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Biogeography of Mediterranean amphipods associated with *Posidonia oceanica* meadows

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Although amphipods are key components of the macro-fauna associated with *Posidonia oceanica* meadows, no studies focussed to date on the structure and diversity of such assemblages across the Mediterranean Sea. Here, we studied whether amphipod diversity reflects generalised biodiversity patterns across the Mediterranean by applying a network approach using incidence data mined from literature to infer biogeographic modules and the biogeographic roles of associated localities. We tested if the observed pattern reflected a geographic component and was linked to species range extension by means of a multivariate analysis. Modularity highlighted four distinct modules characterized by a geographic subdivision driven by the main Mediterranean biogeographic divides, and differences in species diversity along a NW-SE gradient. Modules showed both different biogeographic roles of associated localities and different range extension of amphipod species associated with different modules. The biogeographic roles of localities provided by modularity identified the highest amphipod diversity in the Central-Western Mediterranean, followed by Tunisian coasts. This pattern may be explained by a mix of species persistence and post-Last Glacial Maximum expansion, as suggested by the paleogeographic history of the host seagrass and the ecology of associated amphipods.

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ABSTRACT

Although amphipods are key components of the macro-fauna associated with *Posidonia oceanica* meadows, no studies focussed to date on the structure and diversity of such assemblages across the Mediterranean Sea. Here, we studied whether amphipod diversity reflects generalised biodiversity patterns across the Mediterranean by applying a network approach using incidence data mined from literature to infer biogeographic modules and the biogeographic roles of associated localities. We tested if the observed pattern reflected a geographic component and was linked to species range extension by means of a multivariate analysis. Modularity highlighted four distinct modules characterized by a geographic subdivision driven by the main Mediterranean biogeographic divides, and differences in species diversity along a NW-SE gradient. Modules showed both different biogeographic roles of associated localities and different range extension of amphipod species associated with different modules. The biogeographic roles of localities provided by modularity identified the highest amphipod diversity in the Central-Western Mediterranean, followed by Tunisian coasts. This pattern may be explained by a mix of species persistence and post-Last Glacial Maximum expansion, as suggested by the paleogeographic history of the host seagrass and the ecology of associated amphipods.

INTRODUCTION

The Mediterranean Sea is a recognized hot-spot of biodiversity. Although the volume of this semi enclosed sea is only 0.3% of ocean waters, it hosts about 17,000 species, which represent between 4 to 18% of the global macrofauna, depending on the phylum considered (Coll et al., 2010; Bianchi et al., 2012). This high diversity, together with the significant rate of endemisms, boosted biodiversity studies leading to some generalized patterns to emerge as, for example, the latitudinal gradient of species richness decreasing from north-eastern to south-western regions (Lejeusne et al., 2010). This gradient is correlated with clines of both environmental variables (mainly temperature and salinity) and production levels, together with general water mass circulation and the location of barriers (Coll et al., 2010). All these ecological and biological features allowed identifying biogeographic sectors within the Mediterranean Sea, each characterized by both different biota and ecological parameters (Bianchi et al., 2012). However, data on the pattern and mechanisms of species co-occurrence across the whole Mediterranean basin are limited to a relatively small number of organisms (Arvanitidis et al., 2002; Gerovasileiou & Voultsiadou, 2012). Moreover, geographically widespread and ecologically broadly adapted groups have been only seldom studied according to habitat-related subdivisions (Sevastou et al., 2013). In this work, we focused on the crustacean amphipods associated with *Posidonia oceanica* meadows, since they represent one of the most relevant components of the vagile fauna of the main foundation species of the Mediterranean Sea.

The endemic *P. oceanica* (L.) Delile represents the dominant and most widely distributed seagrass in the Mediterranean Sea, where it plays a fundamental role as an ecosystem engineering supporting important ecosystem functions, including oxygen production, habitat providing, food supplying and reduction of coastal erosion (Boudouresque, Mayot & Pergent, 2006). The complexity of this multi-layered and three-dimensional habitat allows a great variety of associated fauna to live into the canopy, rhizomes and mattes, making the meadows a strikingly biodiversity-rich habitat even within the Mediterranean hot-spot (Buia, Gambi & Zupo, 2000). Among the vagile fauna, crustacean amphipods are one of the dominant groups, being present with high abundance and diversity of species (Mazzella, Scipione & Buia, 1989; Gambi et al., 1992; Sturaro et al., 2015). Amphipods are considered a key ecological component of this habitat, due to their role in transferring energy across the system as grazers, predators or detritivores, and representing an important trophic resource for higher predators such as fish (Pinnegar & Polunin, 2000; Zakhama-Sraieb, Ramzi-Sghaier & Charfi-Cheikhrouha, 2011; Michel et al., 2015; Bellisario et al., 2016).

Despite the relevance of *P. oceanica* meadows and associated amphipod assemblages (which are both sensitive environmental indicators), a comprehensive study on the biogeographic pattern of amphipod species organization in *P. oceanica* meadows at whole basin scale is still lacking. Available data concern check-lists and local studies based on diversity index, focusing either on species richness or the number of endemisms (Gambi et al., 1992; Diviacco, 1998; Como et al., 2008; Scipione & Zupo, 2010; Bedini et al., 2011; Zakhama-Sraieb, Ramzi-Sghaier & Charfi-Cheikhrouha, 2011; Sturaro et al., 2015). Since the aim of this work is to detect significant biogeographic subdivisions in the distribution of amphipods sampled on *P. oceanica* meadows along the Mediterranean coasts, and to verify if they follow the generalized biodiversity patterns recorded for other groups, we applied a network approach based on modularity to take the most from distribution data mined form literature.

