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PSEUDOSTERTAGIA BULLOSA (NEMATODA: TRICHOSTRONGYLOIDEA) IN ARTIODACTYL HOSTS FROM NORTH AMERICA: REDESCRIPTION AND COMMENTS ON SYSTEMATICS

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ABSTRACT: A relationship for *Pseudostertagia bullosa* within the trichostrongyloids has been enigmatic or unresolved. Studies of the synlophe in males and females of *P. bullosa* revealed a tapering system anterior to the deirids and a pattern of parallel ridges extending to near the caudal extremity in both lateral and median fields. Structurally, the synlophe differs considerably from that seen among the Cooperiinae and exhibits homoplasy with respect to ridge systems among some Ostertagiinae. Other structural characters due to symplesiomorphy, homoplasy or because they represent autapomorphies do not serve to reveal the putative relationships for *P. bullosa* with other trichostrongyloids. Although somewhat equivocal, the 2-2-1 pattern of the bursa and position of rays 2 and 3 suggest an association with the Cooperinae, as postulated by Durette-Desset and others. *Pseudostertagia bullosa* appears to be a species that has survived in the pronghorn, *Antilocapra americana*, a relictual pecoran artiodactyl that occurs in xeric regions of western North America; pronghorn are the sole remnant of the late Tertiary radiation for Antilocapridae across North America. *Pseudostertagia bullosa* may occur in mixed infections with a number of ostertagiines in the abomasa of mule deer (*Odocoileus hemionus*) and domestic sheep (*Ovis aries*) in regions of sympatry for pronghorn and these artiodactyl hosts.

Pseudostertagia bullosa (Ransom and Hall, 1912) was originally described based on nematodes in the abomasa of domestic sheep, *Ovis aries* Linnaeus, that were collected at various sites from Colorado during 1911 (Ransom and Hall, 1912). Specimens of this trichostrongyle have subsequently been reported in pronghorn, *Antilocapra americana* (Ord), bighorn sheep, *Ovis canadensis* Shaw, and mule deer, *Odocoileus hemionus* (Rafinesque) across the western plains and deserts extending from New Mexico to Montana and eastward to South Dakota (Hoberg et al., 2001). The distribution of *P. bullosa* appears to be limited to the western Nearctic, and the species should be considered endemic to the North American nematode fauna of ruminants.

Originally described as a species of *Ostertagia* Ransom, 1907, it was referred to the subgenus *Pseudostertagia* Orloff, 1933; the subgenus was later elevated to generic rank by Travassos (1937). The monotypic *Pseudostertagia* has been of uncertain affinities within the trichostrongyles. Considered to be an ostertagiine (e.g., Ransom, 1907; Orloff, 1933), *P. bullosa* was relegated to the Trichostrongylinae Leiper, 1908, and tribe Ostertagiea Skrjabin and Schulz, 1937, in the classification adopted by Skrjabin et al. (1954). Subsequently, *Pseudostertagia* was placed in Trichostrongylidae and Libyostrongylinae Durette-Desset and Chabaud, 1977, in taxonomic revisions and reviews by Durette-Desset and Chabaud (1977, 1981) and Durette-Desset (1983, 1985). In contrast, Gibbons and Khalil (1982) considered *Pseudostertagia* to have uncertain affinities, but possibly related to the ostertagiines. Hoberg and Lichtenfels (1994) recognized the genus within the inclusive Libyostrongylinae but did not examine the phylogenetic relationships for constituent members of the subfamily. Extensive phylogenetic analysis of characters from comparative morphology led Durette-Desset et al. (1999) to identify *Pseudostertagia* as the basal member of the Cooperiinae Skrjabin and Shikhobalova, 1952, in their Cooperiidae (Skrjabin and Shul'ts, 1937). Putative relationships for *Pseudostertagia* have yet to be addressed, however, within the context of multilocus analyses of DNA sequence data (e.g., Chilton et al., 2001; Gouÿ de Bellocq et al., 2001).

The resolution of the taxonomy and systematics for this monotypic genus has been hindered by the incomplete nature of previous descriptions and by the presence of morphological characters that may be contradictory. Thus, affiliation of *P. bullosa* within various taxa among the Trichostrongyloidea remains equivocal. In the current study, we provide detailed descriptions of the synlophe and cervical region, along with structural aspects of the genital cone. These new assessments of characters based on comparative morphology are examined in the context of prevailing concepts for phylogeny among the Trichostrongyloidea (e.g., Hoberg and Lichtenfels, 1994; Durette-Desset et al., 1999). Additionally a range of new morphometric data are presented for characters not previously assessed in the original description.

MATERIALS AND METHODS

Specimens examined

A listing of specimens of *P. bullosa* examined in the current study along with their host and geographic distributions is summarized in Table I. Adult nematodes were studied either as temporary whole mounts cleared in phenol-alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol) or as cross-sections prepared by hand with a cataract knife and mounted in glycerin jelly; 2 males and 3 females were prepared by sectioning. Specimens were examined with bright-field illumination or interference-contrast microscopy.

Characters studied

Characteristics of the synlophe were defined according to Durette-Desset (1983) and Hoberg and Lichtenfels (1992, 1994). Bursal ray patterns were determined and described using the system of Durette-Desset and Chabaud (1981). The numbering system for papillae of the genital cone and rays of the copulatory bursa followed Chabaud et al. (1970).

Protocols for measurements of the ovejectors and specifically the sphincters have not been standardized, although Lichtenfels et al. (2003) outlined criteria that are generally applicable within the Trichostrongyoloidea. We present measurements of the sphincters based on 2 distinct methods. (1) Among some trichostrongyloids, the junction of the vestibule and sphincter is not consistently discernable, and often the vestibule has been counted as part of the sphincter (see Lichtenfels et

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TABLE I. Host, locality of collection, and numbers of specimens examined in a comparative study of *Pseudostertagia bullosa.*

Specimen*	Host	Locality	Number examined ♀	♂
16083†	*Ovis aries*	Colorado	4	6
45542	*Ovis aries*	South Dakota	8	7
45552	*Antilocapra americana*	Montana	3	3
46202	*Antilocapra americana*	North Dakota	4	6
59039	*Ovis aries*	Nebraska	6	8
59380	*Antilocapra americana*	South Dakota	6	—
59399	*Antilocapra americana*	South Dakota	1	—
87024	*Odocoileus hemionus*	Montana	10	11
87026	*Odocoileus hemionus*	Montana	6	7
87029	*Odocoileus hemionus*	Montana	3	7

* Accession numbers, U.S. National Parasite Collection, USDA, ARS, Beltsville, Maryland.
† Type specimens from Ransom and Hall (1912); generally in poor condition.

al., 1993). In this instance, determination of the dimensions of the infundibula and sphincters of the ovejectors uses the edge of the muscular portion of the sphincter as a dividing line following the convention established by Lichtenfels et al. (1993). (2) In contrast, we also present the measurement for complete length of each sphincter, representing the combined length for the bipartite sphincter where sphincter-1 (distal muscular bulb) and sphincter-2 (muscular cylindrical component, demarcated from and terminating proximal to the distal vestibule) are combined (Lichtenfels et al., 2003). Although the latter is the correct measurement for the sphincter, many authors continue to recognize and measure only the muscular bulb. Measurements are in micrometers unless indicated otherwise and are given as (n =) followed by a range with the mean in parentheses. Taxonomy is consistent with Durette-Desset et al. (1999) in order to achieve clear comparisons across groups.

