Relative scaling and colouration of cercopodes in *Branchipus schaefferi* (Crustacea: Branchiopoda)

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Abstract: In the crustacean *Branchipus schaefferi* cercopods are brightly coloured caudal rami at the end of the abdomen. The specific life environment of *B. schaefferi* (highly turbid puddles) may suggest that besides their mechanical function, cercopods may also play an ornamental role to attract a potential mate. We performed standardized major axis regression analyses of cercopod length against body length to determine the allometry. Each allometric slope of cercopod was compared with the median of slopes of non-sexual body parts: eye diameter, epipodit, and antennulae lengths. We also examined the relative differences in cercopods colouration between males and females within a population. Cercopods scaled slightly more positively when compared to both median slope of non-sexual traits as well as to typically used slope value 1. The difference in cercopods colouration between the sexes showed a strong sexual dimorphism. Sexual dimorphism in colouration and slightly positive allometry of cercopods support the hypothesis that this organ might be under the sexual selection and function as an ornament advertising fitness of an individual or a signal of sexual maturity of a male in highly turbid water.

Key words: allometry; isometry; sexual selection; secondary sexual traits; fairy shrimp; sexual dichromatism; sexual dimorphism

Introduction

Strong sexual dimorphism is one of the characteristic features of crustacean fairy shrimp *Branchipus schaefferi* Fischer, 1834 (Branchiopoda: Anostraca). Its presence is most obvious in body traits involved in copulation: genitalia and bizarre male antennae forming a clasping organ. They are important diagnostic features used in species determination, since their morphology is strongly species-specific (Brendonck 1995; Brendonck & Belk 1997). In addition to the copulatory traits, sexual dimorphism also occurs in the colouration of cercopods – paired appendages located at the end of the abdomen, brightly red in males. Moreover, besides the cercopods, opalescent blue ovisacs of females are the sole characters which possess strong colouration. Other parts of the body are white or light grey in this fairy shrimp species. The obvious question is: why has this animal evolved such colouration pattern?

Unfortunately, not much is known on visual abilities of fairy shrimps. On the basis of scarce research on mating behaviour of fairy shrimps we can assume that sex (more precisely: copulatory traits) is recognized by sight sense and copulation is preceded by visual evaluation of a potential mate (e.g., Wiman 1981). Few studies on Anostraca mating behaviour also suggest that (at least to some extent) fairy shrimps are able to recognize the size of a mate. On the other hand, males engaged in mating and copulation with the same sex were also observed (e.g., Forbes et al. 1992; Feigenbaum & Switzer 2007; Sugumar 2010).

Multicelular animals (besides some aphid and spider mite species – Altincicek et al. 2012; Moran & Jarvik 2010) are only able to modify the chemical structure of carotenoids, but can not synthesize them (Goodwing 1984). A few studies focused on biochemical factors influencing colouration in some Anostraca species. Glichrist (1968) describes differences in colouration between males and females (including its intensity in case of cercopods), however, her estimations are purely qualitative. She also provides data on the difference in relative abundance of carotenoids (and their complexes) between sexes in *B. schaefferi* (*B. stagnalis* in the original text). Moreover, there is some evidence for the differences in relative abundance of carotenoids in Anostraca on age and species level. Murugen et al. (1995) suggested that even though carotenoids are distributed in the whole body in *Thamnocephalus platyurus* Packard, 1877, there are different ratios of cis- and trans-canthaxanthin in different parts of the animal body. Moreover, they noted that composition of carotenoids changes between larval stages and matu-
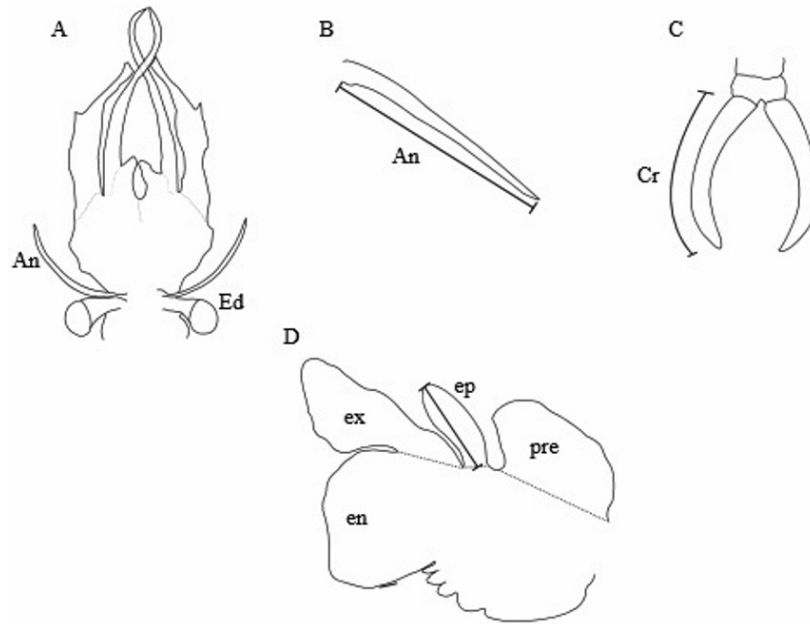
Fig. 1. Sexual and somatic traits of male *B. schaefferi*. A – head dorsal view, B – antennula lateral view, C – cercopodit ventral view, D – thoracic appendage. Abbreviations for measurements: An – antennula; Cr – cercopodit; Ed – eye diameter; en – endopodit; ep – epipodit; ex – exopodit; pre – preepipodit. A – after Daday de Deés (1910), redrawn and changed; B–D – original.