Recently, new methods deriving from network analysis have been successfully applied to identify bioregions, with modularity and map equation outperforming classic clustering methods (Carstensen & Olesen, 2009; Vilhena & Antonelli, 2015; Bloomfield, Knerr & Encinas-Viso, 2018). In particular, modularity (i.e., the tendency of a network to subdivide in densely connected modules) has proved powerful in detecting clusters of areas and species that are associated with each other (i.e., biogeographical modules, *sensu* Carstensen et al., 2012; 2013). This approach is able to provide not only a more efficient identification of the boundaries between bioregions, but also relevant insights into the processes driving the assembly of communities (Bloomfield, Knerr & Encinas-Viso, 2018). Indeed, one of the main advantages of modularity is the possibility to evaluate the importance of each node (e.g., assemblage) in terms of network connectivity, by measuring specific metrics related to the number of links within and between modules. These metrics, which in a biogeographic context are named local and regional topological linkage (*sensu* Carstensen et al., 2013), can be used as indirect estimators of richness and endemisms, so discriminating biogeographic regions (Bloomfield, Knerr & Encinas-Viso, 2018).

In this work, we used a network approach based on modularity to detect significant biogeographic subdivisions in the distribution of amphipods sampled on *P. oceanica* meadows along the Mediterranean basin. We discussed the results obtained in the light of the current knowledge on the biogeographic distribution and ecological features of amphipod species and of the paleo-geographic history of both Mediterranean Sea and *P. oceanica* seagrass, in the aim to provide insights on how amphipod diversity could have spread within the Mediterranean basin.

MATERIALS & METHODS

Study area and starting dataset

An extensive survey of the literature was conducted in order to obtain all available information on the presence of amphipods on *P. oceanica* meadows across different regions of the Mediterranean Sea (a list of the data sources is reported in Supplemental Materials & Methods S1 and reference therein). The available data covered a large portion of the Mediterranean basin (Fig. 1), and were distributed in regions characterized by different geographic, hydrological and geological features, as well as by differences in the potential connectivity due to the general circulation models (Bianchi & Morri, 2000; Bianchi, 2007; Berline et al., 2014). Data selection followed specific criteria aimed at obtaining comparable data, although from different Authors, with particular regard to possible taxonomic issues (for more information see Supplemental Materials & Methods S1). The final dataset included 152 amphipod species from 28 localities: 9 located in Tunisia, 16 in Italy (Tyrrhenian, Adriatic and Ionian Sea), 2 in Spain and 1 in Corsica (France) (Supplemental Materials & Methods S1).

Information about the biogeographic distribution of observed amphipods were obtained from Bellan-Santini & Ruffo (2003), which classified the over 400 species of the Mediterranean amphipod benthic fauna in twelve macro categories on the basis of their current distribution: WM, West Mediterranean; EM, East Mediterranean; Adr, Adriatic Sea; ME, Mediterranean endemics; Afr, African coasts from Ceuta to Cap Vert; Ib, Iberian coasts; Fr, French coasts; Br, British coasts; Norw, Norwegian coasts; Arct, Arctic Sea; Ind-P, Indo-Pacific Ocean; Cosm, Cosmopolite. Here, species with an Atlantic distribution were clumped in two main categories from the five proposed by Bellan-Santini & Ruffo (2003): ATL, Atlantic Sea (Iberian, French and British coasts) and NATL, North Atlantic Sea (Norwegian and Arctic regions), so that our final distribution comprised nine different categories.

Network analysis

To provide insights into the biogeographic distribution of assemblages (Kivelä, Arnaud-Haond & Saramäki, 2015), a thresholding approach was used in order to identify groups of localities having stronger similarity in terms of community composition. The observed pattern of incidence should influence species co-occurrence, so that species with narrower distributions (e.g., present in few samples) should have the tendency to co-occur with a smaller number of species, while species with wider distributions should be present across most localities and so co-occur with many different species.

Data were ordered as a species/incidence matrix, whose entries represent the presence of species (rows) at each locality (columns). A weighted network, where localities represent nodes and links the strength of connections, was built using the Jaccard index on the species/incidence matrix, to derive a similarity distance matrix of species co-occurrence. Values ranged from 0, when two localities were identical in amphipod composition, to 1, when they shared no taxa, so that links with higher weights indicated low similarity between localities, and vice versa.

A thresholding approach was then applied in order to identify closely related localities by finding the critical value describing the threshold similarity among pairs, i.e. percolation network. Among many possible approaches, percolation networks are becoming increasingly used in ecological studies since they allow identifying relationships among populations or critical scales in landscape ecology, with the advantage of not requiring any *a priori* knowledge of a threshold value (Rozenfeld et al., 2008; Fletcher et al., 2013; Bellisario, 2018). This value was measured by removing distances following a decreasing order and starting by the one with the largest distance (i.e., most dissimilar localities), until the network reaches the threshold value beyond which it becomes fragmented into disconnected clusters. The identification of this value is obtained by calculating the average cluster size $\langle L \rangle$, that is, the average number of localities belonging to an l-size cluster, as a function of the last threshold distance value beyond which links were removed (Stauffer & Aharony, 1992):

$$\langle L \rangle = \frac{1}{N} \sum_{l<l_{\max}} l^2 n_l \tag{1}$$

where N is the total number of localities not included in the largest cluster ($l_{\max}$) and n_l is the number of clusters containing l localities. Basically, each time a distance value is removed from the network, localities are redistributed in clusters of different sizes, from largest to smallest. This procedure is therefore iterated until the critical threshold is identified in the transitional region characterized by a strong decrease in $\langle L \rangle$, where the network becomes disconnected (for more information about percolation theory, refer to Stauffer & Aharony, 1992). Here, we used the methodology described in Rozenfeld et al. (2008) and implemented in the package 'sidier' (Muñoz-Pajares, 2013) of R (R Development Core Team, 2013).

