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Morphology of the synlophe and genital cone of *Parostertagia heterospiculum* (Trichostrongylidae) with comments on the subfamilial placement of the genus

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Abstract

The genus *Parostertagia* is referred to the Cooperiinae with reference to a suite of shared characters of the synlophe and genital cone postulated as derived homologies for the subfamily. The genus has traditionally been relegated to the Graphidiinae or Ostertagiinae, but unique structural attributes of the synlophe and genital cone indicate close affinities with the Cooperiinae. The synlophe of *Parostertagia heterospiculum* is characterised by a relatively low number of ridges in the cervical zone (16–20 at the excretory pore), a frontal axis of orientation, sequential increase in ridges posteriorly, lateral addition of ridges, minuscule lateral-most ridges and hypertrophy of specific ridges in the lateral field of females. The genital cone with a tripartite “0” papilla, a pair of dorsal cloacal papillae (a character potentially homologous with lateral protuberances in some cooperiines, but reported for the first time in this study), and paired “7” papillae supporting a small accessory bursal membrane appear unique among the trichostrongylids. Additionally, *Parostertagia* was found to possess minuscule cervical papillae and a rudimentary (or reduced?) cephalic and cervical expansion. Inclusion of *Parostertagia* within the Cooperiinae is based on shared characters with this subfamily (postulated synapomorphies) rather than differences that may be utilized to separate this genus from members of other subfamilies of the Trichostrongylidae.

Introduction

Parostertagia heterospiculum Schwartz & Alicata, 1933 was described for trichostrongylid nematodes (Trichostrongylinae Leiper, 1912) from the intestines of collared peccaries *Tayassu tajacu angulatus* (Cope) in southern Texas (Schwartz & Alicata, 1933). Beyond the original description there have been few reports of this trichostrongylid (Samuel & Low, 1970).

The classification of *P. heterospiculum* is unresolved. Following the original description Travassos (1937) reduced the genus *Parostertagia* to

a synonym of *Hyostrogylus* Hall, 1921, while retaining the latter within the Trichostrongylinae. Skrjabin *et al.* (1952, 1954) considered *Parostertagia* to be valid (based on the absence of a telamon and cervical papillae) and referred it to the Tribe Ostertagiae (Trichostrongylinae), while placing *Hyostrogylus* in the Tribe Hyostrogylea (Cooperiinae Skrjabin & Shikhobalova, 1952). However, Chabaud (1959) supported the synonymy of these genera along with classification among the trichostrongylines. Later Durette-Desset & Chabaud (1977, 1981), and Durette-Desset (1983, 1985) rejected all previous conclusions and in-

cluded *Parostertagia* and *Hyostromgylus* among the Graphidiinae Travassos, 1937. In contrast, Gibbons & Khalil (1982a) considered *Parostertagia* and *Hyostromgylus* to share affinities with the Ostertagiinae Lopez Neyra, 1947. However, they suggested that the paucity of knowledge for details of the morphology of the genital cone, cervical papillae and cephalic capsule precluded accurate determination of the taxonomic position of *Parostertagia*.

Elucidation of the systematic position of *Parostertagia* is critical with respect to the evaluation of hypotheses for evolutionary affinities of the Graphidiinae and Ostertagiinae (see Durette-Desset & Chabaud, 1977, 1981; Durette-Desset, 1985; Jansen, 1989). Additionally, development of a broader understanding of the phylogenetic relationships of the Trichostrongylidae, the most economically significant nematode parasites of ruminants, may result from morphological studies of *Parostertagia* and associated genera. Detailed morphological evaluations are necessary to identify new characters and to recognise postulated synapomorphies (for the family, subfamily and generic levels) in support of phylogenetic analyses and the development of a natural classification for the trichostrongylids.

In the study reported herein, *Parostertagia heterospiculum* is redescribed based on the type-series and voucher specimens from the type-host. Novel structural attributes of the synlophe, genital cone and bursa are reported.

Materials and methods

Specimens were studied as temporary whole-mounts cleared in phenol-alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol), and examined with differential interference contrast light microscopy. Transverse sections were prepared free-hand with a cataract knife and embedded in glycerine jelly. Sections were used to study the structure of the synlophe in the cervical zone (including the region of the oesophago-intestinal junction), anterior half, midbody and posterior region of a male (sections prepared to the

level proximal to the spicules) and four females. Photographs of sections are oriented with dorsal surface toward the top of the plate and anatomical left and right corresponding to the left and right of the figure. Three female specimens were also examined with scanning electron microscopy (SEM), according to procedures in Lichtenfels *et al.* (1988). Throughout the manuscript, measurements are presented in micrometres unless specified otherwise.

The current study focused on the configuration of the synlophe and genital cone; other mensural and structural characters are included where they are considered to differ from the original description or where they have been incompletely described. The numbering of the genital papillae and bursal rays follows the methodology developed by Chabaud *et al.* (1970), and the orientation of the synlophe follows concepts presented by Durette-Desset (1985). The term "cuticular strut" follows Lee (1965). Terminology for the cephalic papillae follows Chitwood (1950); arrangement of papillae is described according to de Coninck (1942, 1965). For measurements of the infundibula and sphincters of the ovejectors the edge of the muscular portion of the sphincter was used as a dividing line between them, and the fluffy coat surrounding the sphincter and the portion of the infundibulum overlapped by the muscles of the sphincter were ignored. Because the separation of the vestibule from the sphincter was not possible to discern reliably, the vestibule was included as a component of the sphincter. The measurement for length of the anterior and posterior sphincter thus constitutes the distance from the distal end of the sphincter (muscular bulb) to the vulva. Structure of the spicules was determined from whole mounts and dissected specimens.

Specimens examined: All material is accessioned in the US National Parasite Collection (USDA, Beltsville, Maryland): (1) Holotype male and allotype female, USNM Helm Coll. No. 30165, and paratypes, USNM 30166, including 6 males and 9 females from *Tayassu tajacu angulatus* (Cope) collected by J.H. Cooper near Raymondsville, Texas (see Schwartz & Alicata, 1933); (2) voucher

Table I. Morphometrics of males and females of *Parostertagia heterospiculum*.

Character	Current study			Original description ¹		
	n ²	Range ³	Mean	n ²	Range ³	Mean
<i>Males</i> (number examined = 15)						
Body length	10	4,581–5,335	5,042	5	4,400–5,500	4,720
Oesophagus length	9	416–520	467	5	452–490	472
Oesophago-intestinal valve	–	–	–	–	–	–
Nerve-ring ⁴	–	–	–	5	160–169	166
Excretory pore ⁴	10	237–291	273	5	205–235	220
Cervical papillae ⁴	17	273–349	317	–	not observed	–
Left spicule length ⁵	14	112–126	117	5	117–125	122
Right spicule length	14	99–122	112	5	112–121	115
Bursal ray pattern	10	2-3	–	–	not specified	–
Genital cone dorsal enlargement	10	absent	–	–	not specified	–
Gubernaculum length	10	55–68	62	5	62–72	68
Dorsal ray length	11	104–125	115	–	–	–
<i>Females</i> (number examined = 20)						
Body length	20	4,779–6,427	5,861	5	4,600–5,800	4,260
Oesophagus length	15	481–651	530	5	468–510	489
Oesophago-intestinal valve length	11	46–59	53	–	–	–
Nerve-ring ⁴	10	163–234	209	5	144–180	157
Excretory pore ⁴	11	195–302	276	5	195–240	202
Cervical papillae ⁴	18	280–355	327	–	not observed	–
Tail length	19	92–130	110	5	90–115	104
Vulva position ⁶	20	72–79%	77%	5	77–81%	79%
Posterior sphincter length	20	184–313	224	–	–	–
Anterior sphincter length	20	211–314	243	–	–	–
Posterior infundibulum length	17	92–125	109	–	–	–
Anterior infundibulum length	15	98–132	115	–	–	–
Ovejector length	15	631–844	699	5	421–483 ⁷	439

¹ Schwartz & Alicata (1933).

