Phylogeography of *Dictyota fasciola* and *Dictyota mediterranea* (Dictyotales, Phaeophyceae): unexpected patterns on the Atlantic-Mediterranean marine transition and taxonomic implications (#34112)

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Phylogeography of *Dictyota fasciola* and *Dictyota mediterranea* (Dictyotales, Phaeophyceae): unexpected patterns on the Atlantic-Mediterranean marine transition and taxonomic implications

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The Atlantic-Mediterranean marine transition is a fascinating biogeographic region, but still very poorly studied from the point of view of seaweed phylogeography. *Dictyota fasciola* and *D. mediterranea* (Dictyotales, Phaeophyceae) are two currently recognized sister species that share a large part of their distribution along the Mediterranean Sea and the Atlantic Ocean, representing a unique study model to understand the diversification processes experienced by macroalgae during and after Messinian at this marine region. In this study, we sampled 102 individuals of *D. fasciola* and *D. mediterranea* from 32 localities along their distribution range and sequenced the mitochondrial *cox*1 and the chloroplast *rbc*L-*rbc*S DNA regions for all the samples. Our data do not support the occurrence of two sister species but a morphologically variable and highly genetic diverse species or a complex of species. Most of the observed genetic diversity corresponds to the Mediterranean populations, whereas the Atlantic ones are much more homogeneous. The early-diverged lineages inferred from both mtDNA and cpDNA phylogenetic reconstructions were constituted by samples from the Mediterranean Sea. Together, these results suggest that the Mediterranean Sea acted as a refugium for the *D. fasciola - D. mediterranea* lineage during the geologic and climatic changes occurred on the region since the Miocene, subsequently dispersing to the Atlantic Ocean.

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Abstract

The Atlantic-Mediterranean marine transition is a fascinating biogeographic region, but still very poorly studied from the point of view of seaweed phylogeography. *Dictyota fasciola* and *D. mediterranea* (Dictyotales, Phaeophyceae) are two currently recognized sister species that share a large part of their distribution along the Mediterranean Sea and the Atlantic Ocean, representing a unique study model to understand the diversification processes experienced by macroalgae during and after Messinian at this marine region. In this study, we sampled 102 individuals of *D. fasciola* and *D. mediterranea* from 32 localities along their distribution range and sequenced the mitochondrial *cox*1 and the chloroplast *rbc*L-*rbc*S DNA regions for all the samples. Our data do not support the occurrence of two sister species but a morphologically variable and highly genetic diverse species or a complex of species. Most of the observed genetic diversity corresponds to the Mediterranean populations, whereas the Atlantic ones are much more homogeneous. The early-diverged lineages inferred from both mtDNA and cpDNA phylogenetic reconstructions were constituted by samples from the Mediterranean Sea. Together, these results suggest that the Mediterranean Sea acted as a refugium for the *D. fasciola* - *D. mediterranea* lineage during the geologic and climatic changes occurred on the region since the Miocene, subsequently dispersing to the Atlantic Ocean.

Introduction

In the last decades, the increase of DNA sequencing data has been a key step to achieve a better understanding of biodiversity, constituting the basis of modern fields like integrative taxonomy and molecular systematics (Dayrat, 2005; Will et al., 2005; Hajibabaei et al., 2007; Maddison, Schulz & Maddison, 2007; Schlick-Steiner et al., 2010). This source of information is particularly important to improve our knowledge of organisms such as macroalgae, frequently showing poor diagnostic phenotypical characters (Verbruggen, 2014). In this way, many studies based on DNA have contributed to recognizing phenotypically cryptic seaweed species (Leliaert et al., 2014 and references therein) or to redefining classifications of some lineages, establishing evolutionarily natural groups (Brodie & Lewis, 2007, and references therein). The advances in these fields also served as a basis to phylogeography, a discipline where seaweeds have experienced increasing relevance during recent years (Hu, Duan & Lopez-Bautista, 2016).
Comparative phylogeography on diverse marine organisms has demonstrated to be a useful tool to unravel evolutionary and ecological patterns across marine provinces and biodiversity hotspots (Bowen et al., 2016). However, the relevance of seaweed studies on some geographical regions such as the Atlantic-Mediterranean transition is still very poor compared to other organisms like animals or land plants (Patarnello et al., 2007; Hu, Duan & Lopez-Bautista, 2016). For instance, most data concerning the diversification processes on this region during the key Miocene-Pliocene boundary come from marine animals (e.g. crustacean, Rastorgueff et al., 2014; echinoderms, Taboada & Pérez-Portela, 2016; or vertebrates, Valsecchi et al., 2005).
According to the most accepted hypothesis, no true marine organisms could have survived in the brackish-water or hypersaline lakes that remained in the Mediterranean Basin during the Messinian Salinity Crisis (MSC; 7.25-5.33 Ma) (Taviani, 2002). Consequently, the Mediterranean Sea would have been recolonized by species occurring in the Atlantic Ocean following the flooding after the MSC (Hsü et al., 1977). In contrast, other studies suggest some true marine enclaves persisted in the deeper areas of the Mediterranean and served as refugia for some "Messinian" species (e.g. Boudouresque, 2004; Sotelo, Morán & Posada, 2009; Reuschel, Cuesta & Schubart, 2010).
Climatic changes during Plio-Pleistocene also had a great impact on the Atlantic-Mediterranean marine transition and the organisms inhabiting this region (Patarnello, Volckaert & Castilho, 2007). Several investigations have reported that latitudinal and sea-level shifts associated with Pleistocene glacial-interglacial cycles fuelled important range changes and vicariance events on Atlantic-Mediterranean marine protists (e.g. Lowe et al., 2012), animals (e.g. Xavier et al., 2011) and seagrasses (e.g. Alberto et al., 2008; Arnaud-Haond et al., 2007). To our knowledge, the only phylogeographic study involving a native seaweed from the Mediterranean Sea focuses on the red coralline algae *Lithophyllum byssoides* (Lamarck) Foslie (Pezzolesi et al., 2017). Based on the genetic differences found among Atlantic and Mediterranean specimens, the authors suggested that MSC and Plio-Pleistocene climatic changes shaped genetic structure of this species. However, the sampling of the study –restricted to the central Mediterranean populations

(Italy and Croatia) plus two Atlantic specimens from Spain – limited the inference of further phylogeographic patterns.

