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The sister group of the Diplogastrina (Nematoda)

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Summary. This paper presents arguments for a sister group relationship between diplogastrids and bunonematids. Five synapomorphies were found to substantiate this hypothesis. The pattern of genital papillae in *Bunonema pini* is homologized with that of diplogastrids. The question whether the stem species of diplogastrids possessed a bursa or not is discussed.

Key words: *Bunonema*, bursa, genital papillae, haustrulum, pharyngeal sleeve.

Following the phylogenetic analysis of the Nematoda based on 53 small subunit rDNA sequences of Blaxter *et al.* (1998) the sister group of the Diplogastrina has to be sought within the paraphyletic "Rhabditidae". This paper provides arguments in favour of a sister group relationship between Diplogastrina and Bunonematidae. Blaxter *et al.* (1998) could not place *Bunonema* within the cladogram that summarized their analysis as this taxon belongs to the "long branch taxa" (taxa with many substitutions in the examined sequences). In their maximum parsimony analysis *Bunonema* grouped within the "Adenophorea" with a representative of the Monhysteridae. However, there is no doubt that *Bunonema* belongs to the Secernentea. This relationship is substantiated by many apomorphic characters as the phasmids and a dauer larva. The tube-shaped buccal cavity and the glottoid apparatus of bunonematids indicates a close relationship with the "Rhabditidae". Within the "Rhabditidae" the sister group of the monophylum comprising *Bunonema* and diplogastrids is unknown.

Bunonematids always crawl on the left body side that is equipped with five cuticular ridges (Fig. 4c), whereas the right body side bears more or less elaborated cuticular ornamentations (Fig. 1a). Until now, when dealing with *Bunonema*, the major matter of interest was this asymmetric cuticular ornamentation, an apomorphy that evolved as an adaptation to live on substrate surfaces (Sachs 1949). But, the inner anatomy as well as the pattern of the genital papillae in the male tail show plesiomorphic characters that have an astonishing resemblance with diplogastrid features.

MATERIAL AND METHODS

Examined species

Bunonema (*Rhodolaimus*) *pini* Fuchs, 1930: Isolated from material sampled underneath the bark of pines, Grunewald, Berlin.

Bunonema penardi Stefanski, 1914; strain GB 048: Our laboratory received a culture of these nematodes from G. Borgonie, Gent (Belgium).

Ceratoplectus armatus (Bütschli, 1873); det. I. Andrássy: isolated from soil samples, collected by W. Dohle at Mt. Duncan (Penguin, Tasmania).

Demaniella cibourgensis Steiner, 1914; strain SB 318: Isolated from casts of barn owls, collected by T. Chatterjee in Halsdorf/Eiffel (Germany).

Diplogaster schneideri Paesler, 1939; isolated from slime fluxes inhabited by *Nosodendron fasciculare* (Nosodendridae, Coleoptera) of willows in Tübingen and horse chestnut trees in Groß Ziethen, Brandenburg (Germany).

Goodeyus ulmi Goodey, 1930; strain PDL 0020: Collected by D. Hunt beneath the bark of a deceased elm infested by *Scolytus* (Scolytidae) in Harpenden (England).

Pseudodiplogasteroides sp. 1; strain SB257: Isolated by K. Kiontke from decaying saguaro cacti (*Carnegiea gigantea*) near Tucson, Arizona (USA).

Pseudodiplogasteroides sp. 2: Isolated from frass of cerambycid beetles under bark sampled by M. Balke at Cyclops Mountains, Sentani (Irian

Jaya, New Guinea).

Rhabditis (*Pelodera*) *teres* Schneider, 1866; strain EM 437: Collected by F. Schulte in a henhouse in Neuenrade, Westfalen (Germany).

Technique

To examine the morphology live nematodes were placed on a microscope slide provided with a 0.5 mm thick layer of agar (5%). After a coverslip was applied the nematodes were not able to move around and could be examined with a Zeiss Axioplan. For preparing the drawings the nematodes needed to be heat relaxed. The drawings were made using a camera lucida. For examination with a SEM fixed (glutaraldehyde) specimens were dehydrated through an ethanol series and then critical point dried from 100% ethanol using CO_2. The specimens were mounted on stubs, sputtered with gold, and examined with a Phillips SEM 515.

Abbreviations

ad	= anterior dorsal genital papilla
cheil	= cheilostom
cs	= cephalic sensillum
db	= dorsal bulge
df	= dorsal fold
flp	= flap-like process
gr	= grinder
gym	= gymnostom
haus	= haustrulum
ls	= labial sensillum
pd	= posterior dorsal genital papilla
phatu	= pharyngeal tube
phsl	= pharyngeal sleeve
steg	= stegostom
svd	= subventral denticle
v1-7	= subventral genital papillae

RESULTS

In the following description the revised nomenclature for stomatal structures of De Ley *et al.* (1995) is used. The stoma consists of a cheilostom, a gymnostom and a stegostom. The cheilostom represents the inner cuticle of the lips. The cuticular lining of the cheilostom is separated from the rest of the buccal cuticle by a wedge shaped interruption on the epidermal side. The cell membrane of the anterior arcade syncytium projects into this interruption and reaches the buccal lumen (Van de Velde *et al.*, 1994, De Ley *et al.*, 1995). The cuticle of the gymnostom is surrounded by arcade syncytia. The cuticular lining of the stegostom is surrounded by pharyngeal muscle cell processes. In many "rhabditids" the anterior pharyngeal cell processes that surround the stegostom lack myofilaments and form a pharyngeal sleeve (phsl; Fig. 7) that ensheathes the proximal part of a cuticular tube.