REDESCRIPTION

Pseudostertagia bullosa (Ransom and Hall, 1912) Orloff, 1933

(Figs. 1–20)

General redescription: Trichostrongyloidea, uncoiled, single male morphotype. Cephalic vesicle present, poorly developed. The buccal capsule small, rounded, elongate with cuticularized lining. Deirids miniscule, sensillalike, located posterior to excretory pore and subventral gland orifice. Cuticle with prominent synlophe throughout length. Esophagus lacking prominent valve at esophageal–intestinal junction.

Synlophe: Ridge systems bilaterally symmetrical, continuous, extending in median and lateral fields from base of cephalic expansion to near level of bursa in males and anus in females. Ridges with perpendicular orientation; gradient in size is not evident. Interval among 2–3 lateral-most ridges in each field, less relative to those in ventral and dorsal fields. Laterally in cervical fields anterior to deirids, synlophe tapers with ridges terminating adjacent to lateral-most ridges. Ridges originating in the subventral and subdorsal fields; some pairs of ridges extend from ventral-most ridge into left and right lateral fields, becoming parallel posteriad. Laterally, in cervical zone, there are 2–3 continuous, parallel ridges. Anterior to deirids, short pairs of ridges flank a 3-ridge system or the lateral-most ridge. Posterior to deirids, ridges are typically parallel throughout lateral fields from anterior to posterior. Ventrally, in cervical zone,

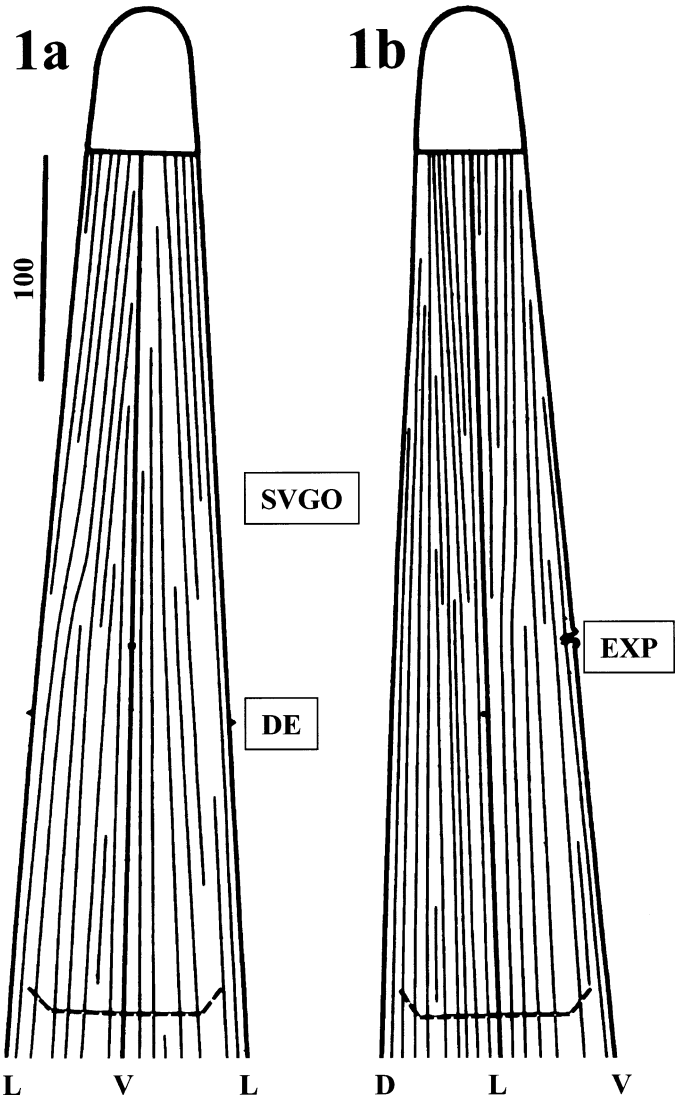
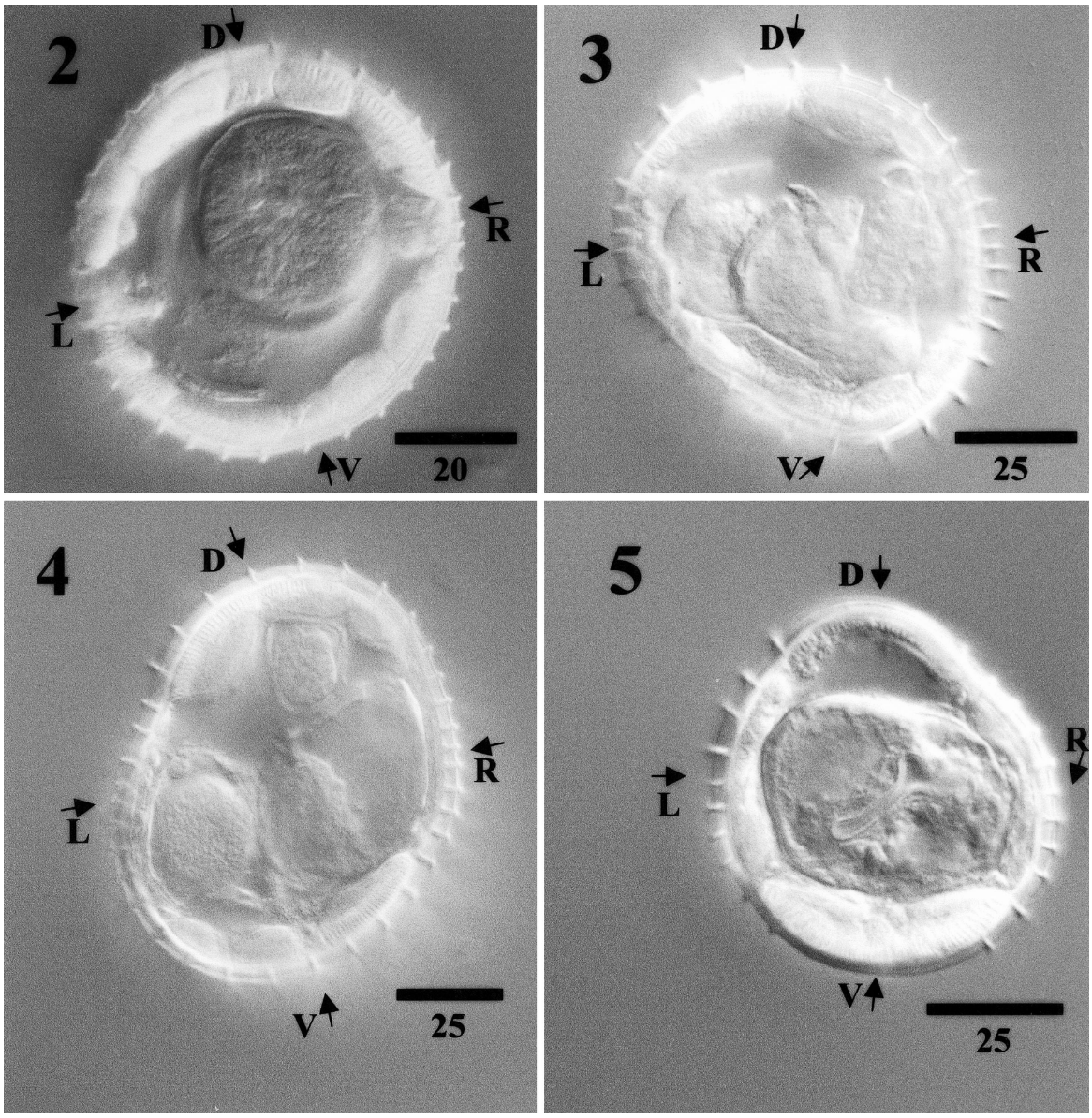