rity, but the exact role of these chemical compounds is largely unknown. In addition, Maeda-Martinez et al. (1995) showed that colour pattern in *T. platurus* is highly dependent on food source, and although carotenoids play an important role in colouration of animals, they are not crucial for development. If, for at least some Anostraca species, carotenoids are not crucial for full development, why animals still do spend energy on metabolism of those compounds?

Taking into account that *B. schaefferi* live in a highly turbid environment (in some puddles turbidity is higher than 1000 NTU, personal observations), we hypothesise that these colourful traits may play an important role in sex recognition in this species. Moreover, cercopods can function as an ornament – a fair signal of male fitness – and thus remain under the strong influence of sexual selection. To our knowledge no research has been conducted to address the role of cercopods in sexual selection and its secondary ornamental function – neither in *B. schaefferi,* nor in other Anostraca species. One possibility to test whether a trait is under such selection is to measure what is its allometry slope (see Eberhard et al. 1998, 2009). The results of such analyses can be then used as a primary indicator of how sexual selection acts on the development of sexual traits and help in formulating hypotheses on their evolution. Knowing the patterns of allometry may also be crucial in designing further experiments investigating the actual relationship between the size of a trait and the reproductive success (Pomfret & Knell 2006).

The present study provides preliminary results on the type of scaling in presumably sexually selected traits in *B. schaefferi*. Our aim was to investigate the character of scaling in coloured cercopods which, according to our hypothesis, should be positive if the

structure functions as an ornament. We also investigated the differences in colouration of cercopods between male and female *B. schaefferi*.

Material and methods

For the allometry analysis male individuals of *B. schaefferi* ($n = 230$) were collected in the same day (to assure the same generation) from 9 puddles along a ca 1 km transect on old tank road ($52.4929°$ N, $16.8748°$ E – $52.4931°$ N, $16.8617°$ E) in Biedrusko military area, in western Poland (Gołdyn et al. 2007, 2012). Since the puddles lay close one to another (from less than one meter to several meters) and the dispersion by wind, animals and human activity is well known in Anostraca (e.g., Vanschoenwinkel 2009, 2011; Rogers 2014; Valls et al. 2016), we assume that each puddle hosts a subpopulation of large *B. schaefferi* metapopulation in this area.

After transporting samples to the laboratory, the animals were anesthetised with carbonated water and preserved in 4% formalin (direct preservation in ethanol causes deformation and shrinkage of animals and their body parts, see Beladjal & Mertens 1999; Rogers 2002; Martin et al. 2016). Each individual was photographed under Olympus SZX 16 stereomicroscope. Body length was measured from the top of the head to the end of the last abdominal segment from the photographs using ImageJ software (Abramoff et al. 2004). Subsequently, the following traits were dissected from each individual, from left and right side of the body: antennulae, eyes, limbs of fifth thoracic segment and cercopods (for detailed information on Anostraca morphology see Dumont & Negrea 2002). All of them (except the eyes) were mounted on microscopic slides in water in order to flatten them out. Eyes were mounted in a standard position in a small hole made in wax dissection plate. Photographs of each trait were taken under SZX 16 Olympus stereomicroscope using Canon D5 Mark II digital camera. Then the structures were measured using ImageJ software (see Fig. 1

