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11   Longitudinal distributional patterns of Peracarida (Crustacea, Malacostraca) in the River

12   Danube

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28

29   **Abstract**

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31   The River Danube plays a central role in the spread of Ponto-Caspian species as a part of the

32   so-called southern invasion corridor (Danube-Main-Rhine system); therefore, changes in its

33   peracarid fauna (comprising the bulk of invasives) merit special attention. The latest

34   international research expedition (Joint Danube Survey 3, 2013) offered an opportunity for

35   updating and synthesizing our knowledge about this group along the Danube, previously

36   based on studies covering only certain river sections and/or dealing with a subset of species.

37   Altogether 17 amphipod, 7 mysid, 3 isopod, and one cumacean species were recorded at 55

38   sites investigated between Ulm (river km 2581) and the Delta. Recent large-distance

39   expansion of additional Ponto-Caspian species was not observed, but three species

40   (*Chelicorophium robustum*, *C. sowinskyi*, and *Echinogammarus trichiatus*) have been able to

41   shorten their distributional gap in the Middle Danube, *E. trichiatus* being recorded for the first

42   time in Serbia. Ponto-Caspian peracarids are still gradually advancing in the German section,

43   as well, implying retreat of native *Gammarus* spp., and impeding the spread of non-Ponto-

44   Caspian invaders. On the contrary, some Ponto-Caspian species seem to have declined in

45   certain river sections; *Dikerogammarus bispinosus* was entirely missing in the Lower Danube,

46   and several species characteristic of the lower reaches had been recorded previously much

47   farther upstream (most notably *Chelicorophium maeoticum* and *Obesogammarus crassus*).

48   The analysis of current and historical distributional patterns revealed that the crucial step in

49   the large-scale spread of Ponto-Caspian species is crossing the section between Baja and the

50   Sava estuary (rkm ~1480-1170) – characterized by an unfavorable combination of relatively

51   strong currents and fine bed material – by passive transport. Presence immediately

52   downstream of this section does not appear to promote further expansion in most of the cases;

53   the source region of large-distance dispersal is most likely the Delta, implying that potential

54   future invaders cannot be identified based on their previous expansion in the lower reaches of

55   the river.

56

57   **Key words**: Amphipoda, Cumacea, Isopoda, Mysida, Ponto-Caspian, range expansion

58

59   **Introduction**

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61   The range expansion of Ponto-Caspian faunal elements has been one of the most significant

62   biogeographical processes in inland waters of the North-Atlantic region in recent times, often

63   having serious consequences on native fauna and ecosystem functioning (Ricciardi &

64   MacIsaac 2000, Bij de Vaate et al. 2002, Ojaveer et al. 2002). The representatives of several

65   metazoan groups are involved, ranging from cnidarians to fish, but in terms of species number

66   peracarid crustaceans dominate; 19 species (13 amphipod, 5 mysid, and one isopod) have

67   extended the limits of their distribution across river basins in Central, Western, or Northern

68   Europe (Bij de Vaate et al. 2002, Bernerth & Stein 2003, Herkül et al. 2009, Hanselmann

69   2010, Grabowski et al. 2012), four of which have established also in the British Isles

70   (Gallardo & Aldridge 2015), and two have appeared even in North America (Witt et al. 1997,

71   Pothoven et al. 2007). In addition, several other species including cumaceans have established

72   in Eastern European reservoirs and lakes mainly by intentional introduction (Grigorovich et

73   al. 2002, Filinova et al. 2008).

74   Europe's second longest – and most international – river, the Danube plays a central role in

75   their spread. Emptying into the Black Sea, it has a direct connection to the Pontic Basin,

76 implying that a high number of Ponto-Caspian endemics are native to the lower reaches of the

77 river (Lyashenko et al. 2012). The first Ponto-Caspian peracarid in the Middle Danube was

78 detected in the 1910s (Unger 1918), and by the 1940s 7 species had established the Hungarian

79 section (Dudich 1947, Borza 2011). The colonization of the Upper Danube was more delayed,

80 starting only in the second half of the 20th century (Kothé 1968), parallel to the growing

81 number of impounded sections favoring the establishment of limnophilous Ponto-Caspian

82 elements. The next step was when in 1992 the Main-Danube canal began to operate,

83 connecting the river to the intertwined Western European waterway network. Since then, the

84 Danube-Main-Rhine system can be referred to as the southern invasion corridor (Bij de Vaate

85 et al. 2002), which – based on the number of species that have passed through – can be

86 regarded as the most important inland invasion route to Western Europe (the other candidate

87 being the central corridor, leading from the Black Sea through the rivers Dnieper, Prypjat,

88 Bug, Vistula, Notec, Oder, and the Mittelland Canal to the Rhine; Bij de Vaate et al. 2002).

89 All of the Ponto-Caspian species that have reached the Upper Danube have already colonized

90 other catchments. The most obvious way forward is the River Rhine; most of the species

91 appeared there within a couple of years after their first record in the German Danube section

92 (Tittizer et al. 2000, Leuven et al. 2009). Several species have continued their expansion in

93 France (Devin et al. 2005, Wittmann & Ariani 2009, Labat et al. 2011, Forcellini 2012), and

94 some also successfully spread eastward using the central invasion corridor, even reaching

95 Poland (Grabowski et al. 2007, Rachalewski et al. 2013a). Due to its earlier connection to the

96 Western European catchments, the appearance of the first Ponto-Caspian invaders overseas

97 can be attributed to the central corridor (Crawford 1935, Cristescu et al. 2004). However, in

98 recent times the southern corridor seems to have taken the leading role in this regard, as well;

99 colonies of *Hemimysis anomala* G. O. Sars, 1907 and *Dikerogammarus villosus* (Sowinsky,

100 1894) could be traced back to the Danube (Audzijonyte et al. 2008, Rewicz et al. 2015), and

101 the recently discovered population of *D. haemobaphes* (Eichwald, 1841) in England also

102 likely derives from the southern corridor inferring from the invasion history of the species

103 (Bij de Vaate et al. 2002, Gallardo & Aldridge 2015).

104 With regard to the role of the Danube in the range extension of Ponto-Caspian species,

105 changes in its peracarid fauna merit special attention. The latest international research

106 expedition, the Joint Danube Survey 3 (JDS3 henceforth) covering the entire navigable course

107 of the river offered an opportunity for updating and synthesizing our knowledge about this

108 group, previously based on studies covering only certain river sections and/or dealing with a

109 subset of species. Accordingly, in the present publication we delineate and interpret the

110 currently observable longitudinal distributions of Peracarida in the river, and provide a

111 synthesis of the biogeographical patterns in the range expansion of Ponto-Caspian species in

112 hope of leading to a better understanding of the processes shaping the fauna of inland waters

113 in the North-Atlantic region.

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115 **Material and Methods**

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117 After 2001 and 2007, the JDS was carried out for the third time between 13 August and 26

118 September 2013. Macrozoobenthos samples were taken at 55 sites of the river between Ulm

119 (river km = rkm 2581) and the Delta (rkm 18, Kiliya branch) using three different methods.

120 (1) Based on the AQEM protocol (Hering et al. 2004), 4-7 samples were collected per site in

121 the littoral zone (0.1-1.5 m depth) by hand net (aperture: 25 x 25 cm, mesh size: 500 μm)

122 representing all habitat types available (multi-habitat sampling, MHS). Each sample consisted

123 of five units covering 25 x 25 cm bottom area. On some occasions, supplementary samples

124 were taken using a Van Veen grab (up to 5 m depth). (2) Deeper parts of the river were

125 sampled using a triangular dredge (aperture: 25 cm, mesh size: 500 μm) towed downstream

126 from a motorboat. Five tows were done per site at locations distributed evenly along the

127 cross-section, each yielding 1-15 dm^3 bed material from depths ranging between 1.1-22.7 m.

128 (3) In order to provide better comparability with previous datasets (JDS2) and to gather data

129 for methodological comparisons, additional samples were taken using the kick and sweep

130 (K&S) sampling technique (EN 27828:1994). Ten subsamples (each covering approx. 25 x 25

131 cm bottom area) representing various habitats were collected at each side of the river in the

132 littoral region up to 2.5 m water depth using a hand net (mesh size: 500 μm). Additional

133 material was gathered from stones, submerged debris, and macrophytes. At the most upstream

134 site only MHS was done.

135 All samples were preserved in 4% formaldehyde solution in the field, and stored in 70%

136 ethanol after sorting. In several cases subsampling was necessary due to the large amount of

137 animals in the samples; altogether ~ 70 000 Peracarida specimens were identified to species

138 level, if possible (usually above 2 mm body length in genera represented by more than one

139 species).

140 Regarding river sections, in the present paper we follow the traditional geographic definition

141 (Upper/Middle Danube: Morava estuary, rkm 1880; Middle/Lower Danube: the lower end of

142 the Carpathian Mountains, ~ Iron Gate I dam, rkm 943).

