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Scanning Electron Microscope Observations of Embryonic Development in *Opisthopatus cinctipes* PURCELL

(Onychophora, Peripatopsidae)

by

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Abstract: In the South African onychophoran, *Opisthopatus cinctipes* PURCELL (Peripatopsidae), the female gives birth throughout the year, unlike other South African Peripatopsidae in which the young are born in batches over a relatively short period. Each female *O. cinctipes* contains up to forty embryos at different stages of development within her uterus. This again differs from other South African Onychophora where the females usually contain up to a maximum of twenty embryos, depending on the species, all at roughly the same stage of development. Embryos were dissected out of the uterus of *O. cinctipes* and their external morphology examined using scanning electron microscopy. Although the morphology of the older embryos is similar to that of all other species, the younger embryos are very different. In *O. cinctipes* it appears, from the external morphology of the early embryos, that segment formation is delayed until the embryo has elongated. This is unlike all other described onychophoran embryos where segment formation occurs as the body is elongating.

1. Introduction:

In all species of Onychophora in which the early embryos have been described, the development of their external body form follows an essentially similar pattern, proceeding in a strict antero-posterior sequence (ANDERSON 1973). As the mouth and anus are being formed, the paired lateral halves of the embryo start to develop as thickened bands on the surface of the blastoderm and soon exhibit paired segmental swellings with intersegmental annuli between them (ANDERSON 1973). The first pair of swellings develop as the two halves of the antennal segment in a preoral position. Behind this, on either side of the mouth the two halves of the jaw segments arise followed by the oral papillae and a series of trunk segments. The number of segmental swellings increases until the appropriate number for the species in question have been formed. The only variations seen in the development of the external form relate to the degree of separation of the segment halves.

In the yolky embryo of the ovoviviparous species *Peripatoides novaezealandiae* (HUTTON) anterior segment halves are widely separated (SHELDON 1889). In the South African viviparous species, *Peripatopsis moseleyi* (WOOD-MASON) and *P. sedgwicki* PURCELL where there is a swollen sac of dorsal extra-embryonic ectoderm, the dorsal separation of the anterior segment halves resulting from the swollen trophic vesicle is extensive but their ventral separation is slight. In the other South African species *P. capensis* (GRUBE) (SEDGWICK 1885 - 88) and *P. balfouri* (SEDGWICK) (MANTON 1949) where a trophic vesicle is not present, dorsal separation of the two halves of the anterior segments is almost eliminated and the embryo develops by the proliferation of segments. In the Brazilian placental viviparous species *Peripatus acacioi* MARCUS and MARCUS development of external form again follows the same pattern, in this case with only relatively narrow bands of ventral and dorsal extraembryonic ectoderm between the segment halves (WALKER & CAMPIGLIA 1988).

As the examples cited above are taken from a world wide distribution, and from both Peripatidae and Peripatopsidae it might perhaps have been anticipated that the early development of external body form would be ubiquitous among the Onychophora. In this paper, scanning electron microscope data is presented that indicates that the external morphology of the early embryo of the South African Peripatopsid, *Opisthopatus cinctipes* PURCELL, differs markedly from what has been considered to be the typical pattern of early onychophoran development (ANDERSON 1973).

O. cinctipes was first described by PURCELL (1899). The genus is endemic to South Africa and two species have been described, *O. cinctipes* and *O. roseus* (LAWRENCE 1947). *O. cinctipes* has a very wide distribution compared to other South African species, its range extending from Cape province north eastwards to the Transvaal following the Escarpment where indigenous forest is found (BRINK 1957). It also has a more extensive vertical distribution than other species being found at altitudes ranging from around 2400 m in the Drakensberg Mountains to sea level (BRINCK 1957). *O. roseus* has only been reported from one locality (BRINCK 1957). PURCELL (1899, 1900) suggested that *O. cinctipes* should be a separate genus connecting the Cape genus Peripatopsis with the mainly American then *Peripatus,* now *Paropisthopatus* found in Chile (RUHBERG 1985). One of the reasons for forming the genus *Opisthopatus* was the presence of structures which PURCELL claimed were reduced seminal receptacula. This observation has been disputed (CHOONOO 1947, RUHBERG 1985), but data will be presented elsewhere (WALKER 1991) that supports PURCELL's claim that reduced seminal receptacula are present in *O. cinctipes.*

Although much is known from the work of SEDGWICK (1885 - 1888) and MANTON (1949) concerning embryonic development within the South African genus *Peripatopsis* very little is known about embryonic development in *Opisthopatus cinctipes* apart from the fact that the female uterus contains up to 40 embryos at different stages of development and that the female gives birth throughout the year (CHOONOO 1947). This is unlike the various species of *Peripatopsis* in which there are fewer embryos and in any female, all the embryos are at roughly the same stage of development and are born in batches (SEDGWICK 1885 - 88, MANTON 1949).

