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Temporal Population Genetics and COI Phylogeography of the Sandhopper *Macarorchestia remyi* (Amphipoda: Talitridae)

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Laura Pavesi, Elvira De Matthaeis, Ralph Tiedemann, and Valerio Ketmaier (2011) Temporal population genetics and COI phylogeography of the sandhopper *Macarorchestia remyi* (Amphipoda: Talitridae). *Zoological Studies* **50**(2): 220-229. In this study we assessed levels of genetic divergence and variability in 208 individuals of the supralittoral sandhopper *Macarorchestia remyi*, a species strictly associated with rotted wood stranded on sand beaches, by analyzing sequence polymorphisms in a fragment of the mitochondrial DNA (mtDNA) gene coding cytochrome oxidase subunit I (COI). The geographical distribution and ecology of the species are poorly known. The study includes 1 Tyrrhenian and 2 Adriatic populations sampled along the Italian peninsula plus a single individual found on Corfu Is. (Greece). The Tyrrhenian population was sampled monthly for 1 yr. Genetic data revealed a deep phylogeographic break between the Tyrrhenian and Adriatic populations with no shared haplotypes. The single individual collected on Corfù Is. carried the most common haplotype found in the Tyrrhenian population. A mismatch analysis could not reject the hypothesis of a sudden demographic expansion in almost all but 2 monthly samples. When compared to previous genetic data centered on a variety of Mediterranean talitrids, our results place *M. remyi* among those species with profound intraspecific divergence (sandhoppers) and dissimilar from beachfleas, which generally display little population genetic structuring. http://zoolstud.sinica.edu.tw/Journals/50.2/220.pdf

Key words: *Macarorchestia remyi*, Talitridae, Cytochrome oxidase I, Population genetics.

The dispersal capability of a species and the ability to persist and reproduce in new areas are important factors in determining its pattern of genetic structuring (De Matthaeis et al. 1998 2000a). For many years, we have been comparatively addressing the issue of how and to what extent different species of marine supralittoral talitrid amphipods that are distributed over the same geographic area but which differ in their respective ecological characteristics are genetically structured. Talitrids, like all amphipods, lack a planktonic larval stage, and active dispersal is limited to short movements along the beach. Over large distances, they can only rely on passive dispersal through floating wrack and wood (De Matthaeis et al. 1998). We showed that at the scale of the entire Mediterranean Sea, species colonizing rocky shores and living in wrack close to the water line (genus *Orchestia* Leach, 1814; beachfleas) are considerably less genetically structured than species confined to sandy beaches at or above the high-water mark (genera *Talitrus* Latreille, 1802; *Talorchestia*, Dana, 1852; and *Deshayesorchestia* Ruffo, 2004; sandhoppers) (De Matthaeis et al. 1998 1999 2000a b, Ketmaier et al. 2003). Our results were recently confirmed by Henzler and Ingólfsson (2007) on Atlantic populations of *Orchestia gammarellus* (Pallas, 1766). It is therefore very likely that the kind of environment inhabited by talitrid species is

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the main determining factor of the degree of intraspecific divergence. Living in wrack cast on rocky shores would enhance the probability of individuals being dragged away by the sea and thus maximize dispersal among populations.

Herein we present a further study case centered on the sandhopper *Macarorchestia remyi* (Schellenberg 1950). Current knowledge on the species' distribution is very poor. *Macarorchestia remyi* was initially found in 1950 in Corsica (Schellenberg 1950) and 5 years later in Sardinia (Ruffo 1960). The species was also reported from a few scattered localities in France, Greece, and Italy (Ruffo 1993), while its occurrence on Atlantic shores is dubious (S. Ruffo, pers. comm. 2008). We intensively surveyed Corsican and Sardinian localities as well as nearby areas but with no success. We suspect that the species has become extinct there owing to the massive human impacts on those coasts. However, we did find *Macarorchestia remyi* in 3 peninsular Italian localities, 1 on the Tyrrhenian coast (Principina a Mare, Grosseto, Tuscany, central Italy) and 2 on the Adriatic coast (Lesina and Varano, Foggia, Apulia, southern Italy). In addition, we obtained a single individual from Corfu Is. (Greece). The species was sampled monthly (for 1 yr) at Principina a Mare and only once in the other localities included in the study. The monthly sampling was part of a companion study aimed at understanding the species life history (Pavesi and De Matthaeis 2009).

Macarorchestia remyi inhabits sandy shores, but its ecology is peculiar in that the species is strictly associated with rotten wood carried by the sea. This association should theoretically lead to high rates of passive dispersal even over large distances. Active dispersal seems to be sporadic and limited to short movements between contiguous pieces of driftwood (Pavesi and De Matthaeis 2009).

