula (Camacho, 2019). Of the 22 species of *Iberobathynella*, 21 belong to three subgenera (Camacho & Serban, 1998): *I. (Iberobathynella)* and *I. (Espanobathynella)*, with five species each, and *I. (Asturibathynella)*, with 11 species. The remaining species, *I. pedroi* Camacho, 2003, does not fit into any of the three subgenera (Camacho, 2003b).

Texanobathynella Delamare Deboutteville, Coineau & Serban, 1975 comprises the second most speciose genus of the tribe. It comprises three North American species from Texas (*T. bowmani* Delamare Deboutteville, Coineau & Serban, 1975; *T. aaronswinki* Camacho & Hutchins, 2021 and *T. coloradoensis* Camacho & Hutchins, 2021) and one from California (*T. sachi* Cho, 1996). This genus is the most

widely distributed of the six, covering a range of about 9000 km that extends from Texas to California.

The third genus of the tribe is *Paraiberobathynella* Camacho & Serban, 1998. It is the second most widely distributed genus of the six, and includes two subgenera and three species: *Pi. (Paraiberobathynella) fagei* (Delamare Deboutteville & Angelier, 1950), whose distribution extends from the south of France to Andalusia in Spain and the Balearic Islands (Mallorca and Menorca-Pretus, 1989); *Pi. (Paraiberobathynella) maghrebensis* (Boutin & Coineau, 1987) from North Africa (Morocco) and *Pi. (Orihuelabathynella) notenboomi* (Camacho, 1989), known only from its locality type in Alicante (Spain).

The fourth genus, *Hexaiberobathynella* Camacho and Serban, 1998, is found in the Iberian Peninsula. It consists in *Hi. mateusi* (Galhano, 1967), whose type locality is in Oporto (Portugal) but its distribution extends widely throughout the central Iberian Peninsula (Camacho, 1988; Camacho & Coineau, 1989; Camacho et al., 1997; Camacho & Serban, 1998; Camacho & Serban, 2000; Camacho et al., 2000; Camacho et al., 2014; Camacho, 2019), and *Hi. hortezuelensis* Camacho & Serban, 1998, whose distribution is restricted to its type locality in a single well in the province of Soria.

The remaining two genera of the Iberobathynellini tribe are *Guadalopebathynella* Camacho & Serban, 1998, from the Iberian Peninsula, and *Californibathynella* (Schminke & Noodt, 1988), from North America.

Schminke and Noodt (1988) considered *Californibathynella californica* (Schminke and Noodt, 1988), a species endemic to California, as a species of *Iberobathynella*, and also synonymized *Texanobathynella*, from Texas, with *Iberobathynella*. However, Schminke (2011), in his monograph of the

family Parabathynellidae, later recognized *Californibathynella* and *Texanobathynella* as valid genera, as well as the subgenera *Paraiberobathynella* (*Paraiberobathynella*) and (*Orihuelabathynella*). However, he did not use other categories, such as the tribe, subtribe and subgenera of *Iberobathynella* that had been erected by Camacho & Serban (1998).

Life underground supposes strong selective pressures and physical isolation. Barriers inhibiting the dispersal of different populations in disconnected habitats (i.e., aquifers, springs, hyporheic habitats or catchment boundaries) can lead to high genetic divergence yet morphological convergence (Finston et al., 2007). Convergent evolution of morphological characters is associated with adaptations to the subterranean environment (Jones et al., 1992; Kane et al., 1994), making the classical (morphological) taxonomy of stygobitic organisms very complex. In the case of Bathynellacea, the taxonomy is complicated, in addition to the extensive morphological simplification observed in the group, by the extreme progenetic development of its species (Schminke, 1981) and the lack of morphological distinguishability between species that could be caused by recent divergence, niche conservatism or morphological convergence (Zagmajster et al., 2018). Due to the increased use of molecular techniques in systematic studies, cryptic species have been detected in almost all taxonomic groups (Knowlton, 1993; Amato et al., 2007; Beheregaray & Caccone, 2007; Le Gac et al., 2007; Heraty et al., 2007; Pfenninger & Schwenk, 2007; Barroso et al., 2010, González-Miguéns et al., 2020), including Bathynellacea (Guzik et al., 2008; Camacho et al., 2011, 2012; Little & Camacho, 2017; Camacho et al., 2018a, b, c). The presence of such species necessitates the use molecular approaches for species delimitation (González-Miguéns et al., 2020).

The main goal of this study is to identify the morphospace of the Iberobathynellini tribe in order to analyse congruence between morphological variability versus morphological homogeneity and genetic divergence, as assessed using both rapidly (mitochondrial cytochrome oxidase subunit I, COI) and slowly (nuclear 18S) evolving genes. Exclusively representatives of the genera of the family that live in the area of the study have been analyzed, excluding other genera of the Parabathynellidae family from all over the world (Australia, India, Asia ...) that were already analyzed globally in previous papers (Camacho et al., 2018; Camacho et al., 2021a), since the study tries to find the precise relationships between the genera of the Parabathynellidae family that inhabit Iberian Peninsula and North America using as outgroup *Hexabathynella*, the only cosmopolitan Bathynellacea genus, because it is very different of rest of genera of Parabathynellidae and has only 6 pairs of legs, a character only shared with the Iberian genus *Hexaiberobathynella*. The specific objectives of this study are to i) define taxonomic units with congruent morphological and molecular data to evaluate the validity of genera and subgenera classifications; ii) assess whether the identified mitochondrial lineages represent cryptic species; iii) provide a plausible phylogenetic hypothesis for the relationships within Iberobathynellini and with the only other two genera of Parabathynellidae, not belonging to the Tribe Iberobathynellini, that inhabit North America and Europe (*Montanabathynella* Camacho, Stanford & Newell, 2009 and *Parabathynella* Chappuis, 1926, respectively, two genera closely related to each other and very different of genera tribe as we already demonstrated (Camacho et al., 2021b)) and iv) propose a plausible paleobiogeographic scenario based on the current distribution and divergence time estimates of the *Iberobathynella* group.

Material and methods

Study area

The study area includes the USA in North America, Morocco in North Africa and the Iberian Peninsula and Balearic Islands in Europe. Species distributions are shown on the map in fig. 1.

Molecular study

Specimen collection for DNA analysis. A total of 208 specimens of Parabathynellidae were collected from Spain, Morocco and Texas from groundwater found in caves, springs, wells or hyporheic zones of streams by A.I. Camacho, B. Hutchins, B.F. Schwartz, A. Brancelj and D. Jaume (supplementary appendix S1).

Of these, 205 belong to the *Iberobathynella* group (16 nominal species and three cryptic species that likely represent new species) from different populations in 30 localities (table 1) and three to the other genera of Parabathynellidae present in North America or/and Europe (*Montanabathynella*, *Parabathynella* and the cosmopolitan genus *Hexabathynella*) (table 1 and supplementary appendix S1). We chose the genus *Hexabathynella* as outgroup for being cosmopolitan, because we had DNA sequences of Spanish species to compare and additionally for being a genus whose species only have six pairs of legs and it is very different of ingroup species. Specimen voucher numbers are provided in table 2 and supplementary appendix S1.

Specimens used in the molecular analysis were stored either in 95% alcohol or in 0.5 ml of digestion buffer (Gilbert et al., 2007) at -20 °C.

DNA extraction, amplification, and sequencing. The DNA extraction, amplification, and sequencing protocols used were as previously described (Camacho et al., 2018c; Camacho et al., 2021a). DNA was extracted

FIGURE 1 World distribution map of species and populations of the “Iberobathynellini tribe” and those of *Montanabathynella* and *Parabathynella*.

from whole specimens or from abdominal tissue, and samples were deposited in the Tissues and DNA Collection of the Museo Nacional de Ciencias Naturales, Madrid (MNCN). Briefly, the abdomen or whole specimen placed in 0.5 ml digestion buffer (Gilbert et al. 2007) and incubated overnight at 55°C with gentle agitation. DNA was then extracted from the digestion buffer using a Qiaquick PCR Purification Kit (QIAGEN), following the protocol used by Alda et al. (2007). A 507 base-pair (bp) fragment of COI was amplified using the primers C1-J-1718 and HCO2198 (Folmer et al., 1994; Simon et al., 1994). For nuclear 18S rRNA, three fragments, totalling 1702 bp, were amplified using the primers 1F and 3R, 3F and 5R, and 5F and 9R (Giribert et al., 1996). The PCR was performed employing standard methods (Camacho et

al., 2018c). PCR products were purified by treatment with ExoSAP-IT (USB Amersham, Buckinghamshire, UK) and sequenced in both directions using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems Inc., Foster City, USA).

All sequences were compiled, assembled and edited using Geneious v. 10.2.4 (<https://www.geneious.com>) (Kearse et al., 2012). Sequences were aligned automatically in MAFFT (Katoh & Toh, 2008), as implemented in Geneious, and the final alignment was checked with Geneious and Mesquite v3.04 (Maddison & Maddison, 2015) for gaps and translate it to see if stop codons were present. Sequence divergences (uncorrected p-distances) of 18S among genera were estimated using PAUP*4.0b 10 (Swofford, 2002).

TABLE 1 Number of specimens used from each locality in COI molecular analyses.

Species	Locality	Specimen number
<i>Iberobathynella andalusica</i> Camacho, 2007	* Varas River, Córdoba	1
<i>I. asturiensis</i> Serban & Comas, 1978	* Infierno Cave, Asturias	9
<i>I. burgalensis</i> Camacho, 2005	* Ojo Guareña Cave (OG53), Burgos	3
<i>I. cantabriensis</i> Camacho & Serban, 1998	CO276 Lobos Cave, Cantabria	3
<i>I. celiana</i> Camacho, 2003	Viar Stream, Castiblanco, Sevilla	1
<i>I. cornejoensis</i> Camacho, 2005	* Redonda Cave, Burgos	2
<i>I. espaniensis</i> Serban & Comas, 1978	* Infierno Cave, Asturias	7
<i>I. imuniensis</i> Camacho, 1987	* Torca Morteros Cave, Burgos	3
<i>I. imuniensis</i>	Bernias Cave, Burgos	14
<i>I. imuniensis</i>	Calleja-Cueto Cave, Cantabria	1
<i>I. imuniensis</i>	Becerral Cave, Cantabria	1
<i>I. imuniensis</i>	Lunada Cave, Burgos	6
<i>I. imuniensis</i>	Torca V142 Cave, Burgos	6
<i>I. magna</i> ?	CO262 Pelacristo Cave, Asturias	4
<i>I. ortizi</i> Camacho, 1989	* Rei Cintolo Cave, Lugo	1
<i>I. parasturiensis</i> Camacho & Serban, 1998	* CO209, Treslajorá Cave, Cantabria	23
<i>I. parasturiensis</i>	CO034, Nava Cave, Cantabria	4
<i>I. parasturiensis</i>	Infierno Cave, Asturias	3
<i>I. sp.- I. sp. nov. 1</i> (cryptic <i>I. magna</i>)	CO314, Pilar Cave, Asturias	4
<i>I. sp. 1- I. sp. nov. 2</i> (cryptic <i>I. imuniensis</i>)	CO314, Pilar Cave, Asturias	12
<i>I. sp. 2- I. cantabriensis</i>	CO209, Treslajorá Cave, Cantabria	13
<i>I. sp. 2 - I. cantabriensis</i>	CO220, Carnero Spring Cave, Cantabria	1
<i>I. sp. 2 - I. cantabriensis</i>	CO314, Pilar Cave, Asturias	1
<i>I. sp. 3- I. sp. nov. 2</i> (cryptic <i>I. imuniensis</i>)	CO314, Pilar Cave, Asturias	1
<i>I. sp. 3- I. sp. nov. 2</i> (cryptic <i>I. imuniensis</i>)	CO069, Pozo Agua Cave, Asturias	14
<i>I. sp. - I. sp. nov. 2</i> (cryptic <i>I. imuniensis</i>)	CO069, Pozo Agua Cave, Asturias	3
<i>I. sp. 4- I. cantabriensis</i>	CO314, Pilar Cave, Asturias	3
<i>I. sp. 4- I. cantabriensis</i>	CO089, Sorios Cave, Asturias	1
<i>I. sp. 5- I. sp. nov. 3</i> (cryptic <i>I. imuniensis</i>)	Fonda Cave, Vizcaya	5
<i>I. sp. 6- I. sp. nov. 3</i> (cryptic <i>I. imuniensis</i>)	Astui Cave, Vizcaya	3
<i>I. sp.- I. sp. nov. 3</i> (cryptic <i>I. imuniensis</i>)	Mina Elvira, Vizcaya	1
<i>Paraiberobathynella cf. fagei</i>	Higuera Cave, Murcia	25
<i>Paraiberobathynella cf. fagei</i>	Pileta Cave, Málaga	11
<i>Pi. maghrebensis</i> (Boutin & Coineau, 1987)	Well, Nador, Morocco	8
<i>Texanobathynella cf. bowmani</i>	Live Oak, Crockett County, Texas	1
<i>T. aaronswinki</i> Camacho & Hutchins, 2021	Sessom Creek, Hays County, Texas	1
<i>T. aaronswinki</i>	* South Llano River, Kimble County, Texas	1

TABLE 1 Number of specimens used from each locality in COI molecular analyses. (cont.)

Species	Locality	Specimen number
<i>T. coloradoensis</i> Camacho & Hutchins, 2021	* Colorado River, Bastrop County, Texas	4
Total		205
Outgroups		
<i>Montanabathynella pecosensis</i>	Live Oak, Crockett County, Texas, USA	1
<i>Parabathynella</i> sp. (<i>Parabathynella</i> cf <i>motasi</i>)	Poltarica spring, Slovenia	1
<i>Hexabathynella sevillaensis</i> Camacho, 2003	* Santiago el Grande Cave, Sevilla	1

* Type locality

Phylogenetic and molecular clock analyses

Phylogenetic analyses using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches were used. We ran analyses with two datasets: (i) COI and ii) COI and 18S. The ML analyses were conducted in IQ-TREE 2.1.1 (Minh et al., 2020). We estimated the best partition scheme with the option MFP+MERGE in ModelFinder (Kalyaanamoorthy et al., 2017), with the following parameters: low perturbation strength (-pers 0.2), number of unsuccessful iterations to stop (-nstop) set to 500 and, to assess node support, 2000 ultra-fast bootstrap replicates. The BI analyses were run in MrBayes 3.2.6 (Ronquist et al., 2012). We ran analyses implementing the partition scheme recovered by ModelFinder with three partitions for both the combined COI and 18S analyses (p1: 18S-coi pos2 = 1–1702, 1703–2207\3; p2: coi pos1 = 1705–2209\3 and p3: coi pos3 = 1704–2208\3) and the COI analyses (p1: coi pos1 = 3–507\3; p2: coi pos2 = 1–505\3 and p3: coi pos3 = 2–506\3). The substitution model space was explored with the reversible-jump model (option lset nst = mixed rates = invgamma; Huelsenbeck et al., 2004). Two independent analyses were run with one cold and three heated chains, each chain

ran for 100 million generations, with the first 25% discarded as burnin. From the resulting trees, a 50% majority rule consensus tree was obtained.

For the divergence time analysis, we used relaxed molecular clocks to reconstruct the divergence time between lineages, and inferred credibility intervals in BEAST 1.8.4 (Drummond et al., 2012). Given the absence of a fossil record for the group, we calibrated the molecular clock based on the time to the most recent common ancestor (TMRCA) of Parabathynellidae [175.7 Ma (95% HPD: 113.4–241.9 Ma); Lognormal Mean, 175.5; STdev, 0.2] that was included in a recent study of seventy specimens of Bathynellacea based on the substitution rates for the nuclear gene 18S (Camacho et al., 2021a). We used an uncorrelated lognormal relaxed molecular clock for each gene analysed to estimate its rate of molecular evolution. As priors, we used broad lognormal distributions with mean general substitution rates retrieved from Papadopoulou et al., (2010). We ran the analyses for 100 million generations, sampling every 10,000 generations. We specified a birth-death process as a speciation tree prior (Kendal, 1948; Stadler, 2009). Convergence

TABLE 2 Specimens of Parabathynellidae Noodt, 1965 used in the phylogenetic analysis (COI+18S).

Order/Species	Locality	Coordinates			Specimen voucher	GenBank accession number	
Bathynellacea Chappuis, 1915		N	E	Z	MNCN/DNA	COI	18S
**1. <i>Iberobathynella andalusica</i>	*Varas River, Adamuz to Pozo Blanco, Córdoba	38.08128	-4.63227	227	29418	OL679928	KC999705
**2. <i>I. asturiensis</i>	*Infierno Cave, Cangas de Onis, Asturias	43.28068	-4.99305	950	54896	MZ128377	MT874908
**3. <i>I. burgalensis</i>	*Ojo Guareña Cave (OG53), Sotoscueva, Burgos	43.03188	-3.65821	724	29520	HQ659859	KC469529
**4. <i>I. cantabriensis</i>	CO276, Torco Lobos Cave, Herrerías, Cantabria	43.28755	-4.50909	492	29372	HQ659853	KC999704
**5. <i>I. celiana</i>	Viar Stream, Castiblanco, Sevilla	37.71374	-5.87772	60	29452	HQ659862	KC469527
**6. <i>I. cornejoensis</i>	*Redonda Cave, Sotoscueva, Burgos	43.03236	-3.62913	668	29946	KP974108	KP999701
**7. <i>I. espaniensis</i>	*Infierno Cave, Cangas de Onis, Asturias	43.28068	-4.99305	950	54893	MZ128378	MT822875
**8. <i>I. imuniensis</i>	*Torca Morteros Cave, Espinosa de los Monteros, Burgos	43.14786	-3.59539	1285	29166	HQ659850	KC469528
9. <i>I. imuniensis</i>	Bernias Cave, Espinosa de los Monteros, Burgos	43.16902	-3.64212	1227	29778	KP974056	KP999690
10. <i>I. imuniensis</i>	Calleja-Cueto Cave, Arredondo, Cantabria	43.29183	-3.62869	649	54926	MZ128379	MT822876
11. <i>I. imuniensis</i>	Lunada Cave, Espinosa de los Monteros, Burgos	43.16993	-3.64993	1285	29994	KP974114	KP999692
12. <i>I. imuniensis</i>	Torca V142 Cave, Espinosa de los Monteros, Burgos	43.14493	-3.67693	1564	54559	KP974119	OL514026
**13. <i>I. ortizi</i>	*Rei Cintolo Cave, Mondoñedo, Lugo	43.39364	-7.36786	409	54616	MF443322	MF436209

TABLE 2 Specimens of Parabathynellidae Noodt, 1965 used in the phylogenetic analysis (COI+18S). (cont.)

