WALTER AND ANDRÉE DE NOTTBECK FOUNDATION
SCIENTIFIC REPORTS

No. 28

Diversity and zoogeography of continental mysid crustaceans

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Academic dissertation in Ecology and Evolutionary Biology,
to be presented, with the permission of
the Faculty of Biosciences of the University of Helsinki,
for public criticism in the Auditorium 1041,
Biocenter 2, University of Helsinki, Viikinkaari 5, Helsinki,
on January 27th, 2006, at 12 noon.

HELSINKI 2006

This thesis is based on the following papers, which are referred to by their Roman numerals:

I Väinölä, R., Audzijonytė, A. & Riddoch, B. J. 2002: Morphometric discrimination among four species of the *Mysis relicta* group. – Arch. Hydrob. 155: 493–515.

II Audzijonytė, A. & Väinölä, R. 2005: Diversity and distributions of circumpolar fresh- and brackish-water *Mysis* (Crustacea: Mysida): descriptions of *M. relicta* Lovén, 1862, *M. salemaai* n. sp., *M. segerstralei* n. sp. and *M. diluviana* n. sp., based on molecular and morphological characters. – Hydrobiologia 544: 89–141.

III Audzijonytė, A., Damgaard, J., Varvio, S.-L., Vainio, J. K. & Väinölä, R. 2005: Phylogeny of *Mysis* (Crustacea, Mysida): history of continental invasions inferred from molecular and morphological data. – Cladistics 21: 575–596.

IV Audzijonytė, A. & Väinölä, R. 2005: Phylogeographic analyses of a circum-arctic coastal and of a boreal lacustrine mysid crustacean, and evidence of fast post-glacial mtDNA rates. – (submitted manuscript).

V Audzijonytė, A., Pahlberg, J., Väinölä, R. & Lindström, M. 2005: Spectral sensitivity differences in two *Mysis* sibling species (Crustacea, Mysida): adaptation or phylogenetic constraints? – J. Exp. Mar. Biol. Ecol. 325: 228–239.

VI Audzijonytė, A., Daneliya, M. E. & Väinölä, R. 2005: Comparative phylogeography of Ponto-Caspian mysid crustaceans shows isolation and exchange among dynamic inland sea basins. – (submitted manuscript).

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Audzijonytė, A. 2006: Diversity and zoogeography of continental mysid crustaceans. – W. & A. de Nottbeck Foundation Sci. Rep. 28: 1–46. ISBN 951-98521-9-0 (paperback); ISBN 952-10-2871-8 (PDF).

Mysid crustaceans are predominantly marine, but there are two important continental exceptions: (i) the descendants of Arctic marine *Mysis* in circumboreal lakes and the Baltic Sea ('glacial relicts' of the *Mysis relicta* group) and in the Caspian Sea, and (ii) autochthonous Ponto-Caspian mysids, with about 20 species in the Black, Azov and Caspian seas. The origin and zoogeographical history of these continental taxa have been subject to much controversy. This thesis applied morphological, molecular and physiological data to analyse the evolution of the continental mysid elements and the importance of various factors that have generated and maintained their diversity at different temporal and spatial scales and at different systematic levels.

The taxonomic part of the study explored the morphological differences among four species of the *Mysis relicta* group, earlier only identified on molecular grounds. Formal taxonomic descriptions of three new species and *M. relicta s. str.,* based on both molecular and morphological characters, were presented. A systematic and distributional analysis suggested that different species have colonised continental waters at different times. The stenohaline European *M. relicta* and the North American *M. diluviana* have probably lived in lakes of the two continents through most of the Pleistocene. The more euryhaline European *M. salemaai* and the circumarctic coastal *M. segerstralei* are more closely related and have penetrated fresh waters later.

The phylogeny of the genus *Mysis* was assessed in a simultaneous analysis of seven molecular and morphological characters sets. The analysis supported the monophyly of the *Mysis relicta* group, of the four Caspian *Mysis* endemics, and of these two continental groups together. The diversification of the *M. relicta* group appears much older than speciation of the morphologically diverse endemic Caspian Sea *Mysis*.

Analyses of mitochondrial DNA variation within each of *M. salemaai* and *M. segerstralei* revealed little large-scale phylogeographic structure, except for a local Beringian lineage in the latter species. Overall, the data suggested efficient (late)glacial long-distance gene flow in the supposedly weakly-dispersing crustaceans, across NW Europe and the Arctic basin, respectively; past exchange of mitochondria between the two species has also been identified. In the Ponto-Caspian autochthonous mysids, similar Late-Pleistocene dispersals across the Caspian, Azov and Black seas were inferred from mtDNA data of three species, while two further species showed distinct inter-basin structuring. Ecological characteristics of the individual species, such as salinity tolerance and vagility, seem to have controlled their responses to paleoenvironmental conditions and ability to disperse along the transient Pleistocene connections among the Ponto-Caspian basins.

Comparisons between post-glacially isolated *Mysis* populations in lakes and the Baltic Sea revealed unexpected patterns of molecular and physiological evolution. A clear-cut organisation of mtDNA variation among Scandinavian populations of *M. salemaai* suggested that post-glacial rates of mtDNA sequence change could be ten times higher than commonly used molecular clocks extrapolated from the sister-species divergences. Inter-population comparisons of a presumably adaptive trait, the spectral sensitivity of the eyes, failed to corroborate a previously postulated direct association between the ambient light environment and visual properties of *M. relicta.*

Overall, there was no congruence in the extent of molecular divergence among co-distributed taxa, either in the 'glacial relicts' or in Ponto-Caspian elements; similar zoogeographical patterns seem to have been created at different time scales.

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1. INTRODUCTION

When and how did all the spectacular forms of nature's diversity originate? This question is probably as old as human cognition, spurring an array of answers in different cultures and different times. The recent upsurge in speciation studies and the results they have generated has brought important understanding of the causes and process of species diversification (Otte & Endler 1989, Barton 2001, Wu 2001, Coyne & Orr 2004). Current molecular analyses allow the identification of genetic factors underlying reproductive isolation (Orr 2001, Turner et al. 2005), conditions required for speciation can be predicted in natural ecosystems or in laboratory experiments (Miyatake & Shimizu 1999, Taylor et al. 2000, Lande et al. 2001), while the development of phylogeography has bridged micro-evolutionary processes with the genealogical organisation of nature (Avise et al. 1987, Riddle 1996, Hewitt 2000). However, the never-ending discussions of species concepts (Wu 2001, Mallet 2001, Mayr 2001) or the geography of speciation (Via 2001, Losos & Glor 2003, Coyne & Orr 2004) demonstrate that some questions have not been resolved since Darwinian times. For example, how many species are there and how can we define them (Hey 2001, Hebert et al. 2003, Will & Rubinoff 2004)? Is sympatric speciation a common phenomenon or a rare exception (Mallet 2001, Losos & Glor 2003, Coyne & Orr 2004)? Do unstable environmental conditions, like Pleistocene glaciation cycles, promote or impede species diversification (Klicka & Zink 1997, Bernatchez & Wilson 1998, Hewitt 2000, Bennet 2004, Johnson & Cicero 2004, Lister 2004, Weir & Schluter 2004)?

Many of the recent important advances in evolutionary studies have been fostered by the application of molecular characters (proteins, DNA), which have generally revolutionised a number of long-established concepts about the diversity, relationships and biogeography of extant organisms. First of all, molecular analyses have uncovered a new world among most, and particularly minuscule organisms, suggesting that traditional morphology based taxonomies have underestimated species diversity by a factor of 10 to 100, or even more (Blaxter 2004). Even for the best studied ecosystems, like the Baltic Sea, and ecologically important macroinvertebrate taxa, molecular approaches have revealed cryptic species and deep intra-specific subdivisions in, for example, the *Mysis relicta* group (Väinölä 1986), *Macoma* bivalves (Väinölä & Varvio 1989a), *Mytilus* bivalves (Väinölä & Hvilsom 1991), *Marenzelleria* polychaetes (Bastrop et al. 1995, Sikorski & Bick 2004) and *Hediste* polychetes (Röhner et al. 1997). Views on the organisation of nature's diversity have also been shattered at nearly all hierarchical levels, for instance as regards the phylogenies of metazoans and arthropods, or the monophyly of Crustacea (i.e. insects are actually 'flying crustaceans') (Halanych 2004, Regier et al. 2005). In biogeographic studies, in turn, the advent of molecular techniques has provided both a more objective way to evaluate species richness and the degree of endemism, as well as crucial information about the timing of taxonomic diversification at various divergence levels (Arbogast & Kenagy 2001, Donoghue & Moore 2003).

1.1. Continental mysids in zoogeographic and evolutionary studies

Mysids (Crustacea, Mysida) are comparatively small crustaceans (5–50 mm), which

carry their developing embryos and young in a brood pouch and hence are also commonly referred to as opossum shrimps. Traditionally (e.g. Mauchline 1980, Müller 1993), mysids (Mysida) have been grouped with lophogastrids (Lophogastrida) into the order Mysidacea and superorder Peracarida, the latter also including orders like Amphipoda, Isopoda, Cumacea. Molecular data have, however, suggested affinities between Mysida and Decapoda, albeit with only a weak support (Jarman et al. 2000, Spears et al. 2005). The current consensus treats Mysida and Lophogastrida as separate orders within the Peracarida (Martin & Davis 2001) (Table 1). Recent estimate of Mysida diversity suggests approx. 1 000 species, of which about 90% are exclusively marine (Wittmann 1998). Of the remaining species, most are confined to a few coastal lakes or caves that were accessible via direct marine inundations (Mauchline 1980). Finally, about 30 currently recognised species from eight genera have attained widespread continental occurrences (Table 1, Fig. 1). These continental distributions of primarily marine organisms with restricted dispersal abilities (i.e. an inability to migrate upstream or to be distributed by natural external agents such as birds) make mysids an interesting object of biogeographic and evolutionary studies (Lovén 1862, Högbom 1917, Sars 1927, Ekman 1953, Segerstråle 1957, Mordukhai-Boltovskoi 1979, Väinölä 1995). Furthermore, continental mysids often occur in large densities and play an important role in aquatic food webs (Tattersall & Tattersall 1951, Mauchline 1980, Salemaa et al. 1986), a fact exemplified by controversial attempts to exploit mysids as a food source via transplantations beyond the natural distribution limits (Ioffe 1963, Lasenby et al. 1986, Nesler & Bergersen 1991).

The continental mysids addressed here belong to two zoogeographical groups that also comprise numerous other invertebrate and vertebrate taxa; their diversification is usually associated with two large-scale paleogeographical events. The first group includes primarily Arctic marine crustaceans, fishes and seals (Lovén 1862, Ekman 1953, Segerstråle 1982), which continental occurrences have likely been defined by Pleistocene glaciations. Arctic continental mysids belong to the genus *Mysis* Latreille, 1802, and encompass four species of the *Mysis relicta* group ('glacial relicts') and four Caspian Sea endemics ('arctic immigrants') (Table 1). The second zoogeographical group comprises Ponto-Caspian taxa, originally endemic to the Black, Azov and Caspian seas. These seas are remnants of the mid-Tertiary (20 Myr) inland Paratethys Sea and throughout their existence experienced a series of environmental and paleogeographic changes that repeatedly separated and connected the basins (Bănărescu 1991). The Ponto-Caspian area harbours rich endemic diversity, particularly in crustaceans (Zenkevitch 1963), and include about 20 currently recognised mysid species, mostly of the diverse genus *Paramysis* Czerniavsky, 1882. The Ponto-Caspian mysids comprise species endemic to the Caspian or Black seas, as well as brackish water taxa represented by disjunct populations in diluted parts of all the three basins (Table 1). This thesis focuses on the latter, shared element, and the term 'Ponto-Caspian' is used in its more narrow sense to exclude species endemic to a single sea (i.e. Caspian or Pontic).

The peculiar occurrences of the continental mysids and of other co-distributed taxa have attracted much interest and speculation on their zoogeographical origin, adaptive history and the ancestor-descendant

Table 1. Taxonomic position of mysids (according to Martin & Davis 2001) and list of species analysed in this thesis. Abbreviations: BLA = Black Sea; AZO = Azov Sea; CAS = Caspian Sea; + = present in the area; – = absent from the area. Distributions in Ponto-Caspian rivers are indicated with + when natural occurrences were known from > 1 000 km upstream.

Class	Malacostraca Latreille, 1802
Subclass	Eumalacostraca Grobben, 1892
Superorder	Peracarida Calman, 1904 (9 orders including Lophogastrida, Amphipoda, Isopoda, Cumacea)
Order	Mysida Haworth, 1825 (4 families)
Family	Mysidae Haworth, 1825 (6 subfamilies)
Subfamily	Mysinae Haworth, 1825 (7 tribes)
Tribe	Mysini Haworth, 1825 (ca 50 genera)

ARCTIC ELEMENTS

Genus ***Mysis*** Latreille, 1802

CONTINENTAL GROUP	
M. relicta Lovén, 1862	North European lakes, Baltic Sea
M. salemaai Audzijonytė & Väinölä, 2005	North European & British Isles lakes, Baltic Sea, Siberian coasts
M. segerstralei Audzijonytė & Väinölä, 2005	Circumarctic coastal, arctic lakes
M. diluviana Audzijonytė & Väinölä, 2005	N North America, continental lakes
M. caspia Sars, 1895	Middle and Southern Caspian Sea, depth 50–400 m
M. macrolepis Sars, 1907	Middle and Southern Caspian Sea, depth 50–400 m
M. amblyops Sars, 1907	Middle and Southern Caspian Sea, pelagic
M. microphthalma Sars, 1895	Middle and Southern Caspian Sea, pelagic
MARINE GROUP	
M. oculata (Fabricius, 1780)	Circumarctic, marine
M.* cf. *litoralis	Circumarctic, coastal
M. litoralis (Banner, 1948)	NE Pacific, coastal
M. polaris Holmquist, 1959	High arctic, under ice
M. mixta Lilljeborg, 1852	N Atlantic, Baltic Sea
M. gaspensis Tattersall, 1954	NW Atlantic, intertidal
M. stenolepis Smith, 1873	NW Atlantic, intertidal

PONTO-CASPIAN ELEMENTS

	Natural distribution in estuaries			**Offshore**	**Rivers**
	BLA	AZO	CAS	CAS	
Limnomysis benedeni (Czerniavsky, 1882)	+	+	+	–	–
Paramysis lacustris (Czerniavsky, 1882)	+	+	+	–	–
P. sowinskii Daneliya, 2002	–	+	+	–	–
P. baeri *sensu lato* Czerniavsky, 1882	+	+	+	+	+
P. ullskyi Czerniavsky, 1882	+	+	+	–	+
P. kessleri G. O. Sars, 1895	+	–	+	+	–
P. intermedia (Czerniavsky, 1882)	+	+	+	–	–