FIGURE 1. *Pseudostertagia bullosa*, cervical synlophe to base of esophagus (dashed line) in a female, with positions indicated for the subventral gland orifice (SVGO), excretory pore (EXP), and deirids (DE); L = lateral, D = dorsal, and V = ventral; scale in micrometers. **a.** Ventral view, showing 3 parallel ridges, with ventralmost being interrupted at the excretory pore. **b.** Lateral view, right, showing tapering pattern anterior to deirids and parallel system extending posteriad from cervical zone.

there are 3 continuous, parallel ridges; ventral-most interrupted at excretory pore. Ventral-most ridge originates at (n = 4) or slightly posterior (n = 6) to base of cephalic vesicle, always anterior to deirids. Variation exists in origin of paired ridges flanking ventral-most ridge; in 5 worms examined, this pair of ridges originated anterior to ventral ridge; in 1 of 5 specimens, pair flanking ventral ridge began at cephalic vesicle. Posterior to esophageal–intestinal junction, 3-ridge lateral fields extend to prebursal papillae in males and anus in females. Median fields terminate anterior to lateral fields with ventral and dorsal-most ridges terminating first within respective fields. In males, the ventral-most ridge terminated at (n = 9) 412–530 (459) anterior to prebursal papillae; dorsal-most ridge at (n = 8) 220–488 (296) anterior to prebursal papillae. In females, dorsal and

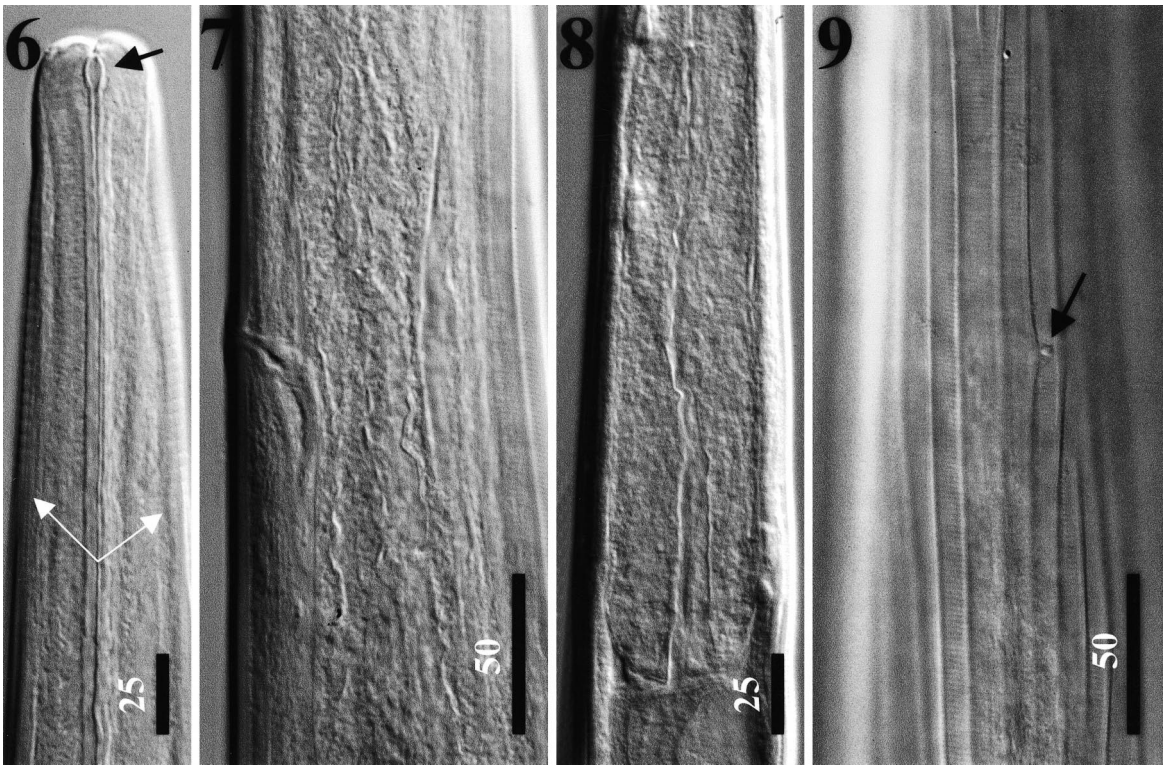


FIGURES 2–5. *Pseudostertagia bullosa*, showing structure of synlophe in a female as revealed in transverse sections; shown in orientation as viewed from posterior with dorsal (D) to top, ventral (V) to bottom, and anatomical left (L) and right (R); scales in micrometers. **2.** Synlophe at level of esophageal–intestinal junction, with 36 ridges; note narrow spacing of 3 lateralmost in left and right fields. **3.** Synlophe at midbody showing 32 ridges. **4.** Synlophe in third quarter showing 36 ridges. **5.** Synlophe at level of anus, showing 21 ridges retained in lateral fields, with prominent gaps dorsally and ventrally; note shieldlike thickening of cuticular inflation anteroventral to anus.

ventral-most ridges terminated (n = 10) 0–250 (155) and (n = 6) 0–225 (140) anterior to anus. Ridges number 20–27 at level of cephalic expansion. Ridges increase in number to 28–34 at level of cervical papillae, 28–38 at base of esophagus, 28–32 at midbody, 28–35 in third quarter. Ridges decrease to 17–16 at level anterior to spicules in males, 14–21 at level of anus in females. Posterior to esophageal–intestinal junction, ridge numbers remain consistent through 87% of body length in males, 90–95% in females.

Male characters: Bursal pattern 2-2-1; prominent and cuticular inflation or bulla present anteroventral to bursa. Ray 2 considerably shorter, thinner relative to ray 3, separate throughout length. Distally, end of ray 3 curves slightly toward tip of ray 2; significant convergence not demonstrated. Distance between distal ends of rays 2 and 3 < separation of rays 3 and 4. Dorsal ray elongate, extending length of bursa, bifurcates at approximately 80% of length from anterior. Posterior to bifurcation, short spur extends laterally from each branch. Additional minute bifurcation sometimes visible at base of each branch of dorsal ray. Externodorsal rays curve ventrally subsequent to origin, positioned ventral to dorsal ray for most of length.

