

Phasmid-like structures in Anguinidae (Nematoda, Tylenchida)

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Summary – Scanning electron and light microscope studies revealed the presence of phasmid-like structures in *Ditylenchus dipsaci*, *D. destructor*, *D. triformis*, *D. convallariae*, *Anguina tritici*, *A. agropyri*, *Heteroanguina graminophila*, *Mesoanguina millefolii*, *M. plantaginis*, and *Subanguina radiculicola*. The structures are dorsal to the lateral fields, in the postmedian portion of the body. In this character, the Anguinidae are close to the families in Tylenchoidea in the classification proposed by Siddiqi (1986); placement in Hexatyliina appears incorrect. The presence of phasmids on the tail in *Psilenchus*, *Atitylenchus* and *Antarctenichus* supports the exclusion of these genera from Tylenchidae as proposed by Maggenti *et al.* (1988).

Résumé – Structures analogues aux phasmides chez les Anguinidae (Nematoda, Tylenchida) – Des observations en microscopie électronique à balayage et en microscopie optique ont révélé la présence de structures analogues à des phasmides chez *Ditylenchus dipsaci*, *D. destructor*, *D. triformis*, *D. convallariae*, *Anguina tritici*, *A. agropyri*, *Heteroanguina graminophila*, *Mesoanguina millefolii*, *M. plantaginis* et *Subanguina radiculicola*. Ces structures sont localisées dorsalement par rapport aux champs latéraux, dans la région postmédiane du corps. Par ce caractère, les Anguinidae sont proches des familles placées dans les Tylenchoidea dans la classification proposée par Siddiqi (1986); leur placement chez les Hexatyliina apparaît inexact. La présence de phasmides dans la région caudale chez *Psilenchus*, *Atitylenchus* et *Antarctenichus* soutient l'idée d'exclure ces genres de la famille des Tylenchidae telle que définie dans la classification proposée par Maggenti *et al.* (1988).

Key-words : *Anguina*, Anguinidae, classification, *Ditylenchus*, hexatyliina, light microscopy, phasmid, scanning electron microscopy, Tylenchidae.

The presence or absence of phasmids and their position on the body of nematodes are of taxonomic significance. Whereas all Tylenchina in the classification of Siddiqi (1986) – with only a few exceptions – appear to possess phasmids or phasmid-like structures, these are absent or have never been detected with certainty in Criconematina and Hexatyliina (Siddiqi, 1986). The genera in the Tylenchina superfamilies Dolichodoroidea and Hoplolaimoidea in this classification generally have phasmids located on tail or in preanal region in the middle of the lateral fields. The genera placed in Tylenchoidea seem to possess phasmid-like structures lying in the postmedian region of the body in a dorsolateral position outside of the lateral fields.

In Anguinidae, which are placed by Siddiqi (1986) in Hexatyliina, phasmids or phasmid-like structures are generally absent (Siddiqi, 1986; Maggenti *et al.*, 1987). However, in his generic diagnoses Golden (1971) reported that phasmids are “indistinct, often invisible” in *Ditylenchus*, and Siddiqi (1971) wrote that phasmids were “indistinct” in the Anguinidae. Phasmids located on the tail had been described and illustrated in several members of the Anguinidae: *Ditylenchus dipsaci* by Thorne (1949) and *D. phyllobius* (Thorne, 1934), *D. nortoni* (Elmiligy, 1971) and *Sychnotylenchus pinophilus*

(Thorne, 1935) in the original descriptions of these species. The presence of phasmids on the tail has been considered to be unproven in subsequent publications, e.g. for *D. dipsaci* by Hooper (1972) and for *D. phyllobius* by Krall (1991). Sturhan and Brzeski (1991) wrote that confirmation on the presence of phasmids in *Ditylenchus* appears necessary.

When studying *Ditylenchus* species for certain morphological characters with light microscope (LM) at high magnifications, we obtained evidence for the presence of phasmid-like structures. Subsequent scanning electron microscope (SEM) studies on several Anguinidae species were conducted to verify our observations. The results of our studies are presented here and the taxonomic consequences discussed.

Materials and methods

For the SEM studies the following nematode species were used :

- *Ditylenchus dipsaci* (Kühn, 1857), isolated from red clover plants originating from Bavaria,
- *D. destructor* Thorne, 1945, from potato tubers from Tartu, Estonia,

– *D. triformis* Hirschmann & Sasser, 1955, kept in agar culture on fungi at Münster, originally from Raleigh, USA,

– *Anguina tritici* (Steinbuch, 1799) infective-stage juveniles from wheat galls from southern Germany,

– *A. agropyri* (Kirjanova, 1955), fixed specimens from Estonia.

The nematodes were fixed at 4 °C in 2 % glutaraldehyde solution buffered with sodium phosphate buffer at pH = 7.2, post-fixed with OsO₄ at room temperature and dehydrated in ethanol, followed by critical point drying in CO₂. Specimens were mounted on specimen stubs using conductive tape and sputter coated with gold. A Hitachi model S-530 scanning electron microscope operating at 20 kV was used for viewing and photography.