In this study, we report on levels of genetic variability and divergence within as well as among the aforementioned 4 populations. We screened sequence polymorphisms in a region of mtDNA coding for a fragment of cytochrome oxidase subunit I (COI). This gene has proven informative at the intraspecific level in amphipods (Henzler 2006, Kelly et al. 2006). More importantly, it has already been used in Mediterranean talitrids both on micro- and macro-geographical scales (Ketmaier et al. 2005a b 2010). Specifically, we tested whether or not the association with rotted wood is conducive to gene flow in *M. remyi*. Under the 1st scenario, we expected to find sharing of haplotypes among populations. The 2nd hypothesis would translate into populations carrying unique haplotypes and ultimately, reciprocal monophyly of geographically localized mtDNA lineages. It is important to emphasize that our sampling, although limited to a few localities, includes 2 increasing geographical levels and hence allows us to test the presence vs. absence of gene flow hypotheses at different geographical scales. As a matter of fact, the 2 Apulian sites are just a few kilometers away from each other, while several hundred kilometers separate the Tuscan, Apulian, and Greek localities.

MATERIALS AND METHODS

Sampling

Samples screened for genetic variation were collected at the 4 following localities (in parentheses are given the abbreviations used to identify the sites): Principina a Mare, Grosseto, Tuscany, central Italy (PRI); Lesina, Foggia, Apulia, southern Italy (LES); Varano, Foggia, Apulia, southern Italy (VAR); and Agios Georgios, Corfu Is., Greece (COR). Sampling sites are shown in figure 1. Animals were collected using an aspirator or by hand and were preserved in 80% EtOH. Males, females and intersexes were identified in the laboratory. Sexes were differentiated by the presence of genital papillae in males and oostegites in females, whereas intersexes were identified by the simultaneous presence of both characters.

Macarorchestia remyi was collected as described in Pavesi and De Matthaeis (2009). It is worth emphasizing that we adopted the same sampling strategy Pavesi and De Matthaeis (2009) used to gather information on the species population demographics at PRI. Hence, we are confident that our samples are representative of the entire population at that site. In total, 208 individuals were analyzed molecularly. This corresponds to 15 individuals for each monthly sampling at PRI (with the following proportions, whenever possible: 5 males, 5 females, and 5 intersexes) for a total of 178 individuals. We collected 14 individuals at LES and 15 at VAR. We found just a single individual at COR.

Genetic analyses

Total DNA extractions were carried out using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) amplifications of a 582-base pair (bp) fragment of the mitochondrial gene coding cytochrome oxidase subunit I (COI) were carried out using the primers reported in Folmer et al. (1994). Double-stranded amplifications were performed as described in Ketmaier et al. (2008); sequences were determined using the Macrogen facility (http://dna.macrogen.com/eng/) and were submitted to GenBank (accession nos.: HQ679919- HQ679937).

Sequences were edited and aligned using Sequencher 4.1 (Gene Code Corporation, Ann Arbor, MI, USA); the alignment was also checked by eye following the guide provided by the reading frame. The following parameters were calculated with PAUP* 4.0b10 (Swofford 2002): base frequencies, numbers of variable and parsimoniously informative sites, the absolute number of substitutions, and the numbers of transversions (Tvs) and transitions (Tis). We used ModelTest (Posada and Crandall 1998) and the Akaike Information Criterion implemented in the program to select the optimal model of sequence evolution; we used a maximum-parsimony (MP) tree generated with PAUP* 4.0b10 as a guide tree to search for the best-fitting model. We then used the settings suggested by the program as those best fitting our dataset to calculate maximum-likelihood (ML) distances. A statistical parsimony network was also constructed using TCS 1.13 (Clement et al. 2000) with a 95% connection limit (Templeton et al. 1992). Genetic diversity (H), the mean number of pairwise differences between all pairs of haplotypes (π), and nucleotide diversity (π_n) were calculated using Arlequin 3.0 (Excoffier et al. 2005). For PRI, these values were calculated for each monthly sampling and on all samples pooled together. We used the same program to test levels of genetic diversity identified by the network search using a hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992). Finally, we used Arlequin to calculate pairwise F_{ST} values (2 different analyses: among monthly samplings at PRI and among PRI, LES, and VAR) and to carry out a mismatch analysis (Schneider and Excoffier 1999) to infer the demographic histories of PRI, LES, and VAR. Monthly samplings at PRI were separately analyzed in the mismatch analysis.