Modularity

After identifying the minimum set of pairwise similarities between localities, we tested for the presence of a significant pattern of aggregation between localities, and if this pattern reflected a geographic component. To this end, we measured the modularity (Q), which is defined as the degree to which a network can be subdivided in aggregated sets of nodes (i.e., modules), where the within-module links are significantly higher than between-module ones (Newman & Girvan, 2004; Fortunato, 2010). Modularity provides a formal description of the pattern of aggregation between species, populations or communities, being able to identify critical scales in specific ecological and evolutionary processes (Fletcher et al., 2013).

Modularity was measured by using the equation originally described by Newman and Girvan (2004):

$$Q = \frac{1}{2m} \sum_{i,j} \left[A_{ij} - P_{ij} \right] \delta(C_i, C_j) \qquad (2)$$

where m is the total number of links in the percolation network (see above), A_{ij} is the matrix expressing the degree of similarity between localities i and j, $\delta(C_i,C_j)$ is a matrix indicating whether i and j are members of the same module and P_{ij} is the probability in the null model that a link exists between i and j. The extent to which links are distributed within and among modules was tested against an appropriate null model, to correct the observed value of Q by null model expectation. Here we used a simulated annealing algorithm (SA) to test for the significance of a modular partitioning by generating 1,000 null matrices having the same degree distribution as the original network. Under the SA algorithm, affiliation of nodes to modules has an accuracy of 90%, and a significant modular structure was find if the empirical Q value lies above the 95% confidence interval for Q in the randomized networks (Guimerà & Amaral, 2005).

We further investigated the role of each locality in the network by measuring its position compared with other localities in its own module and how it relates to localities in other modules. Roles are assigned in a functional space given by the standardized within-module degree (z, the number of links a node has in its own module) and the participation coefficient (p, the number of links a node has with nodes in other modules) (Guimerà & Amaral, 2005). Here, we followed the nomenclature proposed by Carstensen et al. (2012), where z and p are replaced by l (local topological linkage) and r (regional topological linkage). As links in our network relates with pattern of similarity between assemblages, the role of localities (i.e., peripherals, connectors, module and network hubs) allowed for a straightforward description of how amphipod diversity could have spread between different areas of the Mediterranean basin.

Multivariate analysis

To explore to what extent the measured network characteristics (i.e., modularity and nodes topology) were related to the biogeographic distribution of amphipods, we ran a between-group correspondence analysis (BGCA) on the 'sites x species-biogeographic classes' matrix, where groups were given by the identified modules. BGCA performs a classic Correspondence Analysis (CA) of the per-group centres of gravity, providing an ordination of the groups by maximizing the between-group variance (Baty et al., 2006). From the nine species-biogeographic classes derived from the literature (see above), data were aggregated by summing the number of species belonging to each class in a given site.

RESULTS

Modularity

The percolation network showed a co-occurrence similarity threshold of 0.74, which means that sampling sites are expected to share no more than 74% of amphipod species. This leaded to a network structure of 28 localities joined by 104 links, showing a significant modular structure when compared with randomized models ($Q = 0.466 \pm 0.005$, $P < 0.001$). Four distinct modules were identified, characterized by a clear geographic distribution (Fig. 2a).

11 localities constituted the Central-Western Mediterranean module (CWM), which spanned from the Spanish coasts till the central Tyrrhenian ones, and included also Lampedusa (LAM) and Marettimo (MART) Islands. The module showed the higher number of amphipod species and percentage of both module and Mediterranean endemics (Fig. 2b and Table 1).

The Tunisian module (TUN) comprised all 9 localities belonging to the Tunisian coasts, having a relatively high number of species and percentage of module endemics (i.e., species present exclusively in the module) and the lowest number of Mediterranean endemics (Fig. 2b and Table 1). The remaining 6 localities were partitioned in two other modules, Adriatic (ADR, 3 localities) and Ionian (ION, 3 localities), both characterized by lower species number and percentage of module endemics (Fig. 2b and Table 1). The ION module had a relatively high percentage of Mediterranean endemics, whilst ADR showed a high percentage of cosmopolite species (Fig. 2b and Table 1).

Localities in the ADR and ION modules were all classified as peripherals – non-hub connectors (R1 and R2 in Fig. 2c), meaning that amphipod communities in these localities are composed by few local species and by a higher (although not very consistent) number of species having a regional distribution (Fig. 2b). Localities in CWM and TUN modules were classified mainly as hubs, subdivided between provincial and connector hubs (R3 and R4 in Fig. 2c). More than half (54%) of localities in the CWM module can be considered connector hubs, characterized by many local and regional species and most of localities in the TUN module were classified as provincial hubs, so having a large number of local species and few regional ones (Fig. 2c). The pattern of species diversity in the functional space given by l and r showed that high diversity values are found in hub localities only, while lower diversity is observed in all the peripheral localities together with some hubs (including all 9 TUN sites) (Fig. 2c).

