The “Crustacean Seas” — an evolutionary perspective on the Ponto–Caspian peracarids

Melania E.A. Cristescu and Paul D.N. Hebert

Abstract: A spectacular adaptive radiation of crustaceans has occurred in the Black, Caspian, and Aral seas. This study tests several evolutionary scenarios based on the extent of genetic differentiation and the phylogenetic relationships among endemic mysids and gammarid amphipods from the Black and Caspian seas. Molecular phylogenies for these taxa were based on two mitochondrial genes: cytochrome c oxidase subunit I and the large ribosomal RNA subunit (16S), and one nuclear gene, the large ribosomal RNA subunit (28S). The results support the monophyly of the Ponto–Caspian gammarids (genera Dikerogammarus, Echinogammarus, Obesogammarus, and Pontogammarus), suggesting their origin from one colonization event. By contrast, several colonization events preceded the radiation of the Ponto–Caspian mysids (genera Limnomysis and Paramysis). Levels of intraspecific divergence were variable, with mysids showing either no geographic structure or deep genetic splits reflecting a long history of reproductive isolation between populations in marine settings and those in fresh waters. These findings suggest that the diversity of the Ponto–Caspian crustaceans has been underestimated and that species regarded as euryhaline are often composed of distinct evolutionary groups whose taxonomic status should be reevaluated.

Résumé : Il s’est produit une extraordinaire radiation des crustacés dans la mer Noire, la mer Caspienne et la mer d’Aral. Nous évaluons plusieurs scénarios évolutifs basés sur l’étendue de la différentiation génétique et les relations phylogénétiques chez les mysidés et les amphipodes gammaridés endémiques des mers Noire et Caspienne. Les phylogénies moléculaires de ces taxons se basent sur deux gènes mitochondriaux, la sous-unité I de la cytochrome c oxydase et la grande sous-unité d’ARN ribosomique (16S), ainsi qu’un gène nucléaire, la grande sous-unité d’ARN ribosomique (28S). Nos résultats appuient l’hypothèse de la monophylie des gammaridés Ponto–Caspiens (genres Dikerogammarus, Echinogammarus, Obesogammarus et Pontogammarus), ce qui laisse croire qu’ils sont issus d’un même épisode de colonisation. En revanche, plusieurs épisodes de colonisation ont précédé la radiation des mysidés Ponto–Caspiens (genres Limnomysis et Paramysis). Les niveaux de divergence interspécifique sont variables : chez les mysidés, ou bien il n’y a pas de structure géographique, ou alors il y a d’importantes divergences génétiques qui reflètent une longue période d’isolement génétique entre les populations des milieux marins et celles d’eau douce. Ces résultats indiquent que la diversité des crustacés ponto-caspiens a été sous-estimée et que les espèces considérées comme euryhalines sont souvent composées de groupes évolutifs distincts dont le statut taxonomique a besoin d’être réévalué.

Introduction

Many groups of organisms including fishes, mollusks, turbellarians, and crustaceans have radiated in the Ponto–Caspian biogeographic region, which includes the Black, Azov, Caspian, and Aral seas. However, the endemism in varied crustacean lineages (e.g., cladocerans, copepods, cumaceans, decapods, amphipods, and mysids) is significantly higher than in most other animal groups. For example, more than 70 endemic amphipods and approximately 28 mysid species have been described from the Ponto–Caspian region (Cărăuşu 1943; Băcescu 1954; Barnard and Barnard 1983). In fact, the Ponto–Caspian mysid fauna is the most diverse fresh–brackish water mysid fauna in the world (Băcescu 1954; Mauchline 1980). Since most of these endemics are confined to the Caspian Sea, this region has been considered the center for their radiation (Zenkevitch 1963). As a result of this diversity, the Caspian Sea has been nicknamed the “Crustacean Sea”. However, its sibling seas, the Black and Azov seas, also support a substantial number of endemics in their less saline estuaries, peripheral lakes, and rivers. In fact, almost 50% of Ponto–Caspian taxa are shared between these basins and their evolutionary origin is particularly ambiguous.