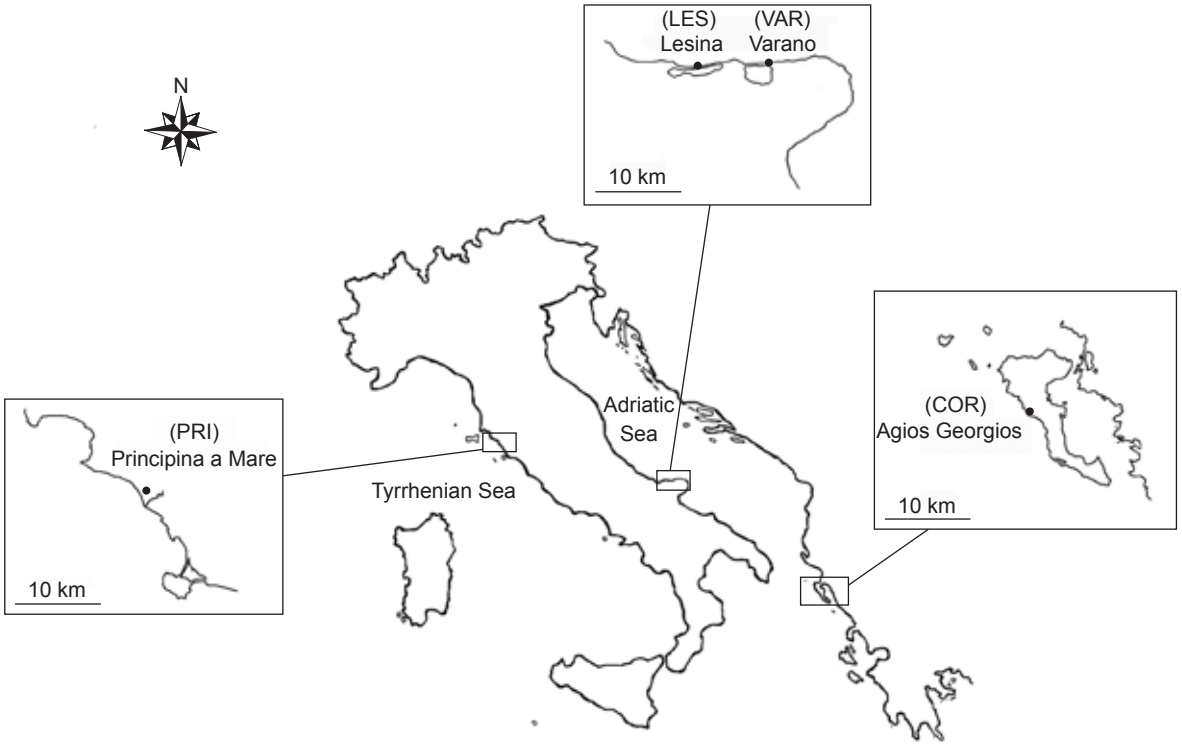

Fig. 1. Sampling localities included in this study.

RESULTS

The sequences obtained for this study defined a total of 19 unique haplotypes in the 208 individuals analyzed. Haplotypes are listed in table 1 along with their absolute frequencies in the different populations (or monthly samples in the case of PRI). The mean nucleotide composition was 29% A, 20% C, 17% G, and 34% T. A χ^2 test showed no deviation of base frequencies from the expected values (p = 1 for all codon positions and for each codon position separately). Estimates of genetic variability are presented in table 2. H ranged between 0.130 (PRI in May) and 0.629 (PRI in Jan.), π was from 0.130 (PRI in May) to 1.276 (PRI in Oct.), while π_n ranged 0.022% (PRI in May) to 0.219% (PRI in Oct.). LES was on average more genetically variable than PRI and VAR. Numbers of transversions (Tvs) and transitions (Tis), and ML distances between all pairs of haplotypes are shown in table 3 (ModelTest suggested the TVM + G model for the sequence evolution-shape parameter α = 0.46; with base frequencies of 0.28 for A, 0.20 for C, 0.16 for G, and 0.36 T, as the best fit of the data). The highest number of Tvs was 10 (H7-PRI vs. H18-VAR), while the maximum number of Tis was 30 (H5-PRI and H9-PRI vs. H18-VAR). D_{ML} ranged between 0.002 (multiple comparisons within LES and VAR) and 0.079 (H7-PRI vs. H18-VAR), with an average value of 0.04 ± 0.51 (standard deviation; SD). At the between-population level, we found 2 degrees of genetic divergence. Mean D_{ML} values for PRI-LES and PRI-VAR were 0.06 ± 0.000 and 0.07 ± 0.010, respectively. The mean D_{ML} value between LES and VAR was much lower (0.01 ± 0.000).

The haplotype network (Fig. 2) sorted the 19 haplotypes in 2 haplogroups. Group I included haplotypes found at PRI + COR, while group II was restricted to Adriatic localities (LES + VAR). Haplotypes differed from 1 to 13 mutations from each other in group I and from 1 to 8 mutations in group II. Group I had a star-like pattern with a common haplotype (H1) shared by most individuals lying at the center of the sub-network and connected by independent mutational steps to haplotypes with much lower frequencies. This haplotype included specimens from all 12 monthly samples at PRI (n = 178), plus the single individual found on COR. There was no structuring by

Table 1. Absolute frequencies of haplotypes (H) in the different localities/samplings included in the study. M, males; F, females; I, intersexes; PRI, Principina a Mare; COR, Corfù; LES, Lesina; VAR, Varano. Sampling localities are shown in figure 1

