Marsupial extension in terrestrial isopods (Crustacea, Isopoda, Oniscidea)

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Abstract

In Oniscidea, the marsupium is a ventral pouch where the offspring develop independently of an external water source. The marsupium is formed by five pairs of overlapping oostegites that develop in the females during their reproductive period. In this study, ovigerous females of 35 species were dissected, their oostegites were extracted, and the intra-marsupial offspring were counted. Two marsupium forms were recognized: distended, in which the oostegites protrude distally in relation to the sternites; and non-distended, in which the oostegites are parallel to the sixth and seventh sternites. *Armadillidium nasatum*, *A. vulgare*, *Pudeoniscus birabeni*, *Circoniscus gaigei* and *Cubaris murina,* conglobating species with a non-distended marsupium, and *Neotroponiscus daguerri* and *N. carolii*, non-conglobating species with a distended marsupium, have a concavity on the ventral floor of the 6th and 7th pereionites, here called the marsupial extension. This is the first record of a marsupial extension which extends beyond the area formed by the oostegites in Oniscidea.

Key words: Conglobation, marsupium, oostegites, woodlice.

Introduction

Among the several adaptations that have allowed isopods to occupy terrestrial environments is the marsupium, an incubator pouch that allows the eggs to develop independently of an external source of water (Hoese, 1984). In Oniscidea, there are two types of marsupium (Hoese, 1984). The amphibious marsupium, found in *Ligia* spp., is open anteriorly and posteriorly. It has internal circulation of water by capillary action through the 6th and 7th pereiopods. The terrestrial type is closed, and provides mechanical protection for the developing offspring (Hoese, 1984; Hoese and Janssen 1989). The marsupium is formed during the parturial molt (Suzuki and Yamasaki, 1989), when five pairs of oostegites appear and grow from the coxae of pereiopods 1-5, protruding toward the median longitudinal axis of the ventral body surface, and overlapping each other (Patane, 1940). Each oostegite consists

of a thin translucent and impermeable cuticle (Patane, 1940; Hoese, 1984). Both the constitution and the way that the oostegites are organized and linked allow the expansion of the marsupial space to accommodate the offspring. In Crinocheta, the cotyledons are responsible for nutrition and oxygenation of the offspring inside the marsupium (Akahira, 1956; Hoese and Janssen, 1989). During comparative studies on morphological patterns of the cotyledons in several species of terrestrial isopods, a new marsupial configuration was observed, here called a marsupial extension. This study aimed to: (i) identify which species among those studied possess this marsupial extension, and (ii) to count the number of individuals housed inside this cavity.

Materials and Methods

Ovigerous females of 35 species from 11 families of terrestrial isopods, from the Collection of Crustaceans of the Zoology Department - UFRGS and from donations and collections, were analyzed. The females were fixed in Dubosc fixative (Humason, 1972), and were dissected and photographed before and after the removal of the oostegites and of the offspring contained in the marsupium. The offspring were counted and classified according to their stage of development, following Milatovič *et al.* (2010): egg with undifferentiated embryo (S1 to S8), differentiated embryo (S9 to S18, when the appendix, eyes and segmentation are visible in the vitelline membrane), and manca (S19, when the isopod is already free of the membrane).

Results and Discussion

Among the 35 terrestrial isopod species examined, seven species possessed the marsupial extension (Table I). The marsupial extension is a concavity on the sternites of the 6th and 7th pereionites. This concavity starts from the fifth sternite, where the last pair of oostegites closes the marsupium (Figs. 1C, 1D). Inside this cavity there is a portion of the developing offspring, which are in the same developmental stage as the rest of the offspring in the marsupium. The proportion of the offspring in the extension is very significant, usually more than 10% and up to 25% of the total fecundity (Table II). For example, one female *Armadillidium nasatum* Budde-Lund, 1885 had 116 eggs in the marsupium and 23 eggs in the marsupial extension (Table II). The number of offspring inside the extension seems to be proportional to the total fecundity, which is in turn related to the female size (Quadros *et al.*, 2009).

