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# Villastrigo, A

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# Cryptic lineages, cryptic barriers: Historical seascapes and oceanic fronts drive genetic diversity in supralittoral rockpool beetles (Coleoptera: Hydraenidae)

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Abstract:	Morphologically cryptic lineages confound many estimates of global biodiversity and are often discovered in ecologically specialised taxa, subject to strong morphological constraint. Such a situation may apply in many extreme environments, including supralittoral rockpools, where dramatic fluctuations in water availability and salinity impose strong selection pressures on the inhabitants. Here we explore the genetic diversity and phylogeography of supralittoral rockpool Ochthebius beetles in the Eastern Atlantic and Western Mediterranean, using a combination of mitochondrial and nuclear markers and dense geographical sampling of the three recognised widespread species. Our results point to the existence of morphologically cryptic lineages within all currently named taxa and suggest that the distribution of these is linked to both historical and contemporary marine hydrogeography; a combination of ocean currents and winds apparently driving the spatial patterns observed. The main contemporary barrier to dispersal for Ochthebius is located around the Ibiza Channel, whilst the Messinian Salinity Crisis appears to have been the ultimate driver of lineage diversification in these insects. Our results show that oceanographic processes do not just shape the evolution of fully marine species, but also impact significantly on the terrestrially-derived inhabitants of the coastal zone.



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# Abstract

Morphologically cryptic lineages confound many estimates of global biodiversity and are often discovered in ecologically specialised taxa, subject to strong morphological constraint. Such a situation may apply in many extreme environments, including supralittoral rockpools, where dramatic fluctuations in water availability and salinity impose strong selection pressures on the inhabitants. Here we explore the genetic diversity and phylogeography of supralittoral rockpool Ochthebius beetles in the Eastern Atlantic and Western Mediterranean, using a combination of mitochondrial and nuclear markers and dense geographical sampling of the three recognised widespread species. Our results point to the existence of morphologically cryptic lineages within all currently named taxa and suggest that the distribution of these is linked to both historical and contemporary marine hydrogeography; a combination of ocean currents and winds apparently driving the spatial patterns observed. The main contemporary barrier to dispersal for *Ochthebius* is located around the Ibiza Channel, whilst the Messinian Salinity Crisis appears to have been the ultimate driver of lineage diversification in these insects. Our results show that oceanographic processes do not just shape the evolution of fully marine species, but also impact significantly on the terrestriallyderived inhabitants of the coastal zone.

Keywords: cryptic species, rocky shores, dispersal, Mediterranean

#### 1. Introduction

Despite much discussion of global biodiversity in recent decades, we still lack an accurate picture of the number of species on earth, but can be confident that most multicellular organisms are arthropods, in both terrestrial and aquatic habitats. Like most authors, Stork (2018) argued that described species represent a small fraction of those extant, estimating that there are over 7 million species of arthropods on earth, the majority of which are both undescribed and insects. One of the reasons behind our uncertainty is the existence of morphologically cryptic species, which have been shown to occur widely across taxa and biogeographical regions (Adams et al., 2014; Fiser et al., 2017; Pfenninger & Schwenk, 2007), particularly in the light of molecular genetic analyses (e.g., Aguilar et al., 2017; Blair & Bryson, 2017; Müller et al., 2018; Puillandre et al., 2012). Whilst a relative lack of morphological divergence may sometimes reflect recent evolutionary divergence (e.g., Zúñiga-Reinoso & Benítez, 2015), the morphological stasis of cryptic species can also persist for long periods when taxa are subject to strong ecological constraints (Cai et al., 2019; Cerca et al., 2020; Fiser et al., 2017; Pfingstl et al., 2019).

Morphological stasis may occur in harsh environments where stabilizing selection can lead to a reduced range of phenotypic traits (Bickford et al., 2007). This is the case with many organisms inhabiting extreme environments such as caves (Zhang & Li, 2014), hypersaline waters (Arribas et al., 2013), mountain summits (Adams et al., 2014) or the intertidal zone (Pfingstl et al., 2019; 2021). Ecomorphological constraints also operate on aquatic organisms more generally, however, particularly through their hydrodynamics, something which increases the likelihood of cryptic species complexes being present in such habitats (Céréginho et al, 2018; Domenici et al., 2007; Jami et al., 2021; Knowlton, 1993). Whilst lineage divergence without population isolation certainly does occur, particularly in marine

scenarios (e.g., Bowen et al., 2013; Faria et al., 2021), speciation is thought much more likely to occur when there is an external barrier to gene flow, at least initially (Barraclough, 2019; Haak et al., 2019; Stock et al., 2019). Limitations to dispersal, and so gene flow, between populations may be rather cryptic themselves; marine systems providing good examples, including oceanic current systems which can be stable enough to act as geographical barriers for passively dispersing organisms. Examples of cryptic taxa apparently diverging as a result of such processes are varied, and include red algae (Billard et al., 2015), cave mysids (Rastorgueff et al., 2014) and chthamalid barnacles (Wares, 2020). In addition to obligately marine organisms, similar patterns have been demonstrated in some groups occupying the marine-terrestrial ecotone, both terrestrial and aquatic (Hurtardo et al., 2014; Santamaria et al, 2016; 2017; Greenan et al., 2018; Mbongwa et al., 2019; González-Castellano et al., 2020), many of which also involve morphologically cryptic lineages.

Supralittoral rockpools are complex and dynamic habitats, whose relatively simple communities reflect the challenges of living in these highly fluctuating environments (Vecchioni et al., 2021). Supralittoral pools undergo dramatic changes in water level, due to alternating cycles of rain and desiccation, and are also exposed to wide fluctuations in salinity as a result of such dilution and concentration events, coupled with occasional inundation by storms and spring tides (Powlik, 1999). Organisms permanently inhabiting such rockpools have to cope with dramatic changes in temperature and salinity, which present considerable physiological challenges (Gaines & Denny, 2007; Powlik & Lewis, 1996). Whilst the harsh environment of supralittoral pools may select for morphological stability, as discussed above, these habitats have, until recently, not been considered to harbour significant cryptic diversity in their inhabitants, since the ephemeral and spatially patchy nature of pool availability would be expected to select for strong dispersal abilities (see Bilton et al., 2001; Ribera, 2008), resulting in high levels of gene flow.

The depauperate fauna of supralittoral rockpools, coupled with their position between marine and terrestrial realms, means that these habitats have been relatively neglected in ecological and evolutionary studies. It is clear, however, that the fauna of these habitats is relatively uniform in many parts of the world (e.g. Orchymont, 1932; Alternat et al., 2012), pools generally supporting a combination of microcrustacea, dipteran larvae (mostly Chironomidae) and in some regions, including the Western Palaearctic, adults and larvae of ochthebiine beetles (Coleoptera: Hydraenidae). Of these, rockpool Ochthebius Leach, 1815 have received considerable attention in recent years, including research on their systematics. behaviour and physiology in various parts of the world (e.g., Bilton, 2021; Jäch & Delgado, 2017; Mirón-Gatón et al., 2022; Ribera & Foster, 2018; Ribera & Hernando, 2019; Sabatelli et al., 2016; 2021; Porretta & Urbanelli, 2012; Urbanelli, 2002; Urbanelli & Porretta, 2008; Villastrigo et al., 2020). The genus includes more than 500 species, and members of a number of lineages have shifted from inland waters to occupying supralittoral rockpools, including two in the western Palaearctic (Sabatelli et al., 2016; Villastrigo et al., 2019). Most *Ochthebius*, including rockpool taxa, are able to fly but their active dispersal capabilities may be limited by their small body size and weak flight (Urbanelli, 2002, unpublished observation), meaning that, like many small insects, they are likely to be largely subject to passive dispersal in air currents (see Bilton et al., 2001). Previous studies have argued for possible sympatric speciation in rockpool Ochthebius, with only minute morphological differences in male genitalia but high genetic divergence based on mitochondrial markers (Antonini et al., 2010; Audisio et al., 2010). More recently, Ribera & Foster (2018) described Ochthebius lanthanus Ribera & Foster, 2018 from Gran Canaria, which is morphologically

indistinguishable from its sister species *Ochthebius algicola* Wollaston, 1871 from Madeira, despite their estimated divergence time of ca. 14-15 MA (Villastrigo et al., 2020). Indeed, Sabatelli et al. (2016; 2021) and Villastrigo et al. (2019) have suggested that such cryptic species might be more common in these beetles, whose constrained morphology may hide a multitude of independent evolutionary lineages. Whilst work to date suggests this may indeed be the case in rockpool *Ochthebius* (e.g., Antonini et al., 2010; Audisio et al., 2010; Urbanelli, 2002; Urbanelli et al., 1996; Sabatelli et al., 2021), our understanding of the extent of this cryptic diversity and, crucially, what may drive it, remains very limited.

Here we use dense geographical sampling to study supralittoral rockpool *Ochthebius* populations from the Eastern Atlantic and Western/Central Mediterranean coasts, exploring phylogeographical patterns and the distribution of major genetic lineages. We employ a combination of mitochondrial and nuclear markers to determine the genetic diversity and phylogeographic structure of *Ochthebius*, and test whether historical and contemporary marine geographic barriers have driven genetic isolation between extant populations. Understanding the genetic diversity of these populations and their geographical subdivision sheds new light on the evolutionary biology of supralittoral rockpools and the influence the sea has on their inhabitants.

#### 2. Material and Methods

#### 2.1. Study area, study groups, and taxon sampling

Our study focussed on the *Ochthebius* beetles inhabiting rockpools of the Eastern Atlantic and Western/Central Mediterranean. We employed dense geographical sampling of populations in Iberia, in order to accurately characterise phylogeographic variation within *Ochthebius* as well as detect the distributional limits of genetic lineages. Members of two distinct groups of

*Ochthebius* are found in rockpools in this region: the *quadricollis* species group of the subgenus *Ochthebius* s. str., the so-called '*Calobius*' lineage, and the subgenus *Cobalius* (see Villastrigo et al., 2019). The former contains one species inhabiting the whole region, from the Eastern Atlantic to Central Mediterranean, *O. quadricollis* Mulsant, 1844, which overlaps with the frequently microsympatric, morphologically cryptic, *O. urbanelliae* Audisio, Trizzino & De Biase, 2010 on Italian Tyrrhenian coasts. *Cobalius* currently includes three species in the study area, *O. subinteger* Mulsant & Rey, 1861 and *O. lejolisii* Mulsant & Rey, 1861, considered endemic to Mediterranean and Atlantic coast, respectively, as well as the recently described *O. evae* Villastrigo, Hernando, Millán & Ribera, 2020. *O. evae* belongs to a newly recognised lineage within the subgenus *Cobalius* (the *biltoni* species group) whose flightless members inhabit rock crevices (Villastrigo et al., 2020) rather than pools. *Ochthebius evae* is known only from a very limited number of specimens and localities, in southern Iberia and Morocco, and as a consequence we do not consider this species further here. All known localities of the three species studied, including data gathered for this work, are mapped in Figs. S1-S3.