Order/Species	Locality	Coordinates			Specimen voucher	GenBank accession number	
Bathynellacea		N	E	Z	MNCN/DNA	COI	18S
Chappuis, 1915							
**14. <i>I. parasturiensis</i>	*CO209, Treslajorá Cave, Peñarrubia, Cantabria	43.26725	-4.59085	1132	29561	KP974005	KP999698
15. <i>I. parasturiensis</i>	CO34, Nava Cave, Lamasón, Cantabria	43.25928	-4.50022	492	29612	KC974021	KC999697
16. <i>I. parasturiensis</i>	Infierno Cave, Cangas de Onis, Asturias	43.28068	-4.99305	950	54897	OL679948	MT822878
17. <i>I. sp.</i> - <i>I. sp. nov. 1</i> (cryptic <i>I. magna</i>)	CO314, Pilar Cave, Peñamellera Baja, Asturias	43.29583	-4.53996	538	29708	KP974069	KP999707
18. <i>I. sp. 1</i> - <i>I. sp. nov. 2</i> (cryptic <i>I. imuniensis</i>)	CO314, Pilar Cave, Peñamellera Baja, Asturias	43.29583	-4.53996	538	29709	KP974071	OL514025
19. <i>I. sp. 2</i> - <i>I. cantabriensis</i>	CO209, Treslajorá Cave, Peñarrubia, Cantabria	43.26725	-4.59085	1132	29573	KC974102	KC999725
20. <i>I. sp. 2</i> - <i>I. cantabriensis</i>	CO220, Carnero Spring Cave, Lamasón, Cantabria	43.28680	-4.47784	156	29734	KP974107	OL514027
21. <i>I. sp. 2</i> - <i>I. cantabriensis</i>	CO314, Pilar Cave, Peñamellera Baja, Asturias	43.29583	-4.53996	538	29755	OL679930	OL514020
22. <i>I. sp. 3</i> - <i>I. sp. nov. 2</i> (cryptic <i>I. imuniensis</i>)	CO314, Pilar Cave, Peñamellera Baja, Asturias	43.29583	-4.53996	538	29473	HQ659866	KC999728
23. <i>I. sp. 3</i> - <i>I. sp. nov. 2</i> (cryptic <i>I. imuniensis</i>)	CO069, Pozo del Agua Cave, Peñamellera Baja, Asturias	43.28383	-4.52318	374	29705	KC974079	KC999713
24. <i>I. sp. 4</i> - <i>I. cantabriensis</i>	CO314, Pilar Cave, Peñamellera Baja, Asturias	43.29583	-4.53996	538	54544	OL679935	OL514021
25. <i>I. sp. 6</i> - <i>I. sp. nov. 3</i> (cryptic <i>I. imuniensis</i>)	Astui Cave, Cestona, Vizcaya	43.21756	-2.18939	131	29978	KP974110	KP999710

TABLE 2 Specimens of Parabathynellidae Noodt, 1965 used in the phylogenetic analysis (COI+18S). (cont.)

Order/Species	Locality	Coordinates			Specimen voucher	GenBank accession number	
Bathynellacea		N	E	Z	MNCN/DNA	COI	18S
Chappuis, 1915							
26. <i>I. sp.- I. sp. nov.</i> 3 (cryptic <i>I.</i> <i>imuniensis</i>)	Mina Elvira, Galdames, Vizcaya	43.27623	-3.08539	511	29971	MZ128380	KP999708
27. <i>Paraiberobathynella</i> cf. <i>fagei</i>	Higuera Cave, Pliego, Murcia	37.97836	-1.50170	496	29672	KC469539	KP999735
28. <i>Paraiberobathynella</i> cf. <i>fagei</i>	Higuera Cave, Pliego, Murcia	37.97836	-1.50170	496	54550	OL679938	OL514022
**29. <i>Paraiberobathy-</i> <i>nella</i> cf. <i>fagei</i>	Pileta Cave, Benaolján, Málaga	36.69133	-5.26981	729	54587	KP974043	KC999742
**30. <i>Paraiberobathy-</i> <i>nella</i> <i>maghrebensis</i>	Well, Nador, Morocco	34.95238	-2.59795	276	29935	KC469542	KC469532
**31. <i>Texanobathynella</i> <i>aaronswinki</i>	*Sessom Creek, Hays County, Texas	29.88996	-97.93596	177	54862	MZ128381	MT822879
32. <i>Texanobathynella</i> <i>aaronswinki</i>	South Llano River, Kimble County, Texas	30.45342	-99.80060	520	54866	OL679945	OL514024
**33. <i>Texanobathynella</i> <i>coloradoensis</i>	*Colorado River, Bastrop County, Texas	30.11187	-97.32775	94	54867	MZ128382	MT874910
**34. <i>Montanabathy-</i> <i>nella pecosensis</i>	*Live Oak Creek, Crockett County, Texas	30.74220	-101.6739	655	54864	—	MT822881
**35. <i>Parabathynella</i> sp. (<i>I. cf. motasi</i>)	Poltarica Spring, Slovenia	45.88681	-14.76842	272	54669	MF443326	MF436211
**36. <i>Hexabathynella</i> <i>sevillaensis</i> (OUTGROUP)	*Santiago el Grande Cave, Constantina, Sevilla	38.03013	-5.90429	358	29545	HQ659869	KC469526

*Type locality; ** Specimens used in the combined analysis (morphological and molecular data)

and performance of each MCMC run were evaluated using Tracer 1.7.1, and the effective sample size (ESS) for each parameter was estimated. The results were summarized and annotated in a maximum clade credibility tree (MCC) generated in TreeAnnotator 1.8.4. (Drummond et al., 2012) with the first 25% of

trees discarded as burnin. All analyses were performed in the CIPRES Science Gateway cluster (Miller et al., 2010) using BEAGLE (Miller et al., 2010; Ayres et al., 2012).

A haplotype network for COI was reconstructed in POPART (Leigh & Bryant, 2015), which can convert a BI nexus file into a

haplotype genealogy (<http://datadryad.org/resource/doi:10.5061/dryad.4n4j1>).

Morphological analysis

For the morphological analysis, we studied specimens of Parabathynellidae that were previously collected, prepared and mounted by A.I. Camacho for more than 30 years and that were deposited in the Arthropod Collection (ARTP) of the MNCN in Madrid, Spain (Perina & Camacho, 2016; Camacho et al., 2017). We achieved the following specific objectives:

1. We reviewed the taxonomic literature on the Iberobathynellini tribe and provided an updated systematic account and comparison with the genera *Parabathynella* and *Montanabathynella* (the only other genera of the Parabathynellidae family present in the study area).
2. We analysed the main morphological features that define the Iberobathynellini tribe as currently understood (Camacho & Serban, 1998, 2000; Camacho, 2003a, b, 2005). Differences and similarities among subtribes, genera and subgenera are listed in table 3 (as were defined in the taxonomic reorganization of Camacho & Serban, 1998).
3. We compared the morphology of the eight European and North American genera (six of the Iberobathynellini tribe, *Parabathynella* and *Montanabathynella*) of Parabathynellidae to understand the extent of variability among its 39 species *Hexabathynella sevillaensis* Camacho, 2005 (the only cosmopolitan genus), was used as outgroup) (table 4). Drawings are included to facilitate the comparison.
4. We defined the morphospace of the Iberobathynellini tribe based on the character states of each species for 28

selected characters (table 5), and the phylogenetic relationships based on a matrix of morphological features (table 6) to assess the validity of taxonomic subcategories (subtribe and subgenus).

Species of Parabathynellidae show observable morphological variability. Characters used to distinguish genera can be observed after a full dissection and preparation of the body parts (Perina & Camacho, 2016). Most of the structures require examination under a compound microscope and/or a 100× oil immersion objective.

Variability among the genera, and differences and similarities among the subgenera (table 3) and species (table 4), of the Iberobathynellini tribe are illustrated through drawings of the antenna, mouthparts (labrum, mandibles, maxillule and maxilla), thoracopod I, thoracopod VIII of males and females, uropods and furcal rami of several representative species of the six genera (and five subgenera) under study and the two species of *Montanabathynella* and *Parabathynella* that have been included for comparison (figs. 9–17).

Abbreviations used

Morphology

AI	antennule
AII	antenna
Endp	endopod
Exp	exopod
Md	mandible
Mx.I	maxillule
Mx.II	maxilla
Sgt	segment
Symp	sympod
Th I–VIII	thoracopods I–VIII
Urp	uropod

TABLE 3 Distinctive taxonomic characters of the Iberobathynellini tribe. Iberobathynellina subtribe: *Iberobathynella* Schminke, 1973, subgenera *I. (Espanobathynella)*, *I. (Asturibathynella)* and *I. (Iberobathynella)*; *Guadalopebathynella* Camacho & Serban, 1998 and *Californibathynella* Camacho & Serban, 1998; Paraiberobathynellina subtribe: *Paraiberobathynella* Camacho & Serban, 1998, *Pi. (Paraiberobathynella)*, *Pi. (Orihuelabathynella)* subgenera and *Texanobathynella* Delamare Deboutteville, Coineau & Serban, 1975) genus; Hexaiberobathynellina subtribe: *Hexaiberobathynella* Camacho & Serban, 1998
Abbreviations: H = homonomous; IH = inhomonomous; N = normal; Npr = not pronounced; Pr = pronounced; sgt = segment

Subtribes	Iberobathynellina			Paraiberobathynellina		Hexaiberobathynellina	
Genera	<i>Iberobathynella</i>			<i>Paraiberobathynella</i>		<i>Hexaiberobathynella</i>	
Subgenera	<i>I. (A.)</i>	<i>I. (E.)</i>	<i>I. (L.)</i>	<i>Guadalopebathynella</i>	<i>Californibathynella</i>	<i>Texanobathynella</i>	<i>Hexaiberobathynella</i>
Number of thoracopods	7	7	7	7	7	7	6
Labrum: teeth	N	N	N	Hook-like	N	N	N
MxI: teeth distal endite	6	7	7	6 (hook-like)	7	7	6
MxII: setae segment 1	0-1	1	1	1	1	1	0
ThI: exopod	1 sgt	1-2 sgt	2 sgt	2 sgt	1 sgt	3 sgt	1 sgt
ThII-VII: exopod	2 sgt	2-3 sgt	2 sgt	2 sgt	2 sgt	3-4 sgt	2 sgt
Endopod:							
Plumose setae	1	1	2	1	1	1	1
Uropod: sympod	IH	IH-H	H	IH	IH	H	IH
Endopod (setae)	0-3	0-3	3	1	3	1	2
Exopod (setae)	3-5	4-6	5-8	5	4	5	4
Anal operculum	Pr-Npr	Pr-Npr	Npr	Npr	Pr	Npr	Pr

Acronyms

MNCN	Museo Nacional de Ciencias Naturales de Madrid (Spain)
CSIC	Consejo Superior de Investigaciones Científicas (Spain)
ARTP/MNCN	Arthropod Collection of the MNCN
MNCN/ADN	Tissues and DNA Collection of the MNCN.

There are currently 33 nominal species in three subtribes: Iberobathynellina with three genera and subgenera and 24 species; Paraiberobathynellina with two genera and subgenera and seven species and Hexaiberobathynellina with one genus and two species.

Tribe Iberobathynellini *Molecular data of specimens from the type locality and **molecular data of specimens from non-type localities.

Subtribe Iberobathynellina Camacho & Serban, 1998

Genus *Iberobathynella* Schminke, 1973

Subgenus *Iberobathynella* (*Asturibathynella*) Camacho & Serban, 1998

**I. (A.) asturiensis* Serban & Comas, 1978. Spain.

I. (A.) cavadoensis (Noodt & Galhano, 1969). Portugal and Spain.

***I. (A.) celiana* Camacho, 2003. Spain.

*& ***I. (A.) cornejoensis* Camacho, 2005. Spain.

I. (A.) guarenensis Camacho, 2003. Spain.

*& ***I. (A.) imuniensis* Camacho, 1987. Spain.

I. (A.) lamasonensis Camacho, 2005. Spain.

**I. (A.) ortizi* Camacho, 1989. Spain.

*& ***I. (A.) parasturiensis* Camacho & Serban, 1998. Spain.

I. (A.) rouchi Camacho & Serban, 1998. Spain.

I. (A.) serbani Camacho, 2003. Spain.

Subgenus *Iberobathynella* (*Espanobathynella*) Camacho & Serban, 1998

**I. (E.) andalusica* Camacho, 2007. Spain.

**I. (E.) burgalensis* Camacho, 2005. Spain.

***I. (E.) cantabriensis* Camacho & Serban, 1998. Spain.

**I. (E.) espaniensis* Serban & Comas, 1978. Spain.

*& ***I. (E.) magna* Camacho & Serban, 1998. Spain.

Subgenus *Iberobathynella* (*Iberobathynella*) Schminke, 1973

I. (I.) barcelensis (Galhano, 1970). Portugal.

I. (I.) gracilipes (Braga, 1960). Portugal.

I. (I.) lusitanica (Braga, 1949). Portugal.

I. (I.) paragracilipes Camacho & Serban, 1998. Spain.

I. (I.) valbonensis (Noodt & Galhano, 1969). Portugal and Spain.

I. pedroi Camacho, 2003. Spain.

Genus *Guadalopebathynella* Camacho & Serban, 1998.

G. puchi Camacho & Serban, 1998. Spain.

Genus *Californibathynella* Camacho & Serban, 1998.

C. californica (Schminke & Noodt, 1998). USA.

Subtribe Paraiberobathynellina Camacho & Serban, 1998

Genus *Paraiberobathynella* Camacho & Serban, 1998

Subgenus *Paraiberobathynella* (*Paraiberobathynella*) Camacho & Serban, 1998.

***Pi. (Pi.) fagei* (Delamare Deboutteville & Angelier, 1950). France, Spain.

***Pi. cf (Pi.) maghrebenensis* (Boutin & Coineau, 1987). Morocco.

Subgenus *Paraiberobathynella* (*Orihuelabathynella*) Camacho & Serban, 1998

Pi. (O.) notenboomi (Camacho, 1989). Spain.

TABLE 4

Taxonomic characters of the Iberobathynellini Tribe Camacho & Serban, 1995 and other species of the North American and European genera of the Parabathynellidae family. 1. *Iberobathynella asturiensis* Serban & Comas, 1978; 2. *I. parasturiensis* Camacho & Serban, 1998; 3. *I. cavadoensis* Noodt & Galhano, 1969; 4. *I. ortizi* Camacho, 1989; 5. *I. rouchi* Camacho & Coineau, 1987; 6. *I. imuniensis* Camacho, 1987; 7. *I. serbani* Camacho, 2003; 8. *I. guarenensis* Camacho, 2003; 9. *I. celiana* Camacho, 2003; 10. *I. lamasonensis* Camacho, 2005; 11. *I. cornejensis* Camacho, 2005; 12. *I. pedroi* Camacho, 2003; 13. *I. espaniensis* Serban & Comas, 1978; 14. *I. cantabriensis* Camacho & Serban, 1998; 15. *I. magna* Camacho & Serban, 1998; 16. *I. burgalensis* Camacho, 2005; 17. *I. andalusica* Camacho, 2007; 18. *I. gracilipes* (Braga, 1960); 19. *I. paragracilipes* Camacho & Serban, 1998; 20. *I. lusitanica* (Braga, 1949); 21. *I. valbonensis* (Noodt & Galhano, 1969); 22. *I. barcelensis* (Galhano, 1970); 23. *Hexaiberobathynella mateusi* (Galhano, 1967); 24. *Hi. hortezuelensis* Camacho & Serban, 1998; 25. *Guadalopebathynella puchi* Camacho & Serban, 1998; 26. *Californibathynella californica* (Schminke & Noodt, 1998; 27. *Paraiberobathynella fagei* (Delamare Deboutteville & Angeller, 1950); 28. *Pl. notenboomi* (Camacho, 1989); 29. *Pl. maghrebenis* (Boutin & Coineau, 1987); 30. *Texanobathynella bowmani* Delamare Deboutteville, Serban & Coineau, 1975; 31. *T. sachi* Cho, 1996; 32. *T. cf bowmani*; 33. *T. aaronswinki* Camacho & Hutchins, 2021; 34. *T. coloradoensis* Camacho & Hutchins, 2021; 35. *Montanobathynella pecosensis* Camacho & Hutchins, 2021; 36. *M. salish* Camacho, Stanford & Newell, 2009; 37. *Parabathynella notasi* Dancau & Serban, 1963; 38. *P. badenwuerttembergensis* Fuchs, Hahn & Cho, 2012; 39. *P. cf stygia*; 40. *Hexabathynella sevillensis* Camacho, 2005. Abbreviations: A = absent; AI = antennule; AIH = antenna; B = barbed; Endp = endopod; Exp = exopod; Frc = f urca; H = homonomous; IH = inhomonomous; Md = mandible; MxI = maxillule; MxII = maxilla; Npr = not pronounced; Not exc = does not exceed; P = plumose; Pr = pronounced; sgt = segment; S = smooth; Sm = small; Symp = sympod; ThI = thoracopod 1; ThVIII = thoracopod 8; Urp = uropod; W = wrinkled. * Hook shape.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
AI: segments	7	7	7	7	7	7	7	6	6	7	7	7	7	7	7	7	7
aesthetascs sgt 5	1	1	2	2	2	2	1	–	–	1	1	1	2	2	2	2	2
AIH: segments	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Labrum: teeth	8	8	8	8	8	8	8	10	8	8	8	8	8	8	9	8–9	8
Md: teeth																	
<i>pars incisiva</i>	5–7	4	5	5	7	6	5	6	–	5	4	6	4–5	5–6	5–8	7	8
<i>pars molaris</i>	7	5	7	6	7	7	6	5	–	6	5	6	5–8	6	7–9	6–8	6
Palp/distal end	Exceed	Not exc	Not exc	Exceed	Not exc	Not exc	Not exc	Not exc	–	Not exc	Not exc	Not exc	Exceed	Not exc	Not exc	Not exc	Not exc
MxI: teeth distal endite	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7
MxII: setae sgt 1	0	0	0	0	1	1	0	0	1	0	1	0	1	1	1	1	1
setae sgt 2	4	4	4	4	4	4	4	4	4	4	3?	4	4	4	4	4	4
setae sgt 3	14	14	15	14	16	14	15	15	15	15	14	15	16	15–16	16	15	15
ThI: Exp: sgt & (setae)	1(1+2)	1(1+2)	1(1+2)	1(1+2)	1(1+2)	1(1+2)	1(1+2)	1(1+2)	1(1+2)	1(1+2)	1(1+2)	1(1+2)	2(2+2)	1(1+2)	2(2+2)	2(2+2)	1(1+2)
Endp (setae)	1(1-2-2-3)	2(2-2-3)	1(1-2-2-3)	1(1-2-2-3)	2(2-2-2-3)	2(2-2-2-3)	1(1-2-2-3)	1(1-2-2-3)	2(2-2-2-3)	1(1-2-2-3)	2(2-2-2-3)	2(2-2-2-3)	2(2-2-2-3)	2(2-2-2-3)	2(2-2-2-3)	2(2-2-2-3)	2(2-2-2-3)
plumose setae	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

