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Spatial and temporal genetic structure of *Velella velella* (Hydrozoa, Porpitidae) and its predator *Janthina pallida* (Gastropoda, Epitoniidae)

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ABSTRACT

The by-the-wind sailor *Velella velella*, is a cosmopolitan, pleustonic colonial hydrozoan, commonly in the Mediterranean Sea. It forms offshore aggregations that occasionally strand on the coasts, following strong wind conditions. Colonies are often found associated with *Janthina pallida*, a pleustonic gastropod that floats thanks to the secretion of a bubble raft, and which prey on them. Because of the massive occurrence of these species along the Italian coasts, and their often neglected ecological roles, it is pivotal to increase the knowledge of their genetic structure to improve monitoring activities and to provide elements to further understand the functioning of Mediterranean pleustonic zooplankton. Analyses of the genetic variability of *V. velella* and *J. pallida,* stranded on the beach in springs 2016 and 2017 at ten locations along the Ligurian and Tyrrhenian seas, show the existence of panmictic Mediterranean populations. Nevertheless, *V. velella* showed high genetic variability with a high number of singleton haplotypes, while *J. pallida* showed a recent population expansion. Considering the ecological and socio-economic importance of gelatinous zooplankton in today's changing marine ecosystems, these results lay the basis for better understanding the ecological role and population dynamics of pleustonic species.

KEYWORDS: Hydrozoa, Mollusca, stranding, molecular markers, Mediterranean Sea

1. INTRODUCTION

Many marine planktonic species are subjected to periodic blooms that usually depend on their peculiar explosive reproductive strategies, often coupled with the insurgence of specific environmental conditions (including temperature, pH, presence of upwellings, …) and variations in biological constrains (e.g., changes in the abundance of preys and predators) (e.g., Evans & Parslow 1985, Graham et al. 2001, Findlay et al. 2006, Suthers et al. 2019, Helm 2021). Such copious and rapid growth in population size plays an important role in the functioning of marine ecosystems and it is now understood that the occurrence of these phenomena is an essential component of a good and healthy ecosystem (Boero et al. 2008, Boero 2013, Suther et al. 2019, Jones et al. 2021).

Pleustonic zooplankton include many species that float on the water-air interface, dragged by wind and superficial currents. Widespread distribution and seasonal bloom events commonly characterize them. One of the most typical pleustonic species is the by-the-wind sailor *Velella velella* (Linnaeus, 1758), a circumtropical hydrozoan belonging to the family Porpitidae (Schuchert 2010), characterized by a floating polymorphic colony (Figure 1a) (Larson 1980, Ricketts & Calvin 1997, Bouillon et al. 2004, Schuchert 2010). The life cycle of *V. velella* is little known. It includes the release of many tiny, zooxanthellate, epipelagic medusae, which supposedly generate planula larvae (never observed to date) (Woltereck 1904, Schuchert 2010, Helm 2021).

Large-scale offshore blooms of *V. velella* (Figure 1b) occur seasonally in the Western Mediterranean Sea, reaching a peak of abundance in spring; this mass occurrence, combined with specific environmental factors, often result in pulse strandings along the coasts from April to June (Boero et al. 2016, Pires et al. 2018), with average densities ranging from about 600 to 11400 colonies m^{-2} (Betti et al. 2019).

V. velella strandings are often associated with the beaching of other pleustonic organisms, like the violet sea-snails *Janthina* spp. These gastropods are holoplanktonic organisms living upside down on the lower side of a raft created by enveloping air bubbles in a mucus layer (Lalli & Gilmer 1989) (Figure 1b). The reproduction is gonochoric, with aphallic males that produce sperms that swim into the female's genital tract, where fertilization occurs (Graham 1954). Fertilized eggs are subsequently glued to the raft; embryos develop into planktonic trochophore larvae and later into juvenile veligers before becoming fully grown adults (Beu 2017). A massive exceptional stranding of *Janthina pallida* (Thompson 1841), with average densities ranging from 13 to 2400 individuals m^{-2}, was recently reported along the Ligurian coasts (Betti et al. 2017). *Velella velella* and *J. pallida* are linked by a prey-predator interaction (Lepoint et al. 2016, Helm 2021), therefore the feeding activity of the gastropod may play a role in controlling the impact that dense aggregations of *V. velella* have on fish recruitment and macroplanktonic assemblages (Purcell et al. 2015).

Considering the lack of empirical data on the genetic structure and diversity of these two pleustonic species, mitochondrial markers (COI and 16S) were used to 1) evaluate the genetic variability and structure of these species along the Ligurian and Tyrrhenian coasts and in two different years, and 2) evaluate the phylogenetic relationship of the two species with their close relatives.

Recognizing the evolutionary history and genetic patterns of connectivity of the populations of the investigated species is pivotal to better understand the processes that drive their very complex reproductive strategies (especially in Epitoniidae gastropods) and their prey-predator specificity. Although herein we did not analyze nuclear markers, the results of this work can be used as a first attempt to monitor changes in the parameters of population genetic and to better understand the processes driving spatio-temporal dynamics of blooms in the Mediterranean Sea (Abboud et al. 2018).