2. Materials and Methods:

Specimens of *Opisthopatus cinctipes* were collected from Vernon Crookes, National Park and in Durban. Animals were maintained at 18° C until required in covered glass dishes containing some damp cotton wool to maintain humidity. To prepared embryos for scanning electron microscopy (SEM) freshly dissected female reproductive tracts were placed in phosphate buffer and opened to release the embryos. The membrane surrounding the embryos were dissected away using fine forceps. Some of the smaller embryos were left associated with the uterus surrounding them and dissected further after fixation and drying. All material was fixed in half strength Karnovsky's fixative for 1 hour, dehydrated through an ethanol series, placed in amyl acetate and dried with a Samdri 780 critical point drier. Specimens were mounted on aluminium stubs, coated with gold/palladium and examined using a Cambridge 100S scanning electron microscope.

3. Results:

In each of the female *O. cinctipes* examined, about forty embryos at different stages of development were present. Oocytes and gastrula stages are found in the proximal region of the uterus nearest the ovary. Progressing down the uterus is a series of embryos of increasing age to mature, fully pigmented foetuses located distally near the vagina. In fresh material, the younger embryos in the proximal half of the uterus are very transparent and have the texture of fluid filled bags. A thin, red pigmented line, the gut, is seen running the length of the embryo. All elongated embryos are folded ventrally within their surrounding membrane.

The earliest stage observed in material for SEM is probably a morula as some cell boundaries can be detected (Fig. 1). In the next stage observed the embryos has the shape of a cylinder (Fig. 2) about 200 - 250 µm in length and about 30 µm in width. On its outer surface cell boundaries can be clearly distinguished. This specimen was broken during preparation and the central gut lumen can be seen (Fig. 2). The next stage is perhaps the most unexpected. The embryo has elongated and is about 1,75 mm in length (Fig. 3). There is no evidence of segmental swellings with segment boundaries between them and there is no evidence of lateral segment halves separated by dorsal and ventral extra-embryonic ectoderm. The anterior end can only by distinguished from the posterior end by the presence of the antennae which are starting to form (Fig. 3). There is no swelling of the preoral region (Fig. 3). In a slightly older specimen (Fig. 4) the first indications of segmental appendages are starting to appear as small bumps located to either side of the ventral midline. Those at the anterior end of the body, the jaws, just below the mouth opening, and the oral papillae appear larger than those of the posterior walking legs (Fig. 4). As the embryo gets older these small bumps enlarge to form distinct limb rudiments (Figs 5, 6). The antennae have elongated further (Fig. 5).

Development now proceeds as in all other species and the embryos grow until they are between 3 - 4 mm in length at birth. The antennae, oral papillae and walking legs elongate by forming a series of annuli (Fig. 7). The jaws sink into the mouth which is surrounded by the developing lips (Fig. 7). Laterally the body wall shows signs of annulations but the ventral surface is smooth with a distinct mid-ventral groove (Fig. 7). As development proceeds, these mid-ventral swellings diminish in size (Fig. 8) and in the mature foetus the ventral organs are seen as small smooth areas in the mid-ventral line (Fig. 9). The foetuses become pigmented once the macropapillae and the adult cuticle has developed over the body surface (Fig. 9). Even fully formed foetuses within the uterus are folded ventrally and are still surrounded by a layer of egg membrane. From the SEM observations it appears that the body surface is overlain by an additional layer as the detail of the surfaces of the macropapillae, spines and claws are obscured (Fig. 10). This layer is probably an embryonic cuticle (WALKER & CAMPIGLIA 1988, 1990) and is present over the surface of the *O. cinctipes* embryos once they have elongated. Using SEM the surface of the embryo illustrated in Fig. 3 is seen as a smooth continuous layer with no cell boundaries.

4. Discussion:

The data presented here demonstrate that early development of the embryos of *O. cinctipes* appears, at least from the external morphology, to follow a different route from the early development seen in all other species of Onychophora in which development has been described (ANDERSON 1973). In *O. cinctipes* there is no sequential addition of segments posteriorly accompanying elongation of the embryos. There are no apparent lateral segment halves separated by extra-embryonic ectoderm, and there is no swelling of the pre-oral antennal segment, a feature of all other embryos previously described. Indications of segmental organisation and segmental appendages only appear after elongation of the body. Also the segmental appendages first appear in their finite position rather than appearing laterally and migrating to a more ventral position as seen in the embryos of *P. acacioi* (WALKER & CAMPIGLIA 1988). The appearance of fresh material is also very different. The early embryos of *P. acacioi* are firm, opaque structures while those of *O. cinctipes* are more fluid and transparent. Another difference is that the embryonic cuticle forms at a much earlier stage of development in *O. cinctipes* than that of *P. acacioi* (WALKER & CAMPIGLIA 1988). Also the barbed projections (WALKER & CAMPIGLIA 1988) covered by embryonic cuticle found at the ends of the walking legs in embryos from the Peripatidae are absent from *O. cinctipes* and other South African Peripatosidae that I have examined (WALKER, unpublished observation).

