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RESEARCH ARTICLE

Microplate tectonics and environmental factors as distribution drivers in Western Mediterranean freshwater planarians

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Abstract

Aim: Species biogeography mainly focuses on palaeogeographical events, while environmental factors are generally overlooked despite their importance in species diversification. Here, we use an integrative approach to understand how palaeogeographical and environmental processes shape species distribution and focus on freshwater planarians as the model system.

Location: Western Mediterranean.

Taxon: *Dugesia*.

Methods: We inferred the phylogenetic relationships of most known *Dugesia* species in the area using six molecular markers. We then estimated their divergence times and reconstructed their ancestral distribution ranges. We also performed environmental niche modelling analyses using *Dugesia subtentaculata* as a model to evaluate the effects of several hydro-environmental variables and the likely existence of interspecific competition on *Dugesia* distributions.

Results: Our results provide a new phylogenetic scheme for *Dugesia* from the Western Mediterranean and show that the time splits between the lineages and their putative ancestral distribution ranges are correlated with microplate tectonic dynamics within the region during the Oligocene–Miocene period. Our environmental niche modelling analyses indicate that the type of land cover and the slope of the terrain are the most important abiotic factors driving the distribution of *Dugesia* from this region. Finally, we found a partial niche overlap between *D. subtentaculata* and two other common planarian species from the Iberian Peninsula.

Main conclusions: The microplate tectonic dynamics of the Western Mediterranean during the Oligocene–Miocene period, together with the position of the mountain ranges and posterior climate changes, may have played crucial roles in driving the biogeographical history of *Dugesia* in this region. Moreover, both interspecific competition and changes in fluvial characteristics driven by human activities may affect the current diversity and distribution of *Dugesia* in the Western Mediterranean. This study highlights the importance of integrating different types of information to study the biogeographical history of a species.

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KEYWORDS
abiotic factors, biogeography, *Dugesia*, interspecific competition, Mediterranean hotspot, niche modelling

1 | INTRODUCTION

The Mediterranean Basin is one of the most biologically rich regions on Earth (Mittermeier et al., 2011). The region has a rather complex palaeogeographical history that includes various tectonic processes, in addition to changes in sea level (Hsü et al., 1973; Krijgsman et al., 1999; Mather, 2009; Rosenbaum et al., 2002). Plate tectonic movements in the Western Mediterranean during the Oligocene–Miocene period are among the most widely studied processes, as they not only resulted in the creation of most of the Western Mediterranean islands, but also shaped the entire contemporary Western Mediterranean coastline (Rosenbaum et al., 2002).

The main tectonic process of the Western Mediterranean during the Oligocene–Miocene period began around 25 Mya when the land mass that is today northeastern Iberia and southern France detached from the continent. It migrated south and subsequently broke into several smaller fragments known as microplates. These microplates presently correspond to the Kabylies, the Betic region, the Riff, Calabria, Corsica, Sardinia and the Balearic Islands, and they split from each other at different time periods while migrating across the Mediterranean Sea until reaching their present positions. In addition to the large geographical consequences of this tectonic process, it is an important driver of the current distribution and diversity of many animal species from the Western Mediterranean region, including snails, lizards, earthworms, spiders and planarians (Bidegaray-Batista & Arnedo, 2011; Lázaro et al., 2011; Mendes et al., 2017; Opatova et al., 2016; Pérez-Losada et al., 2011; Pfenninger et al., 2010).

In addition to tectonic movements, environmental factors can also shape the distribution of species (Monge-Nájera, 2008). These types of factors can be divided into two main categories, biotic and abiotic. Those abiotic factors specific from each species' habitat, such as the type of soil for edaphic organisms or the water characteristics for aquatic ones, can provide relevant hints into the biogeographical history of a species (Bailey et al., 2018). Concerning biotic factors, interspecific competition plays an essential role in shaping the distribution of species. For example, competition theory predicts that when two sympatric species share identical niches, one will eventually exclude the other, or selective pressure will cause niche differentiation, allowing both species to coexist geographically (Brown & Wilson, 1956; Chase, 2011).

Freshwater planarians (Platyhelminthes, Tricladida) are free-living organisms that depend on the continuity of freshwater bodies to survive and disperse, and they are susceptible to desiccation (with few exceptions) and high salinity (Vila-Farré & Rink, 2018). To disperse, they use an active gliding movement along the submerged substrate mediated by ventral cilia, rather than swimming or passively dispersing through the water current (Ball & Reynoldson, 1981). For these reasons, planarians in general, and particularly freshwater planarians, are considered to be ideal model organisms for studying processes that have shaped the diversity and distribution of species, as exemplified in several studies (e.g. Lázaro et al., 2011; Solà et al., 2013).

The genus *Dugesia* is the most species-rich freshwater planarian genus in the Western Mediterranean with 16 known species, and all but two (*D. sicula* and *D. gonocephala*) are endemic to this region (Figure 1; Table S1). Previous studies have found that Western Mediterranean species are the sister clade of Eastern Mediterranean species (Lázaro et al., 2009; Solà et al., 2013). However, *D. sicula* is an exception; although it is present throughout the Mediterranean, it belongs to an African group of *Dugesia* that recently colonised the area (probably mediated by human activities) (Lázaro et al., 2009; Lázaro & Riutort, 2013). Unfortunately, the phylogenetic relationships within the Western Mediterranean clade of species remain unresolved, mainly because of the limited genetic information contained in molecular markers used and incomplete taxon sampling. Therefore, the palaeogeographical processes that may have shaped its present diversity and distribution have not yet been clarified. In addition, there is limited information available for evaluating the roles of different environmental factors on the species distribution of *Dugesia* (Roca et al., 1992).

Interspecific competition for food has been proposed as an essential factor that regulates population density and influences its distribution in some planarian species (Vila-Farré & Rink, 2018). A recent study in the Iberian Peninsula showed that *Dugesia* species can coexist with other freshwater planarian genera and occasionally with other *Dugesia* spp. (Leria et al., 2020). Of all Western Mediterranean *Dugesia*, the distribution range of *Dugesia subtentaculata* has been more widely studied (Leria et al., 2020). This fact, together with its likely competition with *Polycelis felina* and *Dugesia sicula* (Leria, 2019, and M. Riutort personal communication) make *D. subtentaculata* an excellent model for studying the impacts of both abiotic and biotic environmental factors on *Dugesia* distributions.

