Amazonia versus Pontocaspis: a key to understanding the mineral composition of mysid statoliths (Crustacea: Mysida)

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SUMMARY

We have determined the mineral composition of statoliths in 169 species or subspecies (256 populations) of the family Mysidae on a worldwide scale. Including previously published data, the crystallographic characteristics are now known for 296 extant species or subspecies: fluorite (CaF_2) in 79%, vaterite (a metastable form of crystalline $CaCO_3$) in 16%, and non-crystalline (organic) components in 5%, the latter exclusively and throughout in the subfamilies Boreomysinae and Rhopalophthalminae. Within the subfamily Mysinae vaterite or fluorite were found in three tribes, whereas other three tribes have fluorite only. The exclusive presence of fluorite was confirmed for the remaining seven subfamilies. Hotspots of vaterite were found in Amazonia and the Pontocaspis, in each case with reduced frequencies in main and tributary basins of the Atlantic and N-Indian Ocean. Vaterite is completely absent in the remaining aquatic regions of the world. In accordance with previous findings, fluorite occurred mainly in seawater, vaterite mostly in brackish to freshwater. Only vaterite was found in electrolyte-poor Black Water of Amazonia, which clearly cannot support the high fluorine demand for renewal of otherwise large fluorite statoliths upon each moult. Vaterite prevails in Diamysini, distributed over most of the area once occupied by the Tethyan Sea. It also prevails in Paramysini with main occurrence in the Pontocaspis, where fossil calcareous statoliths in the stable form of calcite are known from Miocene sediments of the brackish Paratethys. Four Recent genera from three tribes are heterogeneous in that they comprise both vaterite- and fluorite-precipitating species. Previous hypotheses are expanded to cover greater geographic and time scales, proposing that fluorite-bearing marine ancestors penetrated freshwaters in Tethyan and Paratethyan basins, where they developed precipitation of vaterite. This gave their successors predispositions for shifting into separate evolutionary lines from fluorite to vaterite precipitation and *vice versa*.

INTRODUCTION

Mysid shrimps of the family Mysidae are exceptional among invertebrates by having endogenous static bodies (statoliths), analogous to otoliths of most vertebrates. Mysid statoliths are renewed upon each molt and are again exceptional due to their large relative size. Biology textbooks long held that the Mysidae are unique in the animal kingdom due to endogenous static bodies mineralized with fluorite (CaF_2). The first identification of vaterite (a metastable crystal phase of $CaCO_3$) statoliths in a mysid species was made by Ariani et al. (1981) for *Diamysis bahirensis* (G.O. Sars 1877), later partly transferred to *D. mesohalobia* Ariani & Wittmann 2000, from certain brackish water populations in the Mediterranean. The peculiar crystal habit of vaterite is also recognizable in scanning electron micrographs of statoliths published by Almeida Prado-Por (1981), who, however, did not identify the mineral upon first descriptions of *D. bahirensis hebraica* Almeida Prado-Por 1981, and *D. bahirensis sirbonica* Almeida Prado-Por 1981 - now acknowledged (Ariani and Wittmann 2000) at species level as *D. hebraica* and *D. sirbonica*. Finally, Franco et al. (1989) reported that certain mysid species have non-crystalline (organic) statoliths.

Ariani et al. (1993) showed that fluorite is present in a great majority of extant mysid species on a worldwide scale. Nonetheless, a small number precipitate vaterite, and an even smaller number have non-crystalline (organic) static bodies. Within a given species, the mineral composition of statoliths does not differ between populations or subspecies. In regions where both mineral types are found, vaterite is more likely to occur in fresh- or brackish water, fluorite in seawater. According to Voicu (1981), the function of the static organ of mysids in seawater may demand precipitation of a mineral with greater specific gravity compared to the less dense media in fresh- and brackish waters, namely 3.2 in fluorite versus 2.7 in calcite (the latter value based on fossil statoliths). For living specimens of two species of *Schistomysis* Norman 1892, densities of 2.89 for fluorite and 2.21 for vaterite were estimated by Wittmann and Ariani (1996). These values take into account that statoliths also contain organic matrix, water, impurities, and trace elements. The authors estimated that an enormous metabolic effort is required to precipitate fluorite in seawater: the concentration factors with respect to the seawater mass in average statocysts of *Schistomysis spiritus* (Norman 1860) are 610 for calcium and 183,000 for fluoride. The extent to which fluorine is acquired by feeding, such as ingestion of the freshly shed exuvia (containing both statoliths) upon moulting as sometimes observed by Astthorsson (1980) in laboratory-kept *Neomysis integer* (Leach 1814), is unknown. A rough estimate by Wittmann and Ariani (1996) suggests that mysid fluorite statoliths contribute to a turnover of at most 570 Ky for overall fluoride in the oceanic system. Despite this considerable metabolic effort, several freshwater species precipitate fluorite: the Black Sea endemic *Diamysis pengoi* (Czerniavsky 1882) and the cave-dwelling *Troglomysis vjetrenicensis* Stammer 1933, from the Adriatic coast are important examples. Non-crystalline statoliths occur exclusively and throughout the subfamilies Boreomysinae and Rhopalophthalminae. These subfamilies are split basally from the remaining subfamilies within the Mysidae, based on morphological traits (Hansen 1910) and genetic data (Meland and Willassen 2007, Meland et al. 2015). Boreomysinae is also the subfamily with the oldest known (Triassic) fossils of Mysidae (San Vicente and Cartanyà 2017). This is paralleled by ontogenetic findings that an organic matrix appears prior to mineralization of vaterite statoliths upon each moult (Ariani et al. 1982). Statocysts are completely lacking in the morphologically and genetically equally remote family Petalophthalmidae within the order Mysida.