Ponto-Caspian mysids not analysed in this study:
Caspian Sea endemics – *Paramysis loxolepis*, *P. incerta*, *P. eurylepis*, *P. inflata*, *P. grimmi*, *Schistomysis elegans*, *Caspiomysis knipowitschi, Diamysis pusilla*
Black Sea endemics – *Paramysis kroyeri*, *P. pontica*, *P. agigensis*, *P. kosswigi, Diamysis pengoi*
Ponto-Caspian species – *Katamysis warpachowskyi*, *Hemimysis anomala*

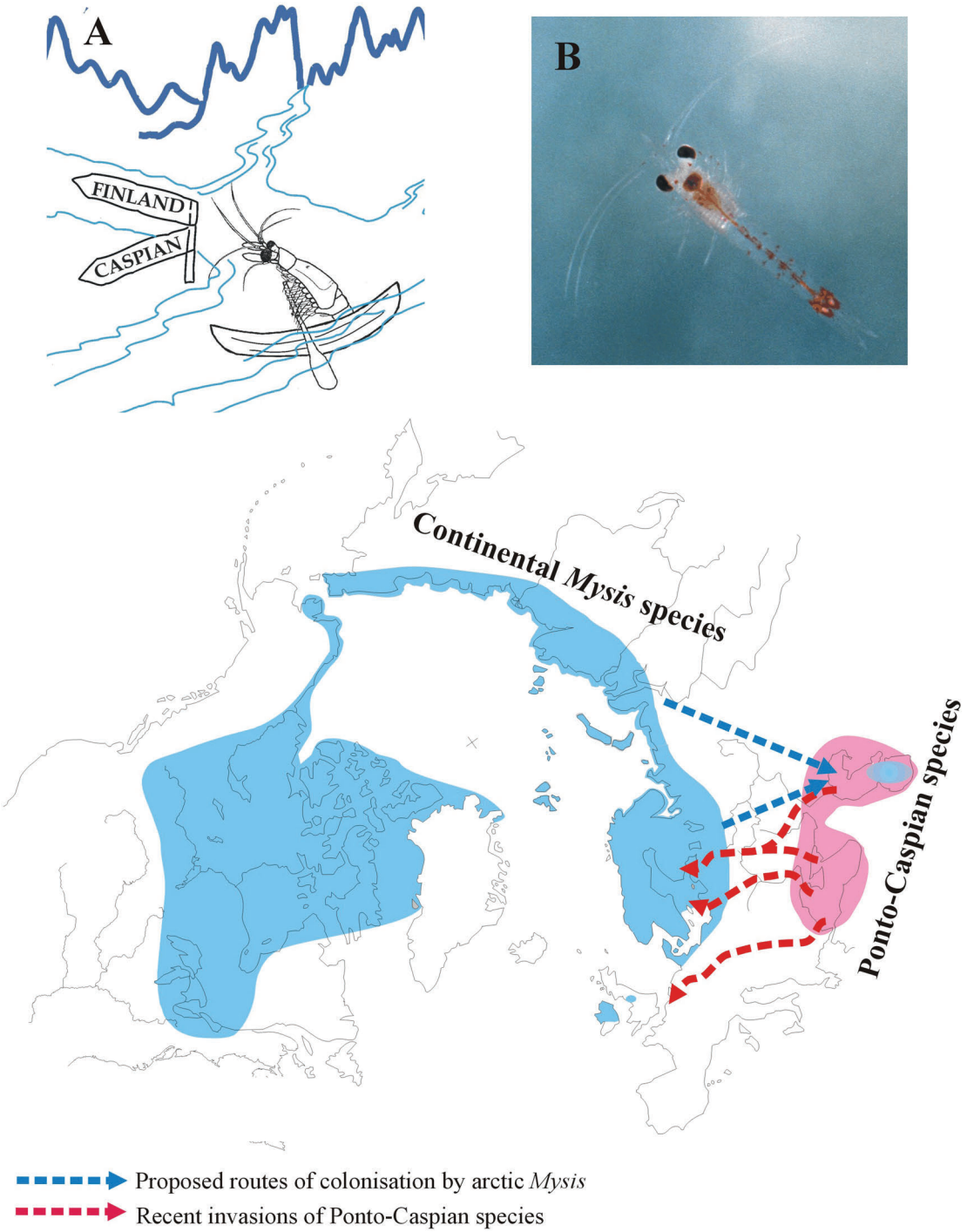

Fig. 1. Distributions of the continental mysids (polar view). **A.** A hypothetical ancestor of the continental *Mysis*. **B.** A recent Ponto-Caspian invader into Northern Europe *Hemimysis anomala* (photo: Alexander Gorbunov).

relationships involved (e.g. Lovén 1862, Högbom 1917, Sars 1927, Derzhavin 1939, Ekman 1953, Holmquist 1959, Mordukhai-Boltovskoi 1979, Väinölä 1995). While most of these studies have focused on one of the two components, these lines of research have several uniting aspects:

1) Zoogeographical interest in factors that define distributions of fauna with limited dispersal abilities. Relying on direct lentic connections for their dispersal, mysids may act as suitable markers of areas that were accessible via past marine inundations or periglacial lakes, events that were also important in shaping the diversity and biogeography of numerous other taxa.

2) Two different biogeographic hypotheses invoked to explain disjunct marine-continental or brackish water occurrences in the two groups, i.e. recent dispersal (< 0.1 Myr) and ancient vicariance (10–30 Myr).

3) Effects of Pleistocene climatic oscillations on the diversification and distributions of species in different geographical areas that were either directly covered by ice-sheets or affected by corresponding changes in temperature, humidity and drainage patterns.

4) Comparative analysis of speciation and adaptive evolutionary rates in sympatric and allopatric populations, isolated at different times and in different geographical areas.

Finally, the two zoogeographical groups also came into contact in the course of past and currently on-going range changes between the two zoogeographical regions (Fig. 1). The arctic *Mysis* element has given rise to a small endemic species flock in the Caspian Sea (Sars 1895, 1907), whereas the autochthonous Ponto-Caspian mysids are currently invading aquatic ecosystems of Northern Europe as a result of human activities (Leppäkoski et al. 2002).

As to the origin of the continental *Mysis* occurrences, an initial view advocated their Late Quaternary descent from landlocked populations of the marine *Mysis oculata* (Fabricius, 1780) (Lovén 1862, Ekman 1919, Thienemann 1925, Hutchinson 1967). Under this transformation hypothesis, forms that were phenotypically intermediate between the marine *M. oculata* and the freshwater *M. relicta* were expected in intermediate brackish water salinities (Olofsson 1918, Ekman 1919) as suggested, for example, in the original description of the coastal Northwest Atlantic species *M. gaspensis* Tattersall, 1954. The four endemic Caspian *Mysis* taxa were considered to have derived from the 'glacial relict' *M. relicta* (*sensu lato*) that was transported to the Caspian Sea during drainage shifts of ice-dammed lakes (Derzhavin 1939, Ekman 1953, Tarasov 1997). The idea of close similarity between *M. oculata* and *M. relicta* (*sensu lato*), as well as between *M. relicta* and Caspian *Mysis* was, however, mostly based on zoogeographical tradition (Thienemann 1950, Zenkevitch 1963) rather than on character analyses. On the other hand, the morphological and ecological diversity of the continental *Mysis*, particularly of the four Caspian endemics, is considerable, with body lengths ranging from 8 to 25 mm, nectobenthic and entirely pelagic life styles, and reduction of eyes in two species (Derzhavin 1939). Indeed, the authors that assessed their morphological characteristics in more detail proposed an ancient, possibly mid-Tertiary (ca 30–40 Myr) origin of the Caspian *Mysis*, when a direct marine connection between the current Arctic ocean and the Ponto-Caspian basin was still open (Sars 1927, Holmquist 1959); the boreal freshwater *Mysis* were considered of similar age.

For the disjunct inter-basin distributions

of the brackish water Ponto-Caspian mysids in the estuaries of the Black, Azov and Caspian seas, an immigration view has similarly advocated a recent dispersal from the Caspian Sea via transiently-established connections in Middle and Late Pleistocene times (Beklemishev 1923, Birshtein 1935, Mordukhai-Boltovskoi 1960). It was believed that throughout the Pleistocene, environmental changes in the Black and Azov seas were too drastic to allow the survival of ancient fauna, and the taxa shared among the Ponto-Caspian region were thus referred to as Caspian (Mordukhai-Boltovskoi 1979, Zubakov 1988, Reid & Orlova 2002). The alternative vicariance view, in turn, argued that current disjunct distributions of the brackish water species are remnants from the Tertiary Sarmatian (ca 10 Myr) or Pontian (ca 6 Myr) seas, which connected the Ponto-Caspian basins to form a large water body; the fauna in question were termed Sarmatian or Pontian relicts (Sars 1907, Derzhavin 1939, Ekman 1953, Weish & Türkay 1975). River deltas and lagoons were seen as long-term refugia enabling their survival during unfavourable environmental conditions (Starobogatov 1970, Grigoriev & Gozhik 1976).

1.2. Molecular markers in studies of continental mysids

The initial application of molecular markers led to the reconsideration of many traditional concepts about the systematics and zoogeography of continental mysids. Allozyme studies of *Mysis* revealed: (i) deep molecular subdivisions within the phenotypically uniform circumpolar *Mysis relicta* taxon, indicating presence of four distinct species (*M. relicta* spp. I–IV; Väinölä 1986, Väinölä et al. 1994); (ii) relatively ancient separation of the *M. relicta* group from Caspian endemic *Mysis* (Väinölä 1995); (iii) recent, possibly Late Pleistocene, diversification of the Caspian *Mysis* species flock (Väinölä 1995); and (iv) little congruence in marine-continental divergence depths between *Mysis* and other similarly distributed 'glacial relict' crustaceans (Väinölä & Varvio 1989b, Väinölä et al. 2001). Molecular analyses of Ponto-Caspian crustaceans generally rejected the hypothesis of Late Pleistocene dispersals between disjunct Black and Caspian Sea populations, suggesting that despite transient contacts among the basins, faunal exchange was limited (e.g. Cristescu et al. 2003, 2004). As with 'glacial relicts', little congruence in the depth of divergence could be seen among co-distributed Ponto-Caspian invertebrate taxa (Cristescu et al. 2003, Therriault et al. 2004).

While molecular markers have so far given new important insights into the diversification of continental mysids, many old questions remain unresolved and an array of new ones was opened. For example, neither the monophyly of the *M. relicta* group, nor that of all continental *Mysis* could be corroborated by allozyme data. The relationships among the *M. relicta* group species have also remained unresolved, and the lack of formal taxonomic descriptions has impeded the appreciation of the new diversity in ecological studies. The estimated age of the marine-continental split in *Mysis* (3–15 Myr) does not fit any known paleogeographic events that might have allowed a continental invasion, and a sister group to the continental *Mysis* has not be confidently identified among the marine taxa. The resolution of intra-specific relationships and population history has been limited, for example regarding the post-glacial colonisation routes of 'relict' mysids in the

Baltic Sea basin; due to requirement for fresh material for allozyme analysis, the diversity in remote geographical areas has remained unknown. In the Ponto-Caspian region, sampling has generally remained fragmented, particularly in the Azov and Caspian seas, and limited to a few crustacean and mollusc taxa. Virtually nothing is so far known about the molecular diversity of mysid crustaceans in disjunct brackish-water populations of the region. Comparing their phylogeographies with those of other co-distributed crustaceans and molluscs having different dispersal abilities should allow broader phylogeographic generalisations about the Ponto-Caspian species history. Finally, molecular characters alone give only a one-sided view of the diversity and evolutionary dynamics of organisms. Comparison and integration of phenotypic and molecular information is important for more informed taxonomic decisions and could greatly improve understanding of species adaptations in nature and finally of factors affecting the diversity itself.

2. OUTLINE OF THE THESIS

This thesis analysed importance of various factors in origin and maintenance of diversity on different temporal and spatial scales, using continental mysid crustaceans as a model group, and applying data from morphological (I, II, III, VI), molecular (II, III, IV, VI), and physiological (V) analyses. The patterns of diversity were assessed at different systematic levels – interspecific (I, II, III, V), intra-specific (IV, VI, V), as well as the boundaries between these two (IV, VI) – and on different geographical scales – among zoogeographical zones (marine, boreal lakes, Ponto-Caspian), among circumpolar lakes of the Northern Hemisphere (I–IV), among drainages of the Ponto-Caspian region (III, VI), as well as among different populations in Europe (IV), and the Baltic Sea drainage (V). More specifically, the study analysed morphological and molecular differentiation among the *Mysis relicta* group species (I, II) and presented their formal taxonomic description (II). Combined analysis of morphological and molecular data was used to resolve the long-standing question on origin of the continental *Mysis* species and the affinities (monophyly) of the 'glacial relict' taxa in the circumpolar boreal lakes and 'arctic immigrants' in the Caspian Sea (III). At the intra-specific level the effects of Pleistocene paleogeography and species ecological characteristics on distributions of genetic lineages and molecular diversity were studied in comparative phylogeographic analyses of a European boreal and a circumarctic coastal *Mysis* species (IV), and of seven Ponto-Caspian mysids taxa (VI). Finally, the importance of species and population history and of environmental factors on adaptive evolution in two European mysid species was assessed in a physiological study of their visual properties (V).

3. MATERIAL AND METHODS

3.1. Samples

Most mysids analysed in this thesis belong to two geographically widespread genera – the primarily arctic marine genus *Mysis* Latreille, 1802 and its representatives in the continental waters, and the speciose Ponto-Caspian-Atlantic genus *Paramysis* Czerniavsky, 1882. Another, monotypic Ponto-Caspian genus *Limnomysis* Czerniavsky, 1882 is also included (Table 1). The three genera are traditionally assigned

to the tribe Mysini (Müller 1993), but the monophyly of this taxon has been questioned on the basis of molecular 18S rRNA data (Remerie et al. 2004). The phylogenetic position of the genera analysed in this thesis was assessed from an approx. 850 bp fragment of the nuclear 18S rRNA gene, using data from Remerie et al. (2004) and new data from *Mysis* (III) and *Paramysis* (unpubl.). The analysis was further suggestive of the paraphyly of Mysini (although with no significant node support) and showed relatively close relationships among *Mysis*, *Paramysis* and *Limnomysis* (Fig. 2).