The present study aimed to disentangle how palaeogeographical processes and environmental factors interact to drive the biogeographical history of species. With this objective, we focused on *Dugesia* freshwater planarians from the Western Mediterranean as a model and aimed to answer the following questions: (1) Did the microplate tectonics of the Western Mediterranean play a role in the biogeographical history of *Dugesia*? (2) Which hydro-environmental factors were more important in driving the distribution of *Dugesia* species? (3) Can interspecific competition influence the current diversity and distribution of the genus? We used information from six molecular markers to infer a time-calibrated phylogeny that included most of the known *Dugesia* species from the Western Mediterranean, and we reconstructed the ancestral ranges of their distributions. We also conducted an environmental niche modelling analysis for *D. subtentaculata* from the Iberian Peninsula to evaluate the influence of different hydro-environmental factors on its distribution and the

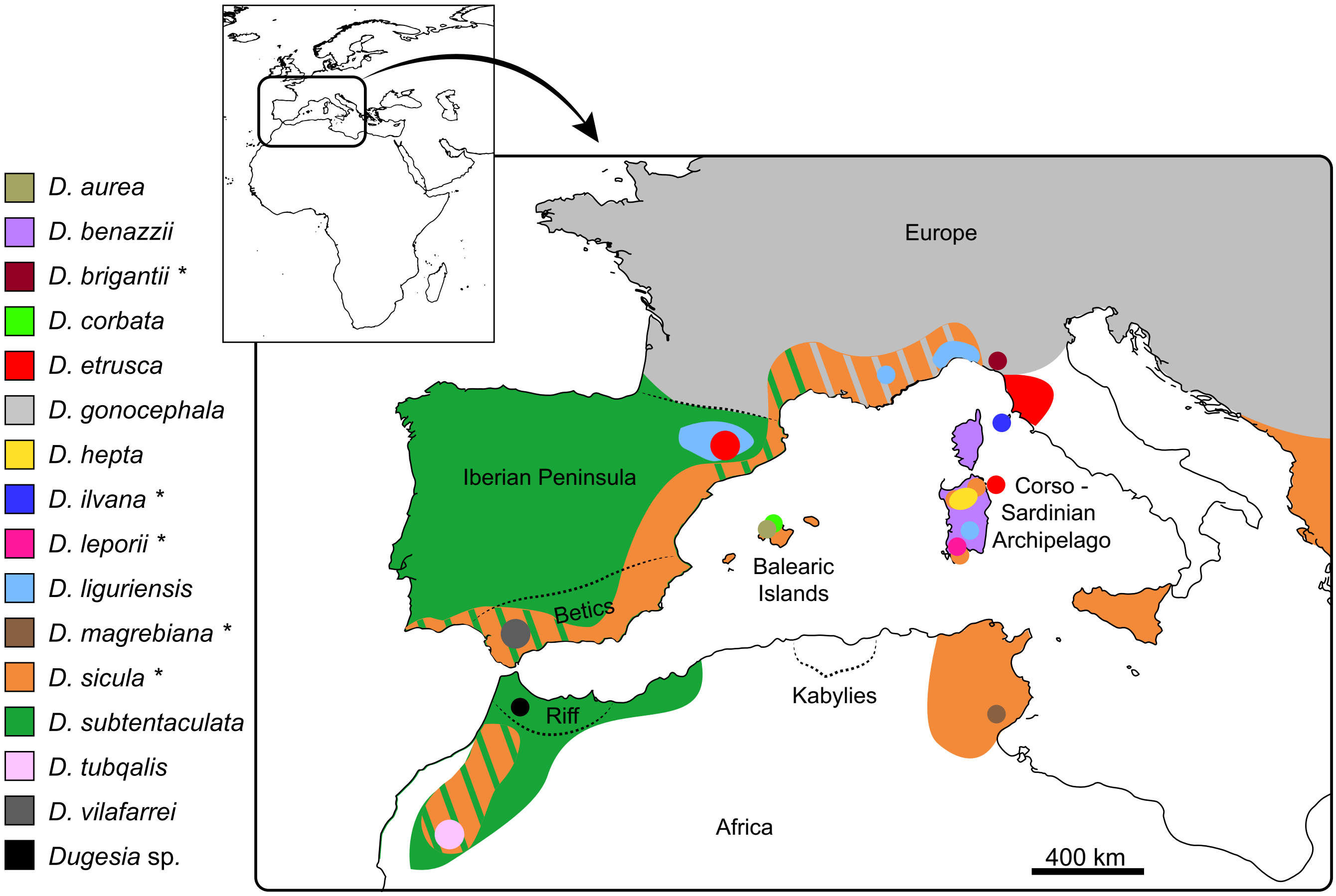

FIGURE 1 Distribution map of all known *Dugesia* species from the Western Mediterranean region. The outlined geographical regions correspond to the different areas included in the analysis of ancestral range reconstruction (excepting Greece, which was used for the outgroup). The geographical distribution of those species known to occur in many different localities from extensive regions (viz., *D. benazzii*, *D. gonocephala*, *D. sicula* and *D. subtentaculata*) has been represented with a coloured area instead of specific points. Species marked with an asterisk have not been included in the present study. References for the distribution of each species are detailed in Table S1. Map projection: geographical

likely existence of interspecific competition between *D. subtentaculata*, *D. sicula* and *P. felina*.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling

The present study used molecular data for 11 of the 15 autochthonous *Dugesia* species from the Western Mediterranean (Figure 1; Table S2). *D. brigantii* and *D. leporii* have not been found since their initial description, and *D. ilvana* and *D. maghrebiana* are found in regions that were difficult to sample; therefore, these four species were precluded from this study. Furthermore, *D. sicula* was not included in the molecular analysis because although it occurs in the area, it does not belong to the Western Mediterranean *Dugesia* clade (Lázaro et al., 2009; Solà et al., 2022). We also used molecular information for five *Dugesia* species from the eastern Mediterranean region as an outgroup.

Please see the Extended Materials and Methods in the Supplementary Information for more extensive descriptions of the methods described in the following subsections.

2.2 | DNA sequences and datasets

For the analyses, we used previously published sequences corresponding to six different molecular markers per species: (a) *28S ribosomal RNA gene* (28S), (b) *internal transcribed spacer 1* (ITS), (c) *18S ribosomal RNA gene* (18S), (d) *cytochrome c oxidase I* (Cox1), (e) an anonymous marker (Dunuc3) and (f) a *disulfide isomerase* (Dunuc5) (Leria et al., 2020; Solà et al., 2022). Only eight sequences were newly obtained for the present study, and they corresponded to Cox1 of *D. tubqalis* and 18S of *D. aurea*, *D.corbata*, *D. subtentaculata* and *D. vilafarrei* (see Supplementary Information for details on sequence amplification).

All individuals were represented by a single sequence per molecular marker, except for *D. subtentaculata*, which was represented by two different Cox1 sequences originating from one individual (Peralejos de las Truchas) that corresponded to the two most divergent Cox1 haplotypes within that individual. It has been suggested that this intraindividual genetic diversity is a consequence of fissiparous reproduction (Leria et al., 2019). GenBank accession numbers are listed in Table S2.

The DNA sequences of the different markers were arranged in two concatenated datasets, named Dataset 1 and Dataset 2, which differed only with respect to the Cox1 sequence of the *D. subtentaculata* individual from Peralejos de las Truchas. Datasets 1 and 2 include the haplotypes MK385877 and MK385895, respectively.