² Number measured.

³ Measurements in micrometres.

⁴ Measured from anterior extremity.

⁵ Left spicule is always greater in length.

⁶ Expressed as percentage of body length from anterior extremity.

⁷ Excluding the infundibula.

specimens including 10 males and 11 females, USNM Helm. Coll. No. 66260, collected by W.M. Samuel and W.A. Low on the King Ranch, Kleberg County, Texas (see Samuel & Low, 1970).

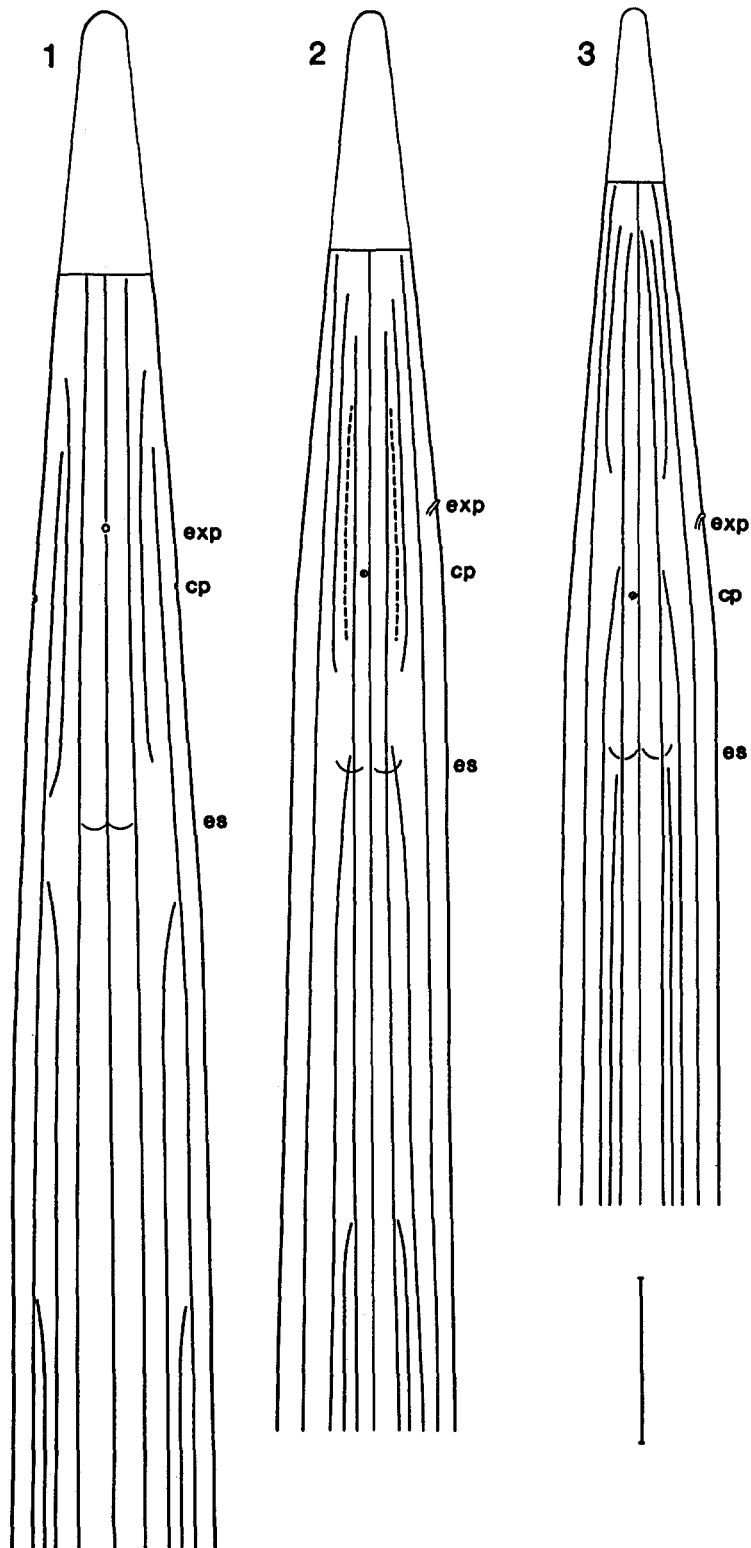
Results

Examination of material available to us revealed few inconsistencies in mensural or structural characters with respect to the original description (Table I; see Schwartz & Alicata, 1933). Structural characters that were incompletely defined in the original or subsequent taxonomic works (Durette-Desset, 1978; Gibbons & Khalil, 1982)

or those not previously considered, (including the synlophe, genital cone, spicules, copulatory bursa ovejectors and cephalic vesicle) are described below.

Description

Synlophe. The synlophe in males and females is composed of a bilaterally and dorsoventrally symmetrical system of prominent ridges extending from the base of a poorly defined cephalic expansion to a position posterior to the prebursal papillae and anus, respectively (Figs 1–3, 7, 9, 10). Individual ridges typically have a “beaded” appearance, attributable to the structure of under-



Figs 1–3. Diagrammatic views of synlophe in cervical region of *Parostertagia heterospiculum*. 1. Synlophe in cervical zone of female, showing typical pattern in ventral field. 2. Synlophe in cervical zone of male, showing typical pattern in right lateral field; dashed lines indicate position of occasional additional pair of accessory ridges. 3. Synlophe in cervical zone of male showing lateral field and pattern with antieriad shift. *Abbreviations:* exp, excretory pore; cp, cervical papillae; es, base of oesophagus. *Scale-bar:* 100 μ m.

lying struts. The primary pattern of the synlophe is composed of 3 uninterrupted, parallel ridges in each dorsal and ventral field (the ventral-most is discontinuous at the level of the excretory pore), 3 uninterrupted, parallel ridges in each lateral field (a fine lateral-most ridge is bordered by 2 prominent lateral ridges), and sequential symmetrical addition, beginning in the cervical zone, of accessory ridges that are convergent anteriorly at their origins on each lateral field (Figs 1–3,7). Among the primary continuous ridges, 3 dorsal and 3 ventral and occasionally the 2 lateral-most attain the base of the cephalic expansion.