	PRI																																				COR			LES			VAR		
H	May			June			July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.											
	M	F	I	M	F	I	M	F	I	M	F	I	M	F	I	M	F	I	M	F	I	M	F	I	M	F	I	M	F	I	M	F	I	M	F	I	M	F	I	M	F	I	M	F	I
1	10	4		4	6	1	1	4	2	5	4	2	3	4	5	5	4	2	2	4	3	4	5	2	2	2	4	5	4	3	5	5	3	2	5	4	1								
2														1																															
3	1			1			1	2	1		1	1		1			1	2	3	1	2			2	3	1	1			3			1	2		1									
4				2	1			1				1	1													2							1												
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14																																										1			
15																																								1					
16																																								1	1	1			
17																																											4	6	1
18																																													1
19																																											1		

Table 2. Estimates of genetic variability in *Macarorchestia remyi*. Gene diversity (H), mean number of pairwise differences between all pairs of haplotype (π), and nucleotide diversity (π_n). Population codes match those in table 1; *n* indicates the sample size. Values in the last column are given as percentages

Sampling		*n*	H	π	π_n
May	PRI (monthly samplings)	15	0.130	0.130	0.022
June		15	0.447	0.476	0.081
July		15	0.484	0.566	0.097
Aug.		15	0.447	0.476	0.081
Sept.		15	0.275	0.714	0.122
Oct.		15	0.447	1.276	0.219
Nov.		15	0.514	0.514	0.088
Dec.		14	0.385	0.407	0.069
Jan.		15	0.629	0.724	0.124
Feb.		15	0.264	0.264	0.045
Mar.		15	0.257	0.267	0.045
Apr.		14	0.363	0.363	0.062
PRI (pooled)		178	0.392	0.488	0.085
LES		14	0.838	1.181	0.310
VAR		15	0.467	1.524	0.261

date of samples in the group; the most variable month (July; 6 haplotypes) also had the highest number (3) of exclusive haplotypes. Each monthly sampling contained 2-6 haplotypes (Table 1). Haplotypes in group II were chiefly sorted by their respective geographic origin. Intersexes had the highest number of haplotypes in both clusters (I: *n* = 8, all of which were from PRI; II: *n* = 7).

Table 4 shows the results of the hierarchical AMOVA; genetic heterogeneity was equally apportioned within populations and among groups. Pairwise F_{ST} values are shown in table 5. A 1st analysis was conducted on PRI monthly samplings only; 6 comparisons produced statistically significant F_{ST} values. All remaining pairwise comparisons within PRI were low and non-significant. A 2nd one compared the 3 localities: PRI (monthly samples grouped together), LES, and VAR. Pairwise F_{ST} values confirmed the deep phylogeographic break between the Tyrrhenian and Adriatic locations, with comparisons between the 2 areas yielding F_{ST} values of about 0.980 that were highly statistically significant. Owing to its sample size (*n* = 1), COR was not considered in the pairwise F_{ST} analysis.

Results of the mismatch analyses (Table 6) supported a model of sudden demographic

Table 3. Maximum-likelihood genetic distances (below the diagonal) and absolute numbers of transversions (Tvs)\transitions (Tis) (above the diagonal) among *Macarorchestia remyi* haplotypes

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1		2\2	0\1	0\1	0\2	1\0	4\3	1\0	0\2	0\1	3\25	3\23	4\25	3\24	4\26	3\25	3\26	6\28	3\27
2	0.007		2\3	2\3	2\4	1\2	4\1	3\2	2\4	2\3	5\27	5\25	6\27	5\26	6\28	5\27	5\28	8\28	5\29
3	0.002	0.009		0\2	0\1	1\1	4\4	1\1	0\3	0\2	3\26	3\24	4\26	3\25	4\27	3\26	3\27	6\29	3\28
4	0.002	0.009	0.003		0\3	1\1	4\2	1\1	0\3	0\2	3\26	3\24	4\26	3\25	4\27	3\26	3\27	6\29	3\28
5	0.003	0.011	0.002	0.005		1\2	4\5	1\2	0\4	0\3	3\27	3\25	4\27	3\26	4\28	3\27	3\28	6\30	3\29
6	0.002	0.005	0.003	0.003	0.005		3\3	2\0	1\2	1\1	4\25	4\23	5\25	4\24	5\26	4\25	4\26	7\28	4\27
7	0.012	0.009	0.014	0.011	0.016	0.011		3\3	4\5	4\4	7\27	7\25	8\27	7\26	8\28	7\27	7\28	10\28	7\29
8	0.002	0.009	0.003	0.003	0.005	0.003	0.011		1\2	1\1	4\25	4\23	5\25	4\24	5\26	4\25	4\26	7\28	7\27
9	0.003	0.011	0.005	0.002	0.007	0.005	0.016	0.005		0\3	3\27	3\25	4\27	3\26	4\28	3\27	3\28	6\30	3\29
10	0.002	0.009	0.003	0.003	0.005	0.003	0.014	0.003	0.005		3\26	3\24	4\26	3\25	4\27	3\26	3\27	6\28	3\28
11	0.057	0.066	0.059	0.059	0.061	0.059	0.070	0.059	0.061	0.059		0\2	1\2	0\1	1\3	0\2	0\3	3\5	0\4
12	0.052	0.061	0.054	0.054	0.056	0.054	0.065	0.054	0.056	0.054	0.003		1\2	0\1	1\3	0\2	0\3	3\5	0\4
13	0.059	0.068	0.061	0.061	0.063	0.060	0.072	0.061	0.063	0.061	0.005	0.005		1\1	0\1	1\0	1\1	4\3	1\2
14	0.054	0.063	0.057	0.057	0.059	0.056	0.058	0.056	0.059	0.056	0.002	0.002	0.003		1\2	0\1	0\2	3\4	0\3
15	0.061	0.070	0.064	0.064	0.066	0.063	0.075	0.063	0.066	0.063	0.007	0.007	0.002	0.005		1\2	1\2	4\4	1\3
16	0.056	0.066	0.059	0.059	0.061	0.059	0.070	0.059	0.061	0.059	0.003	0.003	0.002	0.002	0.003		0\1	3\3	0\2
17	0.059	0.068	0.031	0.061	0.064	0.061	0.073	0.061	0.064	0.061	0.005	0.005	0.003	0.003	0.005	0.002		3\2	0\1
18	0.070	0.075	0.073	0.073	0.075	0.073	0.079	0.072	0.076	0.070	0.014	0.015	0.012	0.012	0.014	0.011	0.009		3\1
19	0.061	0.071	0.064	0.064	0.066	0.063	0.075	0.063	0.067	0.064	0.007	0.007	0.005	0.005	0.007	0.003	0.002	0.007	