Table 1. Slopes, confidence intervals (CI), P values for correlation test (P cor), R^2 value, mean, minimum and maximum of measurements as well as number of measurements (n) for the anostracan cercopodits and somatic traits (all measurements in mm)

Trait	Slope	CI	P cor	R^2	Mean	Min	Max	n
Cercopodit	1.41	1.33–1.5	< 0.01	0.8	2.04	0.97	3.40	208
Antennula	1.01	0.95–1.08	< 0.01	0.76	2.03	0.61	3.01	219
Epipodit	0.93	0.85–1.0	< 0.01	0.68	0.71	0.45	1.12	201
Eye diameter	1.04	0.98–1.11	< 0.01	0.8	0.49	0.28	0.71	226

for details). In some cases the traits measured were damaged or in position that precluded solid measurement. Such measurements were removed from the statistical analyses.

We divided the measured traits into two groups: cercopods and somatic (non-sexual) traits. We hypothesised that brightly red cercopods are an ornament or a sex signalling structure. Somatic traits were included in the analyses as a reference.

Since population abundances were often low, we decided to combine the measurements from all collected individuals into one dataset. The reason for this procedure is that all puddles are in close proximity, thus the genetic pool should be constant (i.e., they came from the same metapopulation) and the animals were of the same age, since puddles fill with water exactly at the same time. If the populations were to be analysed separately, regression analysis might be biased by low sample size (ca. 30 individuals) due to outliers, and using a sample of over 200 individuals makes analyses less prone to low sample source of bias (since increasing sample size lowers the variation, see also Sokal & Rohlf 2012). On the other hand, one may argue that even though hatching occurs at the same time in particular puddles, populations are still subject of different microclimatic conditions (e.g., puddles that are shaded for most of the day will tend to have lower temperature) or different biotic pressure (predation or food base). These differences in habitats may result in different growth rates, but we regard them as different expression of genotype from the same pool. Moreover, we assumed that the predicted allometrical relationship between traits and body size after reaching maturity does not change with individuals age. Under this assumption the size of particular trait is a function of overall body size only.

All data from the measurements were log-transformed and checked for allometries using standardized major axis regression (SMA) (Warton et al. 2006). First, we calculated slopes of somatic traits and cercopodes regressed against body length. Then, using standard procedures described in Warton et al. (2006, 2011), we checked if the slope values of traits predicted to be under sexual selection scale were different from the median slope of somatic characters (as suggested by Eberhard et al. 2009). Finally, we checked if there is a common slope between somatic traits and cercopods (Warton et al. 2006, 2011).

For both males and females ($n = 10$ in both sexes) we checked colour surface ratio to overall cercopod surface ratio using ImageJ software (Abramoff et al. 2004). To check if the colour surface differs from overall surface we used approximate Wilcoxon-Pratt signed-rank test. All the analyses were performed using R software with SMATR package (R Development Core Team 2011; Warton et al. 2011).

Results

There was no common slope between *B. schaefferi* populations in case of cercopods ($P < 0.05$), and visual inspection of diagnostic plots showed deviations from heteroscedascity and normality above the acceptable level (Zuur et al. 2009; Sokal & Rohlf 2012). This situation is common for low level samples, resulting in under- or overestimation of regression slopes, thus we followed the procedure of combining all populations into one as explained in the Materials and methods section. The highest values of allometric slopes among all the traits measured (Table 1) characterized the cercopod ($b = 1.41$). The lowest values were detected for the epipodit ($b = 0.93$). The median slope for all somatic traits was 1.01. Slope of cercopod differed significantly ($P < 0.01$) from this value. Eventually, there was no common slope between cercopods and somatic traits (Fig. 2).

Mean ratio of coloured surface to overall surface in female cercopods was 0.56 (SD = 0.1, min = 0.44, max = 0.74, $n = 10$). Male cercopods were coloured evenly on the whole surface and the ratio was always 1 (see Fig. 3 for qualitative comparison). The difference in coloured surface between males and females was significant at $P = 0.002$ ($Z = 2.803$), with large effect size ($r = 0.63$).