The following characters are found in bunonematids and the stem species pattern of diplogastrids (Fürst von Lieven & Sudhaus, 2000):

1. The buccal cavity consists mainly of an elongated tube but it lacks a pharyngeal sleeve (compare Figs 1b, 2a, 5a, and 9a, b with Fig. 7). That means that the whole tube must be a product of the arcade syncytia and therefore represents the gymnostom. Reports of the existence of a pharyngeal sleeve in Bunonematidae (*e.g.* Fuchs 1930 for *Bunonema pini*) must be due to observational errors. In his careful revision of bunonematids based on the observation of live specimens Sachs (1949) stated that a pharyngeal sleeve can not be found.

2. The cuticularized lining of the gymnostomatal tube is interrupted dorsally by a longitudinal fold of thinner cuticle (df; Figs 1b, 2a, b, 5a, b, 6, 9a, b).

3. This fold is caudally connected with a dorsal bulge of the stegostom (db; Figs 2a, b, 5b, 6, 9a, b). In phylogenetically early offbranching diplogastrids the bulge bears a flap-like process (flp; Figs 5a, b, 6, 9a, b). The flap-like process is an apomorphy of the Diplogastrina (Fürst von Lieven 2001).

4. The cuticular lining of each subventral sector of the stegostom bears a denticle (svd; Figs 1a, b, 2a, b, 5a, b).

5. The third precloacal genital papilla (= first bursal ray in *Bunonema*) of the male tail lies laterally compared to the subventral position of the first and second (v3d; Figs 4c, 9c, 10, 12, 13, 14a, b, c).

6. The metacorpus forms a pronounced round-shaped first bulb (1st bulb; Fig. 1c).

7. The haustrulum, a specialized region of the pharyngeal lumen, is located behind the grinder of the second bulb (gr; Fig. 1c), and demarcated by interradial cuticularizations (Doncaster 1962). In *Bunonema* and *Pseudodiplogasteroides* these cuticularizations are elongated interradial rods (haus; Figs 1c, d, 3) that look identical in both groups. If one depicts the terminal bulb of *Bunonema* without the grinder, it is not distinguishable from that of *Pseudodiplogasteroides* (compare Fig. 1d with 3).

8. Each branch of the female gonad is reflexed at the border between ovary and oviduct which after Lorenzen (1994) is the antidromous condition (arrows; Fig. 4a).

