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New Morphological Characters for Identifying Individual Specimens of *Haemonchus* spp. (Nematoda: Trichostrongyloidea) and a Key to Species in Ruminants of North America

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NEW MORPHOLOGICAL CHARACTERS FOR IDENTIFYING INDIVIDUAL SPECIMENS OF *HAEMONCHUS* SPP. (NEMATODA: TRICHOSTRONGYLOIDEA) AND A KEY TO SPECIES IN RUMINANTS OF NORTH AMERICA

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ABSTRACT: The large stomach worms *Haemonchus contortus, Haemonchus placei*, and *Haemonchus similis* are important pathogens of cattle and sheep. This paper describes characteristics of surface cuticular ridges (synlophe), which for the first time provide morphological criteria for identifying individual adult specimens of either sex. The diagnostic patterns of the synlophe on the anterior half of specimens can be observed at 400× in temporary mounts on glass slides. The synlophe can be studied in cleared preserved specimens or in living or freshly thawed frozen specimens mounted in water. The synlophe of *H. contortus* has 30 ridges in the region of the posterior half of the esophagus, 4 fewer than *H. placei* and *H. similis*. The 4 extra ridges of *H. placei* and *H. similis* are consistently located bilaterally to the 3 ventralmost and the 3 dorsalmost ridges. The 4 extra ridges of *H. similis* extend to the end of the synlophe posterior to midbody, but in *H. placei* they extend posteriorly only to the end of the anterior quarter of the nematode. A key is included to the 3 species of *Haemonchus* parasitic in domestic sheep and cattle using characteristics of spicules, female reproductive system, female tail, and the synlophe.

Three species of the genus *Haemonchus* Cobb, 1898, are known to occur in domestic and wild ruminants of North America. They are common parasites of domesticated ruminants and have achieved a worldwide distribution in part due to the movement of their economically important hosts. These 3 species of large stomach worms are among the most pathogenic nematodes of sheep, cattle, and goats worldwide, causing significant production losses due to morbidity, mortality, costs of treatment, and suboptimal use of contaminated pastures (Gibbs and Herd, 1986).

Haemonchus contortus (Rudolphi, 1803) Cobb, 1898, primarily a parasite of domestic sheep, *Ovis aries*, also infects numerous other domestic and wild ruminants.

Haemonchus placei (Place, 1893) Ransom, 1911, is primarily a parasite of domestic cattle (*Bos taurus*) but it has been found also in domestic sheep, white-tail deer (*Odocoileus virginianus*), and pronghorn antelope (*Antilocapra americana*). *Haemonchus placei* is not universally accepted as a separate species (Gibbons, 1979). However, Lichtenfels et al. (1986) described differences between *H. placei* and *H. contortus* in the percentage of the body covered by the synlophe, which made it possible to identify most populations to species if at least 10 specimens are examined. This paper describes newly

recognized features of the synlophe that can be used to determine the species of individual specimens of either sex of *H. contortus* and *H. placei*.

The third species of the genus *Haemonchus* parasitic in North American ruminants is *H. similis* Travassos, 1914. This species has been reported in domestic cattle in a few southern states of the U.S.A., Central and South America, Asia, and several Atlantic and Pacific islands (Donald, 1963), in *Bos indicus* in Tanzania and Uganda (Gibbons, 1979), domestic sheep in Brazil (Lins de Almeida, 1935), white-tail deer in Florida (Dinaburg, 1939), and in numerous other ruminants and localities (Doss et al., 1985). In addition to differences in the synlophe, distinctive characteristics of the spicules, female reproductive tract, and female tail allow *H. similis* to be separated from both *H. contortus* and *H. placei*.

An illustrated, dichotomous key to males and females of *H. contortus, H. placei*, and *H. similis* is presented. This key makes it possible to identify individual specimens of all 3 species of large stomach worms present in North American ruminants.

Seven additional species of *Haemonchus* (review by Gibbons, 1979) that parasitize wild ruminants of Africa and/or the camel (Dutt and Sahai, 1966) were not included in the present study.

MATERIALS AND METHODS

A list of nematodes studied is presented (Table I) including host names, geographic localities, number of

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TABLE I. Specimens of *Haemonchus* spp. studied by nematode species, host species, locality, and nematode sex.