The four basins in the Ponto–Caspian region formed some 6 million years (Mys) ago following disintegration of the Paratethys Sea, an arm of the more extensive Tethys Sea (Dumont 1998). The shared occurrence of species in the Black and Caspian seas could be explained by either these late Miocene – early Pliocene vicariance events that sepa-
rated the basins or more recent (late Pleistocene) dispersal from the Caspian Sea into the Black Sea. This matter is unresolved. Several authors have suggested that most Ponto–Caspian lineages originated long before the present basin configuration, possibly during the late Miocene (Băcescu 1940; Barnard and Barnard 1983; Dumont 2000), while others argued for a more recent Pliocene or Pleistocene origin (Mordukhai-Boltovskoi 1964; Grigorovich et al. 2003).

The Ponto–Caspian mysids have been partitioned into nine genera: Acanthomysis, Caspiomysis, Diamysis, Hemimysis, Katamysis, Limnomysis, Mysis, Paramysis, and Schistomysis (Băcescu 1940, 1954). Väinölä (1995) showed that the pelagic flock of Caspian Mysis (Mysis microphthalma, Mysis amblyops, and Mysis caspia) reflects a recent intralacustrine radiation, but their relationships with the other endemic mysid genera have not been investigated. The present study addresses this gap in knowledge by examining the genera Paramysis and Limnomysis. Not only is Paramysis the most speciose of the nine Ponto–Caspian mysid genera, but it also contains species such as Paramysis kroyeri, which occurs in salinities ranging from fresh–brackish coastal lakes (salinity < 4‰) to the intertidal and subtidal zones of the Black Sea (salinity = 18‰).

The Ponto–Caspian amphipods belong largely to the families Gammaridae, Pontogammaridae, and Corophiidae, which are each represented by several speciose genera (Cărauşu 1943; Barnard and Barnard 1983; Martin and Davis 2001). The morphological radiation of Ponto–Caspian amphipods has been linked to their fossorial adaptations because most species are burrowers. Although a general evolutionary trend towards increased specialization for burrowing is evident, the phylogenetic affinities among Ponto–Caspian gammarids are unclear. It has been suggested that the nonfossorial genus Echinogammarus is primitive because its gnathopods resemble those of the Gammarus group (Barnard and Barnard 1983). Dikerogammarus is thought to be derived from the Ponto–Caspian Echinogammarus lineage, since its first gnathopod lacks the palmar spination typical of Echinogammarus while retaining the Gammarus type of first antenna (Barnard and Barnard 1983). Other genera such as Obesogammarus and Pontogammarus share additional derived characters such as setae on the bases of periopods 5–7 and setae on the posterior margins of periopods 3 and 4. While Obesogammarus has the primitive condition of setal tufts, Pontogammarus has a single row of coalesced setae. Within Pontogammarus, a further subdivision has been proposed by Karaman and Barnard (1997), who suggested the removal of Pontogammarus maeoticus from the genus because of the presence of a plesiomorphic longer inner ramus on uropod 3 establishing the genus Euxinia. We employ the original nomenclature. Although generally accepted, the morphological phylogeny for this group is based on shared derived states (synapomorphies). It seems likely that the assemblage of primitive features and shared derived characters has been altered by convergent evolution. Furthermore, most diagnosable characters are affected by phenotypic plasticity.

Despite more than a century of taxonomic investigations, the mechanisms of speciation along with the factors responsible for the speciation of the Ponto–Caspian crustaceans remain controversial. There is, for example, no information on whether species flocks descended from a single ancestor or arose through the recurrent colonization of each basin. Similarly, the timing of speciation events is poorly understood.

The present study examines the extent of genetic differentiation and phylogenetic relationship among six species of mysids and eight species of gammarid amphipods from the Black and Caspian seas. Additionally, taking these crustaceans as an example, this study explores the tempo and the mode of speciation responsible for the high species richness in the Ponto–Caspian area.