Dictyota fasciola (Roth) J.V. Lamouroux is a relatively common species of eulittoral pools and the shallow subtidal zones in the NE Atlantic and the Mediterranean Sea. *Dictyota mediterranea* (Schiffner) G.Furnari is a rarer species, endemic to the coasts of the Mediterranean Sea where it occupies a similar habitat to that of the preceding species. As occurs in the majority of *Dictyota* species, these two taxa are notoriously difficult to identify based on morphological, anatomical, or reproductive characters. In this way, *D. mediterranea* was formerly reduced to a synonym of *D. fasciola* by Feldmann (1937) on the basis of similarities in color, width of the axes, and shape of the apices. However, subsequent authors does not follow this opinion and consider *D. mediterranea* as a different species (Coppejans, 1983; Ribera et al., 1992; Pena, Gómez & Crespo, 2004; Cormaci et al., 2012). At present, *D. mediterranea* is look as an accepted taxonomical entity (Guiry & Guiry, 2019). Indeed, *D. mediterranea* shows a terete thallus at the base and the apex – but complanate in the middle part – and a multilayered medulla; whereas axes of *D. fasciola* are all complanate, and a multilayered medulla is restricted to the basal parts of the thallus (Pena, Gómez & Crespo, 2004; Cormaci et al., 2012). Previous molecular phylogenetic studies indicated that these species are closely related (Tronholm et al., 2010), but results of the same study pointed out a noticeable genetic differentiation among them. The divergence between *D. fasciola* and *D. mediterranea* was estimated to occur c. 6.5 Ma [10 Ma – 4 Ma; 95% highest density probability) according to a time calibrated multigene phylogeny of the genus *Dictyota* (Tronholm et al., 2012), partially overlapping with the start of the MSC (Krijgsman et al., 1999). Based on these former data, Tronholm et al. (2010) speculated that *D. fasciola* - *D. mediterranea* lineage would have an Atlantic origin, subsequently colonizing the Mediterranean basin – either before or after the divergence of both species – after the MSC. As the only example of two sister *Dictyota* species occurring along the Mediterranean Sea and the Atlantic Ocean, these two taxa represent a unique study model to understand the phylogeographic processes experienced by macroalgae during and after Messinian at this marine region.

In this study, we use a broad sampling along the distribution range of these *Dictyota* species to investigate their diversification process. Based on the sequences obtained from two variable mitochondrial (*cox*1) and chloroplast (*rbc*L-*rbc*S) DNA regions, we address three main goals. First, we aim to validate the taxonomic differentiation among *D. fasciola* and *D. mediterranea* observed in previous phylogenetic studies of the genus. Second, we will test whether our phylogeographic data fit well to the former “Atlantic to Mediterranean” hypothesis proposed to explain the evolutionary history of this lineage. Finally, we discuss the contribution of our results to the knowledge about the Atlantic-Mediterranean transition during the Messinian and the Plio-Pleistocene periods.

Materials & Methods

Sampling and sequencing

We sampled 102 individuals of *D. fasciola* (67 specimens) and *D. mediterranea* (35 specimens) from 32 sampling sites along their main distribution range (see Tronholm et al., 2010) in the Mediterranean Sea and the Atlantic Ocean (Table 1; Fig. 1). Specimens were identified first in the field and later in the laboratory. Representative samples from all localities were preserved on herbarium sheets and deposited in the BCN-Phyc (Centre de Documentació de Biodiversitat Vegetal, Universitat de Barcelona, Spain) and GENT (Ghent University, Belgium) herbaria. Geographic coordinates for each sampling site are shown in Table S1. The CTAB method (Doyle & Doyle, 1987) with modifications (Soltis et al., 1991; Cullings, 1992) was used to extract total genomic DNA from silica-dried material derived from fresh tissue. The mitochondrial *cox*1 and the chloroplast *rbc*L-*rbc*S regions were amplified and sequenced for all the samples. Amplification procedure was performed as described in Aragay et al. (2017). Direct sequencing of the amplified DNA segments was performed with Big Dye Terminator Cycle Sequencing v 3.1 (PE Biosystems, Foster City, California, U.S.A.) at the Unitat de Genòmica, Centres Científics i Tecnològics, Universitat de Barcelona (CCiTUB) on an ABI PRISM 3700 DNA analyser (PE Biosystems). The sequencing primers used were the same as the amplification ones. Sequences were edited and assembled using Chromas Lite v 2.01 (Technelysium PTy, Tewantin, Queensland, Australia) and Bioedit v 7.0.9 (Ibis Biosciences, Carlsbad, California, U.S.A.), aligning with Clustal W (Thompson, Higgins & Gibson, 1994) and finally adjusting by hand. GenBank accession numbers are given in Table S1.