Biogeographical discussions by Ariani et al. (1993) and by Wittmann and Ariani (2011) pointed to a largely Parathethyan origin

of those species of *Paramysis* Czerniavsky 1882, and *Diamysis* Czerniavsky 1882, that precipitate vaterite. These species are (at least partly) distributed in the Ponto-Caspian area, wherefrom calcareous fossil statoliths are also known (Voicu 1981). Until just recently, the cave-dwelling *Antromysis cenotensis* Creaser 1936, was the only known vaterite-precipitating species (Ariani et al. 1993) in the W-Atlantic (Gulf of Mexico). The scenario of the Ponto-Caspian as the supposedly only global hotspot of vaterite precipitation was changed by findings of Wittmann (2017, 2018), who demonstrated that vaterite occurs in seven species of the tribe Diamysini from freshwater tributaries of the Amazonas and one additional species from brackish waters along the coast of Brazil. The wealth of new data on the global scale presented here updates which biogeographical regions and which taxonomic entities (from subspecies to subfamily) are involved precipitating specific minerals. This contribution also addresses the related adaptive implications in the family Mysidae.

MATERIALS AND METHODS

Based on material collected during four decades, the mineral composition of statoliths was determined in 256 populations belonging to 169 species or subspecies, including supplements to previously published data on 64 populations of 43 taxa. For details including new and already published data on a total of 487 populations of 296 taxa see Supplementary Table. Most materials were collected from 1974-2015 by K.J. Wittmann during sampling campaigns in marine and continental waters of the Antarctic, W- and E-Atlantic, Mediterranean, Marmora, Black Sea, and Red Sea basins; and by A.P. Ariani during research trips to Mediterranean and NE-Atlantic waters from 1965-2011, and to Madagascar in 1999. Extensive materials were gained by exchange of collection materials with Torleiv Brattegard (Bergen), Gwen Fenton (Hobart), and Masaaki Murano (Tokyo); and with the meanwhile deceased Mihai Băcescu (1908-1999) (Bucharest), Thomas E. Bowman (1934-2013) (Washington), and John Mauchline (1933-2013) (Oban). Additional important contributions were made by academic and citizen scientists listed in the acknowledgements below. Further materials were studied in situ or based on loans from museum collections from Bergen, Berlin, Bucharest, Frankfurt am Main, Hamburg, Helsinki, Jerusalem, Leiden, London, Munich, Oslo, St. Petersburg, Rio de Janeiro, Tenerife, Turin, Vienna, and Washington; and from collections of the fisheries institute (INPA) of Manaus, Brazil, and the Southeast Regional Taxonomic Center (SERTC), Charleston, SC.

Body size was measured from the tip of the rostrum to the end of the telson, without spines. Statolith diameter was calculated as the geometric mean of apparent length and width in ventral view. The mineral composition of statoliths, previously largely analyzed by X-ray diffraction (Ariani et al. 1981, 1983, 1993) was determined generally on five (range 1-25, depending on availability) statoliths per population with a combination of optical and chemical examinations according to Wittmann et al. (1993). This powerful method distinguishes all types of composition (fluorite, vaterite, calcite, non-crystalline) previously found in statoliths of 210 living (listed in Supplementary Table) and two fossil species (Ariani et al. 1993, Wittmann et al. 1993), but cannot guarantee the detection of mineral types so far unknown for statoliths. To date, this method has yielded identical results to X-ray diffraction techniques (Franco et al. 1989, Ariani et al. 1993).

Scanning electron micrographs were obtained with a JEOL JSM-5310 on statoliths coated with gold, using 1-3 statoliths of *Boreomysis microps* G.O. Sars 1883, *Euchaetomera tenuis* G.O. Sars 1883, *Mysidium antillarum* Wittmann in Wittmann & Wirtz 2019, *M. triangulare* Wittmann in Wittmann & Wirtz 2019, *M. gracile* (Dana 1852), *Parvimysis* sp. B, and *Surinamysis*

aestuaria Wittmann 2017. Microanalysis was performed in EDS (Energy Dispersion Spectrum) on statoliths of *B. microps* and *Parvimysis* sp. B.

Taxonomic notes including entries in the Supplement: *Prionomysis* sp. 1 was described by Fenton (1985) without establishing a taxon. The description of *Longithorax* sp. A is in press (Wittmann 2019). *Heteromysis* sp. B, *Mysidella* sp. A, *Mysidetes* sp. A, and *Parvimysis* sp. B remain to be described. All these entities are here treated at species level.

Using the package SPSS Statistics 20.0, binary logistic regression analysis was performed on the data matrix in the Supplement, excluding entries from non-indigenous populations. The dependent variable was mineral composition with vaterite and fluorite as nominal factors. Only three tribes showed both minerals, whereby only two tribes had sufficient sample numbers to perform the analysis. The numerical codes given in Table 2 for tributary (sea) basins, and modal values of depth, salinity, and body size were arranged as initial covariates. Non-significant ($P > 0.05$) covariates were backward eliminated.