2. MATERIAL AND METHODS

We collected specimens of both species along the Ligurian and Tyrrhenian coasts during April and May 2016 and 2017. Sixty-nine individuals of *Velella velella* were collected (38 in 2016 and 31 in 2017), 50 along the Ligurian coasts and 19 along the Tyrrhenian ones. Contextually, 76 specimens of *Janthina pallida* (60 in 2016 and 16 in 2017) were collected in the Ligurian area (Table 1, Figure 1c). Specimens from the Ligurian Sea were collected from the beaches of Arenzano, Santa Margherita Ligure and Livorno, and from a bank one nautical mile (NM) off the Genoa harbor; the sampling sites are stretched along about 150 km long NW Italian coasts. The Tyrrhenian sites included the beaches of Passoscuro (Lazio) and Cagliari (Sardinia Island) (Table 1), around 250 km and 500 km distant from the closest Ligurian site (Livorno), respectively (Figure 1c). All the collected organisms were preserved in 80% ethanol alcohol.

Genomic DNA was extracted from preserved samples using the CetylTrimethylAmmonium Bromide (CTAB) protocol (Winnepennickx et al. 1993), with minor modifications to optimize DNA extraction quality and yield.

Before choosing the markers to use in this paper, we tested several universal primers amplifying both mtDNA genes for both species and we found that COI successfully could be amplified in all *V. velella* individuals and 16S in *J. pallida* individuals. In fact, like all non-model organisms, there were very few markers and sequences available in the literature for *V.velella* (10 sequences available on GenBank) and *J. pallida* (no sequences available, only 14 for *Janthina* genus). Additionally, for both species, no previous studies were performed to test the population genetic variability of 16S and COI markers. Moreover, previous studies on several scyphozoans used COI sequences to detect population genetic structure at various spatial scales (e.g. Lee et al. 2013; Dawson et al. 2015).

Here, a portion of the mitochondrial region codifying for the Cytochrome Oxidase subunit I (COI) was amplified for *V. velella* using the universal COI primers LCO1490 and HCO2198 (Folmer et al. 1994) and thermocycle conditions outlined

in Ortman et al. (2010). For *J. pallida*, a portion of the mitochondrial 16S ribosomal DNA was amplified using universal 16S primers developed by Palumbi (1996). For both species, PCR was performed in a total volume of 25 µl, including 2.5 µl buffer 10X, 2.5 µl $MgCl_2$ 25 mM, 0.5 µl dNTPs 10 mM, 1.2 µl of each primer 10 µM, 0.1 µl of Taq polymerase 5 U/µl and 1 µl of DNA. The PCR products were purified, and both strands were sequenced by Macrogen Inc. (Seoul, Korea) using the same PCR primers for the sequencing reaction. Chromatograms were manually checked, edited and trimmed with MEGA7 (Kumar et al. 2016) and aligned with CLUSTALW.

To assess genetic variability within and between populations, DnaSP v6 (Rozas et al. 2017) was used to calculate the number of haplotypes, haplotype diversity and nucleotide diversity for each sample. Departures from selective neutrality and population equilibrium were futher tested with Tajima's D (Tajima 1989) and Fu's Fs tests (Fu 1997) using DnaSP. Pairwise mismatch distribution (Roger & Harpending 1992) were compared to those expected under Rogers' (1995) population expansion model and Excoffier's (2004) range expansion model (Excoffier & Lisher 2010).

Hapview (Barrett et al. 2005) was used to graphically represent the relationship between haplotypes and their frequency. Estimation of pairwise Φ_{ST} values and their significance was performed using Permutation tests (1000 permutations) in Arlequin v3.5 (Excoffier & Lischer 2010) to detect genetic variability among samples and between years. For pairwise Φ_{ST} estimates, false discovery rate corrections (Benjamini & Hochberg 1995) were applied in order to adjust significance levels for multiple comparisons.

COI and 16S sequences of the target species and close-related species available in Genbank and Bold were included in the analysis (Table S1, Online Resource 1). All the obtained sequences were available in the Online Resource 2 and deposited in NCBI GenBank (Accession numbers: from OM491246 to OM491314 for *Velella velella* and from OM522756 to OM522831 for *Janthina pallida*).

For phylogenetic analyses, jModelTest (Posada 2008) was used to select the best nucleotide substitution model for both species: General Time Reversible model (GTR) for *V. velella* and Tamura 3-Parameter model with gamma distribution of variable substitution rates among sites (T92 + G) for *J. pallida* (Rodriguez et al. 1990, Tamura 1992). Phylogenetic trees were generated using MEGA7, with a maximum likelihood approach and 1000 bootstrap permutations. For Bayesian inference, MrBayes v.3.2.6 software (Ronquist et al. 2012) was used to calculate posterior probabilities of branch nodes and infer tree topologies. The MCMC chain length was set at 100000 generations, with sampling every 1000 generations and a burn-in value of 250. Reconstructed trees were visualized in FigTree v.1.4.3.