143 Although the number of sites investigated was lower than in JDS1 and JDS2, the sampling

144 methods applied and the special attention devoted to identification make the present one the

145 most detailed river-wide dataset on peracarids of the Danube. In the present publication we

146 analyze presence-absence data per site based on the pooled sample of all methods (Annex 1);

147 the full dataset is available at: http://www.icpdr.org/wq-db. The results of the survey on the

148 longitudinal distribution of Ponto-Caspian gobies (Pisces: Gobiidae) are discussed by Szalóky

149 et al. (2015).

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Results

Altogether 28 Peracarida species were recorded during the survey representing four orders (17 Amphipoda, 7 Mysida, 3 Isopoda, 1 Cumacea; Table 1). Seven of them were present along the entire course of the river without biogeographically meaningful gaps ("ubiquitous", Fig. 1). *D. villosus* was the most prevalent among all species, occurring at all investigated sites, and *C. curvispinum* was the second, missing only at the most upstream location. Beside them, three other amphipods (*O. obesus*, *E. ischnus*, *D. haemobaphes*), one mysid (*L. benedeni*), and one isopod (*J. sarsi*) could be categorized as ubiquitous.

Three species had a wide distribution similarly to those mentioned above, but with biogeographically interpretable gaps in between ("disjunct", Fig. 1). *C. robustum* and *C. sowinskyi* were missing in certain parts of the Middle Danube (the former between rkm 1367-1159, the latter between rkm 1630-1216), but they were common in the remaining reaches of the river. *E. trichiatus* was detected in three rather separate sections; the Upper Danube, the Iron Gate I reservoir, and the Delta.

Four species were associated with the upstream part of the river (Fig. 1). *G. fossarum* and *P. coxalis* occurred only at the most upstream sampling site, whereas *G. roeselii* was found at two locations of the German section. *D. bispinosus* was common in the Upper and Middle Danube, but it was totally missing in the lower reaches (downstream of rkm 1200).

The distribution of ten species was limited to the lower reaches of the river (Fig. 1), among which *P. lacustris* was the most widespread, occurring downstream of the Tisza estuary (~ 1200 rkm). Three others; *E. sarsi*, *P. intermedia*, and *P. robustoides* also had a relatively wide distribution, penetrating almost 700 rkm into the river. The range of the remaining six species was more restricted; the most upstream record of *P. bakuensis* and *S. scabriusculus* was

175   around rkm 200, while *E. warpachowskyi*, *O. crassus*, *P. ullskyi*, and *U. spinicaudatus* were

176   found only in the Delta during the survey.

177   Four species occurred in the river relatively rarely and without biogeographical determination

178   („sporadic", Fig. 1). *K. warpachowskyi* was present at 15 sites scattered along the whole

179   course of the river, while another mysid, *H. anomala* was found only at one location. *A.*

180   *aquaticus* and *N. hrabei* were each recorded at two rather distant sites.

181

182   **Discussion**

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184   Present distributional patterns

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186   There is no surprise among the species with a ubiquitous occurrence; they all are successful

187   invaders with wide distributions even outside the Danube catchment (Tittizer et al. 2000, Bij

188   de Vaate et al. 2002). It should be noted, however, that although they were found sporadically

189   during the survey, *H. anomala* and *K. warpachowskyi* also have a more-or-less continuous

190   distribution within the river as suggested by literature data (Wittmann 2002, 2007, 2008,

191   Borza et al. 2011). The reason for their scarcity in the material could be that they reach their

192   peak abundances in semi-enclosed embayments not investigated during the survey (Borza et

193   al. 2011).

194   The disjunct distribution of *C. robustum* and *C. sowinskyi* was first observed during the

195   previous Joint Danube Survey (Borza et al. 2010) and was later confirmed by other materials

196   (Borza 2011). Our results indicate that both species have narrowed their distributional gaps to

197   some degree; *C. robustum* reached the upstream part of the Serbian section (rkm 1367, as

198   compared to rkm 1533 in 2010; Borza & Puky 2012), whereas *C. sowinskyi* was recorded for

199   the first time downstream of the Hungarian capital (rkm 1630, previously only upstream of

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200   the city, at rkm 1669; Borza 2011). In the light of its recent rapid downstream spread it is

201   reasonable to presume that the gap of *C. robustum* will soon disappear. On the contrary, in the

202   case of *C. sowinskyi* only time will tell whether the new record is the first sign of nascent

203   range expansion (after several years of stasis), or merely a result of occasional downstream

204   drift.

205   *E. trichiatus* followed a similar expansion scenario as *C. robustum*; i.e., it also reached the

206   Upper Danube recently via jump dispersal (Weinzierl et al. 1997) and subsequently spread

207   downstream, the latest non-native record being not far from the dam of the Gabčíkovo

208   reservoir at Čunovo (Borza 2009). In contrast to the expectations, the present survey yielded

209   no records further downstream in the Hungarian section; indicating that its spread stopped or

210   considerably slowed down, at least. On the contrary, the species was found for the first time in

211   the Iron Gate I reservoir, representing its first record for Serbia.

212   The distributional pattern of *D. bispinosus*; i.e., its total absence within its native range is

213   arguably the most curious case of all. The previous JDSs gave similar results; the species was

214   missing approximately downstream of the Tisza estuary (rkm 1252 and 1216 in JDS1 and

215   JDS2, respectively) with a single record from the Delta in 2007 (Kiliya branch, rkm 18; Graf

216   et al. 2008). Literature data suggest that it was still rather common in the lower Danube in the

217   second half of the 20th century (Popescu-Marinescu et al. 2001), but there are some more

218   recent records, as well (Popescu-Marinescu & Năstăsescu 2005, Petrescu 2009). It is always

219   hard to judge the absence of a species, but data at hand indicate that *D. bispinosus* has at least

220   seriously declined during the past decades within its native range, which is rather surprising

221   taking its successful range expansion in Western Europe during the same period into account.

222   Based on records of the three JDSs, native *Gammarus* species in the German section are

223   retreating parallel to the gradual expansion of Ponto-Caspian invaders. In 2001 only

224   *Gammarus pulex* (Linnaeus, 1758), *G. fossarum*, and *G. roeselii* were present at the most

225 upstream sampling site (Ulm, rkm 2581; Bernerth et al. 2002), whereas in 2013 the section

226 was dominated by *D. villosus*, and *G. pulex* was not found. Similarly, *G. roeselii* disappeared

227 at the second site (Kelheim, rkm 2415) by 2013, while the number of Ponto-Caspian species

228 increased from 2 to 8 (Bernerth et al. 2002). Although such changes are indeed regrettable,

229 the total extinction of native species is not likely; smaller tributaries still inhabited by them

230 show that niche segregation is possible at the regional scale. Nevertheless, the process seems

231 to be slowly going on, indicating that the equilibrium has not been reached yet either because

232 of the relatively recent appearance of *D. villosus* in that river section, or perhaps due to long-

233 term changes affecting the outcome of the interaction.

234 Our finding of a single specimen of the Mediterranean invader *P. coxalis* represents only the

235 second record in the Danube. The first occurrence was detected in 2002 at rkm 2218

236 (unpublished data from the Federal Institute of Hydrology, Koblenz), more than 350 km

237 downstream of the present site, indicating that although it has been present for a rather long

238 time, it has not been able to establish viable populations in the river. Another non-Ponto-

239 Caspian immigrant, the North-American amphipod *Crangonyx pseudogracilis* Bousfield,

240 1958, first recorded in the river during the previous survey (Graf et al. 2008) was missing this

241 time. Similarly to native species, invasive Mediterranean and North American peracarids are

242 also negatively affected by the presence of Ponto-Caspian species; they had been successful in

243 Western Europe primarily before Ponto-Caspian invaders appeared (Tittizer et al. 2000), and

244 subsequently their density decreased (Bernauer & Jansen 2006, Leuven et al. 2009). In the

245 Danube, however, Ponto-Caspian species had arrived before them, which apparently

246 precludes their establishment. *C. pseudogracilis* might be least affected; it prefers smaller

247 stagnant or slowly flowing waters and occurs in large rivers only occasionally. Although

248 waterways might contribute to its spread, the main means of its large-scale dispersal is

249 assumed to be ectozoochory (Tittizer et al. 2000, Gerdes & Eggers 2007, Rachalewski et al.

250 2013b), which might allow its further expansion in the Danube basin. *P. coxalis* is also more

251 prevalent in smaller waters (Kaiser 2005, Eggers 2013), but large rivers and canals are the

252 main promoters of its spread (Van der Velde et al. 2000), so its expansion eastwards might be

253 considerably slowed down by its exclusion from the Danube. The North-American amphipod

254 *Gammarus tigrinus* Sexton, 1939 is more dependent on large rivers, so its conflict with Ponto-

255 Caspian species – especially *D. villosus* – is even more pronounced (Dick & Platvoet 2000).

256 Accordingly, although it was present in the River Main during the 1980-90s (Tittizer et al.

257 2000, Bernerth et al. 2005), it has not been able to establish in the Danube after the opening of

258 the Main-Danube canal, and has since declined in that river, as well (Bernerth et al. 2005).