2.3 | Molecular data analyses

The DNA sequences of each gene were separately aligned using MAFFT online (Katoh & Standley, 2013) with the automatic model selection option. The existence of sequence saturation was evaluated using the program DAMBE (Xia et al., 2003; Xia & Lemey, 2009; Xia & Xie, 2001). The phylogenetic relationships between *Dugesia* species were inferred using Bayesian Inference implemented in MrBayes 3.2 (Ronquist et al., 2012) and maximum likelihood implemented in RaxML 7.0.3 (Stamatakis, 2006) and IQ-TREE 1.6.12 (Nguyen et al., 2015). Two maximum likelihood methods were used because they use different sequence evolution models to infer the trees, resulting in different phylogenetic outputs (see Extended Methods in the Supporting Information for further details about the models). The approximate times of divergence between the lineages were estimated using the software BEAST v.1.8.4 (Drummond et al., 2012). The well-known biogeographical event corresponding to the Mid-Aegean Trench (fragmentation of the Aegean Peninsula into oriental and an occidental regions between 9 and 12 Mya) was used as a calibration point (Dermitzakis & Papanikolaou, 1981). Finally, to reconstruct the ancestral geographical ranges of the different *Dugesia* species from the Western Mediterranean region, the previously obtained time-calibrated phylogenies were input into the software RASP 4.2 (Yu et al., 2015). We assigned nine different areas for this analysis: (A) Africa pro parte, (Ba) the Balearic Islands, (Be) Betics, (E) Europe pro parte, (G) Greece, (I) the Iberian Peninsula pro parte, (K) Kabylies, (R) the Riff and (S) Corso-Sardinian Archipelago (see the divisions in Figure 1). To reduce the length of names, we excluded the 'pro parte' epithet from the area names that refer to a part of Africa, Europe and the Iberian Peninsula (see Supplementary Information for details about such areas).

2.4 | Environmental data analyses

Information about the distribution of the three species included in the environmental analyses (viz., *D. subtentaculata*, *D. sicula*, and *P. felina*) was obtained from the literature (Table S3). The environmental niche of each species was modelled using the software Maxent 3.4.0 (Phillips et al., 2017; Phillips & Dudík, 2008) with information about six non-correlated hydro-environmental variables extracted from the HydroATLAS database (Linke et al., 2019) (Table S4). In the case of *D. subtentaculata*, we inferred an additional model including the obtained potential distribution of *D. sicula* and *P. felina* as variables to analyse the impact of these species on the distribution of *D. subtentaculata*. Finally, we used ENMTools v1.3 (Warren et al., 2010) to assess the degree of niche overlap between *D. subtentataculata* and the other two species in the Iberian Peninsula by calculating Schoener's D statistic (where 0 indicates completely different niches and 1 indicates an identical niche) (Schoener, 1968). We also used ENMTools to perform identity tests to see whether the obtained D metrics were significantly different from those assuming no niche differentiation.

3 | RESULTS

3.1 | Phylogenetic relationships

The sequences of the six molecular markers used in the present study comprised a total aligned length of 5439 characters with no significant levels of substitution saturation (Table S5). The phylogenetic trees obtained using MrBayes, RaxML, and IQ-TREE for the two datasets showed the same topology (Figure 2; Figures S1–S3). As expected, the Western Mediterranean species formed a highly supported monophyletic group (Western clade) in relation to the Eastern Mediterranean species (Eastern clade) (Figure 2). The Western clade was split into clade A (*D. gonocephala*, *D. etrusca* and *D. liguriensis*) and clade B (the remaining western species). Clade B was divided into a clade corresponding to the Sardinian species *D. hepta* and *D. benazzii* (clade B1), and another clade, including the species from Africa (*D. tubqalis* and *Dugesia* sp. 1), the Balearic Islands (*D. corbata* and *D. aurea*) and the Iberian Peninsula (*D. vilafarrei* and *D. subtentaculata*) (clade B2). The first species to diverge within clade B2 was *D. tubqalis*, followed by *Dugesia* sp. 1 from Morocco. The two species from Mallorca (*D. aurea* and *D. corbata*) constituted a monophyletic clade with a sister group relationship with *D. vilafarrei* and *D. subtentaculata*. Most of these clades were highly supported independent of the method used, except for clade B, which received low support in all methods, and the group constituted by *D. subtentaculata* and *D. vilafarrei*, which showed low support values with the likelihood methods (Figure 2).

3.2 | Divergence time estimation

The topology of the time-calibrated trees obtained with BEAST for both datasets was identical to that obtained with MrBayes, RaxML and IQ-TREE (Figure 2; Figures S1-S3). The mean age estimates and confidence intervals of the nodes were highly similar between the two datasets (Table S6). The only node markedly different between the two datasets corresponded to the split within *D. subtentaculata* (node 12), and it was inferred as being double the timing of Dataset 1 in Dataset 2 (0.82 and 1.69 Mya, respectively). For the remaining nodes, we referred to the ages estimates of Dataset 2, as it includes the most divergent Cox1 haplotype of *D. subtentaculata*, and thus provides a better representation of the species' genetic diversity. The split between the Western and Eastern clades was inferred at a mean age of 25.85 Mya. Within the Western clade, clades A and B split at around 23.09 Mya, whereas the divergence between clade B1 and clade B2 dated back to 20.52 Mya. The first split

