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When DNA puts ecological works back on the right track: genetic assessment and distribution patterns of mudsnail populations in the Evros Delta lagoons

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With 3 figures and 4 tables

Abstract: The present study aims to assess the distribution patterns of mudsnail populations in the Evros Delta lagoons and to investigate the key environmental variables affecting them. As a correct species assignment is crucial for ecological studies, the data presented here are substantiated using DNA sequences from the mitochondrial genome to verify the systematic status of the mudsnail taxon in question. A comparative molecular analysis involving almost all brackish-water taxa of Hydrobiinae indicates that the populations from the Evros Delta belong to the Pontic species *Ventrosia maritima*, a taxon that was not reported before from the Mediterranean. Moreover, it appears that in previous studies, the mudsnail populations from the Evros Delta were misidentified. Principal Component Analyses using environmental variables defined station groups among which *V. maritima* showed a significant variation in density. Mudsnail specimens were absent at stations with an immediate access to the sea (salinity 32–34 PSU, very fine to fine sand, organic matter 0.3–1.4 %), they showed intermediate densities (up to 3,000 ind./m^2) in innermost lagoonal parts (34–35 PSU, very fine sand, organic matter 1.0–1.4 %) and relatively high densities (up to 7,000 ind./m^2) only in isolated parts (15–17 PSU, fine sand, organic matter 0.5–0.8 %). Contact with the sea, which is strongly correlated with seawater renewal rate and hydrodynamism, was found to be the most important factor affecting mudsnail distributions. The data presented, being the first detailed ecological information available for *V. maritima*, are compared with the available information for other mudsnail taxa. The present study not only lays the foundation for future comparative eco-genetic works of mudsnail taxa, it also stresses the absolute need for the routine usage of molecular markers in

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ecological studies involving frequently utilized but systematically poorly understood invertebrate species.

Key words: Hydrobiidae, *Ventrosia maritima*, spatial variation, PCA, mtDNA, phylogenetic inference, Aegean Sea.

Introduction

Ecological studies of benthic invertebrate species have started at the beginning of the twentieth century. Since then, benthic communities and/or selected taxa have been widely used as keystone species for ecological characteristics, as bioindicators in environmental impact studies (both rapid bioassessment and long-term monitoring), and for the study of carbon cycling, energy balance, nutrition fluxes and biogeochemical interactions. Many benthic species show a strong habitat selection and even closely related taxa often react differentially on environmental variables, which makes them so interesting for ecologists. However, in order to correctly interpret fine-scale ecological differences or changes, a correct species assignment is absolutely crucial. This might be particularly true for mudsnail taxa of the subfamily Hydrobiinae (Rissooidea: Hydrobiidae), which are notoriously difficult to determine because of the cryptic character of many of its radiations (i. e. shell morphology and soft body anatomy is essentially identical among congeners). This makes a correct species determination based on shell-morphological or anatomical characters in the field and even in the laboratory basically impossible. To give an example, in one of his numerous landmark studies, Barnes (1993) investigated the ecology of the north-western European mudsnail taxon *Hydrobia acuta neglecta* (as *Hydrobia neglecta*). One of the populations studied from the French Atlantic Coast (Lostrouc'h, Finistère) occurred in a highly unusual habitat (open marine setting), and showed marked differences in its reproductive strategy and sex ratio. Subsequent molecular studies (Wilke et al. 2000, Wilke & Pfenninger 2002) indicated that this particular population did not belong to the Boreal *H. a. neglecta* but to the Lusitanian *H. glyca*. The latter taxon can tolerate high salinities and open water conditions much better than the former one, which would explain the observed differences. However, the anatomy of these two taxa is very similar (Wilke et al. 2002) and *H. glyca* had not been reported before from northwestern France. Therefore, in the absence of molecular markers, Barnes (1993) could not possibly have realized that he had likely compared populations from two different species.

It should be noted that only four brackish-water representatives of the Hydrobiinae are known to occur in northern Europe, but at least eight taxa are reported from the Mediterranean and Black Sea (Wilke 2003). Therefore, the problem is likely to be worse in southern Europe. Also, taking into account that our knowledge of the ecology of the Hydrobiinae in the coastal brackish water habitats of the Mediterranean and Black Sea is very restricted (see MARAZANOF 1969, CHUKCHIN 1978, BRITTON 1985, LARDICCI et al. 1997, etc.), a study of the distribution of these taxa in a Mediterranean lagoon system, on the basis of a correct species determination, obviously is of a special interest.

The Evros Delta in the Northern Aegean Sea is of notable natural history and recreational interest and is protected as a wetland of international value according to the RAMSAR convention. Three poikilohaline shallow lagoons (Laki, Monolimni, Drana), the various parts of which are characterized by a graduated contact with the sea, can be found in the delta area. In the Evros Delta area the occurrence of *Hydrobia a. acuta* (DRAPARNAUD 1805) was previously reported by ecological studies (KEVREKIDIS et al. 1996, GOUVIS et al. 1997, KEVREKIDIS 1997).