Among the species that have the marsupial extension, a different marsupial form was also noted. The most common marsupial form in Oniscidea is a distended type, i.e., in which the oostegites protrude distally in relation to the sternites (Figs. 1A, 2A). Among the species examined, 30 have the distended marsupium (Table 1). On the other hand, the conglobating species *A. nasatum, A. vulgare* (Latreille, 1804), *Circoniscus gaigei* Pearse, 1979, *Cubaris murina* Brandt, 1833 and *Pudeoniscus birabeni* Vandel, 1963 have a non-distended marsupium, where the oostegites are parallel to the sixth and seventh sternites (Figs. 1B; 2B; Table 1). A comparison of the internal space of the marsupia revealed that in the distended marsupium, the oostegites bend outwards when the growing embryos require more volume (Fig. 1A). However, in those species with a marsupial extension, the oostegites bend only slightly, and the sternites arch into the body cavity to provide more space for the embryos (Fig. 1B). This leads to a displacement of the female's internal organs (Fig. 1B). Possibly, this displacement results in compression of the organs, which in advanced stages may cause females to cease feeding. In fact, all females with a marsupial extension and non-distended marsupium examined showed a full gut only when they were carrying eggs with undifferentiated embryos (Fig. 2E). Females carrying embryos and mancas, when dissected, showed an empty gut, with no trace of food (Fig. 2F). Females with a distended marsupium, regardless of the intra-marsupial

Table I. Configuration of the marsupium in terrestrial isopods and the presence of a marsupial extension and the conglobating ability. N = number of ovigerous females examined.

Family/species	Marsupium				Conglobation
	N	Distended	Non-distended	With extension	
Armadillidae					
Cubaris murina Brandt, 1833	2		X	X	X
Armadillidiidae					
Armadillidium vulgare (Latreille, 1804)	20		X	X	X
Armadillidium nasatum Budde-Lund, 1885	49		X	X	X
Balloniscidae					
Balloniscus glaber Araujo & Zardo, 1996	5	X			
Balloniscus sellowii (Brandt, 1833)	53	X			
Bathytropidae					
Neotroponiscus carolii Arcangeli, 1936	1	X		X	
Neotroponiscus daguerrii (Giambiagi de Calabrese, 1939)	5	X		X	
Dubioniscidae					
Novamundoniscus meridionalis (Araujo & Buckup, 1994)	3	X			
Novamundoniscus gracilis Lopes & Araujo, 2003	1	X			
Philoscidae					
Atlantoscia floridana (van Name, 1940)	17	X			
Atlantoscia rubromarginata Araujo & Leistikow, 1999	1	X			
Benthana araucariana Araujo & Lopes, 2003	2	X			
Benthana cairensis Sokolowicz, Boelter & Araujo, 2008	20	X			
Benthana convexa Lemos de Castro, 1958	4	X			
Benthana longicornis Verhoeff, 1941	4	X			
Benthana longipenis Lemos de Castro, 1958	4	X			
Benthana picta (Brandt, 1833)	1	X			
Benthana taeniata Araujo & Buckup, 1994	6	X			
Benthana itaipuensis Campos-Filho & Araujo, 2011	2	X			
Burmoniscus meeusei (Holthuis, 1947)	4	X			
Littorophiloscia denticulata (Ferrara & Taiti, 1982)	1	X			
Platyarthridae					
Trichorhina acuta Araujo & Buckup, 1994	5	X			
Trichorhina argentina Vandel, 1963	3	X			
Trichorhina heterophthalma Lemos de Castro, 1964	3	X			
Trichorhina tomentosa (Budde-Lund, 1893)	2	X			
Porcellionidae					
Agabiformius lentus (Budde-Lund, 1885)	5	X			
Porcellio dilatatus Brandt, 1833	11	X			
Porcellio laevis Latreille, 1804	4	X			
Porcellio scaber Latreille, 1804	15	X			
Porcellionides pruinosus (Brandt, 1833)	10	X			
Porcellionides sexfasciatus (Budde-Lund, 1885)	8	X			
Pudeoniscidae					
Pudeoniscus birabeni Vandel, 1963	2		X	X	X
Scleropactidae					
Circoniscus gaigei Pearse, 1917	2		X	X	X
Trachelipodidae					
Nagurus cristatus (Dollfus, 1889)	4	X			
Nagurus nanus (Budde-Lund, 1908)	5	X			

Table II. The distribution of the offspring in terrestrial isopods that have a marsupial extension. Fecundity is given as the total number of offspring per female and the number of offspring inside the marsupial extension (within parenthesis). The letters indicate the developmental stage: eg = egg with undifferentiated embryo; em = differentiated embryo; m = manca (see Material & Methods for details).