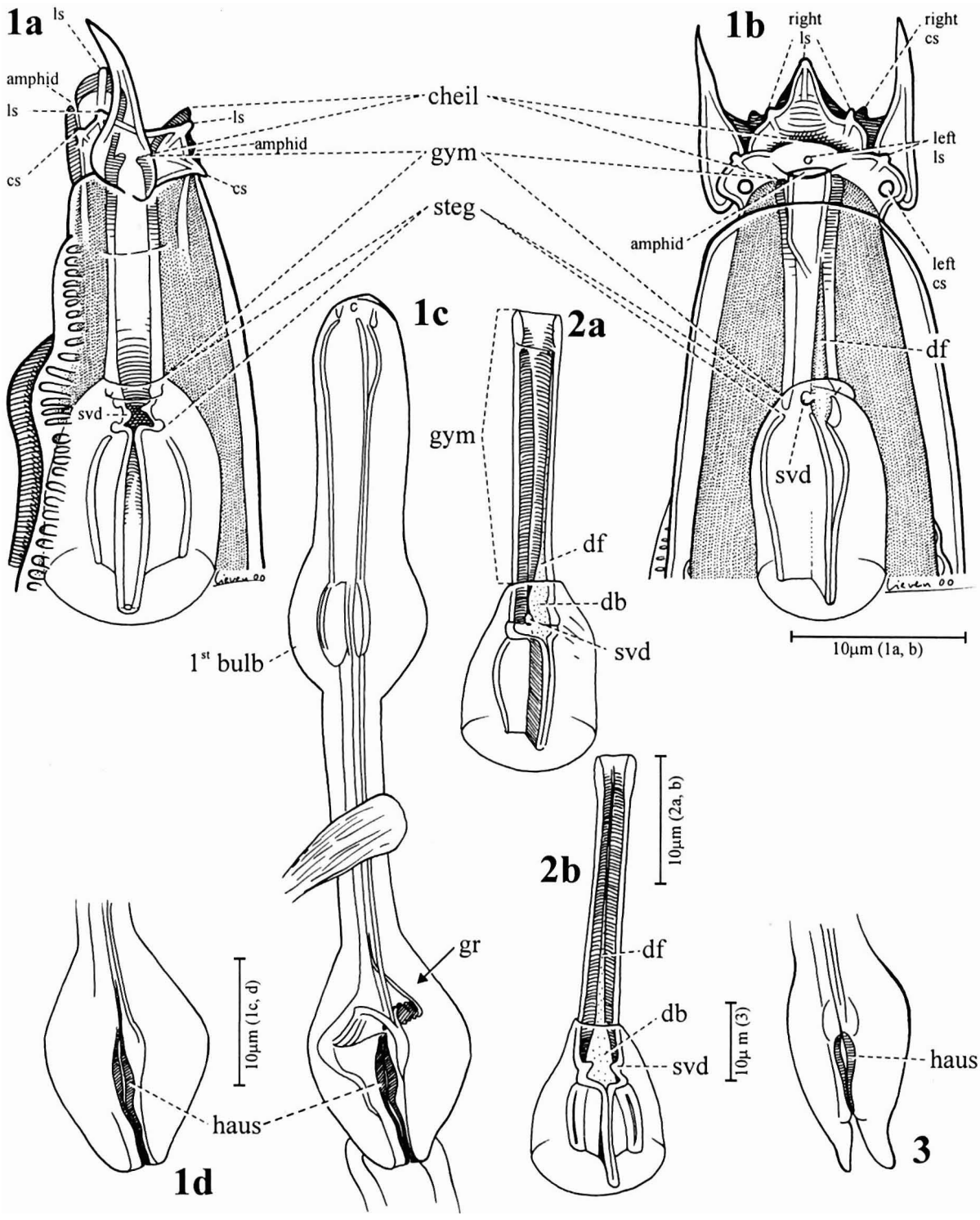

Fig. 1. *Bunonema pini*, female. 1a: Anterior end, ventral view. 1b: Anterior end, left lateral view. 1c: Pharynx, left lateral view. 1d: Terminal pharyngeal bulb, drawn without grinder. **Fig. 2.** *Bunonema penardi*, hermaphrodite, buccal cavity. 2a: Left lateral view. 2b: Ventral view. **Fig. 3.** *Pseudodiplogasteroides* sp. 1, female, terminal pharyngeal bulb.

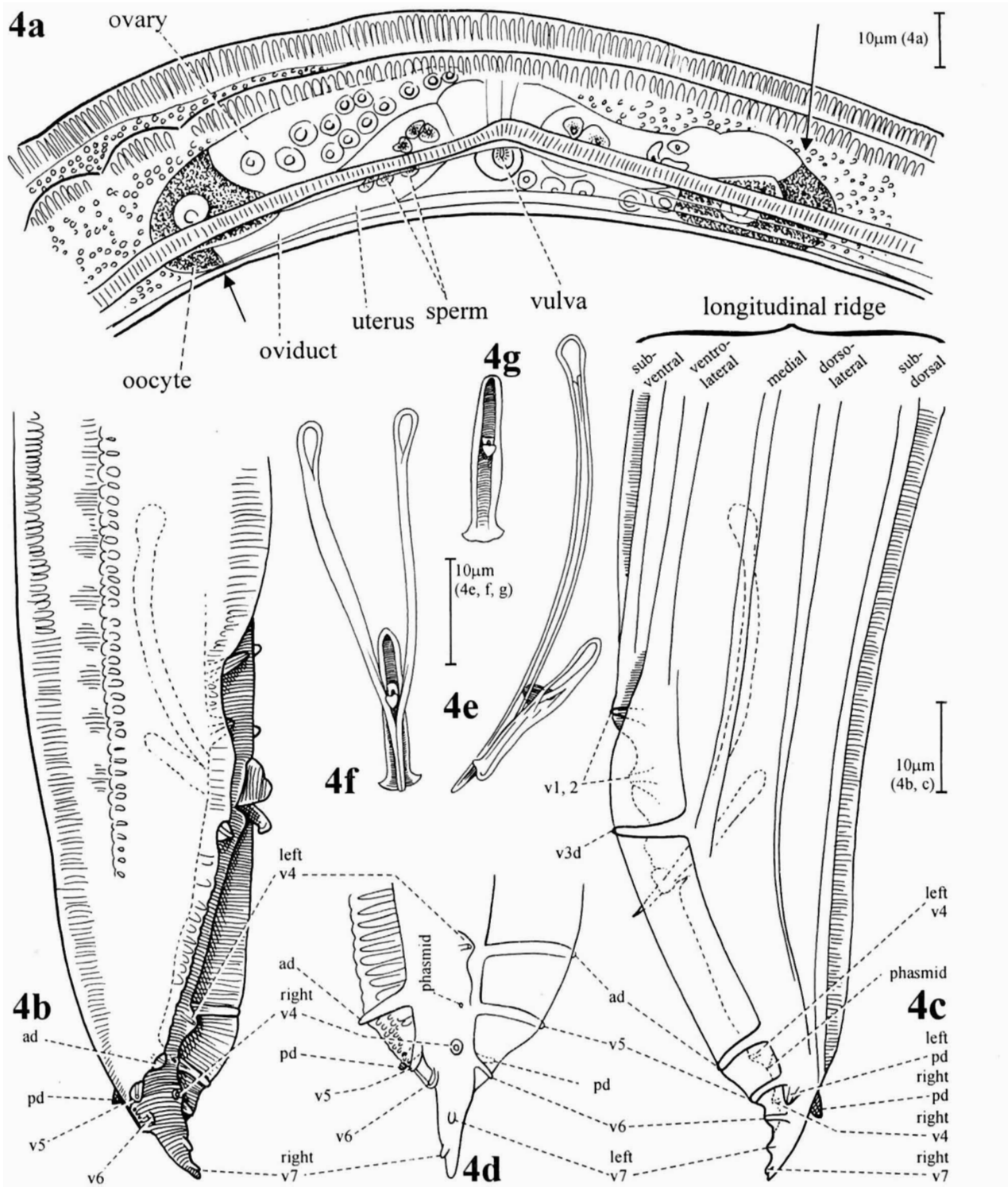

Fig. 4. *Bunonema pini.* 4a: Female, genital tracts, left subventral view. 4b-d: Male tail. 4b: Right lateral view. 4c: Left lateral view. 4d: ventral view. 4e, f: Spicula and gubernaculum. 4e: left lateral view. 4f: ventral view. 4g: Gubernaculum, ventral view.