Phylogenetic analyses of *D. fasciola* and *D. mediterranea*

Molecular phylogenetic reconstruction within the *D. fasciola* – *D. mediterranea* group was performed by Bayesian inference (BI) with MrBayes v 3.2 (Ronquist et al., 2012), independently for both chloroplast and mitochondrial markers. *Dictyota guineënsis* (Kützing) P.Crouan & H.Crouan was chosen as outgroup according to unpublished phylogenetic analyses at the genus level (Olivier de Clerck, Ghent University, pers. comm.). Despite *Dictyota* presents maternal inheritance for chloroplast and mitochondrial organelles (Motomura, Nagasato & Kimura, 2010), we analysed separately both datasets since their DNA loci may undergo independent evolution, potentially generating incorrect phylogenetic inferences based on concatenated datasets (Degnan & Rosenberg, 2009). Partitioning strategies were selected with Partitionfinder v 2.1.1 (Lanfear et al., 2016). A partitioning scheme with 3 partitions organized by codon position was chosen for the mitochondrial genic region *cox*1 (SYM+G, HKY and HKY+G models for the first, second and third positions, respectively), while one single partition (HKY+G model) was applied for the chloroplast *rbc*L-*rbc*S intergenic spacer. Two independent Markov chain Monte Carlo (MCMC) analyses with four Metropolis-coupled chains each were run for 10 million generations, sampling every 1000 generations. The first 25% of the trees were discarded as “burn-in”, after confirming that the average standard deviation of the split frequencies was < 0.01, and the potential scale reduction factor approached 1.0 for all parameters. The remaining trees were pooled to construct 50% majority-rule consensus trees that approximate the posterior distribution of the phylogenetic reconstructions, and to obtain clade posterior probabilities. We also performed maximum likelihood (ML) analyses using RAxML-HPC v.8 (Stamatakis, 2014), partitioning by codon position *cox*1 dataset and one single partition for *rbc*L-*rbc*S. We employed the GTRCAT nucleotide substitution model for all partitions, with the default settings for the optimisation of individual per site substitution rates. The best-scoring ML tree with clade support values was obtained from 10 independent runs, with 1000 rapid bootstrap replicates each run. All these analyses were performed within the CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010), and the resulting summary trees were visualised in FigTree v.1.4.2 (https://github.com/rambaut/figtree).

Genetic variability of *D. fasciola* and *D. mediterranea*

For analyses taking into account phylogeographic structuring of populations, the samples were assigned to three main biogeographic marine regions (i.e. Atlantic Ocean, West Mediterranean and East Mediterranean; Coll et al., 2010). Haplotype minimum-spanning networks (Bandelt, Forster & Röhl, 1999) were reconstructed using PopArt (Leigh & Bryant, 2015), independently for each marker under study, using default settings (i.e. parameter ε = 0) to consider multifurcations and/or reticulations in a phylogenetic network approach.

Haplotype (Hp) and nucleotide (p) diversities were calculated separately for each marker using DnaSP v 5.0 (Rozas & Rozas, 1995). Haplotype richness (R(n)) was computed with RAREFAC (Petit, El Mousadik & Pons, 1998) a software that uses a rarefaction approach to standardize the haplotype richness to a fixed sample size to facilitate comparisons across groups of samples. In this case, the rarefaction value (n = 18) was set according to the sample size of the smallest group of populations (i.e. East Mediterraean group).

Results

Phylogenetic analyses of *D. fasciola* and *D. mediterranea*

Both the mitochondrial *cox*1 and the chloroplast *rbc*L-*rbc*S sequences showed a noticeable level of polymorphism among the 102 samples of *D. fasciola* and *D. mediterranea* analysed in this study. Specifically, 60 and 46 variable sites were observed for the mtDNA (584bp) and the cpDNA (510bp) markers, respectively. The trees inferred from Bayesian (Fig. 2) and ML (Fig. S1-S2) approaches resulted on congruent topologies. These phylogenetic reconstructions inferred the existence of several highly supported monophyletic lineages (PP > 0.95; BS > 90) within the complex of *D. fasciola* and *D. mediterranea*. The analysed specimens were not clustered in two clades according to their taxonomic assignation, but subdivided in multiple nested lineages which did not correspond to a clear-cut differentiation between both species. While some of these lineages were exclusively constituted by specimens of one of the species, a few comprised samples of both *D. fasciola* and *D. mediterranea* intermixed. Specifically, early diverging clades of the trees were mainly constituted by *D. mediterranea* specimens (with a few *D. fasciola* samples intermingled) while more derived clades were basically composed of *D. fasciola* specimens (with one or two *D. mediterranea* samples admixed). Comparing the trees obtained from mtDNA (Fig. 2A) and cpDNA (Fig. 2B), their topology showed overall congruence, except for a few (i.e 4 out of 102) samples which appeared in non-equivalent clades. From a geographic point of view, the Atlantic specimens of *D. fasciola* were all clustered in highly derived clades of both the trees inferred from mtDNA and cpDNA markers. However, these derived clades also contained several samples from the Mediterranean Sea, including a few representatives of *D. mediterranea*.

Genetic variability of *D. fasciola* and *D. mediterranea*

The number of haplotypes found in our study was 22 for *cox1* region and 18 for *rbcL-rbcS* region. The minimum spanning networks of both markers revealed a similarly complex evolutionary structure (Fig. 3), with some groups of closely related haplotypes (connected by one-two mutation steps) loosely distanced to other groups of haplotypes (>3 mutation steps). The geographic distribution of the haplotypes among the different regions did not show a clear pattern. Only the two (cpDNA) or three (mtDNA) haplotypes present on the Atlantic region were all closely related among them, whereas those from the Western and Eastern Mediterranean appeared distributed all over the network. As occurred on the phylogenetic trees, the haplotype networks did not show a simple taxonomic pattern congruent with a clear differentiation involving two species (Fig. S3).

The result of genetic variability analyses is summarized in Table 2. Haplotype diversity (Hd) values was slightly higher for *cox*1 than for *rbc*L-*rbc*S, while nucleotide diversity (π) was very similar among the chloroplast and the mitochondrial regions. From a phylogeographic point of view, the samples from the Mediterranean Sea contained higher genetic variability – in terms of number of haplotypes, haplotype diversity and nucleotide diversity – than those from the Atlantic Ocean (Table 2). Haplotype richness calculated after rarefaction $R_{(18)}$ was also several times higher in each of the Mediterranean groups than in the Atlantic one. Regarding the genetic variability within the Mediterranean groups, the Western samples showed more haplotypes (15 and 12 for mtDNA and cpDNA, respectively) than the Eastern ones (8 haplotypes for both mtDNA and cpDNA). However, the rest of genetic diversity indexes resulted in similar values among both regions of the Mediterranean Sea. In all cases, the results derived from both the mitochondrial and the chloroplast markers yielded congruent patterns of genetic variability.