The material of *Mysis* was obtained from samples collected for various previous studies in 1980–1996 (Kinsten 1986, Väinölä 1992, 1995, Väinölä et al. 1994), and from additional samples collected for this work. Reference material of most samples is deposited in the Finnish Museum of Natural History. Additional material from samples stored at zoological museums in Europe and Canada was also included. The taxonomical and distributional analysis of the *Mysis relicta* species group (I, II) was based on altogether approx. 300 samples from various continental and coastal localities of the Northern Holarctic. Of these, approx. 240 samples (5–20 specimens per sample, if available) were analysed for morphological and morphometric characters and 66 samples (197 specimens) for mtDNA variation. Allozyme character data from approx. 200 samples was also used (Väinölä 1986, Väinölä et al. 1994). Part of the material (44 samples, 169 specimens) was used for mtDNA phylogeographic study of two *M. relicta* group species – *M. salemaai* and *M. segerstralei* – on the circumarctic and North European scales (IV). Analyses of spectral light sensitivities of the eyes of two European 'glacial relict' mysid species – *M. relicta* and *M. salemaai* – were conducted on samples from three sites in the Baltic Sea and from two lakes in southern Finland (V). The sampling was designed to test the effect of environment on visual adaptations and included sympatric occurrences of different species (same environment – different taxa) and allopatric occurrences of the same species in different light conditions (different environments – same taxon). Phylogenetic analysis of the genus *Mysis* (III) included all known species of the genus (Table 1); 1–30 individuals from each species were analysed for morphological and molecular (mitochondrial and nuclear DNA, and allozyme) variation. The Ponto-Caspian mysid diversity and phylogeography was studied using mitochondrial COI gene sequence variation from seven mysid taxa belonging to two genera (*Paramysis* and *Limnomysis*), collected in 18 localities across the region in 1991 and 2000–2004 (VI).

Samples newly collected for molecular and morphological analyses were stored deep frozen (-80 C°) or in ethanol

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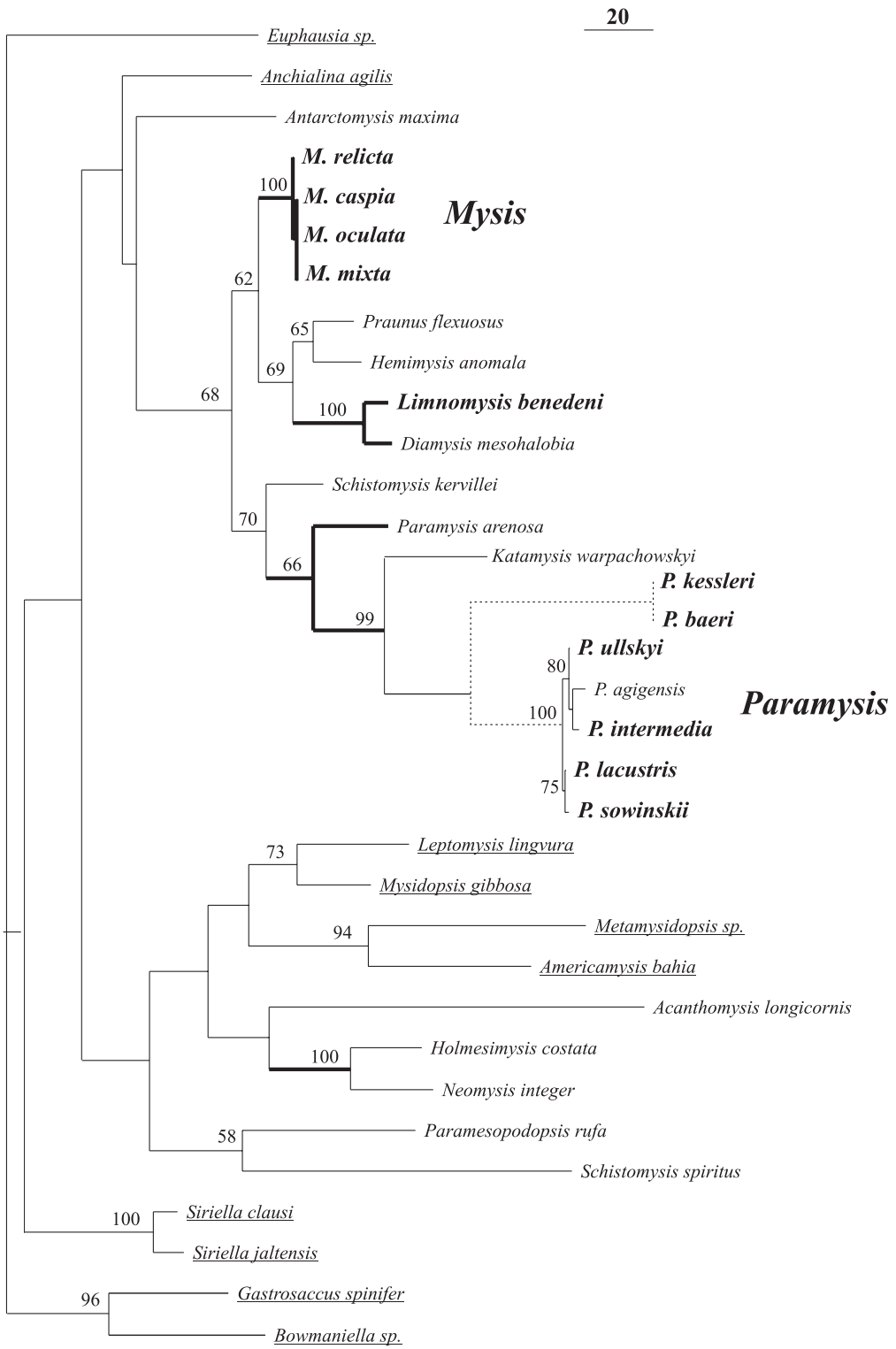
Fig. 2. Single MP tree (1246 steps) of various Mysida, based on approx. 850 bp of the nuclear 18S gene, aligned using default parameters in ClustalW (see description of methods in section 3.3.2.1); gaps were treated as the 5th character state. *Mysis* and *Paramysis* data are from this study, other sequences are from Remerie et al. (2004). Thick branches indicate nodes also supported by direct optimisation analysis (gap insertion costs 1 or 2 relative to base change costs). Bootstrap support is shown above the branches; scale bar indicates the number of inferred changes. Continental mysids analysed in this study are listed in **bold**; taxa that do not belong to the tribe Mysini are underlined. No 18S data of *P. kessleri* and *P. baeri* was available and their position is approximately inferred from the analysis of 28S gene (unpubl.).


20
Euphausia sp.
Anchialina agilis
Antarctomysis maxima
M. relicta
M. caspia
M. oculata
M. mixta
Mysis
Praunus flexuosus
Hemimysis anomala
Limnomysis benedeni
Diamysis mesohalobia
Schistomysis kervillei
Paramysis arenosa
Katamysis warpachowskyi
P. kessleri
P. baeri
P. ullskyi
P. agigensis
P. intermedia
P. lacustris
P. sowinskii
Paramysis
Leptomysis lingvura
Mysidopsis gibbosa
Metamysidopsis sp.
Americamysis bahia
Acanthomysis longicornis
Holmesimysis costata
Neomysis integer
Paramesopodopsis rufa
Schistomysis spiritus
Siriella clausi
Siriella jaltensis
Gastrosaccus spinifer
Bowmaniella sp.
100
62
65
69
68
100
70
66
99
80
100
75
73
94
100
58
100
96

(80–96%). Samples for vision analyses (V) were transported to laboratory and kept in aquaria until analysis (Lindström & Nilsson 1988). Samples obtained from museum collections included both formalin and ethanol fixed material and were mostly used for morphological assessment (II, III). Part of the material, collected in the 1950s–1960s and fixed and stored in strong ethanol (Holmquist 1963, 1975) nevertheless turned out to be usable for molecular analyses (IV).

3.2. Laboratory analyses

3.2.1. Morphological characters

For morphological and morphometric analyses specimens were inspected and measured under a dissecting microscope (10×–50× magnification) and from slide mounts using light microscopy (including phase contrast microscopy) (I, II). Computerised digital image analysis software was used to measure 26 characters used in the morphometric study of the *Mysis relicta* group (I). Qualitative morphological variation in *Mysis* was also assessed using scanning electron microscopy (II, III). All external body parts were screened for taxonomically and phylogenetically informative variation. The final selection of characters useful for species identification included about 20 morphological traits (II). For the phylogenetic analysis of the genus *Mysis* 33 morphological characters were selected, namely those deemed stable within species and amenable to coding into discrete states (III).

3.2.2. Molecular characters

Molecular analyses were conducted from sequences of mitochondrial and nuclear DNA. Although recently criticised (e.g. Ballard & Whitlock 2004, Thalmann et al. 2004, Hurst & Jiggins 2005), mitochondrial DNA remains the most widely used marker in phylogeographic and lower-level phylogenetic studies (e.g. Avise 2000, Hewitt 2004). The advantages of mtDNA include its relative ease of amplification owing to a large number of copies and to availability of universal primers (e.g. Folmer et al. 1994), predominantly uniparental inheritance and hence general absence of recombination, and a comparatively fast rate of evolution. In this thesis sequences of a 600–630 bp segment of the mitochondrial protein coding cytochrome c oxidase subunit I gene (COI) were used to explore the phylogeographic histories of two species in the *M. relicta* group (IV), and of seven Ponto-Caspian mysid taxa (VI). For phylogenetic analysis of the genus *Mysis* (III) a larger set of molecular characters was assembled, including partial mtDNA sequences of COI, cytochrome B (CytB) and the large subunit rRNA (16S) genes, and nuclear DNA sequences of the partial small subunit rRNA gene (18S) and the entire internal transcribed spacer 2 region (ITS2) of the rRNA operon. Allozyme character data from previous studies (Väinölä 1986, 1992, 1995) were also incorporated into the taxonomic and phylogenetic studies (II, III). Laboratory procedures are described in (III), (IV) and (VI).

3.2.3. Eye spectral sensitivity

Spectral sensitivities of the eyes of two mysid species were studied using electroretinogram (ERG) analyses (V). The spectral sensitivity of an organism with compound eyes, as typical for many arthropods, is a result of light absorption of both visual

pigments and of intraocular filters (screening pigments) present in the ommatidia (Goldstein & Williams 1966, Goldsmith 1978). The visual pigment consists of a protein (opsin) and a chromophore (vitamin A), which in crustaceans, including *Mysis*, can be either in the form of retinal (A1) or 3,4-dehydroretinal (A2) (Jokela-Määttä et al. 2005). The spectral sensitivity of *Mysis* can therefore vary either due to amino-acid changes in the opsin protein, due to switch from chromophore A1 to A2 (which causes a shift of spectral sensitivity towards longer wavelengths), or by interference of screening pigments. In the ERG analysis the response of eye to light of different wavelengths is measured directly by inserting an electrode into an eye; the method thus assesses a 'total' response that includes light absorption of both the visual and screening pigments. In this respect the ERG analysis is different from, e.g. microspectrophotometry (MSP), where spectral sensitivity is recorded from an isolated visual pigment only. Generally it is considered that screening pigments in a dark-adapted eye are withdrawn and will not affect the spectral sensitivity; results from various measures of spectral sensitivity have thus been compared directly (e.g. Archer et al. 1999). Yet, in a number of organisms a pronounced difference has been found between the wavelengths of maximum sensitivity measured from the eye and from the visual pigment separately, suggesting that screening pigments may affect vision also in a dark-adapted state (Goldsmith 1978, Frank & Widder 1999, Jokela-Määttä et al. 2005). It remains to be tested which approach gives results that are closer to the actual vision of an organism in nature.

3.3. Data analyses

3.3.1. Morphometric data

Morphometric data on populations of the four *Mysis relicta* group species (I) were analysed using two common multivariate statistical approaches, principal component analysis (PCA) and canonical variate analysis (CVA, also called discriminant analysis) (Pimentel 1979). PCA is useful in summarising the main patterns of multivariate data on fewer dimensions (principal components), whereas CVA helps to identify variables that allow best differentiation among *a priori* defined groups (i.e. variables with small within-group but large between-group variance). A major problem with traditional linear morphometric measurements is to account for ontogenetic size variation in organisms of variable sizes at maturity. In such measurements all variables are generally influenced by the overall size, i.e. all dimensions of body increase in the course of growth. In PCA of linear measurements most of the size variation is summarised in the first principal component (PC1), which typically also accounts for the largest proportion of the total variance (70–95%) and is strongly and positively correlated with all other variables; it has been therefore suggested that PC1 can be used as a summary measure of 'size' (e.g. Teissier 1938 cited in Cadrin 2000). A simple separation of PC1 as 'size' has nevertheless been criticised and in fact all methods proposed to account for size effects in traditional morphometric analyses have either biological or statistical limitations (Thorpe 1983, Rohlf 1990, Cadrin 2000). In this study, we nevertheless assumed PC1 to account for most of the size variation, and other components to embody information about 'shape'. Effects of size were tested

by comparing CVA of raw measurements to results of same analysis performed on PCA scores with PC1 either included or excluded ('size-in' and 'size-out', Thorpe 1983). CVA was carried using the four species (identified by allozyme characters) as *a priori* identified groups, or alternatively using populations as groups with no prior species assignment. The latter approach helped to asses whether populations cluster into the four taxonomic units. A pair-wise discriminant function to identify two sympatrically occurring European species was developed and its applicability was tested with an independent data set.

3.3.2. Molecular data

3.3.2.1. Phylogenetic analysis. The phylogenetic parsimony analysis of the genus *Mysis* (III) was conducted on seven different data sets, including five DNA sequences (mitochondrial and nuclear), a set of 33 morphological characters, and a set of 8 allozyme loci. In addition to assessing relationships among *Mysis* species, the study explored two currently debated methodological aspects of phylogenetic analysis, i.e. the alignment of length-variable DNA regions (*a priori* alignment and direct optimisation) and the combining of different data sets into a simultaneous analysis.

Alignment of length-variable DNA regions (ITS2 in case of *Mysis*) is one of the major challenges and a recent bone of contention in phylogenetic analyses. On the one hand, the independence of alignment and tree search (*a priori* alignment) has been advocated as a sound scientific method to postulate primary homologies (*sensu* de Pinna 1991), i.e. alignment, and then to test them in a subsequent phylogenetic analysis (Simmons 2004). On the other hand, such an independent treatment of data has been blamed for logical inconsistency because it introduces a number of assumptions about character state changes, i.e. insertion-deletion events that are not revised in the course of the analysis (reciprocal illumination *sensu* Hennig 1966). Moreover *a priori* alignment is also typically produced on a basis of a guide tree, which logically should be, but is typically not, the same as the tree obtained from phylogenetic analysis (Schulmeister et al. 2002). The proposed alternative approach, direct optimisation (DO), discards primary and secondary homologies and introduces a topology and parameter dependent dynamic homology (Wheeler 1996). Here a search for an optimal topology and optimal alignment (according to the chosen optimality criterion, i.e. parsimony or likelihood) is conducted simultaneously and homologies of nucleotide bases are allowed to change in order to minimise incongruence (Wheeler 1996, Wheeler et al. 2003). Such a process finally assumes as few as possible DNA changes, and logically trees obtained using DO are shorter or equal to trees from *a priori* alignments of the same data (Wheeler 2001). But exactly for this reason direct optimisation has been criticised as 'automated scheme to purge the data of homoplasy' (Simmons 2004). On the other hand, while use of primary and secondary homologies is a good theoretical framework in morphological analysis, it is hardly applicable to length-variable DNA data, where the proposed criteria to identify primary homologies (i.e. topographical and structural similarity, change during ontogeny; de Pinna 1991) cannot be applied. To some extent the primary homology statements could be strengthened from an inferred secondary structure, if this is known with the sufficient certainty (e.g. for rRNA, Wuyts et

al. 2001, 2002). However, secondary structures suggested for the internal transcribed spacer regions (ITS1 and ITS2) seem to vary a lot and their broader applicability remains to be tested (Mukha et al. 2002, Young & Coleman 2004). Moreover, the inconsistency of *a priori* alignments also pertains to the fact that different parameters (e.g. gap insertion versus base change costs) are typically used during the alignment and the tree search and their effects on the obtained topology are not explored (Wheeler 2001).