RESULTS

Fluorite (CaF_2) was found in 115 taxa (species and subspecies), vaterite (a metastable form of crystalline $CaCO_3$) in 46, and non-crystalline (organic) components in eight taxa. The remaining entries in Tables 1, 2 and Supplementary Table are derived from previously published data. In accordance with the results of Ariani et al. (1993), Wittmann et al. (1993), and Wittmann and Ariani (2011, 2012), there were no differences among subspecies, populations, and/or individuals belonging to the same species. However, one museum specimen (not included in present figures and tables) of *Diamysis lagunaris* Ariani & Wittmann 2000, from a pooled sample taken in Mediterranean brackish waters before

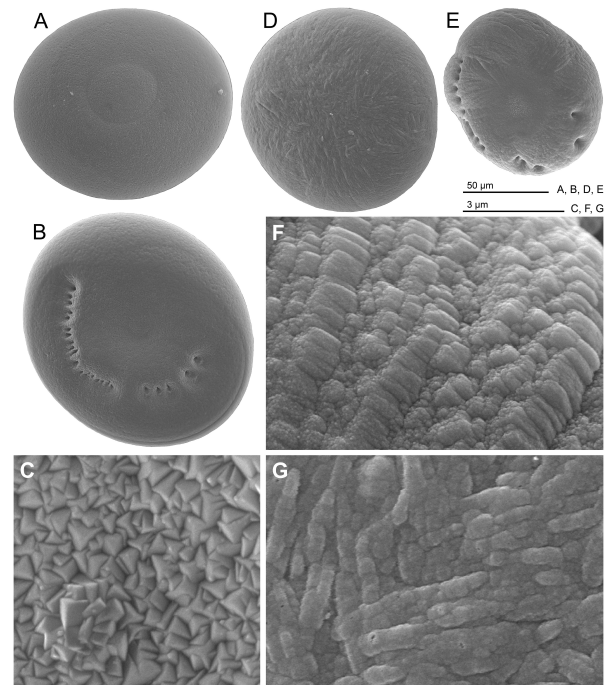1877 yielded a combination of vaterite and calcite.

Figure 1. Statoliths mineralized with fluorite (A-C) versus vaterite (D-G) in species of Mysidae. Dorsal (A, D) and ventral (B, E) face of statoliths are given in toto. Mineral habits of fluorite (C) versus vaterite (F, G) are visible on the statolith surface. The fluorite statoliths belong to *Mysidium gracile* (A), *Mysidium triangulare* (B), and *Mysidium antillarum* (C), the vaterite statoliths to *Surinamysis aestuaria* (D, G) and *Parvimysis* sp. B (E, F). Photos by A.P. Ariani and R. de Gennaro.

Scanning electron microscopy revealed the cubic habit (Fig. 1C) of fluorite at the statolith surface in *Euchaetomera tenuis*, *Mysidium antillarum*, *M. triangulare* and *M. gracile*. In contrast, sheet-like vaterite aggregates (see also discussion) consisting of stacked smaller sheets in perpendicular orientation (Fig. 1F) were found in statoliths of *Parvimysis* sp. B, less distinct sheet-like structures in *Surinamysis aestuaria* (Fig. 1G). Qualitative microanalysis confirmed that the statoliths of *Parvimysis* sp. B contain $CaCO_3$.

Table 1. Composition of statoliths in subfamilies and tribes of the family Mysidae.

| Taxonomic rank | | Number of taxa (species plus subspecies)[1] | | |
Subfamily	Tribus	non-crystalline	fluorite	vaterite
Boreomysinae		10	0	0
Rhopalophthalminae		4	0	0
Siriellinae		0	23	0
Gastrosaccinae		0	19	0
	Anchialinini	0	3	0
	Gastrosaccini	0	16	0
Erythropinae		0	42	0
	Amblyopsini	0	7	0
	Arachnomysini	0	1	0
	Erythropini	0	28	0
	Pseudommini	0	6	0
Leptomysinae		0	45	0
	Afromysini	0	6	0
	Leptomysini	0	20	0
	Mysidopsini	0	19	0
Mysinae		0	71	48
	Anisomysini	0	13	0
	Diamysini	0	7	26
	Hemimysini	0	7	2
	Mysini	0	22	0
	Neomysini	0	15	0
	Paramysini	0	7	20
Palaumysinae		0	1	0
Heteromysinae		0	28	0
	Heteromysini	0	22	0
	Harmelinellini	0	1	0
	Mysidetini	0	5	0
Mysidellinae		0	3	0
	Total	14	232	48

[1] for data sources and references see Supplement.