Genital cone with prominent accessory bursal membrane positioned dorsal to cloaca. Accessory bursal membrane, large, rounded, not incised; suture extending from anterior to posterior



FIGURES 6–9. *Pseudostertagia bullosa*, showing structural characters in cephalic and cervical zone; scales in micrometers. **6.** Cephalic extremity showing cuticular thickening of buccal capsule (black arrow) and posteriad extent of cephalic vesicle (white arrows). **7.** Excretory pore in lateral view. **8.** Base of esophagus at esophageal–intestinal junction, showing absence of valve. **9.** Cervical zone, lateral view showing sensillalike cervical papilla (arrow).

bisects caudal region. Paired 7 papillae, short relative to length and diameter of accessory bursal membrane; situated ventrally, curving both ventrally and laterally from origin. Unpaired 0 papilla slightly ventral and proximal to accessory bursal membrane situated on a triangular platelike structure adjacent and ventral to cloaca. Proconus absent.

Spicules nearly identical in length, thin, unbranched. Distal tips of spicules filamentous, lacking prominent membranes, maintaining ventral curve whether spicules are retracted or extruded from cloaca. In specimens with spicules extruded from body, distal third of the shaft curved dorsally. Gubernaculum triangular, robust, heavily chitinized with multiple processes anteriad and posteriad; laterally, appearing keellike, maximum length evident along ventral edge.

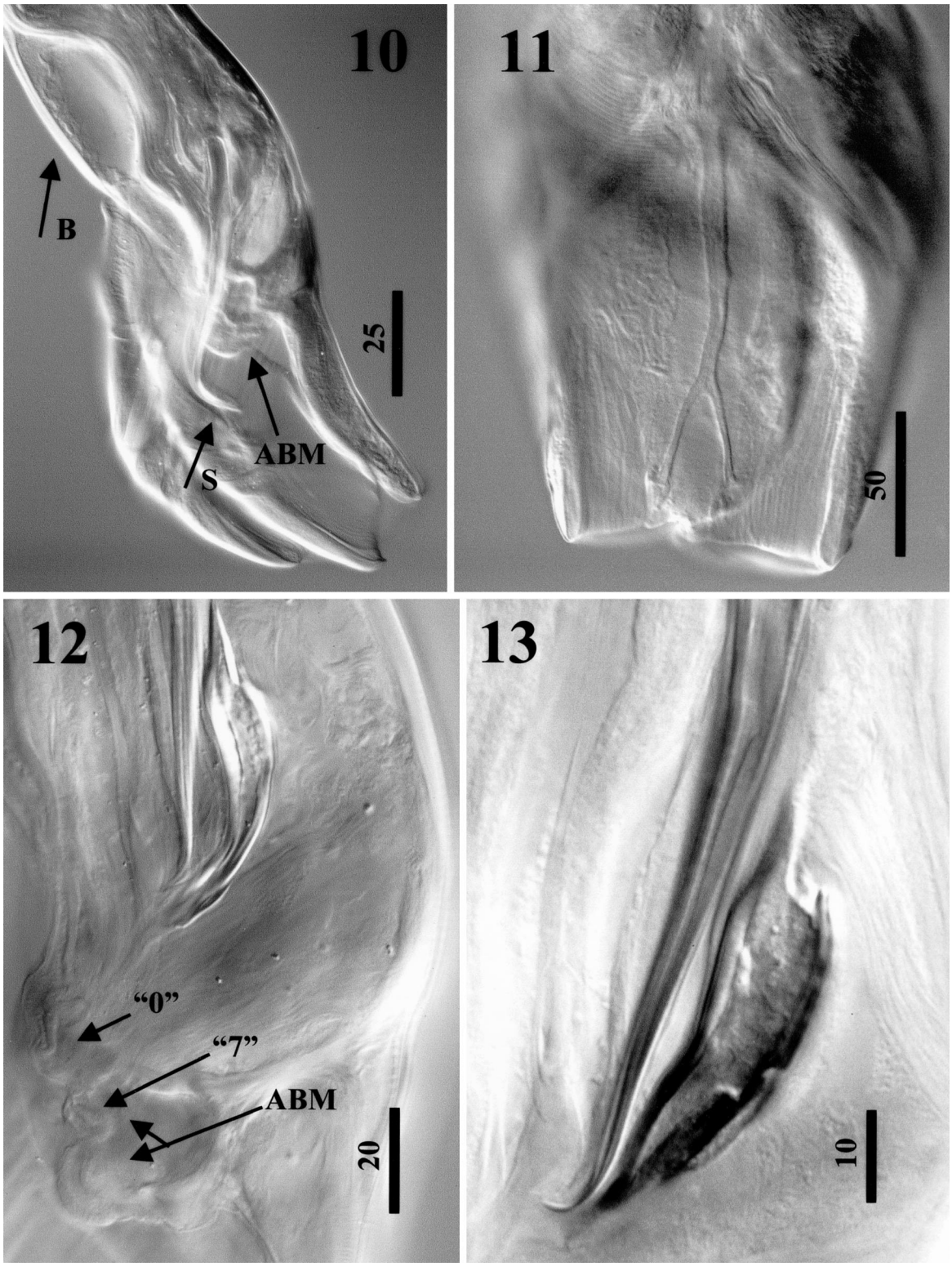
Female characters: Amphidelphic with paired ovejectors composed of central common vestibule, bipartite sphincters, and elongate infundibula extending posteriad and anteriad. Sphincter-1, muscular, bulblike; sphincter-2, cylindrical, with well-demarcated junction represented by muscular constriction at proximal end; continuous with distal vestibule. Length of anterior sphincter > posterior. Anterior and posterior infundibula similar in length. Vulva, transverse, curved, disposed ventrally; vulval flaps and fans not observed laterally or ventrally. Anus surrounded by region of ventral cuticular inflation. Tail elongate, digitate, terminally with bulbous caudal extremity lacking cuticular annulations.

