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A third *Dikerogammarus* invader is located in front of Vienna

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Two species of the genus *Dikerogammarus* are known as successful invaders of Western European rivers: *D. haemobaphes* and *D. villosus*. By allozyme analysis, we found a "subspecies" of *D. villosus* reproductively isolated from *D. villosus* and *D. haemobaphes* in a natural syntopic population in the Hungarian Danube. This taxon clearly merits species status and should be termed *D. bispinosus*. Further genetic surveys revealed a pure *D. bispinosus* population in the Danube at the Morava mouth and two individual in the harbour of Linz/Austria. Further upstream migration is indicated similar to the Pontocaspian distribution.

1 The genus *Dikerogammarus* in the Danube and Rhine system

The genus *Dikerogammarus* STEBBING 1899 is originally known from the Pontocaspian region. Species of this genus live in the Black Sea, the Azov Sea and the Caspian Sea, and penetrate into rivers and lakes in the Aralo-Pontian basin (BARNARD & BARNARD 1983). Recently, two species have invaded the rivers of Western Europe. *Dikerogammarus haemobaphes* (EICHWALD 1841) was found for the first time in the upper Danube in the 1960s (Austrian part, VORNATSCHER 1965) and in 1976 (German part, TITTIZER 1996). Only about 20 years later, *D. villosus* (SOVINSKY 1894) was reported for the first time from the upper Danube: in 1989 from the Austrian part (NESEMANN & al. 1955) and in 1992 from the German part (TITTIZER 1996). After the opening of the Main-Danube Canal across the main watershed in 1992 both species invaded rapidly the Rhine drainage system. As soon as 1993 *D. haemobaphes* reached the Main, a tributary of the Rhine (SCHLEUTER & al. 1994) and 1994 the Rhine (SCHÖLL & al. 1995). A short time later *D. villosus* appeared in the Rhine system by crossing the canal: 1994 reports are documented from the Main and upper and lower Rhine (G. Haas, pers. comm.; BIJ DE VATE & KLINK 1995). It is likely that the larger *D. villosus* displaced *D. haemobaphes* in the Rhine system by competition and probable direct predation (VAN DER VELDE & al. 2000). At present *D. haemobaphes* is hardly found in the Rhine, whereas *D. villosus* has successfully invaded the Rhône system (own observation; A. Bauer, pers. comm.) and the

large rivers in northern Germany (GRABOW & al. 1998). *D. haemobaphes* on the other hand is actively expanding in Poland (JAZDZEWSKI & KONOPACKA 2000).

2 Genetic analyses of *Dikerogammarus* populations in the Danube and imlpications

We report here that a third riverine *Dikerogammarus* species already has colonised the middle and upper Danube: *D. bispinosus*. Our genetic analyses clearly demonstrate the species status of this taxon. It was first described as subspecies of *D. villosus* by MARTYNOV (1925) from the lower Dnjepr. In the following it was thus considered mainly as a subspecies (CARAUSU & al. 1955, PONYI 1956, STRASKRABA 1962); only recently some authors referred to it as a species (BARNARD & BARNARD 1983, JAZDZEWSKI & KONOPACKA 1988).

At one of our sampling localities, in the Hungarian Danube at km 1708 near Szob, all three *Dikerogammarus* species co-occured, but did not hybridise as revealed by allozyme electrophoretic analysis. The lack of hybrid genotypes, i.e. heterozygotes combining fixed diagnostic alleles at one of the loci (MPI), strongly indicates a reproductive isolation among *D. haemobaphes*, *D. villosus* and *D. bispinosus* in this syntopic population. In a subsequent sequence analysis at the mitochondrial loci 16S and COI a strong differentiation was found among the three taxa ranging between 11 % and 20 % Kimura two-parameter distances. The *D. bispinosus* mitochondrial haplotypes were found at four locations: in August 1997 in the Danube near Szob (Hungary), in 1997 in Lake Balaton near Tihany peninsular (Hungary), in October 1998 in the Danube at Devin (Morava mouth, Slovakian-Austrian border) and in April 1998 in the harbour of Linz (Austria). Whereas the Szob and Balaton populations were mixed populations, the Devin population was pure *D. bispinosus* in high abundance. In Linz only two individuals of *D. bispinosus* were found on a ship, demonstrating the dispersal abilities (REINHOLD 1999).

Our report of the abundant and pure *D. bispinosus* population at the Austrian border and a simultaneous record (1998) from the German Danube at the mouth of the river Isar (RACHL & POTEL pers. comm.) indicate a third potential *Dikerogammarus* invader for Western Europe. Further upstream colonisation of the Danube and invasions across the canals to other upper reaches of Western European rivers can be suspected in the future, because this species is reported from upper reaches in the natural range of PontoCaspian rivers (STRASKRABA 1962, JAZDZEWSKI & KONOPACKA 1988). Its dispersal behaviour may be as individualistic as with *D. haemobaphes* and *D. villosus*.

3 Morphological identification of *Dikerogammarus bispinosus*

D. bispinosus can be morphologically distinguished from *D. villosus* according to the (subspecies) description of CARAUSU & al. (1955) and PONYI (1956, 1958). *D. bispinosus* has two main spines on the pointed dorsal protuberances on urosomes 1 and 2 in contrast to three to five spines in *D. villosus*. In addition both the peduncle and flagellum of antenna 2 has dense and long setation in *D. bispinosus*. In *D. villosus*, where only the flagellum has long and dense setation. These features apply mostly to males; in females and juveniles they are less diagnostic.

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