Collection number*	Species	Host	Locality	Number studied	
				Male	Female
39960	*Haemonchus contortus*	*Ovis aries*	Florida	1	2
64756	*H. contortus*	*O. aries*	Georgia	0	1
70001	*H. contortus*	*O. aries*	Georgia	5	5
33156	*H. contortus*	*O. aries*	Maine	4	4
69990	*H. contortus*	*O. aries*	Maryland	1	2
70404†	*H. contortus*	*O. aries*	Michigan	5	5
70405‡	*H. contortus*	*O. aries*	Michigan	5	5
70406§	*H. contortus*	*O. aries*	Michigan	5	5
47825	*H. contortus*	*O. aries*	Nebraska	4	4
40172	*H. contortus*	*O. aries*	North Carolina	5	4
40659	*H. contortus*	*O. aries*	Texas	1	0
‖	*H. contortus*	*O. aries*	Texas	0	3
‖	*H. contortus*	*O. aries*	Texas	1	1
‖	*H. contortus*	*O. aries*	Texas	0	1
‖	*H. contortus*	*O. aries*	Texas	2	2
58830	*H. contortus*	*O. aries*	Vermont	0	5
56711	*H. contortus*	*O. aries*	West Virginia	3	1
69996	*H. contortus*	*O. aries*	Australia	2	1
81048	*H. contortus*	*O. aries*	Brazil	5	5
#	*H. contortus*	*O. aries*	South Africa	12	10
70374	*H. contortus*	*Bos taurus*	Maryland	25	25
67080	*H. contortus*	*Capra hircus*	Sierra Leone	1	4
67185	*H. contortus*	*Odocoileus virginianus*	Oklahoma	2	2
66540¶	*H. contortus*	*O. virginianus*	Texas	6	0
32146**	*H. contortus*	*Ovibos moschatus*	Washington, D.C.	5	2
70277	*H. contortus*	*Pelea capreolus*	South Africa	5	5
70276	*H. contortus*	*Redunca redunca*	South Africa	5	5
66649¶	*H. contortus*	*Syncerus caffer*	Uganda	3	3
39902	*Haemonchus placei*	*B. taurus*	Florida	2	1
69986	*H. placei*	*B. taurus*	Florida	5	0
70247	*H. placei*	*B. taurus*	Florida	3	2
49243	*H. placei*	*B. taurus*	Hawaii	0	5
69987	*H. placei*	*B. taurus*	Louisiana	5	5
70382	*H. placei*	*B. taurus*	Louisiana	16	10
47217	*H. placei*	*B. taurus*	Louisiana	4	6
58800	*H. placei*	*B. taurus*	Louisiana	3	0
29346	*H. placei*	*B. taurus*	Maryland	3	5
70324††	*H. placei*	*B. taurus*	Maryland	0	1
70372††	*H. placei*	*B. taurus*	Maryland	20	11
70373††	*H. placei*	*B. taurus*	Maryland	10	10
70375††	*H. placei*	*B. taurus*	Maryland	5	5
70377	*H. placei*	*B. taurus*	Maryland	6	6
70385††	*H. placei*	*B. taurus*	Maryland	0	1
19472	*H. placei*	*B. taurus*	Mississippi	4	5
70138	*H. placei*	*B. taurus*	New Jersey	2	1
‖	*H. placei*	*B. taurus*	Texas	3	0
59090	*H. placei*	*B. taurus*	Guyana	3	3
70402	*H. placei*	*B. taurus*	South Africa	8	6
66540¶	*H. placei*	*O. virginianus*	Texas	1	2
‖	*H. placei*	*O. aries*	Texas	0	1
69995	*H. placei*	*O. aries*	Australia	5	5
43625	*Haemonchus similis*	*O. virginianus*	Florida	1	0
34073	*H. similis*	*B. taurus*	Florida	0	4
39902	*H. similis*	*B. taurus*	Florida	9	4
70247	*H. similis*	*B. taurus*	Florida	1	2
70301††	*H. similis*	*B. taurus*	Maryland	7	5
70324††	*H. similis*	*B. taurus*	Maryland	8	2
70375††	*H. similis*	*B. taurus*	Maryland	0	2
70385††	*H. similis*	*B. taurus*	Maryland	20	1
45184	*H. similis*	*B. taurus*	North Carolina	0	3
29412	*H. similis*	*B. taurus*	Puerto Rico	2	2
29462	*H. similis*	*B. taurus*	Texas	1	2
59091	*H. similis*	*B. taurus*	Guyana	3	3
66648	*H. similis*	*Bos indicus*	Uganda	3	3