Discussion

Systematic and taxonomic implications

Bayesian inference and ML trees show the occurrence of several statistically supported groups within *D. fasciola* - *D. mediterranea* complex, which do not seem to correspond to a clear-cut differentiation between the two species. Our data indicate that this group of *Dictyota* harbours more genetic diversity and complexity than previously envisaged. Earlier phylogenetic studies by Tronholm et al. (2010; 2012) analysed several specimens of both species, which were placed in two independent clades in agreement with the taxonomic assignation of the samples. In our study, the expanded sampling along the distribution range of *D. fasciola* and *D. mediterranea*, together with the use of more variable markers, reveal additional lineages structured in a nested topology, which rejects a simple scenario with two monophyletic species.

Additionally, as explained above, several lineages in our phylogenetic reconstructions (Fig. 2) are constituted by samples of both species intermixed. These results may suggest that *D. fasciola* and *D. mediterranea* should not be segregated into the current two taxonomic units, but they could constitute a larger complex of cryptic species. Alternatively, the observed diversity could correspond to a single morphologically variable species, as already proposed by Feldmann (1937). There are well-documented examples of *Dictyota* species showing considerable morphological plasticity (e.g. *Dictyota ciliolata* Sonder ex Kützing, Tronholm et al. (2013); *Dictyota dichotoma* (Hudson) J.V.Lamouroux, Tronholm et al. (2008)) so this could also be the case in the *D. fasciola-D. mediterranea* complex. The concordance among the trees derived from loci located in separate compartments of the genome (i.e. mtDNA and cpDNA; Fig. 2A-B) suggests that this phylogenetic pattern –which disagrees with taxonomic delimitation– is not the product of incomplete lineage sorting processes (Leliaert et al., 2014).

The only possibility to accept the two currently recognized species would suppose considering *D. mediterranea* as a paraphyletic taxon. Anacladogenetic speciation processes – often rendering ancestral paraphyletic taxa– have already been proposed to explain similar phylogenetic patterns (e.g. Hörandl, 2006; Crawford, 2010; Kuchta et al., 2018; Smith et al., 2018). In this scenario, some taxonomy-genetic conflict should be taken into account: a few individuals of both species are nested with individuals of the other species (Fig. 2). Most of these cases occurs in sampling sites where the two species cohabit (e.g. Banyuls-sur-mer, in France; or Ladiko Bay, in Rhodes), so misplacements could potentially be caused by identification problems. However, a careful taxonomic determination was performed on each collected individual. Considering as well the close evolutionary relationship within the members of this group, we speculate that hybridization/introgression events between the different lineages are more likely explanations for these cases of taxonomy-genetic discordance. Future studies encompassing more comprehensive sampling, nuclear variable markers and thorough morphological analyses should be undertaken to disentangle the taxonomy of this *Dictyota* complex.

Phylogeography and diversification within *D. fasciola – D. mediterranea* complex

The hypothesis formulated by Tronholm et al. (2010) to explain the diversification of *D. fasciola* and *D. mediterranea* complex do not fit well with the phylogeographic and genetic differentiation results obtained in our study. The scenario proposed by these authors considered that this group of seaweeds would have an Atlantic origin, colonizing the Mediterranean Sea posteriorly to the MSC. However, the genetic diversity values (Table 2) and the haplotype networks (Fig. 3) unambiguously show that the Mediterranean Sea contains much higher genetic diversity than the Atlantic Ocean. Similarly, the phylogenetic trees indicate that the early diverging lineages are always constituted by the Mediterranean specimens, whereas Atlantic samples are all clustered in a single younger lineage, indicating the derived character of the Atlantic distribution (Fig. 2). Even admitting that our sampling in the Atlantic Ocean is considerably incomplete, the extremely low genetic variability (Table 2) found among sampling sites distanced by several hundred kilometres results striking. These combined phylogeographic evidences suggest that the Mediterranean Sea could be the source area of diversification of the *D. fasciola – D. mediterranea* complex.

According to the time-calibrated phylogeny of the genus by Tronholm et al. (2012), this divergence process could have predated the MSC. By that time, the Mediterranean Sea showed a great geographical complexity, with some sub-basins mainly isolated among them (Piller, Harzhauser & Mandic, 2007). Surviving the MSC in these isolated Mediterranean refugia may have been accompanied by a reduction of population sizes, thereby enhancing divergence in allopatry of the isolated populations (Hörandl & Stuessy, 2010; Calvo et al., 2015). This scenario could explain the notably genetic differentiation observed within the *D. fasciola – D. mediterranea* complex in the Mediterranean Sea, as well as the low variability present in the Atlantic Ocean, which would have been putatively colonized after the reopening of the Gibraltar Strait connection. Our phylogenetic reconstructions are not time-calibrated – so we cannot precisely date when the colonization of Atlantic Ocean occurred – but our results clearly show this happened late in the diversification history of the complex.

