

# Sex ratio and sexual selection in wormshrimps (Crustacea, Amphipoda, Ingolfiellidea)

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## Abstract

Small populations of several species of the groundwater dwelling amphipod genus *Ingolfiella* are found in caves, wells, seabottoms, beaches and riverbed interstitial habitats. To gain insight in the socio-ecology of these elusive species, we used data from collected specimens to explore the relationships between sex-ratios, display of secondary sexual characters and other morphological features, and habitat use. We extracted data on the sex ratios and the presence-absence of secondary sexual characters of 13 species from the literature and through examination of museum material. We found a clearly skewed sex ratio with a preponderance of females, both in the individual species as in the genus as a whole. However, sex ratio and the display of secondary sexual characters were not correlated, nor did these characters correlate with the amount of sexual dimorphism. It remains unknown why so many ingolfiellids have evolved these costly features.

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## Introduction

The literature on the evolutionary relationships of the Metazoa is immense, and furnished by a plethora of alternative (phylogenetic) scenarios (Jenner, 2004; Gittenberger and Gittenberger, 2005). Recently crustaceans have become a popular model

group for testing evolutionary hypotheses (e.g. Klossa-Kilia *et al.*, 2005), especially those related to sexual selection (Bauer, 2001; Charnov, 2004; Charnov *et al.*, 2002; Leite, 2002; Weeks and Bernhardt, 2004; Weeks *et al.*, 2002, 2006). In many species females are larger than males, partially due to a fecundity advantage for large females combined with a mating advantage of small males in scrambles over females (Anderson, 1994). How sexual selection operates in sessile species, or species that are severely restricted in their ability to move, is still not adequately solved. We chose a crustacean group that has been sampled in small quantities for over a century and that provides a set of data that has been largely unused to date. This group, groundwater amphipods in the suborder Ingolfiellidea (wormshrimps) live in a variety of groundwater habitats from freshwater in caves, wells, riverbed sediments, to intertidal and deepsea conditions, and from sub-polar to tropical conditions around the world. No information is available on their sexual behaviour, and the eggs, other than those developing in the ovarium (Siewing, 1963) were never found attached to broodplates or in a marsupium as in other amphipods, or as loose eggs deposited in the sediment.

The interstitial habitat and the organisms that live in this environment of darkness and restricted space form a complex ecosystem. Reported active dispersal in small crustaceans through dense sediment led us to ask questions about how migrating populations in groundwater are build up regarding male and female representatives (Rouch and Danielopol, 1999). This socio-ecological aspect in groundwater crustaceans is of importance when dispersal ability and the potential of founding new populations are investigated. Data from detailed studies on the amphipod *Ingolfiella canariensis* Vonk and Sanchez,

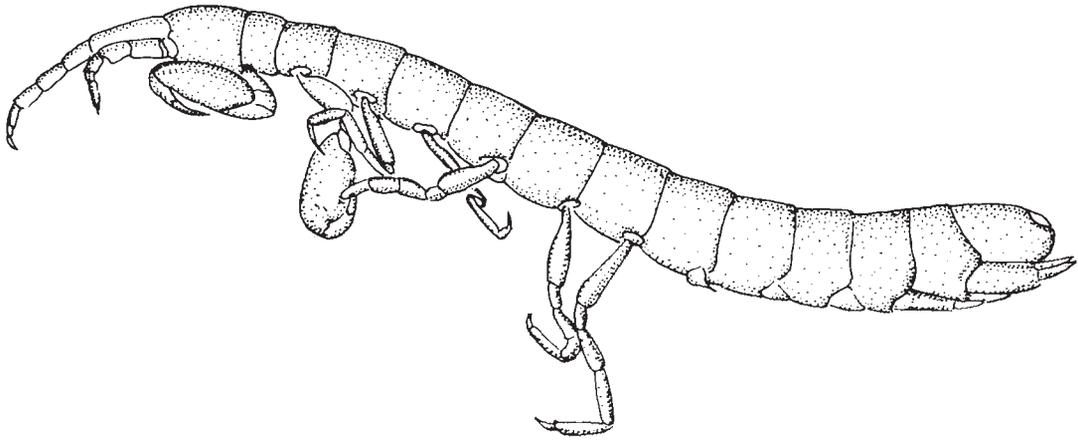


Fig. 1. *Ingolfiella ischitana* Schiecke from subtidal sands in the bay of Naples, Italy. Typical wormshrimp bodyplan.