259 Two further species expanding from the Western Mediterranean, the amphipod

260 *Echinogammarus berilloni* (Catta, 1878) and the isopod *Proasellus meridianus* (Racovitza,

261 1919) encountered Ponto-Caspian species while still being restricted to the Rhine (Tittizer et

262 al. 2000), so their appearance in the Danube basin is even less likely.

263 The presence of *N. hrabei* in the German section of the river was first mentioned by

264 Nesemann et al. (1995), but no exact occurrence data have been published so far. Since *N.*

265 *hrabei* is primarily characteristic of smaller stagnant or slow-flowing waters with decaying

266 plant material, our sporadic record in the main arm of the river can be considered as a result of

267 drift. The location suggests that the wetlands around the Isar estuary might host self-

268 sustaining populations. Previously, the distribution of the species was associated with the

269 extent of the former Paratethys (Nesemann 1993), so the occurrence far beyond the

270 Carpathian basin – parallel with records of *Synurella ambulans* (F. Müller, 1846) (Heckes et

271 al. 1996) – poses an intriguing biogeographical question.

272 Our records of *P. intermedia* at rkm 686 and *P. bakuensis* at rkm 232 represent their most

273 upstream occurrences in the river, indicating a slight headway (Table 2). Contrarily, most

274 other species restricted to the lower parts of the river have previously been reported

275 considerably farther upstream (Table 2; the only exception being *E. warpachowskyi* with all

276 known occurrences restricted to the Delta). What is more, several Ponto-Caspian peracarids

277 previously recorded in the Danube were not found during the survey at all. Most of these are

278 restricted to the Delta, where obviously much more sampling effort would be needed to

279 provide a realistic fauna list. However, there are 10 species which penetrated farther upstream

280 in the Danube according to literature data (Table 2).

281

282 Range expansion of Ponto-Caspian peracarids

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284 Based on their penetration into the Danube, three major groups of Ponto-Caspian peracarids

285 can be distinguished. Firstly, almost half of the approximately 60 species present in the

286 Danube system (Lyashenko et al. 2012) have never been recorded upstream of the Delta,

287 indicating that even if adapted to freshwater, the ability to persist in fluvial environment is not

288 trivial among crustaceans originating from the sea.

289 The second group of species has been able to penetrate several hundred kilometers into the

290 river, but still remained restricted to the lower reaches (Table 2). A priori, the natural

291 obstacles of the Iron Gates section could be assumed to determine their distribution; before its

292 impoundment, the extreme currents (reaching ~ 5 m/s in the 150 m wide Kazan pass) must

293 have represented an insurmountable barrier for all peracarids. However, if we take a look at

294 the most upstream occurrences of the species belonging to his group (Table 2) it becomes

295 evident, that surprisingly the narrows does not have a decisive role; several species never

296 reached this section, while some others were present upstream of the Iron Gates even before

297 the dams were built. The final limit to this type of distribution can be found somewhat more

298 upstream in the Serbian section, as shown by historical records of *C. chelicorne, C.*

299 *maeoticum, O. crassus, O. obesus, P. robustoides*, and *E. sarsi*, and also reflected in the

300   present distribution of *C. robustum*, *C. sowinskyi*, *E. trichiatus*, and *P. lacustris*. The

301   explanation lies most likely in the flow conditions. The section immediately upstream of the

302   Iron Gates is characterized by a low slope comparable to the Lower Danube, and since the

303   construction of the Iron Gate I dam, current velocity is even more reduced (Fig. 2). Upstream

304   of the Sava estuary, however, current speed attains the values characteristic of the Middle

305   Danube (0.8-0.9 m/s). This might present a barrier in itself, but the bed material might further

306   aggravate the situation; until approximately Baja the dominant substrate is sand, which –

307   owing to the relatively strong currents – is very mobile, offering unfavorable conditions for

308   most species.

309   Some of the species have probably been able to colonize the lower reaches actively and have

310   been present there for a long time, as indicated by a considerable genetic divergence between

311   fluvial and estuarine populations of *E. ischnus* in the Dniester and Dnieper rivers, for example

312   (Cristescu et al. 2004). On the other hand, it is likely that passive transport also contributed to

313   the expansion of some species, especially those present upstream of the Iron Gates.

314   In several cases, discrepancy is high between the most upstream occurrences and recent

315   records, leaving an impression of fluctuating ranges. In part, this might be an artefact arising

316   from different sampling effort and different methods used, assuming patchy or simply rare

317   occurrence of certain species. However, it is likely that the range of some species has actually

318   been constricted. Some of the previous records (especially those without subsequent

319   confirmation) might reflect temporary establishment; i.e., the presence of a small population

320   developing as a result of passive transport, but later collapsing (e.g., due to a flood).

321   Nevertheless, there are two relatively well documented cases, that of *C. maeoticum* and *O.*

322   *crassus*, where it seems certain that the species considerably retreated. The reasons are

323   unclear, but a possible explanation for such drastic changes might be the exclusion by a more

324   successful invader, suggested by the fact that both *C. curvispinum* and *D. villosus* appeared

325 later in that section (Băcescu 1948, Karaman 1953; the latter mentioning *C. curvispinum*, but

326 the figures depict *C. sowinskyi*, regarded as a subspecies of *C. curvispinum* at that time).

327 The species having successfully crossed the barrier of the Serbian section represent the third

328 group. They have not only populated the whole navigable course of the river, but all of them

329 have already invaded other catchments, as well. Their establishment also seems more stable

330 compared to the species of the previous group; no retreats have been observed in terms of

331 distance to the sea, but *C. sowinskyi* and *D. bispinosus* serve as intriguing examples for

332 decline in more downstream parts of their range. The large distances involved and the lack of

333 records in the intermediate river sections leave jump dispersal by ships as the only viable

334 explanation for their arrival (e.g., Wittmann 2002). Remarkably, the target region of invasions

335 went through a distinct change during time; previously all species appeared in the Middle

336 Danube, but since the mid-1990s all four novel invaders were first detected in the Upper

337 Danube, or even outside the Danube basin, as in the case *C. robustum* (Weinzierl et al. 1997,

338 Wittmann et al. 1999, Wittmann 2002, Bernerth & Stein 2003). This might be in connection

339 with the presumable rearrangement in shipping activity after the opening of the Main-Danube

340 canal, but unfortunately no comprehensive datasets allowing the analysis of the question are

341 available.

342 Previous invasion success is often used in attempts for identifying potential future invaders

343 (Ricciardi & Rasmussen 1998, Gallardo & Aldridge 2013). Accordingly, it might seem

344 logical to assume a temporal order among the three distributional types. The connection

345 between the estuarine and 'lower section' types is quite evident, but does presence in the Iron

346 Gates section in fact portend large-scale expansion? The case of most early invaders (*D.*

347 *bispinosus*, *D. haemobaphes*, *C. sowinskyi*, *E. ischnus*, and *J. sarsi*) is uncertain, since they

348 were already present at the time of the first faunistic investigations both at the Iron Gates and

349 farther upstream in the Hungarian section. Historical occurrences of *C. curvispinum*, *H.*

350 *anomala*, *K. warpachowskyi*, and *L. benedeni*, and present records of *E. trichiatus* allow the

351 conclusion that they reached the lower Serbian section only after their establishment more

352 upstream in the Danube (Băcescu 1948, Karaman 1953, Wittmann 2007). On the contrary, six

353 species (*C. chelicorne*, *C. maeoticum*, *O. crassus*, *P. lacustris*, *P. robustoides*, and *E. sarsi*)

354 having been reported in the Iron Gates section have not been able to colonize the more

355 upstream reaches of the Danube thus far. Only in the case of three successful invaders has the

356 appearance in this part of the river preceded the large-scale expansion, where the stepping

357 stone role cannot be excluded. However, *C. robustum* and *O. obesus* appeared way before

358 their respective first records upstream, so there is no indication of direct connection. In the

359 case of *D. villosus* the time lag between the first records is much shorter (1968 at the Iron

360 Gates vs. 1975 in the Hungarian section; Popescu-Marinescu 1970 cited by Petrescu 2009,

361 Nosek & Oertel 1980) which might indicate relation, but investigations were rather rare at that

362 time, so the colonization might have happened just as well the opposite way. Here we would

363 like to point out that the first record of *D. villosus* in the Hungarian section is mistakenly

364 dated to 1926 in some recent publications (e.g., Nesemann et al. 1995, Bij de Vaate et al.