Multivariate pattern

The first two principal dimensions of the BGCA accounted for almost 80% of the total inertia (Fig. 3), showing the presence of 3 to 4 main species-biogeographic classes explaining the observed pattern of between-modules variance (Fig. 3). Mediterranean endemic species (ME) accounted for almost 50% of the total variance explained by the first dimension of the BGCA, which was associated with the pattern of distribution of assemblages in the CWM module. Indo-

Pacific and African species accounted for more than 30% of variance along the first dimension (Fig. 3), providing a clear differentiation of the TUN module from all others. Cosmopolite species (COSMP) accounted for 20% of the between-module variance explained by the second dimension of the BGCA, characterizing the pattern of ordination of the ADR module (Fig. 3). With respect to the biogeographic role of localities, provincial and connector hubs seemed to be characterized by both Mediterranean endemics (ME) and species of Indo-Pacific distribution (INDP), while peripheral localities were characterized mainly by cosmopolite species (COSMP), although the overall pattern was not sharply defined (Fig. 3).

DISCUSSION

Modules are biogeographically based

Our data showed that amphipod assemblages are heterogeneous throughout the Mediterranean area, with a maximum of 74% of shared species among sites, and that these differences lay on a geographical base. Despite network methods do not relay on spatial information, the four modules identified consistently correspond to four biogeographic regions of the Mediterranean Sea: Central-Western Mediterranean (CWM), Tunisian (TUN), Ionian (ION) and Adriatic (ADR). Each region is delimited by well-known barriers, such as the Almeria-Oran Front, the Sicily Channel and the Strait of Otranto (highlighted as n. 2, 5 and 6 in Fig. 1). All these barriers have been pointed out as the most relevant in accounting for by ecological and biogeographical heterogeneity across the Mediterranean Sea, and all of them set a quite abrupt change in salinity and temperature regimes of adjacent basins.

The Almeria-Oran Front (AOF) is the range boundary for *P. oceanica* that, being a Mediterranean endemic, is unable to tolerate the low temperatures of the Alboran Sea (Boudouresque, 2004). The less saline and colder Atlantic waters enter this sea through the Strait of Gibraltar, so that salinity and temperature change quite abruptly on the two sides of the AOF, which is the western boundary of the Central-Western (CWM) module. Circulation patterns and changes in temperature and salinity across the Sicily Channel, both concur in partially preventing the dispersal of a number of species across the threshold of the Siculo-Tunisian Straits (Robinson et al., 1992; Coll et al., 2010). In our study, this is the divide between CWM and TUN modules, this latter grouping the sites along the Tunisian coasts characterized by the presence of the jet-like Algerian Current and Atlantic Ionian Stream (Pinardi & Masetti, 2000). The Strait of Otranto delimits the Adriatic Sea, which is a semi-enclosed basin where a number of factors as winds, tides and freshwater runoff from rivers, all determine peculiar low salinity and low winter temperatures (Falco et al., 2000; Lejeusne at al., 2010). This is the boundary between the ADR and ION modules, which are hosted in two basins with a moderate water mass exchange, entering the Adriatic from Ionian Sea through the Albanian side of the Strait of Otranto (Orlic, Gacic & Laviolette, 1992).

Our findings showed that the boundaries between modules are represented by the most effective Mediterranean barriers, in agreement with the geographic patterns highlighted in other organisms studied at the whole basin scale. The areas corresponding to the Western and Eastern Mediterranean and to the Adriatic Sea have been historically considered as different biogeographic provinces, hosting differentiated species assemblages of macrophytes, diatoms and many animal groups (Ignatiades et al., 2009; Gambi, Lampadariou & Danovaro, 2010). Among invertebrates, an analysis of Mediterranean sponge regional diversity showed results comparable with those presented here for *P. oceanica* amphipods, by identifying distinct assemblages from CW Mediterranean, Tunisia, Adriatic and Ionian Sea (Gerovasileiou & Voultsiadou, 2012). Similar results were reported for benthic polychaetes, showing different assemblages in the Western and Central Mediterranean basins and in the Adriatic Sea (Arvanitidis et al., 2002), while deep-sea megafauna showed dissimilar community compositions in Western Mediterranean and Ionian basins (Tecchio et al., 2010).

Modules diversity and differentiation

The differentiation among the four modules depends on different aspects of assemblage diversity and composition. The assemblages characterized by higher diversity were all from CWM module, and in particular were located in the Central Tyrrhenian area (e.g., CHIA, MOR, GIAN, TAV; Fig. 2b), while all localities from ADR and ION modules showed the lowest diversity values, together with a few localities from CWM (MART, MED) and TUN (ZAP, RAF). A decreasing gradient in species richness from north-west to south-east is a generalized pattern in the Mediterranean Sea. Boudouresque (2004) reported an overall animal species diversity 100% greater in the western than in the eastern Mediterranean Sea, and the same trend was recorded for both vertebrates and invertebrates (Coll et al., 2010). As an example, the diversity of deep-sea nematode assemblages decreases with depth but when similar depths are compared a longitude effect appears, with diversity decreasing eastward (Danovaro et al., 2008). A similar pattern was detected in deep-sea foraminifers, whose species richness decreases from western to eastern Mediterranean, likely mimicking the longitudinal cline of organic matter availability on the deep seafloor (Danovaro et al., 2010).