* Collection numbers of specimens deposited in the U.S. National Parasite Collection.
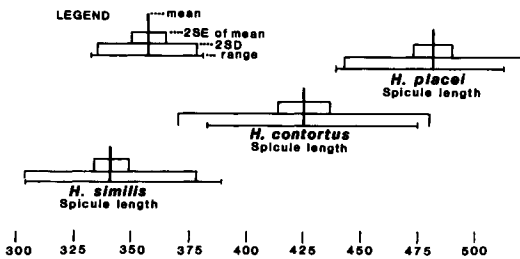† Susceptible strain from G. A. Conder, Kalamazoo, Michigan.

FIGURE 1. Spicule lengths (from data in Table II) of 3 species of *Haemonchus* with 95% confidence bands for mean (±2 SE) and population (±2 SD).

specimens examined, and collection numbers of specimens deposited in the U.S. National Parasite Collection (USDA, ARS, BARC-East, Bldg. No. 1180, Beltsville, Maryland). Frozen specimens from T. M. Craig were used (Zarlenga et al., 1994) in DNA studies and therefore not deposited. Specimens from South Africa loaned for study by J. A. van Wyk were returned to him.

Whole specimens were cleared in phenol–alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol) and studied in temporary mounts. Interference-contrast light microscopy was used to study the synlophe and other characters at a magnification of 400×. Frozen specimens can be studied in water. Living specimens should be studied in saline, and the coverglass should be supported by glass beads if the viability of the specimen is to be preserved. We have not tested the viability of such specimens.

Cross sections were studied as either freehand cuts made with a cataract knife and mounted in glycerine jelly or in paraffin-embedded sections cut at 5 μm with a microtome and stained in hematoxylin and eosin. Measurements of specimens were obtained with the aid of a calibrated ocular micrometer on a compound microscope and are recorded in micrometers unless indicated otherwise. Drawings of the synlophes were prepared free-hand while viewing the specimens at a magnification of 400×.

The description of the ovejectors follows the detailed description of the ovejectors of *H. contortus* by Veglia (1915). The terminology of Veglia (1915) is modified as follows. The elongate cylindrical sphincters of Strongyloidea (see Lichtenfels, 1980) are homologous to the pars ejectrix of Veglia (1915). The anterior portion of the pars ejectrix of *Haemonchus* spp. is sphincter-

shaped, but the pars ejectrix also includes a cylindrical part that is clearly distinguishable from the thinner-walled vestibule (Figs. 13–15). The infundibulum is equivalent to the pars haustrix of Veglia (1915).

Populations of nematodes were identified to species as follows: *H. similis* were distinguished from *H. contortus* and *H. placei* on the basis of spicular structure and length, and vulvar structure and tail length. Then *H. contortus* and *H. placei* were separated on the basis of spicule lengths (Lichtenfels et al., 1988) or on the percentage of the body bearing the longitudinal surface cuticular ridges of the synlophe (percentage of total
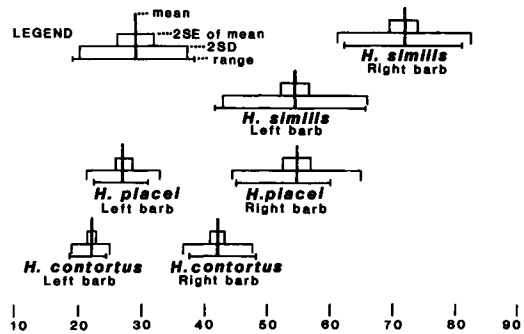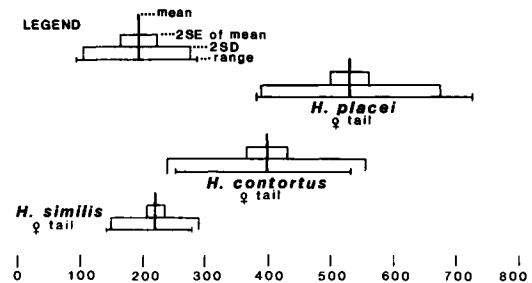

FIGURE 2. Distances (from data in Table II) from spicule barbs to distal tips of spicules of 3 species of *Haemonchus* with 95% confidence bands for mean (±2 SE) and population (±2 SD). See also Figures 10–12.