To understand current phylogeographical patterns and their evolutionary history, we compiled two datasets, examining different temporal and evolutionary scales. Dataset A included all currently recognised lineages within '*Calobius*' and *Cobalius*, all major lineages of the <u>tribe</u> Ochthebiini-tribe, plus a selection of other Hydraenidae (genera *Limnebius* Leach, 1815; *Laeliaena* Sahlberg, 1900 and *Hydraena* Kugelann, 1794) and Ptiliidae taxa as outgroups. This dataset was constructed using sequences from the GenBank database (Sayers et al., 2021), across five commonly used gene markers: two fragments of cytochrome *c* oxidase subunit I, 16S RNA plus tRNA-Leu and the 5' end of NADH dehydrogenase subunit I, an internal fragment of the large ribosomal subunit (28S), and an internal fragment of the

small ribosomal unit (18S). Dataset B mostly comprised samples of the three putative species inhabiting the study area, *O. quadricollis, O. lejolisii* and *O. subinteger*, to which we added existing sequences from GenBank. Beetles were collected mainly during extensive field campaigns from 2016-2019 from the Atlantic and Mediterranean coasts of the Iberian Peninsula and the Balearic Islands, plus samples taken in 2016 and 2017 from Atlantic and Mediterranean coasts of Morocco. All samples were collected by hand in the field using brushes and preserved in absolute ethanol. In total, the dataset A comprised 79 individuals, whilst dataset B included 171 individuals from 57 different localities (see Table S1). Geographical information of localities is available in Table 1 and Fig. 1.

### 2.2. DNA extraction and sequencing

We randomly selected three specimens of each species per population to generate the novel sequences used in dataset B. In total, DNA from 160 specimens was extracted, using a non-destructive protocol following the instructions of Qiagen DNeasy Blood & Tissue Kit (Hildesheim, Germany). Specimens were subsequently dried and kept in the collection of the Aquatic Ecology research group at the University of Murcia. We amplified and sequenced two genes: i) the 3' end of mitochondrial gene cytochrome *c* oxidase subunit I (primers Pat and Jerry, Simon et al., 1994), and ii) a fragment of the nuclear gene wingless gene (primers Wg550F and WgAbRZ, Wild & Maddison, 2008). Sequences were assembled and edited using Geneious v10.1 (Kearse et al. 2012) and were deposited in the ENA database (see Table S1 for a complete list of specimens, localities and accession numbers).

## 2.3. Phylogenetic analyses

Sequences in dataset A were aligned using the G-INS-I algorithm implemented in MAFFT 7.407 (Katoh & Standley, 2013) and concatenated. PartitionFinder v2.1.1 (Lanfear et al., 2016) was used to select the optimal partition scheme and the evolutionary models based on AIC selection criteria, considering initially one partition per gene fragment. A phylogenetic tree was reconstructed using Bayesian Inference in BEAST v1.10.4 (Suchard et al. 2018), using the partition scheme and evolutionary models selected by PartitionFinder, plus one strict clock per partition and Yule speciation process as priors. Four different calibration points were used in the phylogenetic analysis: i) the estimated split of Ptiliidae and Hydraenidae at ca. 165 Ma (95% confidence interval 110-195 Ma) by Mckenna et al. (2019), which is compatible with prior analyses (Hunt et al. 2007; McKenna et al. 2015); ii) the Burmese amber fossil Archaeodraena cretacea Jäch & Yamamoto, 2017, likely to be a member of the crown Hydraenidae (Yamamoto et al., 2017), with a hard minimum bound of 98.79 Ma (see Shi et al. (2012)) and the split between Macaronesian endemic species setting hard maximum bounds in uniform priors as in Villastrigo et al. (2020); iii) the separation between the species of the Canaries and Madeira (14.3 Ma, Machado et al., 2017) and iv) the separation between the species of the islands of Sal and Santiago in the Cabo Verde archipelago (10.3 Ma, Cunha et al. 2017). The analysis was run for 100 million generations, and convergence checked to measure the burn-in fraction using Tracer v1.7.1 (Rambaut et al., 2018). The maximum clade credibility tree was reconstructed using TreeAnnotator v1.10.4 included in Beast v1.10.4.

Dataset B was divided into three subsets, based on each of the putative species, *O. quadricollis*, *O. lejolisii* and *O. subinteger*. Owing to possible nucleotide ambiguities due to the use of Sanger sequences in diploid organisms, haplotypes were inferred using PHASE (Stephens et al., 2001) for 1000 iterations, applying a burn-in of 100 and thinning parameter

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5. To interpret the biogeographical structure of rockpool Ochthebius, we performed a spatial analysis of molecular variation (SAMOVA; Dupanloup et al., 2002), which automatically defines clusters of populations that are homogeneous both geographically and genetically, maximizing differences amongst clusters, using SAMOVA 2.0 (available at http://cmpg.unibe.ch/software/samova2/). We used a variable number of possible groups (k, ranging from 2 to 8) per gene. We selected the homogeneous number of clusters (k) that supported the highest increase of the FCT parameter, relative to the FCT value for k-1 clusters (see Dupanloup et al., 2002). Haplotype networks were estimated using TCS v1.21 (Clement et al., 2000) with gaps as missing data and a fixed connection limit of 95%. Enhanced visualization of TCS networks was performed using tcsBU (Múrias dos Santos et al., 2016), using defined geographical areas based on features known to have driven speciation in other animal groups: the Gibraltar Strait (Lowe et al., 2012), the Almeria-Oran Front (González-Castellano et al., 2020) and the Ibiza Channel (García-Mechán et al., 2012). In addition, we incorporated additional putative boundaries that may promote genetic differentiation: i) the Alboran Sea was divided into two different regions, east and west (Booth-Rea et al., 2018); and ii) the Balearic Islands were divided into two entities corresponding with their extent during the Last Glacial Maximum (LGM), the Pityusic and Gymnesian Islands, respectively (Mora et al., 2017). All these barriers affect not only the connectivity of terrestrial taxa, but also drive the isolation of marine organisms due to the dynamics of oceanic currents (e.g., the Ibiza Channel separates the Iberian Peninsula and the Balearic Islands, where details of oceanic geology result in separate Northern and Balearic Currents, which in turn shape coastal wind patterns, see Pinot et al., 2002). In addition to haplotype networks, gene trees (see Figs. S4 and S5 for COI and wingless genes respectively) were constructed using the raw

sequences in IQTree v2.0 (Minh et al., 2020), including bootstrap and SH-ALRT metrics (both 1000 replicates) under the preferred model selected by IQTree (Chernomor et al., 2016).

#### 3. Results

#### 3.1. Calibrated phylogenetic tree for rockpool Ochthebius

The selected partition scheme included one partition per mitochondrial fragment, plus an additional partition for pooled nuclear genes. Three different evolutionary models were preferred: i) GTR+G+I for the 3' end of cytochrome *c* oxidase subunit I and the pooled nuclear genes, ii) HKY+G+I for the 5' end of cytochrome *c* oxidase subunit I, and iii) TN93+G+I for 16S RNA. The resulting phylogenetic tree (Fig. 2) revealed a similar topology to that in a recent revision of Ochthebiini (Villastrigo et al., 2019), but with different estimated ages for the study groups. *Cobalius* and '*Calobius*' were estimated as originating ca. 30 Ma and ca. 25 Ma respectively, whilst Villastrigo et al. (2019) estimated older ages (ca. 60 and ca. 55 Ma respectively). As expected, we verified that *O. subinteger* and *O. lejolisii* are sister species that diverged ca. 14 Ma from a common ancestor. Each of the putative species studied was divided into distinct, divergent lineages, of considerable apparent age: 1) *O. quadricollis* originated ca. 19 Ma, with three topological splits detected at ca. 17, 7 and 2 Ma respectively; 2) *O. subinteger* originated at 14 Ma, with a single topological split at ca. 6 Ma; and 3) *O. lejolisii* originated 14 Ma, with two relatively recent splits at ca. 2 and 1 Ma respectively.

#### 3.2. Intraspecific genetic variability

We detected moderate genetic variability within each of the studied lineages (Table 2); >6.5% for the 3' end of cytochrome *c* oxidase subunit I in two out of three studied whilst *O. lejolisii* 

displays variability >3%. Variations in the nuclear wingless gene were moderate, in line with expected lower divergence of nuclear genes, reaching 2.47% in *O. quadricollis*, 1.27% in *O. subinteger* and 1.29% in *O. lejolisii*. We observed high haplotype diversity for both COI and wingless genes (greater than 0.7 - see Table 2), together with relatively low nucleotide diversity (especially for wingless - see Table 2).

In SAMOVA (Table 3; see Tables S2-S4 for details on *O. quadricollis*, *O. subinteger* and *O. lejolisii* respectively), we found a considerable increase in FCT values when 3 clusters were defined for *O. quadricollis* (Fig. S6) and *O. lejolisii* (Fig. S7) for both mitochondrial and nuclear genes, and a more complex pattern in *O. subinteger*, with 4 homogeneous clusters with COI but only 3 for wingless (Fig. S8). Detailed results of analyses for each species are now presented in turn.

# 3.3. Ochthebius quadricollis geographic structure and haplotype networks

The best fitted geographical clusters based on SAMOVA (Fig. S6) revealed a common scenario for both genes, with a geographical barrier roughly corresponding with the Ibiza Channel and a second barrier where the Alboran Sea is divided into western and eastern basins, based on anticyclonic oceanic gyres. These barriers match the two oldest topological splits within *O. quadricollis*, separating three different lineages located on the Atlantic coast and the western Alboran Sea, the eastern Alboran Sea up to the Ibiza Channel, including the Balearic Islands, and on coasts north of the Ibiza Channel, including France, Italy and Malta. These same clusters can be seen in the haplotype network for the wingless gene (Fig. 3). In contrast, the mitochondrial network comprises four disjointed clusters, in which populations from southeastern Iberia are grouped either with those from the eastern Alboran Sea or with those from the Balearic Islands. In all the analyses, samples from the Balearic Islands were

associated with populations located south of the Ibiza Channel. Detected geographical barriers did not include either the Gibraltar Strait or the Almeria-Oran front, with either approach.

# 3.4. Ochthebius subinteger geographic structure and haplotype networks

*O. subinteger* was found in coastal rockpools in the Central Mediterranean basin plus the eastern coast of Spain up to Cartagena. The restricted geographical distribution of this species in the study region (Fig. S2) limited the number of samples, and so the estimated number of SAMOVA clusters auto-generated using the wingless gene. As with *O. quadricollis*, populations north and south of the Ibiza Channel were separated in the best-fitted scenarios generated by SAMOVA (clusters with k = 4 for COI and k = 3 for wingless; Fig. S7). This split is also reflected in the haplotype networks (Fig. 4), which also exhibit a notable but unexpected geographical structure, with a split between different populations on the Balearic Islands. Beetles from the Pityusic islands - the ones closest to the Iberian Peninsula - are associated with populations from south-eastern Spain, whilst samples from the Gymnesian islands (i.e., Menorca) are distributed in both networks. The COI of one Menorcan specimen clustered with sequences from north of the Ibiza Channel, whilst the wingless sequence was identical to some from specimens south of this channel.