1993 revealed that sampling spots on marine beaches with coarse, black volcanic sands could hold several specimens at one day but yielded nothing the next day (Vonk and Sanchez, 1993; Vonk, unpubl. data). The hypogean beach environment is very dynamic, even at secluded spots, and the animals move with the sediment, suggesting a fast rate of dispersal in coastal area's. In contrast to this, Danielopol *et al.* (1999), in a study of quantitative micro-spatial distribution, stretching over a year of regular sampling in a confined area in a river system in Southern France, showed how *Ingolfiella thibaudi* Coineau 1968 preferred dense sediment with low porosity and low permeability, suggesting a low capacity to move fast within the interstices. There appears to be marked intergeneric differences in the dispersal abilities, and this in turn may have its effect on the species' socio-ecology. We chose the genus *Ingolfiella* (Fig. 1) with a cosmopolitan distribution to explore these effects.

Data on the exact number of males and females that have been collected were available from 13 species of the 38 species known to date, thus representing almost a third of all known species. Sex ratios are calculated and related to ecological and morphological (secondary sexual) characters. This approach assumes that the number of specimens of each sex that has been collected bears a signal of the real sex ratio. Because determination of sex is not possible in the field it is safe to proceed in the notion that none of the researchers had an *a priori* preference for collecting either male or female specimens.

Furthermore, the method of collection (pumping or scooping up large amounts of water and sieving out all crustaceans), in combination with the small size of the ingolfiellids (<3 mm), makes it unlikely that one of either sex is more likely to be collected than the other.

## Methods

### *Data acquisition*

The data on the sex ratios of *Ingolfiella* were extracted from published papers (Fig. 2). At least six specimens had to be collected in the past in order to be included in our analysis because under the assumption of a one-to-one sex ratio, 6 is the minimal number of individuals to demonstrate a significant departure from this ratio. If data was presented on only a small number of sexed individuals (often just one male and one female), and it was not clear what the sex of the remaining collected specimens of that species was, the study was excluded.

### *Analysis*

For each species we tested whether or not more males or females were collected than a random distribution would show, and we tested whether or not within the genus more species had a preponderance over one of either sex.

For each species we calculated the degree of

sexual dimorphism in body length as presented in the literature, or as the length measured by us from specimens present in the crustacean collection of the Zoological Museum of the University of Amsterdam (ZMA). When a range of lengths were given we used the maximum length in the analysis. Males display an array of secondary sexual characters, mostly in the form of flags or spikes on one of their appendages. We scored the presence of these characters on the pleopods, gnathopods, and uropods, and ranked them based on summation of these characters (0 = none of these characters present, 3 = all three present).

*Ingolfiella* supposedly gained its special features and vermiform appearance in freshwater caves (Siewing, 1963; Vonk and Schram, 2003) and we ranked the different habitats in an evolutionary sequence: from caves (1) to deep sea (3). As we made multiple comparisons, a Bonferroni technique (Sokal and Rohlf, 1995), was used to limit the overall experiment-wise error, with significance assumed if  $P < 0.01$  in a two-tailed test.

**Results**

**Sex ratio and numbers**

For six species significantly more females were collected than males, i. e. *Ingolfiella manni*, *I. canariensis*, *I. fuscina*, *I. britannica*, *I. vandeli* and *I. quokka*. In some species the difference was strongly marked, with for instance 11 times more females than males having been collected in *I. canariensis*, whereas in others the difference, albeit significant, was just two-fold. For none of the species significantly more males than females were collected. Overall, significantly more females were collected than males, i.e. 253 v

Fig. 2. Socio-ecology of Ingolfiellideans (Crustacea, Amphipoda); species are listed by male : female ratio.