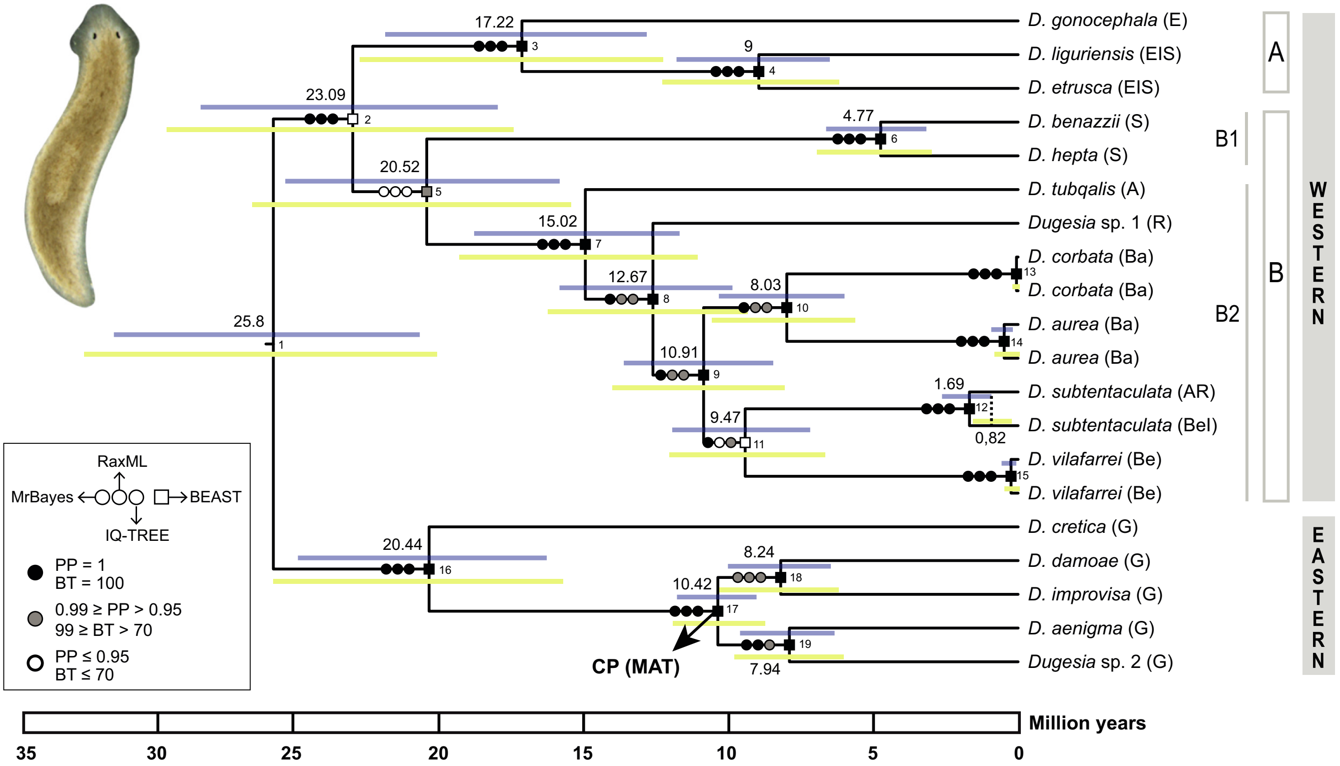

FIGURE 2 Time-calibrated phylogeny of *Dugesia* from the Western Mediterranean obtained with BEAST based on six molecular markers. Summarisation method: maximum credibility. Node bars correspond to the 95% high posterior density intervals of the time estimates (yellow: dataset 1; blue: dataset 2). Values over the nodes correspond to mean time estimates in million years ago obtained in dataset 2. Right values at nodes correspond to the numbering used in Table S6. Coloured dots at nodes indicate the support values obtained in the different phylogenetic analyses (PP: posterior probability; BT: bootstrap). CP (MAT): calibration point used in the BEAST analysis. Boxes and letters on the right indicate the main clades. Scale bar represents time in million years. Letters A–S in brackets indicate the distribution of each species (A: Africa, Ba: Balearic Islands, Be: Betics, E: Europe, G: Greece, I: Iberian Peninsula, R: Riff and S: Corso-Sardinian Archipelago). Photograph: *Dugesia subtentaculata* from the Iberian Peninsula (0.7 cm in length)

within clade B2 (divergence between *D. tubqalis* and the remaining species within this clade) occurred at approximately 15.02 Mya. Subsequently, the divergence between *Dugesia* sp. 1 from Morocco and the group including *D. corbata*, *D. aurea*, *D. vilafarrei* and *D. subtentaculata* was estimated to have occurred at around 12.67 Mya. The clade, including the species from Mallorca (*D. corbata* and *D. aurea*), began to diverge from the ancestors of *D. vilafarrei* and *D. subtentaculata* at around 10.91 Mya. Finally, the divergence of *D. corbata* and *D. aurea* dated back to 8.03 Mya, slightly after the split between *D. vilafarrei* and *D. subtentaculata*, which was inferred to have occurred at around 9.47 Mya.

3.3 | Ancestral range estimation

The results obtained with BioGeoBEARS for the two datasets were nearly identical, and both indicated that the DEC model best fitted our data (Table S7). These analyses also indicated that there were no significant differences between the performances of models DEC and DEC+j (*p*-value of 1 in the likelihood ratio test). The resulting ancestral range estimation under the DEC model for Dataset 2 is shown in Figure 3 (all values mentioned below refer to this dataset). All the estimated ranges of node 2 (ancestor of the Western clade) included Europe plus the Balearic Islands, the Corso-Sardinian Archipelago, the Betic region, the Riff region and/or the Iberian Peninsula. Among these inferred ancestral ranges, the range showing the highest probability was that constituted by Europe, the Balearic Islands and the Corso-Sardinian Archipelago (approximately 20%), while the probabilities of the remaining area combinations accounted for <10% each. Subsequently, the estimated range of node 3 (ancestor of clade A) corresponded to Europe (61.1%), Europe plus the Iberian Peninsula (13.1%) and the last two regions plus the Corso-Sardinian Archipelago (10.6%). The latter mentioned range was the most probable for node 4 (54.9%), followed by the Corso-Sardinian Archipelago plus the Iberian Peninsula (30.2%), or plus Europe (10.5%). Different from nodes 2, 3 and 4, the inferred range of node 5 (ancestor of clade B) was limited to two possible area combinations outside continental Europe: the Balearic Islands plus the Corso-Sardinian Archipelago and the Riff (48.5%), and the Balearic Islands plus the Corso-Sardinian Archipelago and the Betics (47.9%). The estimated range of node 6 (ancestor of clade B1) was exclusively the Corso-Sardinian Archipelago (99.9%), whereas the estimated range of node 7 (ancestor of clade B2) was the region constituted by the Balearic Islands, the Betics and the Riff (96.1%).

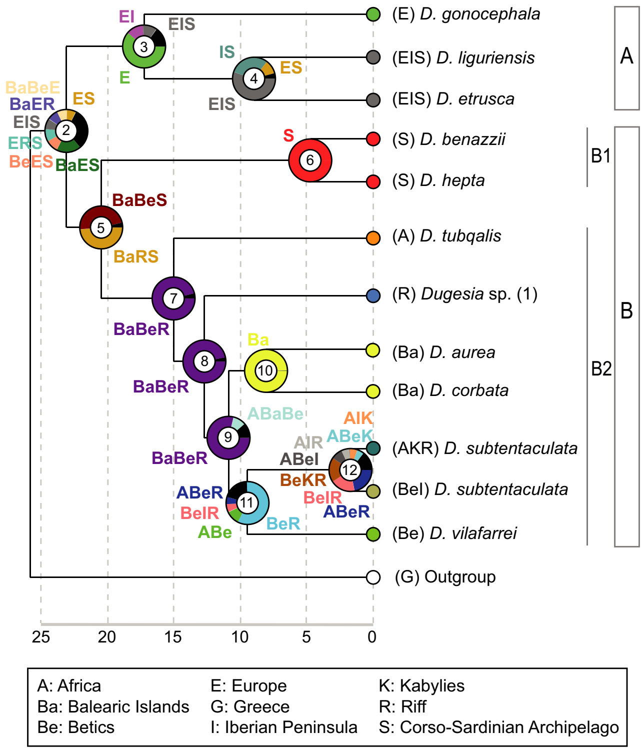

FIGURE 3 Ancestral ranges of *Dugesia* from the Western Mediterranean estimated with BioGeoBEARS (model DEC) on the BEAST time-calibrated phylogeny. Letters A–S indicate the different geographical ranges included in the analysis. Pie charts at nodes represent the estimated relative probability of the different ancestral ranges of the lineages, with each colour denoting a different geographical range (see Table S8 for detailed probabilities). Numbers at nodes are used to refer to each node in the text. Scale bar represents time in million years

This latter-mentioned region was also estimated as the most probable range for node 8 (97%). In the case of node 9, although the range constituted by the Balearic Islands, the Betics and the Riff was estimated to be the most probable one (78.7%), the range constituted by the Balearic Islands, the Betics and Africa showed 9.8% probability. Finally, while the ancestor of *D. aurea* and *D. corbata* (node 10) was estimated with a high probability as within the Balearic Islands (99.6%), the ancestor of *D. vilafarrei* and *D. subtentaculata* (node 11) was inferred to be from the Betic–Riff region (58.14%) or in other areas, including the Betic region and different regions of Africa and the Iberian Peninsula. A similar range reconstruction was found for the ancestor of *D. subtentaculata* (node 12), which included many area combinations of the Iberian Peninsula and the Betics plus different regions of Africa. The detailed percentage probabilities of the reconstructed ancestral ranges of all nodes are shown in Table S8.