It is widely accepted that phylogenetic inference should be conducted on a wide range of characters, the more the better. However, how the different data sets should be treated – analysed separately or together in a simultaneous analysis – has not been settled so far. On the one hand, evaluating repeatability of clades from separate analysis of data partitions allows assessing confidence in the obtained topology through independent investigations (e.g. Chen et al. 2003). On the other hand, combining data partitions into simultaneous analysis is preferable because separate analyses of small data sets suffer from large sampling error and typically yield unresolved topologies (Cummings et al. 1995, Schulmeister et al. 2002). The underlying assumption of simultaneous analysis is expectation of homoplasy being distributed more or less randomly across data partitions – the very fact doubted by advocates of separate analysis (Naylor & Adams 2001). The philosophical arguments remain disputed (e.g. Chen et al. 2003, Kluge 2004), but the emerging consensus is that exploration of data – whether in separate or simultaneous analysis – should constitute an important step in identifying potentially misleading signals in the data partitions (Wheeler 1995, Giribet 2003, Lecointre & Deleporte 2004, but see Grant & Kluge 2003).

In this study exploration of data was performed using both separate and simultaneous analysis. While identifying conflicts among data from separate analysis typically involves comparison of tree topologies, the simultaneous analysis provides a framework to compare support of data sets for each node by using partitioned Bremer support (PBS) values (Baker & DeSalle 1997). Moreover, it also enables to reveal the so-called hidden partitioned Bremer support (HPBS; Gatesy et al. 1999) which is not seen in separate analysis. For example, if a data set I has 5 characters supporting grouping A(B,C) and 4 characters supporting grouping (A,B)C, whereas data set II has 1 character for grouping A(B,C) and 10 characters for grouping (A,B)C, the consensus from the two separate analyses will yield unresolved topology (A,B,C) (Fig. 3). However the A(B,C) grouping is in total supported by 6 characters, whereas (A,B)C by 14 characters. Thus the data

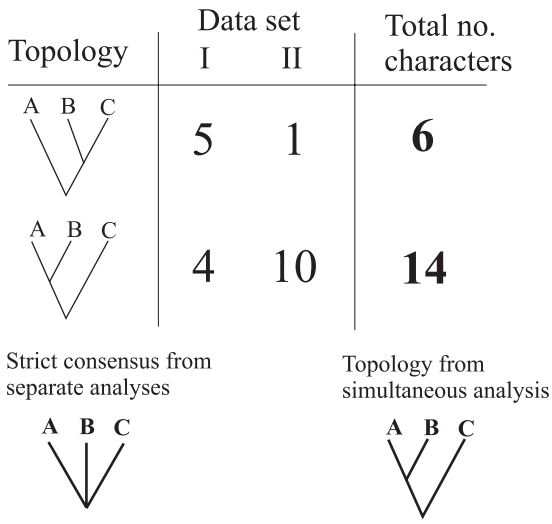


Fig. 3. Results of separate and simultaneous analyses of two data sets that have different degree of support for two alternative topologies.

set I has a 'hidden' support of 4 characters for the topology (A,B)C that can be detected in simultaneous, but not separate analyses. The formal procedure to calculate PBS and HPBS values is described in (III). Another way to assess contributions of data partitions in simultaneous analysis is sensitivity analysis (*sensu* Wheeler 1995). Here tree searches are conducted using a varying set of the analysis parameters (e.g. gap insertion-nucleotide base substitution costs, transitions-transversion costs, different weights of the data) and the stability of the nodes in the obtained topology is evaluated. Stable nodes are judged as reliable (Giribet 2003).

3.3.2.2. Phylogeographic analysis. In phylogeographic analyses of two *M. relicta* group species (IV) and of seven Ponto-Caspian taxa (VI), relationships among sampled units were assessed both in terms of haplotype genealogies and at the population level.

Relationships among all haplotypes were first assessed from neighbour-joining (NJ) trees constructed on uncorrected (p) or Kimura-2-parameter distances with gamma correction for rate heterogeneity (K2P+Γ) (e.g. Nei & Kumar 2000). Parsimony analyses were then conducted on smaller data sets. Analyses of closely related intra-specific haplotypes however often yield large numbers of equally parsimonious trees and unresolved consensus summaries. To deal with specific problems of intra-specific relationships, i.e. small amount of overall variation to reliably infer topologies, presence of ancestral haplotypes, multifurcations and reticulation, phylogeographic analyses are increasingly switching from trees to networks (Posada & Crandall 2001). While there are different methods to construct networks and they do differ in performance (Cassens et al. 2003), by far the most common is the statistical parsimony method (Templeton et al. 1992) also applied in this thesis. This method uses the coalescence theory (Hudson 1990) to make predictions about the expected amount of variability in a sample of haplotypes (assuming an equilibrium population). The analysis starts by defining a number of steps (changes) between two haplotypes, which can be considered parsimonious with a defined (e.g. 95%) probability. This parsimony limit depends on the length of the analysed sequence and the total amount of genetic variation (θ) in the data set and is estimated through Bayesian analysis (from the coalescence theory, for mitochondrial data $\theta = 2N_f u$, where N_f is the effective population size of females and u is the mutation rate) (Templeton et al. 1992, Clement et al. 2000). Once the connection limit (in terms of number of steps) has been defined, all haplotypes that differ by fewer steps are assembled into a network using the parsimony optimality criterion.

Population level relationships were analysed using a matrix of population pairwise Φ_{st} distances (which reflect shared haplotypes in the samples as well as divergences among them), which was analysed in an explicit spatial context using spatial analysis of molecular variance (AMOVA, SAMOVA) (Excoffier et al. 1992, Dupanloup et al. 2000) (IV, VI). AMOVA partitions molecular variance into components among predefined (e.g. geographical) groups of populations (Φ_{ct}), among populations within the groups (Φ_{st}), and among all population (Φ_{st}). The *a priori* definition of groups can be avoided in SAMOVA, where optimal grouping of populations is searched iteratively on the basis of molecular and geographical distances among them.

Levels of molecular diversity in popu-

lations and intra-specific groups were assessed using standard diversity indices such as haplotype (h) and nucleotide (π) diversities (Nei 1987). Deviations of the patterns of molecular diversity from the expectations in equilibrium situations (i.e. neutrality and constant population size) were assessed using Tajima's D and Fu's F statistics and mismatch distributions (Tajima 1989, Rogers & Harpending 1992, Fu 1996, Schneider & Excoffier 1999). Under directional selection, after selective sweeps or after a population expansion the numbers of segregating sites and of rare alleles in a population is excessive compared to the average number of differences between the sequences and hence Tajima's D and Fu's F statistics have negative values; effects of balancing selection or population subdivision are opposite (Simonsen et al. 1995). Mismatch distributions provide a straightforward summary of the average coalescence depths in the sample, i.e. average distance in mutation generation units (τ) from the most recent common ancestor. In an equilibrium population all but one of the coalescences of the sampled haplotypes are expected to occur within half-time to the most recent common ancestor of the sample; the last coalescence of the two remaining lineages will take another half of the time (Hudson 1990). This means that most haplotypes are derived from two long separated lineages and the distribution of pair-wise differences in the sample will be bimodal. In contrast, in an expanding population majority of haplotypes coalesce prior, but not after the expansion event, producing a star phylogeny and a single peak in the distribution of pair-wise differences (Rogers & Harpending 1992). While mismatch distributions are widely used in phylogeographic studies, there are several reservations as regards their utility. For instance, strong heterogeneity in mutation rates will also cause unimodal distributions resembling the case of expansion (Schneider & Excoffier 1999), but its effect on Tajima's D values will be opposite (Aris-Brosou & Excoffier 1996). On the other hand, strong population subdivision after the demographic and range expansion and relatively low levels of gene flow among the demes will produce complex gene genealogies with short and long branches; in such cases population expansion will not be detected using the standard statistics even if global size of the species increased by several orders of magnitude (Ray et al. 2003). Generally, summary statistics require large sample sizes to confidently reject neutrality / population equilibrium (Simonsen et al. 1995) and do not make full use of the haplotype genealogies; a number of other coalescence based methods have been developed for demographic inferences (Emerson et al. 2001). Yet, regardless of the method, effects of selection and demographic changes are practically indistinguishable using data from a single locus, as is the case with mitochondrial DNA (Galtier et al. 2000). The interpretation of the mismatch distributions in such studies is therefore only suggestive.

4. RESULTS AND DISCUSSION

4.1. Diversity of the 'glacial relict' element – the *Mysis relicta* species group (I, II, III)

Mysid crustaceans conventionally attributed to *Mysis relicta* Lovén, 1862 have a broad circumpolar distribution in boreal and subarctic lakes of the previously glaciated continental areas of Europe and North America and in estuarine and coastal regions of the arctic seas (Jägerskiöld 1912,

Segerstråle 1957, Holmquist 1959). The variation in morphological and biological characteristics of this zoogeographically peculiar taxon has been studied extensively (e.g. Lovén 1862, Sars 1867, Smith 1873, Czerniavsky 1882, Samter & Weltner 1900, Kane 1901, Lönnberg 1903, Linko 1908, Ekman 1919, Holmquist 1949, 1959, Johnson 1964). Yet no taxonomic subdivisions were maintained through most of the 20th century, even though morphological differences among various populations were documented (Holmquist 1959, Johnson 1964). The view on phenotypic differentiation was, however, largely guided by the prevalent idea of gradual morphological transformation from a presumed marine ancestor *M. oculata* to the freshwater *M. relicta,* initially proposed by Lovén (1862), and by a search for the corresponding morphological transformation. As no correlation between salinity and morphology could be found, the attention was finally focused on the absence of intermediate forms rather than on the existence of differences in itself. Application of allozyme characters, however, readily disclosed deep systematic differentiation within the *M. relicta* group (Väinölä 1986, Väinölä et al. 1994), but the documented species diversity remained largely overlooked in ecological studies. The aim of this work was to place the recognised *M. relicta* group diversity in a formal taxonomic framework, to find diagnostic morphological characters that could facilitate the identification of the taxa, to characterise their distributions on the circumpolar scale, and to infer relationships among the four species.

Both quantitative morphometric and qualitative morphological analyses demonstrated consistent differentiation among the four species in the *M. relicta* group. In the morphometric approach (I) 97–100% identification efficiency was achieved using pair-wise discrimination functions based on 3–10 best-discriminating characters each. These chosen characters were partly the same as those assessed by earlier authors (Ekman 1919, Holmquist 1949, 1959, Johnson 1964), and included the numbers of spine-setae on telson, the depth of telson cleft, the numbers and lengths of short and long spine-setae on the maxilla, and the relative carapace length. In the qualitative morphological assessment (II) taxonomically diagnostic differences were also recorded in the setation of the second maxillipede, mandibular palp and finer details of setation of maxilla, but following a broader assessment, less weight was finally put on the number of lateral spine-setae on telson and shape of its cleft.

The formal taxonomical species diagnoses (II) were based on three sets of characters, i.e. (i) nine allozyme loci (Väinölä 1986, Väinölä et al. 1994), (ii) sequence of a 634-bp mitochondrial COI gene segment (GenBank accession numbers AY920491–920494), and (iii) about ten main morphological traits. Binomial Linnaean names were assigned to the four species to replace the earlier used provisional names *M. relicta* spp. I–IV (Väinölä 1986, Väinölä et al. 1994): *M. relicta* Lovén, 1862 (= *M. relicta* sp. I), *M. salemaai* Audzijonytė & Väinölä, 2005 (= sp. II), *M. segerstralei* Audzijonytė & Väinölä, 2005 (= sp. III) and *M. diluviana* Audzijonytė & Väinölä, 2005 (= sp. IV). The new morphological diagnosis also allowed identification of museum material not suitable for molecular analyses; altogether the distributions of the four species were surveyed from about 300 samples across the northern Holarctic (Fig. 4).

M. relicta (s. str.) is the prevalent *Mysis* species in lakes of Northern Europe and peripheral parts of the brackish Baltic Sea.

