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9 Success factors and future prospects of Ponto-Caspian peracarid (Crustacea: Malacostraca)  
10 invasions: is 'the worst over'?

11

12 Péter Borza<sup>1\*</sup>, Thomas Huber<sup>2</sup>, Patrick Leitner<sup>2</sup>, Nadine Remund<sup>3</sup>, Wolfram Graf<sup>2</sup>

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14 <sup>1</sup>Danube Research Institute, MTA Centre for Ecological Research, Karolina út 29-31, 1113  
15 Budapest, Hungary

16 <sup>2</sup>Working Group on Benthic Ecology and Ecological Status Assessment, Institute for  
17 Hydrobiology & Water Management, Department of Water, Atmosphere & Environment,  
18 BOKU - University of Natural Resources and Applied Life Sciences, Max Emanuel-Strasse  
19 17, A-1180 Vienna, Austria

20 <sup>3</sup>Info fauna – CSCF, Passage Maximilien-de-Meuron 6, CH-2000 Neuchâtel, Switzerland

21

22 E-mail: [borza.peter@okologia.mta.hu](mailto:borza.peter@okologia.mta.hu)

23 \*Corresponding author

24

25 Abstract

26

27 Ponto-Caspian peracarids (amphipods, isopods, mysids and cumaceans) represent one of the  
28 most successful groups of aquatic invaders comprising several high-impact species, such as  
29 *Chelicorophium curvispinum*, *Dikerogammarus villosus*, or *Hemimysis anomala*. In the  
30 present study we made the first attempt to compare biological traits and the environmental  
31 preferences of invasive and non-invasive members of the group based on both literature and  
32 field data (Joint Danube Survey 3, 2013) with the goal of identifying factors linked to  
33 invasion success and drawing conclusions on future invasion risks. Both datasets indicated  
34 substrate preference as an important factor in spontaneous range expansion; all invasive  
35 species are lithophilous, whereas the majority of non-invasives are psammo-pelophilous. The  
36 remaining seven presently non-invasive lithophilous species deserve special attention when  
37 considering potential future invaders; however, due to their rarity and possible negative  
38 interactions with earlier colonists we consider the probability of their expansion in the  
39 foreseeable future as low. Their potential expansion could most likely be of minor  
40 consequence anyway, since no considerable functional novelty can be attributed to them in  
41 addition to species already present. In this limited context (regarding habitats dominated by  
42 hard substrates and not considering the potential further spread of already invasive species) it  
43 might be justified to conclude that 'the worst is over'. Nevertheless, impending navigation  
44 development projects both in the Danube-Main-Rhine and Dnieper-Pripyat-Bug-Vistula  
45 systems might favour the future spread of non-lithophilous species, which might imply a new  
46 invasion wave of Ponto-Caspian peracarids.

47

48 Keywords

49 Amphipoda, colonization rate, Cumacea, Isopoda, Mysida, substrate preference

50

51 Introduction

52

53 Predicting future invasions by identifying traits of species determining invasion success is a  
54 fundamental endeavor of applied ecology (Williamson and Fitter 1996; Kolar and Lodge  
55 2001; Heger and Trepl 2003). Initial attempts at finding features universally predisposing  
56 species to be an invader concluded that there might be inherent limitations to generalization  
57 (Williamson 1999). However, it also emerged that not all invasions are idiosyncratic, and  
58 carefully designed studies (e.g., distinguishing among stages of the invasion process) might  
59 identify informative traits (Kolar and Lodge 2001). How specifically these traits can be  
60 defined depends on the scope of the study; a meta-analysis comprising all major groups of  
61 organisms ever investigated in this context was only able to demonstrate the universal  
62 importance of climate/habitat match, history of invasive success, and the number of  
63 arriving/released individuals (Hayes and Barry 2008). Another meta-analysis restricted to  
64 plants was able to link invasiveness to more informative but still composite traits related to  
65 performance, such as physiology, leaf area allocation, shoot allocation, growth rate, size, and  
66 fitness (Van Kleunen et al. 2010b). More accurate predictions can be made if one focuses on a  
67 specific taxon in a given region (e.g., fish in the North American Great Lakes; Kolar and  
68 Lodge 2002), and it might even be possible to successfully model the potential range and  
69 impact of single invader species in yet unaffected areas (e.g., Kulhanek et al. 2011).  
70 Data allowing an in-depth analysis of invasion risks are hard or often impossible to obtain  
71 (e.g., propagule pressure in accidental introductions); accordingly, most of the studies deal  
72 with a few well-known taxa (i.e., plants, birds, and fishes), and deliberate introductions are  
73 strongly overrepresented (Kolar and Lodge 2001; Hayes and Barry 2008). Nevertheless, due  
74 to the scale-dependent nature of the issue, specific studies on less tractable but similarly  
75 important groups of invaders are indispensable in order to provide predictions as accurate as  
76 possible.

77 Ponto-Caspian peracarids represent one of the most successful groups of aquatic invaders,  
78 comprising several high-impact species such as the 'Caspian mud shrimp' *Chelicorophium*  
79 *curvispinum* (G.O. Sars, 1895), the 'killer shrimp' *Dikerogammarus villosus* (Sowinsky,  
80 1894), or the 'bloody-red mysid' *Hemimysis anomala* G.O. Sars, 1907 (Van den Brink et al.  
81 1993; Dick et al. 2002; Ricciardi et al. 2012). Studies dealing with factors of their invasion  
82 success so far have concentrated on the comparison with native species, and concluded that  
83 life history traits, such as short generation time and high fecundity might be the main factor of  
84 their superiority (Devin and Beisel 2007; Grabowski et al. 2007a). Although this approach  
85 might reveal important aspects of the explanation of their success, *per se* it does not allow  
86 predictions to be made (Van Kleunen et al. 2010a).

87 In the present study, we make the first attempt to compare biological traits and the  
88 environmental preferences of invasive and non-invasive Ponto-Caspian peracarids based on  
89 both literature and field data, with the goal of identifying factors linked to invasion success  
90 and making conclusions on future invasion risks.

91

92 Material and methods

93

94 Historical context

95

96 The expansion of Ponto-Caspian peracarids toward Western and Northern Europe has been  
97 promoted mainly by two major inland waterways connecting their native region to other  
98 catchments, the so-called southern (Danube-Main-Rhine system) and central corridors  
99 (Dnieper-Pripyat-Bug-Vistula-Notec-Oder system connected to German rivers by the Midland  
100 Canal). The third, northern corridor (Volga-Neva system) has not played a significant role in  
101 this context (Bij de Vaate et al. 2002). After colonizing several interconnected catchments in

102 continental Europe, some of the species were also able to further extend their range to the  
103 British Isles and even to North-America (Ricciardi and MacIsaac 2000; Pothoven et al. 2007;  
104 Gallardo and Aldridge 2015).

105 Along the River Danube, Ponto-Caspian peracarids began to expand around the beginning of  
106 the 20<sup>th</sup> century, parallel to the start of regular mechanized ship traffic; by the middle of the  
107 century seven species had established in the middle section of the river (Fig. 1). In the  
108 following decades, colonization rate decreased until in 1992 the Danube was connected to the  
109 Rhine basin via the Main-Danube canal. Soon after, species which have previously colonized  
110 the middle and upper sections of the Danube appeared in the Rhine, and four additional  
111 species began to expand in the system (Fig. 1). After this hectic period, however, events  
112 apparently slowed down again; presently, large-scale expansions have been detected for more  
113 than a decade.