3.4 | Environmental niche modelling

The environmental niche models for *D. subtentaculata*, *D. sicula* and *P. felina* from the Iberian Peninsula yielded AUC values higher than 0.8 with both the training and testing data (Table S9). This indicated that the model had a high predictive power for all of the species distribution cases. The mean output pictures of the Maxent model showed that the habitat suitability of *D. subtentaculata* was at its maximum along the northern coast of the Iberian Peninsula (Figure. S4). In contrast to *D. subtentaculata*, the habitat suitability for *D. sicula* was high in different regions of the Mediterranean coast on the Iberian Peninsula, whereas habitat suitability of *P. felina* was at its maximum along the Pyrenees and in certain regions on the northern coast of the Iberian Peninsula (Figure S4).

In the case of *D. subtentaculata*, the contributions from environmental variables to the model were roughly balanced; the highest was associated with land cover (23.9%), followed by the slope (17.9%), natural water discharge of the river (16.5%), temperature (15.5%), lithology (14.5%) and then precipitation (11.8%). The most suitable environmental conditions for this species were as follows: rivers with a low natural water discharge (mean annual less than 200 m^3 per second) running on sedimentary rocks either within broad-leaved deciduous tree cover or sparse herbaceous/shrub cover on terrain slopes of approximately 15°, with a mean annual temperature of approximately 13°C, and a relatively high precipitation regime (mean annual around 1200 mm) (Figure 4).

For the species *D. sicula* and *P. felina*, the slope was the environmental variable contributing the most to their distribution models (29.1% and 48.5%, respectively), whereas the remaining variables showed different percentage contributions to the model depending on the species (Table S10). The most suitable environmental conditions for these species are shown in Figure 4.

The distribution model inferred for *D. subtentaculata*, including the obtained potential distribution of *D. sicula* and *P. felina* as additional variables, also yielded high AUC values for both the training and testing data (0.932 ± 0.017 and 0.890 ± 0.043, respectively). In this analysis, the potential distribution of *P. felina* was the variable that contributed the most to the model (36.6%), whereas the potential distribution of *D. sicula* was a variable that contributed the least (3.9%). The analysis also showed that the habitat suitability of *D. subtentaculata* was at its maximum when the habitat suitability of *P. felina* ranged from 0.5 to 0.9 and when the habitat suitability of *D. sicula* was either close to 0 or around 0.6 (Figure S5).

Schoener's D niche overlapping statistics were 0.4533 between *D. subtentaculata-D. sicula*, and 0.5485 between *D. subtentaculata-P. felina*, indicating a certain degree of niche overlap between the two species pairs, particularly between *D. subtentaculata* and *P. felina*. However, the results of the identity tests showed that both D values were significantly different than the ones obtained assuming no niche differentiation (*p*-values lower than 0.001).

4 | DISCUSSION

We propose a novel phylogenetic scheme for *Dugesia* from the Western Mediterranean and exclude the possible impact of intraindividual genetic diversity on the divergence time estimates of the genus. Based on this information, we provided a biogeographical

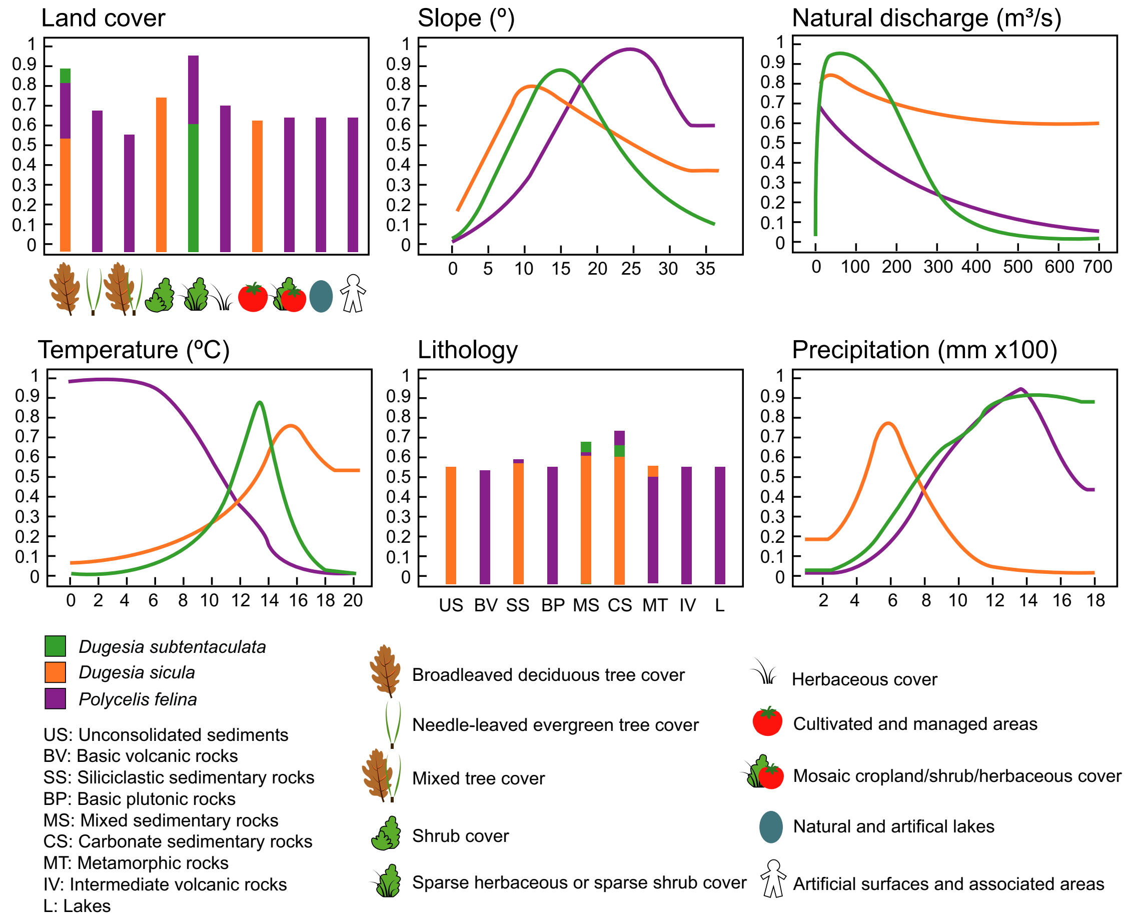

FIGURE 4 Relative effect of different hydro-environmental variables on the potential distribution of the species *D. subtentaculata*, *D. sicula* and *P. felina* in the Iberian Peninsula, obtained with Maxent. Y-axis: habitat suitability (from 0 to 1); X-axis: variation range of each variable. For the variables corresponding to the Land cover and the Lithology, only those categories showing habitat suitability higher than 0.5 have been represented

scenario for *Dugesia* based on the interplay of microplate tectonics, abiotic environmental factors and species interactions. This work contributes to our understanding of how different factors interact to shape species diversity and distribution over time.