Comparisons with prior studies

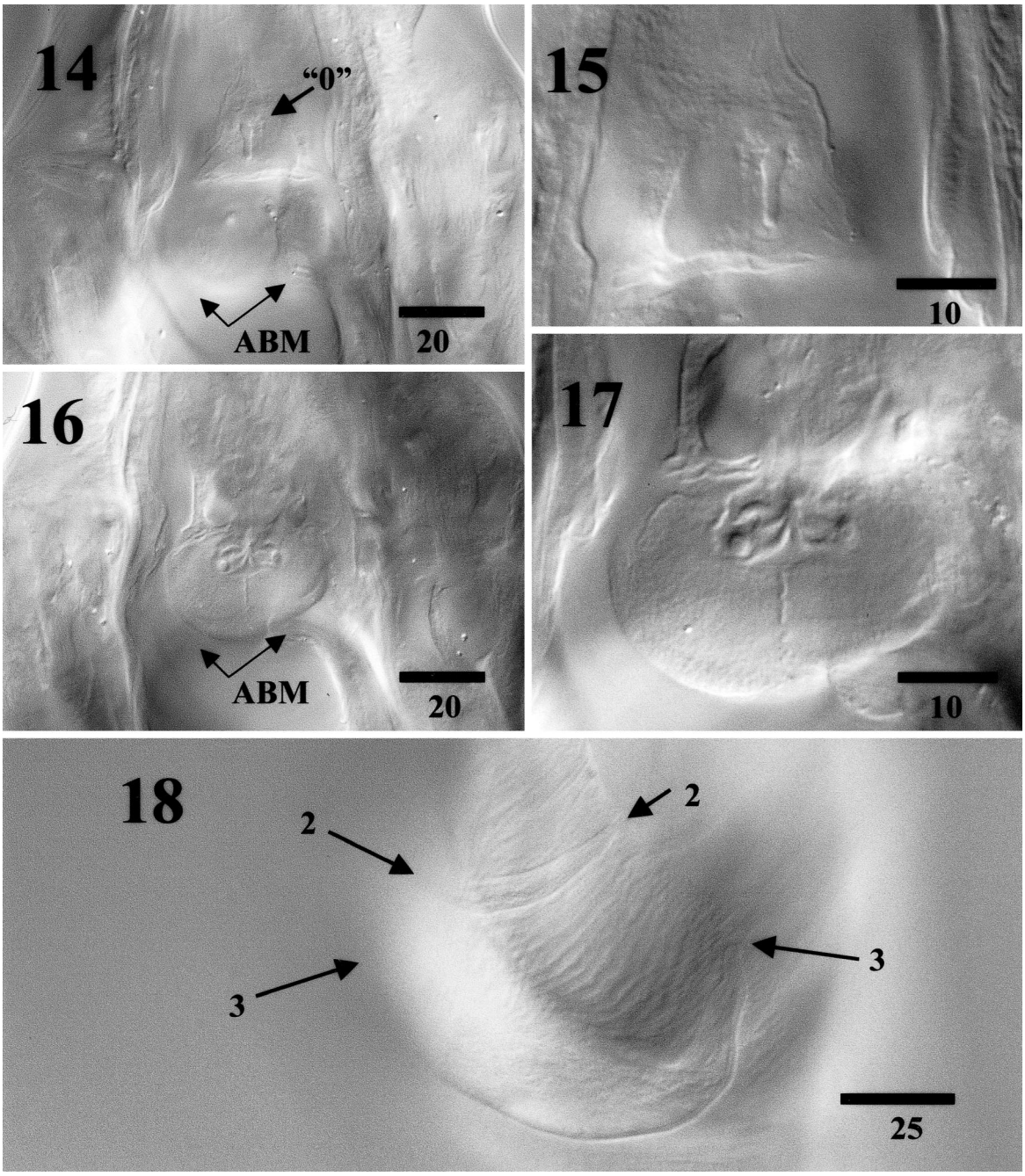
Measurements from the current study are generally within the range of values presented in the original description (Ransom and Hall, 1912) (Table II). Total body length and esophageal length in the original description fall toward the upper end of ranges presented in the current study. The number of specimens on which the original description was based could not be determined with certainty. New meristic data are presented for esophageal characters, dimensions of the component parts of the ovejectors (with distinct 2-part sphincters), and cervical characters. Details of the synlophe, genital cone, and accessory bursal membrane had not been previously evaluated; cervical papillae were reported as absent in the original description (Ransom and Hall, 1912). The buccal capsule typically is small, rounded, and elongate with a thick cuticularized lining consistent with redescriptions by Durette-Desset et al. (1999). The gubernaculum is as described by Olsen (1950) and represents a complex structure unique to this genus.

Comparisons with other genera and species

Structural attributes for specimens of *P. bullosa* include many that represent the putative plesiomorphic condition among the Trichostrongyloidea in the context of current phylogenetic hypotheses (e.g., Hoberg and Lichtenfels, 1992, 1994; Durette-Desset et al., 1999). In general, a parallel and continuous synlophe in both males and females that is perpendicular



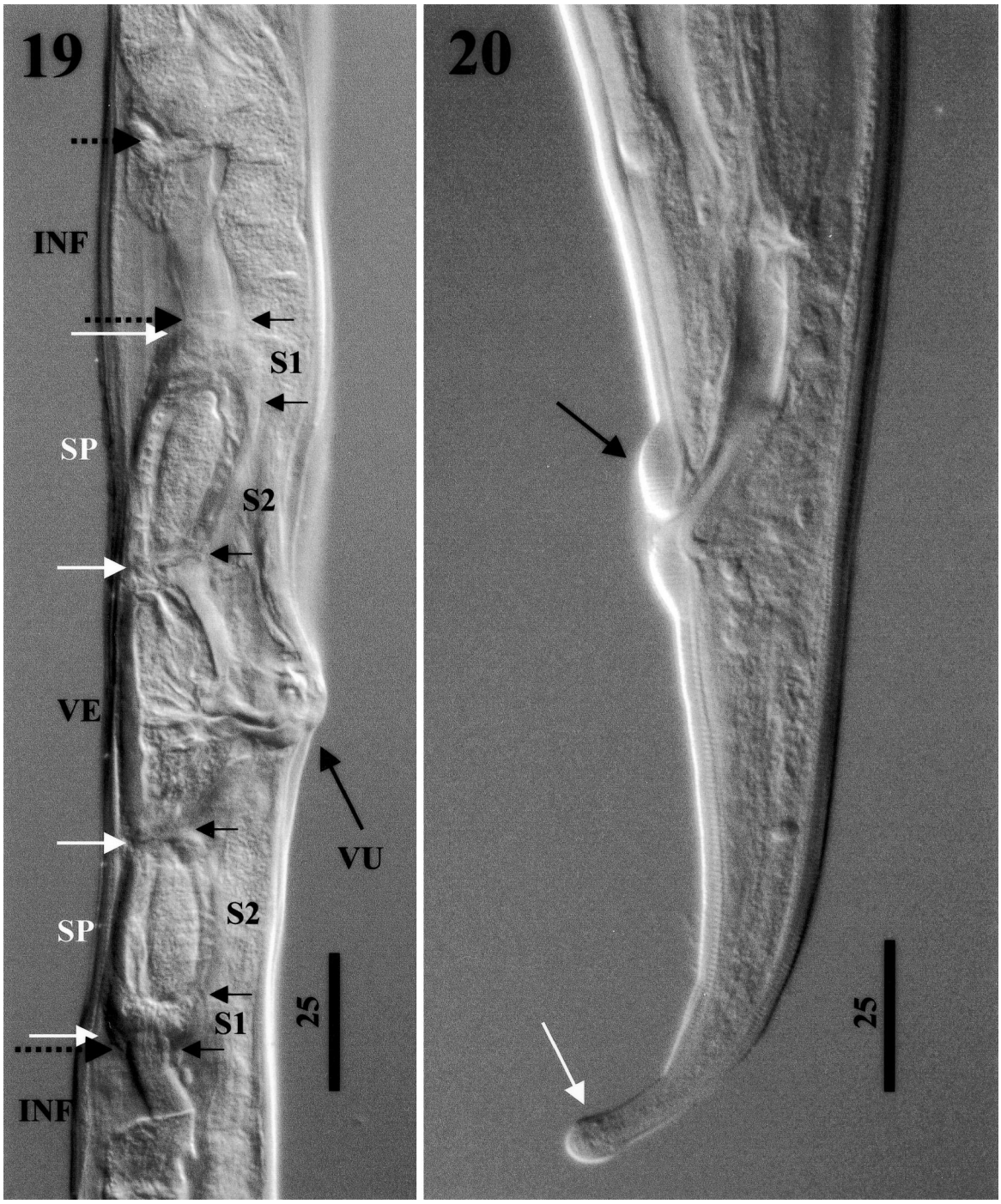
FIGURES 10–13. *Pseudostertagia bullosa*, male, showing structure of the bursa and genital cone; scales in micrometers. **10.** Bursa, lateral view, showing bulla (B), prominent accessory bursal membrane (ABM), and dorsally curved spicule tips (S). **11.** Dorsal ray in dorsal view; note elongate narrow ray with distal bifurcations. **12.** Genital cone, lateral view, showing position of 0 papillae, 7 papillae, and rounded ABM. **13.** Spicule tip and gubernaculum in left lateral view; note unbranched spicule tip and complex gubernaculum with anteriorly and posteriorly directed processes and keellike structure dorsally.