114 Along the central corridor, *C. curvispinum* and *Chaetogammarus ischnus* (Stebbing, 1899),  
115 were first found outside their native range in the early 20<sup>th</sup> century, when ship traffic used to  
116 be the most active (Grabowski et al. 2007b; Karatayev et al. 2008). After World War II, a dam  
117 was built on the Dnieper-Bug canal allowing only occasional ship traffic (Karatayev et al.  
118 2008); still, *Dikerogammarus villosus* and *D. haemobaphes* (Eichwald, 1841) were able to  
119 reach Poland via this route around the millennium (Grabowski et al. 2007b). Beside them,  
120 several other species have expanded their range within the Dnieper basin mainly (but not  
121 exclusively) as a result of deliberate introduction (Mastitsky and Makarevich 2007;  
122 Semenchenko and Vezhnovetz 2008; Pligin et al. 2014). Ponto-Caspian species were also  
123 transported to the Baltic states in the 1960-70s; four mysid and three amphipod species  
124 established in the Baltic region after having been released in Lithuanian or Estonian reservoirs  
125 and lakes (Arbaciauskas 2002; Herkül et al. 2009).

126

127 Literature data

128

129 Pontic and Ponto-Caspian peracarid species occurring in freshwater were considered as  
130 potentially invasive (Table 1). Although they might have the potential of range expansion  
131 (Grabowski et al. 2012), primarily freshwater species (i.e., *Gammarus* spp. and *Niphargus*  
132 spp.) were not included in the analysis, since they have markedly different ecological and  
133 biogeographical characteristics. In the present paper we use the term 'invasive' in a broad,  
134 purely biogeographical sense (i.e., species which have considerably widened their  
135 distributional range in recent times), without referring to abundance, or ecological/economic  
136 impact. We regarded species having spontaneously crossed the borders of their respective  
137 native catchment (Danube in the southern corridor and Dnieper in the central corridor) as  
138 invasive, but we also discuss deliberate introductions and expansions of smaller magnitude.  
139 The species list for the southern corridor is presented after Lyashenko et al. (2012) with slight  
140 modifications (*Dikerogammarus bispinosus* Martynov, 1925, *Diamysis pengoi* (Czerniavsky,  
141 1882), and *Pontogammarus aestuarius* (Derzhavin, 1924) added; *Chaetogammarus behningi*  
142 Martynov, 1919 omitted for synonymy with *C. ischnus*, and *Hemimysis serrata* Băcescu,  
143 1938 omitted for lack of evidence for occurrence in freshwater). The species list for the  
144 central corridor was compiled after Dediu (1980), Komarova (1991), Pligin et al. (2014), and  
145 Vasilenko and Jaume (2015). Regarding amphipod taxonomy we conformed to Lowry and  
146 Myers (2013) with the modifications of Hou and Sket (2016). We note that the classification  
147 of the Ponto-Caspian complex is far from being settled; further substantial rearrangements can  
148 be expected from molecular results (Cristescu and Hebert 2005). For this reason, we did not  
149 include taxonomic/phylogenetic information in the analysis.

150 Coherent datasets could be gathered only for a few basic species traits. Body lengths (average  
151 size of mature females in the summer generations, if available) were compiled after Băcescu

152 (1951), Băcescu (1954), Căraușu et al. (1955), and after species descriptions for species not  
153 included in these. Size data were ordered into four classes (1: ]0,5] mm, 2: ]5,10] mm, 3:  
154 ]10,15] mm, 4: ]15,∞] mm) to decrease incoherency. The substrate preference of mysids and  
155 amphipods has been classified in the most straightforward way by Dediu (1966; 1980),  
156 comprising five categories (litho-, phyto-, psammo-, pelo-, and argyllophilous). We adopted  
157 this system and completed the list for the species not dealt with in those publications after  
158 descriptions of Căraușu et al. (1955), Gruner (1965), and Vasilenko and Jaume (2015). In the  
159 case of *Katamysis warpachowskyi* G. O. Sars, 1893 the classification of Dediu (1966)  
160 contradicted to other observations from both the native and non-native range (Băcescu 1954;  
161 Wittmann 2002; Borza 2014); therefore, we included both opinions as a compromise. Salinity  
162 tolerance was characterized based on field observations in three categories (freshwater,  
163 oligohaline, mesohaline) after Băcescu (1954), Căraușu et al. (1955), Dediu (1980),  
164 Komarova (1991), and Vasilenko and Jaume (2015). Since apparently all species occur in  
165 freshwater as well as in oligohaline waters (the sole exception being perhaps *D.bispinosus*;  
166 Căraușu et al. 1955), only tolerance to mesohaline conditions (>5 ‰) was considered in the  
167 analysis.

168

169 Field data

170

171 The field samples analyzed in the present study were collected during the 3<sup>rd</sup> Joint Danube  
172 Survey between 13 August and 26 September 2013 at 55 sites of the river ranging from Ulm  
173 (river km 2581) to the Delta (river km 18, Kiliya branch). At each site, 4-7 samples consisting  
174 of five units covering 25 x 25 cm bottom area were collected in the littoral zone (0.1-1.5 m  
175 depth) by hand net (aperture: 25 x 25 cm, mesh size: 500 μm) representing all habitat types  
176 available ('multi-habitat sampling'), as defined in the AQEM protocol (Hering et al. 2004).

177 All samples were preserved in 4% formaldehyde solution in the field, and stored in 70%  
178 ethanol after sorting. Sorting was facilitated by fractioning the material on a set of sieves  
179 (mesh sizes: 0.5, 2, 5, 10, 20 mm). In several cases, 2 to 64-fold subsampling of the smallest  
180 one or two fractions was necessary due to the extremely high number of juvenile animals in  
181 the samples. Altogether 41 509 Peracarida specimens were identified to species level  
182 whenever possible (usually above 2 mm body length in genera represented by more than one  
183 species).

184

#### 185 Statistical analysis

186

187 Since regression-based methods could not handle the literature dataset due to the low number  
188 of cases and zero variance in some of the classes, the importance of the variables was assessed  
189 by the more flexible random forest approach (based on conditional inference trees) using the  
190 'cforest' function in the 'party' package (Hothorn et al. 2006) in R 3.2.5 (R Core Team 2016).  
191 When estimating the importance of predictor variables, allowance was made for potential  
192 biases arising from different scale types and from the correlation among them (Strobl et al.  
193 2007; Strobl et al. 2008). Variable importance scores can be used to rank the predictors, but  
194 they are not informative about the strength of the relationship. Therefore, the effect of the  
195 variables with scores amounting to >10% of the highest value was further analyzed with  
196 Fisher's exact tests.