# 3.5. Ochthebius lejolisii geographic structure and haplotype network

The putative species with the broadest geographical distribution is also the one with the most complex genetic structure. Traditionally believed to be an endemic Atlantic taxon, *O. lejolisii* was detected here for the first time in the Mediterranean, in multiple localities throughout south Spain up to the Ibiza Channel. SAMOVA results (Fig. S8) were consistent with 3 clusters in both mitochondrial and nuclear genes, but with no consistent pattern, except the

separation of European Atlantic populations from the rest, with a mixture of Mediterranean and African populations in the case of the mitochondrial marker, whilst the nuclear wingless gene separates European and African populations at Gibraltar Strait. Haplotype networks (Fig. 5) also suggest a complex history, but with COI also revealing a separation of samples from the European Atlantic coast. In the case of wingless, samples from the Atlantic coast of Morocco are separated from those in the Mediterranean, whilst with COI Moroccan samples closest to the Iberian Peninsula (Larache, Asilah and Cap Spartel) are more similar to those in the Mediterranean than others in the Atlantic.

#### 4. Discussion

Our study reveals the presence of cryptic lineages, often with high genetic and haplotype divergence and marked phylogeographic structure in coastal *Ochthebius* populations in the Atlanto-Mediterranean region. Whilst previous studies have also suggested that these rockpool beetles harbour considerable cryptic diversity (e.g., Sabatelli et al., 2016, Villastrigo et al., 2019; 2020), ours is the first investigation to employ detailed enough sampling to identify the drivers of such diversity. In all three species, a combination of historical and contemporary marine hydrogeography appears to shape the distribution of lineages in these small and flying insects, whose diversification has occurred over a range of spatial and temporal scales.

#### 4.1. Calibrated phylogenetic tree

Our calibrated molecular phylogeny reflects the most up-to-date information on the origin and evolution of '*Calobius*' and *Cobalius*. This phylogeny is highly congruent in both topology and timing with previous phylogenetic studies of *Cobalius* (Villastrigo et al., 2020), but

suggests slightly older divergence times than those proposed by Sabatelli et al. (2021). Our analyses also suggest a very different age estimate for '*Calobius*' than previous studies (17 Ma -Sabatelli et al., 2016; 55 Ma - Villastrigo et al., 2019). These differences likely result from the previous use of less precise calibrations (Sabatelli et al., 2016, 2021; Villastrigo et al., 2019), limited taxon sampling (Sabatelli et al., 2016) and the absence of comprehensive phylogenies to contextualise the calibration (Sabatelli et al., 2021). Whilst Sabatelli et al. (2016; 2021) employed sequence divergence rates estimated for a closely related family and Villastrigo et al. (2019) used a secondary calibration point, we opted for an integrative approach including all major lineages within the genus *Ochthebius*, and not only secondary calibration points but also one fossil and two biogeographic events to set hard age constraints (e.g. the geological origin of some Macaronesian islands, see Machado et al. 2017; Cunha et al. 2017), all approaches likely to result in more accurate phylogenetic dating.

#### 4.2. *Phylogeographic structure and geographical patterns*

We consistently detected a geographical barrier in the region of the Ibiza Channel for species inhabiting both sides (*O. quadricollis* and *O. subinteger*). We also found that *O. lejolisii* reaches, but does not pass the Ibiza Channel, the first time this species, long considered an Atlantic endemic, has been detected in the Mediterranean. The location of the Ibiza Channel barrier coincides with the area where the Northern Current and the Balearic Front are responsible for most of the surface-flow characteristics of the Balearic Sea. In all cases, the cluster of populations from south of the Ibiza Channel itself. Oceanic currents in the Channel region are known to influence the phylogeography of a number of fully marine taxa (Schunter et al., 2011; García-Mechán et al., 2012). In the Ibiza Channel, northwestern Mediterranean

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water is redirected northwards, following the Balearic coast, with just a small fraction of the current actually crossing the channel itself (El-Geziry & Bryden, 2010). The Ibiza Channel may shape Ochthebius lineage structure through associated coastal wind currents determining their passive dispersal (Arribas et al., 2012) and/or dispersal of individuals of these salinitytolerant beetles in marine currents themselves (see e.g., Niikura et al., 2015). The degree to which the Ibiza Channel could serve as a geographical barrier in this way will vary seasonally; whilst the Northern Current is affected by an anticyclonic gyre for almost the whole year, limited flow does occur from north to south during winter (Pinot et al, 2002). An additional explanation for the genetic structuring detected around this region may involve the scarcity of suitable habitat on the Iberian coast north of the Ibiza Channel, where rocky coastline gives way to a sandy coast for around 250 km up to the Ebro Delta. The Ibiza Channel may reduce or completely block gene flow, depending on the season, and the absence of suitable habitats in adjacent mainland Iberia enhance the isolation of lineages. Previous studies, whilst much less extensive than ours, have also suggested genetic differences in O. quadricollis populations in this region. Sabatelli et al. (2016) found that populations from Calpe (southern coast of the Ibiza Channel) represent a different molecular entity to those from Italy. Villastrigo et al. (2019; 2020) also detected considerable genetic variation in O. quadricollis, across specimens from the Atlantic coast and Mediterranean coasts of Spain, France and Italy.

The estimated timescale of lineage divergence in *O. subinteger* suggests a role for the Messinian Salinity Crisis (MSC) (e.g., García-Castellanos et al., 2009; Krijgsman et al., 1999) in establishing present genetic diversity patterns. As well as separating the Mediterranean and Atlantic basins, the MSC also connected the Balearic Islands with the Iberian Peninsula (Mas et al., 2018), preventing gene flow between mainland Iberian populations spanning this

region, but favouring it between the Iberian Peninsula and the present-day Balearic Islands. This situation ended in the Zanclean Flood ca. 5.33 MA, when Balearic populations were once again isolated from the mainland. This pattern of connectivity and isolation is reflected in endemic Balearic species whose closest relatives inhabit the Iberian Peninsula, including some *Ochthebius* species (Jäch, 2000) and other animal groups as diverse as spiders (Mora et al., 2017) and mammals (Torres-Roig et al., 2019).

Evidence for the impact of a second geographical barrier was detected only in O. quadricollis, where populations from the Atlantic coast and western Alboran Sea were separated from the rest. The Alboran Sea is divided by two surface water gyres, whose interface may operate as a barrier. Atlantic Ocean waters enter the Mediterranean through the Gibraltar Strait, where their surface component becomes trapped in the permanent western Alboran gyre (García-Ladona, 2017; Maggi and González-Wangüemert, 2015). This barrier coincides with a possible long-term marine refuge for Mediterranean species that provided suitable habitat during the MSC (Booth-Rea et al., 2018), reconnecting with the Atlantic coast after the opening of the Gibraltar Strait ca. 5.3 MA (Andújar et al., 2012). The evolutionary history of O. quadricollis s. lat. spans approximately 20 million years, with two topological splits at ca. 17 and ca. 7 MA, respectively. The first split involves isolation of central Mediterranean basin populations from western ones. The second, more recent, split may be related to the MSC, during which populations in the Alboran Sea and the rest of the Iberian Peninsula would have been isolated in various "patches" or sub-basins within the Mediterranean. Such isolation would have driven genetic differentiation, with the subsequent refilling of the Mediterranean contribution to the current phylogeographic structure through recolonization of coastlines.

This study revealed the presence of *O. lejolisii* in the Mediterranean for the first time, this supposed Atlantic species reaching the Ibiza Channel. The timing of colonization of the Mediterranean by this species is unclear, but recent invasion has been suggested for some coastal taxa such as the gastropod *Nassarius reticulatus* Linnaeus, 1758 (Albaina et al., 2012). *O. lejolisii* and *O. subinteger*, were previously thought to be allopatric, separated by some barrier in southern Spain (e.g., the Gibraltar Strait). Our results instead demonstrate that the two species overlap in part of their distributional range and may indeed occur in true sympatry in southeastern Spain. Both species were found at one of our study sites (Cala Reona, Murcia), but, interestingly, in different years (*O. subinteger* in 2016 and *O. lejolisii* in 2019). These two species are estimated to have diverged ca.15 MYA, but despite their considerable genetic divergence we were not able to detect any unambiguous morphological characters by which they could be distinguished. Jäch (1989) in his morphological revision of *Cobalius* separated *O. lejolisii* and *O. subinteger* on pronotal punctuation. These differences separate specimens from Atlantic versus Mediterranean rockpools but not specimens of the two lineages collected in the Mediterranean.

As previously highlighted, a key trait underlying the genetic structure of coastal *Ochthebius* populations is their dispersal capabilities. Our results suggest that passive dispersal through coastal winds and/or marine currents may be the main way in which these insects disperse over medium to large spatial scales. Long-distance dispersal by wind currents is recognised as an important factor in the colonization dynamics of small arthropods (Gillespie et al., 2012, Beltrán Epele et al., 2021), including many aquatic taxa (Bilton et al., 2001). In coastal *Ochthebius*, another factor that may promote gene flow amongst populations is the action of cyclones (Lionello et al., 2006). In such conditions, supratidal environments are directly exposed to tidal inundation, and resistant stages, including eggs, can be dispersed

in ocean currents, as has been reported in springtails (McGaughran et al., 2010) and mites (Pfingst, 2013). Authors' unpublished observations have revealed that all life history stages of *O. lejolisii* are able to survive for weeks across a wide range of salinities, including that of oceanic water.

Overall, our results demonstrate that surface ocean currents, and their associated winds, may fundamentally shape the evolutionary history of non-strictly marine inhabitants of the coastal zone. We establish the presence of strongly divergent cryptic lineages in two of our study species, moderate divergent cryptic lineages in the third, and show that the distribution of these lineages appears to have been shaped by a combination of historical and contemporary changes to the coastal seascapes of the Atlantic Ocean and the Mediterranean Sea. Whilst we refrain from formal species descriptions at present, it seems highly likely that the lineages we detect correspond to cryptic species, whose distribution should be considered in biodiversity assessments of the coastal zone.

#### **Data Accessibility**

DNA sequences generated during this study are uploaded to the ENA and GenBank databases. Accession numbers are available in Table S1.