FIGURES 14–18. *Pseudostertagia bullosa* male, showing detailed structure of genital cone in ventral view and disposition of bursal rays 2 and 3; scale bars in micrometers. **14.** Genital cone, ventral view, with single 0 papilla anteroventral to cloaca and rounded accessory bursal membrane posterodorsal to cloaca; note the distal ends of the 7 papillae. **15.** Single, digitate 0 papilla situated on triangular cuticular plate anteroventral to cloaca. **16.** Accessory bursal membrane showing general position. **17.** Accessory bursal membrane and divergent 7 papillae; note median suture. **18.** Bursa, right lobe, ventral view, showing disposition of rays 2 and 3; note divergent bases and parallel to slightly convergent tips.

and lacks a gradient is the plesiomorphic state for the Trichostrongyloidea and Molineoidea + Heligmosomoidea within the Trichostrongylina (Gouÿ de Bellocq et al., 2001). *Pseudostertagia bullosa* has a slightly tapering lateral system that becomes entirely parallel posterior to the cervical papillae, a greater number of ridges than found in the Cooperiinae, and also does not have beaded ridges where cuticular struts are visible at the level of light microscopy.

The absence of an esophageal valve in *P. bullosa* and other trichostrongyloids is in contrast with a robust and well-defined valve that constitutes one among several putative synapomorphies for Ostertagiinae (paired 0 papillae, tripartite spicule tips, spicules with characteristic windows, irregular vulval inflations, ♂ polymorphism). Cervical attributes such as the minuscule, sensillalike deirids are also widespread among Trichostrongyloidea, including Cooperiinae and Libyostrongylinae, and



FIGURES 19 and 20. *Pseudostertagia bullosa*, showing structures in females; scale bars in micrometers. **19.** Vulva and ovejectors, right lateral view, showing slight protuberance at vulva (VU); note short infundibula (INF, between dotted arrows), prominent sphincters (SP, between white arrows) composed of distal sphincter-1 (S1) and proximal sphincter-2 (S2), and vestibule (VE); note prominent constriction at junction of sphincter-2 and vestibule. **20.** Tail, left lateral view, showing digitate structure with bulbous tip (white arrow) and cuticular inflation at anus (black arrow).

among the Molineoidea and constitute the plesiomorphic condition for these taxa (Hoberg and Lichtenfels, 1994). A cuticular inflation at the anus is observed in females of *P. bullosa* and in *Libyostrongylus dentatus* Hoberg, Lloyd and Omar, 1995, among the Libyostrongylinae (Hoberg et al., 1995).

Attributes among males of *P. bullosa* are either plesiomorphic or exhibit homoplasy, again in the context of phylogenetic hypotheses for Trichostrongyloidea. Such characters include a single, unpaired 0 papilla, absence of a proconus, and the disposition of the bursal rays. The 7 papillae are reduced in size and modified in form and are similar to those found among species in the genera *Cooperia* Ransom, 1907, and *Libyostrongylus* Lane, 1923; the accessory bursal membrane is similar to that of *L. dentatus*. An elongate dorsal ray with a distal bifur-

TABLE II. Morphometrics (in micrometers; range followed by mean ± 1 standard deviation in parentheses) of males and females of *Pseudostertagia bullosa.*

Character	♂		♀	
Number examined	54		59	
Body length	(43)*5087–7443	(6618 ± 415.9)	(53)7585–10425	(1975 ± 606.4)
Esophagus length	(40)442–575	(505 ± 30.0)	(53)423–625	(544 ± 34.7)
Esophagus width	(42)22–45	(34 ± 7.2)	(53)25–60	(39 ± 7.1)
Esophagus as % body length	(38)6.5–11.2	(7.7 ± 0.76)	(50)5.1–6.8	(5.9 ± 0.4)
Cephalic vesicle length	(32)58–82	(70 ± 5.6)	(37)60–92	(72 ± 6.3)
Subventral esophageal gland orifices†	(46)140–192	(161 ± 11.0)	(47)142–175	(165 ± 6.9)
Nerve ring†	(16)202–265	(225 ± 16.0)	(17)188–238	(218 ± 14.5)
Excretory pore†	(45)248–362	(308 ± 25.0)	(47)252–345	(313 ± 19.4)
Cervical papillae†	(44)292–385	(336 ± 21.0)	(46)275–428	(345 ± 25.3)
Left spicule length	(46)142–170	(155 ± 6.6)		
Right spicule length	(46)142–170	(154 ± 6.2)		
Dorsal ray length	(41)112–155	(134 ± 9.7)		
Dorsal ray % bifurcation‡	(41)73–93	(81 ± 3.7)		
Gubernaculum	(49)52–70	(64 ± 4.1)		
Bursal length§	(39)218–285	(255 ± 16)		
Width at prebursal papillae	(39)90–128	(109 ± 8.3)		
Vulva position†			(53)6670–9075	(7956–549.5)
Vulva position as % body length			(53)85–88	(87 ± 0.9)
Anterior infundibulum length			(38)72–170	(112 ± 17.7)
Posterior infundibulum length			(38)80–138	(107 ± 14.8)
Anterior sphincter length (including vestibule)			(56)138–265	(205 ± 28.3)
Anterior sphincter complete length‖			(39)95–185	(134 ± 20.7)
Posterior sphincter length (including vestibule)			(56)110–275	(188 ± 28.4)
Posterior sphincter complete length‖			(39)62–178	(123 ± 23.6)
Ovejector length#			(29)487–788	(605 ± 68.5)
Tail length			(53)108–170	(140 ± 14.3)

* Numbers of individual specimens examined and measured.
† Measured from anterior.
‡ Percentage from anterior to bifurcation of dorsal ray.
§ Bursa length as determined from prebursal papillae. Measurement following the convention of Lichtenfels et al. (1993) that combines the sphincter and vestibule.
‖ Length from distal end of muscular bulb to muscular constriction defining distal margin of vestibule.
Complete ovejector, combining infundibula, sphincters, and vestibula.

cation can be found in genera within the Cooperiinae, the Libyostrongylinae, and in *Marshallagia marshalli* (Ransom, 1907) within the Ostertagiinae. The unbranched spicules with reduced alae appear most similar to those illustrated in descriptions of *Pararhabdonema longistriata* Kreis 1945, another monotypic genus of uncertain affinity; specimens of *P. longistriata* were not available for examination in the course of this study.