ThIII: epipod	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Exp: sgt & (setae)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)
Endp: plumose setae	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ThIII: epipod	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Exp: sgt & (setae)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)
ThIV: Exp: sgt & (setae)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	3(2-2-2)	2(2-2)	2(2-2)
ThV: Exp: sgt & (setae)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)
ThVI: Exp: sgt & (setae)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)
ThVII: Exp: sgt & (setae)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)
Female ThVIII: cuticle	w	w	s	s	w	s	s	w	S	S	s	s	s	s	s	W	s
Segments	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Teeth (first sgt)	2	2	2	2	2	2	2	2	-	2	2	2	2	2	2	1	2
Setae	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Male ThVIII:																	
Penial región: lobes	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Dentate lobe	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Small lobe	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Endp: setae	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Setae: inner lobe	P	A	A	A	A	A	A	A	A	A	A	P	A	A	A	A	A
Pleopod I	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Urp: sympod	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	H	H	H	IH
Spines	5-6 + 1	8-9 + 1	4-5 + 1	4 + 1	6 + 1	7 + 1	6 + 1	8 + 1	4 + 1	8 + 1	5 + 1	4 + 1	8-12 + 1	7-9	12-13	10-12	7-10 + 1
Exp setae	3B+1P	3B+1P	2B+1P	3B+1P	3B+1P	4B+1P	2B+1P	3B+1P	4B+1P	4B+1P	4B+1P	3B+1P	4-5B+1P	4B+1P	5B+1P	4B+OP	4B+1P
Endp setae	2B	2B	2B	2B	1B+1P+1S	1P	1P	2B	2B+1P	2B	o	2B	1P	1P	1P	o	2B+1P
Exp/endp	=	=	<	<	=	>or=	>or=	>or=	<	<	=	<	>or=	>or=	=	=	=
Furca: spines	8-9	7-8	6-9	7	8-10	6-7	6-7	8	8	7	5	5-6	8-9	7-8	10	7-8	7-10
Setae	1P+1B	1P+1P	1P+1P	1P+1B	1P+1S	1P+1P	1P+1P	1P+1P	1P+1P	1P+1P	1P+1P	1P+1P	1P+1P	1P+1P	1P+1P	1P+1P	1P+1P
Anal operculum	Pr	Pr	Pr	Pr	Npr	Npr	Pr	Pr	Pr	Pr sm	Pr	Pr	Npr	Npr	Npr	Npr	Pr
Minimum-maximum size	1.4-1.8	1.7	0.8-1.6	1.2-1.3	0.8-1.4	1.3-2.2	0.8-1	1.2-1.6	0.9	0.9-1.2	0.9-1.4	0.9-1.2	1.1-1.4	0.9-1.7	1.9	1.2-1.6	1.5-1.9

TABLE 4 (cont.)

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
AI: segments	7	7	7	7	7	7	7	7	7	7	7?	7	6	6	6	7	6
aesthetascs sgt 5	2	2	2	2	2	1	1	2	2	2	2	2	3	3	3	2	2
AIi: segments	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Labrum: teeth	8	8	8	8	8	8	8	8*	8	8	8	9–11	10	10	10	10	9
Md: teeth																	
<i>pars incisiva</i>	10	9–12	7	7–8	6	4–6	5–6	6	5	6–8	6–8	5–9	4	5	4	4–5	6
<i>pars molaris</i>	8–10	8–16	6–8	8–10	10	5–7	7–8	7	5	6–11	10	12	7	6	6	7–8	6
Palp/distal end	not exc	not exc	not exc	not exc	not exc	not exc	exceed	not exc	not exc	not exc	not exc	not exc	not exc	exceed	not exc	not exc	not exc
Mxi: teeth distal endite	7	7	7	7	7	6	6	6*	5+2*	7	7	7	5	5	5	5	5
MxII: setae sgt 1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1
setae sgt 2	4	4	4	4	4	4	4	4	4	3	3	3	–	4	2	3	3
setae sgt 3	16	16	n?–16	15 ² –16	16	14	15	14	16	16	16	15	–	14–15	13	13–14	13–14
ThI: Exp: sgt & (setae)	2(1+2-2)	2(1+2-2)	2(2-2)	2(1+2-2)	2(2-2)	1(1+2)	1(1+2)	2(2-2)	1(2)	3(2-2-2)	2(2-2)	3(2-2-2)	2(2-2)	1(1+2)	2(2-2)	2(2-2)	2(2-2)
Endp (setae)	(2-3-2-3)	(2-3-2-3)	(2-3-2-3)	(2-3-2-3)	(2-3-2-3)	(1-2-2-3)	(1-2-2-3)	(2-2-2-3)	(2-2-2-3)	(2-3-2-3)	(2-3-2-3)	(2-3-2-3)	–	(1-2-1-3)	(2-2-1-3)	(2-2-1-3)	(2-1-0-3)
plumose setae	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ThII: epipod	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Exp: sgt & (setae)	2(1+2-2)	2(1+2-2)	2(1+2-2)	2(1+2-2)	2(1+2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)
Endp: plumose setae	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1
ThIII: epipod	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Exp: sgt & (setae)	2(1+2-2)	2(1+2-2)	2(1+2-2)	2(1+2-2)	2(1+2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	4(2-2-2-2)	3(2-2-2)	4(2-2-2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)
ThIV: Exp: sgt & (setae)	2(1+2-2)	2(1+2-2)	2(1+2-2)	2(1+2-2)	2(1+2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	4(2-2-2-2)	3(2-2-2)	4(2-2-2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)
ThV: Exp: sgt & (setae)	2(1+2-2)	2(1+2-2)	2(1+2-2)	2(1+2-2)	2(1+2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	4(2-2-2-2)	3(2-2-2)	4(2-2-2-2)	3(2-2-2)	2(2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)
ThVI: Exp: sgt & (setae)	2(1+2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	4(2-2-2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)	2(2-2)	3(2-2-2)	2(2-2)	3(2-2-2)
ThVII: Exp: sgt & (setae)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	2(2-2)	0 (o)	0 (o)	2(2-2)	2(2-2)	3(2-2-2)	3(2-2-2)	3(2-2-2)	2(2-2)	2(2-2)	3(2-2-2)	2(2-2)	3(2-2-2)

Downloaded from Brill.com04/26/2023 03:32:19PM
via free access

TABLE 4 (cont.)

	35	36	37	38	39	40
AI: segments	7	7	7	7	7	6
aesthetascs sgt 5	2	2	2	2	1?	2
AII: segments	6	6	5	5	4?	5
Labrum: teeth	4 + 8+4	3 + 12 + 3	9	2-3 + 13 + 3-2	2 + 8+2	1 + 9+1
Md: teeth						
<i>pars incisiva</i>	4	5	4	4	4	4
<i>pars molaris</i>	6	7	4	8	4	6
Palp/distal end	Not exc	Not exc	Not exc	Not exc	Not exc	Not exc
MxI: teeth distal endite	8	9	5	9	6	4
MxII: setae sgt 1	2	2	1	2	2	2
setae sgt 2	5	4	4	5	2	3
setae sgt 3	19	21	12	20	14	13
ThI: Exp: sgt & (setae)	2 (1 + 2-2)	3(2-2-2)	2(2-2)	3(1 + 2-2- 2(2-2)	2(2-2)	1(1)
Endp (setae)	(3-3-3-3)	(4-4-3-3)	(1-2-2-3)	(4-4-4-3)	(1-3-1-3)	(1-1-1-2)
plumose setae	1	2	1	2	1	1
ThII: epipod	P	A	P	P	P	P
Exp: sgt & (setae)	3(1 + 2-2- 2)	5(10)	2(2-2)	5(10)	2(2-2)	2(2-1)
Endp: plumose setae	2	2	1	2	1	1
ThIII: epipod	P	A	P	P	P	P
Exp: sgt & (setae)	4(8)	6(12)	2(2-2)	6(12)	2(2-2)	2(2-1)
ThIV: Exp: sgt & (setae)	5(10)	6(12)	2(2-2)	7(14)	2(2-2)	2(2-1)
ThV: Exp: sgt & (setae)	5(10)	6(12)	2(2-2)	7(14)	2(2-2)	2(2-1)
ThVI: Exp: sgt & (setae)	5(10)	6(12)	2(2-2)	6(12)	2(2-2)	2(2-1)
ThVII: Exp: sgt & (setae)	3(6)	6(12)	2(2-2)	6(12)	2(2-2)	-

Female ThVIII: cuticle	S	S	S	S	S	S	S	S	S
Segments	2 dif.	2	2	2	2	2	2	1	1
Teeth (first sgt)	3+1	2+1	0?	2	0	0	2	2	2
Setae	2S+1P	3P	3S	3B	3S	3B	3S	0	0
Male ThVIII:									
Perianal región: lobes	–	4	3	3	3	3	3	3	3
Dentate lobe	–	P	A	A	A	A	P	P	P
Small lobe	–	P	P	P	P	P	P	A	A
Endp: setae	–	2	2	2	2	2	2	2	2
Setae: inner lobe	A	A	A	A	A	A	A	A	A
Pleopod I	A	A	A	A	A	A	A	P	P
Urp: sympod	1H	1H	1H	1H	1H	1H	1H	H	H
Spines	14+1	14+1	5-7+1	17+1	8+1	8+1	7	7	7
Exp setae	7B+1B	11B+1P	4B	8B+4S	4	4	3B	3B	3B
Endp setae	2B+1P+	2B+1P+	2P+1P+	2B+1P	4	4	2S	2S	2S
	1P	2P	1B						
Exp/endp	=	>	=	=	=	=	>	>	>
Furca: spines	11	14	5	7-8+2	4+2	4+2	3	3	3
Setae	1P+1P	1P+1P	1S+1S	1P+1P	1S+1P	1S+1P	1P+1P	1P+1P	1P+1P
Anal operculum	Npr	Pr sm	Pr sm	Npr	Npr	Npr	Pr L	Pr L	Pr L
Minimum-maximum size	–	2.4-3.0	0.7-1.4	2.3-2.4	1.1-2.5	1.1-2.5	0.7-1.2	0.7-1.2	0.7-1.2

Genus *Texanobathynella* Delamare Deboutteville, Coineau & Serban, 1975

T. bowmani Delamare Deboutteville, Coineau & Serban, 1975. USA.

T. sachi Cho, 1996. USA.

**T. aaronswinki* Camacho & Hutchins, 2021. USA.

**T. coloradoensis* Camacho & Hutchins, 2021. USA.

Subtribe Hexaiberobathynellina Camacho & Serban, 1998

Genus *Hexaiberobathynella* Camacho & Serban, 1998

***Hi. cf. mateusi* (Galhano, 1967). Portugal.

**Hi. hortezielensis* Camacho & Serban, 1998. Spain

Morphological phylogenetic inference and character coding

Our morphological matrix included 28 informative unordered and coded characters (code is provided in supplementary table S1) for all the 33 nominal species included within the tribe Iberobathynellini (100% of taxon sampling). The matrix (table 5) scored a wide variety of external characters, many of which have been previously used in phylogenetic inferences and also considered useful for delimiting species (Camacho & Serban, 2000; Camacho et al., 2000; Guil & Camacho, 2001). Missing characters are coded with a (?), and not applicable ones with (---). Fifteen characters were scored as discrete binary, and thirteen as multistate morphological characters (supplementary table S1). Following the results of Camacho et al. (2018a) and Camacho et al. (2021a) in order to analyse a more complete set of taxa, we also included representative species of the genera of Parabathynellidae present in Europe or North America, *Montanabathynella pecosensis* Camacho &

Hutchins, 2021; *Parabathynella motasi* Dancau & Serban, 1963. *Hexabathynella sevillaensis* was used as outgroup. The morphological matrix was analysed using the Mk model with variable coding and gamma-distributed rate heterogeneity (Lewis, 2001). The analysis was run in MrBayes v.3.2.6 (Ronquist et al., 2012), and consisted of two simultaneous chains of 100,000,000 generations each. A tree was sampled every 10,000 generations, resulting in 10,000 sampled trees. Convergence and mixing among chains were evaluated by checking the average standard deviation of split frequencies (<0.01) and the ESS values for every parameter (>200). A majority consensus tree for each analysis was reconstructed after discarding the first 25% of the sampled trees as burn-in. To calculate the posterior probability of each analysis, we constructed a 50% majority rule consensus tree from the remaining trees, and the percentage of times a clade occurred among the sampled trees was interpreted as its posterior probability.

We also explored the flexibility of the software RevBayes (Höhna et al., 2016) to relax and alleviate some of the assumptions of the Mk model (e.g., character state symmetry) and others sources of bias (e.g., ascertainment bias). For this, we applied the alternative priors suggested in the RevBayes tutorial (https://revbayes.github.io/tutorials/morph_tree/V2.html) to the morphological matrix. This analysis was run with a chain of length of 40 000 generations, and a maximum a posteriori tree was constructed after discarding the first 25% of sampled trees.

Definitions of the morphological character in the phylogenetic analysis

1. Number of AI segments: 6 or 7.
2. Teeth of labrum: normal (plain or concave) or hook like.
3. Number of teeth in distal endite of MxI: 4, 5, 6, 7 or 8.

Characters matrix of species of the Iberobathynellini tribe and of other European and North American genera of the Parabathynellidae family used for the morphological phylogenetic analysis. 1. *Iberobathynella asturiensis*; 2. *I. parasturiensis*; 3. *I. cavadoensis*; 4. *I. ortizi*; 5. *I. rouchi*; 6. *I. imunitensis*; 7. *I. serbani*; 8. *I. guarenensis*; 9. *I. celiana*; 10. *I. lamasonensis*; 11. *I. cornejoensis*; 12. *I. pedroi*; 13. *I. espani*; 14. *I. cantabriensis*; 15. *I. magna*; 16. *I. (burgalensis)*; 17. *I. lusitanica*; 18. *I. gracilipes*; 19. *I. paragracilipes*; 20. *I. valbonensis*; 22. *I. barcelensis*; 23. *Hexaiberoathynella mateusi*; 24. *Hi. hortezuelensis*; 25. *Guadalapebathynella puchi*; 26. *Californibathynella californica*; 27. *Paraiberoathynella fagei*; 28. *Pi. maghrebiensis*; 29. *Pi. notenboomii*; 30. *Texanobathynella bowmani*; 31. *T. sacht*; 32. *T. cf bowmani*; 33. *T. aaronswinkii*; 34. *T. coloradoensis*; 35. *Montanabathynella pecosensis*; 36. *Parabathynella motasi* (37 in Table 4) and 37. *Hexabathynella sevilaensis* (40 in Table 4) outgroup

[illegible]

TABLE 5

Characters matrix of species of the Iberobathynellini tribe and of other European and North American genera of the Parabathynellidae family used for the morphological phylogenetic analysis. 1. *Iberobathynella asturiensis*; 2. *I. parasturiensis*; 3. *I. cavadoensis*; 4. *I. ortizi*; 5. *I. rouchi*; 6. *I. imunitensis*; 7. *I. serbani*; 8. *I. guarenensis*; 9. *I. cellana*; 10. *I. lamasonensis*; 11. *I. cornejensis*; 12. *I. pedroi*; 13. *I. espaniense*; 14. *I. cantabriensis*; 15. *I. magna*; 16. *I. (burgalensis)*; 17. *I. andalusica*; 18. *I. gracilipes*; 19. *I. paragracilipes*; 20. *I. lusitanica*; 21. *I. valbonensis*; 22. *I. barcelensis*; 23. *Hexaiberoathynella mateusi*; 24. *- Hi. hortezuelensis*; 25. *- Guadalupeathynella puchi*; 26. *Californibathynella californica*; 27. *Paraiberoathynella fagei*; 28. *Pi. maghrebenensis*; 29. *Pi. notenboomi*; 30. *Texanobathynella bowmani*; 31. *T. sachi*; 32. *T. cf bowmani*; 33. *T. aaronswink*; 34. *T. coloradoensis*; 35. *Montanabathynella pecosensis*; 36. *Parabathynella motasi* (37 in Table 4) and 37. *Hexabathynella sevillensis* (40 in Table 4) outgroup (cont.)

Species																																						
Ch	1 *	2 *	3	4 *	5	6 *	7	8	9 *	10	11 *	12	13 *	14 *	15	16 *	17 *	18	19	20	21	22	23	24	25	26	27 *	28 *	29	30	31	32	33 *	34 *	35 *	36 *	37 *	
18	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
19	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	1	1	
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	0	
21	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0
22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
23	2	3	2	2	1	3	2	1	2	1	2	0	1	0	1	1	1	0	0	0	0	1	1	0	1	0	1	0	0	2	0	2	3	0	1	2		
24	1	1	1	0	2	2	1	0	1	3	1	2	2	2	3	0	0	0	0	0	0	1	1	2	0	2	2	2	0	0	0	0	0	0	0	0	0	1
25	0	1	1	0	2	2	1	1	3	0	3	2	2	2	0	0	0	0	0	3	1	3	1	3	0	1	1	0	1	0	0	0	0	0	0	0	2	
26	1	1	1	0	0	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	
27	1	1	0	1	2	0	1	0	1	0	1	0	1	1	1	1	1	2	2	2	2	2	1	1	1	0	2	2	2	?	1	?	?	?	1	0	0	
28	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	1	1	

Ch = Characters. *Species used in the combined analysis (species with available molecular data)

TABLE 6 A. Genetic divergence range (%) for COI within and between the genera and subgenera of the *Iberobathynellini* tribe and between these and *Parabathynella*. B. Genetic divergence range (%) for COI between different populations of *Iberobathynella imuniensis*

A.	1.	2	3	4	5
1. <i>I. (Asturibathynella)</i>	11.8–20.5				
2. <i>I. (Espanobathynella)</i>	14.8–19.7	9.3–20.8			
3. <i>Paraiberobathynella</i>	13.0–23.3	13.0–23.1	6.6–9.0		
4. <i>Texanobathynella</i>	16.3–22.5	16.0–22.8	16.4–22.5	12.3–17.2	
5. <i>Parabathynella</i>	18.5–21.4	16.7–21.8	15.4–19.6	21.1–21.9	0.00
B. <i>I. imuniensis</i>	*Morteros	Bernias	Lunada	V142	Becerral
* Morteros	0.2				
Bernias	0.8–1.0	0.0			
Lunada	6.8–7.2	6.8–7.0	0.2		
V142	6.4–6.6	6.4	0.8–1.0	0.0	
Becerral	1.0–1.2	0.2	7.0–7.2	6.6	0.0

* Type locality

4. Number of setae on first segment of MxII: 0, 1 or 2. 5. Thoracopods VII: absent or present. 6. Number of setae on first segment of endopod of ThI: 1, 2 or 3. 7. Number of smooth setae on second segment of endopod of ThI: 1, 2 or 3. 8. Number of setae on the third segment of endopod of ThI: 1, 2 or 3. 9. Number of segments of exopod of ThI: 1, 2 or 3. 10. Number of dorsal setae on first segment of endopod of ThII to VII: 0 or 1. 11. Number of setae on first segment of exopod of ThI: 1, 2 or 3. 12. Number of segments of exopod of ThII and III: 2 or 3 to 4. 13. Number of segments of exopod of ThIV: 2 or 3 to 5. 14. Number of segments of exopod of ThV: 2 or 3 to 5. 15. Number of segments of exopod of ThVI: 2 or 3 to 5.	16. Cuticle of female ThVIII: smooth or wrinkled. 17. Type of inner lobe on male ThVIII: with dense setae or with sparse setae or no setae. 18. Orientation of main axis of outer lobe of male ThVIII: vertical, inclined or curved. 19. Shape of lateral side of outer lobe of male ThVIII: triangular or rectangular. 20. Pleopods: present or absent. 21. Sympod of the uropod: homonomous or inhomonomous. 22. Ctenidia on exopod of uropods: present or absent. 23. Number of barbed setae on exopod of the uropod: 2, 3, 4 or 5 or more. 24. Number of setae on the endopod of the uropod: 0, 1, 2 or 3 or more. 25. Exopod length/endopod length of the uropod: =, <, < or =, or > or =. 26. Anal operculum: protruded or not protruded.