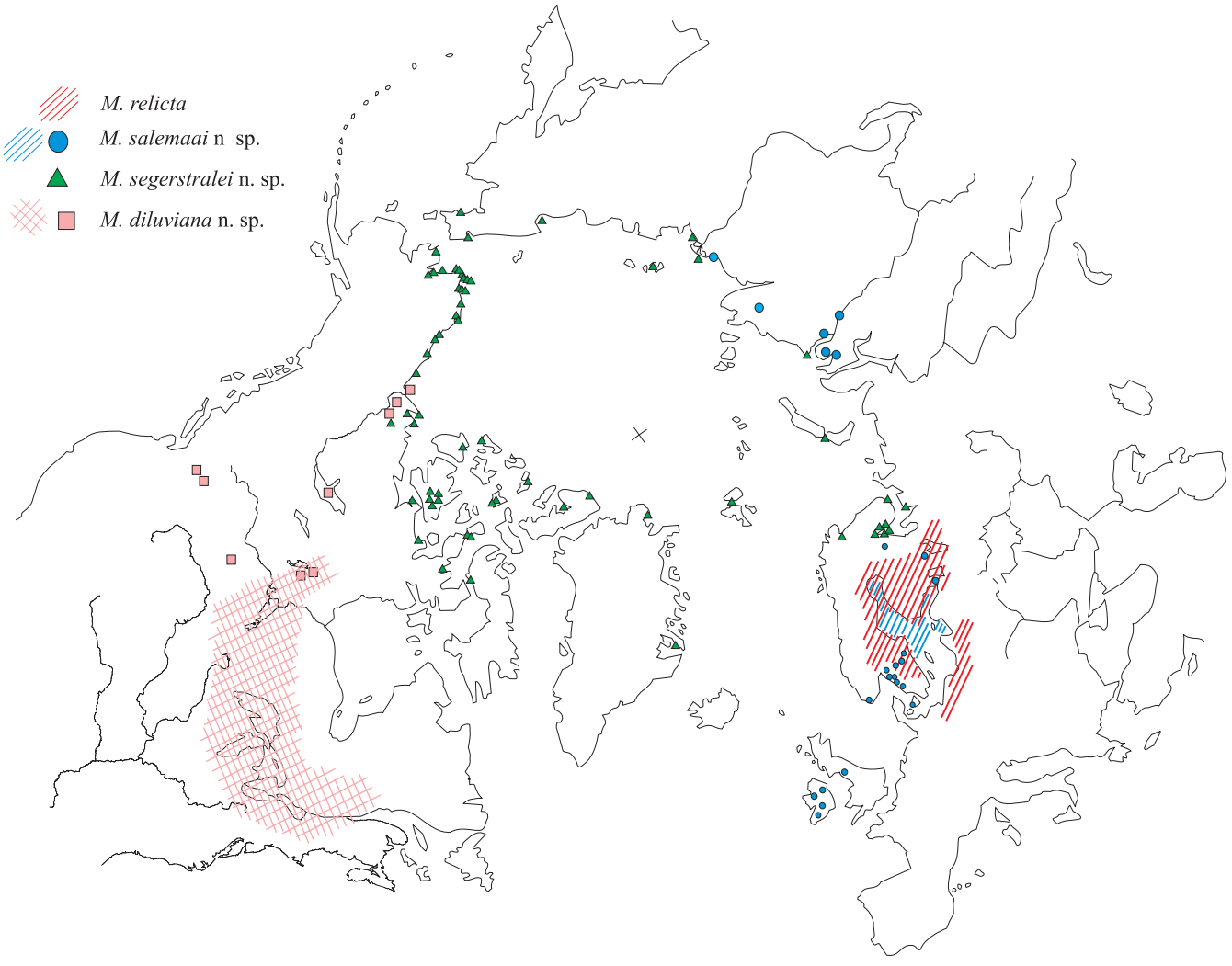


Fig. 4. Circumpolar distributions of the four species of the *Mysis relicta* group. Hatching indicates areas where occurrences of *M. relicta*, *M. salemaai* or *M. diluviana* are common.

M. salemaai inhabits offshore habitats of the Baltic Sea and a range of lakes from the British Isles, Scandinavia and Karelia to coastal northern Siberia. *M. segerstralei* has a circumpolar distribution along the Arctic coasts and islands of Eurasia and North America and also occurs in lakes of these northern regions. *M. diluviana* inhabits continental freshwater lakes of the once-glaciated northern North America. Several contact zones between the four species were identified based on earlier (Väinölä 1986, Väinölä et al. 1994) and the newly obtained data, i.e. sympatric and parapatric occurrences of *M. relicta* and *M. salemaai* in some European lakes and the Baltic Sea, and contact zones (but no sympatric occurrences known so far) of *M. salemaai* and *M. segerstralei* along the European and Siberian arctic coasts, and of *M. segerstralei* and *M. diluviana* in the Mackenzie River delta.

As the four species of the *M. relicta* species group until now were typically treated as a single morphospecies in ecological literature, the question of their monophyly might seem straightforward or even irrelevant. However, neither the allozyme characters (Väinölä 1992, 1995), nor the DNA (COI, CytB, 16S, 18S, ITS2) and morphological data partitions could resolve monophyly of this group when analysed separately (III). The monophyly of the *M. relicta* group was nevertheless supported in a simultaneous analysis of the molecular and morphological data sets and the node was stable under majority of the parameters explored in sensitivity analysis (III). The failure of the morphological data to resolve the monophyly of the seemingly uniform species group should not however imply an absence of phylogenetic signal, but rather reflects a restricted choice of characters suitable for discrete coding. The results confirm the well recognised difficulty of converting mostly quantitative morphological variation into discrete character states, particularly at lower taxonomic levels (e.g. McLeod & Forey 2002).

The importance of methodological aspects of morphological analysis was clearly exemplified by the conflicting results that different approaches gave on the relationships among the four *M. relicta* group species (I, II, III). The quantitative morphometric study (I) indicated distinctness of the coastal *M. segerstralei*, and this species was also suggested as basal to the other *M. relicta* group species in the separate parsimony analysis of morphological data (III) and in allozyme studies (Väinölä et al. 1994). However, the simultaneous parsimony analysis of molecular and morphological data (III) and a broader qualitative morphological assessment (II) supported a close sister group relationship between *M. segerstralei* and *M. salemaai*, while *M. relicta* and *M. diluviana* were considerably more distant. The same was shown by mitochondrial DNA data alone (II, IV), where the *M. salemaai* + *M. segerstralei* complex contained three equidistant and closely (2%) related lineages that did not show a reciprocally monophyletic distribution in the two species, and were contrasted by considerably more diverged *M. relicta* and *M. diluviana* (approx. 8% from each other and from the *M. salemaai* + *M. segerstralei* clade).

4.2. Phylogeny of *Mysis* and history of marine-continental invasions (III, IV)

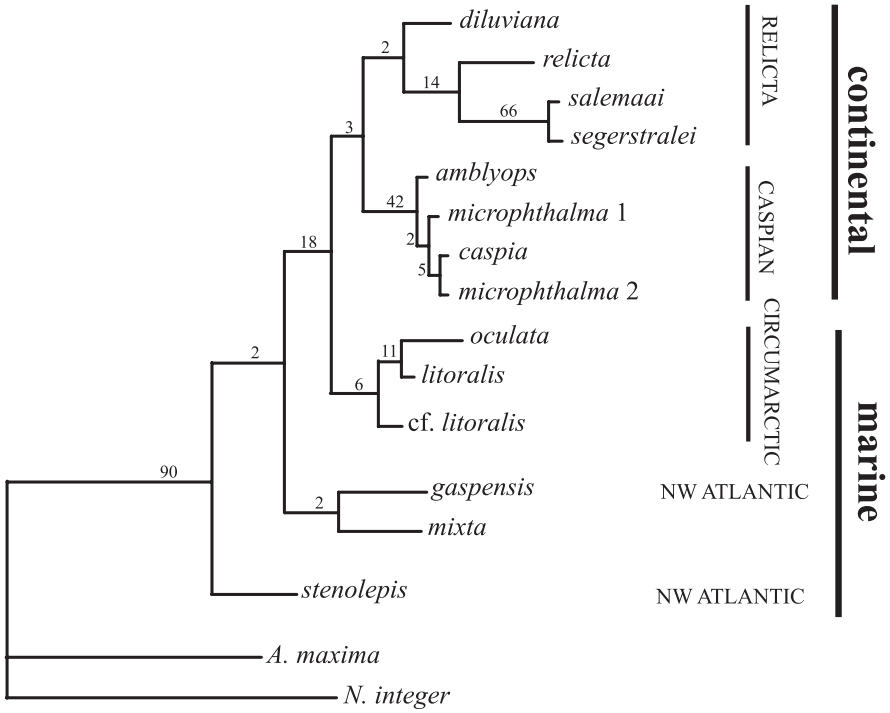
The origins of the primarily arctic marine taxa in circumpolar freshwater lakes and in the deep waters of the continental, brackish Caspian Sea have been a widely discussed

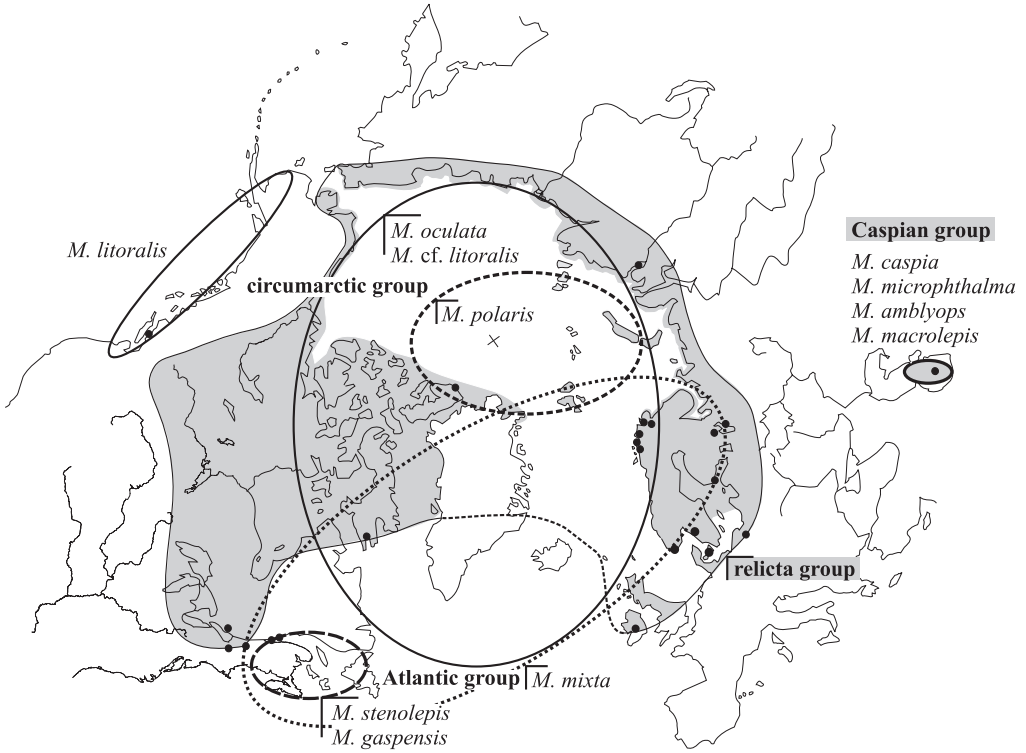
topic in Holarctic zoogeography (e.g. Högbom 1917, Sars 1927, Ekman 1953, Väinölä 1995). The main questions have been: (i) was there a single marine-continental invasion event or several independent invasions, i.e. are the taxa in boreal lakes and the Caspian Sea monophyletic? (ii) when did these continental invasions occur, and were they simultaneous in the different similarly distributed 'relict' genera? (iii) which marine taxa make the sister group ('ancestors') of the continental species?

A simultaneous analysis of 33 morphological characters along with three mitochondrial and two nuclear gene segments supported the monophyly of the continental *Mysis*, i.e. of the *M. relicta* group ('glacial relicts') and the four Caspian Sea endemic species ('arctic immigrants') (Fig. 5). In the direct optimisation analysis a clade of three marine circumarctic taxa (*M. oculata*, *M. litoralis* and a newly disclosed *M.* cf. *litoralis*) was recovered as a sister group to the continental species. Unlike in earlier allozyme character analyses (Väinölä 1992, 1995), the NW Atlantic *M. gaspensis* showed distant relationships to the continental *Mysis*, but grouped closer with another NW Atlantic taxon *M. stenolepis*. As with allozymes, the DNA data however showed deep divergences among the phenotipically relatively uniform *M. relicta* group taxa, but close molecular relationships among the four morphologically diverse endemic Caspian *Mysis* spp. The radiation of the pelagic Caspian *Mysis* flock indeed appears recent, e.g. the entire ITS2 region, often highly variable in interspecies comparisons, was uniform in the analysed Caspian specimens, allozyme loci showed extensive allele sharing (Väinölä 1995), and mtDNA trees did not correspond to the morphological species identities, a pattern congruent with several other cases of mitochondrial-species tree discordance in similar endemic species radiations (Seehausen 2004). Given that the four taxa are largely pelagic, and that no geographic subdivisions of the central and southern Caspian Sea is evident during Pleistocene, the speciation of the Caspian *Mysis* was likely sympatric (Väinölä 1995). Even the stringent condition of resource partitioning, required to infer sympatric speciation (Coyne & Orr 2004), is justified in Caspian *Mysis* species that have different ecologies and food preferences (Bondarenko 1991).

To address the timing of the continental invasions, we must refer to the quantitative molecular divergences and external molecular clock assessments. If the widely cited rate calibration of the invertebrate mitochondrial COI gene at approx. 2–4% per Myr is applicable to *Mysis* (Knowlton & Weigt 1998, McCartney et al. 2000, Wares & Cunningham 2001, but see IV), the split between the circumarctic *M. relicta* species group and the Caspian clade (approx. 13% K2P+Γ corrected divergence, Table 2) could be of 3–6 Myr age, an estimate which also falls within the range suggested from allozyme data (Väinölä 1995). However, so far we lack the evidence for corresponding hydrographic connections that could have allowed immigration of northern taxa into the Caspian basin in those times.

This estimate does not fit either of the earlier proposed time periods, i.e. major Middle and Late Pleistocene glaciations (< 1 Myr) (Arkhipov et al. 1995), generally considered as the agent of continental dispersals (Högbom 1917, Hutchinson 1967, Segerstråle 1982) or mid-Tertiary (ca 30–40 Myr) connection between the current Arctic and the Ponto-Caspian basins (Holmquist 1959). With this in mind, and considering disparate arctic – Caspian divergences in other similarly distributed taxa


diluviana
relicta
salemaai
segerstralei
amblyops
microphthalma 1
caspia
microphthalma 2
oculata
litoralis
cf. litoralis
gaspensis
mixta
stenolepis
A. maxima
N. integer
2
14
66
3
42
2
5
18
11
6
2
90
2
RELICTA
CASPIAN
CIRCUMARCTIC
NW ATLANTIC
NW ATLANTIC
continental
marine


M. litoralis
M. oculata
M. cf. litoralis
circumarctic group
M. polaris
Caspian group
M. caspia
M. microphthalma
M. amblyops
M. macrolepis
relicta group
Atlantic group
M. mixta
M. stenolepis
M. gaspensis

(Table 2), the parsimonious biogeographical hypothesis of a single continental invasion remains poorly corroborated, even if the monophyly of the *Mysis* taxa involved is supported. The same applies to the group of four circumboreal 'glacial relict' taxa as well. On the one hand, the topological relationships among the *M. relicta* group species, inferred by the simultaneous analysis (III), and supported by morphological, molecular and ecological data (II, IV), were in line with the more general pattern of circumpolar zoogeography, i.e. a principal split between the North American continental taxa (*M. diluviana*) versus those in Eurasia, Beringia and the American Arctic (*M. relicta*, *M. salemaai*, *M. segerstralei*) (Bernatchez & Dodson 1994, Kontula & Väinölä 2003, Van Houdt et al. 2005). On the other hand, in quantitative terms ('molecular age') the Nearctic-Palearctic divergences are not concordant among different groups (Table 2). Although a directly comparable molecular clock should not be expected for all the different groups (also see IV), the wide discrepancies still suggest that colonisations to the different continental areas – to the Caspian Sea and to the lakes of the Nearctic and Palearctic – were independent and asynchronous both among different 'relict' genera and within the *M. relicta* group itself. The two stenohaline *Mysis* species, *M. relicta* and *M. diluviana*, have likely lived in freshwaters throughout most of the Pleistocene and independently colonised their ranges in Europe and North America respectively (Väinölä et. al. 1994, II, III). The separation of *M. salemaai* and *M. segerstralei* and continental European invasion of *M. salemaai*, in contrast, took place more recently and its sympatric occurrences with *M. relicta* in the Baltic Sea basin (Väinölä & Vainio 1998, II) represent a secondary contact established in a course of range perturbations during the Quaternary (III, IV). Finally, the coastal circumarctic *M. segerstralei* is zoogeographically not identifiable with the other true continental 'glacial relicts'. This taxon may best represent the continuity of a lineage that survived in diluted northern waters through the Pleistocene ('the marine ancestor'); its current occurrence in coastal lakes is associated with recent marine inundations, similarly known from other coastal marine species (Holmquist 1959, 1973, Mauchline 1980).