The strongly bipartite sphincters characteristic of the ovejectors in *P. bullosa* appear to differ from the condition in genera and species of the Cooperiidae. Exemplars, descriptions. and redescriptions of species from the Cooperiinae (e.g., International Institute for Parasitology, St. Albans, U.K., no. 904, *Cooperia neitzi* Mönnig, 1932), Obeliscoidinae (USNPC 88678, *Obeliscoides cuniculi* (Graybill, 1923)), and Libyostrongylinae (USNPC 83825, *L. dentatus*) lack a well-defined bipartite sphincter and only the distal component, or sphincter-1, is powerfully developed and clearly differentiated (Gibbons, 1981; Measures and Anderson, 1983; Hoberg et al., 1995; Lichtenfels et al., 2003). Additionally, a clear distinction for structure of sphincter-1, sphincter-2, and the vestibule is not evident among species of *Graphidioides* Cameron, 1923, a group currently referred to the Trichostrongylinae (Sutton and Durette-Desset, 1995; Durette-Desset et al., 1999) and is variable but typically poorly demarcated among the Ostertagiinae (Lichtenfels et al., 2003). In contrast, a well-defined 2-part sphincter, with a constriction at the junction of the proximal end of sphincter-2 and the distal vestibule, is characteristic of most genera of Haemonchinae, among *Trichostrongylus* spp., in a putative ostertagiine, *Graphidium strigosum* (Dujardin, 1845) (USNPC 66335) and in *Parostertagia heterospiculum* Schwartz and Alicata, 1933 (USNPC 30166) (Hoberg and Lichtenfels, 1992; Lichtenfels, Pilitt, and Gibbons, 2003).

DISCUSSION

Systematics of *P. bullosa*

A comparative context is provided for new assessments of the synlophe and other morphological characters for specimens of *P. bullosa*. It is apparent that few characters are significant in revealing relationships for *P. bullosa*, as most attributes constitute the plesiomorphic condition within the Trichostrongyloidea based on current phylogenetic interpretations (Hoberg and Lictenfels, 1994; Durette-Desset et al., 1999). Additional characters, including the unique structure of the buccal capsule and gubernaculum, constitute putative autapomorphies for *P. bullosa*.

Comparative or phylogenetic studies of the Trichostrongy-

loidea have recognized *P. bullosa* as an inclusive member of the Libyostrongylinae (e.g., Durette-Desset and Chabaud, 1977, 1981; Durette-Desset, 1985; Hoberg and Lichtenfels, 1994) or among the Cooperiinae following evaluation of putative relationships among an array of genera to reveal higher level systematics (Durette-Desset et al., 1999). Durette-Desset et al. (1999), in contrast with prior opinions (Durette-Desset and Chabaud 1977, 1981), transferred *P. bullosa* from the Libyostrongylinae to the Cooperiinae and retained these subfamilies along with the newly established Obeliscoidinae in the family Cooperiidae.

In this new classification, phylogenetic diagnosis for Cooperiidae was based on a single character, the relative length, position, and spacing of bursal rays 2 and 3 and their degree of convergence distally (character 28 of Durette-Desset et al., 1999). In specimens of *P. bullosa* examined by us and those studied by Gibbons and Khalil (1982), rays 2 and 3 are divergent at their bases; distally, the tips are not strongly convergent; and ray 3 is only marginally ≥ ray 5, clearly depicted in Figure 134 of Gibbons and Khalil (1982). We suggest that interpretation of this putative synapomorphy that provides the basis for inclusion of *P. bullosa* among the Cooperinae is ambiguous at best and may be open to interpretation. In analyses outlined by Durette-Desset et al. (1999), 11 of 48 characters are based on the arrangement of bursal rays and most are not applicable to the Cooperiidae. We suggest that such attributes must be evaluated conservatively given recognition of the problematic or subjective nature of such characters in providing an unambiguous diagnosis for relationships of *P. bullosa* and the Cooperiidae.

Four other traits may determine the position of *P. bullosa* as the basal member of the Cooperiinae or simply may serve to further emphasize the independence of this taxon: (1) the 2-2-1 arrangement of the bursal rays and hypertrophy of the ventral lobe of the bursa (character 46 of Durette-Desset et al., 1999); (2) a ventral cuticular inflation, or bulla, anterior to the bursa (Ransom and Hall, 1912) may constitute a putative autapomorphy; (3) the broad, complex gubernaculum with multiple process directed anteriad and posteriad is unique (Olsen, 1950); and (4) a rounded buccal aperture with esophageal cuticular thickening is recognized as another autapomorphic character for the genus (character 7 of Durette-Desset et al., 1999), although this structure also appears to be present in *P. heterospiculum* (Hoberg and Lichtenfels, 1992). Based on this suite of attributes, the relationships for *P. bullosa* seem ambiguous.

Cuticular ridge systems or the synlophe in specimens of *P. bullosa* are unremarkable and exhibit minimal modification. A parallel, continuous, and unmodified synlophe represents the putative plesiomorphic condition for the Trichostrongyloidea (Hoberg and Lichtenfels, 1992; Hoberg et al., 1993b). Putative synapomorphies for Cooperiinae include (1) convergent addition of ridges in the lateral fields, (2) prominent struts constituting the synlophe, (3) bilateral hypertrophy of the synlophe adjacent to the vulva, (4) sequential increase in numbers of ridges posteriad of the deirids based on addition in the lateral fields, and (5) an axis of orientation other than perpendicular in some taxa. Specimens of *P. bullosa* are characterized by a nearly constant number of ridges posteriad from the deirids and any addition of ridges is not convergent on the lateral field. Thus, the synlophe is structurally inconsistent with Cooperiinae (see Lichtenfels, 1977; Hoberg and Lichtenfels, 1992; Hoberg et al., 1993a). These 2 characters of the synlophe were not considered by Durette-Desset et al. (1999) but may, upon more detailed evaluation among genera and species relegated to the Cooperiinae, contribute to phylogenetic diagnosis for the group; they do not reveal insights about relationships for *P. bullosa*.

The synlophe in *P. bullosa* is also superficially similar to ridge systems described among some Ostertagiinae. Specifically, Lichtenfels et al. (1988) defined the Type I pattern for the tapering lateral synlophe in the cervical zone of *Ostertagia ostertagi* (Stiles, 1892). This pattern is also seen in *Ostertagia bisonis* Chapin, 1925, *Camelostrongylus mentulatus* (Railliet and Henry, 1909), *Longistrongylus sabie* (Mönnig, 1932), *Longistrongylus curvispiculum* (Gibbons, 1973), *Teladorsagia circumcincta* (Stadelman, 1894), *Teladorsagia boreoarcticus* Hoberg, Monsen, Kutz, and Blouin, 1999, and *Sarwaria bubalis* (Sarwar, 1956), and represents an apomorphic state among the ostertagiines (Hoberg and Lichtenfels, 1992; Hoberg et al., 1993b, 1999; E. P. Hoberg and A. Abrams, unpubl. data). Consequently, variation on this theme for a tapering cervical synlophe represents convergence among these ostertagiines and *P. bullosa* (Lichtenfels et al., 1988, 1996; Hoberg et al., 1993c); *Pseudostertagia* clearly lacks the suite of unequivocal synapomorphies that otherwise diagnose the inclusive Ostertagiinae.

Additionally, the synlophes defined for the Haemonchinae (e.g., Lichtenfels and Pilitt, 2000; Hoberg et al., 2002) differ substantially from that in *P. bullosa*. A suite of other characters also clearly distinguishes members of the Haemonchinae (Durette-Desset et al., 1999; Hoberg et al., 2004).