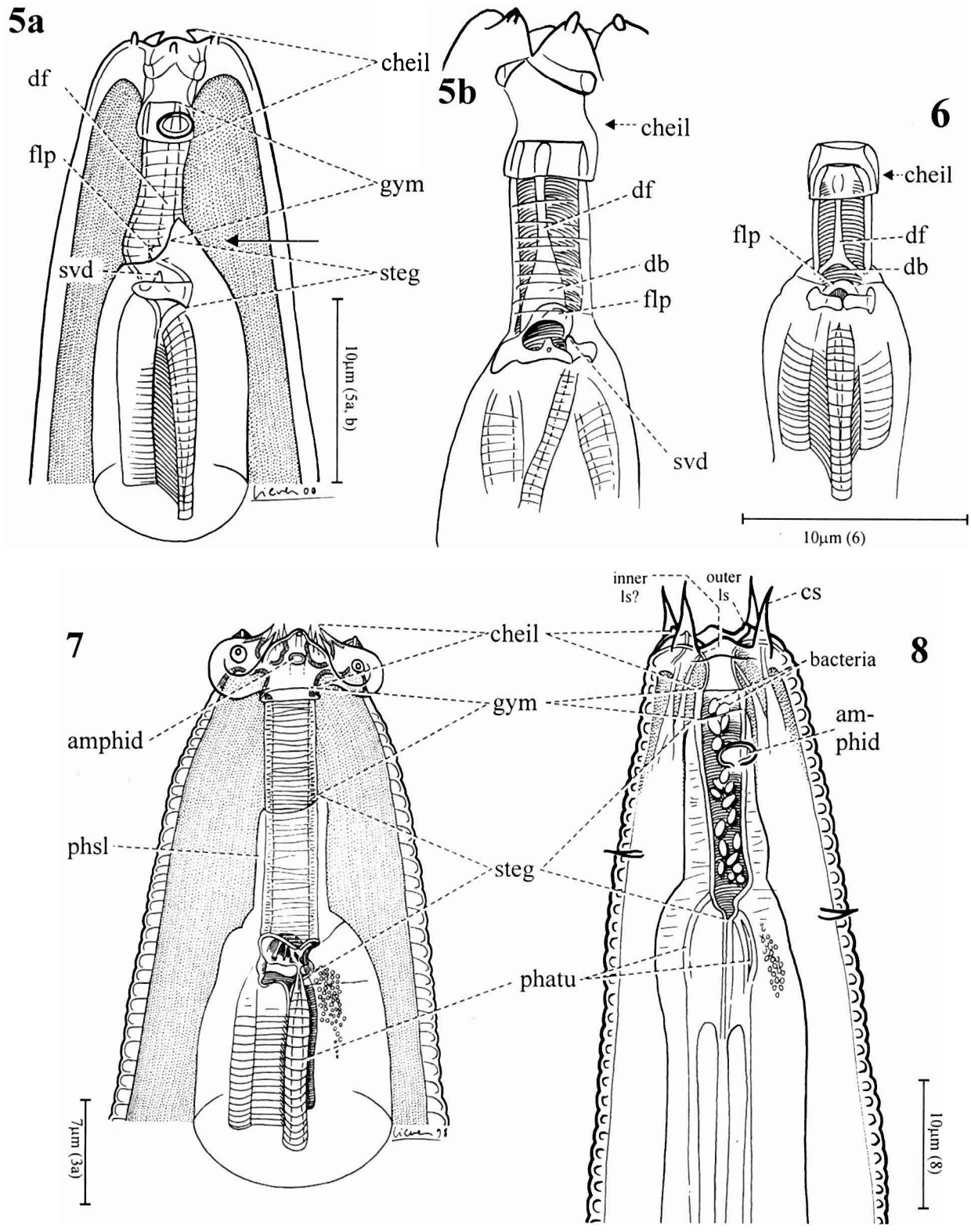

Fig. 5. *Pseudodiplogasteroides* sp. 2, female. 5a: Anterior end, left lateral view. 5b: Buccal cavity, ventral view. **Fig. 6.** *Pseudodiplogasteroides* sp. 1, female, buccal cavity, ventral view. **Fig. 7.** *Rhabditis teres*, female, anterior end, left lateral view. **Fig. 8.** *Ceratoplectus armatus*, female, anterior end, left lateral view.

A further resemblance of bunonematids and diplogastrids concerns the ontogeny:

After Sachs (1949) in *Bunonema* the J2-cuticle of the dauer larva (J3 that is ensheathed by the cuticle of J2) possesses an oily and sticky substance that helps the nematode attach to insect to be transported to a new habitat. In diplogastrids the oily substance covers the J3 after hatching from the J2 cuticle. Despite the discrepancy in the origin of the cuticle that is covered by the oily substance (J2 in *Bunonema*, J3 in Diplogastrina), the occurrence of an "oil sheath larva" in both taxa is remarkable.

DISCUSSION

Points 1-5 can be interpreted as synapomorphies of *Bunonema* and diplogastrids substantiating a sister group relationship of both taxa.