4.1 | Impact of high intraindividual genetic diversity on divergence time estimations

The dating of evolutionary events, such as the timing of species divergence, relies on the production of calibrated phylogenies that are sensitive to the genetic sequences used to build them. High levels of intraindividual genetic diversity have not only been detected in different *Dugesia* species (Dols-Serrate et al., 2020; Lázaro & Riutort, 2013; Leria et al., 2019), but also in several groups of plants and corals (Gill, 1986; Schweinsberg et al., 2015), and these are the result of accumulated somatic mutations during long periods of asexual reproduction. Our results showed that the effect of selecting one or another intraindividual haplotype on the estimated divergence ages was restricted to the species that showed intraindividual genetic diversity, whereas it had no effect on the age estimates for the rest of the species. These results highlight the importance of considering intraindividual genetic diversity when working with asexual organisms at the species level. However, it has no impact in our conclusions regarding the biogeographical history of the genus *Dugesia* that we elucidate in the following subsections.

4.2 | Novel phylogenetic relationships within *Dugesia* from the Western Mediterranean

Our phylogenetic analysis of the Western Mediterranean *Dugesia* species suggests a new evolutionary scenario for this group. A single similar previous analysis (Lázaro et al., 2009) placed *D. subtentaculata* sensu lato (presently divided into *D. aurea*, *D. corbata*, *D. vilafarrei* and *D. subtentaculata* sensu stricto; Leria et al., 2020) as sister to the rest of the western species. In contrast, we propose that the clade constituted by these four species is a derived group within Western Mediterranean *Dugesia*, which is sister to the endemic species from northern Africa (viz., *D. tubqalis* and the putative new species *Dugesia* sp. 1 from Morocco) and together constitute a clade with the two endemic species from Corsica and Sardinia (viz., *D. hepta* and *D. benazzii*) (clade B). In our phylogeny, the first split within Western Mediterranean *Dugesia* corresponds to the separation of the species *D. gonocephala*, *D. liguriensis* and *D. etrusca* (Clade A) and clade B described above. This new and better resolved phylogenetic scenario results from the addition of four molecular markers into the analysis, the increased taxon sampling and the selection of a more closely related outgroup that helped avoid systematic errors (Graham et al., 2002). Nevertheless, a node remains in our tree with low statistical support; it groups the species *D. hepta* and *D. benazzii* with the rest of the species in clade B and could result from rapid diversification between these groups. The other relationships that we determined, such as those within clade A or the phylogenetic relationships obtained between the species that were previously subsumed within *D. subtentaculata*, agreed with the findings of previous studies (Lázaro et al., 2009; Leria et al., 2020).

4.3 | Microplate tectonics played an important role in Western Mediterranean *Dugesia* diversification

Our results indicate that the palaeogeographical events in the Western Mediterranean during the Oligocene–Miocene period were key to shaping the biogeographical history of *Dugesia* in this region (Figure 5). Our ancestral range estimation analysis indicated that the ancestor of the *Dugesia* Western clade was situated in southwestern Europe at around 30 Mya. This result agrees with that of a recent study of the biogeographical history of the entire genus *Dugesia* (Solà et al., 2022), which places the arrival of this ancestral lineage to Europe from Africa during this period through a connection existing between the land masses that today constitute the Italian Peninsula and Tunisia (Hinsbergen et al., 2020). The orogeny of the Alps and the Pyrenees had been ongoing for 30 million years (Dèzes et al., 2004); as the habitat suitability of *Dugesia* likely decreases on steep slopes, we thus hypothesise that both mountain ranges acted as porous barriers to the expansion of *Dugesia* from the Italian Peninsula. In particular, the Alps likely restricted the northward expansion of this ancestral lineage to Central Europe, and the Pyrenees limited its entry to the Iberian Peninsula. This situation likely channelled the dispersion of this *Dugesia* lineage between the two mountain ranges, which is an area constituting part of the landmass that resulted in the first Mediterranean microplate (Figure 5b).

The initial detachment of the first microplate from the continent (approximately 25 Mya) likely resulted in the first diversification event within the Western Mediterranean *Dugesia*, where the ancestor of the species *D. gonocephala*, *D. etrusca* and *D. liguriensis* (which remained on the continent) was isolated from the ancestor of the species presently distributed in the Western Mediterranean Islands, Northern Africa and the Iberian Peninsula (which remained on the microplate) (Figure 5c). This event has also been proposed as a driver of the diversification between the Iberian and Corso-Sardinian species within the genera of freshwater arthropods *Stenasellus* and *Tyrrhenoleuctra* (Fochetti et al., 2009; Ketmaier et al., 2003). In the subsequent fragmentation of the microplate at around 21 Mya, the ancestor of *D. hepta* and *D. benazzi* would have been isolated on the microplate corresponding to Corsica, Sardinia and Calabria, while the ancestor of the other species would have remained on the portion of the landmass including the Balearic Islands, the Betic region and the Riff (Figure 5d), that later collided with Northern Africa at approximately 15 Mya. The short time span between microplate detachment from the continent and its first fragmentation (4 My) may explain the lack of resolution found in this part of the tree. The collision between the rest of the microplate and northern Africa would have facilitated the dispersion of some of the *Dugesia* lineages to this region, and this would have given rise to at least two Moroccan species that are present in our tree (Figure 5e). A similar dispersion event from the Betic–Riff plate to northern Africa has also been suggested for lizards of the genus *Psammodromus* (Mendes et al., 2017). Additionally, pre-Messinian dispersions between the Iberian Peninsula and Northern Africa have been proposed for Cyprinid fish (Levy et al., 2009), which reinforces the hypothesis that land bridges between these regions were accompanied by freshwater connections.

Finally, the fragmentation of the Balearic–Betic–Riff microplate appears to have occurred in two consecutive steps, and this drove the diversification of the species that remained in this region. In the first step, this microplate would have fragmented into the Riff region and the Balearic–Betic region at around 12 Mya, and this would have isolated the ancestors of the two Moroccan species (that would have dispersed to the Riff region during the contact period) from the ancestors of *D. aurea*, *D. corbata*, *D. vilafarrei* and *D. subtentaculata* (that would have remained in the Balearic–Betic region) (Figure 5f). The second and final split of this microplate at around 10 Mya probably isolated the ancestor of *D. aurea* and *D. corbata* in the Balearic Islands, whereas the ancestor of *D. vilafarrei* and *D. subtentaculata* would have remained in the Betic region that became part of the Iberian Peninsula (Figure 5g).