The present study aims to assess the distribution patterns of mudsnail populations in the Evros Delta lagoons and to investigate the key environmental variables affecting them. Given the potential impact of an incorrect species assignment on the interpretation of ecological data, we here substantiate our study utilizing DNA sequences from the mitochondrial genome to clearly demonstrate the systematic status of our taxon in question by performing comparative molecular analyses with other taxa of the Hydrobiinae.

Materials and methods

Study area

The Evros Delta is located at the N. E. part of the Aegean Sea (Fig. 1). Fresh water reaches the delta area through the eastern branch of the Evros River and also, usually from late autumn to early summer, through the western branch of the Evros River and the streams Mikri Maritsa and Loutron (Fig. 1). Three islets and three lagoons were formed in the delta area (Fig. 1). Laki Lagoon, occupying an area of about 1.0 km^2, communicates directly with the sea through two openings (Fig. 1). Drana Lagoon covers an area of 2.2 km^2 (Fig. 1). In the past, Drana Lagoon communicated with the sea through a narrow opening 4 m wide. In order for the Drana Lagoon to be drained, its opening was obstructed in 1987 and since then there has been no direct communication with the sea. Monolimni (or Paloukia) Lagoon, occupying an area of about 1.12 km^2, communicates with the sea mainly through a 15 m wide opening (Fig. 1).

Variations in some physicochemical parameters of water and sediment (depth, water salinity, median diameter, Qd, silt-clay and organic matter content of the sediment) along transects LC and LA and at station LB in Laki Lagoon, along transects I and B in Monolimni Lagoon and at stations DA_1, DA_2, DC and DZ in Drana Lagoon (Fig. 1) in summer 1997 are given in Table 2. The structure of the macrozoobenthic community in Drana and Laki Lagoons in summer 1997 has recently been investigated (KEVREKIDIS et al. 2000, MOGIAS & KEVREKIDIS unpubl. data).

Sampling and laboratory techniques

Sixteen stations that cover all parts of the lagoons Laki, Drana and Monolimni were sampled (Fig. 1). During summer 1997, two or three replicate units of the substrate were collected per station for faunal analyses, using a modified van Veen grab (Larimore 1970). The grab covered a surface of 400 cm^2 (20 × 20 cm) and penetrated to a depth of about 20 cm. The samples were sieved in the field through a 0.5 mm screen and the animals collected were preserved in a 5 % formalin solution. Further details on sampling are given by Kevrekidis et al. (2000) and Mogias & Kevrekidis (unpubl. data). In the laboratory, mudsnails were separated from the remaining macrozoobenthos, decalcified in a 2 N solution of HCl and counted. Up to 20 individuals from each station were used to study the penis type of male specimens, thereby assuring that all

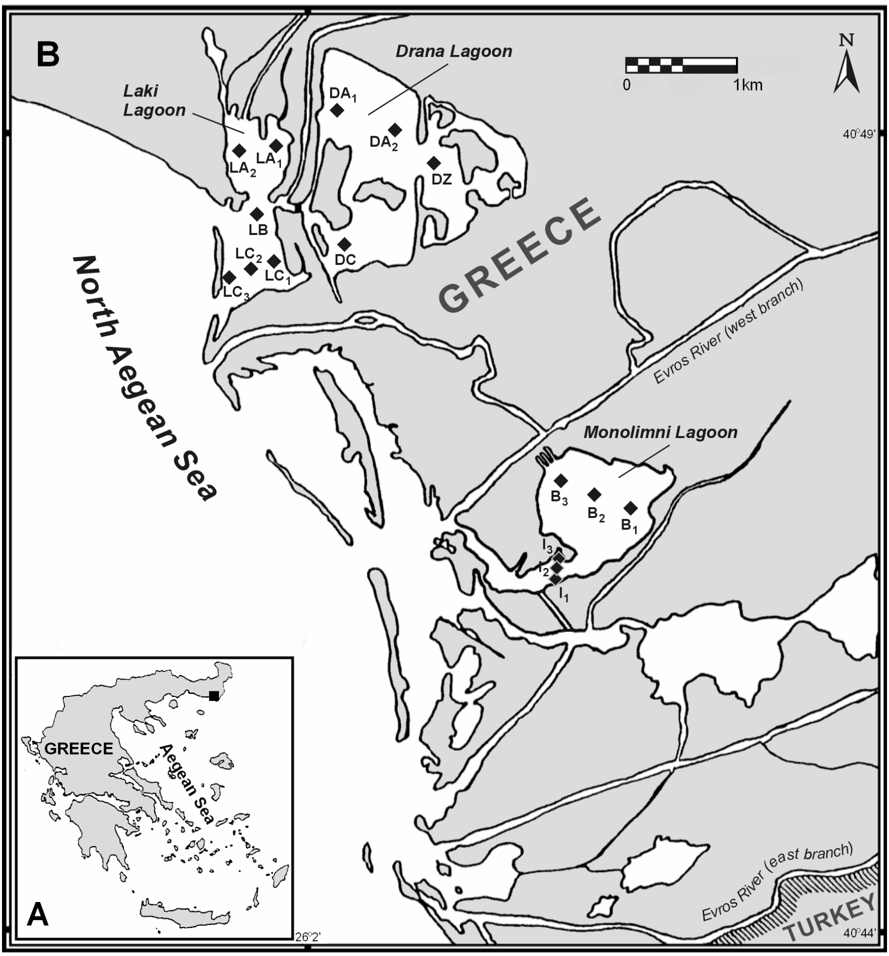

Fig. 1. (**A**) Geographical location of the study site, (**B**) Map of Evros Delta showing the sampling stations.