27. Maximum body size: small (<1.3 mm), medium (1.3–1.9 mm) or large (>1.9 mm).
28. Number of AII segments: 3, 5 or 6.

Combined analysis

A total-evidence analysis was performed by combining the morphological and molecular data. We reduced the terminals of the morphological matrix (table 5, species with *) to those samples for which COI and 18S sequences are available (table 2). The final matrix included 17 terminals (14 species belonging to the *Iberobathynella* group, plus *Montanabathynella pecosensis* and *Parabathynella cf. motasi* and, as outgroup, *Hexabathynella sevillaensis*) and a total of 2320 characters separated in three partitions. The main reason to choose *Hexabathynella* as outgroup is because it is morphologically quite distinct and, according to more complete molecular analysis (Camacho et al., 2021), it results quite different from the ingroup. Besides, *Hexabathynella* is a genus present throughout the world, (four species in North America and 10 in Europe, four in Iberian Peninsula), and has only six pairs of legs, such as *Hexaiberobathynella* (unfortunately we do not have complete COI and 18S sequences from *Hexaiberobathynella* to include in the analysis), all the other known genera in the world have seven pairs of legs. The phylogenetic reconstruction was performed in MrBayes as described above. The best substitution model of each partition was estimated by setting the command *lset nst = mixed*.

Ancestral state reconstructions

To explore the evolution of a subset of the selected characters, we reconstructed their ancestral states under a BI approach. Five characters of interest were selected: 1) the number of segments on the exopod of ThI (character 9); 2) the number of segments on the exopod of ThII–III (character 12); 3) the sculpture of the cuticle in females (character

16); 4) the number of AII segments (character 28) and 5) the number of segments on ThVIII in females (one segment in all tribe species and *Hexabathynella*, and two in *Montanabathynella* and *Parabathynella*). We used the ultrametric tree derived from the BEAST analyses for the reconstructions. A character matrix was assembled for each character based on the data matrix provided in table 4. Analyses were performed in RevBayes (Höhna et al., 2016) following the script provided in the tutorials (https://revbayes.github.io/tutorials/morph/morph_more.html), the Mk model with unequal rates was used for this reconstruction. Analyses were run with a chain of length of 40 000 generations. Results were summarized and plotted using the R package RevGadgets (<https://github.com/revbayes/RevGadgets>).

Results

Molecular results

Phylogenetic analysis. The ML and BI phylogenetic analyses of the two molecular datasets (COI and concatenated COI and 18S) recovered similar topologies. In the concatenated analysis, three main lineages were recovered (fig. 2). The first one (lineage I), comprising the species *Parabathynella cf. motasi* and *Montanabathynella pecosensis*, was highly supported in the ML analysis (ultrafast bootstrap (uBS) = 95). The second (lineage II) includes the three species of *Texanobathynella* (*T. coloradoensis*, *T. cf. bowmani* and *T. aaronswinki*), representing a North American lineage (posterior probabilities (PP) = 1, uBS = 100). The third one (lineage III), though only supported by the BI analysis (PP = 98), represents an Iberian–North African lineage within the *Iberobathynellini* tribe that is comprised of six main clades. The first clade (III.1) (PP = 1, uBS = 99) includes the Andalusian species

FIGURE 2 Bayesian phylogenetic tree based on the concatenated dataset [COI and 18S (2209 bp)] of Parabathynellidae species. Support for each node is provided by the posterior probabilities (PP) and ultrafast bootstrap support values (uBS) with black and grey boxes. Habitat: C = cave; R = river; S = spring; W = well. Spain provinces: BI = Vizcaya; BU = Burgos; CO = Córdoba; LU = Lugo; MA = Málaga; Ma: Morocco; MU = Murcia; O = Asturias; S = Cantabria; SE = Sevilla. Tx = Texas. SI = Slovenia.

I. celiana Viar River (Sevilla) and *I. andalusica* Varas River (Córdoba). The second clade (III.2) (PP = 1, uBS = 99) groups the north-western Iberian species *I. parasturiensis* (Cantabria-Asturias), *I. ortizi* Cintolo Cave (Lugo) and *I. espaniensis* and *I. asturiensis*, both from Infierno Cave (Asturias). The third clade (III.3) (PP = 1, uBS = 98) is comprised of

two specimens of a new species from Vizcaya, *Iberobathynella* sp. nov. 3, *I. burgalensis* OG, though this phylogenetic relationship was not supported. The fourth clade (III.4) (PP = 0.99, uBS = 81) contains two species: *I. cornejoensis* Redonda Cave (Burgos) and *I. imuniensis* (cave populations from Burgos and Cantabria). The fifth clade (III.5) (PP = 1, uBS = 100) includes the species of *Paraiberobathynella* from the southern Iberian Peninsula and Morocco: *Pi. cf fagei* Pileta Cave (Málaga), *Pi. maghrebensis* Nador well (Morocco) and *Pi. cf fagei* Higuera Cave (Murcia). The sixth clade (III.6) (PP = 0.97, uBS = 91) groups *I. cantabriensis* from cave populations in Asturias and Cantabria with two new species of *Iberobathynella*, *Iberobathynella* sp. nov. 1 from Cave CO314 (Asturias) and *Iberobathynella* sp. nov. 2 from Cave CO314 and CO069 (Asturias).

Bayesian analyses of the mtDNA data set (COI) revealed two main lineages within the Iberobathynellini tribe (fig. 3). The first lineage (I), not supported, consists of seven clades containing the species distributed throughout the Iberian Peninsula, and the second (II) of the species from North America. The North American lineage (PP = 1; uBS = 96) that includes the three species of *Texanobathynella* was recovered as the basal clade of the Iberobathynellini tribe. The phylogenetic relationships within the Iberobathynellini tribe were not well-supported based on this data set, however, seven clades were recovered. The relationships of two of the species (*I. burgalensis* and *I. magna*) were not supported. The first clade (2.1) (PP = 1; uBS = 96) includes four species from the northwestern Iberian Peninsula: *I. ortizi* (dark blue) from Lugo (Rei Cintolo Cave); *I. asturiensis* (green) and *I. espaniensis* (garnet) from Asturias (Infierno Cave) and *I. parasturiensis* (light blue) from Cantabria–Asturias. According to the haplotype network, *I. parasturiensis* has the most divergent alleles within this clade. This

species is found in sympatry with *I. asturiensis* and *I. espaniensis* in Infierno Cave. The second clade (2.2) (PP = 1, uBS = 100) includes two specimens of the new species *Iberobathynella* sp. nov. 3 from the caves Astui (purple) and Fonda (orange) (in Vizcaya). The third clade (2.3) (PP = 1, uBS = 95) groups *I. cornejoensis* (dark blue) from Redonda Cave (Burgos) with the more widely distributed *I. imuniensis* from Cantabria (Calleja-Cueto Cave) (purple) and Burgos [V142 and Lunada caves (yellow) and Torca de los Morteros and Bernias caves (red)]. The fourth clade (2.4) (PP = 1, uBS = 97), whose phylogenetic position represents a major difference between the two datasets (mitochondrial vs concatenated), includes *I. andalusica* from Córdoba (Varas River) (blue) and *I. celiana* from Sevilla (Viar Stream, Castiblanco) (green). The fifth clade (2.5) includes the new species *Iberobathynella* sp. nov. 1 from Cave CO314 (red) and *Iberobathynella* sp. nov. 2 from caves CO314/CO069 (orange and purple), which live in sympatry in Cave CO314 (Pilar Cave, Asturias). The sixth clade (2.6) (PP = 1, uBS = 100) only includes the species *I. cantabriensis* from Asturias (Cave CO314; yellow, green and blue) and Cantabria [Cave CO276 (Lobos Cave); purple and CO220 (Carnero Spring Cave); blue]. In the seventh clade (2.7) (PP = 1, uBS = 100), *Pi. cf fagei* from Málaga (Pileta Cave; red) resolved as the sister species of *Pi. maghrebensis* from Morocco (Nador; green) and *Pi. cf fagei* from Murcia (Higuera Cave; light blue) in the southeastern Iberian Peninsula.

Genetic divergence. As the nuclear 18S gene is highly conserved in the species of the Iberobathynellini tribe, genetic divergence (uncorrected p distances calculated using PAUP*4.0b 10) (Swofford, 2002)) between genera, subgenera and species and between these and *Parabathynella*, the other European genus, was only assessed using COI (table 6 and supplementary table S2). Saturation is

FIGURE 3 Bayesian phylogenetic tree based on COI sequences (507 bp) of Parabathynellidae species. Support for each node is provided by the posterior probabilities (PP) and ultrafast bootstrap support values (uBS) with black and grey boxes.

generally observed between genera; however, the results are informative for discussing divergences among species. Base pair changes are observed in the following regions: 163–170; 270–82; 780–794; 1234–1282; 1352–1373; 1401–1432; 1467–1475 and 1578–1586.

The estimated COI divergence between species of *Iberobathynella* is highly variable, ranging from 11.8% to 20.8%. With respect to divergence between populations, nearly the same level of divergence is observed between nearby populations of some of the same species as between those of different genera. For instance, a divergence of 10.0% is observed between two specimens morphologically identified as *I. (E.) cantabriensis* from two nearby populations, Carnero Spring (CO220) and Lobos Cave (CO276), both in Cantabria. This is similar to the divergence observed between two specimens morphologically identified as belonging to different genera (which present strong morphological differences) from two geographically distant populations, i.e., *I. (Asturibathynella) parasturiensis* (from Infierno Cave in northern Spain) and *Paraiberobathynella (Pi.) maghrebensis* (from northern Morocco), which diverge by 13.0%.

Divergence time analysis. The time divergence analysis (fig. 4) reveals a Cretaceous TMRCA of the Parabathynellidae clade, and the divergence of Palearctic from Nearctic species of Iberobathynellini tribe around 76.8 Mya (95% HPD: 57.2–98.6 Mya). The timing of these early divergences agrees with previous results (Camacho et al., 2018b, 2021a; Perina et al., 2019). The TMRCA of the *Iberobathynella* and *Paraiberobathynella* genera dates to the beginning of the Paleogene around 63.7 Mya (95% HPD: 48.2–81.9 Mya), with the main splits within this group occurring during the Middle Eocene [*I. celiana* – *I. andalusica*, 40.8 Mya (95% HPD: 26.9–56.6 Mya); *I. imuniensis*/*I. cornejoensis* – *I. sp. nov.3*/*I. burgalensis*, 39.8 Mya (95% HPD: 28.9–52.8 Mya); *Paraiberobathynella* species – *I.*

cantabriensis – *I. sp. nov. 1*/ *sp. nov. 2*, 47.6 Mya (95% HPD: 35–62 Mya) and *I. parasturiensis*/*I. ortizi* – *I. asturiensis*/*I. espaniensis*, 41.8 Mya (95% HPD: 29.3–55.1 Mya)].

Morphological phylogenetic analysis. The two consensus trees obtained with MrBayes and RevBayes derived from the morphological dataset present the same topology (fig. 5), and show, despite the high support ($PP > 0.95$, as suggested by Alfaro et al., 2003), that Iberobathynellini tribe ($PP = 1$) are not monophyletic. The trees show that phylogenetic relationships amongst the taxa are not fully resolved. Species belonging to *Hexaiberobathynella* group together in a well-supported clade ($PP = 0.96$), suggesting the monophyly of this genus. Intriguingly, none of the currently recognized subgenera of *Iberobathynella* are recovered as a monophyletic group, except for the nominal subgenus *Iberobathynella (Iberobathynella)*, whose five species form a supported clade ($PP = 99$). Species of the genera *Paraiberobathynella* and *Texanobathynella* form a clade, which also includes *Montanabathynella pecosensis*, although support for these relationships is low.

Combined results

The phylogenetic hypothesis derived from the combined analysis of the morphological and molecular datasets (fig. 6), which only included the morphologically defined species, yielded almost the same topology as the tree obtained from the concatenated molecular analyses (compare fig. 6 with fig. 2), with the exception of the phylogenetic position of *I. burgalensis*. In this analysis, *I. burgalensis* resolves as the sister taxon of *I. cantabriensis*; however, this relationship is unsupported.

Ancestral state reconstruction

Bayesian reconstructions of the ancestral states of some characters are illustrated in figs. 7 and 8. All ancestral state reconstruction

FIGURE 4 Temporal estimates derived from a relaxed molecular clock analysis. Divergence times are depicted in the coalescence-based MCC tree. Units of X-axis address Mya and blue bars at nodes indicate the 95% highest posterior density intervals (HPD).

FIGURE 5 Bayesian phylogenetic tree based on the morphological dataset. Support for each node is provided by the posterior probabilities (PP).

analyses reached convergence, with trace-plots showing adequate mixing and ESS values larger than 200 for all parameters. The results for each character are as follows:

1. *Number of aii segments* (Ch. 28) (fig. 7A): The reconstruction indicates three segments as the ancestral condition of

Iberobathynellini, albeit with low statistical support ($PP = 0.5$), and only one transition event, leading to five (*Parabathynella cf motasi*) or six (*Montanabathynella pecosensis*) segments.

2. *Number of segments of the exopod of ThII-iii* (Ch. 12) (fig. 7B): A ThII-III

FIGURE 6 Phylogenetic tree from the combined analyses (morphological and molecular dataset). Support for each node is provided by the posterior probabilities (PP).

exopod with three to four segments was reconstructed as the ancestral state of Iberobathynellini (PP = 0.5). Our results suggest that the presence of only two segments evolved independently at least

three times during the tribe's evolutionary history.

3. *Number of segments of the exopod of ThI* (Ch. 9) (fig. 7C): A ThI exopod with a single segment was reconstructed as the

FIGURE 7 Ancestral State Reconstruction of: (A) Number of segments of AII (Ch.28); (B) Number of segments of exopod of ThII-III (Ch. 12) and (C) Number of segments of exopod of ThI (Ch. 9). Support for each node is provided by the size of circles indicating the posterior probabilities (PP). Coloured box depicted the state of the character.

ancestral state, however with low statistical support (PP = 0.5). In this reconstruction, the presence of two segments evolved independently at least seven times. The presence of three segments evolved once in the lineage leading to the *Paraiberobathynella* clade. In this clade, only *Pi. maghrebenensis* presents two segments, representing one of the

seven transitions to a two-segmented exopod of ThI.

4. *Sculpture of the cuticle in females* (Ch. 16) (fig. 8A): A smooth cuticle in females of the *Iberobathynellini* lineage was reconstructed as the ancestral state (PP = 0.8), with the wrinkled state evolving independently three times, and no reversal events.

FIGURE 8 Ancestral State Reconstruction of (A) Cuticle of female Th VIII (Ch.16) and (B) Number of segments female ThVIII. Support for each node is provided by the size of circles indicating the posterior probabilities (PP). Coloured box depicted the state of the character.

5. *Number of ThVIII segments in females* (fig. 8B): A ThVIII with a single segment appears to be the ancestral condition of the tribe (PP = 0.70). A two-segmented ThVIII evolved in the most recent common ancestor of *Parabathynella cf motasi* and *Montanabathynella pecosensis*.

Morphological results

Systematic account. In this section, a morphological diagnosis of the Iberobathynellini tribe Camacho & Serban, 1998 is provided, and some general considerations regarding previously established lower categories are discussed. Moreover, the relationships of genera within the tribe based on morphological characters are contrasted with the molecular data.

The distribution of the studied species is shown in fig. 1, and the list of the species and populations studied, including information on their habitat and locality, is provided in supplementary appendix S2. Updated diagnoses of rankings higher than species within the Iberobathynellini tribe are detailed in

supplementary appendix S3. These updated morphological diagnoses highlight characters distinctive to the tribe or its genera (table 3) and provide a better understanding of the hierarchy of the taxa within the tribe.

Tribe Iberobathynellini Camacho & Serban, 1998

Diagnosis (after Camacho et al., 2000): AI seven-segmented, very rarely six-segmented. AII three-segmented (fig. 9A-J), small and with four setae on the last segment. Common or special structure (hook-like teeth) (fig. 9S) on labrum, usually with eight main teeth. Strongly inclined connection of the mandible with the cephalon. Usually seven pairs of thoracopods, thoracopod I with a one-, two- or three-segmented exopod (fig. 13A-K) and the rest of the thoracopods with a two, three or four-segmented exopod; coxa of thoracopods with distinct conical projection at inner distal corner; thoracopod I without epipod. Male thoracopod VIII of type “*Iberobathynella*” (fig. 15A-K): large outer lobe, partially fused with basipod; small exopod on latero-external

side of basipod, covered by outer lobe; endopod always present with distal end oriented towards the latero-external side. Pleopods usually absent.

In the Iberobathynellini tribe, the outer lobe of the male thoracopod VIII is well developed in both length and width, and is highly distinctive as a massive element of the lateral face always close to the endopod, in some case covering the distal part of the basipod, a very marked feature in *I. rouchi*; the main axis of the lobe can be vertical to inclined on the postero-distal or antero-distal side; its fusion with the basipod (Serban, 1977) is at the level of the basal parts of the posterior edge of the lobe and the anterior face of the basipod; the exopod is greatly reduced. In Iberobathynellini, the outer lobe (O.lb) is small; in *Parabathynella* (fig. 15M), it is narrow and slightly longer than the exopod and the outer lobe is not fused with the basipod. The exopod is located on the lateral face of the basipod and is covered by the distal part of the outer lobe, however, its small size makes it the most difficult structure to observe in species of the Iberobathynellini tribe.