Discussion

According to Eberhard et al. (2009), positive allometry (hyperallometry) pinpoints that selection favours disproportionally larger trait in larger individuals (and therefore – directional selection favouring large trait is stronger at larger body sizes). Following Bonduriansky & Day (2003), positive allometry occurs when 'directional sexual selection acts on trait size, where viability increases with body size with diminishing returns'. Kodric-Brown et al. (2006) argues that traits functioning as ornaments or weapons are under strong sexual selection and scale always in positive manner. Nevertheless, Bonduriansky (2007) does not agree with this statement, indicating that in many groups ornament traits show negative allometry or isometry. Furthermore, Bertin & Fairbairn (2007) showed that there is no obligatory linkage between positive allometry and direct sexual selection. They reported that there was no static hyperallometry for traits that are well known to be under directional selection in water strider *Aquarius remigis* (Say, 1832). Eberhard et al. (2009) stated that isometric scaling occurs when directional selection acts equally along the body size gradient favouring larger trait sizes. On the other hand, Bonduriansky & Day (2003) proposed that isometric scaling might occur in some other cases, e.g. when there is a directional selection on both body and trait size, but trait size is under
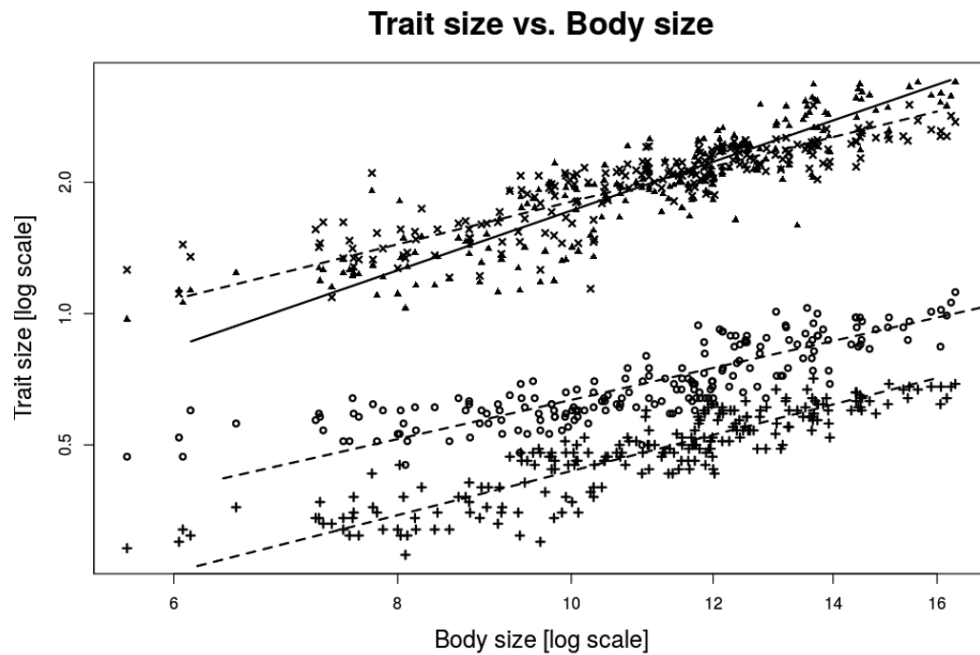
Trait size vs. Body size

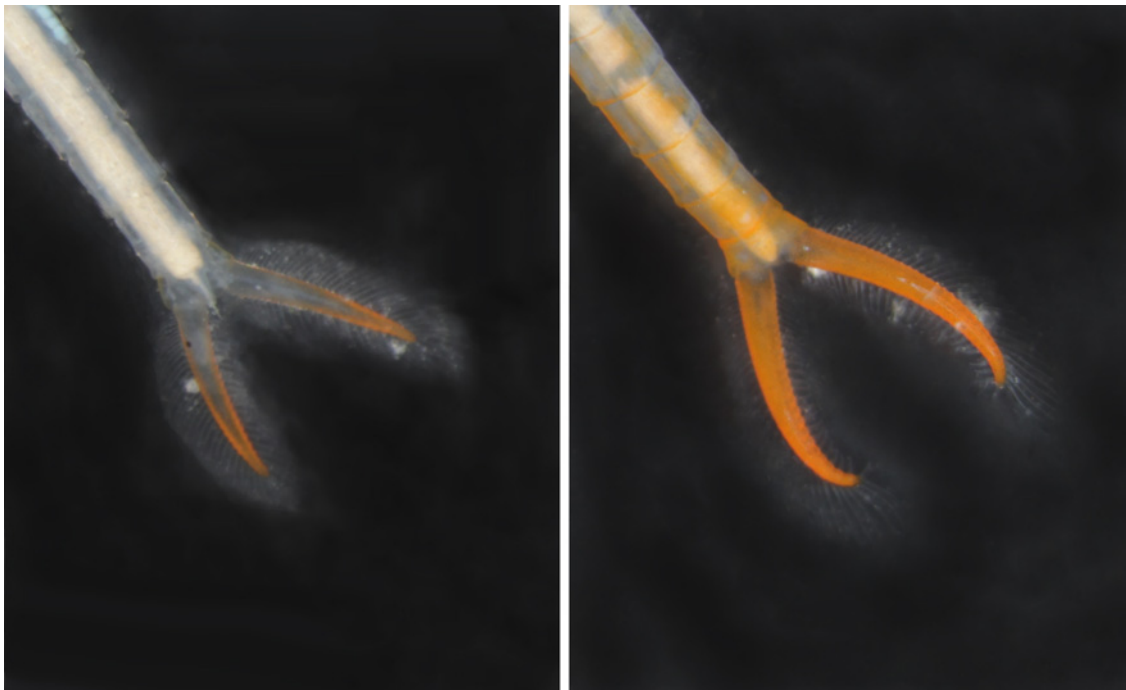Fig. 2. Standardized major axis regression of measured traits.