Materials and methods

Taxon sampling

Fourteen Ponto–Caspian crustacean species, including six mysids and eight amphipods, were analyzed in this study (Table 1). Samples were collected in 1999–2001 from brackish lagoons and estuaries, brackish inland seas, and freshwater ponds and lakes along the northwestern Black Sea coast and in the Caspian Sea (Fig. 1). The samples were sorted and preserved in 90% ethanol shortly after collection. The amphipod Gammarus lacustris and the mysids Tenagonyx australis and Mysis relicta were used as outgroups in the first phase of phylogenetic analyses. To test the monophyly of the Ponto–Caspian gammaroids, we included 16 additional taxa belonging to four gammaridean families (Acanthogammaridae, Gammaridae, Hyalellidae, and Cranogonyctidae). These more inclusive phylogenies were rooted using the isopod Glyptidotea lichtensteini. Most amphipod taxa were represented by several populations in the phylogenetic analyses of the cytochrome c oxidase subunit I (COI) gene, with samples from both the Black and Caspian seas. A phylogeographic survey was also conducted for the mysids Limnomysis benedeni and Paramysis kroyeri at sites within the Black Sea region.

DNA extraction, amplification, and sequencing

Phylogenies were constructed using two mitochondrial genes: COI and 16S ribosomal RNA (tRNA), and one nuclear gene, 28S rRNA. The primer pairs LCOI490 (5′-GGTTCAAAAAATCAAATGATTTAG-3′) and HC02198 (5′-TAAACCTTCAGGGTACCCAAAAATTACA-3′) (Folmer et al. 1994), 16Sr (5′-GCGCTGTTTATCAAAAAACAT-3′) and 16Sbr (5′-CCGGTCTGAACTCACTACG-3′) (Palumbi 1996), and 28Sa (5′-TTGGGACCCCGAATTTGAAGCAT-3′) and 28Sb (5′-GCTGAGGGAAAACTTGGAGGGAAC-3′) (Taylor et al. 1999) were used to amplify a 658-base pair (bp) fragment of the COI gene, with samples from both the Black and Caspian seas. A phylogeographic survey was also conducted for the mysids Limnomysis benedeni and Paramysis kroyeri at sites within the Black Sea region.

Each polymerase reaction (PCR) had a total volume of 50 μL and contained 1–2 μL of DNA template, 5 μL of 10× PCR buffer, 2.0 mmol/L MgCl2, 0.2 mmol/L each dNTP, 5 pmol of each primer, and 1 unit of Taq DNA polymerase. The PCR conditions for COI consisted of one cycle of 94 °C (60 s) followed by five cycles of 94 °C (60 s), 45 °C (90 s), and 72 °C (60 s), 35 cycles of 94 °C (60 s), 51 °C (90 s), 72 °C (60 s), and 72 °C (3 min). The PCR conditions for 16S involved two cycles of 94 °C (30 s), 60 °C (45 s), and 72 °C (45 s) and five cycles of 93 °C (30 s),
Table 1. Species included in the molecular analyses, their geographic distribution, site code, site description, collection date, and GenBank accession Nos.