1. The lack of a pharyngeal sleeve must be regarded as an apomorphy compared to its presence. When a pharyngeal sleeve is present, the gymnostom is shorter compared to the stegostom. A similar length ratio of gymno- and stegostom is found in secernentean outgroup representatives such as Panagrolaimidae, Cephalobidae, and furthermore within the "Adenophorea" in Plectidae, possibly the closest relatives of the Secernentea (Malakhov 1994; Blaxter et *al.* 1998). Therefore, regarding the Secernentea, a short gymnostom combined with a longer stegostom as occurs in species possessing a pharyngeal sleeve must be regarded as the plesiomorpic condition compared to a longer gymnostom that occurs with the lack of the pharyngeal sleeve. (The buccal cavity of diplogastrids is anisotopic. Therefore, as indicated by the arrow in Fig. 5a, the length of the gymnostom must be given as the mean of the lengths measured ventrally and dorsally.) The lack of the pharyngeal sleeve in *Bunonema* and Diplogastrina is an apomorphic condition which allows to assume a sister group relationship of both taxa. (For a discussion of the polarity between the alternatives *presence of sleeve/lack of sleeve* within "*Rhabditis*" see Sudhaus & Kiontke 1996.)

2. The dorsal fold of the gymnostom is hardly visible with light microscopy. Therefore it is not clear if it exists outside the taxa examined here. Regarding this uncertainty this character is suggested as a possible synapomorphy of diplogastrids and bunonematids.

3/4. The combination of a dorsal bulge and subventral denticles in the stegostom is unique among the Secernentea.

5. Fürst von Lieven & Sudhaus (2000) could not designate whether the second or the third precloacal genital papilla originated laterally in the stem species of Diplogastrina. After further investigations it became clear that in the majority of the early offbranching diplogastrids the lateral precloacal papilla is in the third position as in *Bunonema*. In "Rhabditidae" all three precloacal genital papillae originate subventrally or open ventrally on the bursal velum. In a few exceptions (*Caenorhabditis*: Sudhaus & Kiontke 1996; *coarctata*-group and *bütschlii*-group: Sudhaus & Fitch 2001) the first genital papilla opens dorsally. Ventral precloacal genital papillae belong to the stem species pattern of the paraphyletic "Rhabditidae". Therefore the lateral position of the third precloacal genital papilla in *Bunonema* and Diplogastrina is an apomorphic condition.

The polarity of points 6, 7 and 8 is unclear

6. A round-shaped first bulb occurs also in many rhabditid taxa. Regarding *Bunonema* and diplogastrids, the polarity of this character can not be designated as long as the rhabditid sister taxon of their stem species remains unknown.

7. The elongated shape of the cuticularized elements of the haustrulum in *Bunonema* and diplogastrids resembles the posterior cuticularizations of the haustrulum in the Thelastomatidae (as depicted by Leibersperger 1960) and might be a plesiomorphic feature. In Thelastomatidae the cuticularizations of the pharyngeal cuticular lining behind the grinder comprise two subsequent sets of structures. A first set of cuticularizations is represented by adradial triangular plates and a second set by interradial rods. The triangular plates presumably have been lost in the ancestral line of *Bunonema* and diplogastrids.

8. Compared to the homodromously reflexed gonads of many "rhabditids", where the reflexion lies within the growth zone of the ovary, the antidromously reflexed gonad of outgroup representatives is the plesiomorphic condition (Lorenzen 1994; Malakhov 1994). But the taxon formed by *Bunonema* and diplogastrids most likely belongs to the paraphyletic "Rhabditidae". Therefore the stem species of *Bunonema* and diplogastrids probably descends from a species with homodromously reflexed gonads. In this case the antidromous gonads in *Bunonema* and diplogastrids would be apomorphic as they derived secondarily from homodromous gonads. The character polarity of antidromous gonads in *Bunonema* and diplogastrids can not be designated as long as the rhabditid sister taxon of the monophylum formed by both taxa remains unknown.