FIGURE 3. Tail lengths (from data in Table III) of females of 3 species of *Haemonchus* with 95% confidence bands for mean (±2 SE) and population (±2 SD). See also Figures 16–18.

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‡ Ivermectin/benzimidazole-resistant strain from G. A. Conder, Kalamazoo, Michigan.

§ Levamisole/benzimidazole-resistant strain from G. A. Conder, Kalamazoo, Michigan.

‖ All specimens (6 lots) from T. M. Craig, Texas. (1) from Uvalde County, ivermectin/levamisole resistant; (2) original host *Addax nasomaculata*, with experimental infections in lambs; (3) from the Craig ranch; (4) from east Texas, original host *Capra hircus* with experimental infection in lambs, benzimidazole/ivermectin resistant; (5) from Brazos County, raised in cattle; (6) from Boerne, Kendall County. Sheep grazed on pasture with exotic antelope.

Specimens from sheep in South Africa from J. A. van Wyk.

¶ Possible hybrid specimens; short or intermittent subventral and subdorsal ridges.

** Specimens from a National Zoological Park animal. Original locality unknown.

†† Experimental infections in calves in Maryland, originally from tracer calves in Florida.
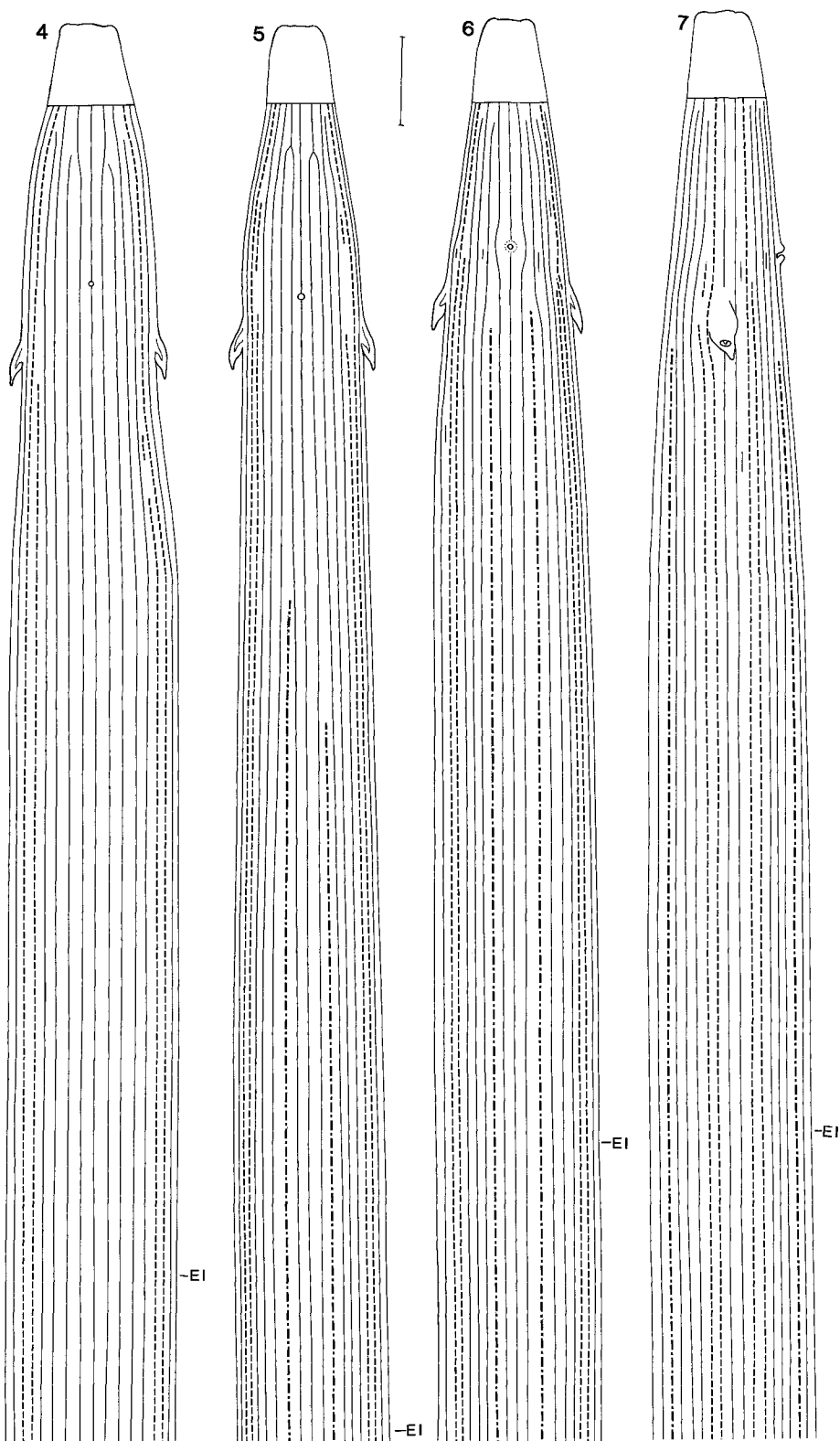
length in males and percentage of prevulvar length in females) (Lichtenfels et al., 1986). Specimens previously identified in the cited earlier studies were used when possible. The numbers of specimens listed in Table I are those used to evaluate the newly described synlophe characteristics.