individuals belong to the same radiation (for details see WILKE 2003). Additionally, snails were collected at stations I_1 and B_2 on July 17, 1999 and freshly preserved in 75 % ethanol (snail : alcohol ratio 1 : 10) for genetic work.

DNA Sequencing and phylogenetic analyses

A total of 11 mudsnail specimens from the Evros Delta were sequenced from sites B_2 and I_1. As the genetic diversity among those individuals was very low (see Results section), only one specimen (DNA isolation # 2218, Genbank accession # AY616140) from site I_1 was used for the phylogenetic studies. Additional taxa for the comparative phylogenetic analyses are listed in Table 1. They include almost all known taxa of the Hydrobiinae (sensu WILKE 2003) with emphasis on the species from the Mediterranean Sea and the Black Sea. As outgroup taxa, representatives of the closely related subfamilies Pyrgulinae (*Pyrgula annulata*, Genbank accession # AY341258) and Pseudamnicolinae (*Pseudamnicola lucensis*, Genbank accession # AF367651) were used.

Table 1. Locality information, DNA isolation numbers and GenBank accession numbers for the representatives of the Hydrobiinae that were used for the comparative phylogenetic studies. Sequences that were previously submitted to GenBank are marked with an asterisk.

Species	Location	Latitude Longitude	DNA isolation #	GenBank accession #
Hydrobia acuta acuta (DRAPARNAUD, 1805)	France, Hérault, Etang du Prévost	43.513° N 3.897° E	653	AF278808*
Hydrobia acuta neglecta MUUS, 1963	Denmark, Jutland, Mariager Fjord, Ajstrup Bugt	56.68° N 10.22° E	848	AF467604*
Hydrobia glyca (SERVAIN, 1880)	Spain, Cádiz, San Fernando, lagoon San Francisco de Asís	36.3937° N 6.1375° W	567	AF467640*
Hydrobia djerbaensis (WILKE, PFENNINGER & DAVIS, 2002)	Tunisia, Médenine, Djerba, Tazdaine, Sebkha de Sidi Garous	33.82° N 11.07° E	692	AF449215*
Hydrobia sp. (see WILKE et al., 2000)	Spain, Balearic Islands, Mallorca, Puerto de Pollença	39.8987 °N 3.0820 °E	1198	AF278821*
Adriohydrobia gagatinella (KÜSTER, 1852)	Croatia, Cetina River estuary near the town Omis	43.44867° N 16.69385° E	2111	AF317843*
Peringia ulvae (PENNANT, 1777)	United Kingdom, Norfolk, Holkham Natural Reserve, Stiffkey Saltmarsh	52.957° N 0.930° E	719	AF118288*
Ventrosia ventrosa (MONTAGU, 1803)	United Kingdom, Norfolk, The Wash, Snettisham lagoon RSPB bird reserve	52.863° N 0.460° E	717	AF118335*
Ventrosia ventrosa (MONTAGU, 1803)	Tunisia, Tunis, Lac de Tunis	36.808° N 10.263° E	1832	AY616137
Ventrosia pontieuxini (RADOMAN, 1973)	Bulgaria, Burgas, bay between Strandscha and Kraimorie	42.430° N 27.513° E	306	AF449216*
Ventrosia maritima (MILASCHEWITCH, 1916)	Ukraine, Crimea, Sevastopol, Kruglaja Buchta	44.61° N 33.45° E	2986	AY616139
Ventrosia truncata (VANETTA, 1924)	USA, New Jersey, Cape May County, Stone Harbor	39.5° N 74.7° W	500	AF449217*
Ventrosia sp.	Tunisia, Médenine, Djerba, Tazdaine, Sebkha de Sidi Garous	33.82° N 11.07° E	686	AY616138
Salenthydrobia ferrerii WILKE, 2003	Italy, Lecce, Torre Chianca, Idume Creek	40.466° N 18.183° E	2453	AF449206*

DNA isolation, PCR and sequencing of the mitochondrial cytochrome oxidase I (COI) gene were done as described in WILKE (2003).

Nucleotide diversities and divergences (corrected according to the K2P-parameter-model) were calculated using MEGA 2 (KUMAR et al. 2000) with standard errors estimated by 1000 bootstrap replications with pairwise deletion of gaps and missing data.