197 We performed redundancy analysis (RDA) to reveal differences in the environmental  
198 preferences among Ponto-Caspian peracarids using the 'rda' function in the 'vegan' package  
199 (Oksanen et al. 2016). We restricted the analysis to the lower section of the river (river km <  
200 685, comprising 13 sites with 56 samples; Fig. 2) where several of the non-invasive species  
201 were present (only *P. lacustris* occurred upstream of this section), or at least could have been



202 present potentially based on previous records (Borza et al. 2015). We used  $\log(x+1)$  and  
203 Hellinger-transformed (Legendre and Gallagher 2001) count data (individuals per sample) in  
204 the analysis, but we show ind./m<sup>2</sup> values in Fig. 3 and 6 for the sake of comparability.  
205 Explanatory variables included substrate type (Table 1), depth, current velocity (measured at  
206 approx. 5 cm from the bottom), pH, conductivity, dissolved O<sub>2</sub>, dissolved organic carbon,  
207 chlorophyll-a, suspended matter, total nitrogen, and total phosphorus concentration. We  
208 performed forward selection (Blanchet et al. 2008a) on the environmental variables with two  
209 different adding limits,  $p = 0.05$  and  $0.01$  (using the ‘ordiR2step’ function in the ‘vegan’  
210 package), and constructed RDA models with each of the two selected variable sets. We tested  
211 the variance explained by the models with ANOVA involving 9999 permutations.  
212 To provide an insight into the autocorrelation structure of the data, we constructed Mantel  
213 correlograms (Borcard and Legendre 2012) using the ‘mantel.correlog’ function in the  
214 ‘vegan’ package about the response variables as well as the residuals of the two RDA models.  
215 The first distance class in the correlograms represents within-site distances, whereas the  
216 subsequent classes were delimited according to the Sturges equation based on river km  
217 distances among sites (12 classes with equal widths of 61.4 river km; the last six are not  
218 shown). P-values of the Mantel correlation coefficients were calculated with Holm-correction.  
219 Since the correlograms did not indicate significant residual spatial autocorrelation (Fig. 5), the  
220 inclusion of a spatial submodel (e.g., asymmetric eigenvector maps, AEM; Blanchet et al.  
221 2008b) was not necessary.

222

223 Results

224

225 Literature data

226

227 A total of 62 peracarid species could be identified as potentially invasive based on our criteria  
228 (Table 2). Overlap was high between the two basins, 59 species being present in the Danube  
229 catchment versus 56 in the Dnieper. In the southern corridor, lithophily proved to be the most  
230 important variable in explaining invasion success, followed by psammophily (its variable  
231 importance score amounting to 15% of the score of lithophily), while all remaining variables  
232 received scores less than 0.01% of the highest. In numbers, all of the 13 invasive species were  
233 lithophilous, whereas 39 out of 46 non-invasive species were not lithophilous, meaning that  
234 the two variables are dependent on each other with a high statistical certainty (Fisher's exact  
235 test of independence,  $p < 0.0001$ ). Psammophily was also strongly associated with invasion  
236 success ( $p < 0.001$ ), but had less explanatory power in terms of numbers (9 out of 13 invasive  
237 species not psammophilous, 40 out of 46 non-invasive species psammophilous), and even this  
238 arose from the strong negative association with lithophily ( $p < 0.0001$ ). Substrate preference  
239 varied strongly among peracarid orders, but the role of lithophily in relation to invasion  
240 success was consistent.

241 In the central invasion corridor the low number of invasive species did not allow the  
242 evaluation of variable importances (all variables were scored zero), but as all four invasive  
243 species are lithophilous (the two variables being dependent at  $p = 0.01$ ), the results are  
244 consistent with the southern corridor.

245

246 Field data

247

248 A total of 22 Ponto-Caspian peracarid species were recorded during the survey of which 21  
249 were present in the section below river km 685 (Fig. 3; *Dikerogammarus bispinosus* was  
250 found only between river km 2258 and 1252). Invasive species tended to occur more  
251 frequently than non-invasive ones and were usually more abundant whenever present (Fig. 3).

252 The forward selection process with  $p = 0.05$  retained six environmental variables, namely  
253 substrate type, pH, conductivity, dissolved  $O_2$ , chlorophyll-a, and total phosphorus  
254 concentration which altogether explained 28.8% of the total variation ( $df = 10$ ,  $F = 3.23$ ,  $p <$   
255  $0.001$ ). With  $p = 0.01$ , the only retained variable was substrate type, accounting for 18.8% of  
256 the variance ( $df = 5$ ,  $F = 3.54$ ,  $p < 0.001$ ). Comparing the results of the two models revealed  
257 that the five physicochemical variables had a minor, individually not interpretable effect on  
258 the ordination of the species (Fig. 4, Appendix 1). Nevertheless, their inclusion eliminated  
259 spatial autocorrelation in the data, which was still present to some degree when substrate  
260 types were considered only (Fig. 5). Consistent differences could be detected between the  
261 substrate preferences of invasive and non-invasive species; the former preferred stony  
262 substrates while the latter were associated mainly with soft sediments (they were not found on  
263 stony substrates at all; Fig. 4, 6). Representatives from both groups occurred on macrophytes  
264 and wood ('phytal'), but invasive species were more abundant on average on these substrates  
265 (Fig. 6). Although the separation between invasive and non-invasive species was not perfect  
266 on the ordination plane (Fig. 4), the main reason for this was the rarity of certain species in  
267 the material (rare species were positioned near the origin). Our dataset does not allow solid  
268 conclusions to be made on the environmental preferences of these species.

269

## 270 Discussion

271

### 272 Factors of invasion success

273

274 Both datasets indicated substrate preference, specifically lithophily as the most important  
275 factor in determining invasion success among Ponto-Caspian peracarids. The two analyses  
276 supplemented each other; literature data showed a comprehensive but somewhat schematic

277 picture about the whole species pool, whereas field data provided a more detailed insight into  
278 the environmental preferences of the most frequent species.

279 The most obvious explanation for the importance of substrate preference is that lithophilous  
280 species have a higher chance of establishment and proliferation outside their native range  
281 because waters here are dominated by stony substrates (gravel, riprap). This explanation is in  
282 accordance with general observations identifying environmental match as the most consistent  
283 factor of invasion success across various groups of organisms (Hayes and Barry 2008). On the  
284 other hand, it also seems reasonable to assume that substrate preference might also affect the  
285 chance of being transported to distant places. Lithophilous species might be more inclined to  
286 attach to hard surfaces of ships, the main means of transport (Reinhold and Tittizer 1999).

287 These two alternatives are not mutually exclusive; in all likelihood both explanations have  
288 some effect on the chance of passing successive stages of the invasion process.

289 Since presently all invasive species are lithophilous but not all lithophilous species are  
290 invasive, preference for hard substrates can be considered as a necessary but not sufficient  
291 prerequisite of invasion success among Ponto-Caspian peracarids. Although the number of  
292 factors included in the analysis was rather low, it is not likely that the consideration of more  
293 variables would change this conclusion, since the importance of habitat match for invasion  
294 success is widely reported and quite evident. Nevertheless, we can presume that some  
295 additional factors of invasion success do exist and accounted for presently non-invasive  
296 lithophilous species. Similarly, although present invasion patterns do not allow much  
297 distinction among non-lithophilous species, invasion potential might vary among them, too.

298 Below we list three factors we consider as potentially relevant in this regard.

299 (1) Invaders already present might impede the establishment of further colonists. Sympatric  
300 members of the Ponto-Caspian peracarid assemblage can be assumed to coexist stably  
301 through resource partitioning, based on their shared evolutionary history (Gallardo and

302 Aldridge 2015). In contrast, the circumstances allowing their coexistence within their native  
303 range might not be provided outside it in all cases. For instance, phytophilous amphipods  
304 (e.g., *P. robustoides*, *O. crassus*, and *C. warpachowskyi*) can be assumed to be able to use  
305 (and actually prefer) stony substrates (Jermacz et al. 2015b), but the presence of lithophilous  
306 species, above all *D. villosus*, might prevent them from doing so (Jermacz et al. 2015a). This,  
307 in the absence of extended macrophyte stands and lentic sandy shoals might impede the  
308 establishment of the newcomers, or even result in the decline of their populations already  
309 present. This mechanism might explain the extinction of *O. crassus* from the Middle Danube  
310 during the 20<sup>th</sup> century concurrent with the appearance of *D. villosus*, and similarly, the  
311 disappearance of *Chelicorophium maeoticum* (Sowinsky, 1898) in the Serbian section of the  
312 Danube and the River Tisza might be linked to the invasion of *C. curvispinum* (Borza et al.  
313 2015).