365 2002, Rewicz et al. 2014). The reason for the confusion is that Dudich (1927) did not indicate

366 the that-time subspecific rank of *D. villosus bispinosus*. Several independent works confirm

367 that *D. villosus* was not present in the Middle Danube even in the 1950s (Dudich 1947,

368 Karaman 1953, Ponyi 1958, Brtek & Rothschein 1964).

369 In conclusion, appearance upstream of the Iron Gates shows little correspondence with large-

370 scale invasion success, establishment in this section does not appear to promote further

371 expansion in the Danube in most of the cases. However, the species pool in the Danube reach

372 adjacent to their estuary might be decisive for large tributaries also colonized by Ponto-

373 Caspian peracarids. In the Drava and Sava only a subset of the third species group is present

374 (Žganec et al. 2009, Borza 2011, Lucić et al. 2015), but the Tisza – allowing further spread of

375　*C. maeoticum* and *P. lacustris* – can be considered as an autonomous side-branch of the

376　southern invasion corridor. The source region of large distance jump dispersal must have been

377　within the Lower Danube in most of the cases at least, but further narrowing is rarely

378　possible. The most informative in this regard is the invasion history of *H. anomala*, the most

379　widespread Ponto-Caspian peracarid at present. This rheophobic species could be

380　characterized by an estuarine distribution before its large-scale expansion began in the 1990s

381　(Wittmann 2007), marking the Delta as the source of its invasion, and indicating at the same

382　time that not only species occurring in the lower reaches should be considered as potential

383　invaders, but – given the chance – estuarine species also might be able to colonize inland

384　waters. Successful introductions or even spontaneous expansions in other rivers also prove

385　that several species restricted to the Delta in the Danube would be able to persist at least in

386　lakes, reservoirs, and other lentic habitats in rivers (Grigorovich et al. 2002). Nevertheless, it

387　might not be entirely random which species are able to realize their potential; there might be

388　traits promoting large-scale invasion success associated with the access to vectors, survival of

389　transport, or the chance of establishment and spread.

390

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392

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References

Audzijonyte, A., Wittmann, K. J. & Väinölä, R., 2008: Tracing recent invasions of the Ponto-Caspian mysid shrimp *Hemimysis anomala* across Europe and to North America with mitochondrial DNA. – Divers. Distrib. **14**: 179–186.

Băcescu, M., 1948: Quelques observations sur la faune benthonique du défilé roumain du Danube: son importance zoogéographique et pratique; la description d'une espèce nouvelle de Mermithide, *Pseudomermis cazanica* n. sp. – Ann. Sci. Univ. Jassy **31**: 240–253.

– 1951: Crustacea: Cumacea. Fauna Republicii populare Romîne, vol. 4(1). – Editura Academiei Republicii Populare Romîne, Bucureşti, pp. 1–95. (in Romanian)

– 1954: Crustacea: Mysidacea. Fauna Republicii populare Romîne, vol. 4(3). – Editura Academiei Republicii Populare Romîne, Bucureşti, pp. 1–126. (in Romanian)

Bernauer, D. & Jansen, W., 2006: Recent invasions of alien macroinvertebrates and loss of native species in the upper Rhine River, Germany. – Aquat. Invasions **1**: 55–71.

Bernerth, H. & Stein, S., 2003: *Crangonyx pseudogracilis* und *Corophium robustum* (Amphipoda), zwei neue Einwanderer im hessischen Main sowie Erstnachweis für Deutschland von *C. robustum*. – Lauterbornia **48**: 57–60.

Bernerth, H., Tobias, W. & Stein, S., 2005: Faunenwandel im Main zwischen 1997 und 2002 am Beispiel des Makrozoobenthos. – In: Faunistisch-Ökologische Untersuchungen

Des Forschungsinstitutes Senckenberg Im Hessischen Main. – Hessisches Landesamt

für Umwelt und Geologie, Wiesbaden, pp. 15–87.

Bernerth, H., Tobias, W., Stein, S. & Turowski, S., 2002: Macrozoobethos. – In: Literáthy, P.,

Koller-Kreimel, V. & Liška, I. (eds.): Joint Danube Survey - Technical Report of the

International Commission for the Protection of the Danube River. – ICPDR, Vienna,

pp. 33–64.

Bij de Vaate, A., Jażdżewski, K., Ketelaars, H. A. M., Gollasch, S. & Van der Velde, G.,

2002: Geographical patterns in range extension of Ponto-Caspian macroinvertebrate

species in Europe. – Can. J. Fish. Aquat. Sci. **59**: 1159–1174.

Borza, P., 2009: First record of the Ponto-Caspian amphipod *Echinogammarus trichiatus*

(Martynov, 1932)(= *Chaetogammarus trichiatus*)(Crustacea: Amphipoda) for the

Middle-Danube (Slovakia and Hungary). – Aquat. Invasions **4**: 693–696.

– 2011: Revision of invasion history, distributional patterns, and new records of Corophiidae

(Crustacea: Amphipoda) in Hungary. – Acta Zool. Acad. Sci. Hung. **57**: 75–84.

Borza, P. & Boda, P., 2013: Range expansion of Ponto-Caspian mysids (Mysida, Mysidae) in

the River Tisza: first record of *Paramysis lacustris* (Czerniavsky, 1882) for Hungary.

– Crustaceana **86**: 1316–1327.

Borza, P., Csányi, B. & Paunović, M., 2010: Corophiids (Amphipoda, Corophioidea) of the

River Danube The Results of a Longitudinal Survey. – Crustaceana **83:** 839–849.

Borza, P., Czirok, A., Deák, C., Ficsór, M., Horvai, V., Horváth, Z., Juhász, P., Kovács, K.,

Szabó, T. & Vad, C. F., 2011: Invasive mysids (Crustacea: Malacostraca: Mysida) in

Hungary: distributions and dispersal mechanisms. – North-West. J. Zool. **7**: 222–228.

Borza, P. & Puky, M., 2012: Current status of epigean aquatic Malacostraca (Crustacea) in

Hungary: accelerating invasions, vulnerable and threatened native species. – Acta

Biol. Debr. Oecol. Hung. **28**: 33–46. (in Hungarian with English summary)

450   Brtek, J. & Rothschein, J., 1964: Ein Beitrag zur Kenntnis der Hydrofauna und des

451   Reinheitzustandes des tschechoslovakischen Abschnittes der Donau. – Biol. Práce **10**:

452   1–64.

453   Crawford, G. I., 1935: *Corophium curvispinum*, GO Sars, var *devium*, Wundsch, in England.

454   – Nature **136**: 685–685.

455   Cristescu, M. E. A., Witt, J. D. S., Grigorovich, I. A., Hebert, P. D. N. & MacIsaac, H. J.,

456   2004: Dispersal of the Ponto-Caspian amphipod *Echinogammarus ischnus*: invasion

457   waves from the Pleistocene to the present. – Heredity **92**: 197–203.

458   Devin, S., Bollache, L., Noël, P.-Y. & Beisel, J.-N., 2005: Patterns of biological invasions in

459   French freshwater systems by non-indigenous macroinvertebrates. – Hydrobiologia

460   **551**: 137–146.

461   Dick, J. T. & Platvoet, D., 2000: Invading predatory crustacean *Dikerogammarus villosus*

462   eliminates both native and exotic species. Proc. R. Soc. Lond. B Biol. Sci. **267**: 977–

463   983.

464   Dudich, E., 1927: Neue Krebstiere in der Fauna Ungarns. – Arch. Balatonicum **1**: 343–387.

465   – 1947: Die höheren Krebse (Malacostraca) der Mittel-Donau. – Fragm. Faun. Hung. **10**:

466   125–132.

467   Eggers, T. O., 2013: Aquatische Neozoen in Fließgewässern des urbanen Raumes der Stadt

468   Braunschweig. – In: Erweiterte Zusammenfassungen der Jahrestagung der Deutschen

469   Gesellschaft für Limnologie (DGL) und der deutschen und österreichischen Sektion

470   der Societas Internationalis Limnologiae (SIL), Koblenz, Germany, 24-28 September

471   2012. – Deutsche Gesellschaft für Limnologie, Hardegsen, pp. 191–196.

472   Filinova, E. I., Malinina, Y. A. & Shlyakhtin, G. V., 2008: Bioinvasions in macrozoobenthos

473   of the Volgograd Reservoir. – Russ. J. Ecol. **39**: 193–197.

474   Forcellini, M., 2012: First record of the Ponto-Caspian invasive crustacean *Chelicorophium*

475   *sowinskyi* (Martinov, 1924) (Amphipoda, Corophiidae) in the French Rhône River. –

476   Crustaceana **85**: 1781–1785.

477   Gallardo, B. & Aldridge, D. C., 2013: Priority setting for invasive species management: risk

478   assessment of Ponto-Caspian invasive species into Great Britain. – Ecol. Appl. **23**:

479   352–364.

480   – – 2015: Is Great Britain heading for a Ponto–Caspian invasional meltdown? – J. Appl. Ecol.

481   **52**: 41–49.