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<td>NE</td>
<td>Finnish Lakes (Väinölä et al. 2001)</td>
<td></td>
<td>AY061796&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gammaracanthus lacustris</td>
<td>Caspian Sea</td>
<td>C</td>
<td>Central Caspian Sea (Väinölä et al. 2001)</td>
<td></td>
<td>AY061797&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gammaracanthus caspius</td>
<td>Circumarctic</td>
<td>NE</td>
<td>Western White Sea (Väinölä et al. 2001)</td>
<td></td>
<td>AY061798&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gammaracanthus aestuariarum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyalellidae</td>
<td>North America</td>
<td>NA</td>
<td>Witt et al. 2003</td>
<td></td>
<td>AY152807&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyalella montezuma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyalella sp.</td>
<td>North America</td>
<td>NA</td>
<td>Witt et al. 2003</td>
<td></td>
<td>AY152783&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyalella sp.</td>
<td>North America</td>
<td>NA</td>
<td>Witt et al. 2003</td>
<td></td>
<td>AY152752&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crangonyctidae</td>
<td>North America</td>
<td>NA</td>
<td>Witt et al. 2003</td>
<td></td>
<td>AY152752&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crangonyx sp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idoteidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyptidotea lichtensteini</td>
<td></td>
<td></td>
<td>Wetzer 2001</td>
<td></td>
<td>AF255781&lt;sup&gt;k&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Classification after Bousfield (1977), Mauchline (1980), and Martin and Davis (2001).
<sup>b</sup>B, Black Sea; BA, Baltic Sea; C, Caspian Sea; BL, Baikal Lake; NE, north Europe; NA, North America.
<sup>c</sup> 16S for mysids and 28S for amphipods.
<sup>d</sup> GenBank accession Nos. AY529017–AY529073 represent taxa sequenced during this project.
<sup>e</sup> GenBank accession Nos. AF052393–AF052394 correspond to specimens to S. Jarman, S. Nicol, N. Elliott, and A. McMinn (Institute of Antarctic and Southern Ocean Studies, University of Tasmania, G.P.O. Box 252-77, Hobart, TAS 7001, Australia, unpublished data).
<sup>f</sup> Genbank accession Nos. AY189478–AY189504 correspond to specimens in Cristescu et al. (2003).
<sup>g</sup> Genbank accession Nos. AY326120–AY326126 correspond to specimens in Cristescu et al. (2004).
<sup>h</sup> GenBank accession No. AF448520 corresponds to specimen in J.E. Ironside, A.M. Dunn, D. Rollinson, and J.E. Smith (Biological Sciences, University of Leeds, Miall Building, Leeds, West Yorkshire LS2 9JT, UK, unpublished data).
<sup>i</sup> GenBank accession Nos. AY061796–AY061801 correspond to specimens in Väinölä et al. (2001).
<sup>j</sup> Genbank accession Nos. AY152752–AY152807 correspond to specimens in Witt et al. (2003).
<sup>k</sup> Genbank accession No. AF255781 corresponds to specimen in Wetzer (2001).
55 °C (45 s), and 72 °C (45 s) followed by 29 cycles of 93 °C (30 s), 50 °C (1 min), and 72 °C (3 min). The temperature profiles for 28S consisted of 35 cycles at 93 °C (30 s), 50 °C (30 s), and 72 °C (1 min). Amplified PCR products were gel purified (2% agarose) using the Qiaex kit (QIAGEN Inc., Valencia, Calif.) and sequenced in one or both directions (for the 28S fragment, both strands were sequenced) using an ABI 377 automated sequencer (ABI, Foster City, California) and the Big Dye terminator 3 sequencing kit (ABI). All sequences obtained during this study

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Table 2. Number of sites available (TS), variable sites (VS), cladistically informative sites (IS), base frequencies, transition to transversion ratio (Ti/Tv), and $\chi^2$ test of homogeneity of base frequencies across ingroup taxa for each data set and each partition.

<table>
<thead>
<tr>
<th>Data set</th>
<th>Best fit model</th>
<th>TS</th>
<th>VS</th>
<th>IS</th>
<th>A</th>
<th>C</th>
<th>G</th>
<th>T</th>
<th>Ti/Tv</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphiopoda COI</td>
<td>TVM + I + G</td>
<td>643</td>
<td>297</td>
<td>284</td>
<td>25</td>
<td>21</td>
<td>18</td>
<td>36</td>
<td>1.3</td>
<td>64.36</td>
</tr>
<tr>
<td>COI + 28S</td>
<td>GTR + I + G</td>
<td>2034</td>
<td>451</td>
<td>294</td>
<td>24</td>
<td>23</td>
<td>27</td>
<td>26</td>
<td>1.5</td>
<td>6.20</td>
</tr>
<tr>
<td>Mysidacea COI</td>
<td>TVM + I + G</td>
<td>633</td>
<td>354</td>
<td>291</td>
<td>22</td>
<td>18</td>
<td>24</td>
<td>36</td>
<td>1.1</td>
<td>235.66</td>
</tr>
<tr>
<td>COI + 16S</td>
<td>HKI + I + G</td>
<td>1121</td>
<td>527</td>
<td>339</td>
<td>25</td>
<td>17</td>
<td>23</td>
<td>35</td>
<td>1.1</td>
<td>85.34</td>
</tr>
</tbody>
</table>

Note: COI, cytochrome c oxidase subunit I; 28S and 16S, ribosomal RNA subunits; TMV, transversional model; I, invariable sites; G, gamma; GTR, general time-reversible model; HKY, Hasegawa–Kishino–Yano model.

have been deposited in GenBank under accession Nos. AY529017–AY529073 (Table 1).