However, as in the case of other Mediterranean organisms (e.g. vertebrates, Domingues et al., 2005; echinoderms, Taboada and Pérez-Portela, 2016; or cnidarian, Pilczynska et al. 2017) we cannot discard the hypothesis that the ancestors of the *D. fasciola-D.mediterranea* complex survived the MSC in the Atlantic Ocean. In this scenario, the arrival of this group of seaweeds to the Mediterranean basin would have happened after the Zanclean re-flooding with Atlantic waters. Assuming the genetic drift occurring at the wave front of an expanding population (Excoffier & Ray, 2008), this phenomenon should have led to higher genetic diversity in Atlantic populations compared to the Mediterranean ones (i.e. exactly the opposite of what was observed in our results). To fit this hypothesis to the low genetic diversity and the derived phylogenetic position of the Atlantic samples found in our study, we should assume the subsequent extinction of most of the relict oceanic diversity after the colonization of the Mediterranean. Several studies have stated that Pleistocene glacial cycles erased Atlantic populations of marine organisms, while the isolated Mediterranean Sea offered a more stable persistence for some of them (e.g. Alberto et al., 2008; Lowe et al., 2012). The habitat fragmentation occurring in the Mediterranean during colder marine regression periods could have further enhanced genetic differentiation processes in this region (e.g. Arnaud-Haond et al., 2007; Rastorgueff et al., 2014). Therefore, a postglacial colonization of the Atlantic from Mediterranean sources would be an alternative or complementary explanation for phylogeographical patterns observed on *D. fasciola-D. mediterranea* complex.

Conclusions

Our results indicate that *D. fasciola* and *D. mediterranea* are not monophyletic species. Conversely, we inferred a complex phylogenetic history challenging previous taxonomic and evolutionary hypotheses on this group of macroalgae. This study also highlights the key role played by the Mediterranean Sea as a refugium for these seaweeds during the major climatic changes occurred since the Miocene in this region of the planet. The limited number of sampling sites included in our study and the fact that some analysed populations consisted of few individuals prevent stablishing more detailed phylogeographic hypotheses. Hence, more research focusing on this *Dictyota* complex -as well as on other algal groups- is needed to unravel the precise evolutionary and biogeographic response of seaweeds to the geological and climatic events that the Mediterranean experienced during and after the Messinian.

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Table 1(on next page)

Summary of sampling locations, geographic circumscription, number of individuals (N) and haplotype information of *D. fasciola* and *D. mediterranea* specimens used in this study.

Table 1. Summary of sampling locations, geographic circumscription, number of individuals (N) and haplotype information of *D. fasciola* and *D. mediterranea* specimens used in this study.

Taxonomic assignation	Geographic region	Sampling site	Code	N	mtDNA haplotype	cpDNA haplotype
Dictyota fasciola	WM	Spain: Alicante, Cabo de Huertas	F-Alac	2	M1	C1/C5
		Spain: Almería, La Isleta	F-Isle	4	M6(1),M18(3)	C12(3)/C13(1)
		Spain: Catalonia, Llançà	F-Llan	5	M1(2),M4(1),M5(1),M7(2)	C1(1),C3(1), C4(2), C8(1)
		Spain: Castellò, Serra d'Irta	F-Cast	6	M1(3),M7(3)	C1(3)/C5(3)
		France: Côte Vermeille, Cerbère	F-Cerb	5	M12	C1
		France: Côte Vermeille, Banyuls-sur-mer	F-Bany	5	M1(2),M12(1),M15(2)	C1
		France: Nice	F-Nice	5	M1(1),M12(4)	C1
		Italy: Sardegna, Isola Rosa	F-SaIR	2	M14	C1(1)/C11(1)
		Italy: Sardegna, Porto Ferro	F-SaPF	4	M12(1),M17(2),M20(1)	C1(1)/C11(3)
	EM	Greece: Central Macedonia	F-CeMa	2	M1(1)M16(1)	C1(1)/C15(1)
		Greece: Karpathos, Agios Nikolaos	F-Karp	2	M1(1),M19(1)	C7(1)/C10(1)
		Greece: Rhodes, Ladiko Bay	F-RhoL	2	M19	C5(1)/C10(1)
		Greece: Rhodes, Fourni	F-RhoF	1	M9	C7
		Italy: Sicily, Aci Castello	F-Sici	1	M11	C1
	ATL	Portugal: Porto Covo	F-Port	3	M1	C1
		Portugal: Madeira, Ponta do Sao Lourenço	F-MaPo	1	M2	C1
		Portugal: Madeira, Reis Magos	F-MaRe	1	M1	C1
		Spain: Cádiz, Tarifa	F-Tari	5	M1	C1
		Spain: Canary Is., Lanzarote, Famara	F-LaFa	1	M1	C1
		Spain: Canary Is., Lanzarote, Puerto del Carmen	F-LaPC	1	M1	C1
		Spain: Canary Is., La Graciosa	F-Grac	1	M1	C1
		Spain: Canary Is., Gran Canaria, Medio Almud	F-GCMA	1	M1	C1
		Spain, Canary Is., Gran Canaria, Maspalomas	F-GCPM	1	M1	C1
		Spain: Canary Is., Tenerife, Punta Hidalgo	F-TePH	3	M1	C1(2)/C2(1)
		Spain: Canary Is., Tenerife, Buenavista	F-TeBu	1	M1	C1
		Spain: Canary Is., El Hierro	F-ElHi	2	M1(1),M3(1)	C1
Dictyota mediterranea	WM	Spain: Alacant, Cabo de Huertas	M-Alac	4	M7(2),M10(2)	C5
		Spain: Mallorca, Alcúdia	M-Mall	1	M7	C5
		Spain: Almería, La Isleta	M-Isle	2	M10	C5
		Spain: Catalonia, Llançà	M-Llan	9	M16	C14(3),C15(6)
		France: Côte Vermeille, Banyuls-sur-mer	M-Bany	8	M21(6),M22(1),M12(1)	C1(1),C16(6),C17(1)
		Italy: Sicily, Capo di Milazzo	M-SiCM	1	M7	C5
	EM	Italy: Sicily, Giardini Naxos	M-SiGN	1	M13	C18
		Italy: Sicily, Aci Castello	M-SiCi	2	M7	C5
		Greece: Rhodes, Ladiko Bay	M-RhoL	3	M1(1), M7(2)	C5
		Greece: Rhodes, Agios Thomas	M-RhoA	2	M9	C5(1),C6(1)
		Greece: Karpathos, Kastellia Bay	M-KarK	1	M8	C9
		Greece: Karpathos, Christou Pigadi	M-KarC	1	M8	C5

Table 2(on next page)

Genetic variability values for each molecular marker in the geographical groups of populations defined in the study.