482   Gerdes, G. & Eggers, T. O., 2007: Erstnachweis von *Crangonyx pseudogracilis* (Crustacea:

483   Amphipoda) im norddeutschen Raum. – Lauterbornia **61**: 141–144.

484   Grabowski, M., Jażdżewski, K. & Konopacka, A., 2007: Alien Crustacea in polish waters–

485   Amphipoda. – Aquat. Invasions **2**: 25–38.

486   Grabowski, M., Rewicz, T., Bacela-Spychalska, K., Konopacka, A., Mamos, T. &

487   Jażdżewski, K., 2012: Cryptic invasion of Baltic lowlands by freshwater amphipod of

488   Pontic origin. – Aquat. Invasions **7**: 337–346.

489   Graf, W., Csányi, B., Leitner, P., Paunović, M., Chiriac, G., Stubauer, I., Ofenböck, T. &

490   Wagner, F., 2008: Macroinvertebrates. – In: Liška, I., Wagner, F. & Slobodník, J.

491   (eds.): Joint Danube Survey 2 - Final Scientific Report. – ICPDR, Vienna, pp. 41–52.

492   Grigorovich, I. A., MacIsaac, H. J., Shadrin, N. V. & Mills, E. L., 2002: Patterns and

493   mechanisms of aquatic invertebrate introductions in the Ponto-Caspian region. – Can.

494   J. Fish. Aquat. Sci. **59**: 1189–1208.

495   Hanselmann, A. J., 2010: *Katamysis warpachowskyi* Sars, 1877 (Crustacea, Mysida) invaded

496   Lake Constance. – Aquat. Invasions **5**: S31–S34.

497   Heckes, U., Hess, M. & Burmeister, E.-G., 1996: Ein Vorkommen von *Synurella ambulans* F.

498   Müller 1846 (Amphipoda: Crangonyctidae) in Südbayern. – Lauterbornia **25**: 95–105.

499   Hering, D., Moog, O., Sandin, L. & Verdonschot, P. F., 2004: Overview and application of

500   the AQEM assessment system. – Hydrobiologia **516**: 1–20.

501   Herkül, K., Kotta, J., Püss, T. & Kotta, I., 2009: Crustacean invasions in the Estonian coastal

502   sea. – Est. J. Ecol. **58**: 313–323.

503   Kaiser, I., 2005: *Proasellus coxalis* (Isopoda, Crustacea) - in Bayern gefunden. – Lauterbornia

504   **55**: 81–82.

505   Karaman, S. L., 1953: Pontokaspische Amphipoden der jugoslawischen Fauna. – Acta Mus.

506   Maced. Sci. Nat. **1**: 21–60. (in Serbian with German summary)

507   Kothé, P., 1968: *Hypania invalida* (Polychaeta Sedentaria) und *Jaëra sarsi* (Isopoda) erstmals

508   in der deutschen Donau. Ein Beitrag zur Verbreitungsgeschichte des pontokaspischen

509   Faunenelements im Donaubecken. – Arch. Hydrobiol. Suppl. **34**: 88–114.

510   Labat, F., Piscart, C. & Fontan, B., 2011: First records, pathways and distributions of four

511   new Ponto-Caspian amphipods in France. – Limnologica **41**: 290–295.

512   Leuven, R. S., Van der Velde, G., Baijens, I., Snijders, J., Van der Zwart, C., Lenders, H. R.

513   & Bij de Vaate, A., 2009: The river Rhine: a global highway for dispersal of aquatic

514   invasive species. – Biol. Invasions **11**: 1989–2008.

515   Lucić, A., Paunović, M., Tomović, J., Kovačević, S., Zorić, K., Simić, V., Atanacković, A.,

516   Marković, V., Kračun-Kolarević, M., Hudina, S., Lajtner, J., Gottstein, S., Milošević,

517   Đ., Anđus, S., Žganec, K., Jaklič, M., Simčič, T. & Vilenica, M., 2015: Aquatic

518   Macroinvertebrates of the Sava River. – In: Milačič, R., Ščančar, J., & Paunović, M.

519   (eds.): The Sava River, The Handbook of Environmental Chemistry. – Springer,

520   Berlin/Heidelberg, pp. 335–359.

521   Lyashenko, A. V., Zorina-Sakharova, Y. Y., Makovskiy, V. V. & Sanzhak, Y. O., 2012:

522   Modern State of the Ponto-Caspian Complex of the Macrofauna of Invertebrates in the

523   Lower Reaches of the Danube River within the Territory of Ukraine. – Hydrobiol. J.

524   **48**: 18–37.

525   Nesemann, H., 1993: Zur Verbreitung von *Niphargus* (Phaenogammarus) Dudich 1941 und

526   *Synurella* Wrześniowski 1877 in der ungarischen Tiefebene (Crustacea, Amphipoda).

527   – Lauterbornia **13**: 61–71.

528   Nesemann, H., Pöckl, M. & Wittmann, K. J., 1995: Distribution of epigean Malacostraca in

529   the middle and upper Danube (Hungary, Austria, Germany). – Misc. Zool. Hung. **10**:

530   49–68.

531   Nosek, J. N. & Oertel, N., 1980: Zoologische Untersuchungen an Aufwüchsen in der Donau

532   zwischen Rajka und Budapest. – Ann. Univ. Sci. Budap. Rolando Eotvos Nom. Sect.

533   Biol. **22-23**: 187–204.

534   Ojaveer, H., Leppäkoski, E., Olenin, S. & Ricciardi, A., 2002: Ecological impact of Ponto-

535   Caspian invaders in the Baltic Sea, European inland waters and the Great Lakes: an

536   inter-ecosystem comparison. – In: Leppäkoski, E., Gollasch, S. & Olenin, S. (eds.):

537   Invasive Aquatic Species of Europe. Distribution, Impacts and Management. – Kluwer

538   Academic Publishers, Dordrecht, pp. 412–425.

539   Paunović, M. M., Jakovcev-Todorović, D. G., Simić, V. M., Stojanović, B. D. & Cakić, P. D.,

540   2007: Macroinvertebrates along the Serbian section of the Danube River (stream km

541   1429–925). – Biologia **62**: 214–221.

542   Petrescu, I., 2009: New mentions of amphipods (Crustacea: Amphipoda) from Romanian

543   waters of the Danube. – Trav. Mus. Natl. Hist. Nat. „Grigore Antipa" **52**: 73–86.

544   Ponyi, E., 1958: Neuere systematische Untersuchungen an den ungarischen *Dikerogammarus*-

545   Arten. – Arch. Hydrobiol. **54**: 488–496.

Popescu-Marinescu, V., 1970: Composition and structure of the biocenosis of the Danube. Benthic biocenosis of the littoral and deep zone. – In: Monografia Zonei Porţilor de Fier. – Editura Academiei Române, Bucureşti, pp. 85–110. (in Romanian)

– (1986) Etappen der Zoobenthos-Entwicklung in der Donau, im Gebiet des "Eisernen Tors", Abschnitt Mraconia. – Rev. Roum. Biol. Sér. Biol. Anim. **31**: 73–80.

Popescu-Marinescu, V. & Năstăsescu, M., 2005: Amphipods (Gammaridae and Corophiidae) from Iron Gates I and II Dam lakes–Danube (Romania), concerning especially 2002 situation. – Trav. Mus. Natl. Hist. Nat. „Grigore Antipa" **48:** 501–521.

Popescu-Marinescu, V., Năstăsescu, M., Marinescu, C., Cutaş, F. & Neagu, E., 2001: Amphipoda (Gammaridae and Corophiidae) from Romanian strech of Danube before and after the construction of Iron Gates I damlake. – Trav. Mus. Natl. Hist. Nat. „Grigore Antipa" **43**: 347–366.

Pothoven, S. A., Grigorovich, I. A., Fahnenstiel, G. L. & Balcer, M. D., 2007: Introduction of the Ponto-Caspian bloody-red mysid *Hemimysis anomala* into the Lake Michigan basin. – J. Great Lakes Res. **33**: 285–292.

Prunescu-Arion, E. & Elian, L., 1965: Beitrag zum Studium der Fauna und der Ökologie der Gammariden im rumänischen Abschnitt der Donau. – Arch. Hydrobiol. Suppl. **30**: 65–79.

Rachalewski, M., Banha, F., Grabowski, M. & Anastácio, P. M., 2013b: Ectozoochory as a possible vector enhancing the spread of an alien amphipod *Crangonyx pseudogracilis*. – Hydrobiologia **717**: 109–117.

Rachalewski, M., Konopacka, A., Grabowski, M. & Bacela-Spychalska, K., 2013a: *Echinogammarus trichiatus* (Martynov, 1932) — a new Ponto-Caspian amphipod invader in Poland with remarks on other alien amphipods from the Oder River. – Crustaceana **86**: 1224–1233.

571 Rewicz, T., Grabowski, M., MacNeil, C. & Bacela-Spychalska, K., 2014: The profile of a

572   "perfect"invader–the case of killer shrimp, *Dikerogammarus villosus*. – Aquat.