#### References

- Adams M, Raadik TA, Burridge CP, Georges A. 2014. Global biodiversity assessment and hyper-cryptic species complexes: More than one species of elephant in the room? *Systematic Biology*, 63, 518–533. <u>https://doi.org/10.1093/sysbio/syu017</u>
- Aguilar A, Maeda-Martínez AM, Murugan G, Obregón-Barboza H, Rogers R, McClintock K, Krumm JL. 2017. High intraspecific genetic divergence in the versatile fairy shrimp *Branchinecta lindahli* with a comment on cryptic species in the genus *Branchinecta* (Crustacea: Anostraca). *Hydrobiologia*, 801, 59–69. <u>https://doi.org/10.1007/s10750-017-3283-3</u>

<ul> <li>Altermatt F, Bieger A, Morgan SG. 2012. Habitat characteristics and metapopulation dynamics of the copepod <i>Tigriopus californicus. Marine Ecology Progress Series</i>, 468, 85-93.</li> <li>Andůjar C, Serrano J, Gómez-Zurita J. 2012. Winding up the molecular clock in the genus <i>Carabus</i> (Coleoptera: Carabidae): assessment of methodological decisions on rate and node age estimation. <i>BMC Evolutionary Biology</i>, 12(1), 1-17.</li> <li>Antonini G, Audisio P, Mancini E, de Biase A, Tronci C, Rossetti G, Trizzino M., 2010. Molecular phylogeography of two Italian sibling species of 'Calobius' (Coleoptera, Hydraenidae, Ochthebiinae) inhabiting mediterranean marine rock-pools. <i>Marine Biology</i>, 157, 371–381. https://doi.org/10.1007/s00227-009-1324-9</li> <li>Arribas P, Abellán P, Velasco J, Bilton DT, Millán A, Sánchez-Fernández D. 2012. Evaluating drivers of vulnerability to climate change: a guide for insect conservation strategies. <i>Global Change Biology</i>, 18(7), 2135-2146.</li> <li>Arribas P, Andújar C, Sánchez-Fernández D, Abellán P, Millán A. 2013. Integrative taxonomy and conservation of cryptic beetles in the Mediterranean region (Hydrophilidae). <i>Zoologica Scripta</i>, 42(2), 182-200.</li> <li>Audisio P, Trizzino M, de Biase A, Rossetti G, Mancini E, Antonini G. 2010. Molecular and morphological evidence of a new sibling species of 'Calobius' (Coleoptera: Hydraenidae of the <i>C. quadricollis</i> complex from peninsular Italy. <i>Italian Journal of Zoology</i>, 77, 29–37. https://doi.org/10.1080/11250000902845738</li> <li>Barraclough TG. 2019. What causes speciation? In <i>The Evolutionary Biology of Species</i>, Oxford University Press, 83-109.</li> <li>Beltrán Epele L, Dos Santos DA, Sarremejane R, Grech MG, Macchi PA, Manzo LM, Miscrendino ML, Bonada N, Cañedo-Argüelles M. 2021. Blowin'in the wind: Wind directionality affects wetland invertebrate metacommunities in Patagonia. <i>Global Ecology and Biogeography</i>, 30(6), 1191-1203. https://doi.org/10.1111/geb.13294</li> <li>Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Win</li></ul>	Albaina N, Olsen J Nassarius nitio colonization p	L, Couceiro L, Ruiz JM <i>lus</i> (Gastropoda): phylog athways. <i>Marine Biolog</i>	, Barreiro, R. 2012. I geographic evidence y, 159(9), 1871-1884	Recent history of the Europea of glacial refugia and 4.
<ul> <li>Andújar C, Serrano J, Gómez-Zurita J. 2012. Winding up the molecular clock in the genus <i>Carabus</i> (Coleoptera: Carabidae): assessment of methodological decisions on rate and node age estimation. <i>BMC Evolutionary Biology</i>, 12(1), 1-17.</li> <li>Antonini G, Audisio P, Mancini E, de Biase A, Tronci C., Rossetti G., Trizzino M., 2010. Molecular phylogeography of two Italian sibling species of 'Calobius' (Coleoptera, Hydraenidae, Ochthebiinae) inhabiting mediterranean marine rock-pools. <i>Marine Biology</i>, 157, 371–381. https://doi.org/10.1007/s00227-009-1324-9</li> <li>Arribas P, Abellán P, Velasco J, Bilton DT, Millán A, Sánchez-Fernández D. 2012. Evaluating drivers of vulnerability to climate change: a guide for insect conservation strategies. <i>Global Change Biology</i>, 18(7), 2135-2146.</li> <li>Arribas P, Andújar C, Sánchez-Fernández D, Abellán P, Millán A. 2013. Integrative taxonomy and conservation of cryptic beetles in the Mediterranean region (Hydrophilidae). <i>Zoologica Scripta</i>, 42(2), 182-200.</li> <li>Audisio P, Trizzino M, de Biase A, Rossetti G, Mancini E, Antonini G. 2010. Molecular and morphological evidence of a new sibling species of 'Calobius' (Coleoptera: Hydraenidae of the <i>C. quadricollis</i> complex from peninsular Italy. <i>Italian Journal of Zoology</i>, 77, 29–37. https://doi.org/10.1080/1125000902845738</li> <li>Barraclough TG. 2019. What causes speciation? In <i>The Evolutionary Biology of Species</i>, Oxford University Press, 83-109.</li> <li>Beltrán Epele L, Dos Santos DA, Sarremejane R, Grech MG, Macchi PA, Manzo LM, Miserendino ML, Bonada N, Cañedo-Argüelles M. 2021. Blowin'in the wind: Wind directionality affects wetland invertebrate metacommunities in Patagonia. <i>Global Ecology and Biogeography</i>, 30(6), 1191-1203. https://doi.org/10.1111/geb.13294</li> <li>Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. <i>Trends in Ecology and Evolution</i>, 22(3), 148-155.</li> <li>Billard F, Reyes J, Mansilla A, Fau</li></ul>	Altermatt F, Bieger dynamics of th 85-93.	A, Morgan SG. 2012. I e copepod <i>Tigriopus ca</i> .	Habitat characteristic lifornicus. Marine Ed	es and metapopulation cology Progress Series, 468,
<ul> <li>Antonini G, Audisio P, Mancini E, de Biase A, Tronci C., Rossetti G., Trizzino M., 2010. Molecular phylogeography of two Italian sibling species of 'Calobius' (Coleoptera, Hydraenidae, Ochthebiinae) inhabiting mediterranean marine rock-pools. <i>Marine Biology</i>, 157, 371–381. https://doi.org/10.1007/s00227-009-1324-9</li> <li>Arribas P, Abellán P, Velasco J, Bilton DT, Millán A, Sánchez-Fernández D. 2012. Evaluating drivers of vulnerability to climate change: a guide for insect conservation strategies. <i>Global Change Biology</i>, 18(7), 2135-2146.</li> <li>Arribas P, Andújar C, Sánchez-Fernández D, Abellán P, Millán A. 2013. Integrative taxonomy and conservation of cryptic beetles in the Mediterranean region (Hydrophilidae). <i>Zoologica Scripta</i>, 42(2), 182-200.</li> <li>Audisio P, Trizzino M, de Biase A, Rossetti G, Mancini E, Antonini G. 2010. Molecular and morphological evidence of a new sibling species of 'Calobius' (Coleoptera: Hydraenidae of the <i>C. quadricollis</i> complex from peninsular Italy. <i>Italian Journal of Zoology</i>, 77, 29–37. https://doi.org/10.1080/1125000902845738</li> <li>Barraclough TG. 2019. What causes speciation? In <i>The Evolutionary Biology of Species</i>, Oxford University Press, 83-109.</li> <li>Beltrán Epele L, Dos Santos DA, Sarremejane R, Grech MG, Macchi PA, Manzo LM, Miserendino ML, Bonada N, Cañedo-Argüelles M. 2021. Blowin'in the wind: Wind directionality affects wetland invertebrate metacommunities in Patagonia. <i>Global Ecology and Biogeography</i>, 30(6), 1191-1203. https://doi.org/10.1111/geb.13294</li> <li>Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. <i>Trends in Ecology and Evolution</i>, 22(3), 148-155.</li> <li>Billard E, Reyes J, Mansilla A, Faugeron S, Guillemin ML. 2015. Deep genetic divergence between austral populations of the red alga <i>Gigartina skottsbergii</i> reveals a cryptic species endemic to the Antarctic continent. <i>Polar Biology</i>, 38, 2021–2034. https://doi.org/10.1007/s0030</li></ul>	Andújar C, Serranc <i>Carabus</i> (Cole node age estim	) J, Gómez-Zurita J. 201 optera: Carabidae): asse nation. <i>BMC Evolutionar</i>	2. Winding up the measurement of methodology, 12(1), 1-	nolecular clock in the genus ogical decisions on rate and 17.
<ul> <li>Arribas P, Abellán P, Velasco J, Bilton DT, Millán A, Sánchez-Fernández D. 2012. Evaluating drivers of vulnerability to climate change: a guide for insect conservation strategies. <i>Global Change Biology</i>, 18(7), 2135-2146.</li> <li>Arribas P, Andújar C, Sánchez-Fernández D, Abellán P, Millán A. 2013. Integrative taxonomy and conservation of cryptic beetles in the Mediterranean region (Hydrophilidae). <i>Zoologica Scripta</i>, 42(2), 182-200.</li> <li>Audisio P, Trizzino M, de Biase A, Rossetti G, Mancini E, Antonini G. 2010. Molecular and morphological evidence of a new sibling species of 'Calobius' (Coleoptera: Hydraenidae of the <i>C. quadricollis</i> complex from peninsular Italy. <i>Italian Journal of Zoology</i>, 77, 29–37. https://doi.org/10.1080/11250000902845738</li> <li>Barraclough TG. 2019. What causes speciation? In <i>The Evolutionary Biology of Species</i>, Oxford University Press, 83-109.</li> <li>Beltrán Epele L, Dos Santos DA, Sarremejane R, Grech MG, Macchi PA, Manzo LM, Miserendino ML, Bonada N, Cañedo-Argüelles M. 2021. Blowin'in the wind: Wind directionality affects wetland invertebrate metacommunities in Patagonia. <i>Global Ecology and Biogeography</i>, 30(6), 1191-1203. https://doi.org/10.1111/geb.13294</li> <li>Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. <i>Trends in Ecology and Evolution</i>, 22(3), 148-155.</li> <li>Billard E, Reyes J, Mansilla A, Faugeron S, Guillemin ML. 2015. Deep genetic divergence between austral populations of the red alga <i>Gigartina skottsbergii</i> reveals a cryptic species endemic to the Antarctic continent. <i>Polar Biology</i>, 38, 2021–2034. https://doi.org/10.1007/s00300-015-1762-4</li> <li>Bilton DT, Freeland JR, Okamura B. 2001. Dispersal in freshwater invertebrates. <i>Annual Review of Ecology and Systematics</i>, 32(1), 159-181.</li> </ul>	Antonini G, Audisi Molecular phy Hydraenidae, ( <i>Biology</i> , 157, 2	o P, Mancini E, de Bias logeography of two Itali Ochthebiinae) inhabiting 371–381. <u>https://doi.org</u> /	e A, Tronci C., Ross an sibling species of mediterranean mari 10.1007/s00227-009	etti G., Trizzino M., 2010. f 'Calobius' (Coleoptera, ine rock-pools. <i>Marine</i> 9-1324-9
<ul> <li>Arribas P, Andújar C, Sánchez-Fernández D, Abellán P, Millán A. 2013. Integrative taxonomy and conservation of cryptic beetles in the Mediterranean region (Hydrophilidae). <i>Zoologica Scripta</i>, 42(2), 182-200.</li> <li>Audisio P, Trizzino M, de Biase A, Rossetti G, Mancini E, Antonini G. 2010. Molecular and morphological evidence of a new sibling species of 'Calobius' (Coleoptera: Hydraenidae of the <i>C. quadricollis</i> complex from peninsular Italy. <i>Italian Journal of Zoology</i>, 77, 29–37. https://doi.org/10.1080/11250000902845738</li> <li>Barraclough TG. 2019. What causes speciation? In <i>The Evolutionary Biology of Species</i>, Oxford University Press, 83-109.</li> <li>Beltrán Epele L, Dos Santos DA, Sarremejane R, Grech MG, Macchi PA, Manzo LM, Miserendino ML, Bonada N, Cañedo-Argüelles M. 2021. Blowin'in the wind: Wind directionality affects wetland invertebrate metacommunities in Patagonia. <i>Global Ecology and Biogeography</i>, 30(6), 1191-1203. https://doi.org/10.1111/geb.13294</li> <li>Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. <i>Trends in Ecology and Evolution</i>, 22(3), 148-155.</li> <li>Billard E, Reyes J, Mansilla A, Faugeron S, Guillemin ML. 2015. Deep genetic divergence between austral populations of the red alga <i>Gigartina skottsbergii</i> reveals a cryptic species endemic to the Antarctic continent. <i>Polar Biology</i>, 38, 2021–2034. https://doi.org/10.1007/s00300-015-1762-4</li> <li>Bilton DT, Freeland JR, Okamura B. 2001. Dispersal in freshwater invertebrates. <i>Annual Review of Ecology and Systematics</i>, 32(1), 159-181.</li> </ul>	Arribas P, Abellán Evaluating dri strategies. <i>Glo</i>	P, Velasco J, Bilton DT vers of vulnerability to c bal Change Biology, 18	, Millán A, Sánchez- limate change: a gui (7), 2135-2146.	-Fernández D. 2012. de for insect conservation
<ul> <li>Audisio P, Trizzino M, de Biase A, Rossetti G, Mancini E, Antonini G. 2010. Molecular and morphological evidence of a new sibling species of 'Calobius' (Coleoptera: Hydraenidae of the <i>C. quadricollis</i> complex from peninsular Italy. <i>Italian Journal of Zoology</i>, 77, 29–37. https://doi.org/10.1080/11250000902845738</li> <li>Barraclough TG. 2019. What causes speciation? In <i>The Evolutionary Biology of Species</i>, Oxford University Press, 83-109.</li> <li>Beltrán Epele L, Dos Santos DA, Sarremejane R, Grech MG, Macchi PA, Manzo LM, Miserendino ML, Bonada N, Cañedo-Argüelles M. 2021. Blowin'in the wind: Wind directionality affects wetland invertebrate metacommunities in Patagonia. <i>Global Ecology and Biogeography</i>, 30(6), 1191-1203. https://doi.org/10.1111/geb.13294</li> <li>Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. <i>Trends in Ecology and Evolution</i>, 22(3), 148-155.</li> <li>Billard E, Reyes J, Mansilla A, Faugeron S, Guillemin ML. 2015. Deep genetic divergence between austral populations of the red alga <i>Gigartina skottsbergii</i> reveals a cryptic species endemic to the Antarctic continent. <i>Polar Biology</i>, 38, 2021–2034. https://doi.org/10.1007/s00300-015-1762-4</li> <li>Bilton DT, Freeland JR, Okamura B. 2001. Dispersal in freshwater invertebrates. <i>Annual Review of Ecology and Systematics</i>, 32(1), 159-181.</li> </ul>	Arribas P, Andújar taxonomy and (Hydrophilida	C, Sánchez-Fernández L conservation of cryptic e). <i>Zoologica Scripta</i> , 42	D, Abellán P, Millán beetles in the Medite 2(2), 182-200.	A. 2013. Integrative erranean region
<ul> <li>Barraclough TG. 2019. What causes speciation? In <i>The Evolutionary Biology of Species</i>, Oxford University Press, 83-109.</li> <li>Beltrán Epele L, Dos Santos DA, Sarremejane R, Grech MG, Macchi PA, Manzo LM, Miserendino ML, Bonada N, Cañedo-Argüelles M. 2021. Blowin'in the wind: Wind directionality affects wetland invertebrate metacommunities in Patagonia. <i>Global Ecology and Biogeography</i>, 30(6), 1191-1203. <u>https://doi.org/10.1111/geb.13294</u></li> <li>Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. <i>Trends in Ecology and Evolution</i>, 22(3), 148-155.</li> <li>Billard E, Reyes J, Mansilla A, Faugeron S, Guillemin ML. 2015. Deep genetic divergence between austral populations of the red alga <i>Gigartina skottsbergii</i> reveals a cryptic species endemic to the Antarctic continent. <i>Polar Biology</i>, 38, 2021–2034. https://doi.org/10.1007/s00300-015-1762-4</li> <li>Bilton DT, Freeland JR, Okamura B. 2001. Dispersal in freshwater invertebrates. <i>Annual Review of Ecology and Systematics</i>, 32(1), 159-181.</li> </ul>	Audisio P, Trizzino morphological of the <i>C. quad</i> 37. <u>https://doi.</u>	M, de Biase A, Rossett evidence of a new siblin <i>ricollis</i> complex from peop org/10.1080/112500009	ti G, Mancini E, Antenna species of 'Calobi eninsular Italy. <i>Italia</i> 02845738	onini G. 2010. Molecular and ius' (Coleoptera: Hydraenida in Journal of Zoology, 77, 29-
<ul> <li>Beltrán Epele L, Dos Santos DA, Sarremejane R, Grech MG, Macchi PA, Manzo LM, Miserendino ML, Bonada N, Cañedo-Argüelles M. 2021. Blowin'in the wind: Wind directionality affects wetland invertebrate metacommunities in Patagonia. <i>Global Ecology and Biogeography</i>, 30(6), 1191-1203. https://doi.org/10.1111/geb.13294</li> <li>Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. <i>Trends in Ecology and Evolution</i>, 22(3), 148-155.</li> <li>Billard E, Reyes J, Mansilla A, Faugeron S, Guillemin ML. 2015. Deep genetic divergence between austral populations of the red alga <i>Gigartina skottsbergii</i> reveals a cryptic species endemic to the Antarctic continent. <i>Polar Biology</i>, 38, 2021–2034. https://doi.org/10.1007/s00300-015-1762-4</li> <li>Bilton DT, Freeland JR, Okamura B. 2001. Dispersal in freshwater invertebrates. <i>Annual Review of Ecology and Systematics</i>, 32(1), 159-181.</li> </ul>	Barraclough TG. 2 Oxford Univer	019. What causes specia sity Press, 83-109.	tion? In <i>The Evolution</i>	onary Biology of Species,
<ul> <li>Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. <i>Trends in Ecology and Evolution</i>, 22(3), 148-155.</li> <li>Billard E, Reyes J, Mansilla A, Faugeron S, Guillemin ML. 2015. Deep genetic divergence between austral populations of the red alga <i>Gigartina skottsbergii</i> reveals a cryptic species endemic to the Antarctic continent. <i>Polar Biology</i>, 38, 2021–2034. https://doi.org/10.1007/s00300-015-1762-4</li> <li>Bilton DT, Freeland JR, Okamura B. 2001. Dispersal in freshwater invertebrates. <i>Annual Review of Ecology and Systematics</i>, 32(1), 159-181.</li> </ul>	Beltrán Epele L, D Miserendino M directionality a Ecology and B	os Santos DA, Sarremej IL, Bonada N, Cañedo-A affects wetland invertebr <i>iogeography</i> , 30(6), 119	ane R, Grech MG, M Argüelles M. 2021. E ate metacommunitie 91-1203. <u>https://doi.c</u>	Macchi PA, Manzo LM, Blowin'in the wind: Wind es in Patagonia. <i>Global</i> org/10.1111/geb.13294
<ul> <li>Billard E, Reyes J, Mansilla A, Faugeron S, Guillemin ML. 2015. Deep genetic divergence between austral populations of the red alga <i>Gigartina skottsbergii</i> reveals a cryptic species endemic to the Antarctic continent. <i>Polar Biology</i>, 38, 2021–2034. <u>https://doi.org/10.1007/s00300-015-1762-4</u></li> <li>Bilton DT, Freeland JR, Okamura B. 2001. Dispersal in freshwater invertebrates. <i>Annual Review of Ecology and Systematics</i>, 32(1), 159-181.</li> </ul>	Bickford D, Lohma Cryptic specie Evolution, 22(	an DJ, Sodhi NS, Ng PK s as a window on diversi 3), 148-155.	, Meier R, Winker K ity and conservation.	K, Ingram KK, Das I. 2007. . <i>Trends in Ecology and</i>
Bilton DT, Freeland JR, Okamura B. 2001. Dispersal in freshwater invertebrates. <i>Annual Review of Ecology and Systematics</i> , 32(1), 159-181.	Billard E, Reyes J, between austra species endem <u>https://doi.org/</u>	Mansilla A, Faugeron S Il populations of the red ic to the Antarctic contin (10.1007/s00300-015-17	, Guillemin ML. 201 alga <i>Gigartina skott</i> nent. <i>Polar Biology</i> , <u>62-4</u>	<ol> <li>Deep genetic divergence sbergii reveals a cryptic</li> <li>2021–2034.</li> </ol>
	Bilton DT, Freelan Review of Eco.	d JR, Okamura B. 2001. logy and Systematics, 32	Dispersal in freshware (1), 159-181.	ater invertebrates. Annual