The LM studies using interference-contrast optics were conducted with preserved nematode specimens, most of them TAF fixed and mounted in glycerine on permanent microscopical slides. In addition to species used for the SEM studies, the following species were available :

– *Ditylenchus convallariae* Sturhan & Friedman, 1965 : type specimens and material collected in the German Alps and in the Tatra Mountains, Poland,

– *Heteroanguina graminophila* (Goodey, 1933) : from *Agrostis* sp. leaf galls, Germany,

– *Mesoanguina millefolii* (Löw, 1874) : isolated from *Achillea millefolii*, Tartu, Estonia,

– *M. plantaginis* (Hirschmann, 1977) : isolated from *Plantago aristata*, USA,

– *Subanguina radicola* (Greeff, 1872) : from soil, Germany,

– *Anguina graminis* (Hardy, 1850) : infective-stage juveniles, isolated from leaf galls of *Festuca rubra*, Germany,

– *A. agrostis* (Steinbuch, 1799) : infective-stage juveniles isolated from *Agrostis* sp. seed galls, Germany.

Results

The SEM studies revealed the presence of phasmid-like structures in *D. dipsaci*, *D. destructor*, *D. triformis*, *A. tritici* and *A. agropyri*. In *D. dipsaci*, distinct structures are located in dorsolateral position well apart from the lateral field (Fig. 1, A-B). In *D. destructor*, the phasmid-like structure is in a similar position (Fig. 1 C; a number of bacteria can be seen attached to the cuticle), and in *D. triformis* it was present closer to the lateral field (Fig. 1 D). In both *A. tritici* and *A. agropyri* the phasmid-like structures were also located close to the lateral fields, which showed irregularities at these sites (Fig. 1, E-F).

With the light microscope it was difficult to identify phasmids in the Anguinidae species and to differentiate these structures from bacteria or any other particles adhering to the cuticle. However, some information on the presence of phasmid-like structures in several species in

addition to those studied by scanning electron microscope could be obtained.

In *D. convallariae* distinct phasmid-like structures were seen in several specimens. They were located dorsal to the lateral fields, about four and a half body widths anterior to the vulva in females and at about 70-75 % of the total body length in males. In a male of *H. graminophila*, a phasmid-like structure was seen 1.5 µm dorsal to the lateral field at 80 % of the body length. In a *M. millefolii* male and in an infective-stage juvenile, phasmid-like structures were observed in a similar position at about 70 % of the body length. In *M. plantaginis*, such structures appeared to be present slightly more than three body widths anterior to the vulva in a female and at about 75 % of the total body length in males. In *S. radicola* females, they appeared between three and four body widths anterior to the vulva. In some other Anguinidae species studied, phasmid-like structures could not be differentiated and located with certainty by LM observation.

Discussion

The discovery by Siddiqi (1978) of phasmids or phasmid-like structures in several genera of Tylenchidae, lying in the postmedian region of the body, in females near the vulva and near the dorsal border of the lateral fields, was considered to be an “epoch-making event” (I. Andrassy and F. G. W. Jones in Siddiqi, 1986). Subsequently, structures in such a position were recorded for many other genera of Tylenchina (see Siddiqi, 1986) and recently also for Aphelenchidae (Baujard *et al.*, 1995). For Siddiqi (1986) these “abnormally located” phasmids or phasmid-like structures have been the basis for recognizing Tylenchoidea as distinct from Hoplolaimoidea and Dolichodoroidea within the suborder Tylenchina in his classification. In the latter two superfamilies phasmids occur in the middle of the lateral fields and are in general located on the tail or in the preanal region.

Siddiqi (1986) used the presence of phasmids or phasmid-like structures as one of the characters differentiating Tylenchina from the suborders Criconematina and Hexatyliina, in which phasmids are absent or have never been detected with certainty (see e.g., the key presented on page 99 of his book and the cladogram on page 75). Ryss (1993) even used the presence and the position of phasmids and phasmid-like structures as a key differential character for the suborders in the order Tylenchida.

The present study has shown that phasmid-like structures in dorsolateral position outside the lateral fields in the postmedian region of the body are not unique to Tylenchidae and other families placed in Tylenchoidea in the classification of Siddiqi (1986). From the results of our study which is based on a number of species, it may be concluded that it is a common character in Anguinidae, where it had been overlooked so far. The pre-