MEGA7 was also used to estimate mean net nucleotide divergences among clades based on uncorrected pairwise distances.

In the case of *V. velella*, the distance-based Automatic Barcode Gap Discovery (ABGD) software (Puillandre *et al.*, 2012) was used for species delimitation. Aligned sequences of both datasets were uploaded on ABGD webserver (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) to sort them into hypothetical species based on pairwise distances by detecting differences between intraspecific and interspecific genetic distances using a range of priors (*Pmin* = 0.001; *Pmax* = 1), while the proxy *X* for the minimum gap width was set to 1.5, with 10 recursive steps and 20 bins.

3. RESULTS

3. 1. Genetic structure and evolutionary traits of Velella velella

A 519 bp fragment of the mitochondrial Cytochrome Oxidase subunit I (COI) gene was sequenced for all 69 specimens of *Velella velella*. The fragments showed 59 variable sites, of which 26 are ascribed as parsimony-informative. The alignment of the sequences revealed 56 haplotypes, with the Are16 location exhibiting the highest number of haplotypes (Figure 2a). Samples generally displayed high haplotype diversity, but low nucleotide diversity (Table 1). The haplotype network highlighted the presence of one main haplotype, shared among five samples. Most of the haplotypes were private and only three haplotypes were shared among two or three samples. The haplotype network did not show any clear geographical and temporal pattern (Figure 2a).

Tajima's (1989) D was negative (-2.158) and significant ($p < 0.05$) such as Fu's (1997) (Fs = -3.79, $p < 0.02$). The nucleotide mismatch distribution among individuals is unimodal evidencing a recent demographic expansion (data not shown).

Pairwise Φ_{ST} values were very low, ranging from -0.141 (between "SMa16" and "Laz17") to 0.083 (between "Off16" and "Laz17"). All pairwise comparisons between samples were not statistically significant ($p > 0.05$) (Table S2, Online Resource 3).

Phylogenetic trees, estimated from maximum likelihood and, Bayesian inference, showed concordant topologies. *Velella velella* sequences from Ligurian and Tyrrhenian seas clustered together and were differentiated from the single representative sequence retrieved from the eastern Pacific Ocean (bootstrap and posterior probability values > 90%). Moreover, this clade (Clade I) was well separated (bootstrap and posterior probability values > 90%) from a monophyletic clade, including individuals from Australia and French Polynesia (Clade II) (Figure 3). Species delimitation analyses (ABGCD) consistently suggested that the two hylogenetic clades correspond to two putative cryptic species. The two clades have a net nucleotide divergence of 0.099 ± 0.012, while the net divergence between *V. velella* clades and *Porpita porpita* (Linnaeus, 1758) clade were 0.15 ± 0.015 and 0.130 ± 0.014 respectively.

3.2. Genetic structure and evolutionary traits of Janthina pallida

A 363 bp fragment of the mitochondrial 16S ribosomal RNA gene was sequenced for all 76 specimens of *Janthina pallida*. Sequence alignment showed 20 variable sites, with 3 of them as parsimony-informative.

The sequence dataset showed 20 haplotypes, with the SMa16 sample having the most haplotypes but the lowest haplotype diversity. By contrast, SMa17 sample showed the highest haplotype and nucleotide diversity (Table 1). The haplotype network was star-shaped, with a central haplotype shared by all populations and many private haplotypes differing from the central one for a maximum of three substitutions (Figure 2b).

Tajima's (1989) D was negative (-2.578) and significant ($p < 0.001$) such as Fu's (1997) ($Fs = -5.252$, $p < 0.02$). The nucleotide mismatch distribution among individuals is unimodal evidencing a recent demographic expansion (data not shown).

Pairwise Φ_{ST} index values ranged from -0.057 (between Are17 and Nol17) to 0.077 (between SMa17 and SMa16). All pairwise comparisons between samples were not statistically significant ($p > 0.05$) (Table S3, Online Resource 3).

Maximum likelihood and Bayesian inference phylogenetic trees showed similar topologies. Sequences from the Ligurian *J. pallida* clustered together, but differentiation from the congenerics, *Janthina* cf. *prolongata* (Swainson, 1822) (accepted as *Janthina globosa* Swainson, 1822) and *Janthina umbilicata* (D'Orbigny, 1841) was not highly supported. Conversely, these three species were clearly differentiated from a cluster made by *Janthina janthina* (Linnaeus, 1758) and *Janthina exigua* (Lamarck, 1816) (Bootstrap values > 90%) (Figure 4). Phylogenetic results were supported by the net nucleotide divergence. In fact, the pairwise differences between *J. pallida, J.* cf. *prolongata* and *J. umbilicata* were equal to 0.033 ± 0.009 and 0.05 ± 0.01, respectively; while between *J. pallida, J. janthina* and *J. exigua* were equal to 0.025 ± 0.008 for both pairwise comparison.