Phylogenetic analyses

Sequences were aligned using the SeqApp 1.9 sequence editor and Sequencher (Gene Codes Corporation, Ann Arbor, Michigan). The estimates of sequence divergence in the amphipod data set were corrected using Tamura and Nei’s (1993) method. LogDet distances (Lake 1994; Gu and Li 1996) were used to correct for inequalities of base composition in the COI amino acid sequences. Maximum likelihood (ML) and neighbor joining (NJ) methods were employed using a branch-and-bound or a heuristic search algorithm with 100 replicates, with sequences added at random and tree bisection–reconnection branch swapping. The stability of phylogenetic hypotheses was assessed using the goodness-of-fit ($\chi^2$) test implemented in PAUP*.

Results

Patterns of divergence

Pairwise sequence divergences were highly variable in both data sets and for all three genes. As expected, higher divergences were found for the fast-evolving COI gene than for the rRNA genes. For amphipods, Tamura–Nei nucleotide distances at COI ranged from 17% to 28% for comparisons between species, while divergences between “conspecific” populations from the Black and Caspian seas ranged from 2% to 7%. The mysids showed even higher divergences at COI with distances ranging from 19% to 49% (the lower values reflected intrageneric comparisons). Despite the very high nucleotide distances at COI in Paramysis, Poisson-correlated amino acid distances ranged from just 0% to 2%. Base frequencies were unequal, with the mitochondrial genes possessing a higher A–T content than the nuclear gene (Table 2). Base composition for the COI gene was homogeneous across the amphipod taxa ($p = 0.99$) but was very heterogeneous among the mysids ($p < 0.001$) (Table 2). Among the three genes analyzed, the nuclear 28S gene had the highest transition to transversion ratio (1.6). The results of the partition homogeneity tests were not significant, as $p$ values were greater than 0.05 in both groups (amphipods: $p = 0.48$; mysids: $p = 0.45$), suggesting congruence in phylogenetic signal among the genes. However, we analyzed the data sets both separately and in combination (Eernisse and Kluge 1993), enabling separate analysis for COI with a more extensive data set. The second round of analyses included a combination of COI and 16S for the mysids and a combination of COI and 28S for the amphipods. To test the monophyly of the Ponto–Caspian gammaroids, we introduced multiple outgroups in a third round of analyses.

Phylogenetic analyses

Mysids

The parsimony analyses of the COI data resulted in four equally parsimonious trees (tree length = 1066, consistency index = 0.55, homoplasy index = 0.45, retention index = 0.81). The topology of the strict consensus MP tree was similar to that of the NJ tree. The tree estimated in the Bayesian phylogenetic analyses differed from the MP and NJ phylo-
genies solely in the placement of *Mysis relicta*. By contrast, *Mysis relicta* was consistently placed as a sister group to *Paramysis* in all phylogenetic reconstructions based on only first and second codon positions (trees not shown). All analyses unambiguously supported the monophyly of the genus *Paramysis* and the affinity between *Mesopodopsis slabberi*, *Neomysis integer*, and *L. benedeni* (Fig. 2). Likewise, ML, BA, MP, and NJ analyses of the combined data set supported the monophyly of the five *Paramysis* species (Fig. 3). The rest of the genera clustered together with *L. benedeni* as sister taxon to the *Mesopodopsis slabberi* and *N. integer* group. Despite inconsistencies in the position of outgroup taxa, neither the data sets nor algorithms supported the monophyly of the Ponto–Caspian mysids with respect to the non-Ponto–Caspian taxa.

**Amphipods**

The strict consensus tree of the five most parsimonious trees (tree length = 900, consistency index = 0.51, homoplasy index = 0.49, retention index = 0.82) for the COI gene had a topology congruent with the trees estimated in both the Bayesian and NJ analyses (Figs. 4 and 5). All phylogenetic reconstructions supported the monophyly of the family Pontogammaridae as well as the monophyly of each of its genera (*Pontogammarus*, *Obesogammarus*, and *Dikerogammarus*). As predicted by morphologically based phylogenies, the genus *Echinogammarus* occupied a basal position. Within the family Pontogammaridae, *Dikerogammarus* was a sister taxon to the cluster formed by *Obesogammarus* and *Pontogammarus*. However, when the mitochondrial (COI) and nuclear (28S) genes were combined, the topology of the trees changed slightly with respect to the position of the genus *Dikerogammarus*, although statistical support for varying arrangements (bootstrap and Bayesian posterior probabilities) was weak. Additional phylogenetic analyses of the COI gene included multiple outgroups from four gammaroid families. The relationship between the ingroup taxa did not change in the BA and NJ analyses and there