The biogeographic role of localities showed how assemblages differ among and within modules, by identifying hubs *vs*. peripheral nodes (i.e., localities). Interestingly, nearly all the assemblages of CWM and TUN modules are classified as hubs (either peripheral or connector), meaning that they share a large proportion of species with many other localities belonging to other modules, besides a relevant number of species among each other. On the contrary, all the assemblages from ADR and ION modules are considered as peripheral or ultra-peripheral nodes, so having assemblages very similar at intra-modular level and highly differentiated with respect to the localities belonging to other modules. Such differentiation is due to the fact that ION and ADR modules are mainly characterized by a sub-set of species found either in the whole basin or in the hubs localities of the CWM module, and at the same time show a very limited number of module endemic (i.e., exclusive) species.

Identified modules differ not only in their species richness and locality role, but also in the biogeographic distribution of the species found in the various assemblages. The less diverse ADR module is mainly driven by cosmopolitan species, while the most diverse CWM assemblages are characterized by Mediterranean endemics and, to a lesser extent, by North-Atlantic species, while Indo-Pacific and African species typify the rich Tunisian assemblages (Fig. 3). In other words, it seems that the lower is the module diversity, the wider is the range of the species associated with. This pattern may be explained in the light of biogeographic considerations.

The presence of species characterized by a regional range in the most diverse CWM module (Mediterranean endemics and, to a minor extent, North-Atlantic species) is in agreement with the historical subdivision in western and eastern Mediterranean biogeographic provinces, which recognizes a concentration of temperate species of Atlantic origin in the western part of the Mediterranean Sea. This is due to the well known and widely described paleogeographic history of the basin, with particular regard to the most recent cycles of Plio-Pleistocene glaciations (Coll et al., 2010). These climatic oscillations induced temperate Atlantic species to periodically enter the Mediterranean Sea where they originated vicariant endemic species as a result of geographic isolation and local adaptation. For this reason the Mediterranean Sea is regarded as a ‘diversity pump’ from the Atlantic, as stated by Bianchi & Morri (2000), and the identification of its biogeographic provinces largely rely on the distribution of Mediterranean endemics (Bianchi & Morri, 2000; Bianchi et al., 2012). Similarly, it has been shown that species originated from warm faunas prevail in the south-eastern part of the Mediterranean basin (Lejeusne et al., 2010). Accordingly, the presence of warm species as a representative of the Tunisian (and Levantine) coasts has been signalled for other vertebrates, e.g., sponges (Gerovasileiou & Voultsiadou, 2012), in agreement with the data reported here for seagrass amphipods.

The assemblages in the geographically confined ADR module are characterized mainly by widely distributed species with cosmopolitan range. This observation, and the fact that only few species are found exclusively in this module, both suggest that this area may be particularly difficult to be colonized, due to both extreme environmental conditions and isolation, so that only vagile and eurhyecious species may enter and establish in this basin. Indeed, the Adriatic Sea is characterized by low salinity and by winter temperatures among the lowest in the Mediterranean, together with a moderate water mass exchange with the neighbouring Ionian Sea through the Strait of Otranto (Orlic, Gacic & Laviolette, 1992; Falco et al., 2000). Moreover, the Adriatic basin was largely dried during the Last Glacial Maximum (LGM, 23K-19K years ago; Rohling et al., 2010), so that a significant part of the Adriatic fauna has only recently re-colonized this basin. Within the poorly vagile group of amphipods, which are brooding species lacking pelagic larvae, cosmopolitan species are generally eurhyaline and eurytherm and prone to passive dispersal (Bellan-Santini & Ruffo, 2003) and so may be those with the higher probability to cross the barriers and settle in the Adriatic peculiar habitat. Accordingly, a recent checklist of opisthobranch Adriatic fauna signalled that the great majority of species had an Atlantic-Mediterranean range, while only few were Mediterranean endemics (Zenetos et al., 2016).

Relationships with *Posidonia oceanica* history

The populations of *P. oceanica* inhabiting the western and eastern parts of the Mediterranean Sea are genetically differentiated, with the central Mediterranean populations around the Siculo-Tunisian Strait characterized by a higher genetic diversity (Arnaud-Haond et al., 2007; Serra et al., 2010). This pattern was interpreted as the result of a secondary contact zone between the western and eastern forms, each one originated by vicariance in glacial refugia during the Last Glacial Maximum (LGM) (Serra et al., 2010). A more recent study has superimposed Ecological Niche Modelling to phylogeographic data, highlighting the southern Mediterranean as the most climatically suitable area during LGM, with particular regard to the central zone. This area was then proposed as the main glacial refugium of the seagrass, thus explaining its higher genetic diversity as due to the long-term persistence in this region (Chefaoui, Duarte & Serrão, 2017).