The division of the tribe into three subtribes (Camacho & Serban, 1998) is based on the diversification of the thoracopods at the exopod level in Iberobathynellina and Paraiberobathynellina, on the different number of thoracopods in Hexaiberobathynellina (table 3), and to organize the taxonomy of the morphogroup and to understand the relationships between the genera.

- Type genus: *Iberobathynella* Schminke, 1973
- Subtribe: Iberobathynellina Camacho & Serban, 1998
 - Paraiberobathynellina Camacho & Serban, 1998
 - Hexaiberobathynellina Camacho & Serban, 1998

The main morphological differences identified among the three subtribes and six genera

(and subgenera) in 1998 are shown in table 3. Morphospace was organized by fitting all known species into the three subtribes and established subgenera.

The number of thoracopods clearly separated the subtribes Iberobathynellina and Paraiberobathynellina from Hexaiberobathynellina, which have seven and six, respectively. More than two segments on the exopod of thoracopods II to VII are only found in the genera of the subtribe Paraiberobathynellina (*Paraiberobathynella* and *Texanobathynella*) and in *I. (E.) magna* (in thoracopods IV and V). The number of teeth in the distal endite of MxI also distinguishes the genera: five in *Texanobathynella*, six in *Hexaiberobathynella* and *Guadalopebathynella*, seven in *Californibathynella* and *Paraiberobathynella*, and in the subgenera of *Iberobathynella*, seven in *Iberobathynella* and *Espanobathynella* and six in *Asturibathynella*. *Guadalopebathynella* shares many character states with the subgenus *Asturibathynella*, but its curved (hook-like) teeth in both the labrum and distal endite of MxI clearly distinguish *G. puchi* from *I. (A.) imuniensis*, with which it is most similar. Other characters are not exclusive to a single subgenus or genus; however, the set of characters allowed the identification of the nine morphotypes corresponding to the six genera and five subgenera (see table 3). But as new species were found, the characters overlapped between subtribes and subgenera and some species such as *I. pedroi* Camacho, 2003 no longer fit into any of the 3 subgenera of *Iberobathynella* (table 4).

Comparative morphological analysis of the Iberobathynellini and non-Iberobathynellini genera from the study area

Table 4 lists the set of character states exhibited by the 33 morphotypes of the Iberobathynellini tribe, and the five species

of *Montanabathynella* and *Parabathynella*, included in the present study. This list highlights the level of variability in the main morphological characters often used to discriminate taxa in taxonomic studies of this group of crustaceans. Below, we briefly review the main characters and discuss the distribution of variability among the nine taxa (genera and subgenera). Of the species of *Iberobathynella*, 21 can be placed into one of the three morphologically established subgenera. However, the species *I. pedroi* displays character states present in the subgenera *Asturibathynella* and *Espanobathynella* and is therefore compared separately.

Species belonging to the Iberobathynellini tribe vary considerably in size, with some barely reaching or exceeding one millimetre: for instance, *I. celiana* measures 0.9 mm; *I. serbani*, 1.0 mm; *I. lamasonensis* and *I. pedroi*, 1.2 mm and *Californibathynella californica*, 1.1 mm. Some species of the subgenera *Asturibathynella* and *Espanobathynella* can reach 2.2 mm and 1.9 mm, respectively. *Paraiberobathynella fagei* and *Pi. notenboomi* can reach 2.8 mm, while the species *I. gracilipes* and *I. paragracilipes*, both from the subgenus *Iberobathynella*, are some of the largest, with some specimens measuring up to 4.0 mm or 3.2 mm, respectively. Large species are also found in *Montanabathynella*, such as *M. salish* Camacho, Newell & Stanford, 2009, which can measure up to 3.0 mm. Finally, some species of *Parabathynella* can measure 2.5 mm.

The variability in the selected morphological structures are described below and illustrated in figs. 9 to 17.

Antennule: Seven-segmented A1 characterizes most species of the tribe (28 species). Two species of *Iberobathynella* (*I. celiana* and *I. guarenensis*) and four of *Texanobathynella* present a six-segmented A1. The five species comprising the genera *Montanabathynella*

and *Parabathynella* also have a seven-segmented A1. The number of setae and aesthetascs on the segments varies among the genera and species.

Antenna (fig. 9): All species of the tribe have three segments (fig. 9A-J) and only setae (four) on the last segment; segment length may vary slightly between species. *Montanabathynella* (fig. 9K) has six segments while *Parabathynella* has five (fig. 9L).

Labrum (fig. 9M-X): The labrum presents as relatively flat (fig. 9M, N, R, T, U), concave (fig. 9O, P, Q, V) or convex (fig. 9S). Most species have eight main teeth on the labrum except *I. guarenensis* (10 teeth); *I. magna* (9 teeth); *Pi. notenboomi* (11 teeth) (fig. 9U) and those of *Texanobathynella* (9 or 10 teeth) (fig. 9V). The labrum in *Guadalopecathynella* is convex (fig. 9S), and has eight hook-like shaped teeth. Compared with the other species, the number of main teeth varies more in *Montanabathynella* (8 to 12 teeth; fig. 9W) and *Parabathynella* (8 to 13 teeth; fig. 9X).

Mandible (fig. 10): The number of teeth in the *pars incisive* varies between four and 12 in *Iberobathynella*: six to 12 in the subgenus *Iberobathynella* (fig. 10F), four to seven in *Asturibathynella* (fig. 10A, B) and four to eight in *Espanobathynella* (fig. 10D, E); *I. pedroi* has six teeth (fig. 10C). Fewer teeth characterize the other genera: *Hexaiberobathynella* has four to six (fig. 10H), *Californibathynella* has five (fig. 10J), *Paraiberobathynella* has six to nine, despite having very large specimens (fig. 10G), and *Texanobathynella* has four to six (fig. 10K). *Montanabathynella* (fig. 10L) and *Parabathynella* (fig. 10M) have only four teeth except *M. salish*, which has five. In the *pars molaris*, the number of teeth is highly variable among species of the tribe, ranging between five and 16; teeth features also varies: they can be with or without denticles, have the two most proximal teeth always joined and have fine setules. Species with the most teeth

FIGURE 9 Antenna (AII) A-L: A. *Iberobathynella lamasonensis*; B. *I. burgalensis*; C. *I. andalusica*; D. *I. paragracilipes*; E. *Guadalopebathynella puchi*; F. *Californibathynella californica*; G. *Hexaiberobathynella hortezuelensis*; H. *Paraiberobathynella notenboomii*; I. *Texanobathynella aaronswinki*; J. *I. pedroi*; K. *Montanabathynella pecosensis*; L. *Parabathynella motasi*. Labrum M-X: M. *I. burgalensis*; N. *I. andalusica*; O. *I. cornejoensis*; P. *I. pedroi*; Q. *I. paragracilipes*; R. *Hexaiberobathynella hortezuelensis*; S. *Guadalopebathynella puchi*; T. *Californibathynella californica*; U. *Paraiberobathynella notenboomii*; V. *Texanobathynella aaronswinki*; W. *Montanabathynella pecosensis*; X. *Parabathynella motasi*.

FIGURE 10 Mandible (Md): A. *Iberobathynella cornejoensis*; B. *I. lamasonensis*; E. *I. pedroi*; D. *I. andalusica*; E. *I. burgalensis*; F. *I. paragracilipes*; G. *Paraiberobathynella notenboomi*; H. *Hexaiberobathynella hortezuelensis*; I. *Guadalopebathynella puchi*; J. *Californibathynella californica*; K. *Texanobathynella bowmani*; L. *Texanobathynella aaronswinki*; M. *Montanabathynella pecosensis*; N. *Parabathynella motasi*.

FIGURE 11 Maxillule (MxI): A. *Iberobathynella lamasonensis*; B. *I. cornejoensis*; C. *I. pedroi*; D *I. andalusica*; E. *I. burgalensis*; F. *I. paragracilipes*; G. *Paraiberobathynella notenboomi*; H. *Hexaiberobathynella mateusi*; I. *Guadalopebathynella puchi*; J. *Californibathynella californica*; K. *Texanobathynella aaronswinki*; L. *Parabathynella motasi*; M. *Montanabathynella pecosensis*.

belong to the subgenera *Iberobathynella* (6 to 16 teeth) (fig. 10F) and *Paraiberobathynella* (6 to 12 teeth) (fig. 10G); *Montanabathynella* has six or seven teeth (fig. 10M), and *P. motasi* has

only four (fig. 10N), whereas *P. badenwuerttembergensis* has eight. All species have a small tooth on the ventral edge between both parts that is variable in size, more or less triangular

FIGURE 12 Maxilla (MxII): A. *Iberobathynella burgalensis*; B. *I. cornejoensis*; C. *I. lamasonensis*; D. *I. andalusica*; E. *I. paragraticipes*; F. *Hexaiberobathynella hortezuelensis*; G. *Paraiberobathynella notenboomii*; H. *Guadalopebathynella puchi*; I. *Californibathynella californica*; J. *Texanobathynella aaronswinki*; K. *Montanabathynella pecosensis*; L. *Parabathynella motasi*.

and, in some cases, with small setae at the base (fig. 10D, F, H). The mandibular palp consists of one segment that is variable in size, with a single seta that either reaches the distal edge of the mandible (fig. 10D, F, J, K), surpasses it (in five species only), as in *P. motasi* (fig. 10N), or does not even reach it (fig. 10G).

Maxillule (fig. 11): In the proximal endite, all species have four claws (spine-like) of different sizes. The number of teeth in the distal endite varies between six and seven in *Iberobathynella* but is constant in all species of each subgenus: six teeth in *Asturibathynella* (fig. 11A, B) and seven in *Espanobathynella*

FIGURE 13 Thoracopod 1: A. *Iberobathynella lamasonensis*; B. *I. cornejoensis*; C. *Iberobathynella burgalensis*; D. *I. andalusica*; E. *I. paragracilipes*; F. *Hexaiberobathynella hortezuelensis*; G. *Paraiberobathynella notenboomi*; H. *I. pedroi*; I. *Guadalopebathynella puchi*; J. *Californibathynella californica*; K. *Texanobathynella aaronswinki*; L. *Montanabathynella pecosensis*; M. *Parabathynella motasi*.

FIGURE 14 Female Th VIII: A. *Iberobathynella asturiensis*; B. *I. parasturiensis*; C. *I. cavadoensis*; D. *I. ortizi*; E. *I. rouchi*; F. *I. imuniensis*; G. *I. serbani*; H. *I. guarenensis*; I. *I. lamasonensis*; J. *I. cornejoensis*; K. *I. pedroi*; L. *I. espaniensis*; M. *I. cantabriensis*; N. *I. magna*; O. *I. burgalensis*; P. *I. andalusica*; Q. *I. paragrácilipes*; R. *I. lusitanica*; S. *I. valbonensis*; T. *I. barcelensis*; U. *Hexaiberobathynella hortezuelensis*; V. *Hi. mateusi*; W. *Guadalopebathynella puchi*; X. *Californibathynella californica*; Y. *Paraiberobathynella fagei*; Z. *P. notenboomi*; AA. *Texanobathynella sachi*; BB. *Texanobathynella aaronswinki*; CC. *Montanabathynella salish*; DD. *M. pecosensis*; EE. *Parabathynella motasi*.

FIGURE 15 Male Th VIII: A. *Iberobathynella burgalensis*; B. *I. lamasonensis*; C. *I. cornejoensis*; D. *I. andalusica*; E. *I. pedroi*; F. *Californibathynella californica*; G. *I. paragracilipes*; H. *Hexaiberobathynella hortezuelensis*; I. *Paraiberobathynella notenboomi*; J. *Guadalopebathynella puchi*; K. *Texanobathynella bowmani*. L. *Montanabathynella pecosensis*; M. *Parabathynella motasi*. Abbreviations: Bsp, basipod; D.lb, dentate lobe; Endp, endopod; Exp, exopod; I.lb, inner lobe; O.lb, outer lobe.

FIGURE 16 Uropod: A. *Iberobathynella andalusica*; B. *I. burgalensis*; C. *I. cornejoensis*; D. *I. lamasonensis*; E. *I. pedroi*; F. *Texanobathynella bowmani*; G. *I. paragracilipes*; H. *Guadalopebathynella puchi*; I. *Paraiberobathynella notenboomi*; J. *Hexaiberobathynella mateusi*; K. *Californibathynella californica*; L. *Montanabathynella pecosensis*; M. *Parabathynella motasi*.

(fig. 11D, E) and *Iberobathynella* (fig. 11F), and also in *I. pedroi* (fig. 11C). *Paraiberobathynella* and *Californibathynella* each have seven teeth (fig. 11G, J); *Hexaiberobathynella* and

Guadalopebathynella have six (fig. 11H, I); *Texanobathynella* has five (fig. 11K); *Montanabathynella* has eight to nine (fig. 11M) and *Parabathynella* has five (as in *P. motasi*,

FIGURE 17 Furcal rami: A. *Iberobathynella cornejoensis*; B. *I. lamasonensis*; C. *I. andalusica*; D. *I. burgalensis*; E. *I. paragracilipes*; F. *I. pedroi*; G. *Guadalopebathynella puchi*; H. *Hexaiberobathynella mateusi*; I. *Paraiberobathynella notenboomi*; J. *Californibathynella californica*; K. *Texanobathynella bowmani*; L. *T. aaronswinki*; M. *Montanabathynella pecosensis*; N. *Parabathynella motasi*.

fig. 11L) to six or nine. The three subterminal smooth setae on the outer distal margin, common in the family Parabathynellidae, are of different lengths, which differ among the species of the tribe.

Maxilla (fig. 12): The three segments comprising maxilla differ in size in the different species, however, the third segment is always the longest, and the second, the shortest. In the proximal segment, most species of the

tribe present a single seta (fig. 12A, B, D, E, F-I, J); the two species of *Hexaiberobathynella* lack setae (fig. 12H), as do the species *I. asturiensis*, *I. parasturiensis*, *I. cavadoensis*, *I. ortizi*, *I. lamassonensis* (fig. 12C), *I. serbani* and *I. guarenensis*, all of the subgenus *Asturibathynella*, and *I. pedroi*. The two species of *Montanabathynella* have two setae (fig. 12K), as do the species of *Parabathynella* except *P. motasi* (fig. 12L), which has one. In the second segment, species of *Iberobathynella* have four setae (fig. 12A-D), as do those of *Guadalopebathynella* (fig. 12H), *Hexaiberobathynella* (fig. 12F) and *Californibathynella* (fig. 12I), whereas *Paraiberobathynella* has three (fig. 12G); *Texanobathynella* has two, three or four (fig. 12J); *Montanabathynella* has four or five (fig. 12K) and *Parabathynella* has two, four or five (fig. 12L). The number of setae in the third segment of MxII (or third and fourth together) varies very little, between 14 and 16, among genera and species of the tribe (fig. 12A-J) (some specimens of different species of *Texanobathynella* present 13 setae). *Montanabathynella* presents between 19 and 21 setae (fig. 12K), and *P. motasi* (fig. 12L) and *P. stygia* Chappuis, 1926 have 12 and 14, respectively, but *P. badenwuerttembergensis* Fuchs, Hahn & Cho, 2012 has 20.

Thoracopod I (fig. 13): All species of the tribe lack an epipod on this leg (fig. 13A-K), as do the species in the genera *Montanabathynella* (fig. 13I) and *Parabathynella* (fig. 13M). The basipod has a smooth seta in the distal outer margin. The exopod may have one segment (in 18 species) (fig. 13A, B, D, F, H, J), two segments (fig. 13C, E, H, I, K), (in 14 species) or, in the case of two species of the *Paraiberobathynella* (fig. 13G) three segments. Irrespective of segment number, the exopod is always shorter than the endopod. Normally, setation consists of three setae in the first or single segment (except in *Californibathynella*, which only has two distal setae in its single segment) (fig. 13J) and two terminal setae (of which, one is always

plumose) in the second and, when present, third segments (fig. 13G); clusters of ctenidia present at the base of setae and on the posterior side (fig. 13C, E, G). Endopod consists of four segments of different sizes: the first is about half the length of the second and third, which are similar in length, and the fourth is very small; on the fourth segment, species of the Iberobathynellini tribe and those in the genera *Montanabathynella* and *Parabathynella* all present two terminal (claw-like) setae of different lengths and a shorter subterminal seta; on the third segment all genera have two distal setae (except *Texanobathynella*, which has only one or no internal seta; fig. 13K), the internal one resembles to a small spine; on the first and second segments, the setation is variable, with different species sharing setal formulas: the formula (1-2-2-3) is shown by some species of the subgenus *Asturibathynella* (fig. 13A) and by *Hexaiberobathynella* (fig. 13F); (2-2-2-3), by species of the subgenera *Asturibathynella* (fig. 13B) and *Espanobathynella* (fig. 13C) and by *Guadalopebathynella* (fig. 13I) and *Californibathynella* (fig. 13J); (2-3-2-3), by *Iberobathynella* (fig. 13E) and some species of the subgenera *Espanobathynella* (fig. 13D) and *Paraiberobathynella* (fig. 13G); in *Texanobathynella*, there are three different combinations among the five species: (1-2-1-3) (fig. 13K), (2-2-1-3) and (2-1-0-3). *Montanabathynella* (fig. 13L) and *Parabathynella* (fig. 13M) present other combinations with more setae in the first three segments (see table 4 for details). The internal seta of the second endopod segment is always plumose.

Thoracopods ii-vii: All species of the Iberobathynellini tribe have epipod on thoracopods II to VII, and one outer distal smooth seta on the basipod; *Montanabathynella* lacks an epipod on thoracopods II and III. The exopod, which is always shorter than the four segments of the endopod, consists of two segments in most species of the tribe, except *I. magna* (three segments in thoracopods IV and

v) and species of *Paraiberobathynella* (three or four segments) and *Texanobathynella* (three segments in some thoracopods) (see table 4); *Montanabathynella* can have between two and six segments, and in these cases, the exopod is longer than the endopod, and *Parabathynella* has two segments in all thoracopods, except in *P. badenwuerttembergensis*, which has two, six or seven segments in thoracopods IV and V; two terminal barbed setae are commonly present on each segment and, on the last one, one of the setae is plumose; ctenidia groups present at the base of the setae and on the posterior side. The endopod consists of four segments of different sizes: the first is about half the length of the second and third, which are similar in length, and the fourth is very small; segment one short and without setae except in the species of the subgenus *Iberobathynella* that have a terminal inner plumose seta (also found in species of *Montanabathynella* and *P. badenwuerttembergensis*); segment two with two groups of lateral ctenidia and two setae, one plumose and one smooth (absent on thoracopod VII and sometimes thoracopod VI); segment three with one tiny seta (except in *Texanobathynella*) and segment four with one seta and two similar strong claws.