Although our results are in general agreement with Rosenbaum's palaeogeographical model for microplate tectonics, the final result disagrees. Rosenbaum's model suggests that the Balearic Islands were isolated from the other plate fragments at around 25–21 Mya, while our results point to a connection between the Balearic Islands and the Betic region until 10 Mya. The biogeographical importance of the connection between the Balearic Islands and the Betics was proposed by Colom (1978). Importantly, this connection until 10 Mya has already been proposed in other phylogeographical studies (Bidegaray-Batista & Arnedo, 2011; Chueca et al., 2015), and it agrees with alternative tectonic models that suggest the union of the Balearic Islands to the Betic–Riff microplate lasted until the early Tortonian (at around 11 Mya) or that different periods of connection existed between these two microplates until this period (Dèzes et al., 2004; Schettino & Turco, 2011; Hinsbergen et al., 2020).

Finally, although the Sardinian populations of *D. etrusca* and *D. liguriensis* were not included in the present study, our results provide insights into their biogeographical history. These species could have dispersed to the microplate corresponding to Corsica–Sardinia–Calabria when this landmass came into contact with the

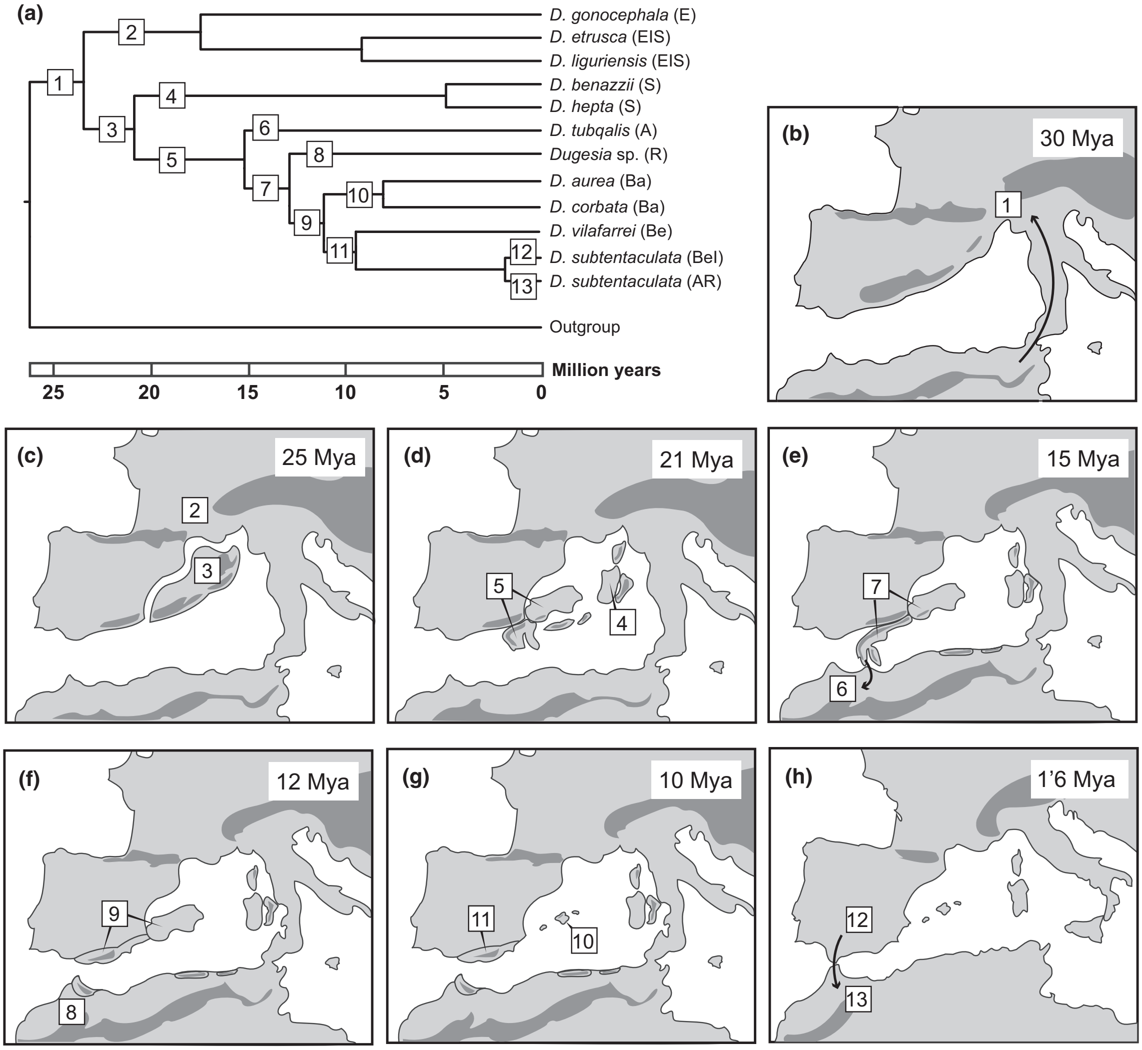

FIGURE 5 Biogeographical history of *Dugesia* from the Western Mediterranean region proposed in the present study. (a) Schematic representation of the phylogenetic relationships between *Dugesia* species under a temporal framework. Letters A–S in brackets indicate the current distribution of each species (A: Africa, Ba: Balearic Islands, Be: Betics, E: Europe, I: Iberian Peninsula, R: Riff and S: Corso-Sardinian Archipelago). (b–h) Palaeogeographical reconstructions based on Dèzes et al. (2004), Rosenbaum et al. (2002), Schettino and Turco (2011). Framed numbers from 1 to 13 indicate the geographical location and temporal framework of each *Dugesia* lineage. Dark grey in pictures (b–h): main orogenic regions

Italian Peninsula (from 18 Mya until the opening of the Tyrrhenian Sea at around 10–6 Mya) (Rosenbaum et al., 2002), and this is a biogeographical pattern that has been proposed for different taxa (Bidegaray-Batista & Arnedo, 2011; Carranza et al., 2008). Alternatively, both species could have dispersed more recently from the continent to the island during any of the land connection periods that occurred during the Messinian Salinity Crisis or during the low sea level events that occurred until the Pleistocene (Ketmaier & Caccone, 2013), as suggested for other groups (Fromhage et al., 2004; Novo et al., 2015). These recent periods of land connection may also explain the dispersion of some *D. benazzii* populations between Corsica and Sardinia. Recent analyses have shown that *D. benazzii* may represent a species complex (Dols-Serrate et al., 2020) with an intricate biogeographical history. In summary, further studies, including the insular populations of these species, are necessary to provide more information about the biogeographical history of the Corso-Sardinian Archipelago.

4.4 | Abiotic environmental factors define *Dugesia* distribution after major diversification events

The present study is the first to use specific hydro-environmental variables to model the distribution of freshwater planarian species. Our results show that the type of land cover, the slope of the terrain and the natural water discharge of the river likely played major roles in driving *Dugesia* distributions. The slope of the terrain has previously been identified as a key factor in explaining the distribution of the freshwater planarian species *Crenobia alpina* in Wales (Lock & Reynoldson, 1976). Similarly, the current velocity (which is directly related to the terrain slope) has also been reported as an important factor driving the distribution of different freshwater planarian species in several springs of the Pyrenees (Roca et al., 1992), including *Dugesia* species, although the authors suggested that slope was not a determinant in explaining the occurrence of *Dugesia* in the analysed springs.