The mineral composition of statoliths was strongly related to the taxonomic framework (including previously published data in Table 1; details in Supplementary Table): the subfamilies Boreomysinae (n = 14 populations of 10 species) and Rhopalophthalminae (4, 4) exhibited only non-crystalline (organic) statoliths. Microanalysis yielded a strong peak of calcium, although no minerals were recognizable, in statoliths of *Boreomysis microps*. Vaterite was found only in three tribes of the subfamily Mysinae: the Diamysini showed vaterite (Vtr) in 26 taxa (species and subspecies) versus fluorite (Fl) in seven taxa; this relation was 20 Vtr + 7 Fl in Paramysini and 2 Vtr + 7 Fl in Hemimysini. Each of these three tribes contained heterogeneous genera with respect to mineral precipitation: *Hemimysis* G.O. Sars 1869, as the only genus of the tribe Hemimysini, showed vaterite in two taxa, fluorite in seven taxa; within the Paramysini this relation was 19 Vtr + 1 Fl in *Paramysis* and 1 Vtr + 3 Fl in *Schistomysis*; within Diamysini it was 13 Vtr + 3 Fl in *Diamysis*. The remaining Paramysini showed vaterite in the monotypic genus *Katamysis* G.O. Sars 1893, versus only fluorite in three species of *Praunus* Leach 1814. The Diamysini showed only vaterite in *Antromysis* Creaser 1936, *Indomysis* W.M. Tattersall 1914, *Limnomysis*

Czerniavsky 1882, *Parvimysis* Brattegard 1969, and *Surinamysis* Bowman 1977; only fluorite in *Gangemysis* Derjavin 1924, and *Taphromysis* Banner 1953. Only fluorite was present in the remaining three tribes (Anisomysini, Mysini, Neomysini) of the subfamily Mysinae and in the remaining seven subfamilies with mineralized statoliths in the family Mysidae (Table 1).

The geographical distribution of mineralized statoliths is here considered excluding data from non-indigenous populations (Table 2; Supplementary Table): the incidence of vaterite statoliths is mainly related to specimens from Ponto-Caspian and Amazonian populations. All seven species examined from Amazonia showed vaterite. The corresponding values in fresh and brackish waters of the Caspian basin are ten species with vaterite versus four species with fluorite. Statoliths from the Black Sea showed vaterite in 16 versus fluorite in seven species, together representing all species known from this basin. Among the twelve Black Sea species at least occasionally occurring in freshwater, only *Diamysis pengoi* and *Mesopodopsis slabberi* (Van Beneden 1861) showed fluorite statoliths, all remaining species vaterite. Among 15 meso- to euhalobious species, six showed fluorite, the remaining nine vaterite. Vaterite was detected in eight, and fluorite in other eight species from anhaline to polyhaline waters of the Marmora basin. This relationship is 18 versus 47 species or subspecies in anhaline to metahaline waters of the Mediterranean, three versus 68 in the E-Atlantic including the Baltic, four versus 34 in the W-Atlantic including Caribbean and Gulf of Mexico, and one versus 19 in the Indian Ocean (excl. Red Sea). Only fluorite was found in ten species from the Red Sea, 49 from the Pacific, three from the Arctic Ocean, and 14 from the Circum-Antarctic Sea (Table 2).

Table 2. Sea basin and tributary distribution of statolith composition in Mysidae.

Item[4]	Main regions	Subdivisions and tributaries	Number of taxa (species plus subspecies)[1,2,3]		
			non-crystalline	fluorite	vaterite
1	Arctic		0	3	0
2-5	W-Atlantic		0	34	11
2		Gulf of Mexico	0	5	1
3		Caribbean	0	13	2
4		Amazonia	0	0	7
5		main basin	0	19	1
6	E-Atlantic	main basin	10	68	3
7-9	Ponto-Mediterranean	(incl. Black Sea)	1	50	31
7		Mediterranean	1	47	18
8		Marmora Sea	0	8	8
9-10	Ponto-Caspian		0	11	19
9		Black Sea	0	7	16
10		Caspian	0	4	10
11-12	Indian Ocean		2	28	1
11		Red Sea	1	10	0
12		all excl. Red Sea	1	19	1
13	Pacific		2	49	0
14	Antarctic		1	14	0
		Total	14	232	48