Glacial refugia have been repeatedly pointed out as hot-spots and melting pots of diversity, not only in terrestrial environments but also in marine habitats (Hewitt, 1999; 2004; Maggs et al., 2008) and this diversity may concern both genetic lineages and community richness in agreement with the postulated relationship between habitat stability and community diversity (Hewitt, 2000; Ives & Carpenter, 2007). Within this frame, both the high diversity and the hub role of TUN module assemblages can be explained by their localization in a refugial area, as proposed by Chefaoui, Duarte & Serrão (2017). However, the same pattern (high diversity and central hubs) characterized the CWM assemblages, which are richer in species and share an even greater percentage of their species with other modules. This finding however may be only apparently in contrast with the low presence probability of *P. oceanica* in the northern Mediterranean during LGM, if we consider that amphipods living on *P. oceanica* are not exclusively found in this habitat.

Amphipods are indeed able to actively choose their substratum and this habitat preference reflects mainly on the relative abundance of different species/genera on the various seagrass or sand substrates, more than on their obligated presence/absence (Sanchez-Jerez et al., 1999; Vázquez-Luis, Sanchez-Jerez & Bayle-Sempere, 2009). A possible scenario is therefore that the northward seagrass range expansion triggered by the climate warming after LGM prompted the migration of part of the associated fauna as expected. At the same time, the north-western Mediterranean was likely already inhabited by a local pool of amphipod species derived from the Atlantic, according to the ‘biodiversity pump’ mechanism, and hence adapted to temperate climatic conditions and able to survive during LGM. Under this hypothesis, it is expected that the CWM assemblages would include many module endemics (i.e., species found only in this area) represented by species with Mediterranean and/or Atlantic distribution (i.e., ME and ATL according to biogeographic classes). Also, CWM and TUN module should share a quite high number of species (i.e., those originated in the southern refugia and migrated northward with the seagrass). Our analyses showed that all these expectations were verified, suggesting that the high richness of present amphipod fauna in CWM would be originated by the blending of the resident cold-adapted Atlantic-Mediterranean endemic species, with the spreading warm-adapted southern species reaching this area together with *P. oceanica* after the end of LGM.

Although community and genes may evolve in different times, the study of the phylogeography of amphipod species with different degrees of association to *P. oceanica* and belonging to different biogeographic classes could allow verify this hypothesis.

CONCLUSIONS

To our knowledge, this study is the first trying to shed light on the pattern of amphipod assemblages associated with *P. oceanica* meadows in the Mediterranean Sea, showing the usefulness of network analysis and, in particular, modularity in detecting biogeographic subdivisions and in identifying the biogeographic role of localities. The results obtained provided a new perspective on the less studied southern Mediterranean, which may gain a relevant place in the origin of the basin biodiversity as a LGM refuge, besides confirming the known role of the Central Western Mediterranean area as a 'biodiversity pump' from the Atlantic. The finding that the distribution of amphipod diversity stems from complex interactions not only between present and past geographic barriers and local species adaptation, but also with the biogeography of the host plant, suggests the need to carry out further comparative phylogeographic studies on amphipod species with different degrees of association with *P. oceanica* and belonging to different biogeographic classes.

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Figure 1

Overview of the Mediterranean Sea with reference localities

Arrows indicate the main circulation patterns and red lines the main barriers: 1, Gibraltar Strait; 2, Almeria-Oran Front; 3, Ibiza Channel; 4, Balearic Front; 5, Sicily Channel; 6, Otranto Strait. For acronyms, see Supplemental Materials & Methods.

