Proteonematalycus wagneri Kethley reveals where the opisthosoma begins in acariform mites

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**Corresponding Author:** Samuel J Bolton, PhD
Florida Department of Agriculture and Consumer Services
Gainesville, Florida UNITED STATES

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**Abstract:**
It is generally thought that the anterior border of the opisthosoma of acariform mites is delimited by the disjugal suture, but the intersegmental furrows of Proteonematalycus wagneri Kethley instead indicate that this suture intersects the metapodosoma. Accordingly, the anterior border of the opisthosoma is between segments D and E. Therefore, segments C and D are metapodosomal and not opisthosomal. The disjugal suture is probably an apomorphic border that is only present in oribatids. Homology between the disjugal suture and the anterior border of the opisthosoma has never been supported with evidence other than the superficial appearance of tagmosis. The alternative homology can be accommodated by a model in which the proterosoma warps. The metapodosoma can readily undergo elongation because it is not constrained by the warp. This would explain why there is a large interval between legs II and III in most mites with elongate bodies.

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Proteonematalycus wagneri Kethley reveals where the opisthosoma begins in acariform mites

S. J. Bolton*

1 Florida State Collection of Arthropods, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida, USA

*samuel.bolton77@gmail.com

Abstract

It is generally thought that the anterior border of the opisthosoma of acariform mites is delimited by the disjugal suture, but the intersegmental furrows of Proteonematalycus wagneri Kethley instead indicate that this suture intersects the metapodosoma. Accordingly, the anterior border of the opisthosoma is between segments D and E. Therefore, segments C and D are metapodosomal and not opisthosomal. The disjugal suture is probably an apomorphous border that is only present in oribatids. Homology between the disjugal suture and the anterior border of the opisthosoma has never been supported with evidence other than the superficial appearance of tagmosis. The alternative homology can be accommodated by a model in which the proterosoma warps. The metapodosoma can readily undergo elongation because it is not constrained by the warp. This would explain why there is a large interval between legs II and III in most mites with elongate bodies.

Introduction

Mites comprise two superorders: Parasitiformes and Acariformes. Almost all mites have lost the furrows that delineate their body segments. However, the dorsal setae of acariform mites are typically arranged in transverse rows, which correspond with the underlying body segments [1]. There are conflicting interpretations concerning the relationship of two of these
segments, C and D (bearing setae c and d), to the metapodosoma, which bears legs III and IV (Fig. 1A). Reuter [2] homologized segments C and D with the metapodosoma. More recently, the same interpretation was put forward by Weigmann [3] (Fig. 1B). Van der Hammen instead homologized segments C and D with the first two segments of the opisthosoma [4] (Fig. 1C, D), which was based on his hypothesis that the anterior border of the opisthosoma is represented by the disjugal suture (Fig. 1E). Many if not most acarologists accord with van der Hammen’s hypothesis by referring to the structures associated with segments C and D (principally setae and plates) as ‘opisthosomal’. The alternative term ‘hysterosomal’ (the region of the body that is behind the sejugal furrow), is neutral with respect to either hypothesis.

Grandjean based a widely cited model on van der Hammen’s hypothesis to explain the body segmentation of acariform mites [5]. According to this model, the dorsal region of the podosoma is dramatically reduced, causing the body regions on either side to be pulled into areas that were occupied by the podosoma (Fig. 1C). Klompen et al. also adhered to van der Hammen’s hypothesis when they suggested an amendment to Grandjean’s model [6]. They infer that the whole of the prosoma has warped somewhat evenly (Fig. 1D), which explains how the dorsal surface of the podosoma can be completely diminished. These interpretations are illustrated with an animation, which is accessible via the following link:

https://zenodo.org/record/5512807#.YUOPiflKhaQ

Proteonematalycus wagneri Kethley, a rare species of mite that is only known from sandy habitats, is exceptionally useful for investigating the arrangement of body segments in Acariformes. Unlike other basal acariform mites in which the furrows that delimit the segments are restricted to the dorsum, some of the furrows of P. wagneri completely encircle the hysterosoma, clearly revealing which segments are associated with the metapodosoma.
This mite was examined with a scanning electron microscope (SEM) and a light microscope in order to confirm that these furrows are intersegmental.