[1] for data sources and references see Supplement
[2] includes multiple assignments of species to different regions
[3] non-indigenous populations excluded
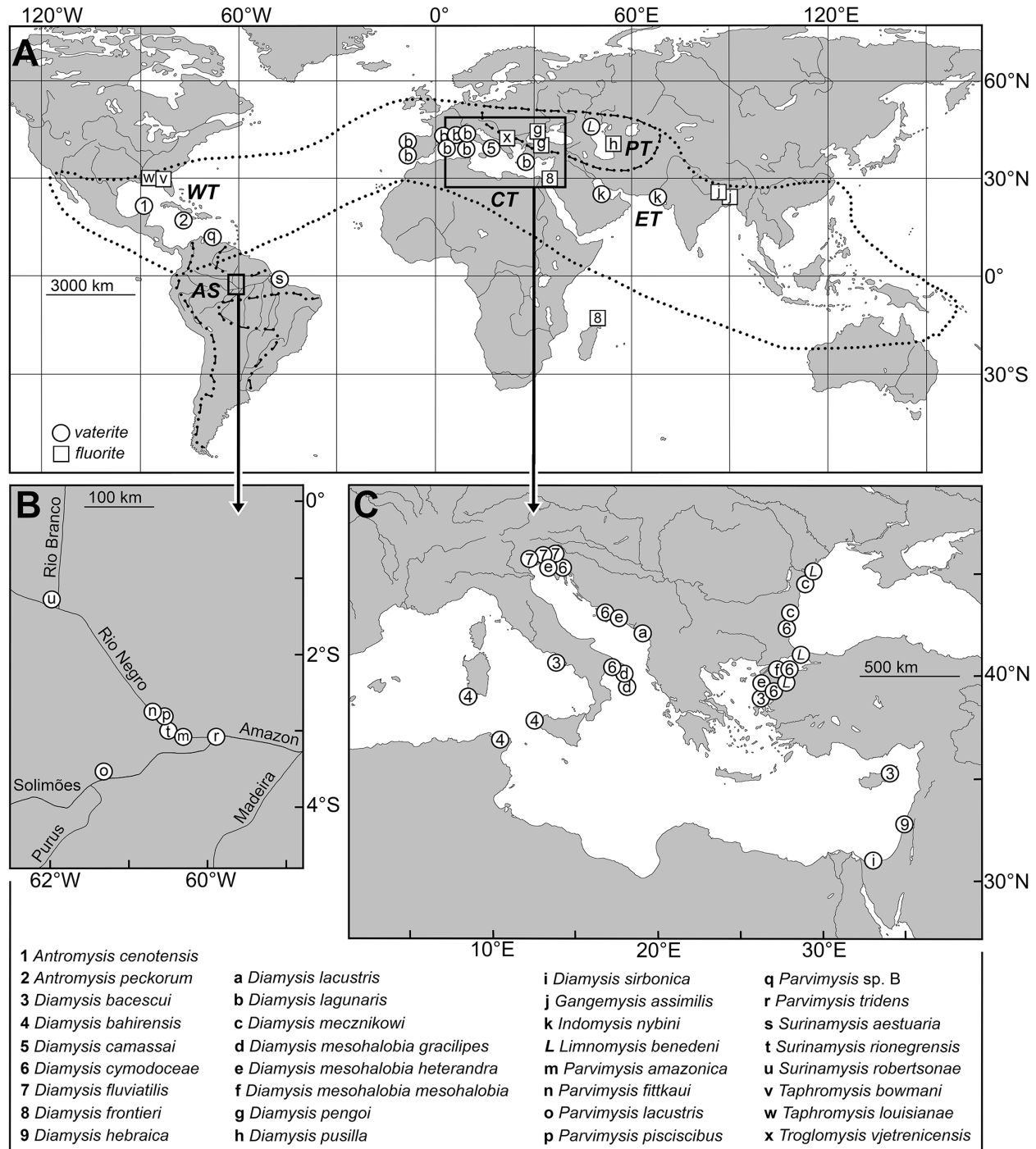[4] item numbers used as arbitrary code for binary logistic regression analysis

Figure 2. Geographic and taxonomic distribution of vaterite (squares) versus fluorite (circles) in mineralized statoliths within the tribe Diamysini. Maximum extensions of the Paleocene Tethys (dotted line) and the Oligocene Paratethys (dashed line, PT) are semi-schematically projected over the extant distribution of continents according to Hou and Li (2017). WT, CT, and ET indicate western, central and eastern parts of the Tethys. Dash-dotted lines indicate the marine transgression over vast parts of South America, according to Ramos and Alemán (2000), leading to the formation of the Amazon Sea (AS) during the Mid-Miocene (12 Ma b.p.). Overlapping symbols are slightly shifted or omitted; non-indigenous populations not shown (original data set in Supplementary Table). Scale bars indicate distances along the equator (maps A, B) and along the latitude 40°N (map C).

The prerequisites for binary logistic regression analysis were met by only two tribes: salinity and distribution among tributary (sea) basins proved to be significant predictors of mineral composition in the Paramysini (P ≤ 0.044 and P ≤ 0.007, respectively; n = 77 populations) and in the Diamysini (P ≤ 0.005 and P ≤ 0.019; n = 81). Depth and body size were not significant. The probability of vaterite precipitation increased with decreasing salinity and with increasing basin code (Table 2) in both tribes. The regression coefficients were -0.207 ± s.e. 0.103 and 3.286 ± 1.227, respectively, for Paramysini versus -0.063 ± 0.026 and 0.504 ± 0.180 for Diamysini. The resulting regression equations correctly predicted the minerals in 92% of the actual samples in Paramysini, 85% in Diamysini.

DISCUSSION

Structure of mineralized statoliths

The structure and mineral composition of the here examined statoliths (Fig. 1; Supplementary Table) fit well with data on 61 species given by Wittmann et al. (1993). Fluorite always shows a cubic habit (Fig. 1C), whereas vaterite forms scale-, needle-, rod-, and lens-like aggregates (Ariani et al. 1993, Wittmann et al. 1993). As shown above for *Parvimysis* sp. B, also sheet-like structures (Fig. 1F) occur in certain vaterite statoliths. Among the great variety of vaterite morphs, astoundingly similar mineral aggregates, in part forming roughly statolith-shaped particles, were obtained by Hou and Feng (2006) in glycine-containing aqueous solutions by subtle variations of experimental conditions.

We were unable to confirm previous determinations by Voicu (1974, 1981) of calcite in *Paramysis kessleri* Grimm [in Sars 1895] and *P. kroyeri* (Czerniavsky 1882) based only on stoichometrical methods. Freshly fixed specimens of both species yielded vaterite in our samples, as already shown by Ariani et al.

(1993) using X-ray diffraction and supported by the present methodology.