Samples were selected to represent as wide a geographic and host range as possible. Measurements were made of a few specimens from each of a large number of individual hosts because variation among samples from individual hosts was expected to be greater than variation within one sample (Lichtenfels, unpubl. data). Observed differences in size of various structures among the 3 species (Tables II, III) were compared statistically by calculating 95% confidence bands for the samples (±2 SD) and for the sample means (±2 SE) (Steel and Torrie, 1960). The results were illustrated graphically (Figs. 1–3) as described by Lichtenfels et al. (1988).

RESULTS

Size differences

Morphometrics of *H. contortus*, *H. placei*, and *H. similis* are given by sex in Table II (males) and Table III (females). Among males of the 3 species, the most useful size differences are spicule lengths (Fig. 1) and distances of the barbs from the distal end (Fig. 2). Figure 1 shows that the mean spicule lengths of all 3 species differ significantly from each other (95% confidence level), but that overlapping ranges of spicule lengths prevent identification of individual specimens. The relative position of the distal barbs of the spicules (Figs. 2, 10–12) of *H. similis* can be used to distinguish unequivocally males of this species from those of *H. contortus* and *H. placei*. Of the 3 species, only in *H. similis* are the barbs of both the left and right spicules greater than 10% of the spicule length from the distal tips. The barb of the left spicule of *H. similis* is significantly farther (20–21% of spicule length) from the distal tip than in the other 2 species (both about 11%). The barb of the right spicule of *H. similis* is significantly farther from the distal tip (13–17% of spicule length) than in *H. contortus* and *H. placei* (both about 5% of spicule

length), but the difference in range of measurements between *H. similis* and *H. placei* (Fig. 2) is not statistically significant (see overlapping bars for 2 SD). Thus, the barbs of the right spicules of *H. placei* and *H. similis* can be distinguished by the percentage of total length from the distal tip to the barb but not by actual measurements because of the significantly greater spicule lengths (Fig. 1) of the latter species. The means of the distances from spicule barb to distal tip can also be used to separate *H. placei* and *H. contortus* males, but the overlapping ranges of measurements prevent the use of this character for identification of individual males of these species.