573   Invasions **9**: 267–288.

574 Rewicz, T., Wattier, R., Grabowski, M., Rigaud, T. & Bącela-Spychalska, K., 2015: Out of

575   the Black Sea: Phylogeography of the Invasive Killer Shrimp *Dikerogammarus*

576   *villosus* across Europe. – PloS one **10**: e0118121.

577 Ricciardi, A. & MacIsaac, H. J., 2000: Recent mass invasion of the North American Great

578   Lakes by Ponto–Caspian species. – Trends Ecol. Evol. **15**: 62–65.

579 Ricciardi, A. & Rasmussen, J. B., 1998: Predicting the identity and impact of future biological

580   invaders: a priority for aquatic resource management. – Can. J. Fish. Aquat. Sci. **55**:

581   1759–1765.

582 Russev, B., 1966: Das Zoobenthos der Donau zwischen dem 845. und 375. Flusskilometer. I:

583   Zusammensetzung, Verteilung, und Ökologie. – Izv. Zool. Inst. Muz. Bulg. Akad.

584   Nauk. **20**: 55–131. (in Bulgarian with German summary)

585 Szalóky, Z., Bammer, V., György, Á. I., Pehlivanov, L., Schabuss, M., Zornig, H., Weiperth,

586   A. & Erős, T., 2015: Offshore distribution of invasive gobies (Pisces: Gobiidae) along

587   the longitudinal profile of the Danube River. – Fundam. Appl. Limnol. **187**: doi

588   10.1127/fal/2015/0768.

589 Tittizer, T., Schöll, F., Banning, M., Haybach, A. & Schleuter, M., 2000: Aquatische Neozoen

590   im Makrozoobenthos der Binnenwasserstraßen Deutschland. – Lauterbornia **39**: 1–72.

591 Unger, E., 1918: Occurrence of *Corophium devium* in the Danube. – Állatt. Közlem. **17**: 148–

592   149. (in Hungarian)

593 Van der Velde, G., Rajagopal, S., Kelleher, B., Muskó, I. B. & Bij de Vaate, A., 2000:

594   Ecological impact of crustacean invaders: general considerations and examples from

595   the Rhine River. – Crustac. Issues **12:** 3–34.

Weinzierl, A., Seitz, G. & Thannemann, R., 1997: *Echinogammarus trichiatus* (Amphipoda) und *Atyaephyra desmaresti* (Decapoda) in der bayerischen Donau. – Lauterbornia **31**: 31–32.

Witt, J. D., Hebert, P. D. & Morton, W. B., 1997: *Echinogammarus ischnus*: another crustacean invader in the Laurentian Great Lakes basin. – Can. J. Fish. Aquat. Sci. **54**: 264–268.

Wittmann, K. J., 2002: Weiteres Vordringen pontokaspischer Mysidacea (Crustacea) in die mittlere und obere Donau: Erstnachweise von *Katamysis warpachowskyi* für Ungarn, die Slowakei und Österreich mit Notizen zur Biologie und zum ökologischen Gefährdungspotential. – Lauterbornia **44**: 49–63.

– 2007: Continued massive invasion of Mysidae in the Rhine and Danube river systems, with first records of the order Mysidacea (Crustacea: Malacostraca: Peracarida) for Switzerland. – Rev. Suisse Zool. **114:** 65–86.

– 2008: Weitere Ausbreitung der pontocaspischen Schwebgarnele (Crustacea: Mysida: Mysidae) *Katamysis warpachowskyi* in der oberen Donau: Erstnachweis für Deutschland. – Lauterbornia **63**: 83–86.

Wittmann, K. J. & Ariani, A. P., 2009: Reappraisal and range extension of non-indigenous Mysidae (Crustacea, Mysida) in continental and coastal waters of eastern France. – Biol. Invasions **11**: 401–407.

Wittmann, K. J., Theiss, J. & Banning, M., 1999: Die Drift von Mysidacea und Decapoda und ihre Bedeutung für die Ausbreitung von Neozoen im Main-Donau-System. – Lauterbornia **35**: 53–66.

WoRMS, 2014: Peracarida. Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=1090 on 2014-12-05.

620   Žganec, K., Gottstein, S. & Hudina, S., 2009: Ponto-Caspian amphipods in Croatian large

621   rivers. – Aquat. Invasions **4**: 327–335.

622   Captions

623

624   Fig. 1. A: Sampling sites on the Danube during JDS3 (site codes are shown for the sake of

625   comparability with the online dataset; numbers are not necessarily consecutive). Shaded area:

626   Danube catchment, ISO codes of riparian countries: DE – Germany, AT – Austria, SK –

627   Slovakia, HU – Hungary, HR – Croatia, RS – Serbia, RO – Romania, BG – Bulgaria, MD –

628   Moldova, UA – Ukraine. B: Distribution of Peracarida species in the Danube during JDS3. ○:

629   multi-habitat sampling, ◊: dredge, ×: kick-and-sweep, neighbouring records are connected

630   with grey line.

631

632   Fig. 2. Mean current velocity and bed material grain size (84% percentile) in the Danube

633   during JDS3.

634

635   Table 1. Peracarid taxa recorded during JDS3. Classification follows the World Register of

636   Marine Species (WoRMS 2014), taxa above family level indicated only if relevant.

637

638   Table 2. Peracarid species penetrating into the lower reaches of the Danube (ordered by their

639   most upstream occurrence ever). ~: estimated value based on geographic name. *: Wittmann

640   (2007) recorded a single specimen of *P. lacustris* near Vienna (rkm 1930).

641

642   Annex 1. Records of peracarids during JDS3 (2013). A: species with occurrences at more than

643   2 sites. CC – *Chelicorophium curvispinum* (G. O. Sars, 1895); CR – *Chelicorophium*

644   *robustum* (G. O. Sars, 1895); CS – *Chelicorophium sowinskyi* (Martynov, 1924); DB –

645   *Dikerogammarus bispinosus* Martynov, 1925; DH – *Dikerogammarus haemobaphes*

646   (Eichwald, 1841); DV – *Dikerogammarus villosus* (Sowinsky, 1894); EI – *Echinogammarus*

647   *ischnus* (Stebbing, 1899); ET– *Echinogammarus trichiatus* (Martynov, 1932); ES – *Euxinia*

648   *sarsi* (Sowinsky, 1898); JS – *Jaera sarsi* Valkanov, 1936; KW – *Katamysis warpachowskyi*

649   G. O. Sars, 1893; LB – *Limnomysis benedeni* Czerniavsky, 1882; OO – *Obesogammarus*

650   *obesus* (G. O. Sars, 1894); PB – *Paramysis bakuensis* G. O. Sars, 1895; PI – *Paramysis*

651   *intermedia* (Czerniavsky, 1882); PL – *Paramysis lacustris* (Czerniavsky, 1882); PR –

652   *Pontogammarus robustoides* (G. O. Sars, 1894); SS – *Schizorhamphus scabriusculus* (G. O.

653   Sars, 1894). B: species with occurrences at 1 or 2 sites. Coordinates are indicative of the sites

654   (i.e., cross-sections of the river); individual samples were collected at slightly different

655   localities. [1]: Kiliya arm, [2]: Sulina arm, [3]: Sf. Gheorghe arm.