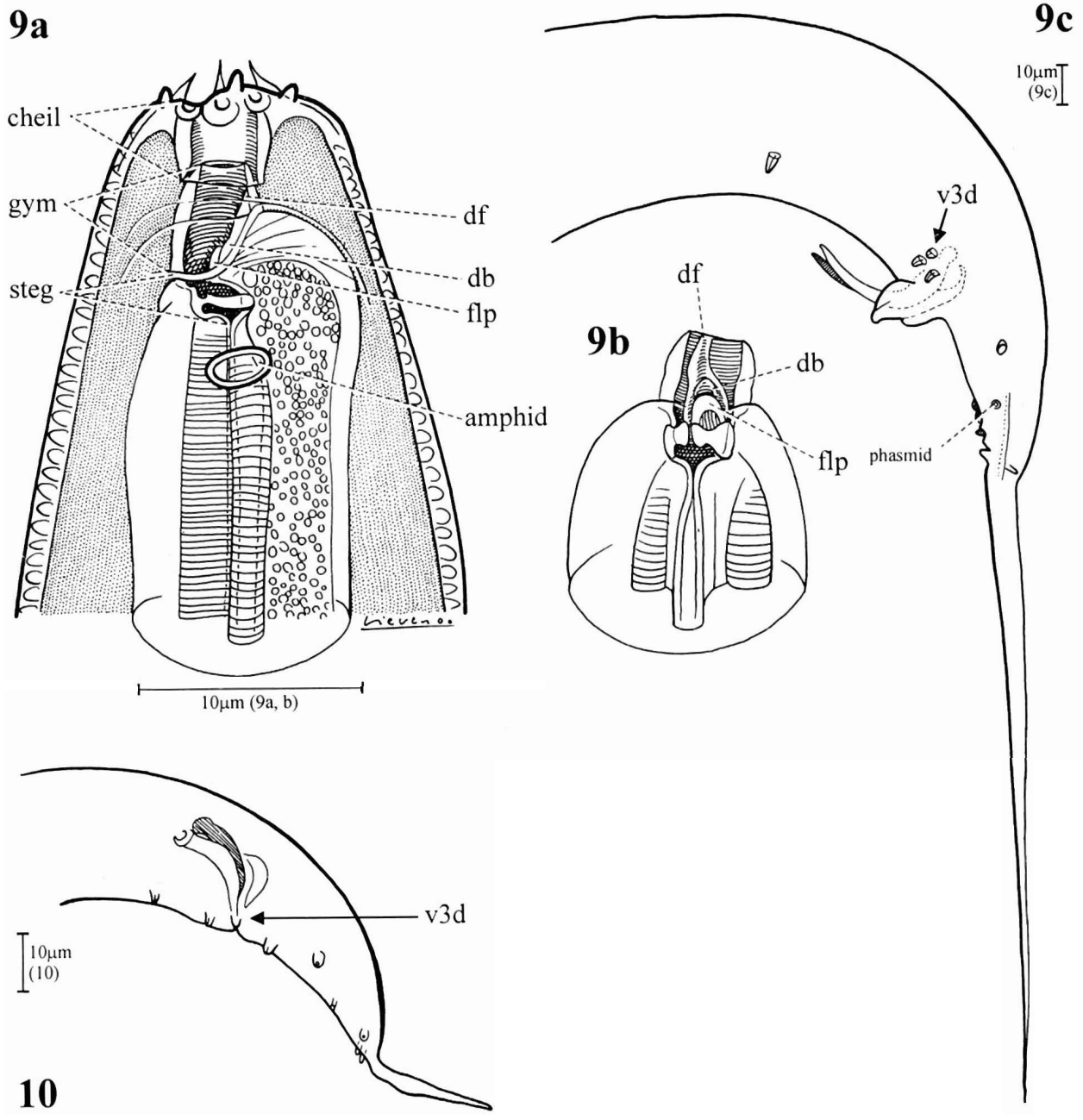

Fig. 9. *Demaniella cibourgensis.* 9a: Female, anterior end, left lateral view. 9b: Female, buccal cavity, ventral view. 9c: Male tail, left lateral view. **Fig. 10.** *Pseudodiplogasteroides* sp. 2, male tail, left lateral view.

Homologization of the genital papillae pattern of diplogastrids and bunonematids (compare Fig. 12 with Fig. 13)

In "Rhabditidae" all three precloacal genital papillae lie subventrally, whereas in the postcloacal group two originate laterally or open dorsally on the bursal velum, one anteriorly (ad) and one posteriorly (pd). Following a nomenclature developed by D. Fitch, K. Kiontke and W. Sudhaus, ad and pd are distinguished from the other papillae by labelling all subventral papillae, precloacal and postcloacal, as "v1, v2,...v7" (numbered from anterior to posterior). In *Bunonema* and diplogastrids the third precloacal papilla (v3) that originates laterally shall be named v3d.

In the stem species pattern of diplogastrids the pattern of genital papillae is characterized as follows (position of cloaca is given by a slash, that of the phasmid by "ph"):