314 (2) Propagule pressure, a strong determinant of invasion success (Hayes and Barry 2008;  
315 Simberloff 2009), can be expected to be correlated with abundance within the donor region.  
316 Accordingly, some of the species might simply be too rare to have a realistic chance of being  
317 transported over long distances in numbers high enough to develop a persistent colony.  
318 According to Dediu (1980), several species occur generally in very low numbers (1-10  
319 ind./m<sup>2</sup>), while the density of some others might reach the magnitude of tens of thousands  
320 ind./m<sup>2</sup>. Furthermore, some of the species have only a few known occurrences which often  
321 date back to several decades ago (Lyashenko et al. 2012).

322 (3) All of the species included in the list of potential invaders have been recorded in  
323 freshwater; however, low salinity might be suboptimal for some of them, decreasing their  
324 chance of ever expanding their ranges in inland waters. Moreover, considering that saltwater  
325 can intrude the deltas of rivers, occasionally up to several tens of kilometers in the artificially  
326 deepened Sulina arm of the Danube, for example (Bondar 1983), sporadic occurrences in

327 freshwater might not prove independence of saltwater in all of the cases. Thus, detailed  
328 studies of their autecology might identify some of the species included in the list as an  
329 occasional visitor in freshwaters.

330

331 Future prospects

332

333 We acknowledge that lithophily is not the only factor affecting invasion success among  
334 Ponto-Caspian peracarids; nevertheless, it is worthwhile to consider it with regard to future  
335 invasion prospects since it is not trivial among the species. In the southern invasion corridor,  
336 the distribution of only seven lithophilous species (six amphipods and one mysid; Table 3)  
337 remained restricted to the lower reaches of the river as yet, which deserve special attention  
338 when considering potential future invaders. However, due to their rarity (several of them have  
339 not been recorded for decades; Table 3) and possibly to other factors (e.g., negative  
340 interactions with invasives) we estimate the probability of their large-scale expansion in the  
341 foreseeable future as low. Even if some of them became invasive after all, the effect on  
342 lithophilous assemblages could most likely be minor, since no considerable functional novelty  
343 can be attributed to them in addition to the species already present. Of course, they can be  
344 expected to occupy different niches, which might imply changes in resource utilization either  
345 by the consumption of previously unused resources or competition for used ones, but this kind  
346 of impact is not comparable to the functional novelty represented by the appearance of the  
347 first corophiid (Van den Brink et al. 1993), large predatory gammarid (Dick et al. 2002), or  
348 zooplanktivore mysid (Ketelaars et al. 1999) in a given ecosystem. Therefore, in this limited  
349 context; i.e., regarding habitats dominated by hard substrates and not considering the potential  
350 further spread of already invasive species, it might be justified to conclude that 'the worst is  
351 over'.

352 Apparently, the system has reached a steady state where the pool of lithophilous species has  
353 run out (or it is close to it, at least), and non-lithophilous species are not able to expand (over  
354 large distances, at least). Nevertheless, most invasions in the history of the corridor occurred  
355 in bursts parallel to major developments in navigation (Fig. 1). Is there something that could  
356 disrupt the *status quo* and might induce a new invasion wave? Since considerable economic  
357 interests are involved, further development of the conditions of shipping on the Danube is  
358 continuously on the agenda, for example in the form of deepening the shipping channel,  
359 which would allow larger classes of ships to pass (Anonymous 2016a). This might imply  
360 increasing ship traffic, shortening of travel times, and a rearrangement in the importance of  
361 traffic hubs both in the donor and recipient regions, which in the end might allow further  
362 species to spread. Another issue is the possible construction of dams in the Middle Danube,  
363 which might become inevitable one day due to sinking ground water levels in the Great  
364 Pannonian Plain. This could result in a more-or-less continuous cascade of reservoirs  
365 throughout the upper and middle river sections which might allow the spread of psammo-  
366 pelophilous species, as exemplified by Eastern European large rivers, where, besides several  
367 deliberate introductions, some of the species began to expand spontaneously (Grigorovich et  
368 al. 2002; Filinova et al. 2008; Semenchenko et al. 2015). The secondary spread of several  
369 species introduced into the Baltic region as well as the recent appearance of *P. lacustris* in the  
370 Serbian Danube section and in the River Tisza (Borza and Boda 2013) also indicate that  
371 spontaneous expansion of non-lithophilous species should be dealt with, at least when the  
372 environment is favourable (i.e., it is dominated by soft substrates) and distances are not too  
373 large (in the magnitude of several hundred kilometres). So, when planning such projects,  
374 further invasions of Ponto-Caspian peracarids should be considered among possible  
375 environmental hazards.

376 In the central invasion corridor, the project aimed at widening the bottleneck represented by  
377 the Dnieper-Bug canal is already near the implementation phase (Anonymous 2016b), which  
378 might give a boost to the expansion of Ponto-Caspian species in the near future (Karatayev et  
379 al. 2008). In this region, much more potential remained in lithophilous species; however,  
380 some of them might reach the Baltic basin even sooner from Germany, as in the case of *D.*  
381 *villosus* and *T. trichiatus* (Grabowski et al. 2007b; Rachalewski et al. 2013). The lowland  
382 character of the rivers constituting this waterway (Semenchenko and Vezhnovetz 2008)  
383 combined with a higher vector activity might provide favourable conditions for the spread of  
384 non-lithophilous species, as well. Some of them are already present in the Baltic basin, so  
385 their potential expansion would be of less consequence, but it could imply the colonization of  
386 further areas within the region. On the other hand, several other species are present in the  
387 reservoirs of the Dnieper (Pligin et al. 2014), the possible further spread of which also should  
388 be dealt with under the altered circumstances.

389

## 390 Conclusions

391

392 In our analysis we were able to identify preference for stony substrates as an important factor  
393 of invasion success among Ponto-Caspian peracarids, providing a consistent but not full  
394 explanation for the presently observable patterns, and allowing general conclusions to be  
395 made on future prospects. At the same time, our effort highlighted how insufficient our  
396 present knowledge is about the taxonomy, faunistics, autecology, and interactions of this  
397 important group. In the light of their already significant impact and still high potential for  
398 further expansion, much more effort should be devoted to studying Ponto-Caspian peracarids  
399 within their native range, which could allow us to provide a more precise assessment of future  
400 invasion risks.



401

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415

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622 Captions

623

624 Fig. 1

625 Cumulative number of invasive Ponto-Caspian peracarid species in the course of time along  
626 the southern corridor, based on first records outside the native range. Points are connected  
627 only for the sake of expressiveness. Dashed line illustrates the time having passed until  
628 present (2016) since the last new species was detected. References: 1: Unger (1918), 2: Borza  
629 (2011), 3: Dudich (1927), 4: Borza et al. (2015), 5: Dudich (1930), 6: Sebestyén (1934), 7:  
630 Woynárovich (1954), 8: Nosek and Oertel (1980), 9: Neemann et al. (1995), 10: Weinzierl et  
631 al. (1997), 11: Wittmann et al. (1999), 12: Bernerth et al. (2005), 13: Wittmann (2002).