Prior to the phylogenetic analyses, the computer program Modeltest 3.06 (POSADA & CRANDALL 1998) was used to find the optimal model of DNA substitution. The model selected was GTR + Γ (general time reversible model with Γ distribution), with base frequencies of A = 0.2620, C = 0.1613, G = 0.1857, T = 0.3910; a rate matrix of [A–C] = 0.6348, [A–G] = 12.0490, [A–T] = 1.0774, [C–G] = 1.8052, [C–T] = 13.2629, [G–T] = 1.000; and a Γ distribution shape parameter of $\alpha = 0.1503$.

For phylogenetic reconstruction, we utilized the software package MrBayes 3.0 b4 (HUELSENBECK & RONQUIST 2001). We ran four chains and 1,000,000 sampled generations with the current tree saved at intervals of 10 generations. Finally, a 50 % majority rule tree was constructed from all sampled trees with the first 1000 trees (= 10,000 generations) ignored as burn in.

An additional parsimony-based parametric bootstrapping approach was used to specifically test the robustness of the relationship between the mudsnail taxon from the Evros Delta and its closest relatives (for a detailed description of the test procedure see WILKE & DUNCAN 2004).

Ecological data analyses

A degree scale of contact of the sampling stations with the sea was established (Fig. 1, Table 2). A principal component analysis (PCA) was conducted using the figures for environmental variables (contact with the sea, depth, salinity, silt-clay and organic matter content of the sediment). Variables were first normalized, then the PCA was performed using the PRIMER package developed at Plymouth Marine Laboratory. Spearman's rank correlation coefficient (ρ) was applied to identify correlations between the first or second component detected by PCA and the environmental variables or density of the mudsnail specimens. The significance of the spatial variation in specimen density was tested by Kruskal-Wallis one-way analysis of variance.

Results

Anatomy

All specimens studied had an identical anatomical ground plan. The females were characterized by dark pigmented coils of the oviduct; an oviduct that joins a ventral channel, which is dorsally open to the capsule gland; a single seminal receptacle, the duct of which directly joins the oviduct; and a large, hammer-shaped bursa copulatrix. All males had an awl-like penis bearing a pointed lobe on the left side. These characteristics are indicative of the genus *Ventrosia*. However, due to the cryptic character of many species belonging to

Table 2. Variables of water and sediment at the sampling stations (see also Kevrekidis et al. 2000, Mogias & Kevrekidis, unpubl. data), degrees (D) of contact of the sampling stations with the open sea (for explanation see text) and density (N, ind./m^2) of *Ventrosia maritima* at the sampling stations. Md, median diameter; Qd, quartile deviation.

Station	Water		Sediment				D	N
	Depth (cm)	Salinity (PSU)	Md (µm)	Qd	Silt – clay (%)	Organic matter (%)		
LC_1	45	32.2	133	0.40	10.10	1.37	5	0
LC_2	85	34.3	108	0.65	23.32	0.31	5	0
LC_3	45	33.6	176	0.45	8.81	0.81	5	0
LB	80	33.5	69	1.70	48.23	2.15	5	12.5
LA_1	10	35.1	82	0.75	33.02	1.40	4	5,900
LA_2	40	33.7	101	0.60	22.49	1.01	4	112.5
I_1	35	29.9	164	0.35	2.57	0.49	3	937.5
I_2	230	29.8	67	1.50	48.51	2.10	3	87.5
I_3	30	29.9	91	0.85	35.45	1.89	3	1,350
B_1	45	30.1	63	1.45	49.14	2.09	2	200
B_2	60	30.3	109	0.65	22.08	1.45	2	262.5
B_3	50	31.6	71	0.65	41.73	1.41	2	337.5
DA_1	30	15.6	133	0.35	9.60	0.74	1	2,337.5
DA_2	55	14.8	138	0.55	18.51	0.48	1	8,512.5
DC	25	15.9	183	0.45	6.96	0.70	1	4,337.5
DZ	40	17.0	129	0.80	22.71	0.77	1	12,187.5

the same mudsnail genus, an exact species determination is not possible solely based on anatomical data (see Wilke et al. 2002).

Phylogenetic analyses

The nucleotide diversity within the 11 specimens studied from the Evros Delta was relatively low with 0.0056 ± 0.0017 (= 0.56 ± 0.17 %), strongly indicating that only one species is involved. The cluster pattern in the phylogenetic tree (Fig. 2) shows that the mudsnail taxon from the Evros Delta belongs to the genus *Ventrosia* and that it is very closely related to the two nominal Black Sea species *V. pontieuxini* (Radoman 1973) and *V. maritima* (Milaschewitch 1916). It is, however, not associated with the common *V. ventrosa* (Montagu 1803), which occurs in large parts of the western and central Mediterranean Sea. As some of the nodes within the *Ventrosia*-clade in Fig. 2 are not well supported (i. e. <95 %), the monophyly of the above mentioned three taxa cannot be considered to be absolutely certain. Thus, we tested the alternative hypothesis of non-monophyly of these three taxa using parametric bootstrapping. The hypothesis of non-monophyly can be rejected at $p \leq 0.03$ and we

