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Mating and spermatophore morphology of the freshwater mite *Brachypoda versicolor* (Müller, 1776) (Acari: Hydrachnidia, Aturidae)

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Abstract

Morphology of the spermatophore of *Brachypoda versicolor* (Müller, 1776) has been described for the first time. Males combine two spermatophores into a double spermatophore. Additionally, Halik's (1955) observations on mating behaviour of this species have been confirmed. In both characteristics, *Brachypoda* shows obvious congruence with *Piona* (Pionidae) whose mode of sperm transfer with the aid of a spermatophore cluster is regarded to be more derived (Bücking 2001). The taxonomical classification of *Brachypoda* as belonging to the family Aturidae (e.g. Viets 1987) cannot be maintained; a close relationship of this genus to the Pionidae is probable.

Keywords: *Arrenurus*, *Aturus*, copulation, pairing, phylogeny

1. Introduction

The hygrobatoid water mite *Brachypoda versicolor* (Müller, 1776) is placed in the family Aturidae Thor 1900 (e.g. Cook 1974, Viets 1987). This family contains genera with highly diverse morphological and ecological characteristics, for that it has been regarded as a polyphyletic group (e.g. Smith 1984, Smith & Oliver 1986). Cook (1974: p. 290) has described the Aturidae as a 'dumping ground for those higher water mite genera with heavily sclerotized bodies which do not show the characteristics used to define the remaining families'.

In the searching of the characters which could help me in reconstructing the phylogenetic relationships within the freshwater mites (Acari: Hydrachnidia) (Witte & Olomski 1991, and in prep.), I succeeded in isolating spermatophores of *B. versicolor* and in observing its pairing and insemination. Their mating had been described formerly by Halik (1955), but spermatophores as such have not been examined until now. For that, this study focuses on the morphology of spermatophores of this species.

Plesiomorphically, the Hydrachnidia transfers sperm indirectly by means of stalked droplet spermatophores which are deposited in small groups on substrate (e.g Witte 1991 and Witte & Döring 1999). In the course of evolutionary radiation of the hydrachnids, semi-direct and direct sperm transfer has evolved and morphology of spermatophore has changed. In this paper I will show that the morphology of spermatophores and the procedure of sperm transfer of *B. versicolor* give us a good reason to suppose a closer relationship of this species to the Pionidae than to the other members of the Aturidae, to which the genus *Brachypoda* belongs.

2. Materials and methods

Adults of *Brachypoda versicolor* (Müller, 1776) were collected in the littoral of the meso-eutrophic lake 'Stadtwaldsee' in Bremen, Germany (53° 06' 51" N, 08° 49' 48" E) in the 10th of August 2009. They were found in great numbers in stocks of the Canadian pondweed *Elodea canadensis* Michx. In order to minimize sexual contacts between the adults, males and females were separated sexually with the aid of a stereomicroscope, not later than 6 hours after collection. The adults were maintained in a tap water for 4 ± 3°C and in dark until mating observations.

For mating observations, three females on the one hand and one male of *B. versicolor* on the other hand were placed in small transparent plastic vials (17 mm deep x 12,5 mm in diameter) filled with 3 ml tap water with a whorl of *Elodea*. The bottom was covered with washed sand. After the mites have acclimatized for two hours at room temperature and on the light, the male was placed in the vial of the females to start the copulation. The mites were observed by stereomicroscope Zeiss DR. Photographs were taken with the aid of digital microscopic ocular Bresser 3.0 MP. The observations on the mating behaviour were carried out in the 17th, 18th, 24th and 27th of August 2009, in series of 3–6 parallel tests per day.

In order to obtain spermatophores of *B. versicolor*, males holding spermatophores ready for transferring to the female were anesthetized for 20 sec in -20°C cold absolute ethanol. Tarsi of their third pair of legs bearing spermatophores were cut up by a pair of micro-scissors and were transferred on a slide by a pipet. The spermatophores were removed gently with tiny insect pins and were directly observed by a light microscope Will BX 200. Some spermatophores were stained in 2% carminic acetic acid after they had been fixed by heating the slide briefly over an open flame.