4. DISCUSSION

This study is the first to analyze the genetic variability and structure of two pleustonic species in the Mediterranean Sea: the hydrozoan *Velella velella* and its predator, the gastropod *Janthina pallida*. The results highlight 1) the absence of any significant spatial and temporal effects on the genetic structure of either species and 2) the need to deepen the phylogenetic relationship within the two genera.

Genetic variability and connectivity of pleustonic taxa

In our study area, *V. velella* is characterized by a high number of singleton haplotypes (i.e. haplotype found only in one individual), with low nucleotide variability and a signature of demographic expansion. Despite the low sample size that could explain the high number of singleton, the haplotype network and the genetic pairwise differentiation suggest that this species represents a local panmictic population in the Ligurian and the Tyrrhenian Sea. No significant genetic structure was, in fact, observed among the stranded samples recorded in sites up to 500 kilometers far from each other. These results are consistent with its pleustonic life style (Pires et al. 2018). Partitioning in different banks of specimens belonging to a single offshore population, resulting in multiple strandings, is weather-induced (Betti et al. 2019) with the colonies transported from one location to another through wind and superficial currents. To our knowledge, few studies were performed on the phylogeography of Hydrozoa (Govindarajan et al. 2005, Churchill 2012, Boissin et al. 2018) and all of them analyze the pattern of population structure at a spatial scale greater than our in the Atlantic, Pacific and Indian oceans. Churchill (2012), using mitochondrial cytochrome oxidase I, observed global panmixis in *V. velella* compared to *Porpita porpita*, which showed regional and within-gyre panmixis. In the Mediterranean Sea, meroplanktonic cnidarians (Scyphozoa) showed different phylogeographic patterns with panmixia in *Pelagia noctiluca* (Forsskål, 1775) (Stopar et al. 2010, but see Aglieri et al. 2014) and regional genetic differentiation in *Aurelia aurita* (Linnaeus, 1758) (Ramsak et al. 2012) and *Rhizostoma pulmo* (Macri, 1778) (Ben Faleh et al. 2017).

These contrasting genetic structures partially agree with the life cycles of the species (e.g. holoplanktonic (*V. velella* and *P. nocticluca*) or meroplanktonic (*A. aurita* and *R. pulmo*) and suggest that these categories may predict population genetic structure in macromedusae (Abboud et al. 2018).

In *J. pallida* we observed a low haplotype number and low nucleotide diversity. Moreover, we noted a star-shaped haplotype network (i.e., a common haplotype surrounded by several private haplotypes) and a presence of a single panmictic population, suggesting a recent entrance of this species in the Mediterranean Sea (see below) and in the Ligurian Sea. Indeed, Betti et al. (2017) suggested that the Ligurian massive banks of this species recently beached on Ligurian shores possibly originated from the Atlantic Ocean, as shown by the simultaneous presence of the rare Atlantic buoy barnacle, *Dosima fascicularis* (Ellis & Solander 1786). In this regard, Atlantic surface currents are known to facilitate the transport of adult and larval species through the Strait of Gibraltar (e.g.: Sciberras & Schembri 2007, 2008, Bianchi et al. 2012). Thus, a combination of present-day and historical processes (high gene flow and the homogenizing effect of a recent expansion) may both contribute to the lack of genetic differentiation detected between populations within the North-western Mediterranean Sea. Finally, like *V. velella*, also *J. pallida* has a very slow movement and cannot oppose the sea currents. It is, therefore, possible that currents contribute in uniting these two organisms (Helm 2021) confirming the connectivity observed in both species at the analyzed spatial scale.

211 The genetic structure of the studied *V. velella* populations did not show any significant difference also among sampling
212 years (i.e. individuals stranded in Arenzano in 2016 and 2017). This supports the inter-annual genetic continuity of the
213 populations, and indicates that the two observed blooms originated from the same pool, and were not caused by
214 ingressions of new banks in the area. Part of the larvae released during the 2016 bloom probably remained in the region,
215 representing the inoculum for the following year's bloom in the same area. This behavior, suggested by the study of the
216 *conaria* larval stage (Woltereck 1904), does not exclude the existence of a yet unknown benthic resting stage; indeed,
217 cysts are known to control seasonal planktonic blooms of many marine species (Boero 1994, Belmonte et al. 1997;
218 Ellegaard et al. 2018). Nevertheless, also based on the same results obtained for *J. janthina*, the genetic composition of
219 the populations may change over time, but, likely, at temporal scales much larger than the one studied here. As observed
220 in Aglieri et al. (2014) a spatial-temporal chaotic genetic patchiness can be observed also in highly dispersive
221 holoplanktonic species suggesting that a genetic monitoring at longer scales is needed. Since blooms of the two pleustonic
222 species occur occasionally all along the Italian coasts (Boero et al. 2016; Betti et al. 2017), a more widespread sampling
223 effort and more variable molecular markers will be needed to evaluate which are the sink and source populations so to
224 better understand the processes driving spatio-temporal dynamics of these blooms.
225
226 *Phylogenetic relationships of pleustonic taxa*
227 The presence of two monophyletic clades supports the existence of two cryptic species in *V. velella*. Mediterranean and
228 Californian specimens of *V. velella* clustered in a separated clade (Clade I) compared to the specimens retrieved from
229 Australia and South Polinesia (Clade II). ABGD analysis strongly supported this result that is in accordance with Churchill
230 (2012), who also evidences different structures in *V. velella* corresponding to northern and southern hemisphere. Previous
231 studies in other Hydrozoa (see for example Dawson & Martin (2001) and Boissin et al. (2018)) have already observed
232 the presence of genetically distinct temperate and tropical subpopulations suggesting the existence of geographically
233 putative cryptic species in this group. Although we did not time-calibrated phylogenetic trees, the short length of the
234 branches between the two clades suggests that the distribution of the two putative cryptic species reflects past geological
235 processes and the spreading of ancestor species to the new environments after the last glaciations period (Dawson &
236 Jacobs, 2001, Schroth et al., 2002). Moreover, Schroth et al. (2002), using ecological data, evidenced that, in *Aurelia*
237 *aurita,* climatic adaptation may have forced ecological and phylogenetic divergence during evolutionary history. In the
238 case of *V. velella*, the presence of cryptic species could be related to morphological differences inducing ecological
239 divergence between hemispheres. In the Mediterranean Sea, *V. velella* has a left-handed sail while in the other seas or
240 oceans it can also have a right-hand sail if the coast of the continent that bathes those seas is on the western side or vice