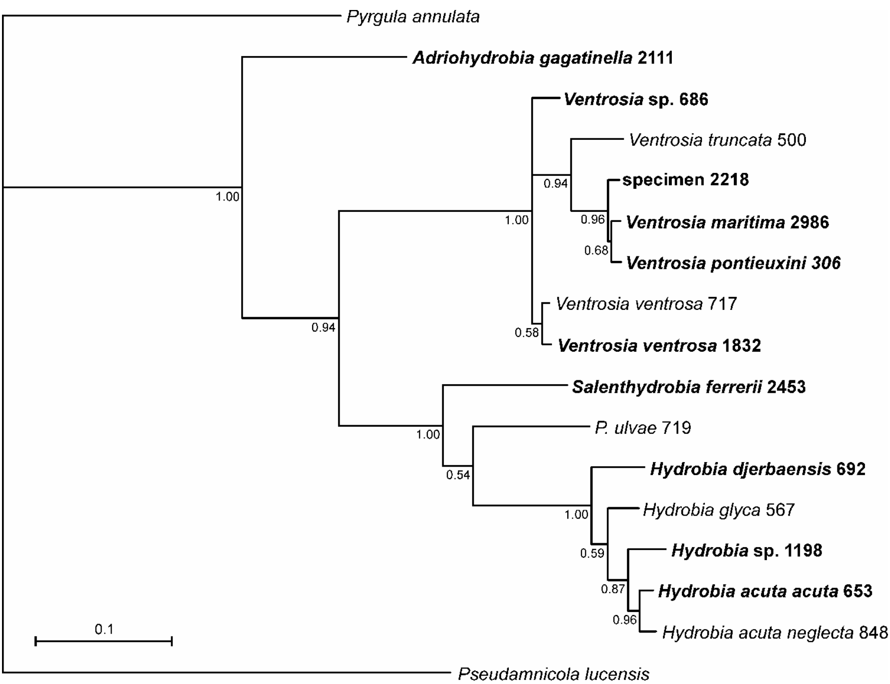

Fig. 2. Bayesian phylogram for taxa of the subfamily Hydrobiinae based on a fragment of the mitochondrial COI gene showing the 50% majority-rule consensus of topologies sampled during the Bayesian search. The scale bar indicates the expected number of substitutions according to the model of sequence evolution applied. Posterior probabilities are provided for each clade. Taxa from the Mediterranean and Black Sea are shown in bold. The taxon from the Evros Delta has the DNA isolation #2218.

therefore can safely assume that the taxon from the Evros Delta forms a monophyletic group together with *V. pontieuxini* and *V. maritima*.

The highest nucleotide divergence within the 11 Evros-specimens studied is with 0.0127 ± 0.0046 in the same range as the average nucleotide divergence among these three taxa (i.e. 0.0130 ± 0.0039 between the Evros specimens and *V. maritima*; 0.0146 ± 0.0044 between the Evros specimens and *V. pontieuxini*; 0.0111 ± 0.0041 between *V. maritima* and *V. pontieuxini*). Moreover, it should be noted that these values are well within the range that was previously established as "typical" intraspecific variation within taxa of the Hydrobiinae (e.g. Wilke & Davis 2000, Wilke et al. 2000). We therefore tentatively consider all three taxa to be conspecific. As the oldest available name for this species is *Ventrosia maritima* (Milaschewitch, 1916), we will use from here on this name for the mudsnail taxon from the Evros Delta.

Spatial variation of *Ventrosia maritima*

Ventrosia maritima in the 16 sampling stations showed a frequency of 81.3 %; it was missing only from stations LC_1, LC_2 and LC_3 (Table 2). The density of this species varied between 12.5 and 12,187.5 ind./m^2 (Table 2). The range of the environmental parameters in the stations, where the mudsnails were found, is the following: depth: 10–230 cm; water salinity near the bottom: 14.8–35.1 PSU; median diameter of the sediment (Md): 63–183 µm; silt-clay content of the sediment: 2.57–49.14 %, and sediment organic matter: 0.48–2.15 %.

The PCA detected two components that jointly account for 79.8 % of the variability in the original data. According to eigenvectors, contact with the sea and organic matter and silt-clay content of the sediment were the most important variables in the first component, while contact with the sea and salinity were the most important ones in the second component (Table 3). In addition, contact with the sea, silt-clay and organic matter were the only examined variables that showed a significant correlation, which was a negative one, with the first component ($\rho = -0.532$ $p < 0.05$, $\rho = -0.850$ $p < 0.01$ and $\rho = -0.817$ $p < 0.001$, respectively, $n = 16$) and contact with the sea and salinity with the second component ($\rho = -0.781$ $p < 0.001$, and $\rho = -0.721$ $p < 0.01$, respectively, $n = 16$). Density of *V. maritima* displayed a significant correlation, which was a positive one, only with the second component ($\rho = 0.528$, $p < 0.05$, $n = 16$), indicating that contact with the sea and salinity were the most important examined variables in affecting the species distribution: as both contact with the sea and salinity decreased, density increased.