3. Results

3.1. Mating behaviour

The males of *B. versicolor* produced spermatophores only in the presence of a female. If a male has touched a female, it took a 'readiness position' on the plant or on the sand. The male raised the posterior part of its idiosoma and the fourth legs which are modified as a grasping organ. With the tarsi of its third legs, it holds the spermatophores in the pocket-like genital opening (Fig. 1). In this 'handstand' position, the male crawled on the substrate with the help of the first and the second leg pairs, searching for the female, or it rested and did not move, waiting for the female. Occasionally, the male vibrated repeatedly with the body.

After having contact with a female, the male grasped it with its fourth legs and positioned the female venter by venter so that the anterior end of the female directs to the posterior end of the male (Figs 2 and 6a). Non-receptive females freed themselves from the male's clasp by rapid legs movements. Receptive females became motionless, occasionally stimulating the male by vibrating touches of its appendages. The front of female's idiosoma could be wedged beneath the overhanging shelf of the male's posterior end in the way Halik (1955) has described. As soon as the pairing partners had achieved the pairing position, the male took the spermatophores with the tarsi of the third legs from his genital pocket and taped them to female's gonopore for few minutes. The pairing was accompanied by repeated heavy vibrations of the male. Mating lasted from several minutes till more than one hour. It was usually ended by brief violent movements of the male.

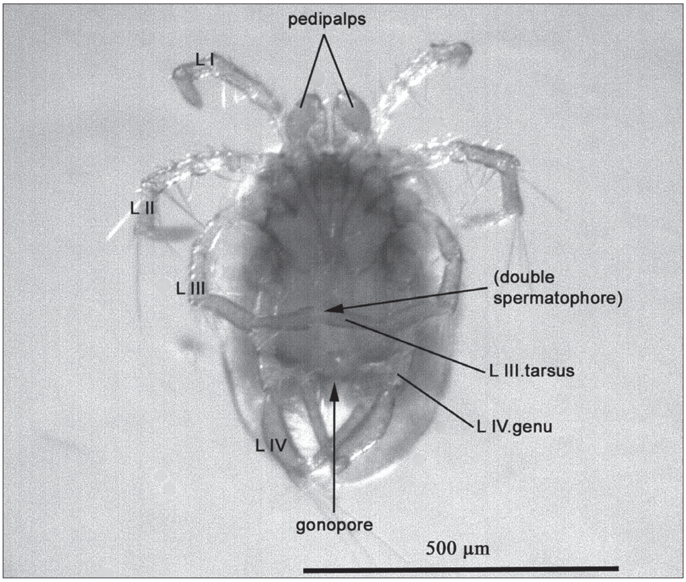


Fig. 1 Male of *Brachypoda versicolor* from ventral view. The male holds with the tarsal claw of its third legs a double spermatophore in its pocket-like genital opening. L I–L IV = leg I–IV of male.

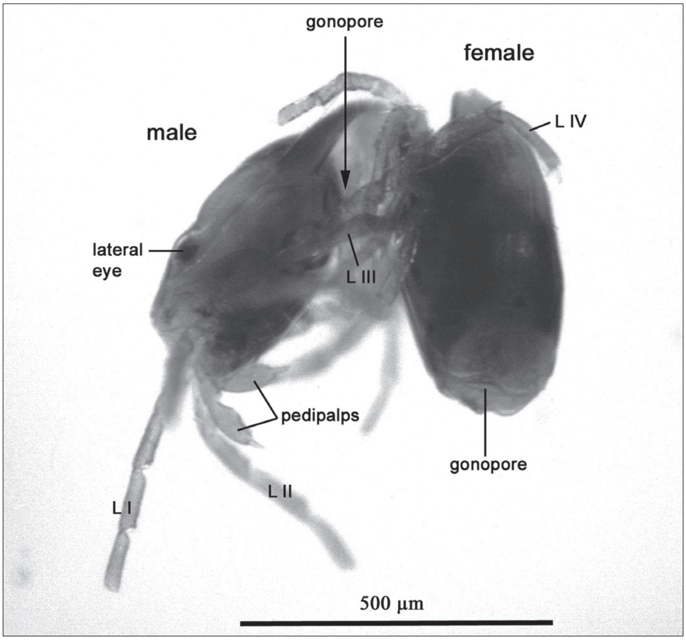


Fig. 2 Male and female of *Brachypoda versicolor* in mating position from lateral view. The male clasps the female with its fourth legs, and holds a double spermatophore with its third legs, ready for placing it into the female’s gonopore. L I–L IV = leg I–IV of male.