In the methodological aspect of the phylogenetic study (III) the direct optimisation analysis generally gave a better resolution than analysis with *a priori* alignment, but overall no strong incongruence was found between the two approaches. The simultaneous analysis of the data partitions was important for the resolution of overall *Mysis* relationships, as different data sets contributed to different nodes. Particularly evidence for the monophyly of the continental *Mysis* taxa and that of the *M. relicta* group was mostly from the 'hidden' support, not seen in separate analyses. In terms of total contribution to the resolution (partitioned Bremer support values), mitochondrial protein coding data appeared to contain most phylogenetic signal (83%). However, the support from these genes was concentrated just on three nodes and grouped taxa where cyto-nuclear discordance, due to intro-

Fig. 5. Phylogeny of *Mysis*. Simultaneous analysis topology from the direct optimisation parsimony analysis of molecular and morphological characters (Bremer support values above the branches), and distributions of the species with sampling sites indicated (for individual relicta group species, see Fig. 4).

Table 2. Mitochondrial sequence divergences (K2P+Γ corrected mean divergence, %) among main zoogeographical zones in arctic continental elements and among different basins of the Ponto-Caspian area. Abbreviations: PAL = Palearctic 'glacial relict' taxa; NEA = Nearctic 'glacial relict' taxa; CAS = Caspian taxa; ARC = arctic marine taxa; BLA = Black Sea populations; AZO = Azov Sea population; – = no data available; * = no natural occurrences in the region.

Arctic elements	**PAL-ARC**	**CAS-ARC**	**PAL-CAS**	**PAL-NEA**
Mysis (COI)	14.0	14.7	13.2	11.5
Gammaracanthus amphipods (COI)[1]	17.5	5.5	17.0	*
Phoca seals (COI+COII+CytB)[2]	0.0	3.5	3.5	*
Myoxocephalus quadricornis fish (CytB+ATPase)[3]	0.5	*	*	0.9
Coregonus lavaretus fish (mtDNA, RFLP)[4]	*	*	*	1.0

Group	**Ponto-Caspian species**	**BLA-CAS**	**BLA-AZO**	**AZO-CAS**
Mysida	*Limnomysis benedeni*	2.0	1.0	1.7
	Paramysis baeri (s. l.)	0.6 (**12**)	0.5	0.6 (**12**)
	Paramysis ullskyi	–	–	1.3
	Paramysis lacustris	1.7	4.9	5.1
	Paramysis kessleri	**0.6**	*	*
Amphipoda	*Pontogammarus robustoides*[5]	3.9	–	–
	Pontogammarus maeoticus[5]	**15.7**	–	–
	Obesogammarus crassus[5]	**2.5**	–	–
	Echinogammarus ischnus[6]	8.3	–	–
Cladocera	*Cercopagis pengoi*[5]	1.3	–	–
	Cornigerus maeoticus[5]	1.5	–	–
	Podonevadne trigodna[5]	1.5	0.0	1.5
Bivalvia	*Cerastoderma glaucum*[7]	**0.0**	*	–
	Dreissena rostriformis[8]	**0.5**	*	*

Mysida data are from this study. Data from other taxa are indicated by superscript numbers that refer to [1]Väinölä et al. (2001); [2]Palo & Väinölä (2006); [3]Kontula & Väinölä (2003); [4]Bernatchez & Dodson (1994), [5]Cristescu et al. (2003); [6]Cristescu et al. (2004); [7]Nikula & Väinölä (2003); [8]Therriault et al. (2004). For Ponto-Caspian taxa all data came from the cytochrome c oxidase I (COI) gene. Comparisons that involve only central and southern Caspian populations are indicated **in bold** figures.

gression or incomplete lineage sorting, appeared likely, i.e. the Caspian *Mysis* spp., *M. oculata* - *M. litoralis*, and *M. salemaai* - *M. segerstralei* clades (III, IV). Overall, the proportion of nodes likely showing mitochondrial tree – species tree discord in *Mysis* (about 25%) was similar to the average in other invertebrate taxa (Funk & Omland 2003), which again underlines the limitations of mitochondrial genes as simple taxonomic tools (DNA barcoding, Hebert et al. 2003). As regards phylogenetics,

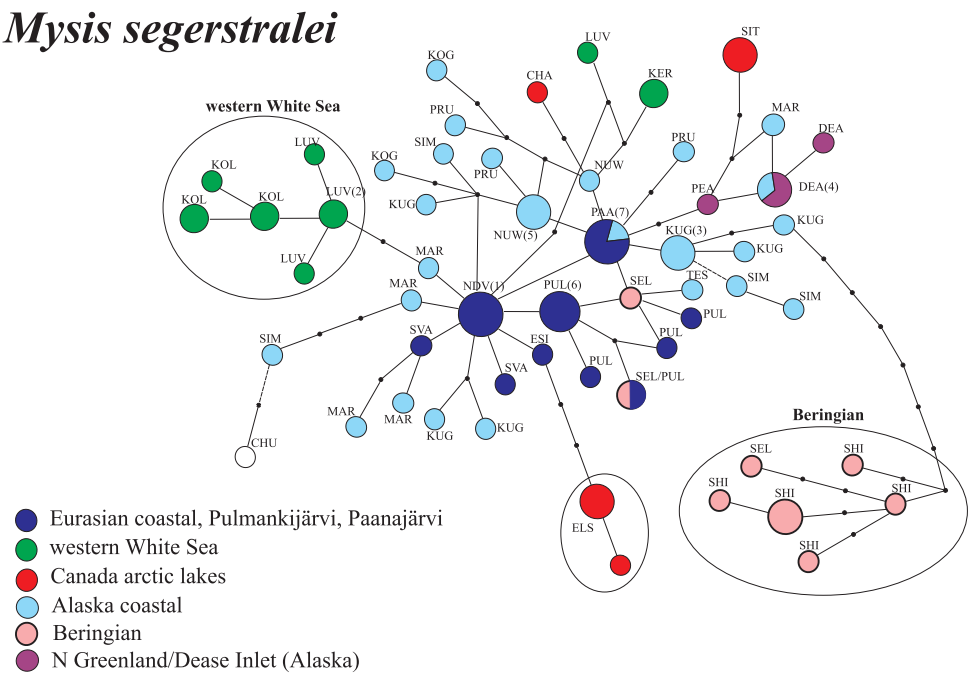
whenever several sets of nuclear and mitochondrial markers are available, data exploration should allow identifying cyto-nuclear conflicts and is deemed crucial in justly presenting the information content and possible misleading signals in the data (Lecointre & Deleporte 2004, but see Grant & Kluge 2003). In *Mysis* such cyto-nuclear incongruence however generally seems to concern sister taxa and would not seriously affect the topology.

4.3. Phylogeographies of a European and a circumarctic mysid: strong regional but weak global structures and fast rates of mtDNA evolution (IV)

It may be theoretically predicted that taxa that have invaded formerly glaciated areas will have reduced levels of genetic diversity and be characterised by few widespread dominant haplotypes, as typical for populations undergoing extensive demographic and range fluctuations (Avise 2000, Hewitt 2000). Indeed phylogeographic studies have generally revealed decreasing levels of genetic diversity towards the north and considerably larger dispersal distances in the northern latitudes (Bernatchez & Wilson 1998, Taberlet et al. 1998, Hewitt 2004). Many aquatic organisms, particularly fishes, seem to have colonised newly deglaciated areas from just a few glacial refugia, their long-distance dispersal facilitated by vast periglacial lakes (Bernatchez & Dodson 1994, Brunner et al. 2001, Hewitt 2004, Van Houdt et al. 2005). For the North European aquatic fauna, the two main refugia most likely were in eastern periglacial lakes and in the North Sea basin and its rivers (Nesbø et al. 1999, Koskinen et al. 2000, Kontula & Väinölä 2001, Nilsson et al. 2001, Vainio & Väinölä 2003, Tonteri et al. 2005). Much less is known about maintenance and structuring of genetic diversity in arctic coastal taxa, particularly on broader circumarctic scales (Weider & Hobaek 2000, Hewitt 2004). Cases of low genetic variability and of considerable allelic richness have both been reported among such organisms, and instances of strong geographic subdivision are contrasted by others with little structure (Bernatchez & Dodson 1994, Weider et al. 1999, Brunner et al. 2001, Hewitt 2004).

Mitochondrial phylogeographies of the European *M. salemaai* and the circumarctic coastal *M. segerstralei* showed a number of deviations from the general expectation. Both species showed high levels of mitochondrial diversity ($h \approx 0.98$; $\pi \approx 0.90\%$) and were characterised by strong regional, but little global genetic structuring. The mtDNA variation of *M. salemaai* showed two unexpected patterns: (a) clear subdivision among six post-glacially (12 kyr) isolated Scandinavian lakes, with monophyletic haplotype groups in each of them, and (b) an even further elevated molecular diversity in four Irish populations, where all analysed specimens ($N = 22$) had unique haplotypes on average 1.1% divergent from each other (Fig. 6). At the same time, no deeper structuring was evident across the studied North European range, and the mean coalescence depth of all Scandinavian haplotypes was only about twice that observed in the post-glacial intra-lake groups.

With the lack of broader structure, the mtDNA of *M. salemaai* failed to corroborate a subdivision between Irish and Scandinavian populations, which was earlier observed in allozyme data and was proposed to reflect a late-glacial disjunction between refugia in the Irish Sea and eastern periglacial lakes respectively (Väinölä et al. 1994). Moreover, while the small allozyme



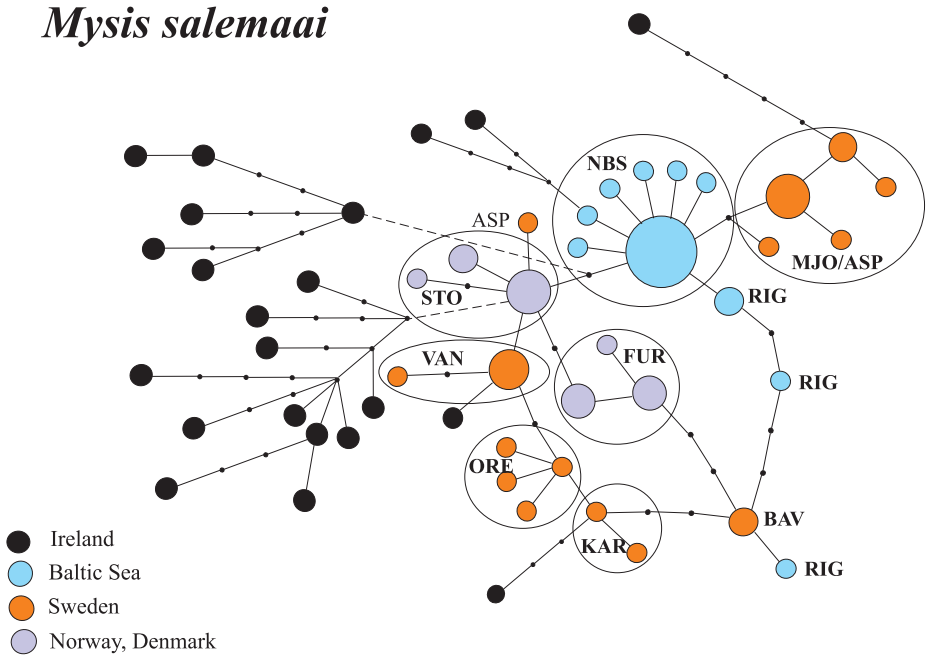


Fig. 6. Statistical (95%) parsimony networks of *Mysis segerstralei* and *Mysis salemaai* mitochondrial haplotypes (approx. 620 bp of COI).

differentiation among Scandinavian lakes ($F_{st} \approx 0.05$) suggested substantial post-glacial effective population sizes of the order of 100 000, the intra-lake monophyly of mitochondrial haplotype groups would imply small effective female population sizes of less than 6 000 individuals. As the mysid sex ratios are typically 1:1 and genetically determined at least in *Mysis* (Mauchline 1980, Väinölä 1998), it is likely that factors other than neutral genetic drift have affected diversities of either of the molecular characters.

In all, mtDNA data *per se* could not confidently identify broader geographical subdivisions in the *M. salemaai* data and so refute the hypothesis of a single European refugium. Yet considering post-glacial colonisation pathways to areas as distant as the Baltic Sea, Southwest Scandinavia and Ireland, neither of the earlier proposed refugia, i.e. eastern periglacial lakes or the Irish Sea appears as a likely candidate. We may propose instead that the geographically intervening North Sea basin would have been the main refugium for *M. salemaai* during the last glaciation – the area that also contributed to post-glacial colonisation of a number of fishes (e.g. Bernatchez 2001, Säisä et al. 2005). Yet the eastern occurrence of *M. salemaai* in a Karelian lake presumably directly derived from the periglacial lakes and not accessible from the Baltic Sea (Väinölä et al. 1994; Fig. 4) indicates that the species did exist also in an eastern refugium, a hypothesis that remains to be tested.

The mitochondrial variation of the circumarctic coastal *M. segerstralei* showed a similar general picture with a few distinct regional clades superimposed on a pattern of little global structure (Fig. 6). A deeply diverged lineage was identified in the Beringian region, and can be associated with the isolation by the repeatedly established Beringian land bridge. Distinct local groups were also found in the western White Sea region and in some Canadian arctic lakes – these clades could have been related with local late glacial refugia that however did not contribute to the broader circumpolar *M. segerstralei* diversity. In contrast, little structure and closely related haplotypes in coastal European, Siberian and Alaskan populations suggest extensive post-glacial gene flow across the entire arctic region, plausibly from the unglaciated Alaskan-North American coasts towards the formerly glaciated European arctic. This is also in line with inferences of similar late- or post-glacial trans-arctic (Pacific/Beringian/NE Atlantic to Barents/White Sea) dispersal suggested for *Macoma* bivalve molluscs, Pacific herring, and Atlantic salmon (Väinölä 2003, Jørstad 2004, Makhrov et al. 2005).