Among other characters, the surface and underlying cuticular region adjacent to vulva lacks modification in specimens of *P. bullosa*. Perivulvar protuberances (character 24 of Durette-Desset et al., 1999) are absent in *Pseudostertagia*, consistent with the proposed plesiomorphic condition (e.g., Hoberg and Lichtenfels, 1992; Durette-Desset et al., 1999). In contrast, they are present as irregular inflations of varying dimensions and orientation in some Ostertagiinae (*Camelostrongylus*, *Cervicaprastrongylus*, *Hyostrongylus*, *Longistrongylus*, *Mazamastrongylus*) (Hoberg et al., 1993a, 1993b) and among *Libyostrongylus* (Hoberg et al., 1995). These are distinguished structurally from the bilateral and symmetrical vulval fans, such as those in some *Cooperia* spp. and *P. heterospiculum*, that are supported by hypertrophied struts of the synlophe (e.g., Hoberg et al., 1993a). It is further apparent that 2 distinct classes of characters are represented by cuticular hypertrophy at the level of the vulva (Hoberg et al., 1993a). Consequently, the character coding proposed by Durette-Desset et al. (1999) for *Parostertagia*, *Libyostrongylus*, the Cooperiinae, and the Ostertagiinae is incomplete.

The structure of the ovejectors in *P. bullosa* shows similarities to that typical among the Haemonchinae but is quite distinct from the condition observed among any of the subfamilies referred to Cooperiidae (Lichtenfels et al., 2003). Considerably greater detail is required across the diversity of the Trichostrongylina before hypotheses about the structural evolution of this attribute can be articulated and evaluated in a phylogenetic context.

All members of Cooperiinae occur in the intestinal tract, a site of localization that is plesiomorphic for the Trichostrongyloidea (Hoberg and Lichtenfels, 1994). *Pseudostertagia* oc-

curs in the abomasum, a site that appears to have been independently colonized during the evolution of Ostertagiinae + Haemonchinae and by species of *Trichostrongylus* Looss, 1905. This constitutes yet another character exhibiting homoplasy, and apparently it has no bearing on understanding the relationships for *P. bullosa*.

The systematic placement of *P. bullosa* remains uncertain based on relationships proposed by either Hoberg and Lichtenfels (1994) or Durette-Desset et al. (1999). Placement within the Cooperiinae is problematic due to the suite of autapomorphic characters and symplesiomorphic attributes of the synlophe shared among the putative inclusive taxa (Hoberg and Lichtenfels, 1992, 1994). Alternatively, the 2-2-1 bursa proposed as the sole synapomorphy for the Cooperiidae (Durette-Desset et al., 1999) is not consistent across the group (including constituent Libyostrongylinae, Obeliscoidinae, and Cooperiinae) and exhibits some homoplasy with the Ostertagiinae (e.g., *Teladorsagia*, *Hyostrongylus*, *Sarwaria*, *Spiculopteragia*, *Mazamastrongylus*). *Pseudostertagia* to some extent remains enigmatic among the trichostrongyloids and may further reflect its isolated status as a typical parasite in the relictual pronghorn, *A. americana*, of North America.

Host associations for *Pseudostertagia*

Subsequent to its original description from domestic sheep, *P. bullosa* was reported in *A. americana*, initially from the National Zoological Park, Washington, D.C. (Price, 1929) and later from Montana and South Dakota in 1944 (Lucker and Dikmans, 1945). Based on their observations of large numbers of *P. bullosa* in *A. americana* relative to the minimal numbers found in *O. aries* and the absence of reports from domestic sheep following its original description, Lucker and Dikmans (1945) regarded this nematode as a characteristic parasite in pronghorn. They advanced the concept that specified presence of infected antelope within the same range as sheep as a prerequisite for infections in the latter host species. Except for the reports of Olsen (1950) and Honess (1951) of *P. bullosa* in *O. aries* from New Mexico and Wyoming, respectively, all records of *P. bullosa* subsequent to the original description have been in association with wild hosts, with the highest prevalence and intensity of infection reported from *A. antilocapra* (Hoberg et al., 2001). Additional host and locality records for *P. bullosa* include *O. canadensis* in New Mexico (Allen, 1955), *A. americana* in North Dakota (Goldsby and Eveleth, 1954), and *O. hemionus* in Montana (Worley and Eustace, 1972). Host and locality records for *P. bullosa* in wild hosts are summarized by Hoberg et al. (2001).

Pseudostertagia bullosa may be expected in mixed infections with various ostertagiines, including *Ostertagia bisonis*, *Ostertagia leptospicularis*, *Ostertagia ostertagi*, *M. marshalli*, and *T. circumcincta* and their minor morphotypes in the abomasum (Hoberg et al., 2001). Based on comparisons of whole specimens, it is distinguished from these parasites by the absence of a basal esophageal valve and the pattern of the cervical synlophe in males and females and by the structure of the bursa and spicules in males (Lichtenfels and Pilitt, 1989, 1991; Hoberg et al., 1993c; Hoberg and Abrams, 2001). In histological section of abomasal tissue, however, the range for numbers of ridges at the midbody overlaps with many of these sympatric ostertagiines: *O. bisonis* (36–41), *T. circumcincta* (29–42), *O. ostertagi* (32–41), *Ostertagia leptospicularis* (32–33), whereas it is substantially greater in *M. marshalli* (51–56). The narrow spacing of ridges in the lateral fields of the synlophe among these species is similar to *P. bullosa*, such that these nematodes cannot be reliably distinguished in typical histological sections.

The restricted geographic range of *P. bullosa* may be determined by the distribution of its apparently specific host, *A. americana*. Pronghorn are relicts of a once diverse antilocaprid fauna that developed through an extensive radiation that was restricted to North America during the Pliocene (Kurtén and Anderson, 1980). Early antilocaprids are first known in the Nearctic during the middle Miocene following range expansion of ancestral pecorans from Eurasia across Beringia. *Antilocapra americana* is the sole extant representative in the Nearctic, although related antilocaprids ranged as far south as Florida during the Pliocene. Assuming a correct placement for *P. bullosa* among the basal Cooperiinae, occurrence of this nematode may indicate an early invasion by members of this subfamily into North America. *Pseudostertagia bullosa* would represent the sole persistent member of a cooperine lineage that is endemic to western North America (Hoberg, 2005); the current distribution of *Cooperia* spp. in the Nearctic is attributable to secondary introduction coinciding with translocation of domestic stock and their parasites after the 1500's (Hoberg et al., 2001; Hoberg, 2005). These observations serve to emphasize the mosaic nature of the parasite fauna in pronghorn and other artiodactyls in the Nearctic (Hoberg et al., 1999; Hoberg, 2005)

The current geographic distribution further suggests that free-living stages of this species of nematode may be adapted to a xeric environment in such a way as to prevent the establishment *P. bullosa* following the translocation of suitable hosts. Such could account for its absence in *O. aries* from more humid regions to the east and in the Pacific Northwest and from black-tailed deer on the west coast of North America and into the northern Rocky Mountains. A similar restriction to xeric conditions may also explain the absence of *Nematodirella antilocaprae* (Price, 1927), an intestinal parasite of *A. americana*, from areas outside the range of its host. In the context of ecological perturbation associated with climate warming, we may anticipate altered host and geographic associations for *P. bullosa* and an array of other nematodes in artiodactyls (Hoberg, 1997, 2005; Hoberg et al., 2004).

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