#P, number of sampling sites; N, number of individuals; Hp, number of haplotypes; Hd, haplotype diversity; $R_{(18)}$, allelic richness after rarefaction; π, nucleotide diversity.

Table 2. Genetic variability values for each molecular marker in the geographical groups of populations defined in the study.

	#P	N	cox1				rbcL-rbcS			
			Hp	Hd	$R_{(18)}$	π	Hp	Hd	$R_{(18)}$	π
Western Mediterranean	11	63	15	0.897	8.05	0.0175	12	0.804	6.27	0.0175
Eastern Mediterranean	9	18	8	0.882	7.00	0.0128	8	0.797	7.00	0.0131
Atlantic	12	21	3	0.186	1.71	0.0003	2	0.095	0.86	0.0002
Total	32	102	22	0.862	9.12	0.0142	18	0.753	7.14	0.0150

Figure 1

Geographic distribution of the samples analyzed in this study (sample code according to Table 1).

The color of the square indicates the geographic circumscription to three main biogeographic marine regions (i.e. Atlantic Ocean, in green; Western Mediterranean Sea, in red; and Eastern Mediterranean Sea, in violet).

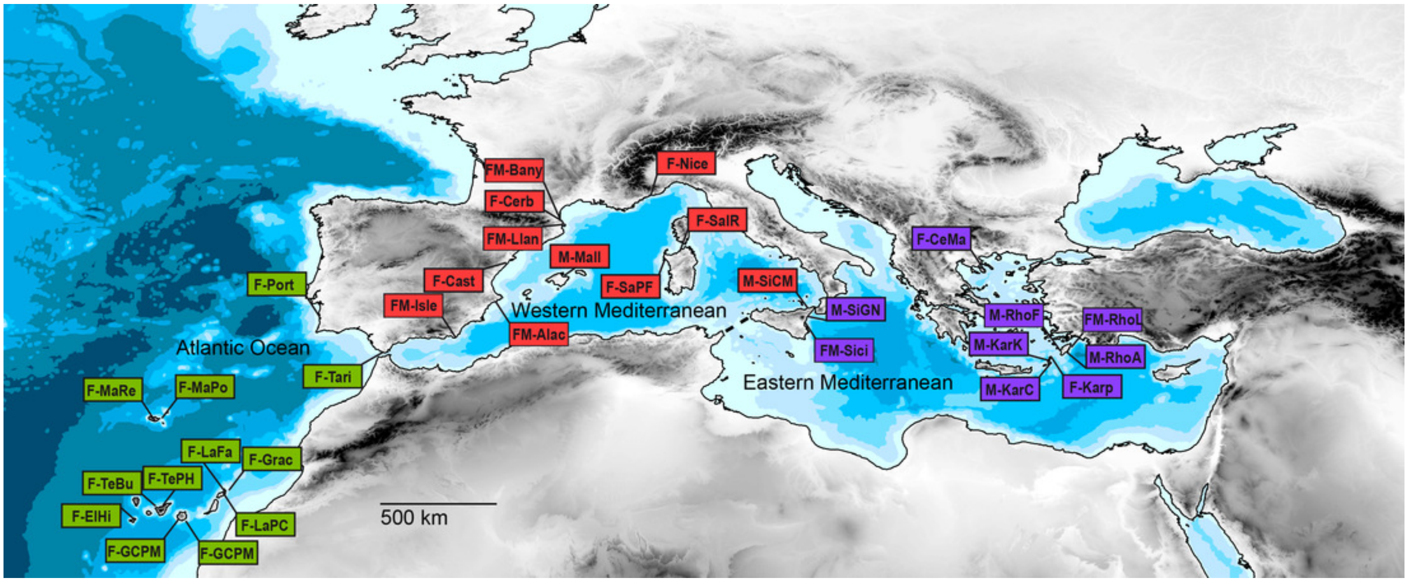
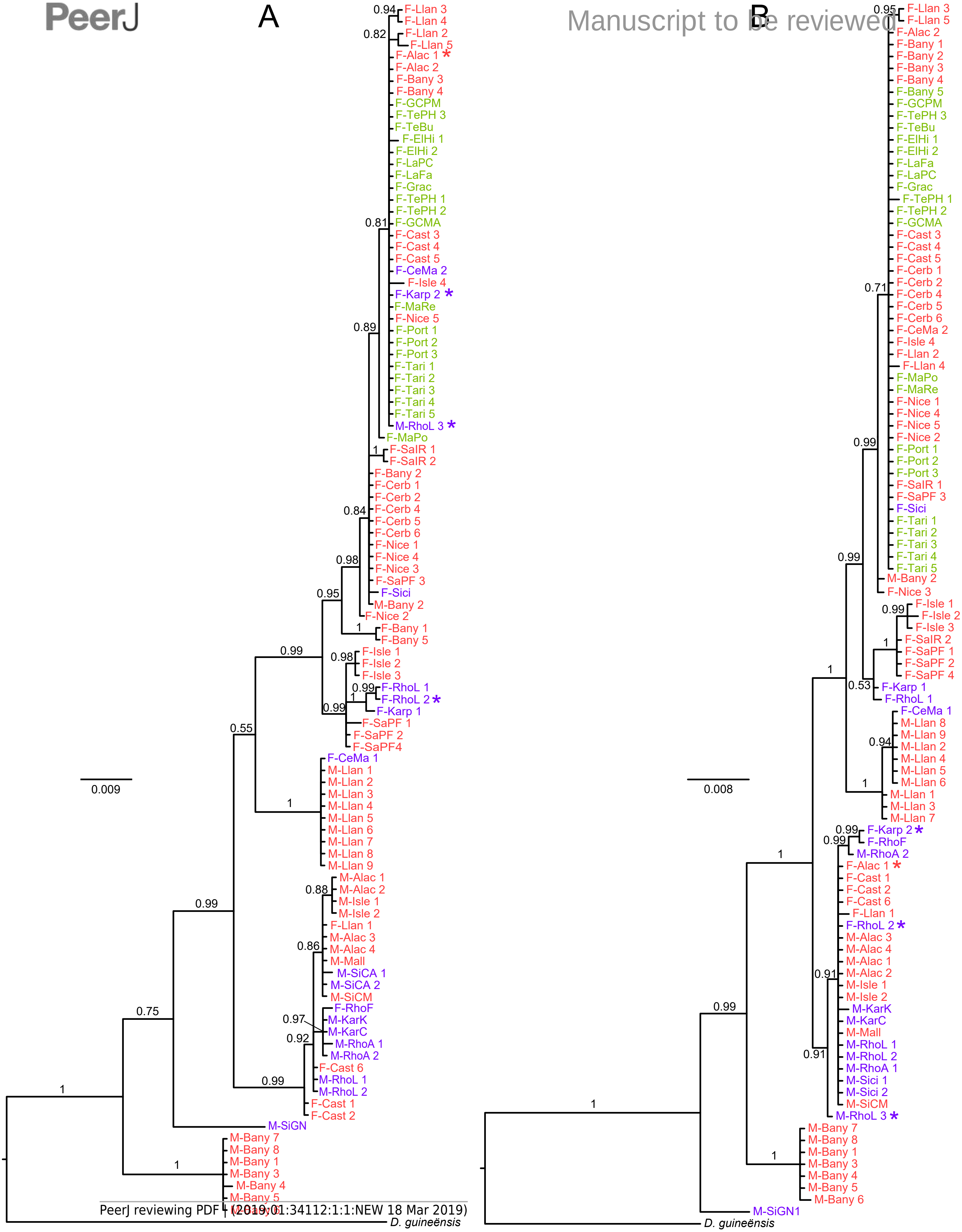