- Bilton DT. 2021. Differentiation of South African coastal rock pool Ochthebius is associated with major ocean currents (Coleoptera: Hydraenidae). *Acta Entomologica Musei Nationalis Pragae*, 61(1), pp.253-260.
  - Blair C, Bryson RW. 2017. Cryptic diversity and discordance in single-locus species delimitation methods within horned lizards (Phrynosomatidae: Phrynosoma). *Molecular Ecology Resources*, 17, 1168–1182. <u>https://doi.org/10.1111/1755-0998.12658</u>
  - Booth-Rea G, Ranero CR, Grevemeyer I. 2018. The Alboran volcanic-arc modulated the Messinian faunal exchange and salinity crisis. *Scientific Reports*, 8, 1–14. <u>https://doi.org/10.1038/s41598-018-31307-7</u>
- Bowen BW, Rocha LA, Toonen RJ, Karl SA. 2013. The origins of tropical marine biodiversity. *Trends in Ecology and Evolution*, 28(6), 359-366.
- Cai C, Lawrence JF, Yamamoto S, Leschen RAB, Newton AF, Ślipiński A, Yin Z, Huang D, Engel MS. 2019. Basal polyphagan beetles in mid-Cretaceous amber from Myanmar: Biogeographic implications and long-term morphological stasis. *Proceedings of the Royal Society B*, 286. <u>https://doi.org/10.1098/rspb.2018.2175</u>
- Cerca J, Meyer C, Stateczny D, Siemon D, Wegbrod J, Purschke G, Dimitrov D, Struck TH. 2020. Deceleration of morphological evolution in a cryptic species complex and its link to paleontological stasis. *Evolution*, 74, 116–131. <u>https://doi.org/10.1111/evo.13884</u>
- Céréghino R, Pillar VD, Srivastava DS, de Omena PM, MacDonald AAM, Barberis IM, Corbara B, Guzman LM, Leroy C, Ospina Bautista F, Romero GQ. 2018. Constraints on the functional trait space of aquatic invertebrates in bromeliads. *Functional Ecology*, 32(10), pp.2435-2447.
- Chernomor O, Von Haeseler A, Minh BQ. 2016. Terrace Aware Data Structure for Phylogenomic Inference from Supermatrices. *Systematic Biology*, 65, 997–1008. <u>https://doi.org/10.1093/sysbio/syw037</u>
- Clement M, Posada D, Crandall KA. 2000. TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, 9, 1657–1659. <u>https://doi.org/10.1046/j.1365-294X.2000.01020.x</u>
- Cunha RL, Assis JM, Madeira C, Seabra R, Lima FP, Lopes EP, Williams ST, Castilho R, 2017. Drivers of Cape Verde archipelagic endemism in keyhole limpets. *Scientific Reports*, 7, 1–11. <u>https://doi.org/10.1038/srep41817</u>
- Domenici P, Claireaux G, McKenzie DJ, 2007. Environmental constraints upon locomotion and predator–prey interactions in aquatic organisms: an introduction. *Philosophical Transactions of the Royal Society B*, 362, 1929-1936.
- Dupanloup I, Schneider S, Excoffier L. 2002. A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, 11, 2571–2581. https://doi.org/10.1046/j.1365-294X.2002.01650.x