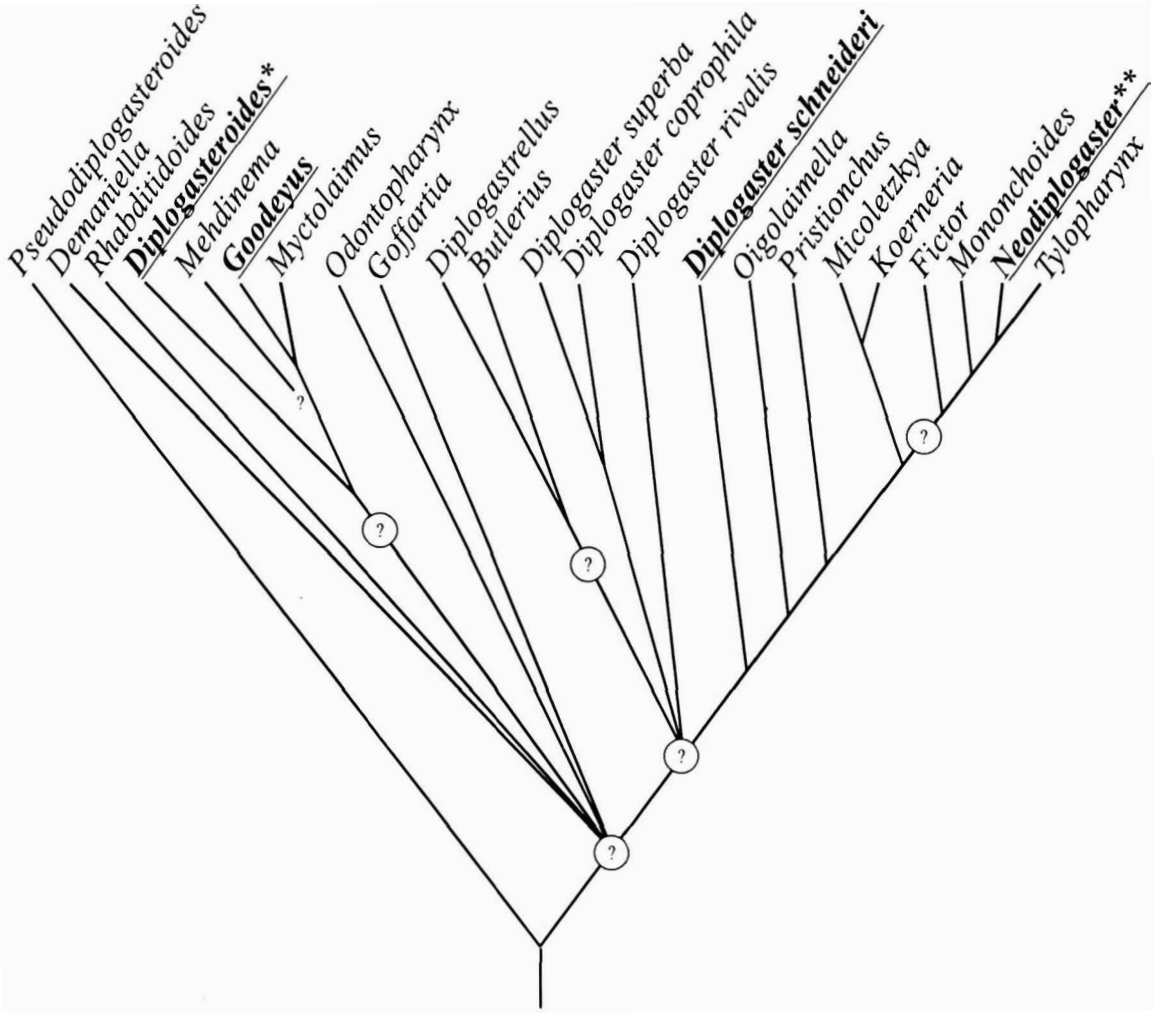

Fig. 11. Phylogenetic relationships within the Diplogastrina, modified after Fürst von Lieven (2001). Taxa in which a bursa occurs are underlined. * *Diplogasteroides leuckarti* is the only *Diplogasteroides* species possessing a true bursa. ** Five out of ten *Neodiplogaster* species possess a noticeable bursa.

v1, v2, v3d / v4, ad, ph, (v5, v6, v7), pd

The parenthesis indicate the conspicous group formed by v5-v7. It is possible to find the homologous papillae of the left side in the asymmetrically altered pattern of *Bunonema pini* by comparison with the bursa bearing diplogastrid *Goodeyus ulmi*:

B. pini : v1, v2, v3d/ ad, v4, ph, v5, pd, v6, v7
G. ulmi: v1, v2, v3d/ v4, ad, ph, (v5, v6, v7), pd

The underlined papillae lie within the bursal velum. The formation of the conspicuous group of v5-v7 that led to the posterior position of pd in diplogastrids clearly is an apomorphy of their stem species whereas the posterior position of v4 in *Bunonema* could be derived from a position more close to the cloaca as in diplogastrids.

Did the stem species of Diplogastrina possess a bursa?

Fürst von Lieven & Sudhaus (2000) regarded the lack of a bursa in diplogastrids as an apomorphic condition. Compared to an alternative scenario this remains the best hypothesis, even with regard to the great resemblance of the association between bursa and papillae in *B. pini* and *Goodeyus ulmi* (with exception of v7).

A bursa in male nematodes consists of a cuticular bursal velum that is supported by at least some of the genital papillae that are then called bursal papillae (Kiontke & Sudhaus, 2000). The phasmids may also become bursal papillae. A criterium to estimate the homology of bursae in different species is the pattern of individual papillae that are incorporated within the velum compared