Relative frequencies of statolith minerals

Ariani et al. (1993) determined the statolith composition for 179 populations of 154 extant species belonging to 55 genera of Mysidae on a worldwide scale. All subfamilies recognized at that time were represented in their study. Later, the taxon Mancomysini Băcescu & Iliffe 1986, was established at subfamily level by Meland and Willassen (2007), subsequently acknowledged as a nomen nudum and renamed to Palaumysinae by Wittmann (2013). The data presented here regard 285 extant species and eleven non-nominotypical subspecies (Supplementary Table). Not counting six informal species entries, this represents 25% of the currently known 1139 species plus 21 subspecies within the Mysidae (excerpt from own database). Fluorite (CaF2) was found in 234 taxa (79%), vaterite in 48 (16%), and non-crystalline (organic) components in 14 (5%) (European taxa over-represented). There was no intraspecific variation of mineral composition in fresh material with normal statolith morphology. As stated above, mineral phase conversion may occur in aged collection material; we consider this to be an effect of spontaneous conversion of metastable vaterite into the most stable $CaCO_3$ polymorph, calcite. Moreover, Ariani et al. (1999) experimentally induced in *Diamysis mesohalobia* (as *Diamysis* sp.) the formation of calcite particles besides or instead of normal vaterite statoliths inside statocysts. In that case the calcite particles did not show normal statolith morphology. Specimens with similar abnormalities are rarely found in nature and are not relevant in the present context.

Table 2 shows the highest frequencies of vaterite among mineralized statoliths in mysids from Amazonian (100%), Caspian (71%), and Pontic (70%) basins. Excluding data from recent range expansions, vaterite is represented by 50% in the Marmora Sea, 28% in the

Mediterranean, 4% in the E-Atlantic (excl. Medit.), 11% in the W-Atlantic including Caribbean and Gulf of Mexico, 5% in the Indian Ocean (excl. Red Sea), and none in the remaining sea basins and oceans of the world (Red Sea, Pacific, Arctic, and Circum-Antarctic oceans). There is a strong gradient of decreasing vaterite frequency with increasing distance from Ponto-Caspian and Amazonian regions, respectively. This fits with the peculiar environmental conditions in both regions favouring vaterite rather than fluorite precipitation. Waters with electrolyte-poor (thus also fluoride-poor) conditions are common across Amazonia (Küchler et al. 2000). The Ponto-Caspian is characterized by generally lower salinity with respect to the adjacent marine areas, i.e. the Marmora plus Mediterranean basins. In addition, the water levels in the Ponto-Caspian basins changed drastically during the Pleistocene, correlated with alternations between freshwater and high-salinity phases (Hsü 1978; Mudie et al. 2002; Ryan et al. 2003).

Biogeography of statolith mineralization

As shown in Figs. 2-4, vaterite statoliths were exclusively found along a longitudinal stripe of tropical to temperate, coastal to continental waters from the Gulf of Mexico, over the Caribbean, Amazonia and 'nearby' Atlantic coasts, E-Atlantic, Mediterranean, Pontocaspis, and Indian Ocean. This traces the course (Fig. 2) of the Mesogeic or Tethyan Sea, which extended up until the Miocene from the Caribbean to Australia. According to Ramos and Alemán (2000), the Amazonian Sea was flooded and connected with the Tethyan arm during the Miocene marine transgression about 12 Ma b.p. (Fig. 2). A Tethyan origin has already been hypothesized based on distributional data for *Diamysis* and related genera by Băcescu (1940, 1981), Ariani (1981), Porter et al. (2008), and Wittmann et al. (2016). Molecular clocks are currently not available to shed additional light on a potential Tethyan origin.

The present data also confirm the prevalence of statoliths mineralized with vaterite in extant Mysidae of the Pontocaspis, as observed by Ariani et al. (1993). Those authors concluded that this mineral type may have its roots in the brackish Paratethys, a Tertiary, northern derivate of the central Tethys. Their hypothesis is mainly based on evidence of fossil calcareous (calcite) statoliths in Miocene (mostly Upper Volhynian - Lower Bessarabian) deposits extending from the Caspian to the Vienna and the Pannonian basins (Voicu 1974, 1981; Fuchs 1979; Maissuradze and Popescu 1987). These fossils, assigned to the living genus *Paramysis* by Voicu (1974), were later transferred to the fossil genus *Sarmysis* Maissuradze & Popescu 1987 - with reference to the Tertiary Sarmatian Sea - by Maissuradze and Popescu (1987). A Tertiary origin of *Paramysis* is also suggested by rough estimates of Audzijonyte et al. (2008: Fig. 5) that genetic divergence of 14 *Paramysis* species, 12 of which we demonstrate to exhibit vaterite (listed in Supplementary Table), could altogether be rooted 12 Ma b.p., subgenera 6-10 Ma, and pairs of sister species 2 Ma.