The question remains, why do the early embryos of *O. cinctipes* differ from those of all other early onychophoran embryos so far described? Differences in early embryonic development within a group are not unprecidented. Modes of specification of basic body pattern in insect embryogenesis have been described by SANDER (1976) as varying from short germ band types to

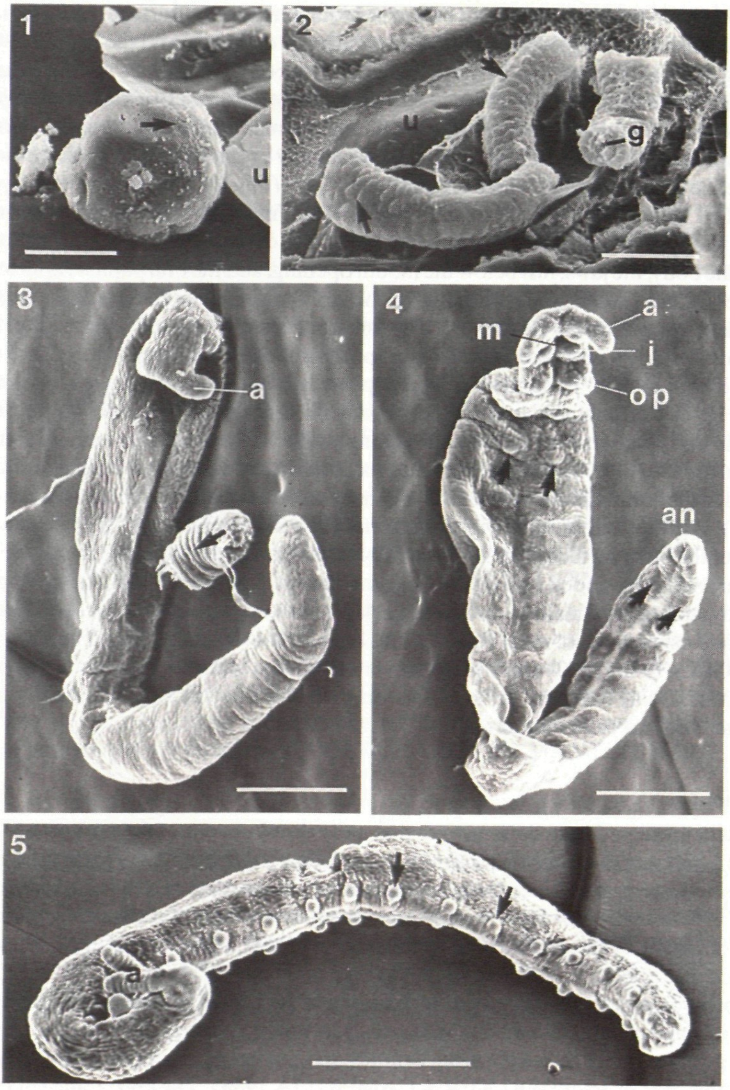

Fig. 1: A morula in which some cell boundaries can be seen (arrow) and remnants of part of the proximal region of the uterus (u). Scale bar = 50 μm.

Fig. 2: A cylindrical embryo showing distinct cell boundaries (arrow) on its outer surface. Where the embryo is broken the gut (g) is seen. (u = remnants of the proximal region of the uterus). Scale bar = 50 μm.

Fig. 3: Elongated embryo with antennae (a) starting to form. An antenna that has broken off from an older embryo is present (arrow). Scale bar = 250 μm.

Fig. 4: An elongated embryo in which segmental appendage buds are starting to appear. The jaws (j) are located beside the mouth (m), and below them are the oral papillae (op). The limb buds (arrows) are seen as small bulges to either side of a mid ventral line and the anus (an) is seen at the posterior end of the body; a = antenna. Scale bar = 250 μm.

Fig. 5: An older embryo in which the antennae (a) are elongating and the rudiments of the walking legs (arrows) are well formed. Scale bar = 0,5 mm.