Species and literature source	Distribution	Habitat type <sup>†</sup>	Secondary sexual characters <sup>††</sup>	Male body length (mm)	Female body length (mm)	Specimens collected (M : F)	Difference in sex ratio (P-value)
Guanche wormshrimp <i>Ingolfiella canariensis</i> Vonk and Sanchez, 1991	Canary Islands	2	3 (1:1:1)	1.80	1.90	4 : 45	<0.001
Rottnest wormshrimp <i>I. quokka</i> Gallego-Martinez and Poore, 2003	Australia	3	3 (1:1:1)	1.06-1.25	0.78-1.54	2 : 14	<0.05
Catalan wormshrimp <i>I. catalanensis</i> Coineau, 1963	Spain	1	3 (1:1:1)	2.1	2.5	1 : 6	
American wormshrimp <i>I. fuscina</i> Dojiri and Steg, 1987	E. Atlantic, N. Caribbean	3	1 (0:0:1)	1.25-1.33	1.43-1.8	5 : 29	<0.001
English wormshrimp <i>I. britannica</i> Spooner, 1960	Britain	3	1 (0:0:1)	1.71	n.d.	3 : 14	<0.01
Common wormshrimp <i>I. manni</i> Noodt, 1961	South America	2	1 (1:0:0)	1.35	1.55	30 : 90	<0.001
Caribbean wormshrimp <i>I. fontinalis</i> Stock, 1977	Curaçao, S. Caribbean	1	3 (1:1:1)	2.50	2.00	2 : 6	
Greek wormshrimp <i>I. vandeli</i> Bou, 1970	Greece	1	2 (0:1:1)	1.50-1.70	1.43-1.87	6 : 17	<0.05
Aruban wormshrimp <i>I. tabularis</i> Stock, 1977	Aruba, S. Caribbean	2	3 (1:1:1)	2.00	2.30	4 : 9	
Tasmanian wormshrimp <i>I. bassiana</i> Lowry and Poore, 1989	Tasmania	3	3 (1:1:1)	1.70-1.90	1.10-1.80	4 : 7	
Seamount wormshrimp <i>I. sandroruffoi</i> Andres, 2004	N. Atlantic	3	2 (0:1:1)	0.90-1.00	0.70-1.40	6 : 10	
Sardinian wormshrimp <i>I. cottarellii</i> Ruffo and Vigna Taglianti, 1989	Sardinia, Mediterranean	1	1 (1:0:0)	2.30-2.50	2.50-2.70	4 : 4	
Balkan wormshrimp <i>I. petkovskii</i> Karaman, 1957	Balkan	2	3 (1:1:1)	1.80-2.10	2.30-2.40	8 : 2	

<sup>†</sup> 1 = caves, riverbed interstitial; 2 = groundwater and beach interstitial, 3 = subtidal seabottom. <sup>††</sup> The presence of secondary sexual characters on the three appendages is indicated between brackets, and list the presence or absence on pleopods, gnathopods and uropods, respectively. See text for details.

79 (binomial test,  $P < 0.01$ ), and significantly more females than males were collected per species (Mann-Whitney U test,  $n_1 = 13$ ,  $n_2 = 13$ ,  $z = 2.67$ ,  $P < 0.01$ ).

At the genus-level, in 11 species more females than males were collected whereas in 1 species the reverse was observed, the difference being significant (binomial test,  $P < 0.01$ ).

Sex ratio is unrelated to the habitat types in which the species are found (Kruskall-Wallis one-way analysis of variance,  $H = 0.19$ ,  $df = 2$ ,  $P = 0.91$ ), and holds no relation to male or female body size (Spearman Rank Correlation,  $R_s = 0.29$ ,  $P = 0.33$  and  $R_s = 0.22$ ,  $P = 0.48$  for males and females, respectively). The female dominance is not an effect of sampling effort, as there is no relation between the number of individuals collected per species and the sex ratio (Spearman  $R_s = 0.38$ ,  $P = 0.20$ ).

The number of individuals collected per species differs between habitats. Most individuals are collected for species occurring in ground water and beach interstitial ( $48 \pm 51$  individuals species<sup>-1</sup>), and less so for species occurring in caves and riverbeds ( $19 \pm 9$  individuals species<sup>-1</sup>) and subtidal seabottom ( $12 \pm 8$  individuals species<sup>-1</sup>); the difference, however, not being significant (Kruskall-Wallis,  $H = 3.57$ ,  $df = 2$ ,  $P = 0.17$ ).