expansion for all populations. Moment estimators of time to the expansion (τ) were 0.51, 2.1, and 5.2 for PRI, LES, and VAR, respectively (corresponding to mismatch observed means of 0.51, 1.81, and 1.52). The probability of observing a less-good fit between the model and observed distribution by chance (P_{SSD}) ranged 0.06 (LES) to 0.82 (PRI). These results translated into unimodal mismatch distributions for each population analyzed (graphs not shown). Within PRI, the hypothesis of a sudden demographic expansion was rejected for the Nov. and Jan. samples only (P_{SSD} = 0.043 and 0.040, respectively).

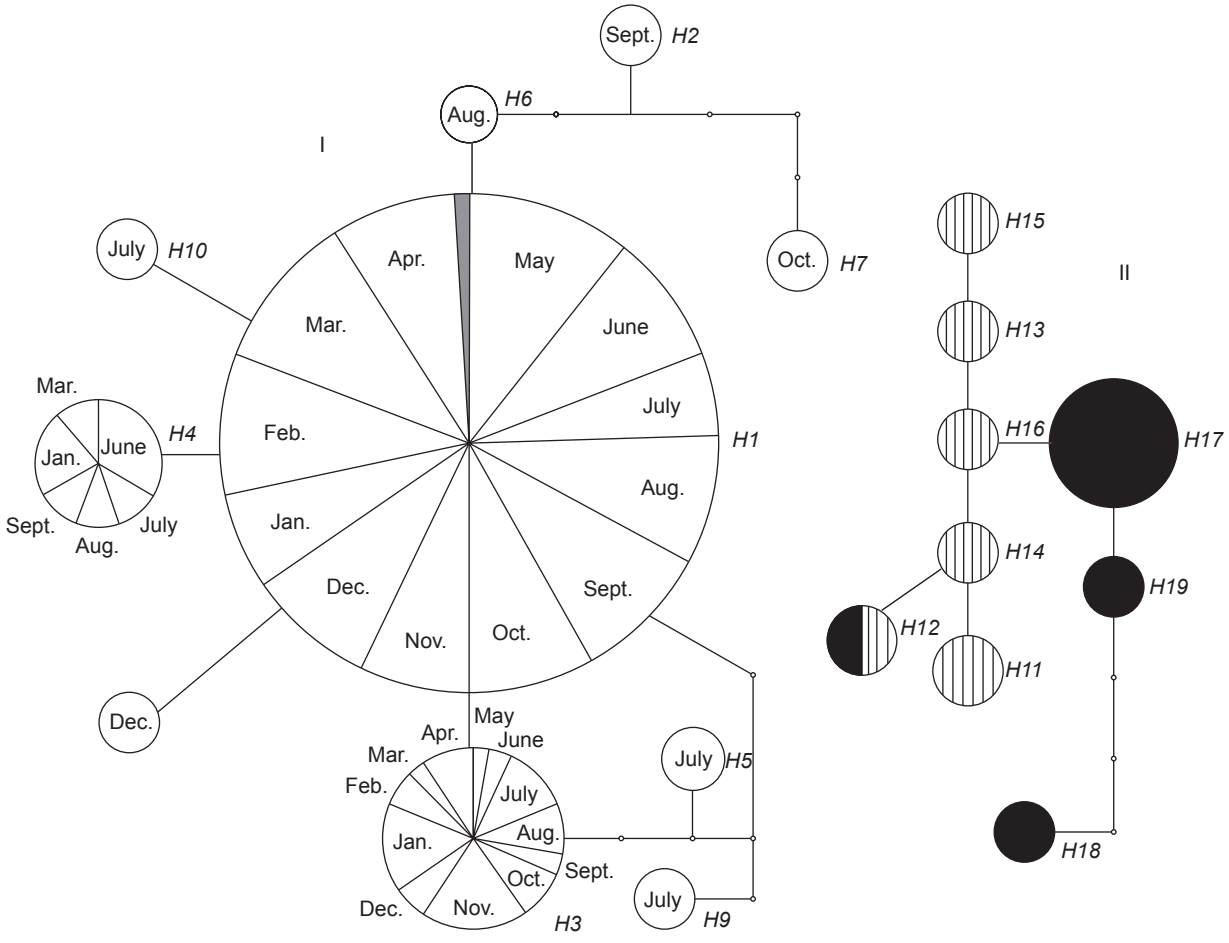

Fig. 2. Haplotype network analysis based on the statistical parsimony (95% criterion) of Templeton et al. (1992). There are 2 major haplogroups (I and II). Group I includes individuals sampled at Principina a Mare (PRI) + Corfù (COR), while group II clustered Adriatic haplotypes only of Lesina (LES) + Varano (VAR). Black dots are missing haplotypes. Solid white, PRI; striped shading, LES; solid black, VAR; solid gray, COR. The size of each circle is proportional to the number of individuals carrying that particular haplotype.

Table 4. Results from the AMOVA. Three groups were considered: Principina a Mare, Lesina, and Varano

Source of variation	Variance component	*p*	Fixation index	Percent (%) variation
Among groups	Va = 0.19	0.014	0.460	46.400
Among populations within groups	Vb = 0.02	0.000	0.080	4.280
Within populations	Vc = 0.20	0.000	0.510	49.320