Fig. 1

659   Fig. 2

660

661
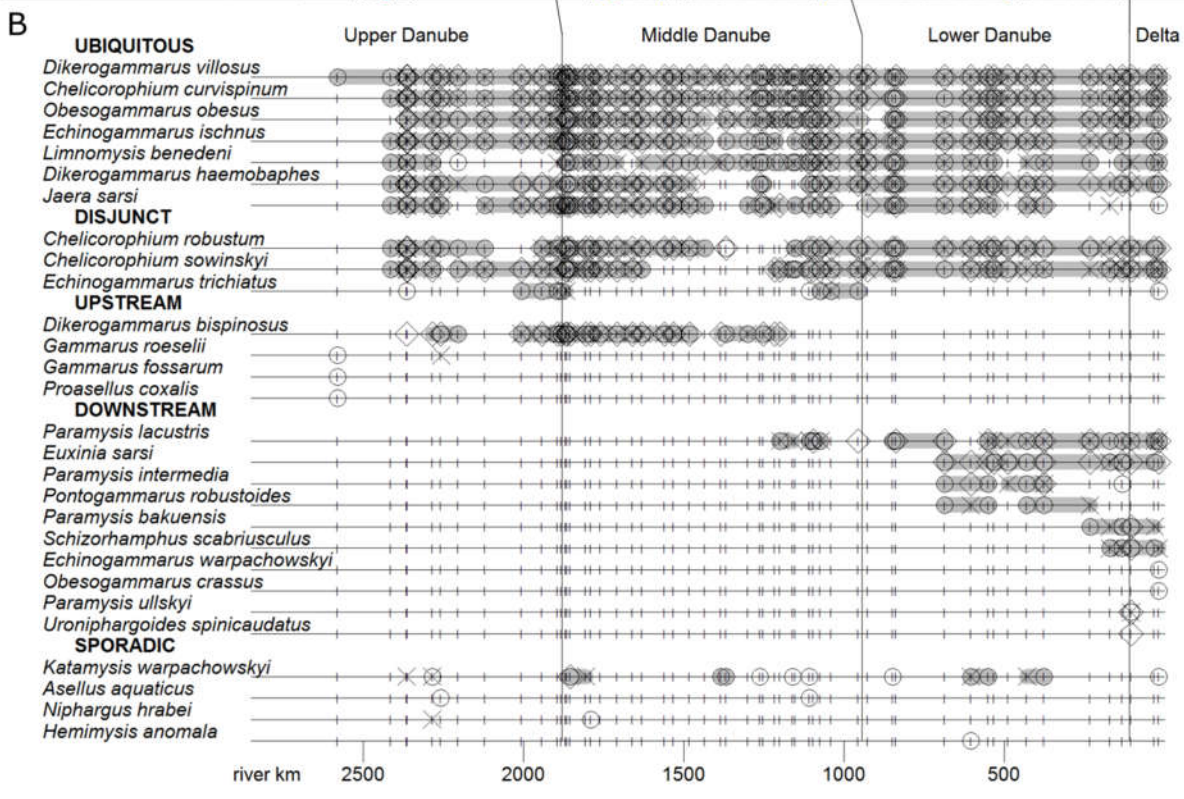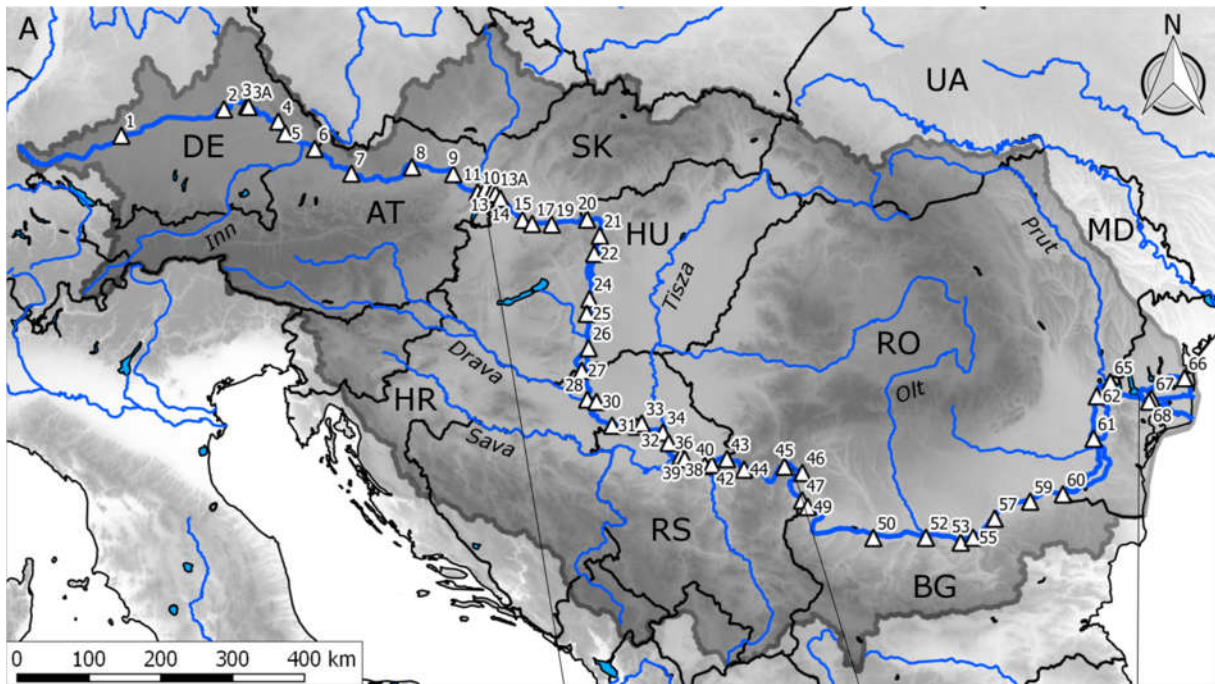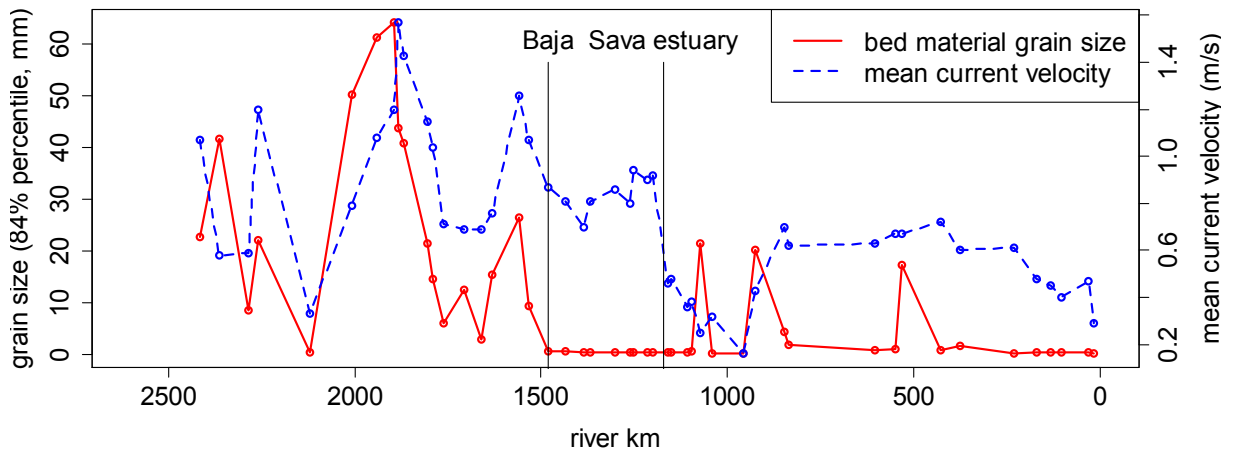
Table 1

Order Amphipoda

Infraorder Corophiida

  Family Corophiidae

Chelicorophium curvispinum (G. O. Sars, 1895)

Chelicorophium robustum (G. O. Sars, 1895)

Chelicorophium sowinskyi (Martynov, 1924)

Infraorder Gammarida

 Parvorder Crangonyctidira

  Family Niphargidae

Niphargus hrabei S. Karaman, 1932

 Parvorder Gammaridira

  Family Gammaridae

Dikerogammarus bispinosus Martynov, 1925

Dikerogammarus haemobaphes (Eichwald, 1841)

Dikerogammarus villosus (Sowinsky, 1894)

Echinogammarus ischnus (Stebbing, 1899)

Echinogammarus trichiatus (Martynov, 1932)

Echinogammarus warpachowskyi (G. O. Sars, 1894)

Gammarus fossarum Koch, 1836

Gammarus roeselii Gervais, 1835

  Family Pontogammaridae

Euxinia sarsi (Sowinsky, 1898)

Obesogammarus crassus (G. O. Sars, 1894)

Obesogammarus obesus (G. O. Sars, 1894)

Pontogammarus robustoides (G. O. Sars, 1894)

Uroniphargoides spinicaudatus (Cărăuşu, 1943)

Order Cumacea

 Family Pseudocumatidae

Schizorhamphus scabriusculus (G. O. Sars, 1894)

Order Isopoda

 Family Asellidae

Asellus aquaticus (Linnaeus, 1758)

Proasellus coxalis (Dollfus, 1892)

 Family Janiridae

Jaera sarsi Valkanov, 1936

Order Mysida

 Family Mysidae

Hemimysis anomala G. O. Sars, 1907

Katamysis warpachowskyi G. O. Sars, 1893

Limnomysis benedeni Czerniavsky, 1882

Paramysis bakuensis G. O. Sars, 1895

Paramysis intermedia (Czerniavsky, 1882)

Paramysis lacustris (Czerniavsky, 1882)

Paramysis ullskyi (Czerniavsky, 1882)