El-Geziry TM, modeller co	Bryden IG. 2010. The circulation pattern in the Mediterranean Sea: issues for onsideration. <i>Journal of Operational Oceanography</i> , 3(2), 39-46.
Faria R, Johann under the s	esson K, Stankowski S. 2021. Speciation in marine environments: Diving urface. <i>Journal of Evolutionary Biology</i> , 34(1), 4-15.
Fišer C, Konec ecological (Switzerlar	M, Alther R, Švara V, Altermatt F. 2017. Taxonomic, phylogenetic and diversity of Niphargus (Amphipoda: Crustacea) in the Hölloch cave system ad). <i>Systematics and Biodiversity</i> , 15(3), 218-237.
Gaines SD, Der California	my MW. 2007. <i>Encyclopedia of tidepools and rocky shores</i> . University of Press.
García-Castella Vicente R. crisis. <i>Natu</i>	nos D, Estrada F, Jiménez-Munt I, Gorini C, Fernández M, Vergés J, De 2009. Catastrophic flood of the Mediterranean after the Messinian salinity <i>tre</i> , 462(7274), 778-781.
García-Ladona the Western	E. 2017. Currents in the Western Mediterranean basin. In Atlas of Bedforms in Mediterranean. Springer, Cham, 41-47.
García-Merchái Rodríguez crustaceans <i>Evolution</i> 6	n VH, Robainas-Barcia A, Abelló P, Macpherson E, Palero F, García- M, Gil de Sola L, Pascual M. 2012. Phylogeographic patterns of decapod at the Atlantic-Mediterranean transition. <i>Molecular Phylogenetics and</i> 52, 664–672. <u>https://doi.org/10.1016/j.ympev.2011.11.009</u>
Gillespie RG, E distance dis 27(1), 47-5	Saldwin BG, Waters JM, Fraser CI, Nikula R, Roderick GK. 2012. Long- spersal: a framework for hypothesis testing. <i>Trends in Ecology and Evolution</i> , 6.
González-Caste Genetic div based on m Atlantic–M <u>https://doi.</u>	llano I, González-López J, González-Tizón AM, Martínez-Lage A. 2020. rersity and population structure of the rockpool shrimp <i>Palaemon elegans</i> icrosatellites: evidence for a cryptic species and differentiation across the lediterranean transition. <i>Scientific Reports</i> , 10, 1–11. org/10.1038/s41598-020-67824-7
Greenan TM, G intertidal L coastline (1	riffiths CL, Santamaria CA, 2018. Phylogeography and cryptic diversity of <i>igia</i> isopods (Crustacea, Isopoda, Ligiidae) across the southern Africa No. e3332v1). <i>PeerJ</i> , 6:e4657.
Haak CR, Powe differentiat <i>Albula gore</i> <u>https://doi.e</u>	er M, Cowles GW, Danylchuk AJ. 2019. Hydrodynamic and isotopic niche ion between juveniles of two sympatric cryptic bonefishes, <i>Albula vulpes</i> and <i>zensis. Environmental Biology of Fishes</i> , 102, 129–145. org/10.1007/s10641-018-0810-7
Hunt T, Bergste Ahrens D, 1 M, Bocak I evolutionar	en J, Levkanicova Z, Papadopoulou A, John OS, Wild R, Hammond PM, Balke M, Caterino MS, Gómez-Zurita J, Ribera I, Barraclough TG, Bocakova L, Vogler AP. 2007. A comprehensive phylogeny of beetles reveals the y origins of a superradiation. <i>Science</i> , 318, 1913–1916.

- Hurtado LA, Lee EJ, Mateos M, Taiti S. 2014. Global diversification at the harsh sea-land interface: mitochondrial phylogeny of the supralittoral isopod genus *Tylos* (Tylidae, Oniscidea). *PloS one*, 9(4), p.e94081.
- Jäch MA, 1989. Revision of the palearctic species of the genus *Ochthebius* LEACH II. The subgenus *Cobalius* REY (Hydraenidae, Coleoptera). *Zeitschrift der Arbeitsgemeinschaft Österreichischer Entomologen*, 41, 41–51.
- Jäch MA, 2000. Revision of the Palearctic species of the genus *Ochthebius* LEACH XVIII. Description of two new species from the Balearic Islands, Spain. *Koleopterologische Rundschau*, 70, 65-67.
- Jäch MA, Delgado JA. 2017. Revision of the Palearctic species of the genus Ochthebius LEACH, 1815 XXXII. Ochthebius (Cobalius) biltoni sp. n. from Sicily (Italy). Koleopterologische Rundschau, 87, 85-88.
- Jami L, Gustafson GT, Steinmann T, Piñeirua M, Casas J. 2021. Overcoming Drag at the Water-Air Interface Constrains Body Size in Whirligig Beetles. *Fluids*, 6(7), p.249.
- Katoh K, Standley DM, 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772– 780. <u>https://doi.org/10.1093/molbev/mst010</u>
- Kearse M, Moir R, Wilson A, Stones-Havas 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. https://doi.org/10.1093/bioinformatics/bts199
- Knowlton N. 1993. Sibling species in the sea. Annual review of ecology and systematics, 24(1), pp.189-216.Krijgsman, W., Hilgen, F.J., Raffi, I., Sierro, F.J., Wilson, D.S., 1999. Chronology, causes and progression of the Messinian salinity crisis. *Nature*, 400(6745), 652-655.
- Krijgsman W, Hilgen FJ, Raffi I, Sierro FJ, Wilson DS. 1999. Chronology, causes and progression of the Messinian salinity crisis. *Nature*, 400(6745), 652-655.
- Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution.*, 29, 1695–1701. <u>https://doi.org/10.1093/molbev/mss020</u>
- Lionello P, Bhend J, Buzzi A, Della-Marta PM, Krichak SO, Jansa A, Maheras P, Sanna A, Trigo IF, Trigo R. 2006. Cyclones in the Mediterranean region: climatology and effects on the environment. In *Developments in earth and environmental sciences* (Vol. 4, pp. 325-372). Elsevier.
- Lowe CD, Martin LE, Montagnes DJS, Watts PC. 2012. A legacy of contrasting spatial genetic structure on either side of the Atlantic-Mediterranean transition zone in a marine protist. *Proceedings of the Natural Academy of Sciences of the United States of America*, 109, 20998–21003. <u>https://doi.org/10.1073/pnas.1214398110</u>

	the genus <i>Laparocerus</i> , with comments on colonisation and diversification in macaronesia (Coleoptera, Curculionidae, Entiminae). <i>ZooKeys</i> , 2017, 1–77. https://doi.org/10.3897/zookeys.651.10097
Ma	aggi C, González-Wangüemert M. 2015. Genetic differentiation among Parastichopus regalis populations from Western Mediterranean Sea: potential effects of its fishery a current connectivity. <i>Mediterranean Marine Science</i> , 16(3), 489-501.
Ma	as G, Maillard A, Alcover JA, Fornós JJ, Bover P, Torres-Roig E. 2018. Terrestrial colonization of the Balearic Islands: New evidence for the Mediterranean sea-level drawdown during the Messinian Salinity Crisis. <i>Geology</i> , 46(6), 527-530.
Mł	bongwa NA, Hui C, Pulfrich A, von der Heyden S. 2019. Every beach an island deep population divergence and possible loss of genetic diversity in <i>Tylos granulatus</i> , a sa shore isopod. <i>Marine Ecology Progress Series</i> , 614, pp.111-123.
Mo spi	cGaughran A, Stevens MI, Holland BR. 2010. Biogeography of circum-Antarctic ringtails. <i>Molecular Phylogenetics and Evolution</i> , 57(1), 48-58.
Mo	eKenna DD, Shin S, Ahrens D, Balke M, Beza-beza C, Clarke DJ. 2019. The evolution genomic basis of beetle diversity. <i>Proceedings of the Natural Academy of Sciences of</i> <i>United States of America</i> , 116, 24729-24737. <u>https://doi.org/10.1073/pnas.19096551</u>
Mo	cKenna DD, Wild AL, Kanda K, Bellamy CL, Beutel RG, Caterino MS, Farnum CW, Hawks DC, Ivie MA, Jameson ML, Leschen RAB, Marvaldi AE, Mchugh JV, Newte AF, Robertson JA, Thayer MK, Whiting MF, Lawrence JF, Slipinski A, Maddison D Farrell BD. 2015. The beetle tree of life reveals that Coleoptera survived end-Permia mass extinction to diversify during the Cretaceous terrestrial revolution. <i>Systematic</i> <i>Entomology</i> , 40, 835–880. <u>https://doi.org/10.1111/syen.12132</u>
Mi	nh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R, Teeling E. 2020. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. <i>Molecular Biology and Evolution</i> , 37, 13 1534. <u>https://doi.org/10.1093/molbev/msaa015</u>
Mi	rón-Gatón JM, Botella-Cruz M, García-Meseguer AJ, Millán A, Velasco J, 2022. The tolerance differs between co-occurring congeneric beetle species in marine supratida rockpools. <i>Marine Ecology Progress Series</i> , 681, 185-196.
Mo	ora E, Paspati A, Decae AE, Arnedo MA. 2017. Rafting spiders or drifting islands? Or and diversification of the endemic trap-door spiders from the Balearic Islands, Wester Mediterranean. <i>Journal of Biogeography</i> , 44, 924–936. <u>https://doi.org/10.1111/jbi.12</u>
Mi	iller MI, Morais DH, Costa-Silva GJ, Aguiar A, Ávila RW, da Silva RJ. 2018. Diversi the genus <i>Rhabdias</i> (Nematoda, Rhabdiasidae): Evidence for cryptic speciation. <i>Zoologica Scripta</i> 47, 595–607. https://doi.org/10.1111/zsc.12304