Table 5. Pairwise F_{ST} values among *Macarorchestia remyi* populations included in the study. (A) Monthly samplings at Principina a Mare (PRI); (B) PRI (monthly samplings grouped together), Lesina (LES), and Varano (VAR). Bold values indicate statistical significance at the $p < 0.05$ level (after Bonferroni's correction in table B)

(A)

	Sampling		1	2	3	4	5	6	7	8	9	10	11	12
1	May	PRI (monthly samplings)	0.000											
2	June		0.066	0.000										
3	July		**0.020**	0.07	0.000									
4	Aug.		0.022	-0.008	0.021	0.000								
5	Sept.		0.004	0.009	**0.111**	-0.014	0.000							
6	Oct.		0.039	0.003	0.015	-0.049	0.009	0.000						
7	Nov.		0.217	0.098	-0.025	0.032	**0.015**	-0.002	0.000					
8	Dec.		-0.005	-0.005	0.051	-0.052	-0.018	-0.053	0.046	0.000				
9	Jan.		**0.206**	0.039	-0.035	0.029	**0.128**	0.008	-0.045	0.049	0.000			
10	Feb.		0.008	0.012	0.053	-0.047	0.008	-0.059	0.026	-0.061	0.041	0.000		
11	Mar.		-0.046	-0.016	**0.135**	-0.018	-0.025	-0.004	0.149	-0.036	0.126	-0.023	0.000	
12	Apr.		0.02	0.01	0.039	-0.049	0.012	-0.064	0.009	-0.061	0.016	-0.073	-0.016	0.000

(B)

		1	2	3
1	PRI	0.000		
2	LES	**0.976**	0.000	
3	VAR	**0.977**	**0.348**	0.000

Table 6. Results of the mismatch analysis in 3 populations of *Macarorchestia remyi*. For Principina a Mare (PRI), the expansion parameter (τ), the mutation parameter before (θ_0) and following (θ_1) expansion, the mismatch observed mean (Obs. mean), and the raggedness index (Ragged.; Harpending 1994) were calculated for each monthly sampling and for all samples pooled together. Values in brackets refer to the 95% consistency index. P_{HARP} is the probability of obtaining a higher value of the raggedness index by chance. P_{SSD} (sum of squared deviations) is the probability of observing a less-good fit between the model and the observed distribution by chance