664 Table 2

Species	Most upstream occurrence (rkm)		Reference
	Present survey	Previously	
Paramysis lacustris (Czerniavsky, 1882)	1199	1300*	Paunović et al. (2007)
Obesogammarus crassus (G. O. Sars, 1894)	18 (Kiliya arm)	~1258	Dudich (1947)
Chelicorophium maeoticum (Sowinsky, 1898)	not found	~1116/ ~178 (Tisza)	Karaman (1953)/ Borza (2011)
Euxinia sarsi (Sowinsky, 1898)	686	~1116	Karaman (1953)
Chelicorophium chelicorne (G. O. Sars, 1895)	not found	~1072	Popescu-Marinescu & Năstăsescu (2005)
Pontogammarus robustoides (G. O. Sars, 1894)	686	1072-943	Popescu-Marinescu et al. (2001)
Echinogammarus placidus (G. O. Sars, 1896)	not found	834	Russev (1966)
Stenogammarus carausui (Derzhavin & Pjatakova, 1962)	not found	834	Russev (1966)
Paramysis ullskyi (Czerniavsky, 1882)	104 (Sf. Gheorghe arm)	747	Russev (1966)
Paramysis intermedia (Czerniavsky, 1882)	686	644	Băcescu (1954), Wittmann (2007)
Pontogammarus maeoticus (Sowinsky, 1894)	not found	552	Russev (1966)
Paraniphargoides motasi (Cărăuşu, 1943)	not found	552	Russev (1966)
Niphargogammarus intermedius (Cărăuşu, 1943)	not found	495	Russev (1966)
Schizorhamphus scabriusculus (G. O. Sars, 1894)	170	450	Băcescu (1951)
Pontogammarus borceae Cărăuşu, 1943	not found	375	Russev (1966)
Pontogammarus aestuarius (Derzhavin, 1924)	not found	~375	Prunescu-Arion & Elian (1965)
Uroniphargoides spinicaudatus (Cărăuşu, 1943)	104 (Sf. Gheorghe arm)	~375	Prunescu-Arion & Elian (1965)
Paramysis bakuensis G. O. Sars, 1895	232	80	Băcescu (1954)
Paramysis kessleri sarsi (Derzhavin, 1925)	not found	150	Băcescu (1954)

665

Annex 1A

Site ID	Date	Lat (N)	Lon (E)	Rkm	CC	CR	CS	DB	DH	DV	EI	ET	ES	JS	KW	LB	OO	PB	PI	PL	PR	SS
1	13-Aug	48.42419	10.02761	2581						*												
2	13-Aug	48.90874	11.90123	2415	*	*	*		*	*	*			*		*						
3	14-Aug	48.97915	12.33248	2365	*	*	*	*	*	*	*	*		*	*	*	*					
3A	14-Aug	48.97389	12.36222	2363	*	*	*		*	*	*			*		*	*					
4	15-Aug	48.82700	12.95435	2285	*	*	*	*	*	*	*			*	*	*	*					
5	16-Aug	48.68595	13.10883	2258	*	*		*	*	*	*			*			*					
6	17-Aug	48.52750	13.68978	2205	*	*	*	*	*	*	*					*	*					
7	18-Aug	48.25643	14.41622	2121	*	*	*		*	*	*			*			*					
8	18-Aug	48.38720	15.54578	2007	*		*	*	*	*	*	*		*			*					
9	19-Aug	48.33132	16.33048	1942	*	*	*	*	*	*	*	*		*			*					
10	20-Aug	48.11658	16.80312	1895	*	*	*	*	*	*	*	*		*		*	*					
11	21-Aug	48.16607	16.95138	1882	*	*		*	*	*	*	*		*			*					
13	21-Aug	48.14224	17.08258	1868	*	*	*	*		*	*	*		*		*	*					
13A	21-Aug	48.10639	17.14056	1860	*	*	*	*	*	*	*			*		*	*					
14	22-Aug	48.04858	17.23590	1855	*	*	*	*	*	*	*			*	*	*	*					
15	23-Aug	47.79092	17.65987	1806	*	*	*	*	*	*	*			*	*	*	*					
17	23-Aug	47.74393	17.84257	1790	*	*	*	*	*	*	*			*		*	*					
19	24-Aug	47.74400	18.20568	1761	*	*	*	*	*	*	*			*		*	*					
20	25-Aug	47.81490	18.86405	1707	*	*	*	*	*	*	*			*		*	*					
21	25-Aug	47.61418	19.10332	1660	*	*	*	*	*	*	*			*			*					
22	26-Aug	47.38657	19.00443	1630	*	*	*	*	*	*	*			*		*	*					
24	28-Aug	46.81755	18.92853	1560	*	*		*	*	*	*			*		*	*					
25	29-Aug	46.63305	18.88267	1532	*	*		*	*	*	*			*		*	*					
26	29-Aug	46.20115	18.92518	1481	*	*		*	*	*	*			*		*	*					
27	30-Aug	45.91557	18.80750	1434	*	*				*	*			*		*	*					
28	31-Aug	45.55618	18.91455	1384	*			*		*					*	*						
30	31-Aug	45.52952	19.07842	1367	*	*		*		*	*				*	*	*					
31	1-Sep	45.23288	19.36178	1300	*			*		*	*			*		*	*					

33

Site ID	Date	Lat (N)	Lon (E)	Rkm	CC	CR	CS	DB	DH	DV	EI	ET	ES	JS	KW	LB	OO	PB	PI	PL	PR	SS
32	2-Sep	45.22340	19.80373	1262	*				*	*	*			*	*	*	*					
33	3-Sep	45.26162	19.88713	1252	*			*	*	*	*			*		*	*					
34	3-Sep	45.14825	20.26202	1216	*			*		*	*			*		*	*					
36	4-Sep	45.01687	20.36708	1199	*		*	*		*				*		*	*			*		
38	6-Sep	44.85318	20.57685	1159	*	*	*			*					*	*	*			*		
39	6-Sep	44.81287	20.64495	1151	*	*	*			*	*			*		*	*					
40	7-Sep	44.72470	21.00068	1107	*	*	*		*	*	*	*		*	*	*	*			*		
42	7-Sep	44.73655	21.12330	1095	*	*	*		*	*	*					*	*			*		
43	8-Sep	44.80508	21.38992	1073	*	*	*		*	*	*	*		*		*	*			*		
44	9-Sep	44.66830	21.68792	1040	*	*	*		*	*	*	*		*		*	*					
45	9-Sep	44.69203	22.39952	956	*	*	*		*	*	*	*				*	*			*		
46	10-Sep	44.60565	22.71053	926	*	*	*		*	*	*			*		*						
47	12-Sep	44.26088	22.68898	847	*	*	*		*	*	*			*	*	*	*			*		
49	13-Sep	44.17505	22.78380	837	*	*	*		*	*	*			*		*	*			*		
50	14-Sep	43.74992	23.89870	686	*	*	*		*	*	*		*			*	*		*	*	*	
52	15-Sep	43.71322	24.80642	604	*	*	*		*	*	*		*	*		*	*			*	*	
53	15-Sep	43.62342	25.40175	550	*	*	*		*	*	*		*	*	*	*	*			*	*	*
55	16-Sep	43.67415	25.61943	532	*	*	*		*	*	*		*	*		*	*			*		
57	18-Sep	43.89015	26.01707	488	*	*	*		*	*	*		*				*		*	*		
59	19-Sep	44.06632	26.65588	429	*	*	*		*	*	*		*	*	*	*	*		*	*	*	
60	19-Sep	44.11870	27.23455	375	*	*	*		*	*	*		*	*	*	*	*		*	*	*	
61	20-Sep	44.77473	27.86305	232	*	*	*		*	*	*		*			*	*		*	*	*	
62	21-Sep	45.30087	27.99493	170	*	*	*		*	*	*		*	*		*	*			*		*
65	22-Sep	45.45785	28.26178	132	*	*	*		*	*	*		*			*	*	*	*	*		*
66	24-Sep	45.39553	29.58547	18[1]	*	*	*		*	*	*	*	*	*	*	*	*			*		*
67	25-Sep	45.19450	28.95933	31[2]	*	*	*		*	*	*		*			*	*		*	*		*
68	25-Sep	45.15953	28.90893	104[3]	*	*	*		*	*			*			*	*		*	*		*

1 Annex 1B

Species	Site ID	Date	Lat (N)	Lon (E)	Rkm
Asellus aquaticus (Linnaeus, 1758)	5	16-Aug	48.68595	13.10883	2258
	40	7-Sep	44.72470	21.00068	1107
Echinogammarus warpachowskyi (G. O. Sars, 1894)	66	24-Sep	45.39553	29.58547	18
Gammarus fossarum Koch, 1836	1	13-Aug	48.42419	10.02761	2581
Gammarus roeselii Gervais, 1835	1	13-Aug	48.42419	10.02761	2581
	5	16-Aug	48.68595	13.10883	2258
Hemimysis anomala G. O. Sars, 1907	52	15-Sep	43.71322	24.80642	604
Niphargus hrabei S. Karaman, 1932	4	15-Aug	48.82700	12.95435	2285
	17	23-Aug	47.74393	17.84257	1790
Obesogammarus crassus (G. O. Sars, 1894)	66	24-Sep	45.39553	29.58547	18
Paramysis ullskyi (Czerniavsky, 1882)	68	25-Sep	45.15953	28.90893	104
Proasellus coxalis (Dollfus, 1892)	1	13-Aug	48.42419	10.02761	2581
Uroniphargoides spinicaudatus (Cărăuşu, 1943)	68	25-Sep	45.15953	28.90893	104

2
3