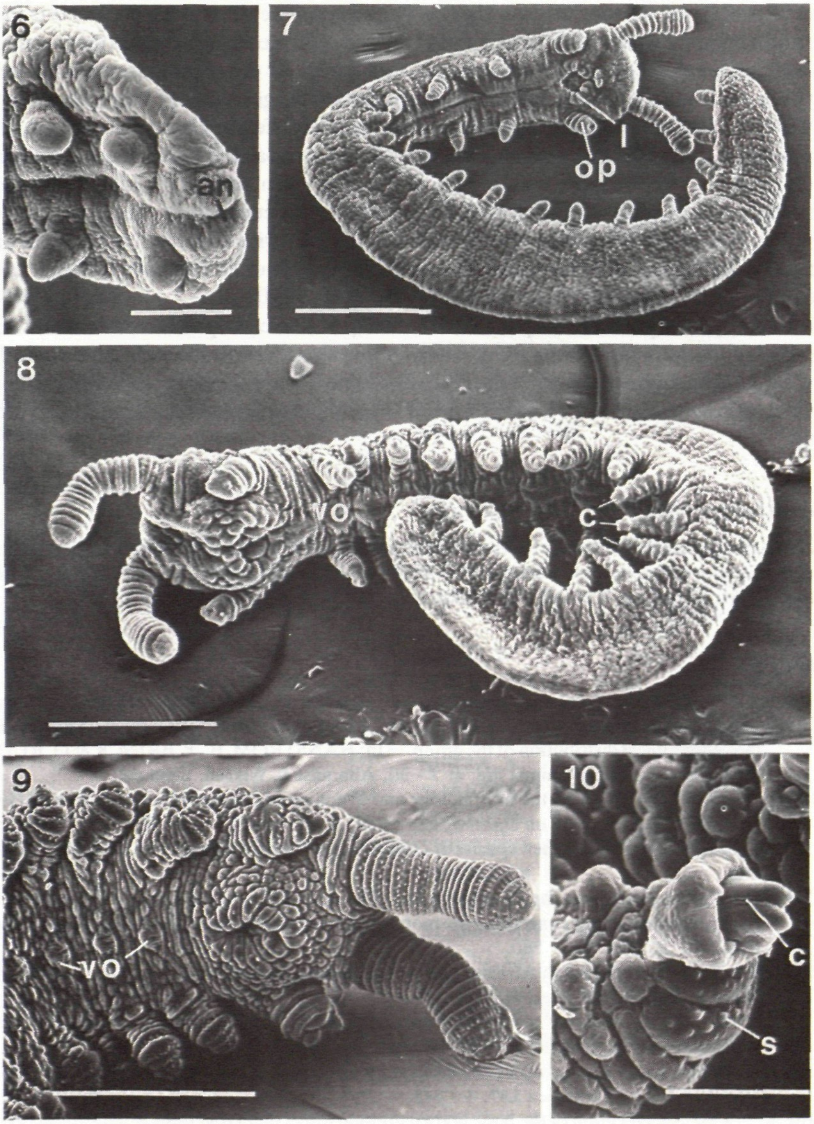

Fig. 6: Posterior end of the embryo illustrated in Fig. 5, showing the walking leg rudiments located to either side of the ventral mid line; an = anus. Scale bar = 100 μm.

Fig. 7: Larger embryo in which the lips (l) surround the mouth, the walking legs are elongating, and annuli are seen on the body surface apart from a smooth band to either side of the ventral midline arrow); op = oral papillae. Scale bar = 0,5 mm.

Fig. 8: Foetus in which the walking legs have completed their elongation and bear claws (c). The body surface is covered with annuli except in the regions of the developing ventral organs (vo). Scale bar = 0,5 mm.

Fig. 9: Anterior end of a mature foetus in which the macropapillae and spines are fully formed; vo = ventral organs. Scale bar = 500 μm.

Fig. 10: Foot of a mature foetus. The lack of detail seen on the spiny foot pads (s) and claws (c) indicates the presence of the embryonic cuticle covering the whole body. Scale bar = 100 μm.

long germ band types. At one extreme, the short band type, the germ anlage represents the procephalon and a budding zone which subsequent to the germ anlage stage produces the metameric part of the body as, for example, in the orthopteran *Tachycines*. At the other extreme is the long germ band type, seen in *Apis*, where the different body regions in the germ anlage are represented proportionally to their relative dimensions in the germ band and no differential growth occurs. All onychophoran embryos described so far in the literature may be comparable to the short germ band type, while *O. cinctipes* may be more comparable to the long germ band type. Light microscope and ultrastructural studies of the early embryos of *O. cinctipes* are currently in progress to elucidate the timing of the formation of internal segment boundaries. Likewise ultrastructural studies are being undertaken to determine whether or not the embryonic cuticle of *O. cinctipes* is similar to that described in *P. acacioi* (WALKER & CAMPIGLIA 1988, 1990).

As the early embryonic development of *O. cinctipes* is so clearly different from that described in other Peripatidae and Peripatopsidae it is now important to look at the early embryonic development of the Chilean representatives of the Peripatopsidae to see if there are similarities with *O. cinctipes* which would substantiate PURCELL's (1899) original rationale for forming the genus *Opisthopatus*.

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