The ecological and statolith characteristics observed in *Paramysis* species of the Mediterranean are explained (Wittmann and Ariani 2011) in the light of the Paratethyan drainage during the Messinian (Upper Miocene) salinity crisis. A Paratethyan origin can also be attributed to the Mediterranean species and subspecies of the genus *Diamysis* based on morphological, ecological, paleogeographical and paleoecological data (Ariani 1981, Ariani and Wittmann 2000). By contrast, all E-Atlantic and Mediterranean populations of *Hemimysis* taxa examined show fluorite statoliths (Fig. 4) and share a deeply incised telson, thus supporting the hypothesis of Ledoyer (1989) that the Mediterranean taxa plus the (fluorite-bearing) Pontic subspecies *H. lamornae pontica* Czerniavsky 1882, could originate from the E-Atlantic. The Ponto-Caspian endemics *H.*

anomala G.O. Sars 1907, and *H. serrata* Băcesco 1938, share vaterite statoliths and differ from the remaining congeners by a terminally truncate or only weakly notched telson; they therefore probably represent a separate phylogeographic branch.
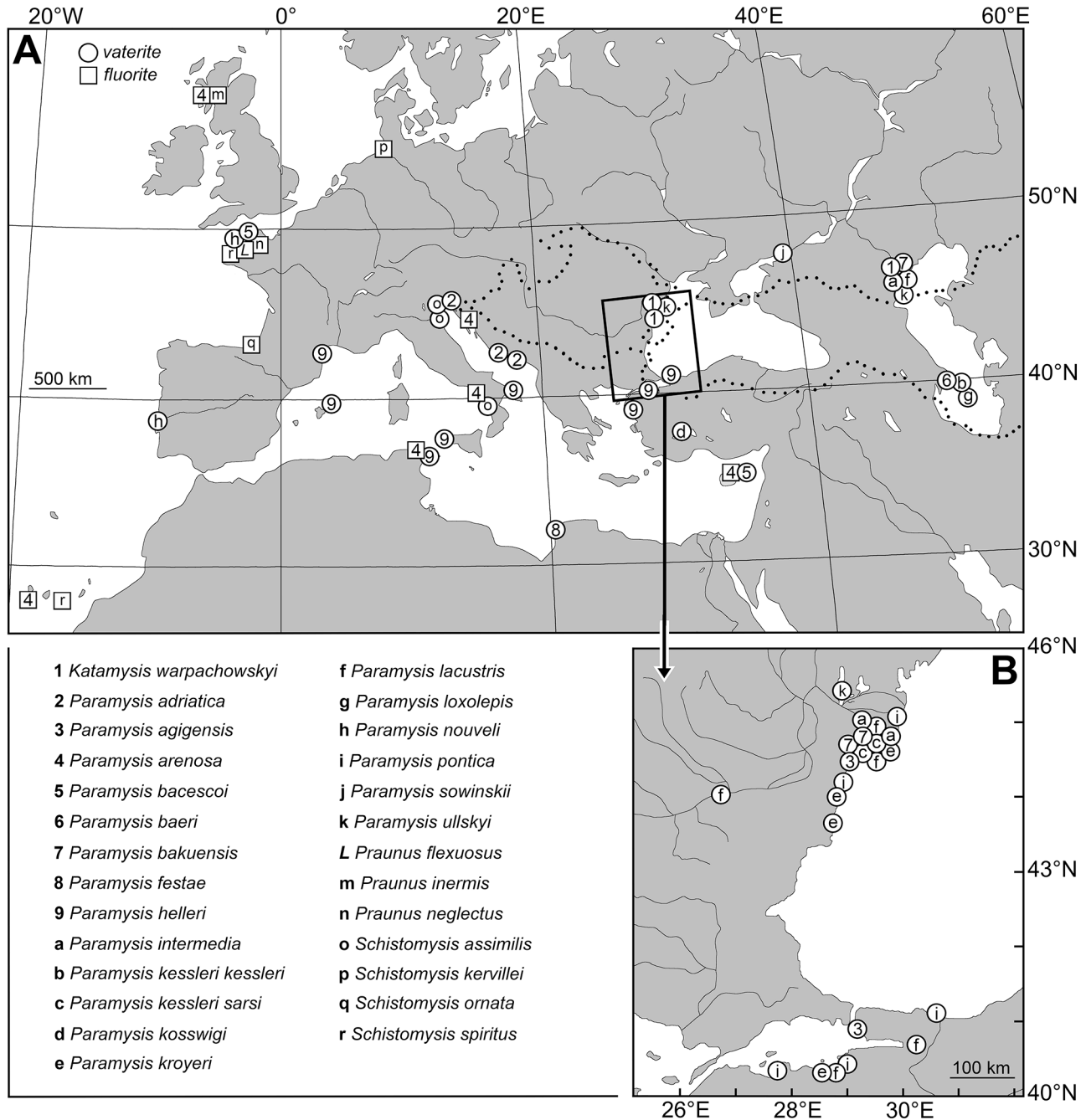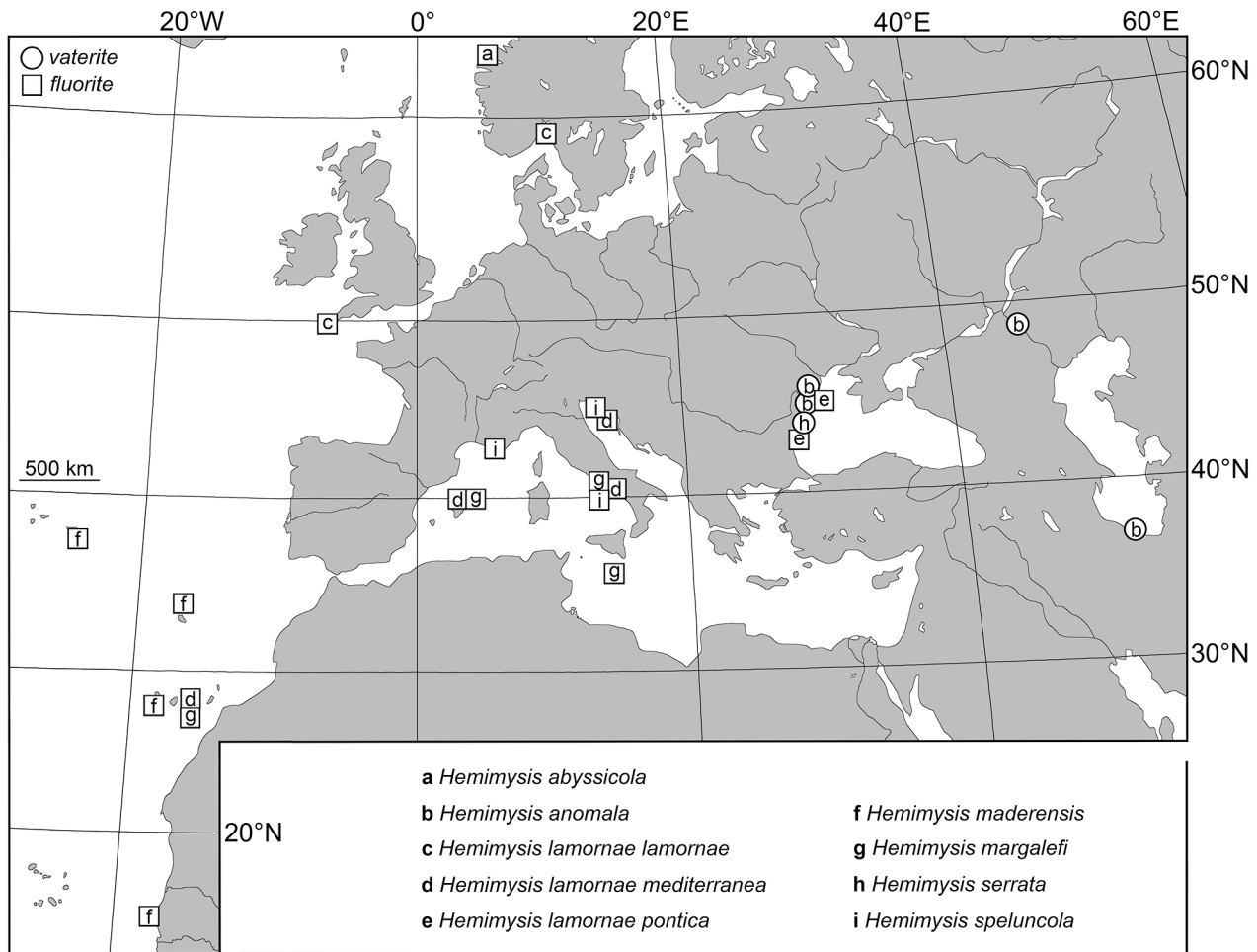

