Observations on *Peripatopsis clavigera* (Onychophora, Peripatopsidae)

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Received 15 September 1987; accepted 15 February 1988

The colouring of live specimens and female reproductive system with associated developmental stages of *Peripatopsis clavigera* are described. An unusually dense population of this rare species was found in Harkerville State Forest, southern Cape region. Colouration of these specimens differs distinctly from that of the four syntypes described by Purcell (1897). Early developmental stages show a trophic vesicle as described for some other *Peripatopsis* species (Manton 1949). Endoparastic worms (Acanthocephala) were found in the haemocoel of one female specimen.

Die kleur van die lewendige organismes en die vroulike geslagsorgane en geassosieerde embrionale ontwikkelingstadiums van *Peripatopsis clavigera* word beskryf. 'n Buitengewoon digte bevolking van hierdie rare spesies is in die Harkerville-staatsbos in die suidelike Kaapse streek gevind. Die kleur van hierdie individue verskil heelwat van die vier sintipes soos deur Purcell (1897) beskryf. Die vroeë ontwikkelingstadiums besit 'n trofiese vesikel wat deur Manton (1949) vir sekere ander *Peripatopsis*-spesies beskryf is. Endoparasitiese wurms (Acanthocephala) is in die hemoseel van een vroulike individu gevind.

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The Onychophora comprise two families: the Peripatidae and the Peripatopsidae. While the Peripatidae have a circumequatorial distribution, the Peripatopsidae are confined to the southern hemisphere. In southern Africa, the two genera with nine species are distributed throughout the present and former indeginous forest areas.

Peripatopsis clavigera (Purcell 1899) is a rare species of South African Onychophora. Since the last specimen was collected in the Garden of Eden, Knysna, 1975 (Ruhberg 1985), it was commonly believed to be extinct. The biology of *P. clavigera* is practically unknown: a short description of live and preserved specimens is given by Purcell (1897, 1899), and a review of all available information concerning distribution, morphology and anatomy by Ruhberg (1985).

In January 1984 an unusually dense population of *P. clavigera* was discovered in Harkerville State Forest between Knysna and Plettenberg Bay. This paper describes the colouring of live specimens and the female reproductive system with associated embryonic developmental stages of this species.

Material and methods

Live specimens, collected in January and October 1984, were kept in glass tanks at 20–25°C and 75% R.H. on a substrate of humus, soil and rotten wood. Whole specimens were preserved in 10% formalin or 70% ethanol. Genital tracts and embryos dissected out of females in insect ringer solution were measured and then fixed in Dubosq-Brazil or 10% formalin. The pH of humus samples collected from beneath rotten logs where animals were discovered was measured.

Results

Locality

In the Cape Province, the distribution of *P. clavigera* appears to be restricted to the forests of Knysna and Tsitsikama (Ruhberg 1985). At Kranshoek (Harkerville District, 34°05'S / 30°02'E), an indigenous forest area, 35 female specimens, were located in and under rotten logs from 80 m altitude to a few meters above sea level. Substrate pH ranged from 3,8 to 6,0 ($n = 6$). As at other localities *Peripatopsis sedgwicki* Purcell 1899 occured sympatrically with *P. clavigera*. *P. sedgwicki* was usually more abundant than *P. clavigera,* however at Kranshoek only a few specimens of *P. sedgwicki* were found.

Pigmentation in live specimens

The dorsal side is medium to dark brown with a black mid-line and orange-red papillae (mostly brown tipped) distributed randomly over the whole dorsal surface and the outer surface of the legs. The ventral side and the inner surface of the legs are pink. The antennae are dark brown to black and the spinous pads are bright green. In four of 29 animals, that part of the head bearing the slime papillae has a lighter pigmented area in the shape of a band of the same orange-red colour found in some of the major papillae. The body colour of those embryos in a stage prior to parturition is light pink; while their antennae, slime papillae and feet are green. Already in this stage the lighter coloured band above the base of the legs can be recognized.

Genital tract and embryos

Onychophora are epimorphic and, except for a few Australasian species, are all viviparous (Siewing 1969). The sexes are separate. In the South African *Peripatopsis* species which do not posess a 'placenta' as described for South American species (Anderson & Manton 1972), the embryos of each brood are all of approximately the same developmental stage. Females undergo parturition once a year after a 'pregnancy' of 12 to 14 months (Purcell 1899; Manton 1949).