**Results**

The integument of *P. wagneri* is extremely soft and fragile, causing it to readily distort when removed from alcohol. Alcohol stored specimens of this species completely shriveled up when viewed with cryo-SEM, which has been used with a high degree of success on other soft-bodied mites [7]. By comparison, mites that were desiccated using ethanol–hexamethyldisilazane (HMDS) provided relatively good images using conventional SEM, although there was still some shriveling (Fig. 2A, B). SEM reveals that each hysterosomal segment is clearly delimited by intersegmental furrows, which fall on either side of a transverse row of setae. A closeup of the integument shows that the striae break up into very fine protuberances along the base of each furrow (Fig. 2B). By uncovering furrows that correspond with the borders of all the hysterosomal segments, SEM removes any remaining ambiguity concerning the homology of the furrows with segmental borders; the drawings in the original species description do not include the furrow between segments *F* and *H* [8].

**Figure 2.** *Proteonematalycus wagneri* Kethley. A. Lateral view of female (SEM; FSCA 00030222). B. Lateral view of segments *F–AN* (SEM; same female as above). C. Lateral view of female (light microscopy; FSCA 00030224), approximately 2 minutes after immersion in PVA. White dotted lines delimit segments *PS–AN*. Small white arrowheads delimit the dorsolateral borders of coxae III and IV (note that there is slight sagging of the integument over the border of coxa III).

Despite the relatively low image resolution, light microscopy demonstrates how *P. wagneri* appears when it has not undergone any shriveling. This species reveals very distinct intersegmental furrows (Fig. 2C). This is an important distinction from mites that exhibit a disjugal suture [9], which can be explained as a non-segmental feature because it bears no real resemblance to an intersegmental furrow; suture intersects a plane whereas an intersegmental furrow is a slight constriction between two segmental humps (Fig. 3A).

**Figure 3.** The metapodosomal-opisthosomal boundary. A. Illustration of the difference between a furrow and a suture. B. Same model as Fig. 1E, but color coded to show that the disjugal suture intersects the metapodosoma.
(yellow). C. A new model for explaining elongate bodied acariform mites. Blue = proterosoma; yellow = metapodosoma; brown = opisthosoma; Fu = furrow; Su = suture; black arrowhead = disjugal suture.

With respect to segmental homology, *P. wagneri* shows that segments *C* and *D* bear legs III and IV, respectively, and so these segments represent the metapodosoma, whereas segment *E* represents the first segment of the opisthosoma. There is no trace of a disjugal suture or furrow.

**Discussion**

**The disjugal suture**

Other than the superficial appearance of tagmosis in some oribatids [4,5], there is no evidence that the disjugal suture forms a boundary between the metapodosoma and opisthosoma. This suture must instead intersect the metapodosoma (Fig.3B). Perhaps the main reason that van der Hammen’s hypothesis has been so widely adopted is that it adds two additional segments to the acariform body (segments *C* and *D* are treated as separate from the metapodosoma), which makes the body segment count of this lineage closer to that of other arachnids [4]. However, this is not a strong argument because there is noticeable variation in the number of body segments among other arachnids; Scorpiones have nineteen body segments, whereas Opiliones have only fifteen [1].

Based on van der Hammen’s hypothesis, the term ‘disjugal’ is often applied to the dorsal part of the sejugal furrow of non-oribatid taxa [12–15]. And in *Neognathus*, longitudinal ridges of tubercles have been mislabeled as disjugal (each ridge cuts between setae *c1* and *c2* and so cannot delineate the opisthosoma) [16]. But a true disjugal suture is probably only present in Oribatida. This lineage has evolved a suite of defensive modifications that are found in association with the disjugal suture [17,18]. It is relatively common for secondarily derived borders to form in association with sclerotization. For example, labidostomatids have a distinctive groove that divides the body into a dorsal and ventral shield [19]. Therefore, it seems likely that the disjugal suture originated as an apomorphic border as a result of sclerotization in oribatids.
The presence of visible body segments, involving intersegmental furrows, is very obvious in some soft-bodied basal acariform taxa [10,11]. This is also the case in other basal arachnid lineages, for example, Mesothelae within Araneae [20,21], and Opilioacarida within Parasitiformes [6,22]. It is therefore appropriate to base the interpretation of acariform body segmentation on *P. wagneri*, which is a basal acariform mite with a complete set of intersegmental furrows along the hysterosoma.