Variation in the synlophe resides in the pattern and distribution of accessory ridges. The typical pattern in the cervical zone includes a pair (rarely 2 pairs) of discontinuous ridges in each lateral field that terminate anterior to the base of the oesophagus (Figs 1–2,7). In the postcervical region, an additional 2 pairs of ridges originate in each of the lateral fields and are uninterrupted posteriorly. Posterior to the midbody, a minimum of 3 pairs of continuous ridges have origins in each of the lateral fields; occasional discontinuous or isolated ridges also occur laterally, particularly in the region of the vulva in females. Thus in typical male and female specimens, there are 16 (seldom 20) ridges at the level of the excretory pore; 16 at the level of the oesophageal valve; 12–14 at the oesophago-intestinal junction (Fig. 11) (dependent on variation in the termination of the discontinuous cervical ridges); 16 posterior to the oesophagus; and 20 through the region of the midbody (Figs 12–13). An anterior shift of accessory ridges in the cervical and postcervical regions may slightly alter the typical pattern in some specimens (Fig. 3). Posterior to the midbody in males there were 24 ridges in the posterior 3/4, 27–28 ridges in the anterior last 1/4 (Fig. 14), and 30–31 (32) in the region adjacent to the proximal level of the spicules; there is a marked reduction in height of the ridges in the lateral fields (Fig. 14). Among females 24 ridges occurred anterior to the vulva. In the region of the vulva, the synlophe becomes irregular and discontinuous, particularly in the ventral field (Figs 15–17). There were 16–24 ridges at the level of the vulva (Fig. 16); posterior

the ridges increase in number to attain a maximum of 28 at the level of the anus (Figs 9–10).

A highly consistent pattern is evident in the origins of accessory ridges, all being convergent on the 3-parallel ridge systems of the left and right lateral fields (Figs 1–3,7,9). The spacing of the lateral ridges is relatively narrow (5–6 μm) (Fig. 7) in contrast to the interval between ridges in the dorsal and ventral fields (about 10 μm). Spacing of the lateral ridges increases posterior to the midbody.

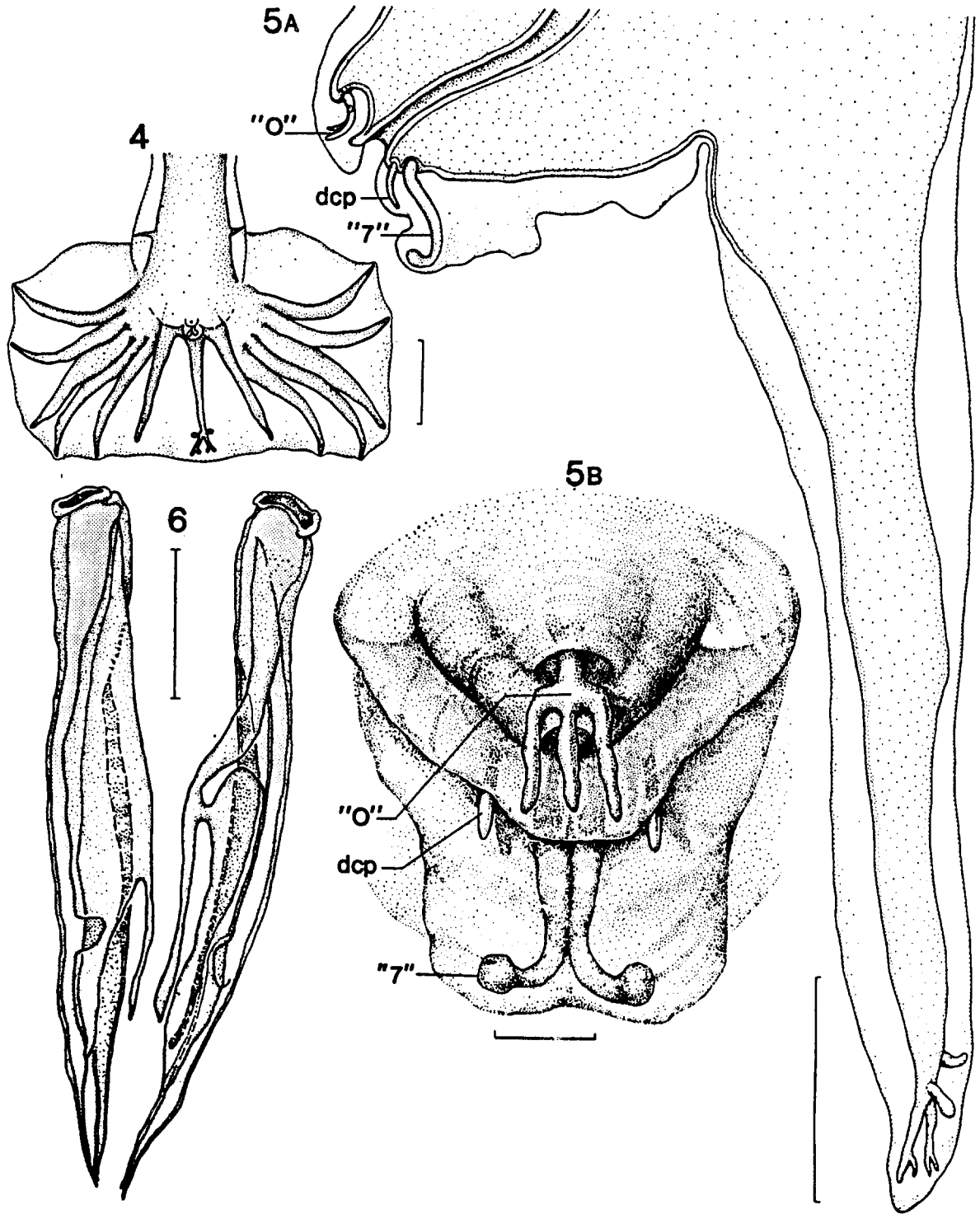
As determined from transverse sections, the synlophe is not perpendicular, rather it exhibits a bilateral and dorsoventral symmetry and an axis of orientation with ridges directed dorsally and ventrally from the lateral fields (Figs 11–13, 16–17). Cuticular struts project into and support each ridge (Figs 11–17). Ridges generally decrease in height posteriorly and the lateral-most are always minuscule with respect to those occurring dorsally and ventrally. Additionally all ridges in the lateral fields become reduced in height in the far posterior in both males and females and the orientation is no longer discernible.

A constituent of the continuous lateral synlophe in females is the bilateral vulval fan (Figs 15–17, 21–22), representing single hypertrophied ridges with massive cuticular struts that curve toward the dorsal field (Figs 15–17). Hypertrophy of some irregular and discontinuous ridges in the left subventral field was also observed at the level of the vulva (Fig. 16).