versa (Edwards 1966). Moreover, a complex flexibility in the zooxanthellae–jellyfish association was observed in this species (Djeghri et al. 2019). *V. velella* can form symbiotic associations with different dinoflagellate genera (*Brandtodinium* and *Scrippsiella* (or *Ensiculifera*)), possibly with a biogeographical pattern (*Brandtodinium* in the Atlantic Ocean and possibly the Mediterranean Sea, *Scrippsiella* (or *Ensiculifera*) in the Pacific Ocean) (Probert et al. 2014, Djeghri et al. 2019). However, a broader geographic sampling effort coupled with additional observational data collection, molecular analysis, and biophysical global ocean modeling (Dawson et al. 2005) is necessary to corroborate this hypothesis.

The phylogeny of *Janthina* spp. generated from mitochondrial 16S sequences is congruent with the molecular phylogeny of Curchill et al. (2011) but partially incongruent with recent morphology-based phylogenies proposed by Beu (2017). The two approaches evidenced a close relationship between *J. pallida* and *Janthina cf. prolongata* (now accepted as *Janthina globosa*), but it remains unclear if *J. pallida* is a daughter species that originated from *J. globosa* (Beu 2017) or vice versa. In fact, *Janthina umbilicata* and *J. globosa* were more similar to each other compared to *J. pallida*, while in Beu (2017) *J. umbilicata* was more distant from *J. globosa* and morphologically more similar to *Janthina exigua*. Fossil data place *J. globosa* in the late Piacenzian or Gelasian (c. 3–2 Ma) to Holocene; while all the other species were placed in the Holocene (last 11,700 years of Earth's history) (Beu 2017). Because of their morphological similarity, an integrative revision of the genus *Janthina* is needed, including not only additional molecular markers but also new three-dimensional (3D) anatomical reconstruction. Conversely, a concordance between morphological and molecular data was observed in the high evolutive distance between *J. pallida* and *J. janthina*. Differences between these two species are evidenced also in their biological traits being *J. pallida* oviparous, while *J. janthina* ovoviviparous (Calabrò et al. 2019). In addition, the two species seem to prefer different preys: while *J. janthina* feeds on *Physalia* spp. and *V. velella*, *J. pallida* seems adapted to prey only on *V. velella* (Helm 2021).

In conclusion, our study reports the persistence, in two seasonally abundant pleustonic species, of single populations, with minimal geographical structure, at least in the North-western Mediterranean Sea. Natural changes through time and space, commonly affecting these organisms, seem not to influence their genetic structure. The obtained information represents the basis for better understanding the ecological role and population dynamics of these important and often neglected pleustonic species in the Mediterranean basin.

DATA AVAILABILITY STATEMENT: All relevant data are within the paper and its Supporting Information files.

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Ethics approval: Organisms used in the manuscript were found dead on the beach

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Table 1: Name of the locations, codes, sampling dates, GPS coordinates, number of sampled individuals, and the genetic variability of *Velella velella* and *Janthina pallida.* Genetic variability is reported as number of individuals (n), number of haplotypes (h), haplotype diversity (hd) with standard deviation (Dev. St.) and nucleotide diversity (Pi).