- Múrias Dos Santos A, Cabezas MP, Tavares AI, Xavier R, Branco M. 2016. TcsBU: A tool to extend TCS network layout and visualization. *Bioinformatics*, 32, 627–628. https://doi.org/10.1093/bioinformatics/btv636
- Niikura M, Honda M, Yahata K. 2015. Phylogeography of semiterrestrial isopod, *Tylos granuliferus*, on East Asian coasts. *Zoology Science*, 32, 105–113. https://doi.org/10.2108/zs140004
- Orchymont A d'. 1932. Discours présidentiel: 'Des «rockpools» submarins et de leur population entomologique'. *Bulletin et Annales de la Société Entomologique de Belgique*, 72, 17-33.
- Pfenninger M, Schwenk K. 2017. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology*, 7, 121.
- Pfingstl T, 2013. Resistance to fresh and salt water in intertidal mites (Acari: Oribatida): implications for ecology and hydrochorous dispersal. *Experimental and Applied Acarology*, 61(1), 87-96.
- Pfingstl T, Baumann J, Neethling JA, Bardel-Kahr I, Hugo-Coetzee EA. 2021. Distribution patterns of intertidal oribatid mites (Acari, Oribatida) from South African shores and their relationship to temperature. *African Journal of Marine Science*, pp.1-11.
- Pfingstl T, Baumann J, Lienhard A. 2019. The Caribbean enigma: the presence of unusual cryptic diversity in intertidal mites (Arachnida, Acari, Oribatida). *Organisms, Diversity and Evolution*, 19, 609–623. <u>https://doi.org/10.1007/s13127-019-00416-0</u>
- Pinot JM, López-Jurado JL, Riera M. 2002. The CANALES experiment (1996-1998). Interannual, seasonal, and mesoscale variability of the circulation in the Balearic Channels. *Progress in Oceanography*, 55(3-4), 335-370.
- Porretta D, Urbanelli S. 2012. Evolution of premating reproductive isolation among conspecific populations of the sea rock-pool beetle Ochthebius urbanelliae driven by reinforcing natural selection. *Evolution*, 66(4), 1284-1295.
- Powlik JJ. 1999. Habitat characters of *Tigriopus californicus* (Copepoda: Harpacticoida), with notes on the dispersal of supralittoral fauna. *Journal of the Marine Biological Association of the United Kingdom*, 79(1), 85-92.
- Powlik JJ, Lewis AG. 1996. Desiccation Resistance in *Tigriopus californicus* (Copepoda, Harpacticoida). *Estuarine, Coastal and Shelf Science*, 43(4), 521-532.
- Puillandre N, Lambert A, Brouillet S, Achaz G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, 21, 1864–1877. <u>https://doi.org/10.1111/j.1365-294X.2011.05239.x</u>
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology*, 67, 901–904. <u>https://doi.org/10.1093/sysbio/syy032</u>

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Rastorgueff PA, Chevaldonné P, Arslan D, Verna C, Lejeusne C. 2014. Cryptic habitats and cryptic diversity: Unexpected patterns of connectivity and phylogeographical breaks in a Mediterranean endemic marine cave mysid. *Molecular Ecology*, 23, 2825–2843. <u>https://doi.org/10.1111/mec.12776</u>

- Ribera I. 2008. Habitat constraints and the generation of diversity in freshwater macroinvertebrates. In: Lancaster J, Briers RA, eds. *Aquatic Insects: Challenges to populations*. Proceedings of the Royal Entomological Society's 24th Symposium, 289–311.
- Ribera I, Foster GN. 2018. Report of Frank Balfour-Browne's collecting in Gran Canaria and Madeira (1932-1933), with the description of *Ochthebius (Cobalius) lanthanus* sp. nov. (Coleoptera, Hydraenidae). *Zootaxa*, 4524(1), 65-76.
- Ribera I, Hernando C. 2019. Notes on the distribution and habitat of *Ochthebius lobicollis* Rey, 1885, a poorly known north-western Mediterranean coastal species (Coleoptera: Hydraenidae). *Fragmenta Entomologica*, 2019; 51 (1): 51-4.
- Sabatelli S, Audisio P, Antonini G, Solano E, Martinoli A, Trizzino M. 2016. Molecular ecology and phylogenetics of the water beetle genus *Ochthebius* revealed multiple independent shifts to marine rockpools lifestyle. *Zoologica Scripta*, 45, 175–186. https://doi.org/10.1111/zsc.12141
- Sabatelli S, Ruspantini P, Cardoli P, Audisio P. 2021. Underestimated diversity: cryptic species and phylogenetic relationships in the subgenus *Cobalius* (Coleoptera: Hydraenidae) from marine rockpools. *Molecular Phylogenetics and Evolution* https://doi.org/10.1016/j.ympev.2021.107243
- Santamaria CA, Bluemel JK, Bunbury N, Curran M. 2017. Cryptic biodiversity and phylogeographic patterns of Seychellois *Ligia* isopods. *PeerJ*, 5, p.e3894.
- Santamaria CA, Mateos M, DeWitt TJ, Hurtado LA. 2016. Constrained body shape among highly genetically divergent allopatric lineages of the supralittoral isopod Ligia occidentalis (Oniscidea). *Ecology and Evolution*, 6(5), pp.1537-1554.
- Sayers EW, Cavanaugh M, Clark K, Pruitt KD, Schoch CL, Sherry ST, Karsch-Mizrachi I. 2021. GenBank. *Nucleic acids research*, 49(D1), D92-D96.
- Schunter C, Carreras-Carbonell J, Macpherson E, Tintoré J, Vidal-Vijande E, Pascual A, Guidetti P, Pascual M. 2011. Matching genetics with oceanography: directional gene flow in a Mediterranean fish species. *Molecular Ecology*, 20(24), 5167-5181.
- Shi G, Grimaldi DA, Harlow GE, Wang J, Wang J, Yang M, Lei W, Li Q, Li X. 2012. Age constraint on Burmese amber based on U-Pb dating of zircons. *Cretaceous Research*. 37, 155–163. https://doi.org/10.1016/j.cretres.2012.03.014
- Simon C, 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 polymerase chain reaction primers. *Annals of the Entomological Society of America*, 87, 651–701. <u>https://doi.org/10.1093/aesa/87.6.651</u>

- Stephens M, Smith NJ, Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics*, 68, 978–989.
- Stock W, Vanelslander B, Rüdiger F, Sabbe K, Vyverman W, Karsten U. 2019. Thermal niche differentiation in the benthic diatom cylindrotheca closterium (Bacillariophyceae) complex. *Frontiers in Microbiology*, 10. <u>https://doi.org/10.3389/fmicb.2019.01395</u>
- Stork NE. 2018. How many species of insects and other terrestrial arthropods are there on Earth?. *Annual Review of Entomology*, 63, 31-45.
- Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution*, 4, 1–5. https://doi.org/10.1093/ve/vey016
- Torres-Roig E, Piñero P, Agustí J, Bover P, Alcover JA. 2019. First evidence of endemic Murinae (Rodentia, Mammalia) in the early Pliocene of the Balearic Islands (western Mediterranean). *Geological Magazine*, 156(10), 1742-1750.
- Urbanelli S. 2002. Genetic divergence and reproductive isolation in the *Ochthebius* ('Calobius') complex (Coleoptera: Hydraenidae). *Heredity*, 88(5), pp.333-341.
- Urbanelli S, Porretta D. 2008. Evidence of reinforcement of premating isolation between two species of the genus *Ochthebius* (Coleoptera: Hydraenidae). *Evolution*, 62(6), pp.1520-1527.
- Urbanelli S, Sallicandro P, De Vito E, Colonnelli E, Bullini L. 1996. Molecular reexamination of the taxonomy of *Ochthebius* (Calobius) (Coleoptera: Hydraenidae) from the Mediterranean and Macaronesian Regions. *Annals of the Entomological Society of America*, 89(5), pp.623-636.
- Vecchioni L, Arculeo M, Cottarelli V, Marrone F. 2021. Range-wide phylogeography and taxonomy of the marine rock pools dweller *Tigriopus fulvus* (Fischer, 1860) (Copepoda, Harpacticoida). *Journal of Zoological Systematics and Evolutionary Research*, 59(4), 839-857.
- Velasco J, Mirón-Gatón J, García-Meseguer AJ, Botella-Cruz M. *In press*. Life cycle differences of two coexisting species of supratidal roockpools: Ochthebius quadricollis Mulsant, 1844 and O. lejolisii Mulsant and Rey, 1861 (Coleoptera, Hydraenidae). *Boletín de la Sociedad Española de Entomología*.
- Villastrigo A, Hernando C, Millán A, Ribera, I. 2020. The neglected diversity of the *Ochthebius* fauna from Eastern Atlantic and Central and Western Mediterranean coastal rockpools (Coleoptera, Hydraenidae). *Organisms, Diversity and Evolution*, 20, 785-801. <u>https://doi.org/10.1007/s13127-020-00463-y</u>
- Villastrigo A, Jäch MA, Cardoso A, Valladares LF, Ribera I, 2019. A molecular phylogeny of the tribe Ochthebiini (Coleoptera, Hydraenidae, Ochthebiinae). *Systematic Entomology*, 44, 273–288. <u>https://doi.org/10.1111/syen.12318</u>

Wares JP. 2020. Small, flat, and gray: Cryptic diversity in chthamalid barnacles in the global context of marine coastal biogeography (Cirripedia: Balanomorpha: Chthamalidae). <i>Journal of Crustacean Biology</i> , 40, 1–16. <u>https://doi.org/10.1093/jcbiol/ruz086</u>
Wild AL, Maddison DR. 2008. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. <i>Molecular Phylogenetics and Evolution</i> , 48, 877–891. <u>https://doi.org/10.1016/j.ympev.2008.05.023</u>
Yamamoto S, Jäch MA, Takahashi Y. 2017. Discovery of the first hydraenid beetle in amber, with description of a new genus and species (Coleoptera: Staphylinoidea: Hydraenidae). <i>Cretaceous Research</i> , 78, 27–33. <u>https://doi.org/10.1016/j.cretres.2017.05.015</u>

- Zhang Y, Li S. 2014. A spider species complex revealed high cryptic diversity in South China caves. *Molecular Phylogenetics and Evolution*, 79, 353-358.
- Zúñiga-Reinoso Á, Benítez HA. 2015. The overrated use of the morphological cryptic species concept: An example with *Nyctelia darkbeetles* (Coleoptera: Tenebrionidae) using geometric morphometrics. *Zoologischer Anzeiger*, 255, 47–53. https://doi.org/10.1016/j.jcz.2015.01.004

# **Figure captions**

Figure 1. Distribution of sampling localities (major figure) and main surface marine currents and potential geographic barriers to dispersal (top-right corner).