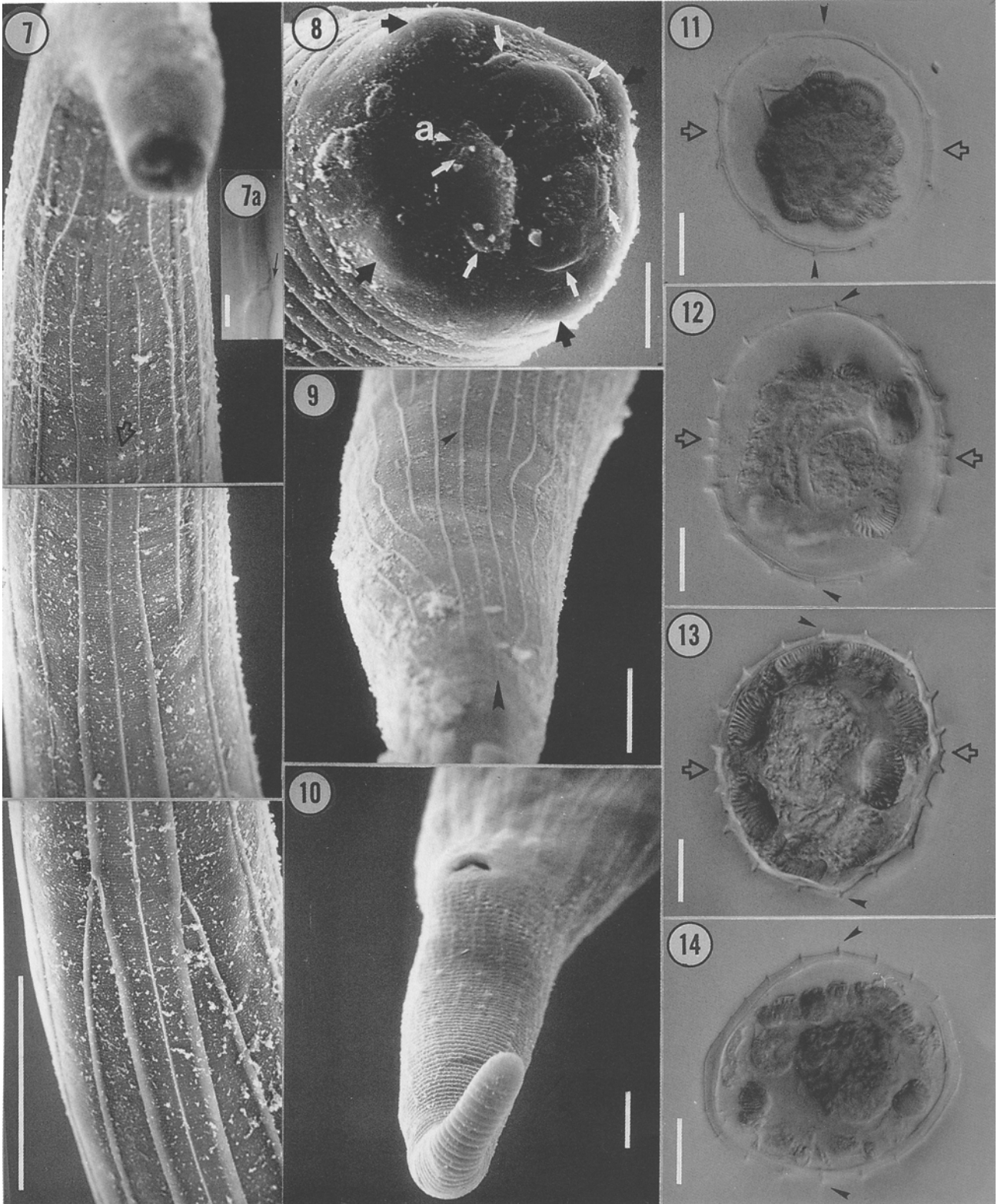
Cephalic vesicle. A reduced cephalic vesicle is evident, extending anteriorly approximately 100–160 μm from the region of termination for the dorsal and ventral synlophe (Fig. 18). Transverse striations of the cuticle are more widely spaced in this region than on the remainder of the nematode. A slight expansion of this zone is also apparent (Fig. 18).

The cephalic extremity has a slight inflation and is characterised by 3 prominent lips, 6 labial papillae of the inner circle and 4 double papillae of the outer circle (Fig. 8).

Cervical papillae. Cervical papillae are located



Figs 4-6. Genital cone and spicules of *Parostertagia heterospiculum*. 4. Bursa in ventral view: note 2-3 pattern with all laterals being divergent from all ventral rays [modified from original figure in Schwartz & Alicata (1933)]. 5a. Genital cone in lateral view showing dorsal ("7") and ventral ("0") raylets and the dorsal cloacal papillae (dcp). 5b. Genital cone in ventral view showing "7" papillae and accessory bursal membrane, "0" papillae with trident structure, and the dorsal cloacal papillae. 6. Spicules in ventral view, showing 2 inner processes on left spicule (arrow indicates position of smaller dorsal process) and single process on right spicule. Scale-bars: 4, 100 μ m; 5a, 6, 25 μ m; 5b, 10 μ m.



slightly posterior to the excretory pore; the left papilla is often anterior to that on the right (Figs 1–3,7). The papillae are minuscule, 1.5–2.0 μm in length, with each appearing as a single sensillum projecting from a pit in the lateral cuticle situated in a position dorsal (right) and ventral (left) to the lateral-most ridges (Fig. 7).

Ovejectors. The ovejectors are relatively long (Table I; Fig. 20), and the anterior sphincter (including the anterior portion of the vestibule) and infundibula are typically longer than the posterior. However, due to variation in these contractile organs the differences were not significant.

Cuticular fan at vulva. A bilateral fan-shaped region, approximately ($n = 15$) 234–351 μm (299) in length, extended posterior and anterior to the vulva in all 20 specimens examined (Figs 21–22). The fan appeared as a tapering inflation of the cuticle consistently arising off the lateral field and attained a maximum width near the level of the vulva. The origin of the inflation in all cases was continuous with a single hypertrophied ridge, in the right and left lateral fields, bordering the lateral-most ridge dorsally. The vulva, consistently in the left subventral field (among 15 specimens) (Fig. 21), was formed by a longitudinal slit along a short, discontinuous accessory ridge arising adjacent to the system of parallel lateral ridges.