Species	Location	Code	Date	Coordinates	N° of individuals	h	hd (Dev. St)	Pi
Velella velella	Noli-Liguria	Nol16	13/04/16	44°12’14”N 08°25’01”E	13	12	0.987 (0.035)	0.00887
	Arenzano-Liguria	Are16	12/04/16	44°24’28”N 08°41’32”E	13	13	1 (0.030)	0.00892
	S. Margherita-Liguria	SMa16	15/04/16	44°20’09”N 09°13’27”E	4	4	1 (0.177)	0.01509
	Offshore-Liguria	Off16	12/04/16	44°22'0.12"N 8°52'21.72"E	8	8	1 (0.063)	0.00860
	Arenzano-Liguria	Are17	01/04/17	44°24’28”N 08°41’32”E	12	10	0.955 (0.057)	0.00928
	Livorno-Toscana	Liv17	07/05/17	43°32'36"N 10°19'1" E	8	8	1 (0.063)	0.01060
	Cagliari-Sardegna	Sar17	08/05/17	39°04′28″N 08°39′57″E	10	9	0.978 (0.054)	0.00818
	Passoscuro-Lazio	Laz17	09/05/17	41°54′08″N 12°09′26″E	1	1	0	0
TOTAL					69	64	0.986 (0.007)	0.00948
Janthina pallida	Noli-Liguria	Nol16	13/04/16	44°12’14”N 08°25’01”E	11	5	0.618 (0.164)	0.00200
	Arenzano-Liguria	Are16	12/04/16	44°24’28”N 08°41’32”E	19	4	0.380 (0.134)	0.00113
	S. Margherita-Liguria	SMa16	15/04/16	44°20’09"N 09°13’27”E	21	6	0.043 (0.134)	0.00131
	Arenzano-Liguria	Are17	01/04/16	44°24’28”N 08°41’32”E	9	3	0.417 (0.191)	0.00122
	Noli-Liguria	Nol17	01/05/17	44°12’14”N 08°25’01”E	8	4	0.643 (0.184)	0.00207
	S. Margherita-Liguria	SMa17	02/05/17	44°20’09”N 09°13’27”E	8	4	0.750 (0.139)	0.00394
TOTAL					76	20	0.497 (0.072)	0.00173

Figure 1. a) *Velella velella* colony floating on the surface. b) *Janthina pallida* as seen from below the surface, with the bubble raft and the pink eggs. c) Map of the sampling sites. Purple dots indicate *Janthina pallida* sampling locations, while blue triangles refer to the sampling sites of *Velella velella*. White scale bar = 1 cm.