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**Fig. 2.** (a) Strict consensus of 19 000 trees estimated in the Bayesian phylogenetic analysis (BA) for the cytochrome *c* oxidase subunit I (COI) gene for mysids. The numbers above the nodes indicate the Bayesian posterior probabilities. (b) Strict consensus of the four most parsimonious trees (tree length = 1066, consistency index = 0.55, homoplasy index = 0.45, retention index = 0.81) obtained using the maximum parsimony (MP) criterion for the COI gene. The numbers above the nodes indicate MP bootstrap support (1000 replicates), and the numbers below the nodes indicate neighbor-joining (NJ) bootstrap percentages (1000 replicates) for the nodes supported by both methods.
was increased statistical support for most of the clades with strong support for the monophyly of the Ponto–Caspian gammaroids, including the genus *Echinogammarus* (Fig. 6).

**Discussion**

**Phylogenetic inferences**

**Ponto–Caspian mysids**

All phylogenetic reconstructions in this study confirmed the monophyly of the genus *Paramysis*. However, these analyses did not fully resolve phylogenetic relationships among its component taxa (*Paramysis baeri*, *Paramysis kessleri*, *Paramysis kroyeri*, and *Paramysis intermedia*). Moreover, at a generic level, no single, completely resolved topology was favored by all analytical approaches. Certainly, MP and BA methods were not decisive owing to the low statistical support for deep nodes and the instability of the position of key taxa such as *Mysis relicta*. Although the relationship between the Ponto–Caspian genera *Limnomysis* and *Paramysis* and the related mysids (*Mysis relicta*, *N. integer*, and *Meso- podopsis slabberi*) was not resolved, the paraphyly of the Ponto–Caspian mysids (genera *Limnomysis* and *Paramysis*) was apparent, suggesting that there have been multiple invasions and radiations in the region.

The high COI divergences of 19%–27% within the *Paramysis* flock contrast with the very low allozyme distances (0.06) found by Väinölä (1995) for members of the Caspian *Mysis* species flock. In fact, a surprisingly high level of sequence divergence (24%) was found between two adjacent brackish (B11) – marine (B4) populations of *Paramysis kroyeri*. This value is higher than the mean COI divergence value of 11.3% among congeneric animal species (Hebert et al. 2003) and far higher than the mean intraspecific divergence of 1%–2% often recorded in the phylogeographic literature (Avise 2000).

**Ponto–Caspian gammaroids**

This study supports the monophyly of the Ponto–Caspian gammaroids (genera *Dikerogammarus*, *Echinogammarus*, *Obesogammarus*, and *Pontogammarus*) as well as the monophyly of each individual genus. In addition, this study confirms the monophyly of the family Pontogammaridae while refuting the monophyly of the family Gammaridae. Since all reconstruction methods and all data sets support a close phylogenetic relationship (sister groups) between members of the family Pontogammaridae and the two *Echinogammarus* species, the assignment of the Ponto–Caspian *Echinogammarus* to the family Pontogammaridae warrants consideration. Furthermore, the genus *Pontogammarus* is paraphyletic when *Pontogammarus* (*Euxinia*) *maeoticus* is treated as a member of the genus *Euxinia*, indicating that its assignment to a new genus is not supported by molecular data. Barnard and Barnard (1983) suggested that the Ponto–Caspian gammaroids originated from a fresh–brackish water *Gammarus*-type ancestor and that the evolutionary path fol-
Fig. 4. (a) Strict consensus of 9400 trees estimated in the Bayesian phylogenetic analysis (BA) for the cytochrome c oxidase subunit I (COI) gene for amphipods. The numbers above the nodes indicate the Bayesian posterior probabilities followed by the neighbor-joining (NJ) bootstrap percentages (1000 replicates) for the nodes supported by both methods. (b) Strict consensus of the five most parsimonious trees (tree length = 900, consistency index = 0.51, homoplasy index = 0.49, retention index = 0.82) obtained using the maximum parsimony (MP) criterion of the COI gene. The numbers above the nodes indicate bootstrap support greater than 50% (1000 replicates).