Female thoracopod viii (fig. 14): The eighth pair of thoracopods is greatly reduced in species of the Parabathynellidae family. In the tribe Iberobathynellini (except the genus *Texanobathynella*) it reduced to a simple segment with a relatively triangular appearance, a wrinkled (in six species of the subgenera *Asturibathynella* and *Espanobathynella*, fig. 14A, B, E, H, I, O) or smooth cuticle (fig. 14C, D, F, G, J-N, P-Z), one, two or three small teeth and without setae (fig. 14A-Z); in *Texanobathynella*, it has a scale-like appearance with small terminal denticles (fig. 14AA, BB). Thoracopod VIII in females of *Montanabathynella* consists of two segments of similar length (basal segment slightly wider than the second one) (fig.

14CC, DD); *Parabathynella* also has two segments but the basal segment is slightly longer than second one (fig. 14EE). In both cases, denticles are present on the first segment, and three setae, on the second.

Male thoracopod viii (fig. 15): Its general appearance is almost square in most species of the tribe (fig. 15A-D, F-H, J), rectangular in *I. pedroi* (fig. 15E) and in species of *Texanobathynella* (fig. 15K) and trapezoidal in *Paraiberobathynella* (fig. 15I); very large (twice as long as wide) in *Montanabathynella* (fig. 15L) and elongated in *Parabathynella* (fig. 15M). In species of the Iberobathynellini tribe, the penial region has three lobes: an outer lobe (O.lb), a dentate lobe (D.lb) and an inner lobe (I.lb). *Montanabathynella* has an additional lobe, the small lobe (S.lb) (fig. 15L), and *Parabathynella* has three lobes, the outer, inner and small lobes, but not a dentate lobe (fig. 15M). In all species of the tribe, the outer lobe of the male thoracopod VIII is well developed, in both length and width, the main axis of the lobe can be vertical or inclined on the posterodistal or anterodistal side, partially fused with the basipod at the level of the basal parts of the posterior edge of the lobe and the anterior face of the basipod (Serban, 1977) and is distinctive as a massive element of the lateral face always close to the endopod. The outer lobe differs in the various species: in the subgenera of *Iberobathynella*, it is rectangular with a sloping main axis in *Espanobathynella* (fig. 15A, D); orientated towards the posterior part and with a curved main axis in *Asturibathynella* (fig. 15B, C) and triangular with vertical main axis in *Iberobathynella* (fig. 15G); and in the other genera, it has a posterior part that is longer than the anterior part and a rectangular lateral side in *Guadalopecathynella* (fig. 15J); a distal part orientated towards the posterior side in *Californibathynella* (fig. 15F); an outer lobe smaller than the dentate lobe,

with a triangular- or trapezoidal-shaped distal end in *Paraiberobathynella* (fig. 15I); triangular with setules on the distal end of the inner lobe in *Texanobathynella* (fig. 15K) and, in *Hexaiberobathynella* (fig. 15H) and anterior part shorter than is shorter than the posterior, a distal part strongly inclined toward the caudal part and a postero-distal inclination on the main axis; the outer lobe in *Parabathynella* is narrow and slightly longer than the exopod, and is not fused with the basipod (fig. 15M). The dentate lobe has between eight and 12 small teeth. The inner lobe, generally larger than the dentate lobe, may have small setules on the distal edge, as in the subgenus *Iberobathynella* (fig. 15G) and in *I. asturienensis* and *I. espaniensis*, or small spinules as in *Texanobathynella* (fig. 15K). The basipod (Bsp) is more or less conical and has a seta on the latero-external face of its distal end. The exopod, which is on the latero-external side of the basipod and covered by the outer lobe, is greatly reduced in all species of the Iberobathynellini tribe, and due to its small size, is the most difficult structure to observe in these species (fig. 15A-K); sometimes with small teeth or denticles; *Parabathynella* has a row of small spines. Endopod always present, with distal end oriented on the latero-external side and with two setae; also present in *Parabathynella* (fig. 15M) and *Montanabathynella* (fig. 15L).

Pleopod: The first pair of pleopods, reduced to simple setae, is only present in *Californibathynella californica*. The other 32 species of the tribe, and those of *Montanabathynella* and *Parabathynella*, do not present pleopods nor simple setae.

Pleotelson: Dorsal margin of pleotelson with anal operculum not pronounced in most species of the tribe (21 species) and pronounced in the rest (13 species), including eight species of the subgenus *Asturibathynella*, one of *Espanobathynella*, *I. pedroi*, the two species of *Hexaiberobathynella* and the one of

Californibathynella. *Montanabathynella salish* and *P. motasi* have a pronounced anal operculum but little protrusion.

Uropod (fig. 16): The sympod is considerably longer than wide in all species of the tribe, and almost always twice as long as the exopod and endopod; it has a row of spines that are equal in size (homonomous), as in the species *I. burgalensis* (fig. 16B), *I. magna*, *I. cantabriensis* and *Pi. notenboomi* (fig. 16I), or it has a row of spines in which the distal one is greater than the rest (inhomonomous) (fig. 16A, C-F, H, J-K); *Montanabathynella* (fig. 16L) and *Parabathynella* (fig. 16M) present an inhomonomous row of spines, similar to most species of the Iberobathynellini tribe; the row of spines can occupy half to two thirds of the sympod or almost its entire length (as in *I. pedroi*, fig. 16E); the number of spines is highly variable, between nine and 27 in the species of the subgenus *Iberobathynella* (fig. 16G) and between five and 13 in the subgenera *Asturibathynella* (fig. 16C, D) and *Espanobathynella* (fig. 16A, B) and in the rest of the tribe species (fig. 16E, F, H-K); species of *Montanabathynella* have 15 spines (fig. 16L) and those of *Parabathynella*, between six and 18 (fig. 16M). The endopod, in lancet form, has approximately the same length as the exopod in almost all species; it may lack setae (only *I. cornejoensis* and *I. burgalensis*, fig. 16B, C) or have one [nine species, including all of *Paraiberobathynella* (fig. 16I) and *G. puchi* (fig. 16H)], two [nine species (fig. 16D, E) including those of *Hexaiberobathynella* (fig. 16J)] or three (17 species, fig. 16A, F, G, K) setae that may be smooth, barbed or plumose and of different lengths. Exopod with apical and subapical setae (between two and five) and with (fig. 16A, C, D, E, G-K) or without (fig. 16B, F) a basal seta on the internal side; *Montanabathynella* (fig. 16L) has between seven and 11 setae, and *Parabathynella*, between four and eight (fig. 16M).

Furcal rami (fig. 17): The general shape is triangular or trapezoidal, with a variable number of spines, from five or six (as in *I. cornejoensis*, *I. pedroi*, *Hi. mateusi*, *Californibathynella* and *Texanobathynella*, fig. 17A, F, H, J-L) to 12 or 13 (as in *I. paragracilipes* and *Pi. notenboomi*, fig. 17E, I), and the two most distal spines are always larger; *Montanabathynella* has 11 to 14 spines (fig. 17M) and *Parabathynella* has five to 10 (fig. 17N). On the dorsal side, the furcal rami have two plumose setae of different lengths.

Discussion

The results of the morphological and molecular analyses of the Iberobathynellini tribe reveal the need to address the complex group of poorly delimited morphological species using integrative approaches. The morphological, molecular and combined cladograms show the non-monophyletic status of some subgroups and a lack of congruence needed to consider the current taxonomic organization as resolved. The existence of cryptic species and the underestimation of the true diversity of this amphiatlantic group of crustaceans are also highlighted.

Taxonomic status of the Iberobathynellini tribe based on morphology

Delamare Deboutteville, Serban & Coineau (1975), in their description of the first species of the Parabathynellidae family found in North America, *Texanobathynella bowmani* from Texas, highlighted its similarity with species of the genus *Iberobathynella*, particularly with respect to the structure of ThVIII in the male, the reduction of AII and the morphology of the labrum, uropod and furca. Subsequently, Schminke & Noodt (1988), who discovered the second North American species, *Iberobathynella californica* from California, considered *Texanobathynella* as synonymous

with *Iberobathynella*. Regarding their new species *I. californica*, they remarked: “*The new species shows the diagnostic features of the genus Iberobathynella: antennules 7-segmented; antennae 3-segmented with (4) setae only on distal segment; maxillae 3-segmented, second segment with characteristic seta on inner surface; coxa of thoracopods i-vii with typical conical projection at inner distal border; sympodite of uropods with row of unequal spines; exopod of uropods with basiventral seta*”. Regarding the differences between *I. californica* and *T. bowmani*, they write: “*Iberobathynella californica and Iberobathynella bowmani, new combination, can be distinguished by the number of spines on the sympodite of the uropods, by the number of segments of the exopods of thoracopods i-vii, by the number of teeth on the labrum, by the number of segments of the antennules, and by the fact that the first pleopods of I. californica are represented by a pair of setae lacking in I. bowmani*”.

Camacho and Serban (1998) later reviewed the group in light of new species found in the Iberian Peninsula and offered a new point of view on the systematics of the group by erecting tribe and subtribe categories, in an attempt to better understand the relationships within the group. These authors, for various reasons, considered the species from California distinctive from *Iberobathynella* and erected the genus *Californibathynella*. They also considered *Texanobathynella* as a valid genus (Camacho & Serban, 1998, 2000). Subsequently Schminke (2011) accepted both of these proposals. At present, the tribe consists of 33 nominal species and three cryptic ones. Here, we have updated information about the character states and their distribution across the morphotypes.

The results of the morphological phylogenetic analysis (fig. 5) does not support the monophyly of the Iberobathynellini tribe with respect to the genera *Parabathynella* and

Montanabathynella (i.e., the other two genera of the family present in Europe and North America, respectively). *Montanabathynella* and *Parabathynella* appear within the Iberobathynellini tribe. In the combined morpho-molecular dataset the tribe appears monophyletic, however the genera without molecular data are excluded. Also, according to this analysis, at the subtribe level, only Hexaiberobathynellina (with its single genus *Hexaiberobathynella*) is monophyletic based on the morphological characters used. The internal relationships among subgenera and species are not well resolved either.

Previous phylogenetic reconstructions of Iberobathynellini based on 19 morphological characters (and 14 species) also did not resolve these internal relationships (Camacho et al., 2000; Guil & Camacho, 2001; Camacho et al., 2006). Those studies used another set of characters that were ordered (polarized) according to criteria that were widely accepted at the time (Schminke, 1974; Schram, 1984; Camacho, 1987; Coineau, 1996; Camacho et al., 2000). They also assumed the reduction of structures (spines, seta, segments, among others) as a common trend (0 = plesiomorphic and 1 or 2 = apomorphic states). These previous studies hypothesized that the phylogenies will gradually become more precise as new species and populations are discovered, allowing for more a robust assessment of kin relationships (Camacho & Serban, 2000; Camacho et al., 2000; Guil & Camacho, 2001; Camacho et al., 2006). However, a great deal of uncertainties remains, despite the fact that new species have been described and included in more recent analyses. The set of characters used here defines an almost complete morphospace with all possible states for each of the characters, without any polarization to avoid the subjectivity of previous assumptions. Despite this, the resolution of some relationships remains very low.

Phylogenetic hypothesis for the Iberobathynellini tribe

The phylogenetic reconstruction of the Iberobathynellini tribe based on morphological data (see fig. 5) does not support the monophyly of the tribe and it also lacks the necessary resolution to shed light on kinships between and within internal groups, and shows the limited validity of the subtribes and subgenera that were erected by Camacho & Serban (1998) to help organize the taxonomy of the group. These categories were useful at the time to understand the relationships within the group formed by the four Iberian genera (*Iberobathynella*, *Guadalopebathynella*, *Hexaiberobathynella* and *Paraiberobathynella*), the two North American genera (*Californibathynella* and *Texanobathynella*) and the African species, *Pi. maghrebensis* (Camacho & Serban 1998). However, these categories are no longer suitable for the tribe: despite they are more species (10) described, also included in our analyses, the previously considered categories are not monophyletic. The phylogenies obtained from the molecular and combined analyses (see fig. 2 and 6) are largely congruent, and also support the invalidation of the subtribe and subgenera categories, even though the combined analysis did not include some information for the genera *Californibathynella*, *Guadalopebathynella*, *Hexaiberobathynella* and the subgenus *Iberobathynella* (*Iberobathynella*). Comparisons between the Iberobathynellini tribe and the other two genera of the family present in North America or Europe, and the cosmopolitan genus *Hexabathynella*, clearly show the monophyly of the tribe and its sister group relationship with the two well-defined genera *Montanabathynella* and *Parabathynella*.

Exclusively representatives of the genera of the family that live in the area of the study have been analyzed, excluding other genera of the Parabathynellidae family from all over the world (Australia, India, Asia ...)

that were already analyzed globally in previous papers (Camacho et al., 2018b; Camacho et al., 2021a). Our study tries to find the relationships between the genera of the Parabathynellidae family that inhabit Iberian Peninsula and North America using as outgroup *Hexabathynella*, the only cosmopolitan Bathynellacea genus. We have also used *Hexabathynella* as outgroup because it is very different from other genera and has only 6 pairs of legs, a character only shared with the Iberian genus *Hexaiberobathynella* belonging to the Iberobathynellini Tribe. Since the two genera are distantly related, this character is most likely the product of convergent evolution.

We previously found several morphotypes inhabiting some of the same caves in northern Spain (C0209 and C0314, among others) that we associated with the nominal species *I. parasturiensis*, *I. imuniensis*, *I. cantabriensis* and *I. magna* (Camacho et al., 2006). Despite the morphological differences observed between the specimens from both caves, our analysis of their CO1 sequences, along with those of specimens of the four species collected from their respective type locality (supplementary table S2), showed that, in Cave C0209, all the specimens belong to only two of the nominal species, namely *I. parasturiensis* and *I. cantabriensis*, and that, in Cave C0314, the specimens correspond to these two species plus two new species, *I. sp. nov. 1* and *I. sp. nov. 2*, which are morphologically identical to *I. magna* and *I. imuniensis*, respectively. The genetic divergence between the new species and their respective nominal species is very high (18.5% and 16.1%, respectively; see supplementary table S2), supporting their designation as cryptic species. After revising the specimens from Fonda and Astui caves, which are morphologically similar to the nominal species *I. imuniensis* but diverge with it by 14.7% to 15.6%, we propose that they belong

to a new species, *I. sp. nov. 3*, unrelated to *I. sp. nov. 2* (genetic distances between 18.1% and 21.3%) (supplementary table S2).

Wide species distribution presented in fig. 1 and supplementary appendix S2 for *Hexaiberobathynella mateusi* (25 populations from Oporto to Castellón, Granada and Jaén passing through Madrid, Guadalajara, Toledo, Soria, Teruel and Huesca), *Iberobathynella imuniensis* (32 populations from Asturias, Cantabria, Burgos to Alava and Huesca) and *Paraiberobathynella fagei* (45 populations, from South France to Cadiz, and to Asturias and Mallorca Island) must be taken with caution since these species could represent cryptic species. Unfortunately the absence of molecular data does not allow to verify the presence of cryptic species and related distributional ranges.

Morphological simplification is extreme in Bathynellacea: due to progenetic development, Bathynellacea reach adulthood at a stage corresponding to the ancestral last larval stage (Schminke, 1981). The absence of morphological distinguishability between subterranean species may be related with three mechanisms: niche conservatism, recent divergence or morphological convergence (Trontelj et al., 2012; Meleg et al., 2013; Zigmajster et al., 2018). A homogeneous geographic distribution of cryptic species, with high CO1 genetic divergences, can occur because these three mechanisms offset each other along spatial or environmental gradients (Eme et al., 2018). Phylogenetic interpretations in cases of convergent evolution can be misleading if they are based only on morphology (Lefébure et al., 2007). To investigate possible morphological convergence, we performed ancestral state reconstructions for five characters representative of morphological differences between groups (see figs. 7 and 8), however, given low support at the nodes, we cannot draw robust conclusions

from these analyses. Some of the preliminary results of our study, along with those of other studies, such as the phylogenetic study of Australian Parabathynellidae by Abrams et al. (2012), contradict paradigms, including the evolutionary hypothesis that species tend to evolve towards a more simplified body structure with reduction of segments, setae and other structures. However, this hypothesis towards simplification, argued as the basis for many phylogenetic reconstruction analyses (for polarity discussion, see Schminke, 1973, 1974; Schram, 1984; Camacho, 1987, Camacho et al., 2000; Coineau, 1996; Hong & Cho, 2009; Bandari et al., 2017), is debatable.

The number of segments of antennule, antenna and exopod of thoracopods are characters that have been previously used to define genera and to assess the oligomerization principle (i.e., serial appendage reduction over time), which is commonly invoked in crustacean systematics (Adamowicz et al., 2007) to infer which character state is plesiomorphic or apomorphic. However, given the high occurrence of convergent morphologies in parabathynellids, it is necessary to determine whether the characters used to define genera and species are homoplastic and therefore useless to infer phylogenetic relationships. Previous molecular analyses on Parabathynellidae from different countries (Camacho et al., 2018b; Camacho et al., 2021a) highlighted the paucity of the data available to create a robust phylogeny for the family. Fresh material from type localities (especially from Africa, South America, Asia) should be collected to obtain sequences that could clarify the relationships amongst genera. Also, there are many unexplored areas in all continents that most likely host new species (Bolivia, Argentina, Chile, Brazil, Perú, Mexico, China, Philippines, Malasia, Thailand, Mozambique, Malawi, Zambia, Syria, Turkey ...).

The reality is that there are very few countries/regions that have been heavily/systematically sampled for interstitial/subterranean fauna. Many European countries have a long speleological “tradition” and many cavers sampled several caves throughout the last century. The arid zones of Western Australia are also heavily sampled due to mining developments and environmental legislation. Unfortunately, for the rest of the world subterranean sampling events are just sporadic and opportunistic.

Updated distribution and a palaeobiogeographic scenario

The Iberobathynellini tribe is distributed across three continents [Europe (Iberian Peninsula), North America (Texas and California) and Africa (the Maghreb, Morocco and Algeria)], with a presumed origin in Laurentia (Camacho, 2003a; Camacho et al., 2018b). In the Iberian Peninsula, the geo-palaeobiological conditions of the Lower Triassic, and an extensive marine domain, favoured the colonization of the continental environment by a common ancestor of the tribe (Camacho et al., 2006). This ancestor may have been present in coastal habitats along the margins of landmasses during the Middle Triassic (238 Mya) (Camacho et al. 2000; Coineau & Camacho, 2013) when the Iberian Peninsula, North America and North Africa were still linked. This scenario is the most plausible explanation for the existence of these phylogenetically closely related species on these three continents, particularly given that they have no effective intrinsic dispersal mechanism (Coineau & Camacho, 2013).