The PCA defined a close relationship between stations LC_1, LC_2 and LC_3, stations LA_1 and LA_2, stations I_3, B_1, B_2 and B_3, and stations DA_1, DA_2, DC and DZ, while LB, I_1 and I_2 remained as independent stations (Fig. 3). The range of the environmental variables and the mean density (± standard error) of *V. maritima* in each of the afore-mentioned station groups or stations is given in Table 4. The mudsnails showed a significant spatial variation (Kruskal-Wallis one-way analysis of variance: $H = 12.748$, $n_1 = 3$, $n_2 = 1$, $n_3 = 2$, $n_4 = 1$, $n_5 = 1$, $n_6 = 4$, $n_7 = 4$, $p < 0.05$). *Ventrosia maritima* was not found, or only

Table 3. Component Weights in PCA.

Variables	Component 1	Component 2 (79.8 %)
Contact with the sea	0.371	0.638
Depth	−0.345	0.325
Salinity	−0.484	−0.482
Silt – clay	−0.499	0.399
Organic matter	−0.510	0.310

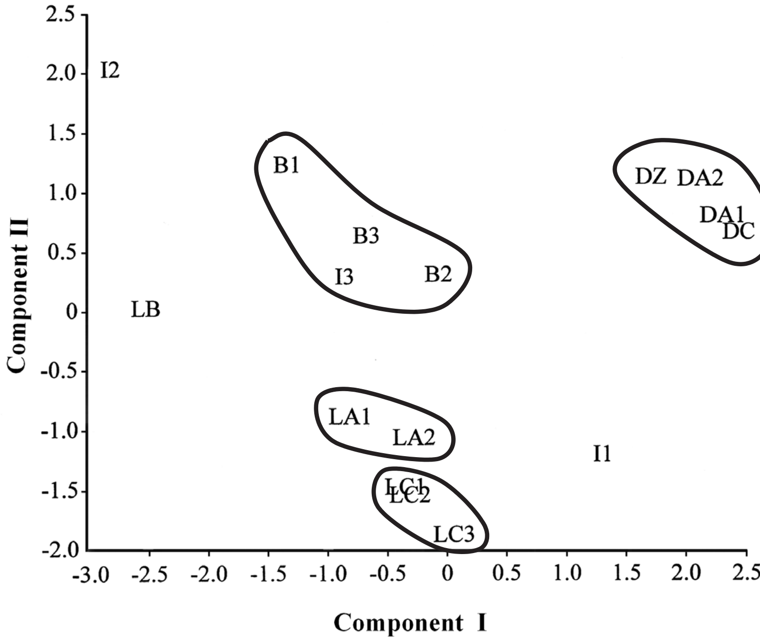

Fig. 3. The first two axes of Principal Component Analysis (PCA) conducted using the figures for environmental variables (contact with the sea, depth, salinity, silt-clay and organic matter content of the sediment). These components jointly account for 79.8 % of the variability in the original data.

Table 4. The range of environmental variables and density of *Ventrosia maritima* (N, mean ± standard error) in each station group. [a]: For explanation see text.

Station group	Contact with sea[a]	Depth (cm)	Salinity (PSU)	Md (µm)	Silt – clay (%)	Org. matter (%)	N (ind./m^2)
LC_1, LC_2, LC_3	5	45–85	32.2–34.3	108–176	8.81–23.32	0.31–1.37	0
LB	5	80	33.5	69	48.23	2.15	12.5
LA_1, LA_2	4	10–40	33.7–35.1	82–101	22.49–33.02	1.01–1.40	3,006.3±2,893.8
I_2	3	230	29.8	67	48.51	2.10	87.5
I_1	3	35	29.9	164	2.57	0.49	937.5
I_3, B_1, B_2, B_3	2, 3	30–60	29.9–31.6	63–109	22.08–49.14	1.41–2.09	537.5±272.3
DA_1, DA_2, DC, DZ	1	25–55	14.8–17.0	129–183	6.96–22.71	0.48–0.77	6,843.8±2,197.1

found in extremely low densities, in stations with the highest contact with the sea (stations LC_1–LC_3, LB) (Table 4). Density was also low at the site I_2, which is characterized by deep waters (Table 4); a black layer was observed on the surface of the sediments at station I_2. The mudsnails displayed a comparatively high density in the innermost station group LA_1–LA_2, where salinity was 34–35 PSU and the sediment very fine sand, with a 22.5–33.0 % silt-clay and 1.0–1.4 % organic matter content, and the highest one at the isolated station group DA_1–DZ, where salinity was lower (15–17 PSU) and the sediment was fine sand with a lower silt-clay and organic matter content (7.0–22.7 % and 0.5–0.8 %, respectively) (Fig. 1, Table 4).