Figure 2(on next page)

Consensus tree based on Bayesian inference of (A) the mitochondrial *cox1* region and (B) the chloroplast *rbcL-rbcS* intergenic spacer.

The color of the labels indicates their geographic origin following the Figure 1. The samples marked with * show incongruent placement between the two phylogenetic reconstructions.


A
0.94
F-Llan 3
F-Llan 4
0.82
F-Llan 2
F-Llan 5
F-Alac 1 *
F-Alac 2
F-Bany 3
F-Bany 4
F-GCPM
F-TePH 3
F-TeBu
F-ElHi 1
F-ElHi 2
F-LaPC
F-LaFa
F-Grac
F-TePH 1
F-TePH 2
0.81
F-GCMA
F-Cast 3
F-Cast 4
F-Cast 5
F-CeMa 2
F-Isle 4
F-Karp 2 *
F-MaRe
F-Nice 5
0.89
F-Port 1
F-Port 2
F-Port 3
F-Tari 1
F-Tari 2
F-Tari 3
F-Tari 4
F-Tari 5
M-RhoL 3 *
F-MaPo
1
F-SalR 1
F-SalR 2
F-Bany 2
F-Cerb 1
F-Cerb 2
0.84
F-Cerb 4
F-Cerb 5
F-Cerb 6
F-Nice 1
F-Nice 4
F-Nice 3
0.98
F-SaPF 3
F-Sici
0.95
M-Bany 2
F-Nice 2
1
F-Bany 1
F-Bany 5
0.99
0.98
F-Isle 1
F-Isle 2
F-Isle 3
0.99
F-RhoL 1
1
F-RhoL 2 *
F-Karp 1
0.99
F-SaPF 1
F-SaPF 2
F-SaPF4
0.55
F-CeMa 1
M-Llan 1
M-Llan 2
M-Llan 3
M-Llan 4
M-Llan 5
M-Llan 6
M-Llan 7
M-Llan 8
M-Llan 9
1
0.009
0.88
M-Alac 1
M-Alac 2
M-Isle 1
M-Isle 2
F-Llan 1
M-Alac 3
0.86
M-Alac 4
M-Mall
M-SiCA 1
M-SiCA 2
M-SiCM
F-RhoF
0.97
M-KarK
M-KarC
0.92
M-RhoA 1
M-RhoA 2
F-Cast 6
0.99
M-RhoL 1
M-RhoL 2
F-Cast 1
F-Cast 2
0.99
0.75
M-SiGN
1
M-Bany 7
M-Bany 8
M-Bany 1
M-Bany 3
M-Bany 4
M-Bany 5
M-Bany 6
1
D. guineënsis
B
0.95
F-Llan 3
F-Llan 5
F-Alac 2
F-Bany 1
F-Bany 2
F-Bany 3
F-Bany 4
F-Bany 5
F-GCPM
F-TePH 3
F-TeBu
F-ElHi 1
F-ElHi 2
F-LaFa
F-LaPC
F-Grac
F-TePH 1
F-TePH 2
F-GCMA
F-Cast 3
F-Cast 4
F-Cast 5
F-Cerb 1
F-Cerb 2