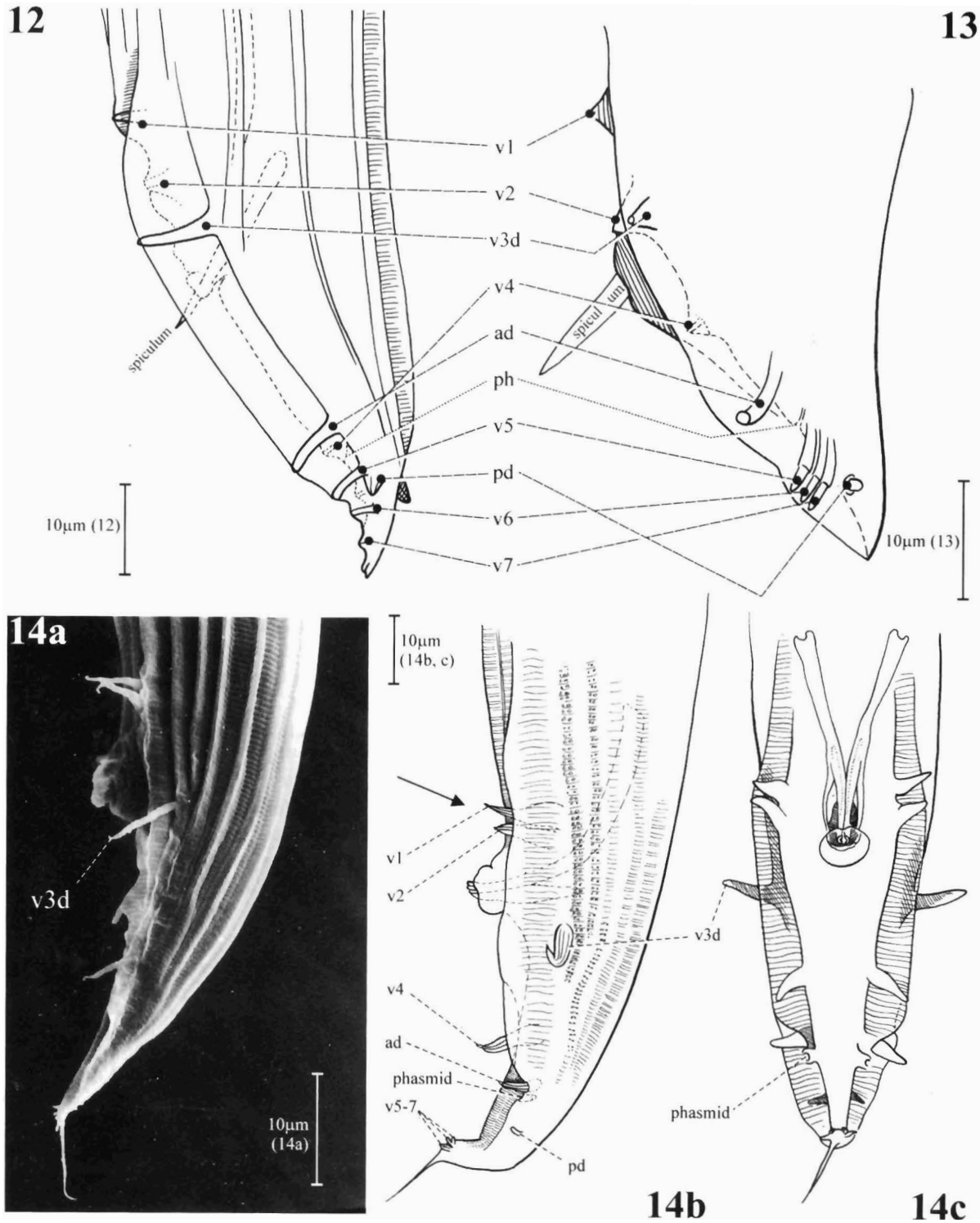

Fig. 12. *Bunonema pini*, male tail, left lateral view. **Fig. 13.** *Goodeyus ulmi*, male tail, left lateral view. **Fig. 14.** *Diplogaster schneideri*, male tail. 14a: SEM image, left lateral view. 14b, c: Drawings after light microscopy. 14b: Left lateral view. 14c: Ventral view.

to those that stand free.

In *B. pini* a velum as an extension of the ventrolateral cuticular ridge is only present on the left hand side. With *B. pterygiosoma* and *B. pusillus* Fuchs (1930) described two bunonematids possessing a symmetrical bursa. It follows that the asymmetry of the bursa originated within the Bunonematidae. Within the bunonematids with asymmetrical bursa, *B. pini* belongs to a group (subgenus *Rhodolaimus*) that represents the most "primitive" condition with similar papillae patterns on both sides (Sachs 1949).

Within the Diplogastrina a true bursa is present in *Diplogaster schneideri, Diplogasteroides leuckarti, Goodeyus ulmi,* and in certain species of *Neodiplogaster* (underlined taxa in Fig. 11). In *D. schneideri* (Figs. 14a-c) the bursal velum is formed by the extended ridges of the body cuticle that cause the striation in many diplogastrids. The genital papillae originate between the ridges. In *Goodeyus* the velum is supported by v3d, ad, and v5-v7. Judging from the figures of Rühm (1956), the papillae v5-v7 are excluded from the formation of the bursa in *D. leuckarti* (the only *Diplogasteroides* possessing a true bursa) and *Neodiplogaster pissodis.* (*N. pissodis* belongs to five out of ten species within *Neodiplogaster* that possess a noticeable bursa.) These differences lead to the *a priori* assumption that the bursae of diplogastrids evolved independently several times within the taxon while the stem species did not possess such a structure.

Alternatively it seems reasonable to homologize the bursa of *Bunonema* with that of *Goodeyus ulmi.* On the basis of the cladogram of Fürst von Lieven & Sudhaus (2000) and Fürst von Lieven (2001) that was reconstructed using mainly stomatal characters, the consequence of the assumtion that the diplogastrid stem species possessed a bursa is that it must have been lost in at least four lines (*Pseudodiplogasteroides*; *Mehdinema*; *Myctolaimus*; stem species of all other *Diplogasteroides* excluding *D. leuckarti*). The number of losses increases if one regards the bursa within *Neodiplogaster* as homologous with the bursa of other diplogastrids. The bursa of *D. schneideri* is clearly an evolutionary novelty of this species. If *D. schneideri* descends from a bursa bearing stem species, this bursa must have been lost and replaced again in the line leading to *D. schneideri.*

After this *a posteriori* discussion the homology hypothesis for the bursae in *Bunonema* and diplogastrids must be rejected as it requires too many additional assumptions. The stem species of the Diplogastrina most likely lacked a bursa. The discussion of a possible homology of the bursa in *Bunonema* and "*Rhabditis*" is beyond the scope of this paper.

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Резюме. В работе продемонстрированы доказательства родственных взаимоотношений между диплогастеридами и бунонематидами. Обнаружены 5 аутапоморфий, подтверждающих эту гипотезу. Выявлены гомологии в расположении генитальных папилл у *Bunonema* и у диплогатерид. Обсуждается вопрос о наличии бурсы у нематод предковой для диплогастерид группы.