Estimation of the mitochondrial COI substitution rate, based on the observed intra-clade diversities in monophyletic haplotype groups in individual post-glacially isolated *M. salemaai* lakes suggested a rate of 0.27% per 10 kyr (27% per Myr), i.e. about 10 times faster than the widely used 1–4% per Myr estimates obtained from sister-species comparisons (Brown et al. 1979, Fleischer et al. 1998, Knowlton & Weigt 1998, McCartney et al. 2000, Wares & Cunningham 2001, Kontula et al. 2003). The estimated rate was in fact similar to that reported from pedigree-level mutation accumulation studies (Denver et al. 2000), assumed to indicate strong purifying selection in natural populations even at silent sites. It may be possible that calibration points used in phylogenetic comparisons do not allow identification of initial fast accumulation of substitutions and cannot be extrapolated to date recent diver-

gences. On the other hand, the fast COI rate in *M. salemaai* might be caused by other reasons (e.g. effects of population size and selection) and requires further studies for broader inferences.

4.4. What factors define visual adaptations in two 'glacial relict' mysids? (V)

It is generally assumed that vision in animals is well adapted to the light characteristics of their environments (Lythgoe 1979, Johnson et al. 2002). In aquatic habitats with small and high amount of organic matter the wavelength of the dominant light can vary from 470 to 700 nm respectively (Kirk 1994) and the spectral sensitivities of organisms living in these conditions are expected to be adapted accordingly. On the other hand, the evolution of vision, just as of any other adaptive trait, is affected by a number of limitations, including functional constraints on the permissible amino-acid changes, physiological trade-offs, efficiency of selection in populations of finite size and phylogenetic history of species and populations (Donner et al. 1990, Douglas & Partridge 1997, Archer et al. 1999, Yokoyama 2000). The interplay of selection and constraints is not well understood, particularly because physiological studies tend to interpret the observed patterns as *a priori* adaptive (e.g. Lythgoe 1979). Well dated isolation of lakes and populations of 'glacial relict' *Mysis*, and variable light conditions in these habitats therefore provide a good model system to explore visual adaptations on post-glacial time scales. Indeed, pronounced difference in spectral sensitivities between two populations from different light environments was taken as an evidence for a rapid post-glacial evolutionary adaptation to the long-wave dominant light environment in a dark humic water lake (Lindström & Nilsson 1988). A difference in spectral sensitivities was also found between inshore and offshore *Mysis* populations in the Baltic Sea that likely represented the two sibling species – *M. relicta* and *M. salemaai* (Lindström 2000). However, as the two species also came from different light environments, the effect of environment versus genetically determined species-specific characteristics remained unclear.

In this study spectral sensitivities were first compared between the two different species *M. relicta* and *M. salemaai*, probably reproductively isolated for at least a million years (Väinölä 1986, II) and currently living in the same (sympatric) or in different (parapatric, allopatric) light environments. Although both *M. relicta* and *M. salemaai* are known from fresh and brackish waters, their different salinity preferences and broad-scale distributions suggest long-term evolution in different salinity regimes (Väinölä et al. 1994, Väinölä & Vainio 1998, II, IV). It could be therefore expected that the more strictly freshwater *M. relicta* should have peak sensitivity at longer wavelengths, as characteristic for species from freshwater habitats with generally higher amounts of organic matter (Jerlov 1976, Lythgoe et al. 1994, Johnson et al. 2002). The results from the Baltic Sea populations of the two species indeed conformed to the prediction: regardless of the current light conditions *M. relicta* had maximum sensitivity at approx. 20 nm longer wavelengths than *M. salemaai*, with estimated wavelength of peak sensitivity $\lambda(S_{max})$ at approx. 565 nm and approx. 545 nm respectively (Fig. 7).

In the second part of the study, spectral sensitivities were compared among four post-glacially isolated populations of *M.*

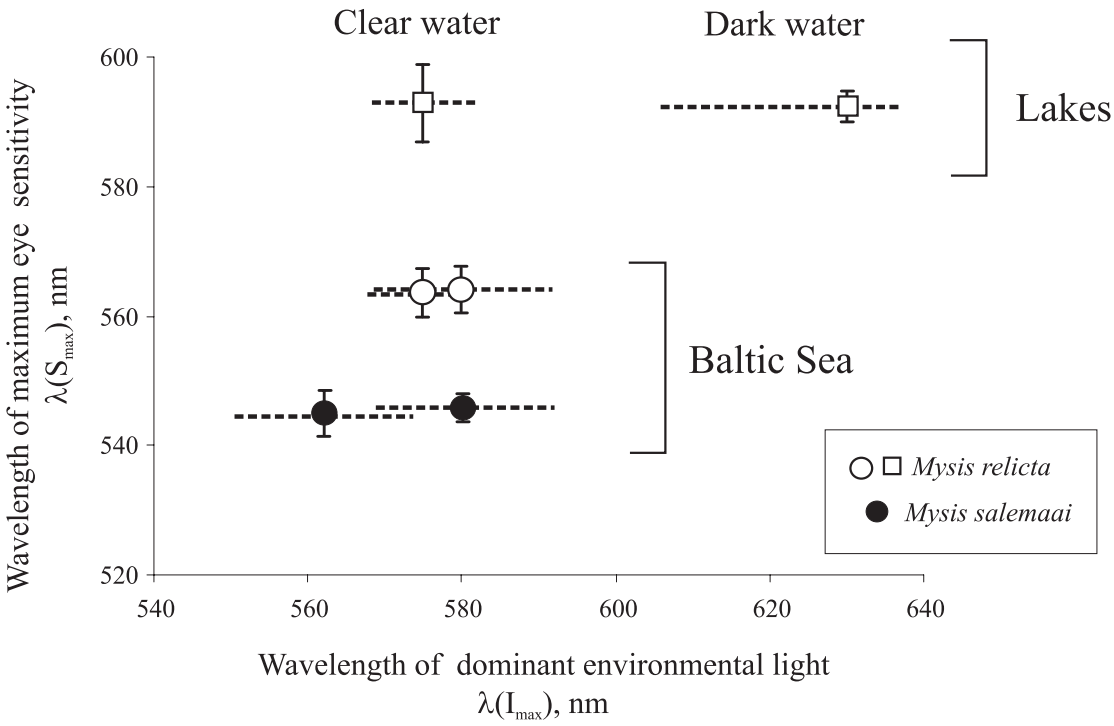


Fig. 7. Wavelength of the maximum spectral sensitivity, $\lambda(S_{max})$ (mean ± SE) of *M. relicta* and *M. salemaai* from six studied populations plotted against the wavelength (peak and range) of the maximally transmitted light in their environments, $\lambda(I_{max})$.

relicta, currently exposed to strongly different light environments. The four populations are likely derived from a single periglacial refugium and isolated for ca 10 kyr (Väinölä et al. 1994). The observed spectral sensitivities however did not follow the expectations in this case. The two lake populations, living in clear and in dark water, were both characterised by a peak sensitivity at longer wavelengths ($\lambda(S_{max}) \approx 590$ nm) that differed clearly from those in the Baltic Sea ($\lambda(S_{max}) \approx 563$ nm) (Fig. 7). The results may suggest that, in contrast to the earlier hypothesis, the long-wave sensitivity is not a post-glacial adaptation, but an ancestral state inherited from periglacial populations, from which both analysed lakes were isolated in the early stages of deglaciation (ca 10 kyr ago). Consequently, the short-wave shift must have occurred later in the Baltic Sea.

The study thus demonstrates that information on the current light environment is not sufficient to explain differences in spectral sensitivities of *Mysis* species and populations and proposes that internal genetic constraints or other environmental factors may affect the rates of adaptation. At the same time, however, a large difference in $\lambda(S_{max})$ between recently isolated Baltic vs. lacustrine *M. relicta* shows that spectral sensitivity shifts can occur relatively fast. Given that the populations in the Baltic Sea and those of each lake have been isolated

from each other for ca 10 kyr, a fundamental question remains unanswered – why has the spectral sensitivity changed in the Baltic Sea, but not in the clear water lake? From the physiological point of view, other things being equal, the short-wave sensitive visual pigments are more advantageous as they are inherently less 'noisy' (Donner et al. 1990); thus the long-wave sensitivity in the clear water lake does not appear adaptive.

4.5. Mysid vicariance-dispersal history among subdivisions of the Ponto-Caspian basin (VI)

The brackish-water fauna of the Ponto-Caspian region has recently come into scientific focus for three reasons: (i) interest in the origin and diversity of endemic species in ancient lakes, such as the Caspian Sea (Martens 1997, Goldman 2003, Schön & Martens 2004); (ii) revived interest in the contrasting views on the biogeography of the Ponto-Caspian region itself (Ekman 1953, Mordukhai-Boltovskoi 1979, Cristescu et al. 2003); (iii) the accelerated flow of human-mediated invasions of Ponto-Caspian species into European and North American aquatic ecosystems and the attempts to apply molecular characters to identify their sources (Cristescu et al. 2001, Leppäkoski et al. 2002). As with the case of 'glacial relicts', the Ponto-Caspian biogeographic arguments centre around (i) the relative importance of vicariance and dispersal events and two alternative temporal scales proposed to explain origin of the disjunct populations, i.e. Tertiary ('Sarmatian relicts' 10–5 Myr) and Late Pleistocene (< 0.1 Myr), and (ii) the congruence of cladogenies and depths of divergence among various co-distributed taxa, e.g. mysids, amphipods, cumaceans, and gastropod and bivalve molluscs.

The comparative phylogeography of seven mysid taxa distributed across the Ponto-Caspian region (comprising the Black, Azov and Caspian Sea basins) revealed highly variable patterns of divergence and suggested that both historical paleogeographical factors and the ecological characteristics of organisms have been important in shaping their molecular diversity. Generally, both the ancient vicariance and recent dispersal scenarios were supported for different species. Three main phylogeographic patterns were distinguished in the data: (a) no deep subdivisions across the entire Ponto-Caspian region; (b) genealogical splits matching geographical borders between the Black + Azov vs. Caspian seas, or between all the three basins; (c) little differentiation among Black + Azov and the northern Caspian Sea, but divergent lineages within the Caspian itself. At least two cryptic species within the Caspian Sea were identified in what was initially treated as *Paramysis baeri*, as demonstrated by approx. 9% uncorrected COI divergence and specific morphological characteristics.

Almost no differentiation across the Ponto-Caspian basin was found in *Paramysis kessleri*. In contrast, the widespread and stenohaline (< 2–3‰ upper salinity limit) *P. lacustris* exhibited strong structuring among the three basins and among the Black Sea rivers. Particularly the Azov Sea clade was distinct from those in the Caspian and Black seas, with COI divergence of about 5%, but no consistent morphological differentiation was recorded to corroborate taxonomic distinction. For this taxon the data thus supported the importance of long-term refugia throughout the Pleistocene period, but not as ancient as the Sarmatian. In other mysid species however no subdivision between Black and Azov sea

populations was recorded. The Azov-Black sea divergence in the stenohaline *P. lacustris* but not in more euryhaline taxa suggests that even during the most desalinated phase of the Black Sea the salinity was too high (> 3–4‰) for inter-refugial exchange in *P. lacustris* (cf. Ryan et al. 1997, Mudie et al. 2002).

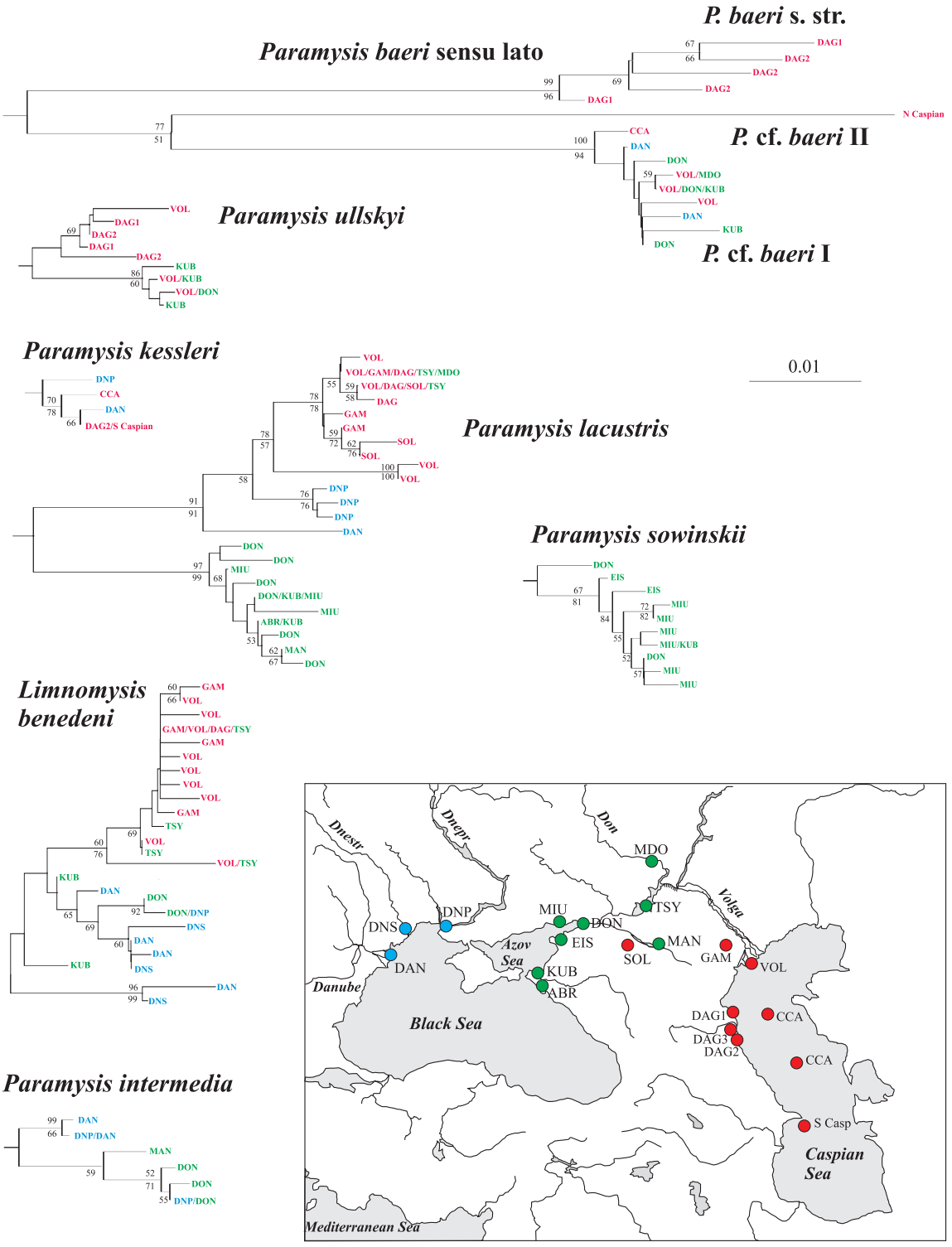
Most mysid species had more or less distinct Black/Azov and Caspian Sea lineages (Fig. 8), a pattern also reported in cladoceran and amphipod crustaceans (Cristescu et al. 2003). However, the geographical distribution of these lineages did not always match the borders among the basins, e.g. in *P. baeri* (*sensu lato*) and *P. ullskyi* the Black/Azov lineage was also found in the delta of the Volga River, draining to the Caspian. Overall, the Ponto-Caspian mysid phylogeography was characterised by discordant depths of molecular subdivisions among co-distributed taxa; this was even more evident when other invertebrate taxa were included in the comparison (Fig. 8; Table 2). The data thus add support to the more general observation that similar phylo- and biogeographic patterns have been formed at completely different times (e.g. Donoghue & Moore 2003). Evidently, in some species gene flow occurred recently across large parts of the Ponto-Caspian region, while populations in other taxa remained isolated. Ecological characteristics of species might have played an important role in shaping these migrations. In case of mysids, species that showed least amount of differentiation among the recently connected Black, Azov and northern Caspian seas, were also those with largest body sizes, and partly known for their ability to disperse upstream (Buchalova 1929, Mordukhai-Boltovskoi 1957).