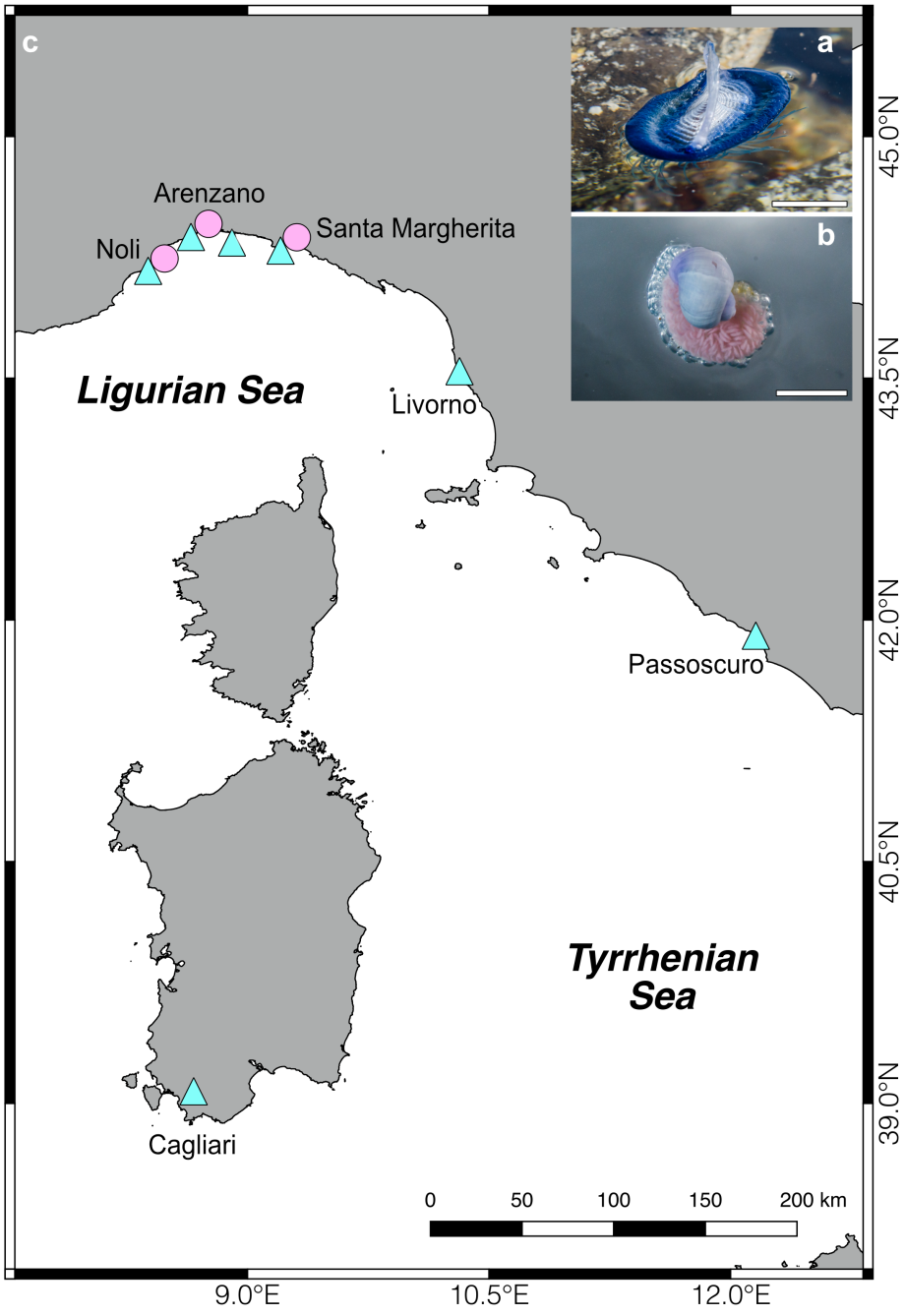

Figure 2. a) Median joining haplotype networks of the individuals of *Velella velella*; b) Median joining haplotype networks of the individuals of the Ligurian populations of *Janthina pallida.* The network is standardized in such way that small circles represent haplotypes present in one individual. Each branch represents 1 mutation between the 2 adjacent sequences and black small circles represent unsampled haplotypes.

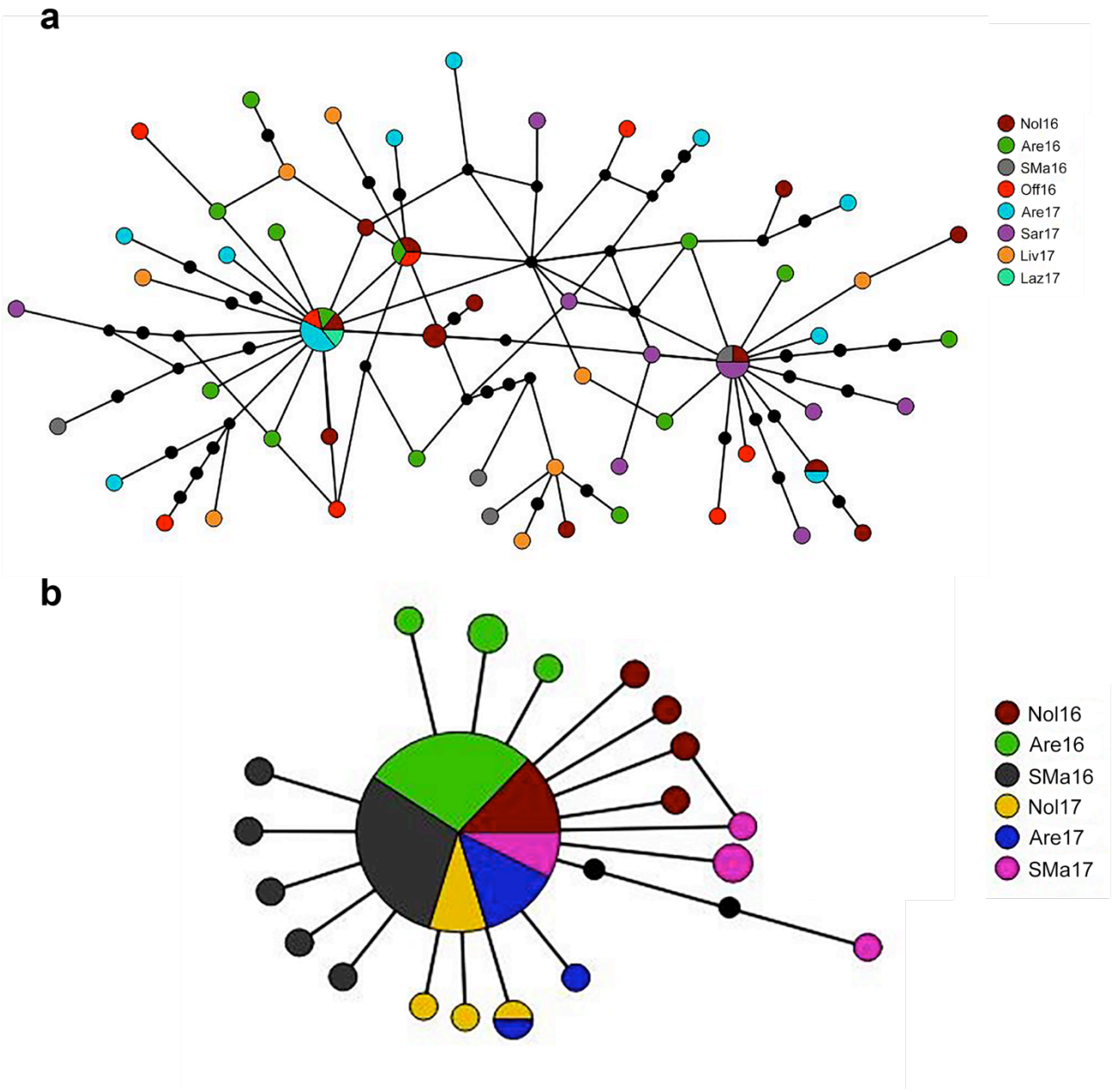

Figure 3. Maximum-likelihood (ML) tree for the COI sequences of *Velella velella* and close-related species. Bayesian inference (BI) tree showed the same topology (data not shown). Posterior probability and bootstrap support values are indicated when > 50% (BI/ML). The tree is rooted using *Obelia* sp., *Campanulariidae* and *Clytia* sp. as outgroups.