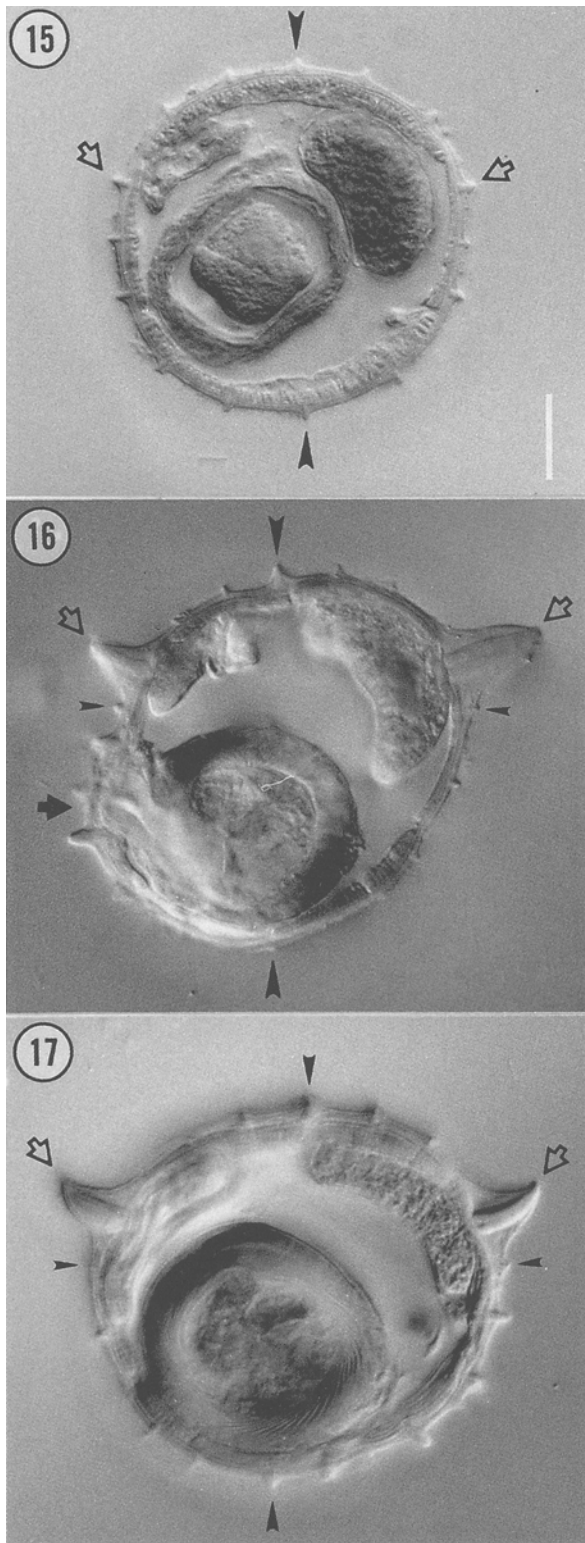
Copulatory bursa. The pattern of the bursa is 2-3; rays 2 and 3 (ventrals) are divergent from rays 4, 5 and 6 (laterals) rays); rays 2 and 3 are not highly convergent and are nearly equal in dimen-

sions (Fig. 4). Ray 9/10 (dorsal) is contained in the membranous dorsal lobe and is supported by a granular, thickened region of the cuticle extending the length of the ray (Fig. 19). The base of ray 8 (externodorsal) is markedly angular in appearance (Fig. 19). The synlophe extends to the base of the bursa; the prebursal papillae are prominent.

Genital cone. The genital cone is complex; a proconus is lacking (Fig. 5a,b). The ventral raylets or “0” papillae are tripartite. The elongate processes of the trident papillae have a common trunk and are situated in a semilunar depression on the ventral aspect of the genital cone adjacent to the orifice of the cloaca (Fig. 5b). A previously unrecognised pair of papillae, which we designate as the dorsal cloacal papillae, reside within a membrane appearing as filiform extensions of the dorsal lip of the cloaca (Fig. 5a,b). These are distinct from the dorsal raylets or “7” papillae which are located dorsally to the dorsal cloacal papillae. The paired “7” papillae are fused at their bases and bifurcate distally, forming a pair of j-shaped structures that support a small accessory bursal membrane.

Spicules. The spicules are asymmetrical, with the left also being slightly longer than the right (Table I; Fig. 6). The left has two inner processes arising from the dorsal and ventral alae, respectively. The shorter right spicule has a single process corresponding with the ventral process of the left spicule (Fig. 6).

Figs 7–14. Synlophe, cephalic, cervical and caudal structures in female by SEM; transverse sections showing synlophe in male. 7. Cervical zone of female showing composite of lateral field with SEM, note minuscule cervical papilla (open arrow). 7a. Detail of cervical papilla in lateral view with light microscopy, note single sensillum in pit (arrow). 8. Cephalic structures with SEM, showing prominent lips, labial papillae of the inner circle (white arrows), paired papillae of the outer circle (black arrows), and an amphid (a); note the broad transverse striation of the anterior cervical zone. 9. Synlophe in region adjacent to tail in female, lateral view; note distribution of ridges around lateralmost ridge (solid pointers). 10. Tail of female showing anus, rounded tip of tail and distribution of the synlophe extending onto the tail region. 11. Transverse section at level of the oesophago-intestinal junction in male; note axis of orientation in fields of lateral ridges and cuticular struts extending into each ridge. Minuscule lateral-most ridges are indicated by open arrows, dorsal and ventral ridges by black pointers. In this view 14 ridges are present, due to the asymmetry in the termination of a pair of accessory ridges in the right lateral field. 12. Transverse section in male midway in anterior second quarter; 20 ridges are present and orientation remains discernable. 13. Transverse section in male at the midbody; 20 ridges are present, orientation is visible and the lateralmost ridges remain minuscule. 14. Transverse section in male in anterior fourth quarter; note diminutive height of lateral ridges. *Scale-bars:* 7, 50 μm ; 7a, 8, 5 μm ; 9–14, 20 μm .