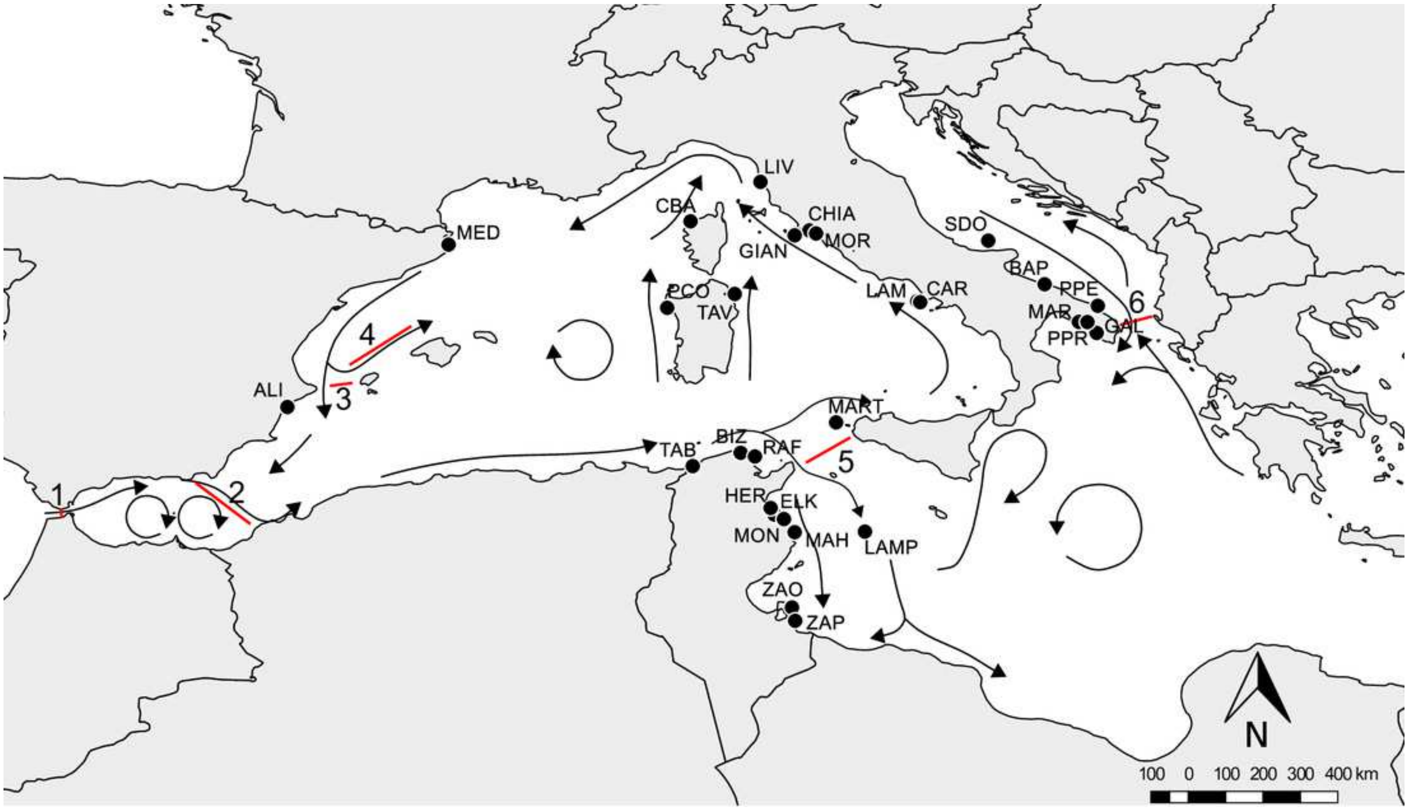

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Figure 2

Spatial distribution of modules, network structure and biogeographic roles of localities.

(a) Spatial distribution of the modular subdivision of localities with colours indicating different modules. (b) Visualization of biogeographic modules and associated amphipod species (black dots). (c) Plot showing the biogeographic role of localities in the topological space given by local topological linkage (*l*) and regional topological linkage (*r*) (revised after Carstensen et al., 2012). Colours of localities correspond to the identified modules, with size proportional to species diversity. R1, Peripheral nodes: few local and regional species; R2, Non-hub connector nodes: few local and many regional species; R3, Provincial hubs: many local and few regional species; R4, Connector hubs: many local and regional species.

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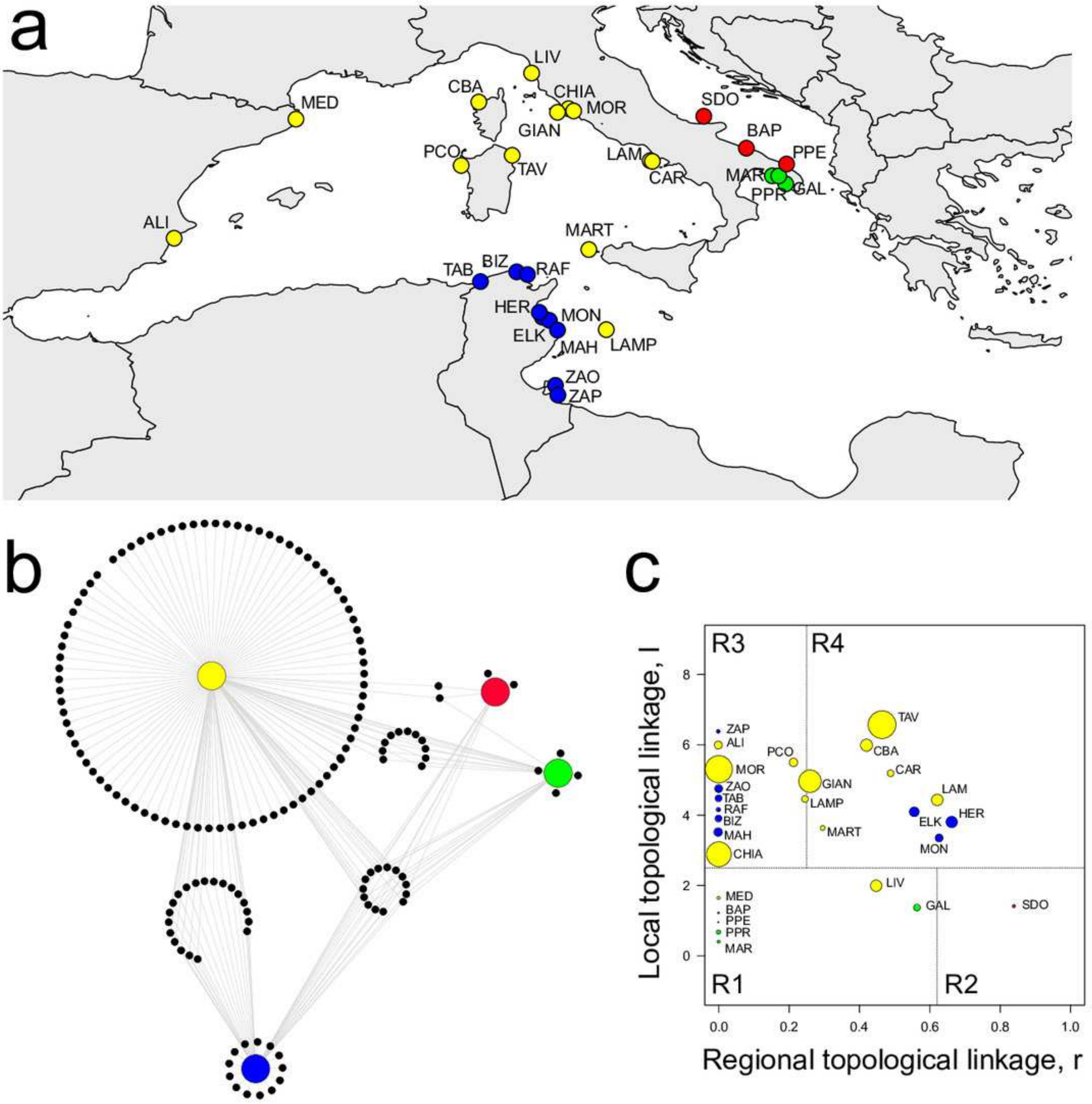
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Figure 3

Between-Group Correspondence Analysis (BGCA) of localities based on the nine species-biogeographic classes according to literature.