632

633 Fig. 2

634 Sampling sites (white triangles) during Joint Danube Survey 3 downstream of river km 685.  
635 The dark shaded area corresponds to the River Danube basin. Country codes: RS: Serbia, RO:  
636 Romania, BG: Bulgaria, MD: Moldova, UA: Ukraine.

637

638 Fig. 3

639 Frequency of occurrence versus average abundance (whenever present)  $\pm$  SE of Ponto-  
640 Caspian peracarid species downstream of river km 685 in the Danube during Joint Danube  
641 Survey 3. Both scales are  $\log_{10}$ -transformed. Black triangles: invasive species, white triangles:  
642 non-invasive species. Abbreviations: Cc: *Chelicorophium curvispinum*, Ci: *Chaetogammarus*  
643 *ischnus*, Cr: *Chelicorophium robustum*, Cs: *Chelicorophium sowinskyi*, C\_sp:  
644 *Chelicorophium* sp., Cw: *Chaetogammarus warpachowskyi*, Dh: *Dikerogammarus*  
645 *haemobaphes*, Dv: *Dikerogammarus villosus*, D\_sp: *Dikerogammarus* sp., Es: *Euxinia sarsi*,  
646 Ha: *Hemimysis anomala*, Js: *Jaera sarsi*, Kw: *Katamysis warpachowskyi*, Lb: *Limnomysis*

647 *benedeni*, Oc: *Obesogammarus crassus* , Oo: *Obesogammarus obesus*, Pb: *Paramysis*  
648 *bakuensis*, Pi: *Paramysis intermedia*, Pl: *Paramysis lacustris*, Pr: *Pontogammarus*  
649 *robustoides*, P\_sp: *Paramysis* sp., Pu: *Paramysis ullskyi*, Ss: *Schizorhamphus scabriusculus*,  
650 Tt: *Trichogammarus trichiatus*.

651

652 Fig. 4

653 Triplot of the RDA model including only substrate type. Samples are not shown for the sake  
654 of perspicuity. Black triangles: invasive species, white triangles: non-invasive species, solid  
655 line: convex hull for invasive species, dashed line: convex hull for non-invasive species.

656 Substrate types (explanation in Table 1): ARG: argyllal, LIT: lithal, PEL: pelal, PPE:  
657 psammopelal, PSA: psammal, PHY: phytal. Abbreviations of species names as in Fig. 3  
658 (specimens identified to genus level are not included).

659

660 Fig. 5

661 Mantel correlograms of the response variables (squares/solid line), the residuals of the RDA  
662 model including six explanatory variables (circles/dashed line), and the residuals of the RDA  
663 model including only substrate type (triangles/dotted line). The distance class at 0 river km  
664 corresponds to within-site distances. Solid symbols indicate significant correlations (\*:  $P <$   
665  $0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ ). Numbers on the top of the graph indicate the number of  
666 pairs involved in the calculation of correlations for each distance class. Symbols are  
667 connected only to visualize the trends.

668 Fig. 6

669 Density of invasive (A) and non-invasive (B) Ponto-Caspian peracarid species on different  
670 substrate types (explanation in Table 1) downstream of river km 685 in the Danube during  
671 Joint Danube Survey 3.

672

673 Table 1

674 Definitions of substrate types used in the study.

675

676 Table 2

677 Checklist of already or potentially invasive Ponto-Caspian peracarid species present in the

678 Danube and Dnieper catchments (see text for definitions). Invasion status: 0: not invasive, 1:

679 invasive, NA: not present in the given catchment.

680

681 Table 3

682 Presently non-invasive lithophilous Ponto-Caspian peracarid species in the southern corridor

683 (Danube-Rhine system). \*: The specific rank of *P. aestuarius* is not universally acknowledged

684 (Dediu 1980); therefore, information on its distribution and ecology is very limited.

685

686 Appendix 1

687 Triplot of the RDA model including six explanatory variables. Samples are not shown for the

688 sake of perspicuity. Black triangles: invasive species, white triangles: non-invasive species,

689 solid line: convex hull for invasive species, dashed line: convex hull for non-invasive species.

690 Substrate types (explanation in Table 1): ARG: argyllal, LIT: lithal, PEL: pelal, PPE:

691 psammopelal, PSA: psammal, PHY: phytal. Abbreviations of continuous variables: con:

692 conductivity, dis: dissolved O<sub>2</sub>, chl: chlorophyll-a, toP: total phosphorus. Abbreviations of

693 species names as in Fig. 3 (specimens identified to genus level are not included).

694 Table 1

Substrate type	Definition	Number of samples	Grouping	
			RDA	Fig. 6
argyllal	silt, loam, clay (inorganic); grain size < 0.063 mm	3	argyllal	
riprap	rocks of variable size, artificial	1		hard
mesolithal	cobbles; grain size 6 cm to 20 cm	7	lithal	
microlithal	coarse gravel; grain size 2 cm to 6 cm	3		
psammal	sand; grain size 0.063-2 mm	7	psammal	
psammopelal	sand and mud	13	psammopelal	soft
pelal	mud (organic); grain size < 0.063 mm	16	pelal	
macrophytes	submerged macrophytes, including moss and Characeae	2		phytal
xylal	tree trunks, dead wood, branches, roots	4		

695

696 Table 2

Order/Family	Species	Invasion status		Size class	Substrate preference (-philous)					Mesohaline
		Danube	Dnieper		Lito-	Phyto-	Psammo-	Pelo-	Argyllo-	
<b>Amphipoda</b>										
Behningiellidae	<i>Cardiophilus marisnigrae</i> Miloslawskaya, 1931	0	0	2	0	0	0	1	0	1
Corophiidae	<i>Chelicorophium chelicorne</i> (G.O. Sars, 1895)	0	0	2	1	1	1	1	1	0

Gammaridae	<i>Chelicorophium curvispinum</i> (G.O. Sars, 1895)	1	1	1	1	1	1	1	1	0
	<i>Chelicorophium maeoticum</i> (Sowinsky, 1898)	0	0	1	1	1	1	1	1	0
	<i>Chelicorophium mucronatum</i> (G.O. Sars, 1895)	0	0	1	0	0	1	1	0	0
	<i>Chelicorophium nobile</i> (G.O. Sars, 1895)	0	0	2	1	1	1	1	1	0
	<i>Chelicorophium robustum</i> (G.O. Sars, 1895)	1	0	2	1	0	1	1	0	0
	<i>Chelicorophium sowinskyi</i> (Martynov, 1924)	1	0	1	1	1	1	1	1	0
	<i>Amathillina cristata</i> G.O. Sars, 1894	0	0	3	1	1	0	0	0	0
	<i>Chaetogammarus ischnus</i> (Stebbing, 1899)	1	1	2	1	1	0	0	0	0
	<i>Chaetogammarus placidus</i> (G.O. Sars, 1896)	0	0	3	1	1	0	0	0	1
	<i>Chaetogammarus warpachowskyi</i> (G.O. Sars, 1894)	0	0	1	0	1	1	0	0	0
	<i>Compactogammarus compactus</i> (G.O. Sars, 1895)	0	0	2	0	0	1	1	0	1
	<i>Dikerogammarus bispinosus</i> Martynov, 1925	1	0	3	1	0	0	0	0	0
	<i>Dikerogammarus haemobaphes</i> (Eichwald, 1841)	1	1	3	1	1	0	0	0	0
	<i>Dikerogammarus villosus</i> (Sowinsky, 1894)	1	1	3	1	0	0	0	0	0
	<i>Euxinia maeotica</i> (Sowinsky, 1894) (= <i>Pontogammarus maeoticus</i> )	0	0	2	0	0	1	0	0	1
	<i>Euxinia sarsi</i> (Sowinsky, 1898) (= <i>Pontogammarus sarsi</i> )	0	0	3	0	0	1	0	1	0
	<i>Euxinia weidemanni</i> (G.O. Sars, 1896) (= <i>Pontogammarus weidemanni</i> )	0	0	2	0	0	1	0	0	0
	<i>Gmelina aestuarica</i> Cărbăușu, 1943	0	0	2	0	1	1	1	0	1
	<i>Kuzmelina kusnezowi</i> (Sowinsky, 1894)	NA	0	3	0	0	0	1	0	0
	<i>Lanceogammarus andrussowi</i> (G.O. Sars, 1896)	0	0	1	0	0	1	1	0	0
	<i>Niphargogammarus intermedius</i> (Cărbăușu, 1943)	0	0	2	0	0	1	0	1	0
	<i>Niphargoides corpulentus</i> G.O. Sars, 1895	0	0	3	0	0	1	1	0	1
	<i>Obesogammarus crassus</i> (G.O. Sars, 1894)	0	0	2	0	1	1	0	0	0
	<i>Obesogammarus obesus</i> (G.O. Sars, 1894)	1	0	2	1	1	0	0	0	0
	<i>Paraniphargoides motasi</i> (Cărbăușu, 1943)	0	NA	2	0	0	1	0	0	0
	<i>Pontogammarus aestuarius</i> (Derzhavin, 1924)	0	NA	4	1	0	0	1	0	0
<i>Pontogammarus borceae</i> Cărbăușu, 1943	0	0	2	0	0	1	0	0	0	
<i>Pontogammarus robustoides</i> (G.O. Sars, 1894)	0	0	3	0	1	0	0	0	0	