Species	Fecundity
A. nasatum	57(15)em; 98(22)eg; 96(12)eg; 53(6)m; 58(11)em; 69(12)m; 56(8)em; 91(15) em; 80(13)em; 70(9)em; 65(7)em; 83(10)em; 87(15)em; 67(6)eg; 83(10)m; 88(7)eg; 75(12)m; 81(10)eg; 99(15)em; 73(13)m; 77(9)eg; 67(8)em; 101(8) em; 45(8)m; 58(7)eg; 48(8)em; 85(8)eg; 72(9)em; 65(8)eg; 66(6)eg; 139(23) eg; 72(9)eg; 65(8)eg; 69(9)eg; 83(10)m; 76(11)em; 79(15)m; 77(9)eg; 58(8)em; 58(10)m; 62(9)m; 61(7)eg; 94(12)m; 61(5)em; 56(6)m; 65(7)em; 72(12)eg; 66(9)em; 79(11)em;
A. vulgare	70(15)m; 74(14)eg; 137(20)m; 61(11)m; 85(12)eg; 96(18)eg; 102(15)m; 78(11)eg; 54(6)m; 52(5)em; 60(5)m; 51(5)em; 69(6)m; 53(5)eg; 67(12)m; 121(26)m; 57(8)m; 93(16)em; 51(9)m; 31(5)em;
C. gaigei	18(5)eg; 18(6) eg;
C. murina	12(3)m; 14(4)m;
N. carolii	8(2)em;
N. daguerri	14(3)eg; 12(3)eg; 12(2)em; 4(2)m; 5(2)m;
P. birabeni	7(3)eg; 7(2)eg;

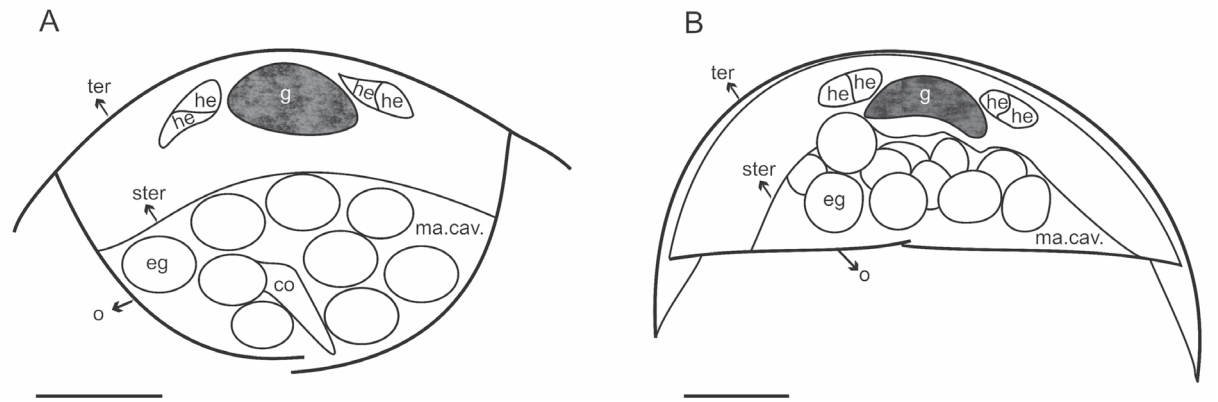

Figure 1. Schematic cross-section of the pereion of terrestrial isopods showing two marsupial forms. A, distended (drawn from *Balloniscus sellowii*). B, non-distended, *Armadillidium vulgare*. co: cotyledon; eg: egg; he: hepatopancreas; hg: hindgut; ma cav: marsupial cavity; o: oostegites; ster: sternite; ter: tergite. Bar: 0.5 mm.

development stage, always showed a full gut (Fig. 2G).

Another important point is that in species belonging to the ecomorphological category of the "rollers" (Schmalfuss, 1984), the lack of a large distention of the marsupium allows ovigerous females to conglobate. However, it is noteworthy that among the species that have a marsupial extension, two, *Neotroponiscus carolii* Arcangeli, 1936 and *N. daguerrii* (Giambiagi de Calabrese, 1939), are non-conglobating species. Information on the presence or absence of the marsupial extension in species in families and genera that were not considered in this study is necessary to understand the evolution of the conglobating ability, which is considered a convergence that evolved within the Tylidae, once in Synocheta, and several times within the Crinocheta (Schmidt, 2002). The marsupial form must

Figure 2. Marsupium in terrestrial isopods. A, distended marsupium in *Balloniscus sellowii.* B, non-distended marsupium in the conglobating *Cubaris murina*. C, manca of *C. murina* inside the marsupial extension. D, empty marsupial extension of *Armadillidium vulgare*, showing the space occupied by mancas upon their removal. E, beginning of the ovigerous period in *A. vulgare*, showing eggs and food inside the gut. F, late ovigerous period in *A. vulgare*, showing the mancas and an empty gut. G, late ovigerous period in *B. sellowii*, showing the mancas and the food inside the gut. Bar: 0.5 mm.

also be considered in future studies on the phylogeny within Oniscidea. To date, no similar structure has been found in Peracarida and for a better understanding of the structure of this extension structure and its implications for the life history of these species, further studies are needed.

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