Fig. 3. *B. schaefferi* male (left) and female (right) cercopodit.

stronger selection. They also noticed that directional selection on traits size and stabilising viability selection on trait to body size ratio would result in proportional scaling.

In our study, the only traits that showed positive allometry were the cercopods. The role of cercopods in selecting a mate in Anostraca is unknown, nevertheless they are often intensively coloured and may function as an ornament. This way, our findings could support the aforementioned model by Kodric-Brown et al. (2006). On the other hand, the reason for the hyperallomet-

ric scaling of this trait could be purely mechanical, and higher values of allometry could be caused by other factors than sexual selection as suggested by Bertin & Fairbairn (2007). Disproportionally larger cercopod in bigger animals could simply be necessary for the effective swimming. Nonetheless, if the cercopod had become hyperallometric due to mechanical reasons, it could have gained its ornamental function as a fair signal of male quality. The advertising colour and ornamental function of the already allometric trait was gained later, in pleiotropic manner, as a consequence of sexual selec-

tion. Consequently, the sexual function of this organ may be considered as secondary. It should be noted that making strong statements while basing on limited research (especially in evolutionary research) can lead to the trap of 'adaptationist storytelling' (Gould & Lewontin 1979; Nielsen 2009) or teleological expectations. Also Ioannidis (2005) and Munafò et al. (2017) suggest that making statements on results from single studies is more likely to be biased. Taking this into account, we conclude that more detailed studies on the mechanics of swimming and direct experiments on natural/sexual selection in Anostraca are needed to unravel the problem of all the functions of cercopod and its colouration.

Nonetheless, strong differences between the sexes in the colouration of cercopods were recorded in our study. The red colour of the cercopod is most probably caused by carotenoids, already reported in Anostraca (e.g., Glichrist 1968; Dararat et al. 2012). Since the production of these colourants is costly for an individual (Lozano 1994), it is evolutionarily more advantageous for females to allocate the resources in egg production than in such ornament. Instead, females seem to advertise their presence and maturity by allocating more carotenoids in structurally coloured ovisacks, probably avoiding the significantly increased costs of second ornament production. This way, brightly red and positively allometric cercopod in males likely remains a good sign of their sex and a fair signal of their quality as potential mates.

Other possible explanation of hyperallometry and coloration of cercopodes is natural selection (which does not necessary exclude sexual selection, and may act parallel to it) on attractiveness for predators. Since astatic waterbodies favourable for anostracans can be separated by long distances, hitchhiking using birds (or other animals) foraging on fairy shrimps fosters passive dispersal of cysts and colonization of new habitats (e.g., Green et al. 2005; Beladjal & Mertens 2009; Muñoz et al. 2013). To our knowledge there is no research on predator preferences for more colourful anostracan individuals in highly turbid environment of puddles inhabited by *B. schaefferi*.

Last but not least, Ben Naceur et al. (2012) showed that physicochemical parameters of water may affect the morphology of the anostracan *Artemia salina* (L., 1758), potentially altering allometry slopes of particular traits. Contrary to most of anostracan species, however, *A. salina* inhabits exclusively brine waters. Those pools are usually less ephemeral and possess higher water density than puddles inhabited by *B. schaefferi,* and thus, growth patterns are most likely different in the two species. Anyhow, influence of environmental factors on our results can't be ruled out and in-depth, experimental study is needed to confirm the connection between cercopode morphology and sexual selection in Anostraca.

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