Discussion

Ventrosia maritima (MILASCHEWITCH 1916) was the only mudsnail species observed in the Evros Delta Lagoons. The present findings represent the first record of the occurrence of this species in the Aegean Sea and, generally, in the Mediterranean Sea, since hitherto, *V. maritima* was only known from the Black Sea Region (GROSSU 1986). In addition, to our knowledge, published detailed ecological data for this taxon is essentially lacking.

The distribution pattern of *V. maritima* in the Evros Delta lagoons suggests that this gastropod is a typical lagoonal species. Similarly, *Ventrosia ventrosa* (MONTAGU 1803) and other hydrobiinids as well [e. g. *Hydrobia acuta acuta* (DRAPARNAUD 1805), *H. acuta neglecta* MUUS 1963], are considered to be typical inhabitants of lentic tideless brackish waters (e. g. MUUS 1967, FENCHEL 1975, GUELORGET & PERTHUISOT 1992, LARDICCI et al. 1997, BARNES 1999). *Ventrosia ventrosa*, which ranges from the Mediterranean Sea through the Iberian Peninsula, North Sea and Baltic Sea to Iceland and the White Sea (WILKE & DAVIS 2000), is usually found in non-tidal lagoons or lagoon-like habitats, in densities that can reach 150,000 ind./m^2, although intertidal marine or brackish water populations have also been recorded (e. g. CHERRILL & JAMES 1985, BARNES 1991, 1999, PROBST et al. 2000). *Ventrosia truncata* (VANETTA 1924), which is found along the Atlantic coast of North America, usually occurs on more or less protected intertidal soft-bottom habitats, but also on exposed ones (e. g. WELLS 1978, MANDRACCHIA & RUBER 1990, FORBES & LOPEZ 1990).

The distribution of *V. maritima* was found to be mainly affected by the contact with the sea and salinity. The taxon was essentially collected only at the innermost localities of the Evros Delta lagoons, displaying its highest densities at the most isolated ones, where low salinities (15–17 PSU) occurred. However, as *V. maritima* was found having a comparatively high density at a salinity of 35 PSU as well, and since salinity was linked to the contact with the open sea, we suggest that the later was the most important factor affecting the distribution of this species. More information is obviously needed to illustrate the role of salinity on the distribution of *V. maritima*. Similarly, as the preference of *V. ventrosa* for sheltered habitats is independent of salinity (see CHERRILL & JAMES 1985), WILKE & DAVIS (2000) suggested that salinity is probably not the limiting factor for *V. ventrosa* but other factors correlated with brackish habitats. Field data and laboratory experiments indicated that *V. ventrosa* tolerates salinities from 3 to 65 PSU (e. g. CHERRILL & JAMES 1985, DRAKE & ARIAS 1995, BERGER & GORBUSHIN 2001). Under laboratory conditions, *V. truncata* (as *Hydrobia totteni*), the North American sister species of *V. maritima*, was active in salinities ranging from 10 to 40 PSU, but was largely inactive in salinities from 0 to 5 PSU (WELLS 1978).

The extreme values of some environmental variables, such as seawater renewal rate and hydrodynamics, possibly produce a gradient along the lagoons and act as threshold to the seaward distribution of *V. maritima*. According to GUELORGET & PERTHUISOT (1992) "confinement", which represents the time of renewal with elements of marine origin (trace elements, vitamins, etc.) at a given location, primarily determines the biological zonation and species distribution in lagoonal ecosystems. They suggested that confinement contributes to the development of typical lagoonal species "for which there is maximal threshold beyond which one or several elements would act as poisons". Moreover, FORBES & LOPEZ (1990) suggested that sediment transport events, common in exposed habitats, negatively affect survival and time available for feeding in mudsnails.

Low oxygen availability, both in the water near the bottom and the surface sediment, was possibly responsible for the low density of *V. maritima* at the deepest sampling site (st. I_2). A black layer, resulting from the activity of sulphur bacteria, was observed in the surface of the sediments of this station. MUUS (1967) noted that black mud is avoided by hydrobiid species. Moreover, under laboratory conditions, LASSEN & KRISTENSEN (1978) observed that *V. ventrosa* was less tolerant to low sulphide concentrations/anoxia than other hydrobiinids [*H. acuta neglecta, Peringia ulvae* (PENNANT 1777)] and that for all these species the time to 50 % mortality decreased with increasing sulphide concentrations.

Ventrosia maritima was found in relatively high abundance on both sediment types, which occurred in the Evros Delta lagoons (fine sand and very fine sand with a higher silt-clay and organic matter content); its highest densities occurred on sediments with 7 to 33 % silt-clay and 0.5 to1.4 % organic matter content. Its congener *V. ventrosa* has been found in a wide variety of sediments (e. g. MUUS 1967, BICK & ZETTLER 1994, DRAKE & ARIAS 1995, GRUDEMO & JOHANNESSON 1999), and displayed its highest abundances on sediments with 9 to 74 % silt content at the Swedish West coast (GRUDEMO & JOHANNESSON 1999). The American *V. truncata* showed higher densities on sediments with 2 % silt-clay and 1.4 % organic content than on sediments with a higher or lower silt-clay and organic content (FORBES & LOPEZ 1990). Additionally, the body size of both these *Ventrosia* species was generally larger on fine-grained than on coarse-grained sediments, though growth was not faster on the former sediment type (FORBES & LOPEZ 1990, GRUDEMO & BOHLIN 2000).