	<i>Shablogammarus chablensis</i> Cărăușu, 1943	0	0	1	0	0	1	1	0	0
	<i>Shablogammarus subnudus</i> (G.O. Sars, 1896)	0	0	2	0	0	1	1	0	0
	<i>Stenogammarus carausui</i> (Derzhavin & Pjatakova, 1962)	0	0	1	0	0	1	1	0	0
	<i>Stenogammarus compressus</i> (G.O. Sars, 1894)	0	0	2	0	0	1	0	0	0
	<i>Stenogammarus deminutus</i> (Stebbing, 1906)	0	NA	1	0	0	1	0	0	1
	<i>Stenogammarus macrurus</i> (G.O. Sars, 1894)	0	0	2	0	0	1	0	0	0
	<i>Stenogammarus similis</i> (G.O. Sars, 1894)	0	0	2	0	0	1	1	0	0
	<i>Trichogammarus trichiatus</i> (Martynov, 1932)	1	0	3	1	0	0	0	0	0
	<i>Turcogammarus aralensis</i> (Uljanin, 1875) (= <i>Obesogammarus aralensis</i> )	NA	0	3	0	0	1	1	0	1
	<i>Uroniphargoides spinicaudatus</i> (Cărăușu, 1943)	0	NA	3	0	0	1	0	0	0
	<i>Yogmelina limana</i> Karaman & Barnard, 1979	0	0	2	0	0	1	1	0	0
Iphigenellidae	<i>Iphigenella acanthopoda</i> (Grimm) G.O. Sars, 1896	0	NA	2	0	0	1	1	0	0
	<b>Mysida</b>									
Mysidae	<i>Diamysis pengoi</i> (Czerniavsky, 1882)	0	0	3	1	1	0	0	0	0
	<i>Hemimysis anomala</i> G.O. Sars, 1907	1	0	2	1	1	0	0	0	1
	<i>Katamysis warpachowskyi</i> G.O. Sars, 1893	1	0	2	1	1	1	1	0	0
	<i>Limnomysis benedeni</i> Czerniavsky, 1882	1	0	2	1	1	0	0	0	1
	<i>Paramysis bakuensis</i> G.O. Sars, 1895	0	0	4	0	0	1	1	0	0
	<i>Paramysis intermedia</i> (Czerniavsky, 1882)	0	0	2	0	0	1	1	0	0
	<i>Paramysis kessleri sarsi</i> (Derzhavin, 1925)	0	0	4	0	0	1	1	0	0
	<i>Paramysis lacustris</i> (Czerniavsky, 1882)	0	0	3	0	0	1	1	0	0
	<i>Paramysis ullskyi</i> (Czerniavsky, 1882)	0	0	3	0	0	1	1	0	0
	<b>Isopoda</b>									
Janiridae	<i>Jaera sarsi</i> Valkanov, 1936	1	0	1	1	0	0	0	0	0
	<b>Cumacea</b>									
Pseudocumatidae	<i>Caspiocuma campylaspoides</i> (G.O. Sars, 1897)	0	0	1	0	0	1	1	0	1
	<i>Pseudocuma cercaroides</i> G.O. Sars, 1894	0	0	1	0	0	1	1	0	1
	<i>Pseudocuma graciloides</i> G.O. Sars, 1894	0	0	2	0	0	1	1	0	1
	<i>Pseudocuma laeve</i> G.O. Sars, 1914	0	NA	1	0	0	1	1	0	1



<i>Pseudocuma tenuicauda</i> G.O. Sars, 1894	0	0	1	0	0	1	1	0	1
<i>Pterocuma pectinatum</i> (Sowinsky, 1893)	0	0	2	0	0	1	1	0	1
<i>Pterocuma rostratum</i> (G.O. Sars, 1894)	0	0	2	0	0	1	1	0	1
<i>Schizorhamphus eudorelloides</i> (G.O. Sars, 1894)	0	0	2	0	0	1	1	0	1
<i>Schizorhamphus scabriusculus</i> (G.O. Sars, 1894)	0	0	1	0	0	1	1	0	1
<i>Volgacuma telmatophora</i> Derzhavin, 1912	NA	0	1	0	0	1	1	0	1