Figure 2. Calibrated phylogenetic tree obtained with BEAST v1.10.4 of *Ochthebius* with focus in *Cobalius* subgenus and *quadricollis* species group (former 'Calobius' subgenus). Numbers in nodes represent posterior probabilities. Calibrations points used in analysis are specified by grey dots.

Figure 3. Haplotype network for *Ochthebius (Ochthebius) quadricollis*. Colours represent the main geographic areas as indicated in the legend.

Figure 4. Haplotype network for *Ochthebius (Cobalius) subinteger*. Colours represent the main geographic areas as indicated in the legend.

Figure 5. Haplotype network for *Ochthebius (Cobalius) lejolisii*. Colours represent the main geographic areas as indicated in the legend.

Figure S1. Currently known localities in which the presence of *Ochthebius (Ochthebius) quadricollis* is confirmed.

Figure S2. Currently known localities in which the presence of *Ochthebius (Cobalius) subinteger* is confirmed.

Figure S3. Currently known localities in which the presence of *Ochthebius (Cobalius) lejolisii* is confirmed.

Figure S4. Phylogenetic tree for the COI gene obtained with IQTree for the studied samples. Numbers in nodes represent support measures (ultrafast bootstrap/SH-like aLRT support).

Figure S5. Phylogenetic tree for the wingless gene obtained with IQTree for the studied samples. Numbers in nodes represent support measures (ultrafast bootstrap/SH-like aLRT support).

Figure S6. Best fitted geographic clusters based on SAMOVA for *Ochthebius (Ochthebius) quadricollis* for the COI and wingless genes.

Figure S7. Best fitted geographic clusters based on SAMOVA for *Ochthebius (Cobalius) subinteger* for the COI and wingless genes.

Figure S8. Best fitted geographic clusters based on SAMOVA for *Ochthebius (Cobalius) lejolisii* for the COI and wingless genes.

# **Table captions**

Table 1. Detailed information on sampling localities, including the number of specimens used for each lineage.

Table 2. General genetic statistics (N = number of samples, h = number of haplotypes, Hd = haplotype diversity,  $\pi$  = nucleotide diversity, Max. Div. = maximum sequence divergence)

Table 3. Distribution of genetic diversity as inferred by analysis of molecular variance (K = best-fitted number of homogeneous groups defined by SAMOVA2)

Table S1. List of material used for molecular analyses, including details of vouchers, localities, collectors, dataset in which they were used and accession numbers. In bold, sequences newly generated for this work.

Table S2. SAMOVA2 detailed information for *Ochthebius (Ochthebius) quadricollis*, including information on the number of homogeneous groups (K), the genetic variability among populations within groups (FSC), among populations (FST), among groups (FCT), and the increase of FCT relative to the FCT value for k-1 clusters ( $\Delta$ FCT).

Table S3. SAMOVA2 detailed information for *Ochthebius (Cobalius) subinteger*, including information on the number of homogeneous groups (K), the genetic variability among populations within groups (FSC), among populations (FST), among groups (FCT), and the increase of FCT relative to the FCT value for k-1 clusters ( $\Delta$ FCT).

Table S4. SAMOVA2 detailed information for *Ochthebius (Cobalius) lejolisii*, including information on the number of homogeneous groups (K), the genetic variability among

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populations within groups (FSC), among populations (FST), among groups (FCT), and the increase of FCT relative to the FCT value for k-1 clusters ( $\Delta$ FCT).

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Table 1. Detailed information of sampling localities, including the number of specimens used for each lineage.

ц	Landar	T - 494 - 1	T	COI			Wingless		
Ħ	Location	Latitude	Longitude	quadricollis	subinteger	lejolisii	quadricollis	subinteger	lejolisii
1	Saint Jean de Luz, France	43.4033	-1.6582			1			
2	Ibarranguelua, Spain	43.4107	-2.6547			1			
3	Rinlo, Spain	43.5592	-7.1055			1			
4	Nigrán, Spain	42.1419	-8.8063	1		1			
5	Luz, Portugal	37.0756	-8.7502	1					
6	Isla de Tarifa, Spain	36.0019	-5.6124	3		3	3		3
7	Cala Milla de Plata, Spain	36.3065	-5.2602	3		3	3		3
8	Nerja, Spain	36.7409	-3.8841	3		3	3		3
9	Cala de Maro, Spain	36.7529	-3.8465			1			
10	Velilla, Spain	36.7449	-3.6616	3		3	3		3
11	Cala Rijana, Spain	36.7092	-3.3923	3		3	3		3
12	El Playazo, Spain	36.8628	-2.0036	3		3	3		3
13	Cala de las Conchas, Spain	37.2841	-1.7310	3		3	3		3
14	Pozo del Esparto, Spain	37.3190	-1.7001	3		2	2		1
15	Cabo Cope, Spain	37.4372	-1.4840	3		3	2		2
16	Cala de las Pulgas, Spain	37.4678	-1.4712	3		3	3		3
17	Percheles, Spain	37.5293	-1.3795	3		3	3		3
18	Cala Reona, Spain	37.6173	-0.7130	4	3	3	3	2	3
19	Punta del Cocedor, Spain	37.7504	-0.7281	4		3	3		3
20	Cabo de Santa Pola, Spain	38.1948	-0.5173	3		3	3		3
21	La Illeta, El Campello, Spain	38.4316	-0.3807	3		3	3		3
22	Calpe, Spain	38.6399	0.0679	1					
23	Moraira, Spain	38.6849	0.1252	3		3	3		3
24	Denia, Spain	38.8335	0.1385	3	3		3	3	
25	Sant Carles de la Rapita, Spain	40.5816	0.5564	3	3		3	3	
26	Cala de Sant Francesc, Spain	41.6793	2.8084	3			3		
27	Sant Feliu de Guixols, Spain	41.7711	3.0300		1				
28	L'Escala, Spain	42.1181	3.1536		3			3	
29	Plateau de Malmousque, Marseille, France	43.2804	5.3479	1	1				

30	Civitavecchia, Italy	42.0542	11.820	1					
31	San Antonio Abad, Ibiza, Spain	39.1136	1.5079	2	2				
32	Caló de ses Agulles, Cabrera, Spain	39.1483	2.9359	1	1				
33	Punta Prima, Menorca, Spain	39.8125	4.2806	3	4		2	1	
34	Cap Corse, Corsica, France	42.9664	9.3503	1					
35	Isola Rossa, Sardinia, Italy	41.0139	8.8705	1	1				
36	Porto Azzurro, Elba, Italy	42.7636	10.393	1					
37	Catania, Sicily, Italy	37.5539	15.149	1					
38	Sant Pawl's bay, Malta	35.9495	14.402	1			1		
39	Gashri, Gozo, Malta	36.0790	14.228	1					
40	Cala Trifa, Morocco	35.3377	-2.960			1			
41	Ghansou, Morocco	35.2046	-3.302	1			1		
42	Ceuta, Spain	35.8946	-5.283	1					
43	Cap Spartel, Morocco	35.7818	-5.931			1			1
44	Asilah, Morocco	35.4647	-6.042			1			
45	Larache, Morocco	35.1976	-6.155	1		1			
46	El Jadida, Morcco	33.0763	-8.665			1			1
47	Ouled Aissa, Morocco	33.0014	-8.738	1					
48	Essaouira, Morocco	31.5142	-9.772	1		1			1
49	Imesouane, Morocco	30.8453	-9.825	1					
50	Tagant, Morocco	30.6492	-9.887			1			
51	Tagazhout, Morocco	30.5464	-9.719	1					
52	Mirleft, Morocco	29.5687	-10.0519	1		1	1		
53	Sidi Ifni, Morocco	29.3465	-10.1977			1			1
54	South Kerry, Ireland	51.9081	-10.3558			1			
55	Skelmorlie, Scotland	55.8394	-4.8918			1			
56	Pembrokeshire, Wales	51.8959	-5.2958			1			
57	Swansea, Wales	51.5686	-3.9872			1			
	Total localities			41	10	34	23	5	20
	Total samples			84	22	65	60	12	49

diversity, $\pi$ = nucleotide diversity, Max. Div. = maximum sequence divergence)	Table 2. General genetic statistics (N = number of samples, h = number of haplotypes, Hd = haploty	'pe
	diversity, $\pi$ = nucleotide diversity, Max. Div. = maximum sequence divergence)	

arversity, <i>n</i> indefeetide arversity, max. Div. maximum sequence arvergence)										
					COI			V	Vingless	
	Ν	Localities	h	Hd	π	Max.	h	Hd	π	Max.
						Div.				Div.
O. quadricollis	84	41	46	0.964	0.04431	8.20%	17	0.875	0.00852	2.47%
O. lejolisii	65	34	29	0.887	0.00891	3.26%	14	0.727	0.00334	1.29%
O. subinteger	22	10	17	0.974	0.03543	6.64%	7	0.808	0.00453	1.27%

Table 3. Distribution of genetic diversity as inferred by analysis of molecular variance (K = best-fitted number of homogeneous groups defined by SAMOVA2)

Species	Gene	K	Among groups (FCT)	Among populations within groups (FSC)	Within populations (FST)
O. quadricollis	COI	3	0.80803	0.78152	0.95806
-	Wingless	3	0.75466	0.62258	0.90740
O. lejolisii	COI	3	0.85110	0.79014	0.96875
	Wingless	3	0.67995	0.40891	0.81082
O. subinteger	COI	4	0.83428	0.10928	0.85239
Ũ	Wingless	3	0.79792	0.27489	0.85347

s 3 0.79792 0.27489 0.85347