The genital tract of the female consists of two tubes divided into the ovary, paired oviducts and paired uteri. In *P. clavigera* the uteri join at the beginning of the segment which bears the 17th pair of legs to form a short vagina leading out between these legs. The ovary is 2,30 to 2,75 mm long, lies freely in the dorsal or lateral part of the haemocoel, and is situated between the 5th and 10th pair of legs. Eggs ready for fertilization measure between 110 µm and 150 µm. They rest on a stalk formed by follicle cells protruding from the ovary surface into the haemocoel. The oviducts are 7–15 mm long, measured up to the first embryo. The uteri measure 65–80 mm in length and lie in the middle or posterior part of the body cavity. The positions of the embryos are indicated by thick swellings and up to four of these may lie adjacent to one another so taking up 2/3 of the transverse space of the haemocoel.

In 10 dissected females the number of embryos per individual varies between 15 and 22 ($\bar{x}$ = 17).

Embryos were sampled in each of two months.

October sample: In five females all embryos were in the same stage of segment formation. The paired lateral halves of the embryos could be seen clearly as segmental swellings on the blastoderm (Figure 1c, 2). A sixth female contained slightly more mature stages in which almost all trunk segments were formed and the limb buds are present (Figure 1a, b). The halves of the embryos, especially the anterior segments, were divided

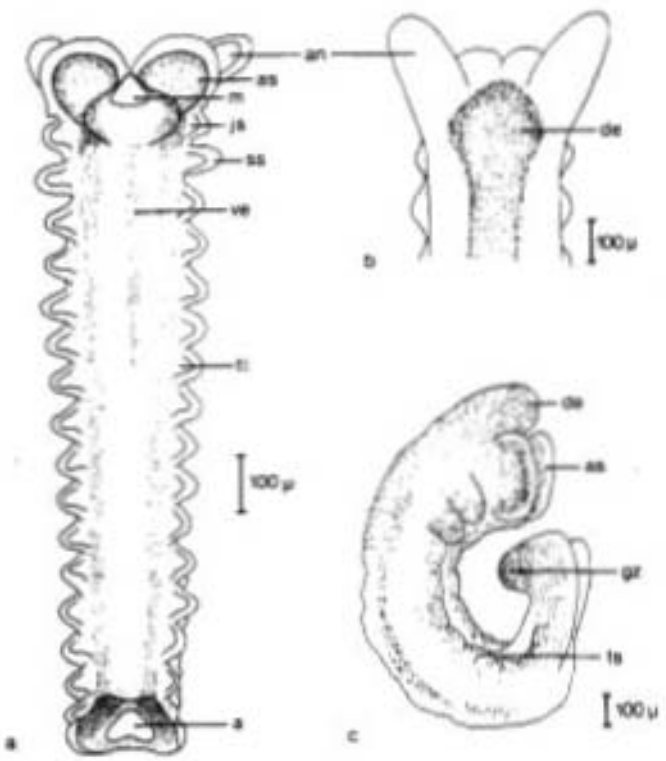

Figure 1 Embryos of October 1984: (a) ventral view, (b) dorsal view of head and first trunk segments, (c) lateral view. a = anus, an = antennae, as = antennal segment, de = dorsal extra-embryonic ectoderm, gz = growth zone, js = jaw segment, m = mouth, ss = slime papilla segment, tl = trunk limb, ts = trunk segment, ve = ventral extra-embryonic ectoderm.

Figure 2 Embryo of October 1984. as = antennal segment, de = dorsal extra-embryonic ectoderm, ts = trunk segments.

by a so-called extra-embryonic ectoderm (Anderson 1973), which is not directly involved in forming embryonic organs. The younger embryos of *P. clavigera* posessed a dorsal extra-embryonic ectoderm, which extended beyond the swellings of the antennal segment (Figure 1c). The more advanced embryos showed a less extended dorsal extra-embryonic ectoderm (Figure 1b). The surface structure of the dorsal extra-embryonic ectoderm differed from the rest of the body in that it was already organized into papillae, in contrast to the smoother surface of the segmental swellings, where papillae had not yet developed.

February sample: The embryos were all in a stage almost ready for birth and the inner organs were completely formed. The integument, including papillae, claws and spinous pads were covered with a kind of membrane (Figure 3), which probably protects the mother from injury by the spiny cilia of the papillae as the first moulting in juvenile animals occurs during or just after birth (Ruhberg 1985).

Parasites

One specimen of *P. clavigera* contained three Acanthocephala lying freely in the haemocoel. While the immature stages of these parasites develop in one or a few invertebrate intermediate hosts, the adults live in the guts of vertebrate hosts. For this reason the specimens found here are likely to be late larval stages, not yet encysted: each had a well-developed proboscis with many hooks (Figure 4) and the inner organs were completely formed.