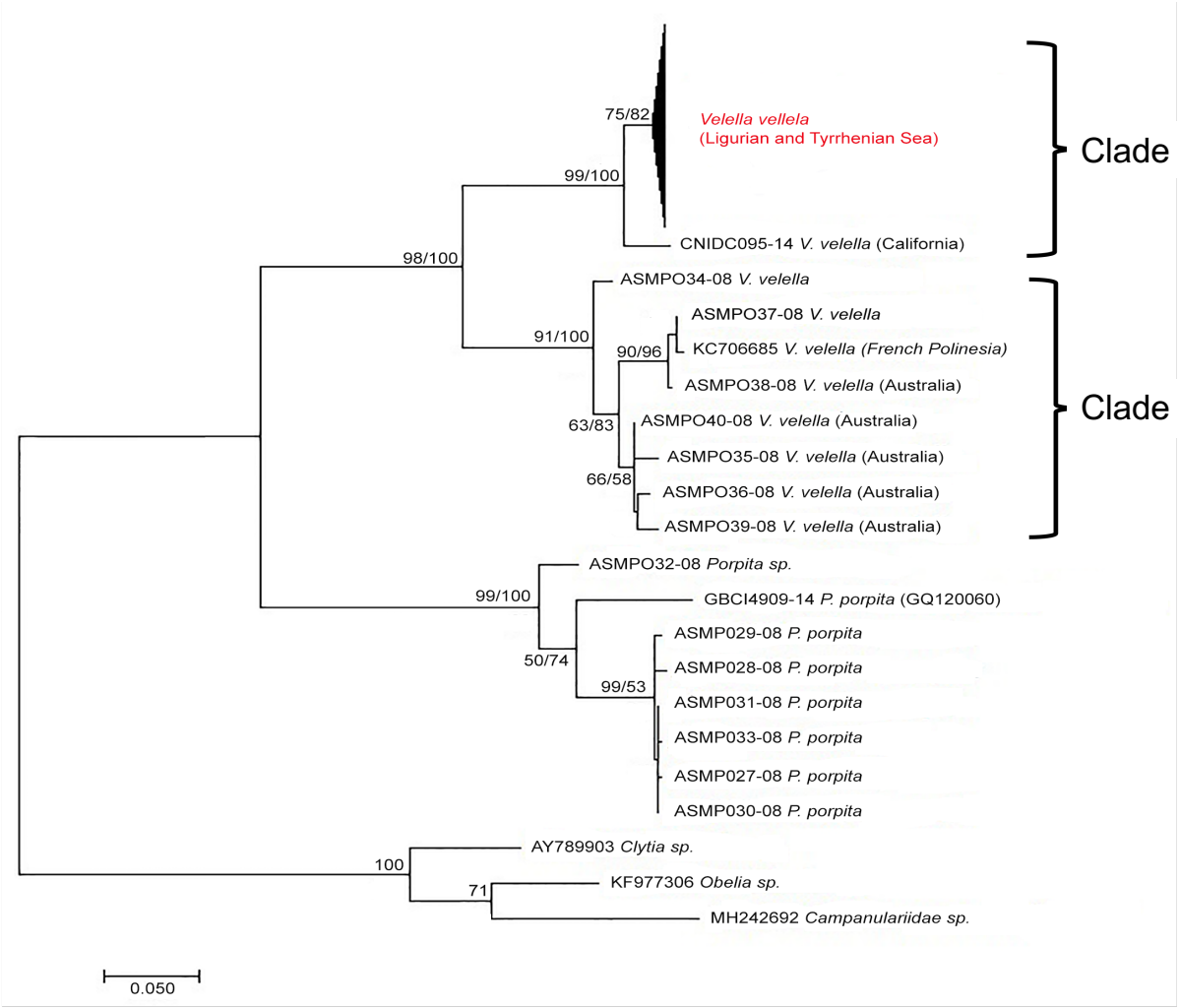

Figure 4. Maximum-likelihood (ML) haplotype tree for the 16S sequences of *Janthina pallida* and close-related species. Bayesian inference (BI) tree showed the same topology (data not shown). Posterior probability and bootstrap support values are indicated when > 50% (BI/ML). The tree is rooted using *Recluzia jehennei, Acrilla acuminata* and *Epidendrium sordidum* as outgroups. * Accepted as *Janthina globosa*.