The natural water discharge of the river may represent an important abiotic factor in the distribution of freshwater planarians, as it influences several key aquatic processes, such as the level of dissolved oxygen, sediment transport and deposition, the water quality, and the habitat type (Bunn & Arthington, 2002; M. Warren et al., 2015). Finally, although the type of land cover may not appear to be an a priori determinant for a freshwater species, we found that it was the most important variable that explains the distribution of *D. subtentaculata* on the Iberian Peninsula. This discovery implies that major historic changes in the vegetation and habitat characteristics within the Western Mediterranean may have played important roles in the biogeographical history of the group.

After the final split of the microplates described above at approximately 10 Mya, global temperatures began to cool, and Western Mediterranean vegetation shifted gradually from evergreen forests to mixed and deciduous tree cover (Jiménez-Moreno et al., 2010). This shift from subtropical to temperate forests was probably favourable for the *Dugesia* lineages inhabiting continental Europe and the western Mediterranean islands at that time, potentially resulting in their geographical expansion. However, in the case of Mallorca, posterior events (such as eustatic sea movements during the Pleistocene; Dumitru et al., 2021) may have resulted in losses of diversity and distributional range. This explains why only two endemic species remain on the island (*D. aurea* and *D. corbata*); they are genetically highly differentiated, and each species is restricted to a single locality.

In contrast to the aforementioned *Dugesia* lineages found in southern Europe and the western Mediterranean islands after the split of the microplates, lineages that had arrived in North Africa from the Betic–Riff microplate (viz., the ancestors of *D. tubqalis* and *Dugesia* sp. 1 from Morocco) encountered a gradual desertification scenario that began in around the Miocene/Pliocene period (Micheels et al., 2009). These unfavourable environmental conditions probably restricted the geographical distribution of the lineages to the forested areas of the region, and it explains why these two species are presently endemic from the Atlas and the Riff, which are the two most humid eco-regions of north-western Africa (Rankou et al., 2013).

Interestingly, some *D. subtentaculata* populations are also present in the Atlas and Riff regions. According to our results, the divergence between the Iberian and the north African populations of this species dates to 1.6 Mya, which indicates that the colonisation of Africa from the Iberian Peninsula possibly happened during some of the low sea level periods that occurred during the Pleistocene (Figure 5h) (Clark et al., 2009). Although there is an extensive evidence of species exchange between Africa and Iberia during this period (Fernández-Mazuecos & Vargas, 2011; Gibert et al., 2003; Kaliontzopoulou et al., 2011; Pleguezuelos et al., 2008; Straus, 2001), most such examples are of terrestrial vertebrates that likely crossed the strait of Gibraltar via rafting. As *Dugesia* species require the continuity of freshwater bodies for survival and dispersal, this indicates that some freshwater connections may have existed between these regions during the Last Glacial Maximum; for example, by river plumes (Gibert et al., 2003).

Although the glacial periods of the Pleistocene may have enabled the increased distribution of some *Dugesia* species (such as the African colonisation of *D. subtentaculata*), the associated permafrost soil extensions likely reduced the diversity and distribution of the *Dugesia* lineages found in continental Europe. This scenario explains why most Western Mediterranean *Dugesia* species diversity is presently found in southern Europe. In contrast, continental Europe is only occupied by genetically similar populations of *D. gonocephala* (Lázaro et al., 2009), which likely expanded northward from southern regions or from microrefugia in central Europe after the glaciations ended. It has been proposed that a similar situation shaped the present genetic diversity and distribution of the freshwater planarian species, *Schmidtea polychroa*, *S. lugubris* and *S. nova* (Leria et al., 2018; Pongratz et al., 2003), in addition to the current biogeographical pattern of many other European species (Hewitt, 2000).

4.5 | Interspecific competition and human habitat transformation as potential *Dugesia* distribution drivers

Environmental niche overlap between species can indicate interspecific competition. In this sense, our results show a certain degree of overlap between *D. subtentaculata* and *P. felina and D. sicula*, particularly between *D. subtentaculata* and *P. felina*. Therefore, the distribution dynamics of the *Dugesia* lineages arriving from Africa to Western Europe at around 30 Mya were likely influenced by the freshwater planarian species that were already found on the European continent, such as the genus *Polycelis* or *Schmidtea* (Lázaro et al., 2011; Leria et al., 2018). In the case of *D. subtentaculata* and *P. felina*, both species may be currently competing, or past interspecific competition may have resulted in niche differentiation associated with certain environmental characteristics that have not been evaluated in the present study, such as trophic, circadian or microhabitat differentiation (Afonso & Eterovick, 2007; Boddington & Mettrick, 1974; Lombardo et al., 2011), and these allowed the two species to coexist in several localities.

Unlike *P. felina*, and refuting our initial hypothesis, our results imply that *D. sicula* may not be presently competing with *D. subtentaculata* in the Iberian Peninsula, as their niches only overlap in limited regions on the eastern coast, and the potential distribution of *D. sicula* does not influence the distribution of *D. subtentaculata*. However, our analyses show that *D. sicula* not only presents high suitability for cultivated and managed areas, but it is also widely tolerant of different water discharge regimes, which may help to explain its recent colonisation across the Mediterranean region that is likely driven by human trade activities (Lázaro & Riutort, 2013). Therefore, the replacement of different *D. subtentaculata* populations from the southern and eastern coasts of the Iberian Peninsula by *D. sicula* may have been driven by changes in the characteristics of the freshwater environments due to human activities, such as agriculture, livestock rearing and urbanisation, rather than by direct competition between the species. For instance, unlike *D. subtentaculata*, *D. sicula* is more frequently found in canalised rivers, wells, plant nurseries and fountains within urban parks (Lázaro & Riutort, 2013). In the southern regions of Europe, the environment is being increasingly transformed from natural habitats to areas managed by humans, and the consequences of human-driven climate changes in these regions are contributing to extreme climate characteristics in the Mediterranean region, such as severe droughts and sudden heavy storms. We, therefore, tentatively predict the favoured expansion of *D. sicula* in this part of the Mediterranean region, as there is a reduction in suitable habitats for the autochthonous *Dugesia* species.

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DATA AVAILABILITY STATEMENT

All sequences have been deposited in GenBank. The alignments are available in DRYAD database: https://doi.org/10.5061/dryad.c866t1g80.

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BIOSKETCH

Laia Leria obtained her PhD in 2019, focused on understanding the processes shaping freshwater planarians' genetic diversity. This manuscript represents an expanded version of her thesis work. She and the other authors collaborate on questions on planarian diversity at the Universitat de Barcelona (MR research team web page: www.ub.edu/geisan) and the Max Planck Institute for Multidisciplinary Sciences (MV departmental web page: https://www.mpinat.mpg.de/640083/miquel-vila-farre).

Author contributions: LL, MVF and MR did the initial study design. LL processed and analysed the data and wrote the manuscript with input from all authors. RR and XF contributed to interpreting the results of the biogeographical analyses. All authors read and approved the final manuscript. Authors declare no conflict of interest.

SUPPORTING INFORMATION

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