Sampling		τ	θ_0	θ_1	Obs. mean	Ragged.	P_{HARP}	P_{SSD}	θ_{PI}
May	PRI (monthly samplings)	2.890 (0.270-2.890)	0 (0.000-0.234)	0.160 (0.000-1703)	0.130	0.560	0.690	0.340	0.130 ± 0.220
June		0.610 (0.000-1.310)	0 (0.000-0.990)	640.620 (0.620-3877)	0.480	0.170	0.500	0.190	0.480 ± 0.490
July		0.670 (0.000-1.330)	0 (0.150-0.990)	640.000 (0.770-3723)	0.570	0.140	0.190	0.270	0.570 ± 0.520
Aug.		0.610 (0.000-1.350)	0 (0.000-0.990)	640.000 (0.617-3877)	0.480	0.170	0.490	0.200	0.480 ± 0.490
Sept.		3.000 (0.470-4.250)	0 (0.000-0.370)	0.310 (0.000-2390)	0.710	0.40	0.660	0.170	0.710 ± 0.640
Oct.		6.000 (2.120-10.00)	0 (0.000-1.940)	0.720 (0.000-5.960)	1.280	0.170	0.750	0.550	1.280 ± 0.950
Nov.		0.790 (0.000-1.570)	0 (0.000-1.380)	701.000 (1.500-5004)	0.510	0.260	0.120	0.043	0.510 ± 0.510
Dec.		0.500 (0.000-1.130)	0 (0.000-0.720)	517.000 (0.370-3445)	0.410	0.180	0.400	0.370	0.410 ± 0.450
Jan.		0.930 (0.000-1.780)	0 (0.000-1.430)	2157.000 (1.960-4907)	0.720	0.230	0.090	0.040	0.710 ± 0.640
Feb.		0.350 (0.030-1.190)	0 (0.000-0.630)	3.340 (0.000-3148)	0.260	0.290	0.470	0.380	0.260 ± 0.350
Mar.		3.000 (0.450-4.250)	0 (0.000-0.340)	0.360 (0.000-2170)	0.270	0.300	0.710	0.470	0.270 ± 0.350
Apr.		0.500 (0.000-1.140)	0 (0.000-0.620)	364.000 (0.670-3313)	0.360	0.210	0.380	0.300	0.360 ± 0.420
PRI (pooled)		0.510 (0.290-0.660)	0 (0.000-0.350)	179.000 (0.800-2514)	0.500	0.170	0.260	0.820	0.490 ± 0.470
LES		2.100 (0.250-3.290)	0 (0.000-2.050)	1242.000 (10.700-8046)	1.810	0.140	0.100	0.060	1.810 ± 1.240
VAR		5.200 (1.430-7.470)	0 (0.000-1.920)	0.910 (0.000-17.980)	1.520	0.290	0.450	0.330	1.520 ± 1.080

DISCUSSION

Phylogeography and genetic divergence

Our data clearly demonstrated that in spite of the theoretical potential for long-distance dispersal via passive transport on rotten wood, *M. remyi* is not able to maintain levels of gene flow sufficient to prevent the accumulation of local differences. This holds valid both locally (Apulian sites) and between the Tyrrhenian and Adriatic basins. It is worth noting that the statistically significant F_{ST} value between LES and VAR (0.348) was comparable to most of the values obtained when comparing Adriatic and Tyrrhenian sites. Indeed, only 1 of the 9 Adriatic haplotypes was shared between LES and VAR. No haplotypes were shared between Adriatic and Tyrrhenian sites, whereas the single individual found on Corfù Is. carried haplotype H1, which was also the most frequent at PRI. This result is difficult to explain given the otherwise profound genetic divergence found in the species. We need to examine a much larger number of individuals from Corfù Is. to assess whether this population is genetically connected to PRI or not.

The pattern of genetic structuring found in *M. remyi* at the mtDNA level closely resembles that of the sandhopper *Talitrus saltator* (Montagu, 1808). De Matthaeis et al. (1995 2000a b), using a set of allozymic markers, revealed the occurrence of 3 genetically distinct groups of populations within this species. These groups are geographically localized and correspond to the 3 major basins of the Mediterranean Sea (Tyrrhenian, Adriatic, and Aegean). The divergence within *T. saltator* was explained by invoking a combination of historical (i.e., paleogeography of the Mediterranean Sea) and intrinsic (low potential for long-distance dispersal) factors (De Matthaeis et al. 1995). We do not have a proper geographical sampling to address these issues in *M. remyi*; nonetheless, the occurrence of a major phylogeographic break between the Tyrrhenian and Adriatic populations speaks in favor of parallel evolutionary trends in these 2 species. De Matthaeis et al. (2000a) obtained similar results for 2 other genera of sandhoppers (*Talorchestia* and *Deshayesorchestia*). *Talitrus*, *Talorchestia*, and *Deshayesorchestia* have comparable ecological requirements in that they all live on sandy beaches above the high-tide mark where they dig up to 0.5 m deep in the sand during daytime to avoid dehydration. On the other hand, various species of beachfleas colonizing rocky shores and living within rotting seaweed (genera *Orchestia* and *Platorchestia* Bousfield, 1982) proved to be much less genetically structured at the same geographical scale (De Matthaeis et al. 2000a b). The most likely explanation for these contrasting results is that sandhoppers and beachfleas have different probabilities of passive dispersal. As a matter of fact, supralittoral amphipods can only rely on floating wrack and wood to move over long distances (De Matthaeis et al. 1998), but the probability of being routinely dragged away by the sea is presumably high for beachfleas, which are abundant in wrack close to the water line, and low in sandhoppers, which occur only at or above the high-water mark.