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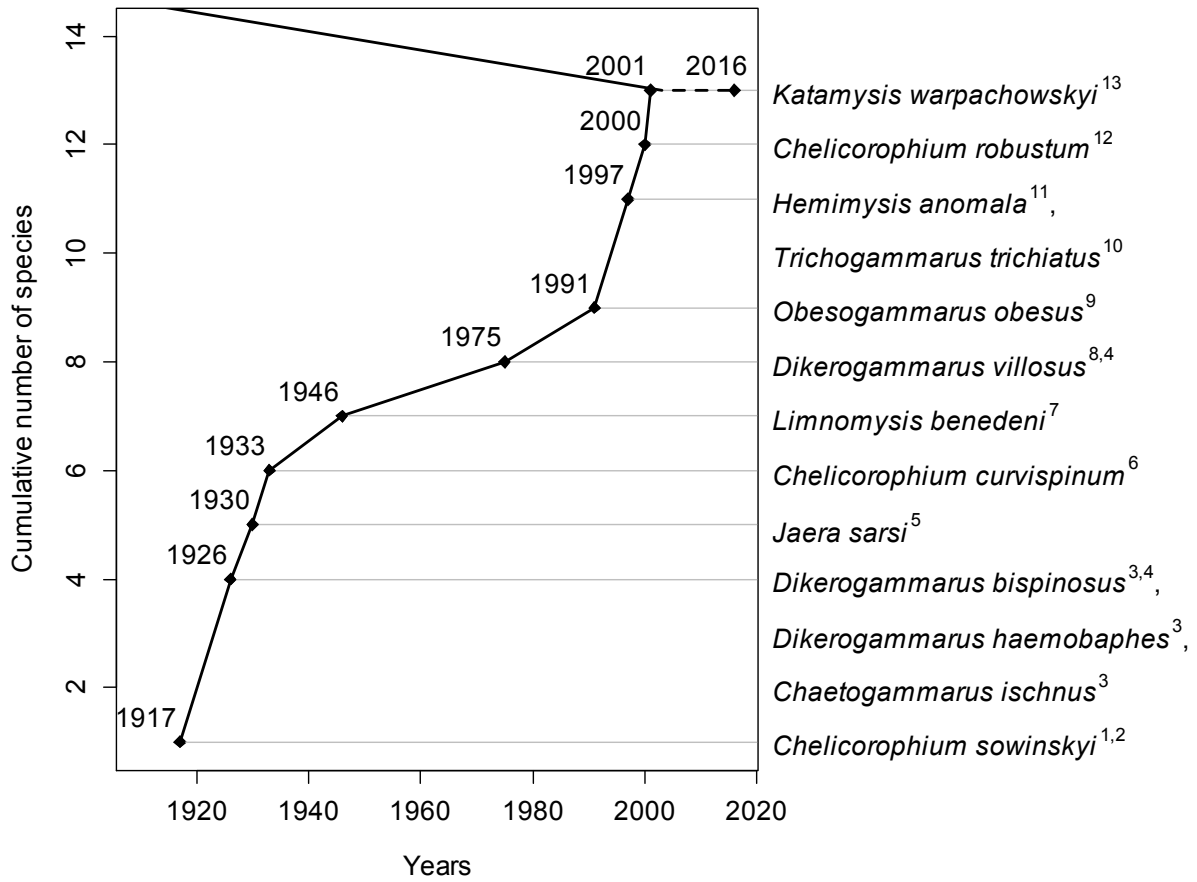
Table 3

Species	Latest records
<i>Amathillina cristata</i> G.O. Sars, 1894	1972-1977: Kiliya branch (Lyashenko et al. 2012)
<i>Chaetogammarus placidus</i> (G.O. Sars, 1896)	1972-1977: Kiliya branch (Lyashenko et al. 2012)
<i>Chelicorophium chelicorne</i> (G.O. Sars, 1895)	2002: Iron Gate I reservoir (Popescu-Marinescu and Năstăsescu 2005); 2003-2009: Kiliya branch (Lyashenko et al. 2012)
<i>Chelicorophium maeoticum</i> (Sowinsky, 1898)	1987-1998: Kiliya branch (Lyashenko et al. 2012)
<i>Chelicorophium nobile</i> (G.O. Sars, 1895)	2003-2009: Kiliya branch (Lyashenko et al. 2012)
<i>Diamysis pengoi</i> (Czerniavsky, 1882)	1985-2008: up to river km 317 (Karl J. Wittmann, unpublished data)
<i>Pontogammarus aestuarius</i> (Derzhavin, 1924)*	1958-1962: up to river km ~375 (Prunescu-Arion and Elian 1965)

700 Figures

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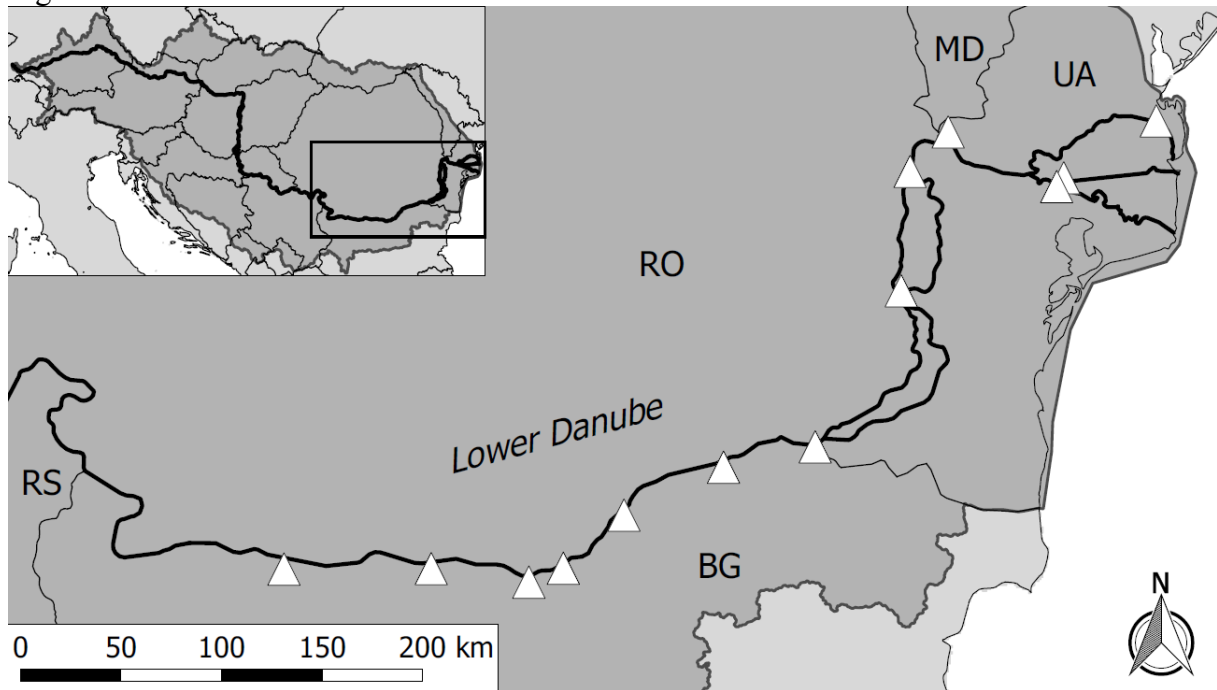
702 Fig. 1



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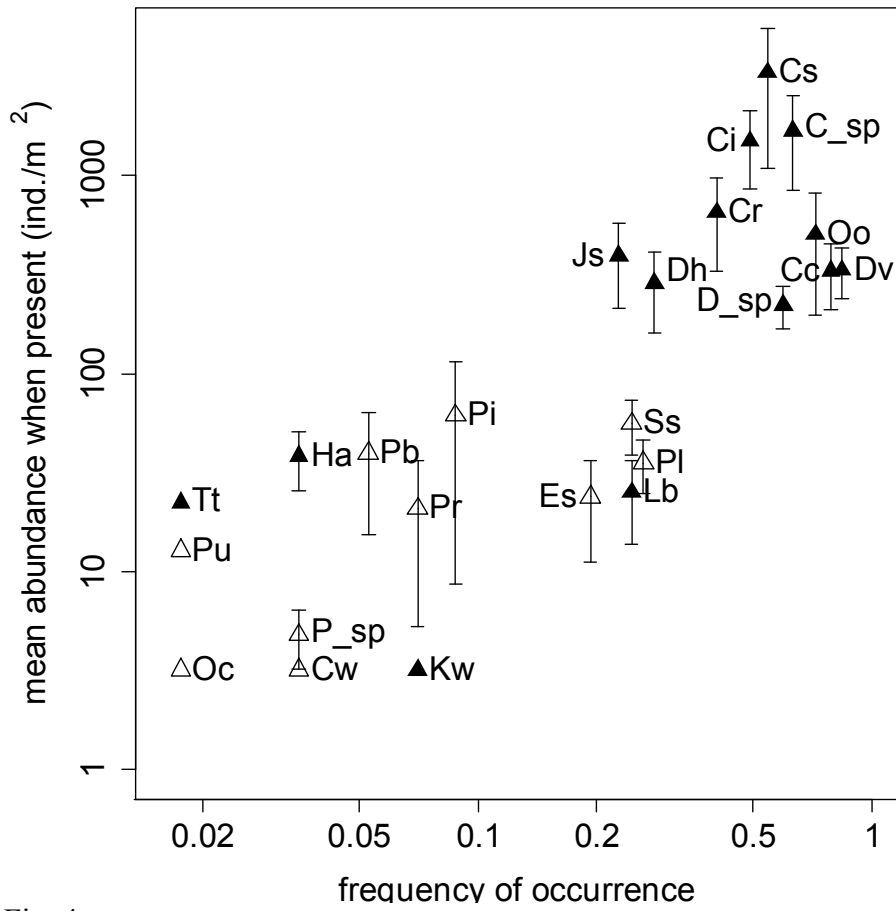
Fig. 2