A disjugal furrow is clearly absent from *P. wagneri*. There appears to be no example of a true disjugal furrow, as opposed to a disjugal suture, in any mite (Fig. 3A). Instead, the intersegmental furrows of all mites that exhibit them are concordant with Reuter [2] and Weigmann’s [3] interpretation of where the opisthosoma begins. Accordingly, the border between the metapodosoma and the opisthosoma is formed by a transverse furrow between segments D and E (Fig. 2A). This is shown by *P. wagneri*. Mites in the family Tarsocheylidae also show this, although they have an incomplete set of intersegmental furrows along the hysterosoma [23]. And these taxa affirm what has already been inferred from other taxonomic groups, namely Siteroptidae [2] and Oribatida [3].

**Model**

The relatively posterior position of the paired eyes of mites has been explained by the warping of the entire prosoma [6] (Fig. 1D). However, *P. wagneri* indicates that the metapodosoma cannot be part of the warp. But warping of the proterosoma still seems likely based on the position of the paired eyes. This would also explain the uneven distribution of legs along the bodies of mites in which segment C has elongated (in most elongate bodied taxa this segment elongates more than any other). Legs I and II are always tightly packed with the palps and chelicerae [23–25] (Fig. 2), which is probably because of a constraint attributable to a proterosomal warp (Fig 3C). But legs III and IV are much more posterior because they belong on segments C and D. These segments can readily elongate because they are not constrained by that warp. It is noteworthy that, primitively, the euarthropod body underwent tagmosis into a head (proterosoma) and trunk (hysterosoma) [26,27]. Warping of the proterosoma may in some way be linked to this tagmosis (Fig. 3C).
Materials and methods

Collection

Proteonematalycus wagneri was collected from foredune sand using heptane flotation [28].

Collection event: U.S.A., Indiana, Porter Co., Indiana Dunes State Park, 41.6780 N 87.0081 W, sand dune (10 cm deep); collector: Samuel Bolton, 16 May, 2013 (FSCA 00030222:

- Female x1, Deutonymph x1, Protonymph x1, larva x1) (FSCA 00030224: Female x1; FSCA 00030225: Deutonymph x1).

SEM

Specimens of P. wagneri were transferred from a storage medium of 95% ethanol into the following series of solvents: 1) absolute ethanol; 2) 50:50 volume HMDS; 3) 100% HMDS. Immersion in each fluid medium lasted approximately five minutes. A minute pin, which had been glued onto a FisherbrandTM plain wooden applicator, was used to maneuver the specimens between solutions. For the final step, the HMDS was left to evaporate. The specimens were then mounted on SEM stubs and sputter coated with approximately 70 nm of gold/palladium using a Denton IV sputtercoater. Micrographs were captured with a Phenom XL G2 Desktop SEM.

Light microscopy

Examination was with a compound microscope (Leica DM2500) equipped with differential interference contrast (DIC) and a digital SLR (Canon EOS 80D). Imaging was with a dry, 10x objective (brightfield). Polyvinyl alcohol (PVA) mounting medium was used to hold the specimens in a relatively still position. Specimens were imaged without a coverslip within minutes of being placed into the PVA. Contrast was heightened by setting the turret of the DIC between the 10 and 40 intervals.

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Jonathan Bremer, at the Florida State Collection of Arthropods, undertook the SEM imaging of *P. wagneri* (Fig. 2A, B). The U.S. National Park Service granted the author permission to collect from the Indiana Dunes National Lakeshore. The following individuals are thanked for their contribution through internal review and/or useful discussion: Erin Powell, Elijah Talamas, Paul Skelley (Florida Department of Agriculture and Consumer Services, Gainesville, Florida, USA) and Hans Klompen (Ohio State University, Columbus, Ohio, USA). The Florida Department of Agriculture and Consumer Services – Division of Plant Industry are thanked for their support on this contribution.

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