KEVREKIDIS et al. (1996) previously found mudsnails (referred to as *Hydrobia acuta*) in the Evros Delta with a frequency of 88.6 % and abundance of 30 to 70,200 ind./m^2 in 35 sampling stations that covered the entire delta area. They were found in stations located from the open sea beach to the innermost, isolated localities of Evros Delta, in salinities varying from 2.5 to > 40 PSU and in sediments having a median diameter of 2 to 208 µm and organic matter content of 0.17 to 3.5 % (KEVREKIDIS et al. 1996). However, the highest densities were mainly found in isolated areas where salinity ranged from 28 to > 40 PSU and sediment was mainly muddy sand with an organic matter content of 0.7 to 2.3 % (KEVREKIDIS et al. 1996). Moreover, throughout the annual cycle, the mudsnails showed a much higher density in an isolated area of the Evros Delta (max. monthly density ~ 375,000 ind./m^2, sal. 24.5–36 PSU, temp. 3.4–26.9 °C, very fine sand, organic matter 0.86–2.54 %) than in a coastal one, which has immediate access to the sea (max. monthly density ~ 730 ind./m^2, sal. 4–25 PSU, temp. 4.3–24.5 °C, fine sand, organic matter 0.37–1.3 %) (GOUVIS et al. 1997, KEVREKIDIS 1997).

The data presented here strongly question the presence of *Hydrobia acuta* in the Evros Delta (see KEVREKIDIS et al. 1996, GOUVIS et al. 1997, KEVREKIDIS 1997). Acknowledging that mudsnail species belonging to different radiations, such as *Ventrosia* and *Hydrobia*, are frequently sympatric in the Mediterranean (WILKE et al. 2000), the previous assumption that *H. acuta* occurs in the Evros Delta was based on a nomenclatural confusion surrounding the two genera *Hydrobia* and *Ventrosia* (see GIUSTI et al. 1998). This problem was recently solved by the ruling of the International Commission of Zoological Nomenclature (see opinion 2034 of the ICZN from 30 June 2003).

In general, there is little information in the literature on the distribution and ecology of mudsnail species in the coastal brackish habitats of the Mediterranean Sea, which are characterized by a great variety and variability of the environmental conditions (e. g. GUELORGET & PERTHUISOT 1992). A fair amount of information only exists for the type species of the family Hydrobiidae, *Hydrobia acuta acuta* (e. g. MARAZANOF 1969, BRITTON 1985, KOUTSOUBAS et al. 2000). Thereby, more data on these subjects, along with confirmation of several records of mudsnail species in the Mediterranean Sea, are needed. The same applies to the Black Sea Region, where the occurrence of several mudsnail species has been reported (e. g. CHUKHCHIN 1978, GROSSU 1986, BEKMURZAYEV 1991, DZHURTUBAEV et al. 1992, MIKHAJLOVA 1992). However, according to our unpublished genetic data, only the presence of *V. maritima* can be confirmed so far.

Compared to the Mediterranean and Black Sea mudsnail taxa, the Atlantic species are studied much more extensively. In a poikilohaline lagoon system on the Atlantic coasts of SW Spain, *H. glyca* (as *H. minoricensis*) was most abundant in a submerged channel, a fact attributed to a lower tolerance to emersion for this species, *P. ulvae* was most abundant in the area with the greatest water renewal, while *V. ventrosa* did not show significant spatial differences (DRAKE & ARIAS 1995). The three north-west European mudsnail species (*P. ulvae, V. ventrosa, H. acuta neglecta*) are rarely sympatric in the macrotidal Atlantic and North Sea, where they are segregated between lagoons (*V. ventrosa, H. acuta neglecta*) and the marine/estuarine intertidal zone (*P. ulvae*), but they often coexist in the microtidal Baltic Sea, where, they display habitat selection with regard to salinity (or possibly to an intertidal-marine to nontidal-lagoonal gradient) (e. g. MUUS 1967, FENCHEL 1975, SIEGISMUND & HYLLEBERG 1987, BARNES 1991, 1999).

Given the general problem of species determination in mudsnail taxa outlined above, there is a real possibility that a number of taxa previously used in ecological studies are misidentified and that some of the contradicting ecological information given for mudsnail species is due to cases of mistaken identity. In this sense, we present here one of the first ecological studies of macrozoobenthos that uses mtDNA data to verify the systematic status of the species of interest. Utilizing molecular data we could:

A) show that our study object previously was very likely misidentified in related studies,
B) unambiguously link systematics, ecology and biogeography, and
C) lay the foundation for future comparative eco-genetic works.

The present study not only demonstrates the potential of combined ecological and genetic data, it also stresses the absolute need for the routine usage of molecular markers in ecological analyses involving frequently used but poorly understood invertebrate species.

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