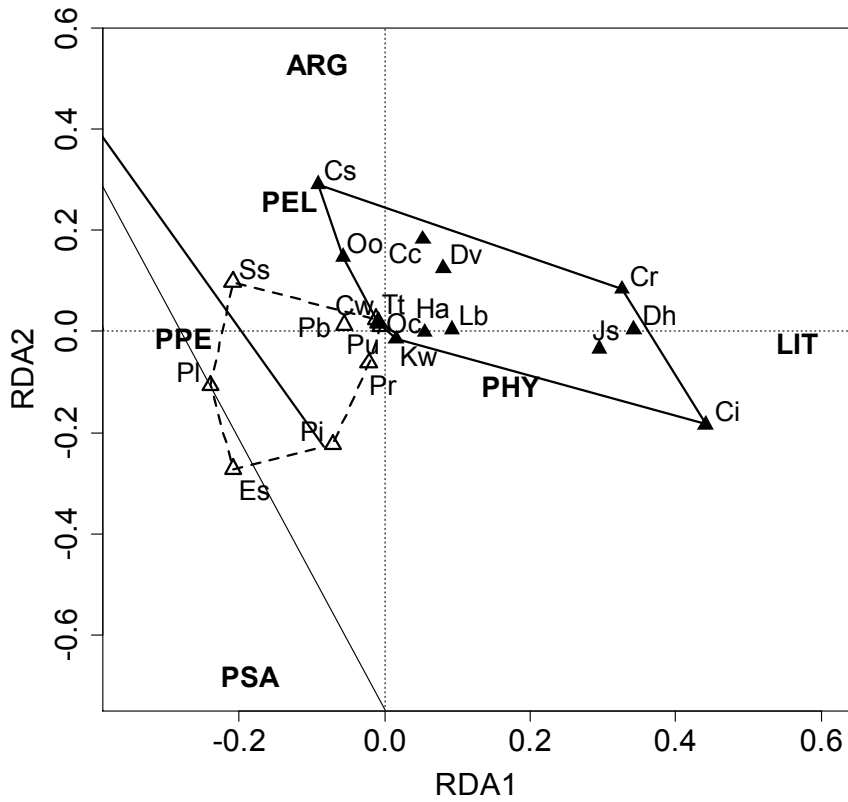
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707 Fig. 3

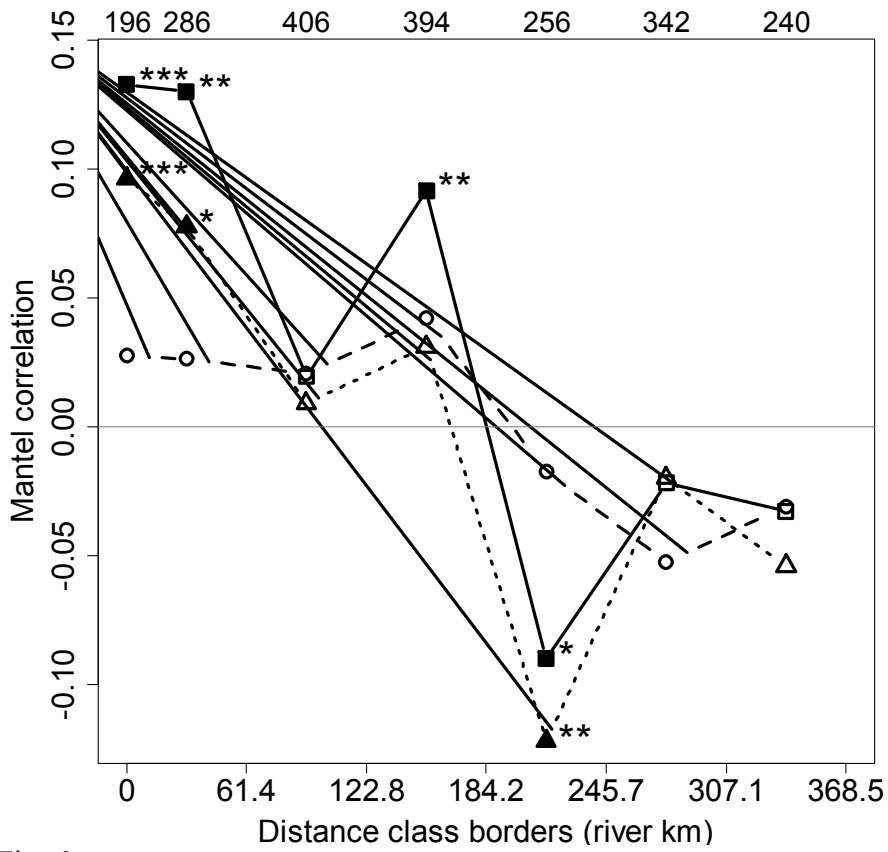


708 Fig. 4  
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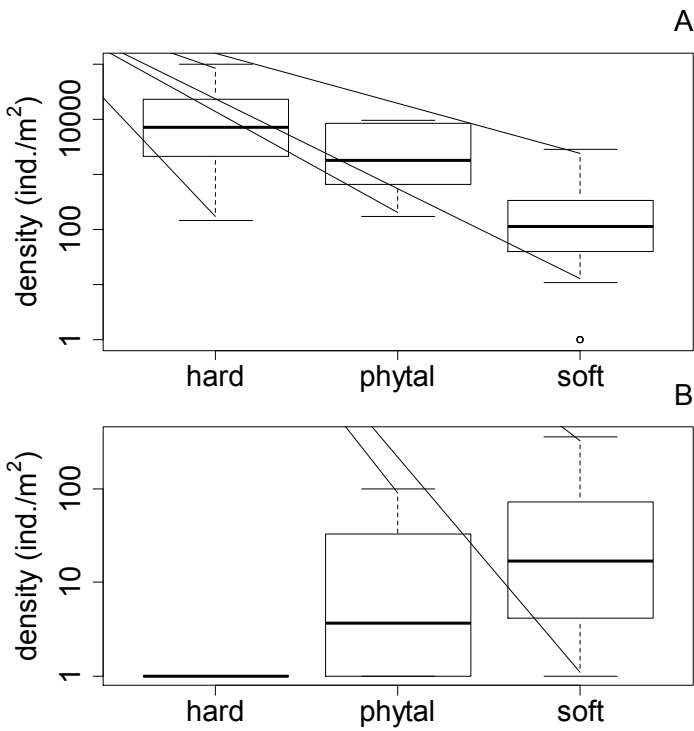


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712 Fig. 5  
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714 Fig. 6  
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