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F-Cerb 5
F-Cerb 6
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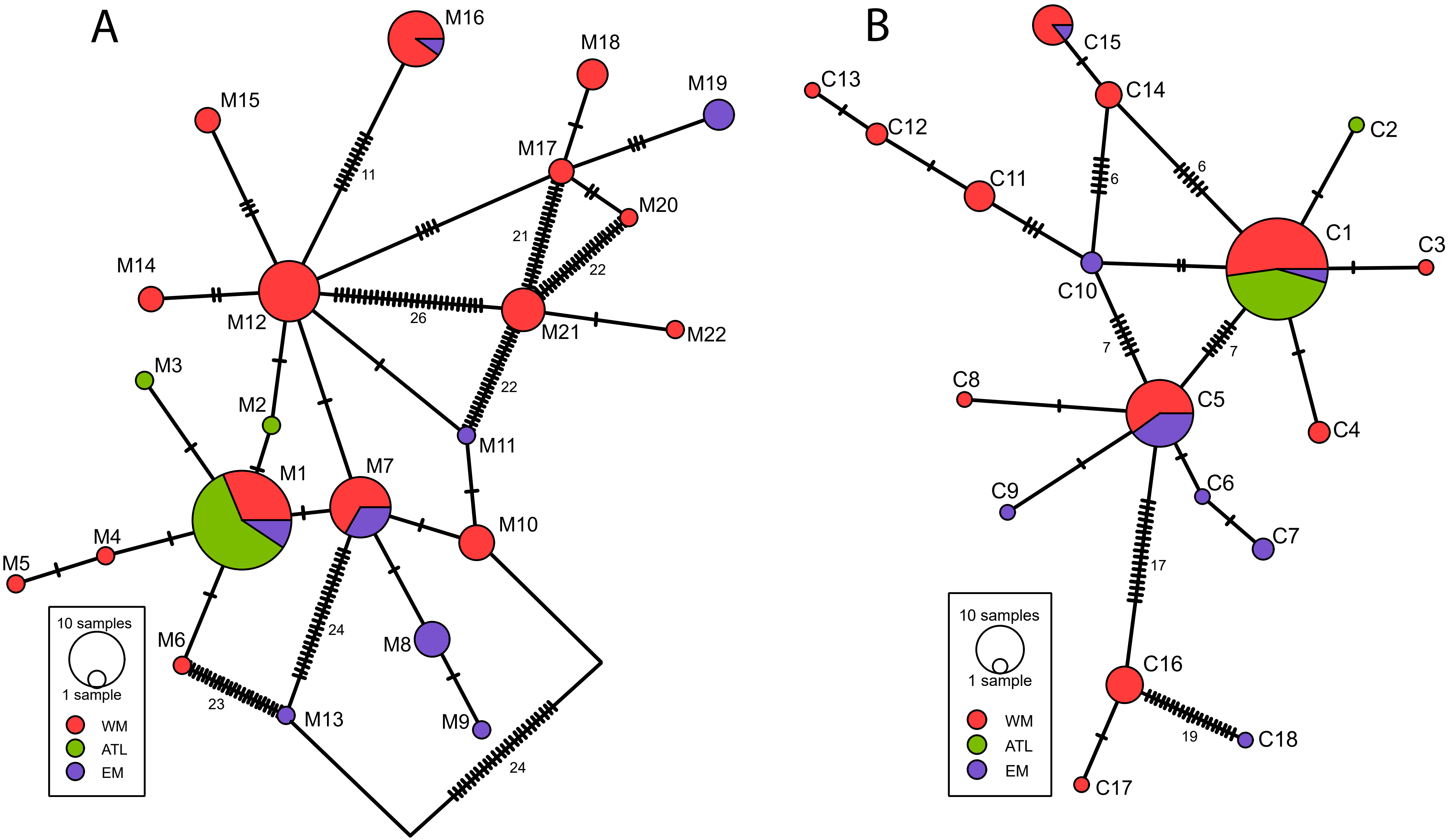
F-Isle 4
F-Llan 2
F-Llan 4
F-MaPo
F-MaRe
F-Nice 1
F-Nice 4
F-Nice 5
F-Nice 2
0.99
F-Port 1
F-Port 2
F-Port 3
F-SalR 1
F-SaPF 3
F-Sici
F-Tari 1
F-Tari 2
F-Tari 3
F-Tari 4
F-Tari 5
0.99
M-Bany 2
F-Nice 3
0.99
F-Isle 1
F-Isle 2
F-Isle 3
1
F-SalR 2
F-SaPF 1
F-SaPF 2
F-SaPF 4
1
0.53
F-Karp 1
F-RhoL 1
F-CeMa 1
M-Llan 8
M-Llan 9
0.94
M-Llan 2
M-Llan 4
M-Llan 5
M-Llan 6
1
M-Llan 1
M-Llan 3
M-Llan 7
0.008
0.99
F-Karp 2 *
0.99
F-RhoF
M-RhoA 2
F-Alac 1 *
F-Cast 1
F-Cast 2
F-Cast 6
F-Llan 1
F-RhoL 2 *
M-Alac 3
M-Alac 4
M-Alac 1
0.91
M-Alac 2
M-Isle 1
M-Isle 2
M-KarK
M-KarC
M-Mall
0.91
M-RhoL 1
M-RhoL 2
M-RhoA 1
M-Sici 1
M-Sici 2
M-SiCM
M-RhoL 3 *
1
0.99
M-Bany 7
M-Bany 8
M-Bany 1
M-Bany 3
M-Bany 4
M-Bany 5
M-Bany 6
1
1
M-SiGN1
D. guineënsis

Figure 3(on next page)

Minimum spanning network representing the haplotypes of *D. fasciola* and *D. mediterranea* sampling inferred from (A) *cox1* and (B) *rbcL-rbcS* markers.

Black stripes represent un-sampled intermediate haplotypes, one base mutation distant. The size of the circles represents the number of individuals and the color indicates their geographic circumscription. The number of unsampled mutation steps are shown when there are more than 5.


A
M1
M2
M3
M4
M5
M6
M7
M8
M9
M10
M11
M12
M13
M14
M15
M16
M17
M18
M19
M20
M21
M22
11
21
22
26
22
24
23
24
10 samples
1 sample
WM
ATL
EM
B
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
C11
C12
C13
C14
C15
C16
C17
C18
6
6
7
7
17
19
10 samples
1 sample
WM
ATL
EM