3.2. Spermatophores

In order to transfer spermatozoa, males of *B. versicolor* combined two spermatophores into a double spermatophore (Fig. 3 and 4), that is comparable with the cluster of spermatophores known from *Piona* (e.g. Böttger & Schaller 1961, Proctor 1992 and Bücking 2001; Fig. 5).

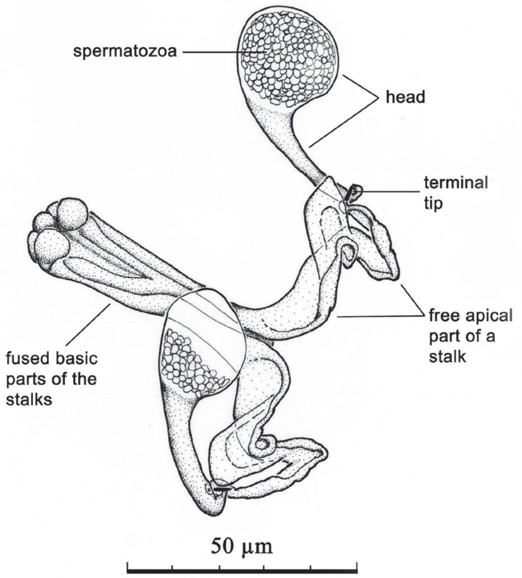


Fig. 3 Double spermatophore of *Brachypoda versicolor* from dorsolateral view.

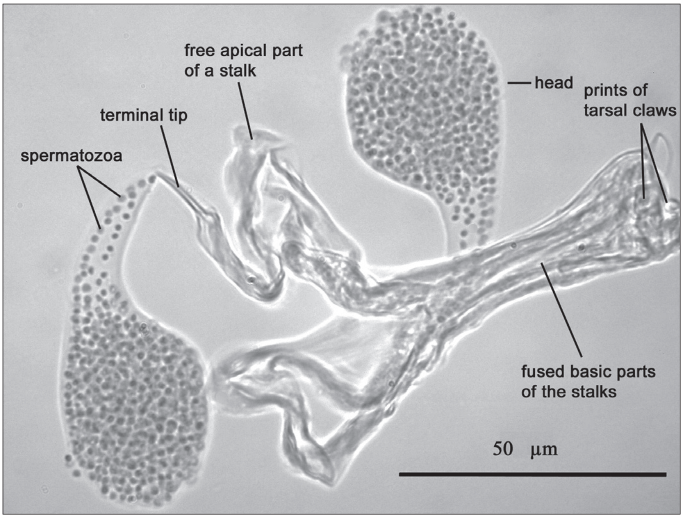


Fig. 4 Double spermatophore of *Brachypoda versicolor* after staining in 2% carminic acetic acid.

The both spermatophores in such a small cluster were fused with their basic parts of their stalks. The common part of the stalks was thickened and its proximal end showed prints –knops and depressions– of the tarsal claws of the third legs (Fig. 4). The free apical parts of the two stalks were bent several times like a pair of horns (Fig. 3). They showed membranous extensions and ended by a needle-shaped tip. On each tip, a tear-shape spermatophore head was situated. There was no chitinous sperm pouch covering the head, like in earlier derivative Euhydrachnidia, e.g. *Parathyas, Hydrachna* or *Limnesia* (Witte 1991). The heads were densely packed with spermatozoa and covered with homogeneous translucent membrane. The spermatozoa are stained intensively by carminic acetic acid (Fig. 4). It could not be justified, weather the double spermatophore is covered by a gelatinous mass like in *Piona*-species, or not.

The length of the stalk of the spermatophores was: the fused basic part 47–54 µm, the free apical part (without the terminal tip) 67–99 µm, and the apical needle-shaped tip 11 µm. Thus, the total length of the stalk was 124–164 µm. The head of the spermatophores was 61–63 µm in length and about 27 µm in diameter.