### Sexual selection

In all species included in the analysis males showed secondary sexual characters. There is no correlation between the amount of secondary sexual characters in males and sex ratio (Spearman  $R_s = 0.11$ ,  $P = 0.70$ ): in those species where there are less males relative to the number of females, the males do not display less secondary sexual characters. The degree of difference in secondary sexual characters in males is not related to the habitat type in which the species is found (Kruskall-Wallis,  $H = 0.60$ ,  $df = 2$ ,  $P = 0.74$ ). Sexual dimorphism reflected in body length holds no relation to sex ratio (Spearman  $R_s = 0.11$ ,  $P = 0.73$ ), nor to the habitat in which the species are found (Kruskall-Wallis,  $H = 2.58$ ,  $df = 2$ ,  $P = 0.27$ ).

Male body length does not differ between the three habitat types recognised; the largest species are found in the subtidal seabottom ( $2.20 \pm 0.38$  mm), with smaller species found in ground water and

beach interstitial ( $1.81 \pm 0.33$  mm) and caves and riverbeds ( $1.44 \pm 0.36$ ) (Kruskall-Wallis,  $H = 5.85$ ,  $df = 2$ ,  $P = 0.06$ ). Female body length, however, does differ between the three habitat types recognised; the largest species are found in the subtidal seabottom ( $2.27 \pm 0.40$  mm), with smaller species found in ground water and beach interstitial ( $2.04 \pm 0.39$  mm) and caves and riverbeds ( $1.64 \pm 0.20$  mm), albeit not significantly so (Kruskall-Wallis,  $H = 6.27$ ,  $df = 2$ ,  $P = 0.04$ ). Pair wise comparisons reveal that only the difference between subtidal seabottom and caves and riverbeds is significant (Mann-Whitney U,  $n_1 = 4$ ,  $n_2 = 4$ ,  $P = 0.01$ ), although females from subtidal seabottoms tend to be larger than those from ground water and beach interstitial (Mann-Whitney U,  $n_1 = 4$ ,  $n_2 = 4$ ,  $P = 0.06$ ).

### Discussion

This study demonstrates unequivocally a clearly skewed sex ratio in wormshrimps, with more females being present, both within the species as in the genus as a whole. This appears not to be related to the morphology of the species, nor to sampling effort. Although we do not have data on the age distribution of wormshrimps, we do not expect that the suborder resorts to an adaptive sex ratio adjustment to temporal variations in population age distributions (Charnov and Hannah, 2002) as there was no relation between sex ratio and the number of individuals sampled. Given the skewed sex ratio it was quite surprising that there was no relation between sex ratio and the amount of secondary sexual characters in males or male body size. Furthermore, we were also not able to demonstrate a difference in sex ratio and the habitat type in which the species is found, and, assuming that the genus probably evolved in a cave environment, the plesiomorph state is not known. However, of the species in our dataset that were assigned to the category 'cave and river interstitial habitats', only *I. cottarellii* is genuinely confined to a cave. Hence, we hypothesise that the ancestral ingolfiellid lived in a one-on-one sex ratio, and, as in *I. cottarellii* (Fig. 2), males showed only a modest amount of secondary sexual characters. Although Siewing (1963) and later Vonk and Schram (2003) indicated that cave species were larger than those found in other habitats (without providing

comparative data), we found that species in caves and riverbeds, or at least the females, were in fact significantly smaller than those in other habitat types. Whether or not this is an artefact of the small sample size in our study or the pooling of male and female body lengths (Vonk and Schram, 2003) remains to be tested.

While we find that females are significantly larger than males there is no indication yet that this is the result of protandry. How males turn into females and then continue to grow larger has been shown in the caridean shrimp *Lysmata* (Bauer, 2001).

The study of sessile species, or species that are severely restricted in their ability to move, including those living in the intertidal range (e.g. Dadon 2005) and inland interstitial spaces (e.g. Karanovic, 2005) is limited. As the study of wormshrimps hitherto has been restricted to anatomical, morphological and taxonomical investigations, we have no knowledge on the function of the secondary sexual characters in life. We have demonstrated that it bears no relation to the sex ratio, nor to the amount of sexual dimorphism (in body length), nor to the habitat in which the species is found. It remains unknown why so many ingolfiellids have evolved these costly features.

We advocate here that wormshrimps, and indeed other groundwater crustaceans, are a suitable taxon to test several socio-ecological theories. Not only holds the grey literature massive amounts of relevant data, ready to be analysed in a contemporary manner, but the lack of behavioural field data clearly hampered our insight in the species' socio-ecology. This is a fruitful avenue along which to proceed with further research. By means of this note, we hope to instigate other workers to pick up this rewarding task.

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