The Mesozoic continental split that broke up Pangea (Schram, 1977) may have been the vicariant event responsible for the divergence of the ancestral clade (Coineau & Camacho, 2013). Continental drift further subdivided the area during the Early Triassic

(245 to 205 Mya; Golonka, 2007), when the Iberian Peninsula and North America started to separate (Yilmaz et al., 1996) and when parts of present-day Portugal, Galicia and Salamanca, as well as California and Texas, had already emerged (Yilmaz et al., 1996), coinciding with the earliest divergence in iberobathynellids (Coineau & Camacho, 2013). The break-up of Laurasia has played a major role in shaping the present-day distribution not only in the Bathynellacea but also in other ecologically similar and phylogenetically distant taxa as subterranean amphipod Metacrangonyctidae (Bauza-Ribot et al., 2012; Pons et al., 2019). The family Metacrangonyctidae lives in caves, wells and other subterranean habitats, as Bathynellacea, in areas covered by the sea in the geological past in several Mediterranean islands (Mallorca, Menorca), and Morocco in North Africa (Pons et al., 2019). The earliest divergences in the Iberobathynellini tribe coincide with the separation of the Iberian Peninsula and Africa, around 100 Mya (Camacho et al., 2000). The ancestor of *Paraiberobathynella* must have lived on the Tethys seashore and, as suggested by its present distribution, colonized emerged land before the Senonian (88.5 to 65 Mya) when the island of Mallorca, the Iberian Peninsula and the Alboran plate were all still part of the same landmass (Yilmaz et al., 1996; Camacho et al., 2000, Camacho et al., 2018b).

Europe and North America were connected by a land bridge during the late Eocene (34 Mya), possible disjunct species between these two areas would have diverged at least at the end of the Eocene (Smith & Donoghue, 2010), in this case we should find young divergences between taxa. This scenario is consistent with our dating for the separation date between *Montanabathynella* (North American genus), *Parabathynella* (European genus) and *Texanobathynella*. (fig. 7).

The presence of Iberobathynellini in the Cantabric and central Iberian Peninsula can be explained by marine regressions. The ancestor is hypothesized to have colonized these areas of transitional karst-marine facies during the Upper Cretaceous (96–65 Mya) (Yilmaz et al., 1996; Camacho et al., 2006). From our molecular clock analysis, the TMRCA of the *Iberobathynella*/*Paraiberobathynella* group and *Texanobathynella* is dated at 76.8 Mya (see fig. 4), consistent with the Upper Cretaceous colonization hypothesis. Vicariance at local level leading to new biogeographical barriers (faults) or local ecological changes likely influenced speciation and extinction events in the tribe, which were followed by small scale dispersal. The faults and thrusting associated with the Alpine Orogeny during the Cenozoic (63.7 Mya) (Yilmaz et al., 1996) could have been the vicariant processes responsible for the various lineages of *Iberobathynella* and their present-day distribution. In the Northern Hemisphere there were geological events during the Neogene (23 Mya) (Graham, 1999) that may have played a role in diversification: uplifting of the Rocky Mountains and erosion of the Appalachian Mountains and the formation of the Mountain ranges in Europe, which began in the Paleogene (65–23 Mya) (Thompson, 2005). The lack of molecular information on the Californian species of the *Texanobathynella* genus prevents us from making assumptions on the divergence on both North American coasts. According to our dating, in the Eocene (40 Mya) *Iberobathynella* species from Andalusia (*I. andalusica* and *I. celiana*) were able to diversify from the rest of species. Also, around 40 Mya, other speciation events within the tribe happened, as the diversification of the species from Burgos (*I. burgalensis*, *I. imuniensis* and *I. cornejoensis*) and País Vasco (*I. sp. nov.* 3).

This scenario is consistent with the results of the divergence time analysis. The presence of

species-rich areas associated with low phylogenetic diversity (i.e., areas inhabited by species with short evolutionary histories that group within the same clade) can reflect a process of recent and rapid in situ speciation. Such areas are known as “cradles of diversity” (Jablonski et al., 2006). Infierno Cave represents one such area: it is inhabited by the three closely related species *I. espaniensis*, *I. asturiensis* and *I. parasturiensis* that began to diverge around 41.8 Mya (95% HPD: 29.3–55.1 Mya) (see fig. 2); the type of diversification that has occurred is an interesting pattern to explore. By contrast, the existence of areas with species displaying higher phylogenetic diversity (i.e., species with long independent evolutionary histories in different clades) suggests decreased extinction rates. These areas are defined as “museums”, as diversity is preserved over time (Jablonski et al., 2006). An example is the Ojo Guareña system (Camacho & Puch, 2021a), where species from different clades occur: *I. burgalensis* and *I. cornejoensis* [TMRCA: 39.8 Mya (95% HPD: 28.9–52.8 Mya)].

The Late Miocene (11.63–5.33 Mya) was characterized by compression along the southeastern margin of Iberia in the Prebetic fold belt and in portions of the Balearic Islands. Westward break out of the Alboran Block opened the North Algerian Basin in its wake. The Alboran Block collided with the southeastern margin of Iberia and the northwestern margin of Africa during the late Oligocene/early Miocene (Yilmaz et al., 1996).

During the Middle Miocene (10.5 Mya) as a consequence of the progressive subduction S-SE of the Iberia (“European lithosphere”) towards the “Backarc stretched area”, which previously belonged to the “Alpine-Betic belt”, new “Alpine-Betic belt” collides, moving towards N-NW. In the North of Africa, the “Apennines-Maghrebides Belt” collides. In this geodynamic framework of the western Mediterranean area, new basins formed

(e.g., Algerian Basin and Provençal Basin). In the western area the “Alboran Sea” and “Valencia Trough” bordered Iberia on the E and S and separated it from Africa (Andeweg, 2002; Rosenbaum et al., 2002; Carminati et al., 2012). During Late Miocene, in the Alboran Sea there was the progressive formation of the Gibraltar Arc (“Alboran arc”) to the approach of the Baetic Belt (“Alpine-Betic belt”) in the South of Iberia and the Rif (“Apennines-Maghrebides Belt”) in the North of Africa (Krisjgman et al., 2018). These tectonic movements in Tortonian times (11 Mya) can explain the speciation of *Paraiberobathynella* the Iberian Peninsula and North Africa (fig. 7), since it is consistent with the divergence time found (about 10 Mya). The lack of molecular data for specimens from Balearic Island populations (*Paraiberobathynella* cf. *fagei*) prevented their inclusion in the phylogenetic reconstruction analyses.

As new species and populations are found, and their molecular data analysed, more information can be gathered on the genetic divergence and phylogenetic relationships of the ancient group, Iberobathynellini tribe, to confirm the evolutionary history that extends at early Cretaceous, allowing for more robust species delimitations and divergence time estimations. These data will help to determine the most plausible palaeobiogeographic scenario for this tribe with an amphiatlantic distribution, and provide a better understanding of the current distribution and the true diversity. There is biogeographical congruence with the Holarctic subterranean Crangonyctidae amphipod, a group of probably Early Cretaceous origin, that show similar distribution pattern (probably Laurasian origin) of old lineages by vicariance (continental level) (Copilas-Ciocianu et al., 2019) as occur also in Metacrangonyctidae subterranean amphipods (Pons et al., 2019). It is interesting to find similar distribution patterns between

subterranean crustaceans of ancient origin, phylogenetically distant but ecologically similar, which support the plausibility of the proposed biogeographic scenario.

Conclusions

A modern systematic revision of the Iberobathynellini tribe or “*Iberobathynella* group” using both molecular and morphological approaches, along with distribution data to delineate species from three continents, is presented here. Despite having information on 33 morphotypes that appear to encompass all the possible morphological variability of the group, and molecular data for 16 nominal and three cryptic species, these data are not sufficient to fully resolve all of the phylogenetic relationships of the group. However, the results of these analyses make it possible to highlight the following conclusions:

1. Subtribe and subgenus are invalid categories so they must be eliminated. However, we keep them in the supplementary material, so that it can be understood why it was necessary to create them when studying the morphological similarities and differences between the species. The systematics proposed in 1998 offered a coherent organization of the morphological variability exhibited by the morphotypes of the tribe at that time.
2. *Paraiberobathynella* needs to be revisited. At the moment we are going to point out that it may be that *Paraiberobathynella* has to be synonymous with *Iberobathynella*, but first we have to obtain DNA from fresh *Pi. fagei* specimens type locality and include them in the phylogeny to see their relationship with *Iberobathynella*. It could be that the populations found in Spain

are cryptic species, morphologically identical to *Pi. fagei*, but in reality they are new *Iberobathynella* species and hence our populations are within a group of *Iberobathynella* species and the *Paraiberobathynella* genus could still be valid.

3. The molecular dating results support the early divergence of this group of ancient subterranean crustaceans and the vicariance by plate tectonics as main factor to explain the *amphi*-Atlantic distribution shown by Iberobathynellini Tribe. Cycles of marine transgressions and regressions, together with vicariance associated with the Alpine Orogeny (faults and thrusting) leading to new biogeographic barriers and local ecological changes may be the processes responsible for the distribution of lineages at local level. The separation of the lineage of the two Andalusian species (*I. celiana* and *I. andalusica*) of the rest of genus *Iberobathynella* is also early.
4. COI p-distances within-between species and genera vary greatly therefore it is not possible to apply a threshold on this group, but assess case by case with multiple evidence (morpho-molecular).
5. There are species morphologically very similar to *I. magna* and *I. imuniensis*, but genetically different, we can ensure the existence of at least three cryptic species.
6. *Texanobathynella* is undoubtedly a valid genus distinct from *Iberobathynella*.
7. *Montanabathynella* and *Parabathynella* are two well-differentiated genera closely related to the tribe Iberobathynellini.

Future taxonomic studies of groundwater animals should be integrative and take advantage of the variety of new techniques for species delimitation.

Testing biogeographical hypotheses using morphological analyses only is a challenge on Bathynellacea due to the extreme morphological simplification. The lack of useful characters to distinguish species and genera might be due to niche conservatism, recent divergence or morphological convergence (Trontelj et al., 2012; Meleg et al., 2013). In addition, the isolation of different populations in disconnected habitats can lead to high genetic divergences (Finston et al., 2007).

Acknowledgements

We gratefully acknowledge C. Puch for help with various aspects of the paper. Thanks to A. Brancelj, D. Jaume, B.T. Hutchins and B.F. Schwartz for providing specimens of various species, and to J.A. Noriega who helped in the Infierno cave sampling. We thank Melinda Modrell who helped us with the English translations, and the anonymous reviewers who provided valuable comments. This work was supported by project PID2019-110243GB-I00 by the MICINN/FEDER. PMP is supported by MICINN-FEDER through contract BES-2016-077777. EKLE is supported by a doctoral scholarship from CONACyT-Mexico (330519/472100).

Supplementary material

Supplementary material is available online at: <https://doi.org/10.6084/m9.figshare.16974721>

References

- Abrams, K.M., Guzik, M.T., Cooper, S.J.B., Humphreys, W.F., King, R.A., Cho, J.-L. & Austin, A.D. (2012) What lies beneath: Molecular phylogenetics and ancestral state reconstruction of the ancient subterranean Australian Parabathynellidae (Syncarida, Crustacea). *Mol. Phylogenet. Evol.*, 64, 130–164. doi:10.1016/j.ympev.2012.03.010.
- Adamowicz, S.J., Menu-Marque, S., Hebert, P.D.N. & Purvis, A. (2007) Molecular systematics and patterns of morphological evolution in the Centropagidae (Copepoda: Calanoida) of Argentina. *Biol. J. Linn. Soc.*, 90, 279–292. doi.org/10.1111/j.1095-8312.2007.00723.x.
- Alda, F., Rey, I. & Doadrio, I. (2007) An improved method of extracting degraded DNA samples from birds and other species. *Ardeola*, 54, 331–334.
- Alfaro, M.E., Zoller, S. & Lutzoni, F. (2003) Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling a bootstrapping in assessing phylogenetic confidence. *Mol. Biol. Evol.*, 20, 255–266.
- Amato, A., Kooistra, W.H., Ghiron, J.H.L., Mann, D.G., Pröschold, T. & Montresor, M. (2007) Reproductive isolation among sympatric cryptic species in marine diatoms. *Protist*, 158, 193–207.
- Andeweg, B. (2002) Cenozoic Tectonic Evolution of the Iberian Peninsula: effects and causes of changing stress fields. *Published PhD thesis*, Vrije Universiteit Amsterdam, the Netherlands, 178 pp.
- Ayres, D.L., Darling A., Zwicky, D.J., Beeril, P., Holder, M.T., Lewis, P.O., Huelsenbeck, J.P., Ronquist, F., Swofford, D.L., Cummings, M.P., Rambaut, A. & Suchard, M.A. (2012) BEAGLE: An application programming interface and high-performance computing Library for statistical Phylogenetics. *Syst. Biol.*, 61, 170–173. <https://doi.org/10.1093/sysbio/syr100>.
- Bandari, E., Shaik, S. & Ranga Reddy, Y. (2017) A phylogenetic review of the genus *Atopobathynella* Schminke, 1973 (Crustacea, Malacostraca, Bathynellacea) with three new species from southeastern India. *J. Nat. Hist.* 51, 2143–2184. doi.org/10.1080/00222933.2017.1360528.
- Barroso, R., Klautau, M., Solé-Cava, A.M. & Paiva, P.C. (2010) *Eurythoe complanata* (Polychaeta:

- Amphinomidae), the 'cosmopolitan' fireworm, consists of at least three cryptic species. *Mar. Biol.* 157, 69–80.
- Bauzá-Ribot, M., Juan, C., Nardi, F., Oromi, P., Pons, J., & Jaume, D. (2012). Mitogenomic Phylogenetic Analysis Supports Continental-Scale Vicariance in Subterranean Thalassoid Crustaceans. *Curr. Biol.* 22(21), 2069–2074. doi.org/10.1016/j.cub.2012.09.012.
- Beheregaray, L.B. & Caccone, A. (2007) Cryptic biodiversity in a changing world. *J. Biol.*, 6, 9.
- Braga, J.M. (1949) Un Bathynellidae (Crust. Syncarida) du Portugal (*Parabathynella lusitanica* sp. n. *Publ. Inst. Zool. "Dr. A. Nobre"*, 40, 1–15.
- Braga, J.M. (1960) Sur une *Parabathynella* (Crust. Syncarida) nouvelle du Portugal. *Publ. Inst. Zool. "Dr. A. Nobre"*, 75, 9–22.
- Boutin, C. & Coineau, N. (1987) *Iberobathynella* sur le continent africain. Implications paléogéographiques. *C. R. Acad. Sci. Paris*, 304, Série III, 13, 355–358.
- Camacho, A.I. (1987) A new subterranean Syncarid (Crustacea) from Spain: *Iberobathynella imuniensis* n. sp. (Bathynellacea, Parabathynellidae). *Arch. Hydrobiol.* 111, 137–149. #8232;
- Camacho, A.I. (1988) Historia, clave de determinación y distribución de género *Iberobathynella* (Crustacea, Syncarida, Parabathynellidae) en la Península Ibérica. *Congreso Ibérico de Entomología*, Actas, 3, 43–56.
- Camacho, A.I. (2003) Four new species of groundwater crustaceans (Syncarida, Bathynellacea, Parabathynellidae) endemic to the Iberian Peninsula. *J. Nat. Hist.*, 37, 2885–2907.
- Camacho, A.I. (2003a) An overview of the distribution of the Parabathynellidae (Crustacea Syncarida Bathynellacea) on the Iberian Peninsula and Balearic Islands. *Graellsia*, 59, 63–78.
- Camacho, A.I. (2003b) Four new species of groundwater crustaceans (Syncarida, Bathynellacea, Parabathynellidae) endemic to the Iberian Peninsula. *J. Nat. Hist.*, 37, 2885–2907.
- Camacho, A.I. (2005) One more piece in the genus puzzle: a new species of *Iberobathynella* Schminke, 1973 (Syncarida, Bathynellacea, Parabathynellidae) from the Iberian Peninsula. *Graellsia*, 61, 123–133.
- Camacho, A.I. (2007) A new species of *Iberobathynella* Schminke, 1973 (Syncarida, Bathynellacea, Parabathynellidae) in the South of the Iberian Peninsula. *Graellsia*, 63(2), 339–347.
- Camacho, A.I. (2019) Diversity, morphological homogeneity and genetic divergence in a taxonomically complex group of groundwater crustaceans: the little known Bathynellacea (Malacostraca). *Bull. Soc. d'Hist. Nat. Toulouse*, 154, 105–160.
- Camacho, A.I. & Coineau, N. (1989) Les Bathynellacea d'Espagne: Répartition et Biogéographie. *Mém. Biospéol.*, 16, 111–124.
- Camacho, A.I. & Puch, C. (2021). Ojo Guareña: a hotspot of subterranean Biodiversity in Spain. *Diversity*, 13, 199. doi.org/10.3390/d13050199.
- Camacho, A.I. & Serban, E. (1998) Tribu des Iberobathynellini nov., diagnoses des taxa nouveaux (Parabathynellidae, Bathynellacea, Podophallocarida). *Trav. Inst. Spéol.* «É. Racovitza», 34, 15–75.
- Camacho, A.I. & Serban, E. (2000) Revisión del grupo *Iberobathynella* (*Iberobathynella*) Camacho & Serban, 1998 (Crustacea, Syncarida, Parabathynellidae) endémico de la Península Ibérica. *Graellsia*, 56, 35–48.
- Camacho, A.I., Bello, E. & Estabrook, G.F. (1997) A statistical approach to the evaluation of characters to estimate evolutionary relationships among the species of the aquatic subterranean genus *Iberobathynella* (Crustacea, Syncarida). *Biol. J. Linn. Soc.*, 60, 221–241.
- Camacho, A.I., Serban, E. & Guil, N. (2000) Phylogenetical review and biogeographic remarks on the interstitial and subterranean freshwater iberobathynells (Crustacea, Syncarida, Parabathynellidae). *J. Nat. Hist.*, 34, 563–585.