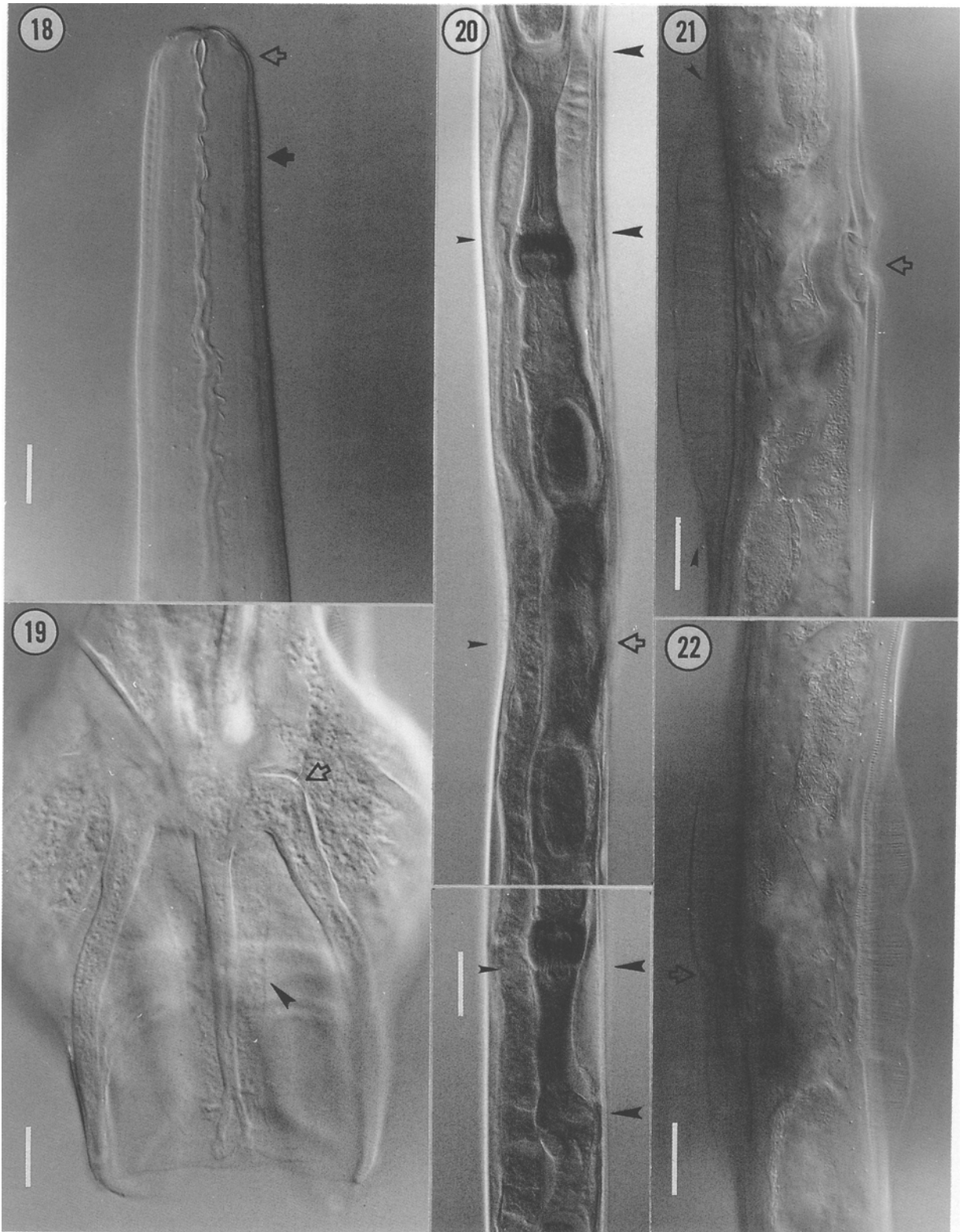


Discussion

The synlophe in males and females of *Parostertagia* has been described as a 20-ridge system, with a perpendicular orientation and hypodermal supports, extending from the base of the cephalic expansion to the bursa and anus, respectively (Durette-Desset, 1978). Gibbons & Khalil (1982a) depicted a system of 20 ridges in the midbody of a male, with the lateral-most ridges being substantially less in height than those in the dorsal and ventral fields. Our observations augment those from previous studies in describing a modified orientation (both bilaterally and dorso-ventrally symmetrical) in males and females (pattern not discernable in far posterior), convergent addition of ridges only in the lateral fields, a sequential increase in the number of ridges posteriorly, consistently minuscule lateral-most ridges, narrow spacing of the lateral fields, hypertrophy of ridges in the lateral field of females, cuticular struts, and a striated or beaded structure of individual ridges. The present study also confirms that there are relatively few ridges comprising the synlophe of *P. heterospiculum* compared to other members of the Trichostrongylidae.

The synlophe of *P. heterospiculum* differs substantially from any so far described among the Graphidiinae and Ostertagiinae, but exhibits numerous similarities to the ridge systems reported among the Cooperiinae. Lichtenfels *et al.* (1988, 1989, 1990) described the synlophe in 13 species of the Ostertagiinae. They recognised two pat-

Figs 15–17. Synlophe of female *Parostertagia heterospiculum*. 15. Transverse section anterior to vulva; note initial hypertrophy of ridges in dorsal lateral fields (open arrows); solid pointers denote dorsal and ventral ridges; 16 ridges are present and the axis of orientation evident in the anterior is no longer discernable. 16. Transverse section at level of vulva; note massive ridges supported by cuticular struts that constitute the fans (open arrows), minuscule lateral-most ridges on the shoulders of the fans (small black pointers), hypertrophied and irregular ridges at level of the left subventral vulva (solid arrow), and dorsal and ventral ridges (large black pointers); 18 ridges are present. 17. Transverse section posterior to vulva; note massive lateral ridges (open arrows), minuscule lateral-most ridges (small pointers) and dorsal and ventral ridges (large pointers); 21 ridges are present. *Scale-bar:* 20 μm .



terns and designated the Type-2 lateral synlophe for *Ostertagia leptospicularis* Assadov, 1953, *O. kolchida* Popova, 1937, *Marshallagia marshalli* Ransom, 1907 and *O. occidentalis* Ransom, 1907, where there were three (or five) parallel, continuous lateral ridges with relatively narrow spacing. Additionally, the ridge systems of the Ostertagiinae are perpendicular, do not increase substantially in number posteriorly, have a greater number of cervical ridges (36–44) and typically do not have hypertrophied ridges in contrast to *Parostertagia*.

Relatively little is known about the synlophe among the Graphidiinae [including *Graphidium* Railliet & Henry, 1909, *Hyostrongylus* and *Parostertagia*, according to Durette-Desset (1983); including *Graphidium* and *Graphidioides* Cameron, 1923, according to Gibbons & Khalil (1982a)]. All are considered to be characterised by a small lateral-most ridge, relatively narrow spacing of the lateral fields, perpendicular orientation and a relatively great number of ridges at the midbody [52 and 68 in males and females of *Graphidium strigosum* (Dujardin, 1845); 26 and 28 in *H. moreli* Durette-Desset & Denke, 1978, referred to *Cervicaprastrongylus* Gibbons & Khalil, 1982 in the Ostertagiinae; and 84 in males of *Graphidioides affinis* Mégnin, 1895)] (Durette-Desset & Denke, 1978; Gibbons & Khalil, 1982b).

In contrast to the Ostertagiinae and Graphidiinae, some of the Cooperiinae have minuscule lateralmost ridges, lateral addition of and increase in the number of ridges posteriorly, origins of ridges that are convergent on the lateral fields, a relatively low number of ridges, prominent cuticular struts or supports underlying each ridge (see Lee, 1965), an orientation (sagittal, with either dorsoventral or ventrodorsal inclination) and hy-

per trophy of individual ridges in the lateral field among some species (Lichtenfels, 1977; Durette-Desset, 1982a, 1983; Gibbons & Khalil, 1982a). The beaded appearance of the synlophe is also particularly evident among *Cooperia* spp. (Lichtenfels, 1977) and is considered to be caused by the linear arrangement of struts constituting the foundation of individual ridges (see Lee, 1965).

In transverse section the structure of the synlophe in *Parostertagia* appears most similar to that of *Cooperioides hamiltoni* (Mönnig, 1932) and *Chabaudstrongylus dubosti* (Durette-Desset & Chabaud, 1974) (see Gibbons & Khalil, 1982a) and some other *Cooperia* spp. (Gibbons, 1981). Orientation in the synlophe is rare among the trichostrongylids, being exhibited only among the Cooperiinae (e.g. *Paracooperia* Travassos, 1935) (Durette-Desset, 1983, 1985). However, the orientation observed in *Parostertagia*, where ridges trend dorsally and ventrally from the lateral fields, cannot be categorised as sagittal or frontal, according to definitions by Durette-Desset (1983, 1985). The configuration that is evident could represent a modification of the usual bilaterally symmetrical pattern observed among several of the Cooperiinae mentioned above (e.g. *Chabaudstrongylus*, *Cooperioides*, *Cooperia*).