4. Discussion

Structures used in reproduction and mating behavioural patterns might be suitable to detect phylogenetic relationships if they are of sufficient complexity. The euhydrachnidian water mite superfamilies 'Hygrobatoidea' (e.g. *Piona*, *Brachypoda* and *Aturus*) und 'Arrenuroidea' (e.g. *Arrenurus*) are rich in species and show a great diversity of structures and behaviour involved in pairing and insemination (for review see Proctor 1992). Although this circumstance was already recognized by earlier authors like Mitchell (1957) it has been insufficiently taken into account in taxonomical classification up to now.

This study shows congruencies in the morphology of the spermatophores and in the mode of insemination between *Brachypoda versicolor*, which belongs to the 'sampling' family Aturidae, and members of the Pionidae, especially *Piona* (Bücking 2001).

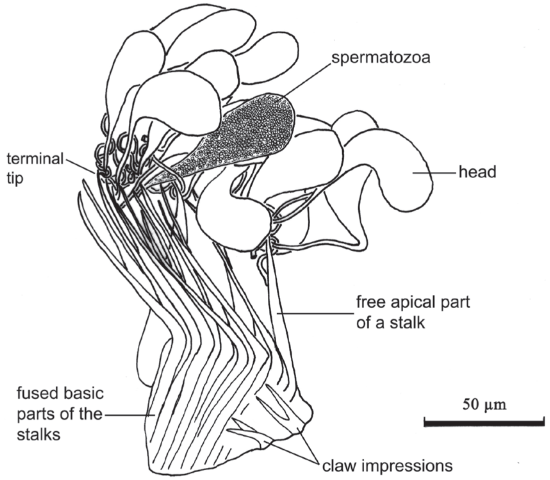
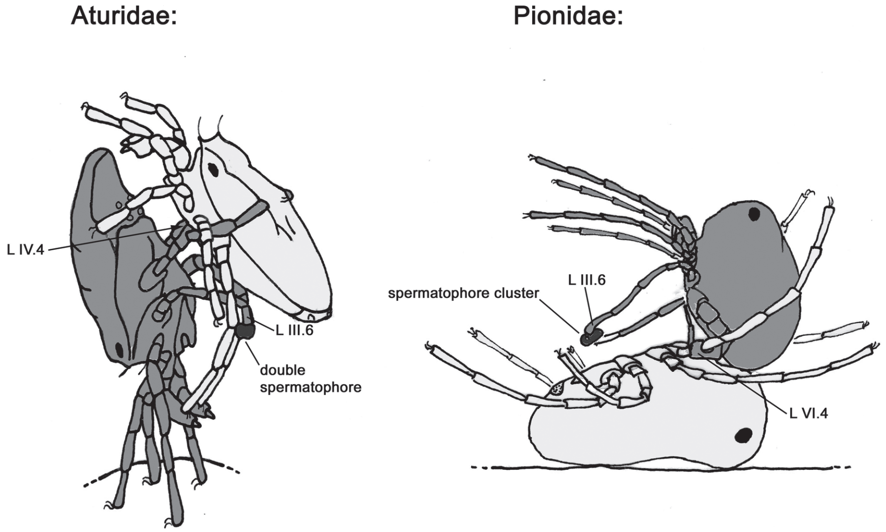
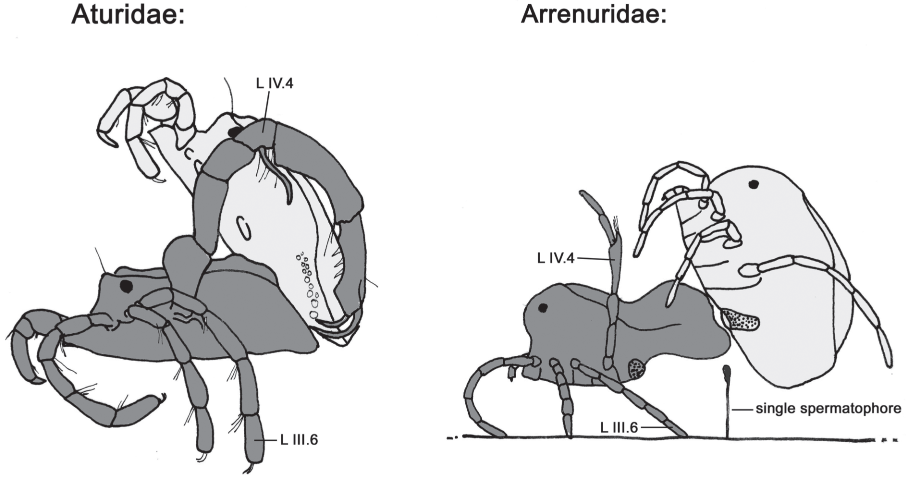