Discussion

The population of *P. clavigera* at Kranshoek Forest was previously unknown and is extraordinary in that only females were found. The existence of males of this species was established by reference to the collections of the British Museum, London, the Museum of Comparative Zoology (Agassiz Museum), Cambridge, Massachusetts and Peter Brinck's private collection,

Figure 3 Part of the body surface of a mature embryo (SEM 500×, February 1984). c = claw, ci = cilium, mp = dorsal major papillae, sp = spinous pad.

Figure 4 Total view of an Acanthocephala, found in the body cavity of a *P. clavigera* specimen (SEM 40×). h = hooks, p = proboscis.

Lund, Sweden. Evidence for the existence of males in the Kranshoek population derives from the presence of sperm in the ovaries of dissected females. It is possible that the males inhabit a slightly different microhabitat (e.g. deeper in the soil) than the females, at least during the period when collections were made.

The colouration of the Kranshoek specimens differs distinctly from that of the four syntypes described by Purcell (1897). After his specimens were in alcohol for two and a half years he described the dorsal and lateral surfaces as green (1899). The ground colour of my specimens also changed to green in 70% ethanol but brown pigment was still present after more than two years.

The pH of the substrate at the Kranshoek collection locality lies in the same range as that reported for the biotope of *Peripatus acacioi* Marcus & Marcus in Brazil (Lavallard, Campiglia-Reimann, Parisi Alvarez & Valle 1975).

The female reproductive system of *P. clavigera* does not differ histologically from that of *P. moseleyi* described by Choonoo (1947), but in position and size some differences are apparent.

With an egg size of 110 µm–150 µm, *P. clavigera* lies between *P. moseleyi* (150 µm–170 µm) and *P. sedgwicki* (65 µm–80 µm) (Manton 1949). The ovary is situated between the 5th and 10th pair of legs in *P. clavigera*, between the 10th and the 21st pair in *P. moseleyi*. Ovary, oviducts and uteri are shorter than in *P. moseleyi* owing to the smaller body size of *P. clavigera*.

Based on the position and the extension, the dorsal extra-embryonic ectoderm of *P. clavigera* embryos can be recognized as a trophic vesicle as described for the embryos of *P. moseleyi* and *sedgwicki* (Manton 1949) (Figure 5). Early and late stages of *P. clavigera* must be examined further to obtain greater detail concerning duration and extension of the trophic vesicle.

The discovery of parasites in *P. clavigera* is unusual as Onychophora are normally free of parasites. No

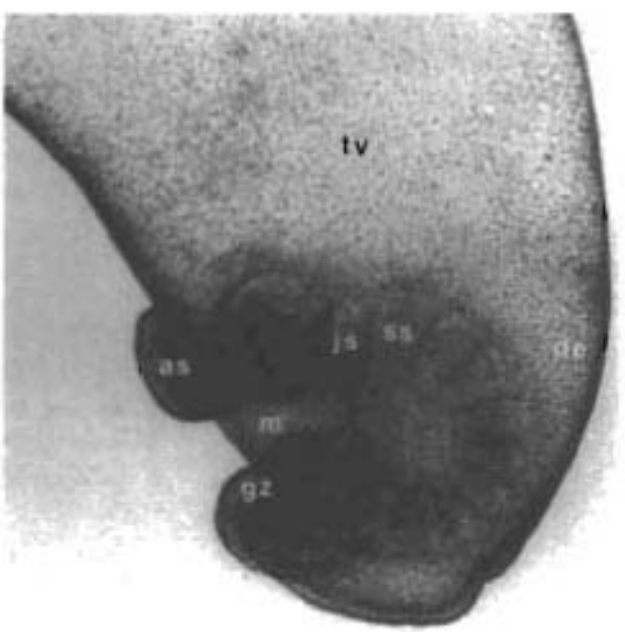

Figure 5 *P. sedgwicki*. Embryo with large trophic vesicle. as = antennal segment, de = dorsal extra-embryonic ectoderm, gz = growth zone, m = mouth, tv = trophic vesicle.

ectoparasites are known. Except for some fungi and the intracellular coccidian *Mantonella peripati,* which lives in the cylindrical cells of the gut (Lawrence 1953), no endoparasites have previously been recorded.

Acknowledgements
I thank Prof. Hugh Paterson, Mr Malcolm Keeping, Ms Anthony Gordon, Dr Al Cannone (Department of Zoology, University of the Witwatersrand) and Prof. Michael Webb (Department of Zoology, University of Stellenbosch) for support in many ways, and Dr. Hilke Ruhberg (Zoologisches Institut der Universität Hamburg) for consultation on Peripatopsidae and their habitats.

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