The bilateral cuticular inflation at the level of the vulva in *Parostertagia* appears structurally similar to that depicted for specimens of *Cooperia neitzi* Mönnig, 1932 and *C. okapi* Leiper, 1935 by Gibbons (1981). Transverse sections at the level of the vulva prepared during the present study revealed enlarged struts associated with several subventral ridges in specimens of the later species. This may confirm the putative homology of these structures in *Parostertagia* and some cooperiines (Hoberg unpublished observations); however,

Figs 18–22. Cuticular and genital structures of male and female *Parostertagia heterospiculum*. 18. Cephalic and anterior cervical zone in male specimen; note buccal capsule (open arrow) and rudimentary cervical and cephalic expansion indicated by inflated region with broad transverse striations of the cuticle (solid arrow). 19. Externodorsal and dorsal ray in dorsal view; note thickened membranous support around dorsal ray (black pointer) and angular base of externodorsals (open arrow). 20. Ovejectors, composite view showing infundibula (between large pointers), and sphincters including the anterior and posterior vestibula (demarcated by small pointers to open arrow at level of vulva); note greater length of anterior sphincter as measured from the vulva and including the vestibula. 21. Cuticular fan at level of vulva in female, ventral view showing right fan (between pointers) and left subventral vulva (open arrow). 22. Cuticular fans at level of vulva, dorsal view showing bilateral structure of fans; vulva is not in plane of focus (arrow). *Scale bars:* 18, 19, 22, 20 μm ; 20, 21, 40 μm .

more detailed observations are necessary. Such cuticular inflations, formed by single hypertrophied ridges are currently unknown among other trichostongylids.

Inflations described among the Ostertagiinae [*Longistrongylus sabie* (Mönnig, 1932), *L. curvispiculum* (Gibbons, 1973), *Mazamastrongylus odocoilei* (Dikmans, 1931) and *M. pursglovei* (Davidson & Prestwood, 1979)], although somewhat similar morphologically, appear structurally distinct from that found in *Parostertagia*. Vulvar inflations in *L. sabie* and these *Mazamastrongylus* spp. are not supported by single hypertrophied struts and are typically independent of the synlophe. A pair of continuous lateral ridges in *L. sabie* are superficial and extend the length of each lateral inflation (Hoberg, unpublished), whereas several ridges may be present on the irregular inflations characteristic of *Mazamastrongylus* spp. (Lichtenfels, unpublished). Thus such cuticular inflations are considered convergent in *Parostertagia* (also in the Cooperiinae) and the Ostertagiinae, with those in the latter group possibly being a derivation or precursor for vulvar flaps typical of *Ostertagia* spp. and related genera (see Michel *et al.*, 1972).

Although a suite of structural components of the synlophe appears to be similar among *Parostertagia*, and some of the Ostertagiinae, Graphidiinae and Cooperiinae, it is necessary to demonstrate that such shared characters are homologous. Only shared derived characters (synapomorphies) are useful in establishing hypotheses for phylogenetic relatedness and form the basis for the development of natural classifications (Hennig, 1966; Wiley, 1981). In this respect, the pattern of convergent addition and sequential increase in the number of ridges posteriorly, the presence of prominent enlarged cuticular struts, hypertrophy of lateral ridges and the axis of orientation appear to represent derived characters unique to the Cooperiinae (postulated synapomorphies; see Durette-Desset, 1985), and indicate that *Parostertagia* should be referred to this subfamily.

However, the limitation in availability of detailed data on the cervical pattern, and other

structural attributes of the synlophe among the subfamilies and genera of the trichostongylids does not currently allow a broad assessment of the evolution of these systems of cuticular ridges. Among the Trichostongylidae, it appears that only for the Cooperiinae and Trichostongylinae are patterns sufficiently defined as to be useful in determining inclusive relationships for genera within these subfamilies. This situation exists due to the many structural components of the synlophe that are widely shared among the Trichostongylidae and Trichostongyloidea (see Durette-Desset, 1985), and as such represent symplesiomorphic characters which are not indicative of relationship at the level of family, subfamily and genus.

Apparently unique elements, tripartite ventral raylets or "0" papilla and filiform dorsal cloacal papillae, are present in the genital cone of *Parostertagia*. The trident "0" papillae were originally included in the figures of the bursa presented by Schwartz & Alicata (1933), but not discussed in the text. Previous studies of the genital cone among the strongyles, including the Trichostongyloidea, have demonstrated a degree of uniformity and homology in the structural configuration of this organ (Andreeva, 1958; Stringfellow, 1970, 1972; Chabaud *et al.*, 1970; Gibbons & Khalil, 1983). Among the trichostongylids, the primitive condition (with reference to strongyloid outgroups) of the cone is represented by a single ventral raylet (Haemonchinae, Cooperiinae, Trichostongylinae, Graphidiinae and Libyostongylinae), whereas a paired "0" papilla appears characteristic of the Ostertagiinae (Gibbons & Khalil, 1982a; Durette-Desset, 1985). Additional structures of the genital cone include a pair of dorsal raylets ("7" papillae) often associated with an accessory bursal membrane (Gibbons & Khalil, 1983; Jansen, 1989) and dorsolateral genital appendages that may be situated adjacent to the cloaca in some groups (Stringfellow, 1970; Gibbons & Khalil, 1983). Chabaud *et al.* (1970) also described papilliform cuticular ornaments that were present in some strongylidans, these latter structures not apparently a component of the genital-cloacal complex.

The “7” papillae and accessory bursal membrane in *Parostertagia* is relatively small but is similar to those reported among the Ostertagiinae and some Graphidiinae (*Graphidioides*) and Cooperiinae (*Paracooperia*) (Andreeva, 1958; Gibbons & Khalil, 1982a, 1983). The dorsolateral position of the dorsal cloacal papillae of *Parostertagia* suggests that they may be reduced genital appendages and possibly homologous to the “lateral protuberances” described for some *Cooperia* spp., although this requires confirmation (Stringfellow, 1970; Gibbons & Khalil, 1983). It is not clear whether the dorsal cloacal papillae contain nerve tracks; such are apparently lacking in these other accessory structures. Additionally, there is no counterpart for the trident configuration of the ventral raylets among those groups that have been examined in detail, although the single “0” papilla of some haemonchiines (*Haemonchus* Cobb, 1898 and *Ashworthius* Le Roux, 1930) apparently contains a tripartite structure described by the terminal nerve tracks (Gibbons & Khalil, 1983).

The structure of the dorsal raylets in *Parostertagia* may be a shared primitive character (symplesiomorphy) among the trichostrongylids, whereas the trident ventral raylets may represent an autapomorphic condition in *Parostertagia*. Consequently, these characters are not useful in elucidation of the systematic affinities of *Parostertagia* within the family Trichostrongylidae. However, homology of the filiform dorsal cloacal papillae with the larger lateral protuberances of *Cooperia* would support recognition of relationship with the Cooperiinae.