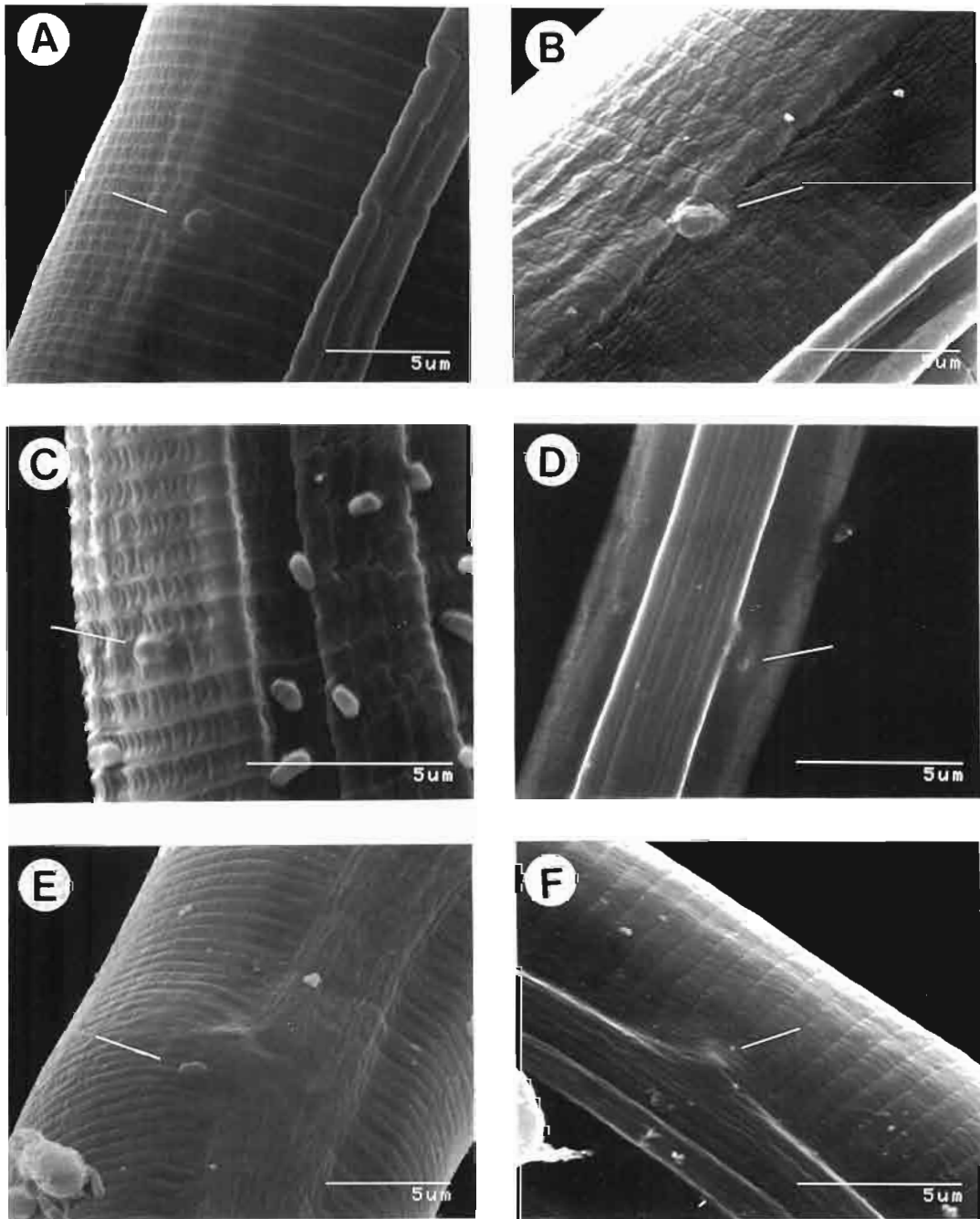


Fig. 1. Scanning electron micrographs showing phasmid-like structures in dorsolateral position close to the lateral field in *Ditylenchus dipsaci* (A, B), *D. destructor* (C), *D. triformis* (D), *Anguina tritici* (E) and *A. agropyri* (F).

vious observations on the presence of phasmids on the tail in some Anguinidae species (see above) appear to be incorrect.

The present observations provide evidence for placing Anguinidae in Tylenchina rather than in the suborder Hexatyline. Maggenti *et al.* (1987) did not recognize Siddiqi's (1986) concept of the suborder Hexatyline and already brought back the Anguinidae to Tylenchina, and Sturhan and Brzeski (1991) concurred with this approach.

Among the genera included in the families Tylenchidae and Anguinidae in the classification presented by Maggenti *et al.* (1987, 1988), *Psilenchus*, *Atetylenchus* and *Antarctenchus* are the only genera in which the position of phasmids on the tail is reported; they are placed into separate Tylenchidae subfamilies, together with other genera possessing phasmid-like structures in dorsolateral, precaudal position. The ancestral position of the phasmid is caudal, and there are other characters supporting the assumption that *Atetylenchus*, *Psilenchus* and *Antarctenchus* represent the most primitive taxa of Tylenchida (Luc *et al.*, 1987; Ryss, 1993). Placement of these genera into a separate, though obviously paraphyletic taxon (Psilenchidae) in Dolichodoroidea (Siddiqi, 1986; Ryss, 1993) instead of keeping them in closer systematic relationship to Tylenchidae and Anguinidae appears more justified. After transferring of Anguinidae to Tylenchoidea in the classification of Siddiqi (1986) this superfamily includes all genera of Tylenchida with phasmid-like structures dorsal of the lateral fields and well anterior to the tail.

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