The mitochondrial phylogeographies disclosed a wave of recent migrations of Caspian *P. lacustris* and *Limnomysis benedeni* to the Azov Sea basin through the Volga-Don canal that was opened in 1950s. The inferred direction of migration was opposite from the proposed dominant invasion flow into the Caspian Sea (Grigorovich et al. 2003). The data also have implications for attempts to trace the origins of recent Ponto-Caspian invaders in European waters using mitochondrial genes (e.g. Cristescu et al. 2001). Such approaches should be efficient in *P. lacustris* and partly in *L. benedeni*, which showed geographically well-structured mitochondrial diversity among basins. In other taxa however mitochondrial data will provide no power to distinguish most of the Caspian, Azov and Black Sea populations.

5. CONCLUSIONS

This thesis has applied combined inferences from molecular, morphological, and physiological data to study factors that have defined the diversity and distributions of continental mysids in two main zoogeographical groups: the continental elements of the primarily arctic marine genus *Mysis,* and the endemic Ponto-Caspian brackish-water taxa. The results are partly in line with earlier data and with theoretical predictions, but the work has also revealed a number of new unexpected patterns that call for further investigations.

Despite the long research tradition on the focal taxa, the application of molecular characters disclosed new cryptic species diversity in both *Mysis* and *Paramysis* genera (III, VI), a typical outcome in modern molecular systematic work. A more detailed analysis also demonstrated morphological differences among the four *Mysis relicta* group species earlier only identified


P. baeri s. str.
Paramysis baeri sensu lato
DAG1
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DAG2
DAG2
DAG1
N Caspian
P. cf. baeri II
CCA
DAN
DON
VOL/MDO
VOL/DON/KUB
VOL
DAN
KUB
DON
P. cf. baeri I
Paramysis ullskyi
VOL
DAG1
DAG2
DAG1
DAG2
KUB
VOL/KUB
VOL/DON
KUB
Paramysis kessleri
DNP
CCA
DAN
DAG2/S Caspian
VOL
VOL/GAM/DAG/TSY/MDO
VOL/DAG/SOL/TSY
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Dnepr
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Volga
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Azov Sea
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MAN
SOL
GAM
VOL
DAN
KUB
ABR
Danube
Black Sea
DAG1
CCA
DAG3
DAG2
CCA
S Casp
Caspian Sea
Mediterranean Sea

on molecular grounds (I, II) and this also turns out to hold for the newly discovered *Mysis* cf. *litoralis* (III) and *Paramysis* cf. *baeri* (VI). In all, a combined appraisal of molecular and morphological differences gave the best insights into delimitation of species boundaries and phylogenetic resolution (I, II, III, VI). For instance, only in a simultaneous parsimony analysis of molecular and morphological characters was clear support obtained for the main hypotheses of the monophyly of the *Mysis relicta* group, that of all continental *Mysis* (*M. relicta* group and Caspian endemics), and recognition of the sister group of the continental taxa in a clade of three (circum)arctic marine species. However, even with seven data sets of molecular and morphological characters, parts of the intra-generic phylogeny remained poorly resolved; for instance, the monophyly of the continental *Mysis* species, although stable, remained weakly supported.

The relationships among the four *Mysis relicta* group species, inferred from the morphological, molecular, and ecological data, were in line with other similarly distributed taxa in having a principal split between Nearctic continental versus Palearctic continental and Holarctic coastal taxa (II, III). The molecular divergence and taxonomic status (intra- to inter-specific) of the Nearctic and Palearctic taxa, however, were not congruent (Table 2) and suggested asynchronous marine-continental colonisations in different 'glacial relicts' and within the *M. relicta* group itself (III, IV). The stenohaline European *M. relicta* and the North American *M. diluviana* are morphologically and molecularly distinct species (I, II, III) that probably existed in freshwaters of the two continents throughout most of the Pleistocene. The euryhaline *M. salemaai* and *M. segerstralei* were more closely related (II, III, IV), which implies a relatively recent colonisation of European freshwaters by the 'glacial relict' *M. salemaai* where it has met the earlier coloniser *M. relicta*.

Post-glacial isolation of 'glacial relict' mysid populations in Fennoscandian lakes provided a model system to analyse patterns of divergence on a Holocene time scale, which in turn brought up a number of unexpected results concerning molecular and physiological evolution (IV, V). The mitochondrial phylogeography of *M. salemaai* (IV) revealed strong genetic structuring among Scandinavian lakes with monophyletic haplotype groups in each of them. The inferred effective population sizes were about 10 times smaller than estimates from nuclear allozyme data, whereas estimates of COI divergence rate were about 10 times greater than the commonly used 1–4% Myr^{-1}. This could mean that molecular clock calibrations based on sister-species divergences fail to account for the initial accumulation of mutations at the intraspecific level and are not suitable for dating recent events. In contrast to clear inter-lake subdivisions, no deeper structuring could be seen in *M. salemaai* that would enable inferences about distinct long-term glacial refugia, typically disclosed in other aquatic species of Northern Europe. The hypothesis of a single refugium for the analysed NW European *M. salemaai*, possibly in the North Sea basin, was not rejected. High molecular diversities and a weak glo-

Fig. 8. Neighbor-joining trees (K2P+Γ distance) of seven Ponto-Caspian mysid taxa based on approx. 600 bp of mtDNA COI gene. A map of the sampling sites in the Ponto-Caspian region is included.

bal structure were also observed in the circumarctic coastal *M. segerstralei* (IV), suggesting that the last glaciation facilitated long distance dispersals across the circumarctic coasts.

The variation in vision properties of *Mysis* eyes showed that species and population histories could also have influenced genetic factors underlying likely adaptive physiological traits. Current light conditions were not sufficient to explain spectral sensitivities of *Mysis* at the inter- and intra-specific levels (V). In the Baltic Sea, the two species *M. relicta* and *M. salemaai* showed consistent spectral sensitivity differences in sympatry and in parapatry. *M. relicta* populations in two lakes however showed peak sensitivities at longer wavelengths than conspecifics from the Baltic Sea, regardless of whether the spectral properties of ambient light in lakes were different from the Baltic. Thus, in contrast to earlier inferences of fast adaptive evolution of long-wave sensitivity in dark-water environments, the long-wave sensitivity could be an ancestral state inherited from precursors in periglacial lakes from which both lake populations were isolated in the early stages of deglaciation. However, it remains unclear why the visual characteristics would subsequently have changed in the clear water Baltic Sea, but not in the clear water lake.

While historical factors, such as paleogeography and population history, have clearly played an important role in shaping current distributions and adaptations of mysids, the ecological characteristics of the animals, particularly salinity tolerance and vagility, in turn have controlled their response to paleoenvironmental conditions. The importance of species ecology was well demonstrated in Ponto-Caspian mysid phylogeography, characterised by a startling lack of congruence in the extent of intra-specific molecular differentiation among co-distributed taxa (VI). Disjunct brackish water populations in the Black, Azov and Caspian seas contained nearly identical mitochondrial haplotypes in some mysid species, but were approx. 1–5% divergent in others. Overall, most taxa did exhibit two main genealogical lineages matching a long-term isolation in Black/Azov and Caspian Sea basins, yet the boundaries between the clades and geographical regions did not always match. Comparative phylogeography of seven mysid taxa and other co-distributed invertebrates suggested a complex pattern of vicariance, dispersals and extinctions in separate species at different temporal scales.

The lack of genealogical subdivisions across a circumarctic range (IV) or the Ponto-Caspian region (VI) in some species of mysids – organisms that are considered to be poor dispersers – showed that long-distance migrations have been common and must be considered as an important factor in biogeography. Further, the lack of congruence in extent of molecular divergence among co-distributed zoogeographic elements, both 'glacial relicts' and Ponto-Caspian taxa, appeared more the rule than an exception (Table 2). The observations suggest that either the rates of molecular evolution vary tenfold in different taxa or that similar zoogeographical patterns have been created repeatedly at different time scales and reflect not zoogeographic congruence, but pseudo-congruence. To evaluate the earlier biogeographic hypotheses, neither any particular period in middle or late Tertiary (30–3 Myr) nor Late Pleistocene (< 0.1 Myr) was supported as an important period of mysid species diversification. Speciation has been a continuous and still on-going process. Furthermore, the low correlation between the amount

of molecular vs. morphological differentiation in *Mysis* implied that, at least at the intra-generic level, divergence in molecular and morphological traits may proceed in an uncoupled manner. Information from either one of the characters is therefore not sufficient to predict the level of variation in the other. Objective assessment of morphological differentiation, however, poses a number of methodological challenges, especially at lower taxonomic levels where phenotypic variation is largely polymorphic and quantitative (I, II, III). Pitfalls of mitochondrial DNA characters are, on the other hand, shown in the frequent mitochondrial-species discord (approx. 25% of *Mysis* species, III, IV). Overall, the results support the recognised, but inconvenient fact that well-grounded taxonomic and phylogenetic inferences require extensive data, with respect to both the characters and the specimens assessed.

6. ACKNOWLEDGEMENTS

Firstly my thanks are undoubtedly for Risto for his supervision of my work and for the support given in so many ways. Thanks for taking time and interest to answer naïve questions of a beginner 'Mysis relicta zealot' from Vilnius, for the possibility to come to Helsinki, where I have got a rather different view of what science is. Despite the inevitable ups and downs along the road to PhD, I have had no doubts that joining your group in Helsinki was the best thing that I could have done at this period of my life. Thanks for always taking time to discuss my meaningful and not so clever questions and ideas, for guiding me to more promising directions and still leaving freedom to find the way myself, for taking efforts and interest to get into details of the analyses and to carefully revise all the manuscripts and, particularly, for always fostering me to understand what I do. Thanks for the nice time in expeditions, and activities outside research. In you I have found a person of a bright mind and a wonderful heart.

Thanks for all the current and past members of 'relict group' – Raisa, Jukka, Tytti, Minttu, Helena, Hannu – for sharing the joys and troubles of a PhD student, for a good company, and for your help and advice in scientific and practical questions. Particularly thanks for my closest companion Raisa – I am fascinated by your positive attitude to people, and by the natural and simple way you can do a greatest favour. Thanks to a number of people in MES lab who have contributed in acquiring the DNA data. I am grateful for Misha, Nikolai and Piotr, my dear colleagues from Russia, for the inspiring time together while hunting mysids, discussing science and life, and for keeping me in touch with the life in the 'wild east'. Nikolai, your humour and calmness during the expedition in Dagestan, even while standing surrounded by armed Russian soldiers (who were guarding Caspian mysids?), was an invaluable support and a great discovery for me. My gratitude to all the colleagues and friends at the Department, Zoological Museum and Tvärminne Zoological Station – without you neither this thesis, nor my life would be complete. Thanks for Ilmari Valovirta for keeping me aware, that world animals consist not only of mysids, but sometimes also of mussels, and for Jaakko Kullberg for learning that Finns can be also talkative. Thanks for all the international and Finnish community at the Department for sharing weekends, vappus, pikkujoulus and other Finnish events. I also thank all the vision research people – Magnus Lindström, Johan Pahlberg, Mirka Jokela-Määttä and Kristian Donner – for broadening my views about what is important and interesting in science, and for getting to know presumably one of the merriest scientific communities. Magnus, I have been impressed by your optimism and enthusiasm, and enjoyed a lot our discussions on topics as different as construction of electroretinogram equipment from coffee cans to medieval music. Thanks for the scientific personnel at the University of Copenhagen and Swedish Museum of Natural History, particularly Jakob Damgaard, Jørgen Olesen and Karin Sindemark, for help in arranging museum loans, guidance in the lab, inspiring and useful scientific discussions, and after-science activities. My gratitude also for Roy Siddall and Carl-Adam Hæggström for correcting English

language and editing the summary of the thesis.

I am grateful to friends and colleagues in Klaipėda and Vilnius for keeping me in touch with Lithuania, for the wonderful company, and for the feeling that somewhere in the world I have a home. Thanks for Kestas Arbaciauskas for supervising my MSc thesis and leading me to discover 'glacial relicts', which I am still in love with. My special thanks to Lars Leonardson from the University of Lund, for the invaluable help to start my research during the undergraduate studies. Thanks also for Magnus Fürst, Björn Kinsten and personnel of the Institute of Freshwater Research in Drottningholm for the support in research and sampling that contributed to this thesis.

Ačiū and spasibo for Arturas and Olga, my family in Finland – thanks to you Helsinki was a home for me from the very first days here. Special thanks to Irina and Marcus, my second family that I was so lucky to find. Irina, these years in Helsinki would have been very different without your friendship; you have contributed so much to my view about what is important in the world, and always reminded me that science is not all that matters. Also the members of the Gaia band gave me a possibility to forget manuscripts and dive into the world of music. Finally, thanks so much for Frank for sharing troubles and happiness of my work in so many ways. I am indebted to everybody, in Finland and outside it, who have contributed to this thesis with samples, with advice, with good ideas, and by making my life more fulfilling. Living in Finland was a great experience to me. In so many ways I have found Finns possessing exactly those qualities that I would like to have myself.

Thanks for Ilkka Teräs and Veijo Kaitala for arranging my studies and practicalities related to the defence. I am very grateful for Christer Erséus and Eric Taylor for the review of this thesis, and Koen Martens for the examination. At last, I express my gratitude for those providing financial support, without which this study would not have been possible first of all. For four and a half years this study was supported by the Walter and Andrée de Nottbeck Foundation, University of Helsinki research funds, Academy of Finland, the EU Commission programme IHP, and Societas pro Fauna et Flora Fennica.

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