Fig. 5. (a) Maximum likelihood (ML) phylogram (–ln likelihood = 7616.56) for the total evidence (cytochrome c oxidase subunit I (COI) and 28S genes) for amphipods. The numbers above the nodes indicate bootstrap support (100 replicates) followed by the Bayesian posterior probabilities. (b) The most parsimonious tree (tree length = 1086, consistency index = 0.59, homoplasy index = 0.41, retention index = 0.51) obtained using the maximum parsimony (MP) criterion of the total evidence (COI and 28S). The numbers above the nodes indicate MP bootstrap support followed by neighbor-joining (NJ) bootstrap percentages for the nodes supported by both methods (1000 replicates). BA, Bayesian phylogenetic analysis.
The Ponto–Caspian gammarids derived from an Echinogammarus-type ancestor. Certainly, the Baikalian group represented in our phylogeny by the genera Acanthogammarus and Eulimnogammarus has no direct evolutionary link with the Ponto–Caspian gammarids. Recent studies of sequence diversity in the nuclear 18S gene in Baikalian amphipods have revealed the presence of two main clades (Sherbakov et al. 1998). One consists of benthic, mostly “unarmed” taxa, while the other includes predominantly taxa with more ornamental complexity (Sherbakov et al. 1998; Sherbakov 1999). More relevant to the present study is the old age of the Baikalian generic lineages, which based on the COI calibration for the Caribbean crabs (Schubart et al. 1998) appear to be as old as the lake itself (approximately 20–30 Mys; Sherbakov et al. 1998; Sherbakov 1999). Similarly, our estimates based on the snapping shrimp COI clock (Knowlton et al. 1993; Knowlton and Weigt 1998) suggest that the age of the Ponto–Caspian lineages is remarkably deep (approximately 8–16 Mys). It is likely that these lineages arose shortly after the origin of the basin (Paratethys and Sarmatian basins).

A consistent and marked genetic subdivision was detected at the “subspecific” level between populations of amphipods from the Black and Caspian seas reinforcing the role of geographic isolation and local refugia in lineage survival and evolution (Cristescu et al. 2003). For example, the level of COI divergence between populations of five amphipods (Pontogammarus maeoticus, Pontogammarus robustoides, Pontogammarus obesus, Obesogammarus crassus, and Echinogammarus ischnus) from the two basins varied from 2.3% to 11%, values that are significantly higher than those typical of intraspecific divergence (Avise 2000).

**Geological and molecular reconciliation: age and sequence of invasions**

When comparing the timing estimated by molecular results with geological evidence, we found that several cladogenesis events among amphipods and mysids appear concurrently and match various geological events. However,
these molecular clock estimates must be treated with the caution always accorded to these types of best estimates. Nevertheless, the COI divergences in this study support the conclusion that members of most peracarid species flocks diversified well in advance of the Pleistocene. While the close evolutionary relationship between members of the Caspian *Mysis* flock points to a recent (middle or late Pleistocene) intralacustrine origin (Văinălă 1995), and the extent of genetic divergence among the *Mysis relicta* species complex indicates a Pliocene radiation (Văinălă 1986; Văinălă et al. 1994; Dooh 2003), our COI data support a middle–late Miocene radiation for the *Paramysis* flock. Furthermore, it appears that the gammarid group radiated almost simultaneously. The timing provided by the COI clock corresponds to the extensive, brackish Paratethys and Sarmatian basins (16–10 and 10.5–8 Mys, respectively). These radiations have spanned a broad stretch of time, with estimates ranging from Miocene at the generic level to late Miocene throughout Pliocene at the congeneric level.

It appears that two major and contrasting evolutionary forces acted upon the gene pool of most Ponto–Caspian crustacean lineages. On one side, we observe an arresting morphological diversity between most members of species flocks (e.g., pontogammarids, paramysids, mysids, and onychopods), which is not necessarily associated with high genetic divergences, and on the other side, we detect sharp genetic subdivisions between morphologically uniform populations that inhabit ecologically distinctive habitats (Cristescu et al. 2003). A reassessment of the taxonomic status of the latter “species” is needed for a more inclusive view about the key evolutionary and ecological factors responsible for the plethora of endemics in the Ponto–Caspian region.

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**References**


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