In BGCA, colours indicate the identified modules and different shapes correspond to the biogeographic role of localities (see top-right box). Dashed arrows indicate the nine species-biogeographic classes: COSMP, Cosmopolite; INDP, Indo-Pacific; AFR, African coasts from Ceuta to Cap Vert; ATL, Atlantic coasts from Spain to Britain; NATL, North Atlantic from Norway to Arctic Sea; WM, West Mediterranean; EM, East Mediterranean; ADR, Adriatic Sea; ME, Mediterranean endemics.

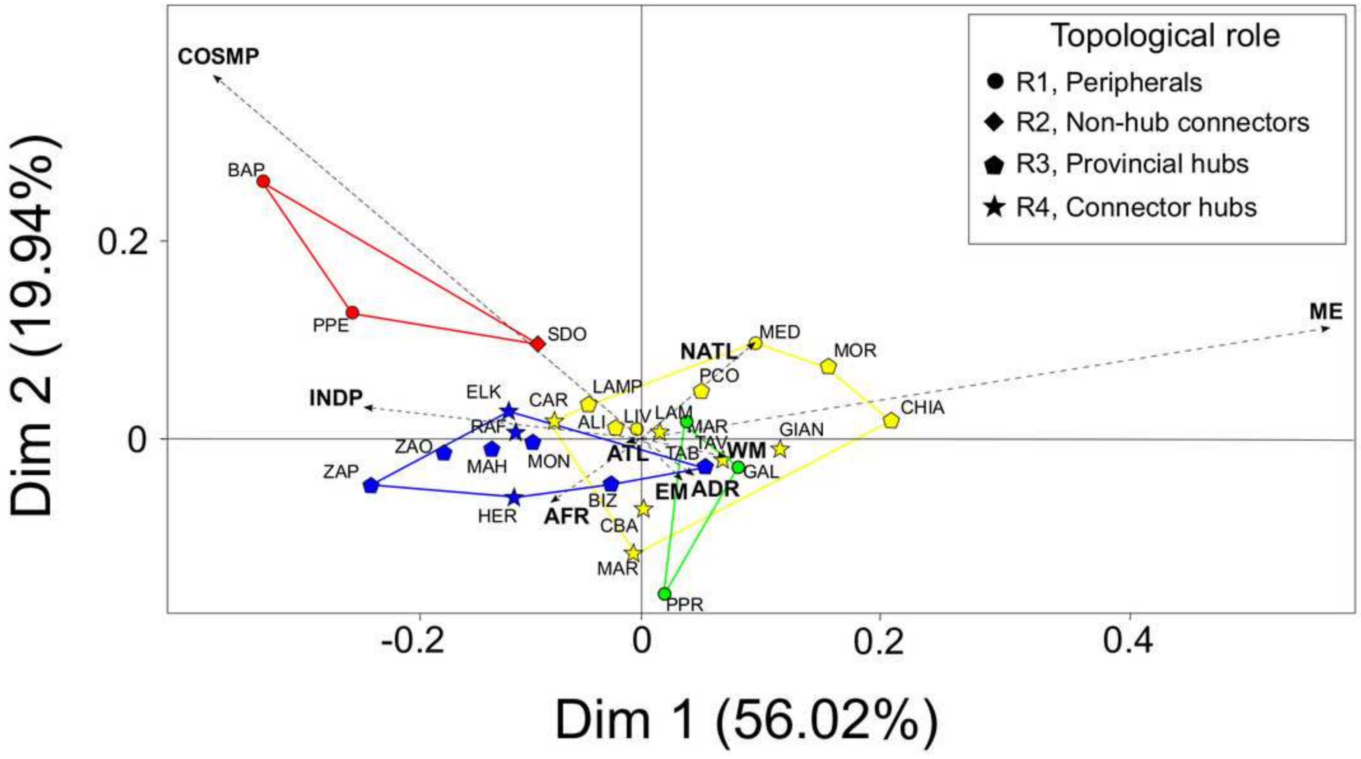

Table 1(on next page)

Modular subdivision of sampling localities.

l is the number of localities in each module; *s* the total number of species. Module endemics is the percentage of species exclusively present in a single module; ME and COSMP are the percentage of species in each module belonging to the Mediterranean Endemics (ME) and Cosmopolite (COSMP) biogeographic classes (see Materials & Methods).

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Module	*l*	*s*	Module endemics (%)	Mediterranean endemics (ME%)	Mediterranean cosmopolite (COSMP%)
CWM	11	133	64.7	16.7	4.8
TUN	9	47	27.6	7.3	7.3
ION	3	26	15.4	13	4.3
ADR	3	11	18.4	9	27.3

1