Figure 3. Geographic and taxonomic distribution of vaterite (squares) versus fluorite (circles) in mineralized statoliths within the tribe Paramysini. Dotted line in map A denotes the Mid-Miocene extension of the Paratethys (simplified following Palcu et al. 2017). Overlapping symbols are slightly shifted or omitted; non-indigenous populations not shown (original data set in Supplementary Table). Scale bars indicate distances along the latitude 40°N.

Figure 4. Geographic and taxonomic distribution of vaterite (squares) versus fluorite (circles) in mineralized statoliths within the tribe Hemimysini (only genus *Hemimysis*). Overlapping symbols are slightly shifted or omitted; non-indigenous populations not shown (original data set in Supplementary Table). Scale bar indicates distance along the latitude 40°N.

The Recent distribution of calcareous (vaterite) statoliths (Figs. 2-4) shows a strong taxonomic component insofar as the tribe Diamysini almost exclusively inhabits the Tethyan realm from the Gulf of Mexico plus Amazonia to the Strait of Malacca (E-Indian Ocean); the tribes Paramysini and Hemimysini exclusively inhabit the E-Atlantic to the Caspian, not considering non-indigenous populations of recently expansive species.

We show here that each of the three vaterite-bearing tribes are heterogeneous by comprising vaterite- as well as fluorite-precipitating genera; this heterogeneity is also evident within the genera *Hemimysis*, *Paramysis*, *Schistomysis*, and *Diamysis*, which are all representatives of the three tribes. In extension of the hypothesis by Ariani et al. (1993), this heterogeneity is explained using the classical concept of homoiology developed by Plate (1902), Riedl (1975), and others: fluorite-bearing marine ancestors likely penetrated freshwaters in the Tethyan (including the Miocene Amazon Sea) and Paratethyan basins, where they developed the precipitation of vaterite. This gave their successors the predispositions for shifting into separate evolutionary lines from fluorite to vaterite and *vice versa*. Accordingly, the vaterite-bearing

species in marine waters are interpreted as offspring of ancestors that returned from freshwater to the sea.

AUTHOR CONTRIBUTIONS

All authors wrote and contributed equally to the paper.

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