- Camacho, A.I., Torres, T., Ortiz, E., Puch, C. & Valdecasas, A.G. (2006) Small-scale biogeographical pattern in groundwater Crustacea (Syncarida, Parabathynellidae). *Biodivers. Conserv.*, 15, 3527–3541. doi: 10.1007/s10531-004-1872-8.
- Camacho, A.I., Dorda, B.A. & Rey, I. (2011) Identifying cryptic speciation across groundwater populations: first COI sequences of Bathynellidae (Crustacea, Syncarida). *Graellsia*, 67, 7–12.
- Camacho, A.I., Dorda, B.A. & Rey, I. (2012) Undisclosed taxonomic diversity of Bathynellacea (Malacostraca: Syncarida) in the Iberian Peninsula revealed by molecular data. *J. Crust. Biol.* 32, 816–826. doi: 10.1163/193724012X638473.
- Camacho, A.I., Dorda, B.A. & Rey, I. (2014) Iberian Peninsula and Balearic Island Bathynellacea (Crustacea, Syncarida) database. *Zookeys*, 386, 1–20.
- Camacho, A.I., Stanford, J.A. & Newell, R.L. (2009) The first record of Syncarida in Montana, USA: a new genus and species of Parabathynellidae (Crustacea, Bathynellacea) in North America. *J. Nat. Hist.* 43(5–6), 309–321.
- Camacho, A.I., Sánchez-Chillón, B., Dorda, B.A. & Rey, I. (2017) The collection of Bathynellacea specimens of MNCN (CSIC) Madrid: microscope slides and DNA extract. *ZooKeys*, 678, 31–36. doi: 10.3897/zookeys.678.11543.
- Camacho, A.I., Hutchins, B., Schwartz, B.F., Dorda, B.A., Casado, A. & Rey, I. (2018a). Description of a new genus and species of Bathynellidae (Crustacea: Bathynellacea) from Texas based on morphology and molecular characters. *J. Nat. Hist.*, 52(1–2), 29–51. doi.org/10.1080/00222933.2017.1401680.
- Camacho, A.I., Mas-Peinado, P., Watiroyram, S., Brancelj, A., Bandari, E., Dorda, B.A., Casado, A. & Rey, I. (2018b) Molecular phylogeny of Parabathynellidae (Crustacea, Bathynellacea), and three new species from Thai caves. *Contrib. Zool.*, 87, 227–260.
- Camacho A.I., Mas-Peinado, P., Dorda, B.A., Casado, A., Brancelj, A., Knight, L.R.F.D, Hutchins, B., Bou, C., Perina, G. & Rey, I. (2018c) Molecular tools unveil an underestimated diversity in a stygofauna family: a preliminary world phylogeny and an updated morphology of Bathynellidae (Crustacea, Bathynellacea). *Zool. J. Linn. Soc.*, 183, 70–96.
- Camacho A.I., Mas-Peinado, P., Ranga Reddy, Y., Bandari, E., Shaik, S., Perina, G., Dorda, B.A., Casado, A. & Rey, I. (2021a) An integrated approach to re-evaluate the validity of the family Leptobathynellidae (Crustacea: Bathynellacea). *Zool. J. Linn. Soc.*, 20. 1–43.
- Camacho A.I., Mas-Peinado, P., Hutchins, B.T., Schwartz, B.F., Dorda, B.A., Casado, A. & Rey, I. (2021b). New stygofauna from Texas, USA: three new species of Parabathynellidae (Crustacea, Bathynellacea). *J. Nat. Hist.*, 55(15–16), 979–1007. doi.org/10.1080/00222933.2021.1928316.
- Carminati, E., Lustrino, M., & Doglioni, C. (2012) Geodynamic evolution of the central and western Mediterranean: Tectonics vs. igneous petrology constraints. *Tectonophysics*, 579, 173–192.
- Chappuis, P.A. (1926) *Parabathynella stygia* n.g. n.sp. nouveau crustacé cavernicole de la Serbie Orientale. *Bull. Soc. St. Cluj*, II, 7–10.
- Cho, J.L. (1996) A new species of the genus *Texanobathynella* from California (Crustacea, Malacostraca, Bathynellacea). *Korean J. Syst. Zool.*, 12(4), 389–395.
- Coineau, N. (1996) Sous-Classe des Eumalacostracés (Eumalacostraca Grobben, 1892). Super-Ordre des Syncarides (Syncarida Packard, 1885). In: J. Forest (Ed.), *Traité de Zoologie, Crustacés*, 7, 897–954.
- Coineau, N. & Camacho, A.I. (2013) *Superorder Syncarida Packard, 1885*. Chapter 50, 357–449. In: J.C. von Vaupel Klein, M. Charmantier-Daures and F.R. Schram, (Eds), (Founded by P.-P. Grassé). *Treatise on Zoology-Anatomy, Taxonomy, Biology. The Crustacea. Traité de*

- Zoologie, Koninklijke Brill nv ISBN 978 90 04 17809 0. Leiden.
- Copilas-Ciocianu, D., Sidorov, D. & Gontcharov, A. (2019) Adrift across tectonic plates: molecular phylogenetics supports the ancient Laurasian origin of old limnic crangonyctid amphipods. *Org. Divers. Evol.* 19, 191–207. doi.org/10.1007/s13127-019-00401-7.
- Dancau, D. & Serban, E. (1963) Sur une nouvelle *Parabathynella* de Roumanie, *Parabathynella motasi* nov. sp. *Crustaceana*, 5(4), 241–250.
- Delamare Deboutteville, C. & Angelier, E. (1950) Sur un type de Crustacé phréaticole nouveau: *Parabathynella fagei* n.sp. *C. R. Hebd. Acad. Sci.* Paris, 231, 175–176.
- Delamare Deboutteville, C., Serban, E. & Coineau, N. (1975) Découverte de la famille des Parabathynellidae (Bathynellacea) en Amérique du Nord: *Texanobathynella bowmani* n.g. n. sp. *C. R. hebd. Acad. Sci., Paris, Sér. D*, 280, 2223–2226.
- Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.*, 29, 1969–1973.
- Eme, D., Zgamaister, M., Delić, T., Fišer, C., Flot, J.-F., Konecny-Dupré, L., Pálsson, S., Stoch, F., Zakšek, V., Douady, C.J. & Malard, F. (2018) Do cryptic species matter in macroecology? Sequencing European groundwater crustaceans yields smaller ranges but does not challenge biodiversity determinants. *Ecography*, 41(2), 424–436. https://doi.org/10.1111/ecog.02683.
- Finston, T.L., Johnson, M.S., Humphreys, W.F., Eberhard, S.M. & Halse, S.A. (2007) Cryptic speciation in two widespread subterranean amphipod genera reflects historical drainage patterns in an ancient landscape. *Mol. Ecol.*, 16, 355–365.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit 1 from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotech.*, 3, 294–299.
- Galhano, M.H. (1967) Sur une nouvelles *Parabathynella* psammique du Portugal. *Publ. Inst. Zool. "Dr. A. Nobre"*, 98, 9–18.
- Galhano, M.H. (1970) Contribução para o conhecimento de fauna intersticial em Portugal. *Parabathynella lusitanica valbonensis* ssp. n. *Publ. Inst. Zool. "Dr. A. Nobre"*, 110, 77–82.
- Gilbert, M.T.P., Moore, W., Melchior, L. & Worobey, M. (2007) DNA extraction from dry museum beetles without conferring external morphological damage. *PLoS ONE* 2, e272.
- Giribert, G., Carranza, S., Baguña, J., Riutort, M. & Ribera, C. (1996) First molecular evidence for the existence of a Tardigrada + Arthropoda clade. *Mol. Biol. Evol.* 13, 76–84.
- Golonka, J. (2007) Late Triassic and Early Jurassic palaeogeography of the world. *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 244, 297–307.
- González-Miguéns, R., Muñoz-Nozal, E., Jiménez-Ruiz, Y., Mas-Peinado, P., Ghanavi, H.R. & García-París, M. (2020) Speciation patterns in the *Forficula auricularia* species complex: cryptic and not so cryptic taxa across the western Palearctic region. *Zool. J. Linn. Soc.*, 190(3), 788–823.
- Graham, A. (1999) Late Cretaceous and Cenozoic history of North American vegetation. New York: Oxford University Press.
- Guil, N. & Camacho, A.I. (2001) Historical Biogeography of *Iberobathynella* (Crustacea, Syncarida, Bathynellacea), an aquatic subterranean genus of Parabathynellids, endemic to the Iberian Peninsula. *Glob. Ecol. Biogeog.*, 10, 487–501.
- Guzik, M.T., Abrams, K.M., Cooper, S.J.B., Humphreys, W.F., Cho, J.-L. & Austin, A. (2008) Phylogeography of the ancient Parabathynellidae (Crustacea: Bathynellacea) from the Yilgarn region of Western Australia. *Invert. Syst.*, 22, 205–216.
- Heraty, J.M., Woolley, J.B., Hopper, K.R., Hawks, D.L., Kim, J.W. & Buffington, M. (2007) Molecular phylogenetics and reproductive incompatibility in a complex of cryptic species of aphid parasitoids. *Mol. Phylogenet. Evol.*, 45, 480–493.
- Höhna, S., Landis, M.J., Heath, T.A., Boussau, B., Lartillot, N., Moore, B.R., Huelsenbeck,

- J.P. & Ronquist, F. (2016) RevBayes: Bayesian phylogenetic inference using graphical models and an interactive model-specification language. *Syst. Biol.*, 65, 726–736.
- Hong, S.J. & Cho, J.-L. (2009) Three new species of *Billibathynella* from Wertern Australia (Crustacea, Syncarida, Parabathynellidae). *J. Nat. Hist.*, 43, 2365–2390.
- Huelsenbeck, J.P., Larget, B. & Alfaro, M.E. (2004) Bayesian Phylogenetic Model Selection Using Reversible Jump Markov Chain Monte Carlo. *Mol. Biol. Evol.*, 21, 1123–1133. doi.org/10.1093/molbev/msh123.
- Jablonski, D., Roy, K. & Valentine, J.W. (2006) Out of the tropics: evolutionary dynamics of the latitudinal diversity gradient. *Science*, 314, 102–106.
- Jones, R., Culver, D.C. & Kane, T.C. (1992) Are parallel morphologies of cave organisms the result of similar selection pressures?. *Evolution*, 46, 353–365.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Haeseler, A. von & Jermini, L.S. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods*, 14, 587–589.
- Kane, T.C., Culver, D.C. & Mathieu, J. (1994) Biotic fluxes and gene flow, pp. 245–259. In: J. Gibert, D.L. Danielopol, & J. Stanford (eds.), *Groundwater Ecology*. Academic Press, New York.
- Katoh, K. & Toh, H. (2008) Recent developments in the MAFFT multiple sequence alignment program. *Briefings Bioinf.*, 9, 286–298.
- Kearse, M., Moir, R. Wilson, A. Stones-Haves, S., Cheung, M., Sturrock, S., Buxton, S. Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649. doi.org/10.1093/bioinformatics/bts199.
- Kendall, D.G. (1948) On the generalized "birth-and-death" process. *Ann. Math. Stat.* 19.1, 1–15.
- Knowlton, N. (1993) Sibling species in the sea. *Ann. Rev. Ecol. Syst.*, 24, 189–216.
- Krisjman, W., Capella, W., Simon, D., Hilgen, F.J., Kowenhoven, T.J., Meijer, P.T., Sierro, F.J., Tulbure, M.A., van der Berg, B.C.J., van der Schee, M. & Flecher, R., (2018) The Gibraltar Corridor: Watergate of the Messinian Salinity Crisis. *Mar. Geol.*, 403, 238–246.
- Le Gac, M., Hood, M.E., Fournier, E. & Giraud, T. (2007) Phylogenetic evidence of host-specific cryptic species in the anther smut fungus. *Evolution*, 61, 15–26.
- Lefebure, T., Douady, C.J., Malard, F. & Gibert, J. (2007) Testing vicariance and cryptic diversity in a widely distributed groundwater amphipod (*Niphargus rhenorhodanensis*). *Mol. Phylog. Evol.*, 42, 676–686.
- Leigh, J.W. & Bryant, D. (2015) POPART: full-feature software for haplotype network construction. *Methods Ecol. Evol.*, 6, 1110–1116. doi.org/10.1111/2041-210X.12410.
- Lewis, P.O. (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. *Syst. Biol.*, 50, 913–925.
- Little, J., & Camacho, A. I. (2017) Morphological and molecular characterisation of a new genus and new species of Parabathynellidae (Crustacea: Syncarida) in Queensland, Australia. *Invert. Syst.*, 31, 208–219.
- Maddison, W.P. & Maddison, D.R. (2015) Mesquite: a modular system for evolutionary analysis. Version 3.04 <http://mesquiteproject.org>.
- Meleg, I.N., Zaksek, V., Cene Fiser, C., Kelemen, B.C. & Moldovan, O.T. (2013) Can environment predict cryptic diversity? The case of *Niphargus* inhabiting Western Carpathian groundwater. *PlosOne*, 8, e76760. [doi: 10.1371/journal.pone.0076760](https://doi.org/10.1371/journal.pone.0076760).
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *2010 gateway computing environments workshop (GCE)*. (p. 1–8). Ieee, 2010.
- Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., Haeseler, A. von & Lanfear, D., (2020) IQ-TREE 2: A new generation of phylogenetic analysis tool. *Mol. Biol. Evol.*, 37, 1530–1540. doi.org/10.1093/molbev/msaa011.

- R. (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.*, 37, 1530–1534. doi. org/10.1093/molbev/msaa015.
- Noodt, W. & Galhano, M.H. (1969) Studien an Crustacea Subterranea (Isopoda, Syncarida, Copepoda) aus dem Norden Portugals. *Publ. Inst. Zool. "Dr. A. Nobre"*, 107, 9–75.
- Papadopoulou, A., Anastasiou, I. & Vogler, A.P. (2010) Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Mol. Biol. Evol.* 27, 1659–1672.
- Perina, G., & Camacho, A.I. (2016) Permanent slides for morphological studies of small crustaceans: Serban's method and its variation applied on Bathynellacea (Malacostraca). *Crustaceana*, 89, 1161–1173.
- Perina, G., Camacho, A.I., Huey, J., Horwitz, P., & Koenders, A. (2019) The role of allopatric speciation and ancient origins of Bathynellidae (Crustacea) in the Pilbara (Western Australia): two new genera from the De Grey River catchment. *Contrib. Zool.*, 88(4), 452–497.
- Pfenninger, M. & Schwenk, K. (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evol. Biol.*, 7, 121.
- Pons, P., Jurado-Rivera, J.A., Jaume, D., Vonk, R. & Bauzá-Ribot, M.M. (2019) The age and diversification of metacrangonyctid subterranean amphipod crustaceans revisited. *Mol. Phylogenet. Evol.* 140, 106599.
- Pretus, J.L. (1989) Noves dades per a la distribució de l'estigofauna balear. *Endins*, 14–15, 61–64.
- Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.*, 61, 539–542.
- Rosenbaum, G., Lister, G.S. & Duboz, C. (2002) Reconstruction of the tectonic evolution of the western Mediterranean since the Oligocene. *Journal of the Virtual Explorer*, 8, 107–130.
- Schminke, H.K. (1973) Evolution, System und Verbreitungsgeschichte der Familie Parabathynellidae (Bathynellacea, Malacostraca). *Akademie der Wissenschaften und der Literatur Mainz, Mathematisch-Naturwissenschaftliche Klasse, Mikrofauuna des Meeresbodens* 24, 1–192.
- Schminke, H.K. (1974) Mesozoic intercontinental relationship as evidenced by Bathynellid Crustacea (Syncarida: Malacostraca). *Syst. Zool.*, 23, 157–164.
- Schminke, H.K. (1981) Adaptation of Bathynellacea (Crustacea, Syncarida) to life in the Interstitial ("Zoea Theory"). *Internat. Rev. Hydrobiol. Hydrog.*, 66, 575–637.
- Schminke, H.K. (2011) Arthropoda: Crustacea: Malacostraca: Bathynellacea: Parabathynellidae. V 21(1), pp 244. In: Flora and Fauna de Korea. Invertebrate Fauna of the World. National Institute of Biological Resources, Ministry of Environment, Republic of Korea.
- Schminke, H.K. & Noodt, W. (1988) Groundwater Crustacea of the Order Bathynellacea (Malacostraca) from North Amerika. *J. Crust. Biol.*, 8, 290–299.
- Schram, F.R. (1977) Paleozoogeography of late Paleozoic and Triassic Malacostraca. *Syst. Zool.*, 26, 367–379.
- Schram, F.R. (1984) Fossil Syncarida. *Transact. San Diego Soc. Nat. Hist.*, 20, 189–246.
- Serban, E. (1977) Sur les péreiopodes VIII males de *Iberobathynella* cf. *fagei* de Majorque (Bathynellacea, Parabathynellidae). *Crustaceana*, 33(1), 1–16.
- Serban, E. & Comas, J. (1978) Contribution à la connaissance du genre *Iberobathynella* Schminke: *I. asturiensis* n. sp. et *I. espaniensis* n. sp., nouvelles espèces d'Espagne (Bathynellacea, Parabathynellidae). *Trav. Inst. Spéol. "É. Racovitza"*, 17, 13–37.
- Serban, E. (1977) Sur les péreiopodes VIII males de *Iberobathynella* cf. *fagei* de Majorque (Bathynellacea, Parabathynellidae). *Crustaceana*, 33(1), 1–16.

- Simon, C.L., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved PCR primers. *Ann. Entomol. Soc. America*, 87, 51–701.
- Smith, S.A. & Donoghue, M.J. (2010) Combining historical biogeography with niche modelling in the *Caprifolium* clade of Lonicera (Caprifoliaceae, Dipsacales). *Syst. Biol.*, 59, 322–341.
- Stadler, T. (2009) On incomplete sampling under birth–death models and connections to the sampling-based coalescent. *J. Theoret. Biol.*, 261, 58–66.
- Swofford, D.L. (2002) PAUP*: Phylogeny Analysis Using Parsimony (*and other methods), version 4.0b10. Sunderland, MA: Sinauer Associates Inc.
- Thompson, J.D. (2005) Plant evolution in the Mediterranean. Oxford University Press.
- Trontelj, P., Blejec, A. & Fiser, C. (2012) Ecomorphological convergence of cave communities. *Evolution*, 66, 3852–3865.
- Yilmaz, P.O., Norton, I.O., Leary, D. & Chuchla, R.J. (1996) Tectonic evolution and paleogeography of Europe. In P. A. Ziegler, & F. Howath (Eds), *Peri-Tethys Memoirs 2: Structure and Prospects of Alpine Bassins and Forelands* (pp. 47–60). Paris, *Mém. Mus. Nat. Hist. Nat.*.
- Zagmajster, M., Malard, F., Eme, D. & Culver, D.C. (2018) Subterranean Biodiversity Patterns from Global to Regional Scales. Chapter, 9, 195–227. Moldovan et al., (eds). In “*Cave Ecology*”, Ecological Studies 235. https://doi.org/10.1007/978-3-319-98852-8_9.

RECEIVED: 16 JULY 2021 | 2 NOVEMBER 2021 |

ACCEPTED: 4 NOVEMBER 2021

EDITOR: R. VONK