Several other characters, including the configuration of the copulatory bursa, cervical papillae and cephalic vesicle, are important to consider. The pattern of the bursa in *Parostertagia* is 2-3 (all ventral rays are divergent from the lateral rays) with rays 2 and 3 of near equal dimensions and being only slightly convergent, the former situation being observed in the family Trichostrongylidae only among the Cooperiinae [although the 2-3 pattern is considered the primitive condition of the bursa for strongylidans, according to Durette-Desset (1985)]. Durette-Desset (1985) accepted a 2-3 pattern (or a 1-3-1), along with the tips of rays 2 and 3 being convergent and ray 2

being substantially smaller than ray 3, as diagnostic characters for the Cooperiinae. Whereas the 2-3 pattern appears typical for the subfamily, there is considerable variation in the degree of convergence and the relative dimensions of these rays among the genera currently referred to this subfamily (see Durette-Desset, 1983; Gibbons & Khalil, 1982a; Gibbons, 1981). In contrast, bursae considered typical of the Ostertagiinae and Graphidiinae are either 2-2-1 or 2-1-2 in the former and 2-2-1 in the later (Durette-Desset, 1985). Additionally, the base of ray 8 (externodorsal) is particularly marked in *Parostertagia* as in some cooperiines, and the elongate ray 9/10 (dorsal ray) is supported by a thickened cuticular membrane, the latter apparently not previously having been observed among the trichostrongylids.

Minute cervical papillae were observed in *Parostertagia* for the first time during the present study. The obscure sensilla-like structures differ from the thorn-like papillae that project from the cuticle in the Ostertagiinae and Haemonchinae and some Graphidiinae (Durette-Desset & Chabaud, 1977; Gibbons & Khalil, 1982a). Sensilla-like papillae are widespread among the trichostrongyloids and probably constitute the primitive condition among the trichostrongylids (and other strongylidans). However, the cervical papillae are particularly obscure in *Parostertagia* and among the Cooperiinae.

A reduced cephalic vesicle is present in *Parostertagia*, and is represented by the region of cephalic cuticle anterior to the synlophe. The vesicle is only slightly inflated and demarcated from the body of the nematode by a broader spacing in the transverse striations of the cuticle. In this respect it appears similar in structure to the cephalic zones of both *Ostertagia* spp. (Gibbons & Khalil, 1982a) and *Cooperia* spp. (Durette-Desset, 1983).

Aside from morphological criteria, an examination of habitat or site of localization may be useful. *Parostertagia heterospiculum* typically occurs in the small intestine, whereas all species among the genera of the Ostertagiinae (see Gibbons & Khalil, 1982a; Durette-Desset, 1989; Jansen, 1989) and Graphidiinae occur in the abomasum or stomach (Skrjabin *et al.*, 1954; Jansen, 1986). Members of the Cooperiinae are usually

Table II. Characters currently used to define the subfamilies Ostertagiinae, Graphidiinae and Cooperiinae.¹

Ostertagiinae	
buccal capsule	absent
cephalic vesicle	present; region with transverse striations
cervical papillae	thorn-like, projecting from body surface
synlophe	(a) high number of ridges (b) ridges of even height (c) perpendicular orientation
genital cone	(a) ventral raylets ("0" papillae) paired (b) accessory bursal membrane well developed (c) lateral protuberances absent
bursa	pattern 2-2-1 or 2-1-2
spicules	relatively short (except <i>Marshallagia</i> , <i>Camelostrongylus</i>)
Graphidiinae ²	
buccal capsule	present
cephalic vesicle	absent
cervical papillae	thorn-like
synlophe	(a) high number of ridges ² (b) ridges of even height (lateral may be slightly smaller) (c) perpendicular orientation
genital cone	(a) ventral raylet single (b) accessory bursal membrane reduced
bursa	(a) pattern 2-2-1 (b) rays 2-3 convergent (c) dorsal ray divided at distal tip
spicules	long, filiform ²
Cooperiinae	
buccal capsule	absent
cephalic vesicle	present
cervical papillae	sensillum projecting from minuscule pit
synlophe	(a) low number of ridges (b) ridges of different height (laterally) (c) convergent addition of ridges laterally (d) orientation present (sagittal) (e) some ridges hypertrophied in lateral fields
genital cone	(a) ventral raylet single (b) accessory bursal membrane modified, often reduced
bursa	pattern 2-3 (ventral and lateral rays divergent) also 1-3-1 in some genera
spicules	relatively short

¹ Characters common to a majority of genera from Gibbons & Khalil (1982a, 1983), Durette-Desset (1982a, 1982b, 1983) and Stringfellow (1970, 1972).

² Following the opinion of Gibbons & Khalil (1982b), where *Hyoststrongylus rubidus* (Hassal & Stiles, 1892) and both *Cervicapraststrongylus moreli* and *C. gabonensis* (Durette-Desset & Chabaud, 1974) (formerly *Hyoststrongylus*) are referred to the Ostertagiinae.

restricted in distribution to the small intestine (Skrjabin *et al.*, 1954). However apparent patterns of habitat selection by parasites, like those of host-distribution must be interpreted with caution in the absence of phylogenetic hypotheses (based on morphological criteria) for the parasite group.

Consideration of the morphological characters presented above allows discussion of the subfamilial placement of *Parostertagia heterospiculum*. Under current classifications, the genus is referred

to the Graphidiinae (Durette-Desset, 1983, 1985; Jansen, 1989) or is considered to have possible affinities with the Ostertagiinae (Gibbons & Khalil, 1982a). An association with the Cooperiinae has not been previously suggested, although Skrjabin *et al.* (1952, 1954) referred *Hyoststrongylus* to this subfamily [this decision is indicated here with respect to opinions that reduced *Parostertagia* as a synonym of *Hyoststrongylus* – see Travassos (1937) and Chabaud (1959)]. Current definitions

for the three subfamilies are presented in Table II and are a combination of characters (particularly the genital cone and synlophe) used in the most recent classifications and keys for the Trichostrongylidae (Gibbons & Khalil, 1982a; Durette-Desset, 1983, 1985) and as such recognise all structural characters that have been used to define these groups (see Jansen, 1989; with reference to the Ostertagiinae).

Under the criteria presented in Table II, *Parostertagia* cannot be referred to either the Ostertagiinae or the Graphidiinae. A unique configuration of the "0" papilla and an axis of orientation in the synlophe, in addition to other characters precludes unequivocal placement of *Parostertagia* among the Ostertagiinae or Graphidiinae. Attributes presented in Table II and discussed in the text indicate that *Parostertagia* has affinities with the Cooperiinae and should be referred to that subfamily. The basis of this opinion resides in the structure of the synlophe (orientation, distribution, pattern, hypertrophy laterally and number of ridges) and genital cone (postulated homology for dorsal cloacal papillae and genital appendages) and the morphology of the bursa (divergent ventral and lateral rays). However, the utility of these structural attributes in elucidation of the relationships between *Parostertagia* and some cooperiines is dependent on whether or not such characters are synapomorphic. Consequently, *Parostertagia* is provisionally placed among the Cooperiinae, pending the availability of detailed evaluations of associated genera among the the subfamilies under consideration.

There continues to be the necessity to re-evaluate characters in current use, provide well-supported hypotheses for homology and the direction of transformation for complex structural attributes [by outgroup comparisons – see Wiley (1981)], and to recognise synapomorphies. Based on these concepts, refined definitions of the families, subfamilies and genera of the Trichostrongyloidea may be obtained. The continued application of unique combinations of shared primitive characters in the definition of taxa does not provide a reliable database for the elucidation of phylogenetic relationships within this group of nematodes or for the

development of hypotheses for host-parasite co-evolution and historical biogeography (see Jansen, 1989).

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