We asked whether a strict association with rotting wood stranded on sandy beaches above the high-tide mark would be conducive to high levels of gene flow or not. Given the data at our disposal, we tentatively concluded that this ecological condition is not sufficient per se to maintain genetic cohesiveness over a broad geographical scale. Rather, we hypothesize that the major factor influencing the genetic structure of talitrids is proximity to the water line and not association with potential carriers.

We also emphasize that the mean level of genetic divergence between the Adriatic and Tyrrhenian populations (6%-7%) is almost double the species-screening threshold (3.75%) adopted in a COI-based barcoding study in the amphipod genus *Hyalella* (Witt et al. 2006). Our figures are also well within the COI range found by Costa et al. (2007) in a variety of crustaceans, including 12 species of amphipods. We are aware that our conclusions on *M. remyi* are not definitive, because they are based on a too sparse of a sampling. Nonetheless, our data suggest that processes of divergence have taken place in the species. Whether or not these 2 gene pools represent truly independent entities (species) or are the extreme of clinal variations is not possible to tell here. Testing this hypothesis would require the analysis of populations intermediate between the Tyrrhenian and Adriatic ones (i.e., potential stepping-stones). These are not known in the literature nor have been found during our samplings. Finally, our conclusions are based on a single mtDNA marker. The analysis of bi-parentally inherited markers might potentially challenge the scenario proposed here. However, we do not believe that this is likely in *M. remyi*. Contrasting patterns in mtDNA and nuclear DNA imply a male-biased gene flow, which seems unrealistic in a species with a female-

biased sex ratio (Pavesi and De Matthaeis 2009). Additionally, the aforementioned resemblance of the genetic structuring of *M. remyi* and *T. saltator* (mtDNA based in the former and nucDNA based in the latter) argues in favor of concordant patterns in markers with different evolutionary properties and thus suggests similar underlying evolutionary processes.

Intra-population variability

PRI, LES, and VAR have comparable levels of genetic diversity, with numbers of haplotypes ranging from 4 (VAR) to 10 (PRI).

At PRI, we observed no preferential distribution of haplotypes with respect to date of sampling or sex (Table 1). However, it is interesting to note that we found the highest number of haplotypes (6) in July, when the population abundance was at a minimum (Pavesi and De Matthaeis 2009). This result can be explained by the occurrence of a breeding peak in early spring (Pavesi and De Matthaeis 2009) that potentially introduces new genetic variants into the population and the effects of which persist even when the population abundance declines. Our mismatch analyses indicated that all but 2 monthly samples at PRI (Nov. and Jan.) had undergone a sudden population expansion. For Nov. and Jan., P_{SSD} values were only borderline significant. We concluded that population stasis is more the exception that the rule in *M. remyi*, at least for the populations examined in the study. The ephemeral persistence in time of the habitat selected by this species (i.e., rotted wood) could explain these results. We therefore envision a scenario in which populations experience cycles of contraction and expansion following the availability of suitable wood strata. The wide fluctuations in the population abundances at PRI recorded by Pavesi and De Matthaeis (2009) further support this hypothesis. Also the significant F_{ST} values among some of the monthly samplings can be explained in light of their temporal instability, as they might not have the chance to persist long enough for haplotype frequencies to become homogeneous. Nonetheless, this phenomenon, although present, should not be regarded as predominant since F_{ST} values were significant in just 6 of 66 possible pairwise comparisons. The demographic trends at PRI are at odds with what was found by Ketmaier et al. (2010) in a single population of *T. saltator* sampled at 4 different points along 3 km of a dynamic sand beach. That study showed that the population was temporally stable with only slight signs of expansion where the impact of tourism on the beach (and hence the risk of population crashes) was the highest.

In this study, we report the 1st genetic data on a talitrid species, *M. remyi*, strictly associated with stranded rotted wood on sand beaches. Owing to this particular ecological characteristic, we hypothesized that the species could potentially achieve high rates of gene flow via passive transport of wood by sea currents. Our mtDNA data clearly demonstrated that this was not the case, as we found a substantial lack of gene flow both locally and on a wide geographical scale. All tested populations conveyed signs of recent sudden expansions in their genetic structure probably due to the scattered and temporally unstable nature of their habitat. The high degree of genetic divergence we found coupled with signs of demographic instability suggests that *M. remyi* is in dynamic equilibrium under low levels of gene flow (Slatkin 1993). Such a scenario implies that if the species becomes locally extinct re-colonization is very unlikely. Furthermore, given the tendency to become geographically and genetically subdivided, any local extinction would translate into a loss of genetic diversity. Stranded wood is often removed from beaches for cleaning purposes. Proper management of sand beaches (and thus of their ecological communities) would have to take into consideration the evolutionary dynamics described in this study.

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