Fig. 5 Spermatophore cluster of *Piona carnea* in lateral view; redrawn from Bücking (2001), modified.



a) *Brachipoda versicolor* (Müller, 1776)

b) *Piona carnea* (Koch,1836)



c) *Aturus scaber* Kramer, 1875

d) *Arrenurus globator* (Müller, 1776)

Fig. 6 Mating position and sperm transfer in the water mite families Aturidae, Pionidae and Arrenuridae from lateral view; redrawn **a**: from Lundblad (1929b), **c**: from Halik (1955), **d**: from Lundblad (1929a), all are modified; **b**: own observation. Males are indicated by dark-grey colour, females are indicated by light-grey colour. L III.6 = tarsus of leg III, L IV.4 = genu of leg IV.

In *B. versicolor* and in *Piona*-species the spermatophores are composed of a bended stalk, terminating by an acute tip and of a spermatozoa-filled head which is situated on this tip (Figs 3–5). A protecting sperm pouch, as it is typical in spermatophores of the earlier derived Euhydrachnidia, e.g. *Parathyas*, *Hydachna* and *Limnesia* (Witte 1991, Witte & Döring 1999, Witte & Olomski 1999), is lacking. From two to more than ten spermatophores are fused with the basic part of their stalks, forming a cluster. During copulation, the male places this 'double spermatophore' (*Brachypoda*) resp. 'spermatophore cluster' (*Piona*, *Pionopsis*, *Tiphys*, *Forelia*) with the claws of its third legs to the female's gonopore (Fig. 2, 6a and b). The claws are modified in favour of this function (Proctor 1992, Bücking 2001, own observations). During mating, the male fixed the female with its modified fourth legs, venter-to-venter facing in opposite directions.

As early as Mitchell (1957) has supposed this mating position, and the sperm transfer with the aid of the third legs is thought by him to be highly characterized for a common ancestral form of Pioninae and Tiphysinae. Mitchell (1957) concluded that the Pionidae are a monophyletic family. Following Mitchell's assumptions, *Brachypoda* belongs to the Pionidae and not to the Aturidae, as they are classified actually.

In contrast to *Brachypoda* and the Pionidae, *Aturus*, giving the name of the entire family Aturidae, takes up the same mating position as *Arrenurus* (Fig. 6c, d): The male carries the female pick-a-back, male's and female's bodies are orientated in the same direction (Lundblad 1929a, b and Böttger & Schaller 1961). In *Brachypoda* and *Piona* as well as in *Aturus* and *Arrenurus* the male grasps the female with its modified fourth legs, but in the latter the female than will be fixed on the male's back with the aid of a gluing secretion produced by the male. The males of *Arrenurus* transfer spermatozoa by single stalked spermatophores, which they deposit on the substrate. After deposition, the males usually position the female above the spermatophore and insert the head of the spermatophore directly or indirectly via a petiole into its gonopore by lowering its venter. In most of the investigated species, the males deposit more than one spermatophore during copulation (Proctor 1992). For the reason of the same pairing position, Proctor (1992) has supposed that the males of *Brachypoda* transfer their spermatozoa by the same manner as *Arrenurus*, with the aid of the single spermatphore, which are deposited on the substrate. Her supposition has been verified nowadays by observations of Peter Martin (University of Kiel, personal communication).

The taxonomical position of *Brachypoda* in the family Aturidae (e.g. Viets 1987) can no longer maintain; a close relationship to the Pionidae is probable. This is substantiated especially by the observations of spermatophores morphology. The results confirm the hypothesis of Witte & Döring (1999) about the evolution of the sperm transfer mechanisms in water mites. According to this supposition, *Brachypoda* and *Piona* on the one hand and *Aturus* and *Arrenurus* on the other hand are of monophyletic origin. Consequently, the family Aturidae with *Brachypoda* and *Aturus* is polyphyletic and the superfamily Hygrobatoidea with *Piona*, *Brachypoda* and *Aturus* is paraphyletic.

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