Females of *H. similis* can be distinguished from those of *H. placei* by their significantly shorter tail length (Figs. 3, 16–18; Table III); and most specimens of *H. similis* can be distinguished from both *H. placei* and *H. contortus* by the presence of a longer vagina (Table III) that usually extends into a vulval flap and opens in the flap (Fig. 15) rather than on or near the body wall as in the other 2 species (Figs. 13, 14).
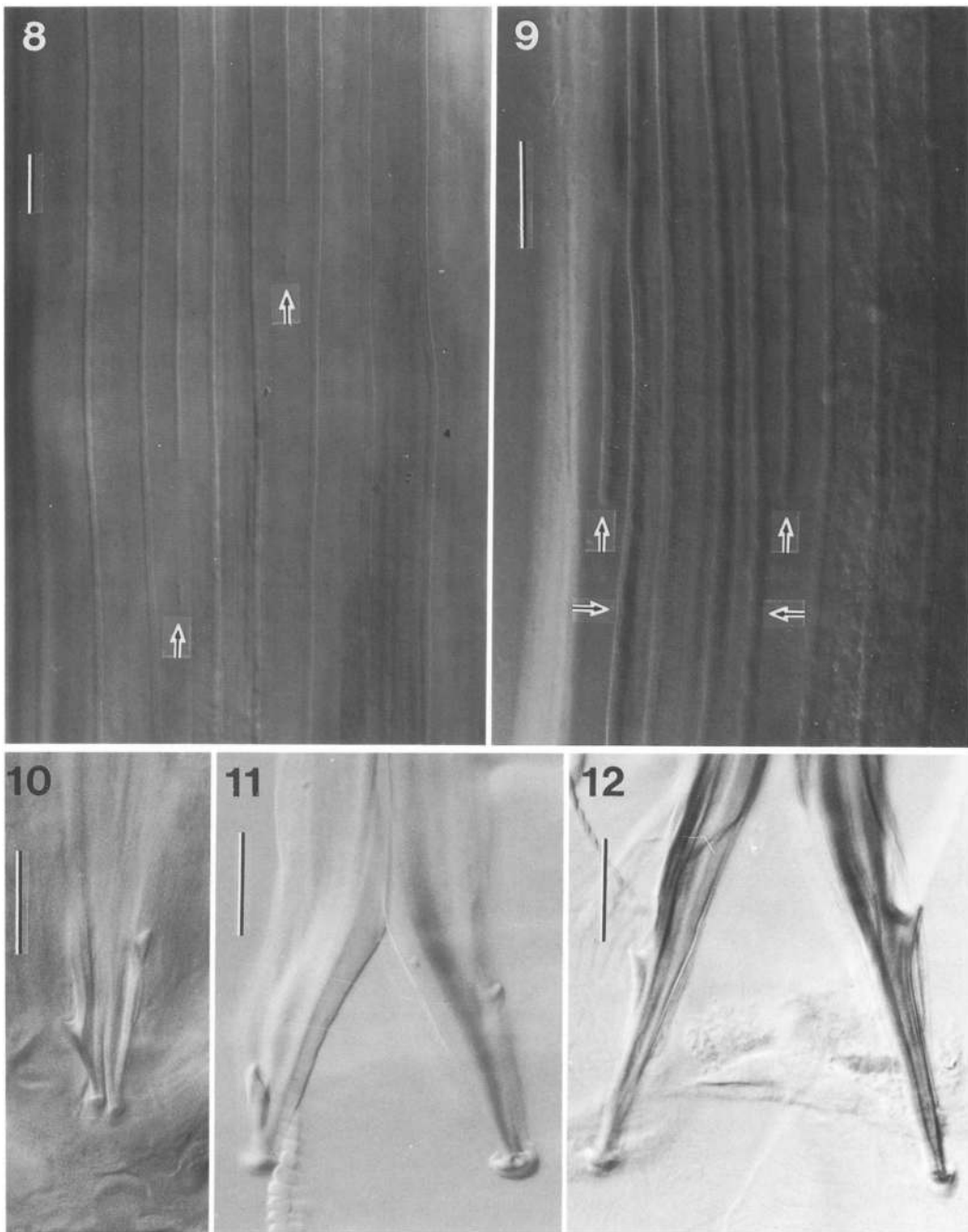
Synlophe

The synlophe in all 3 species is bilaterally and dorsoventrally symmetrical with the result that left and right, and also dorsal and ventral halves, are mirror images of each other. There is no ridge at the lateral line so each of the 30 or 34 ridges is in either the dorsal or ventral field (Figs. 4–7). In all 3 species the third ridge from the lateral chord both dorsally and ventrally is shorter than adjacent ridges and usually extends anteriorly only to the level of the cervical papillae, although in some specimens it ends well anterior to the excretory pore.

Both *H. similis* and *H. placei* bear 17 dorsal and 17 ventral (total 34) longitudinal cuticular ridges in the region of the posterior half of the esophagus (Figs. 5–7, 23, 27). In *H. contortus*,

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FIGURES 4–7. Diagrammatic drawings of the anterior ends of 3 species of *Haemonchus* showing the synlophe patterns in the region of the esophagus (dashed lines, sublateral ridges; dot-dash lines, subventral or subdorsal ridges; EI, esophageal–intestinal junction) (scale bar = 100 μm). Note that in ventral views (Figs. 4–6) because there are no lateral ridges dividing ventral and dorsal fields, the lateralmost lines in these figures represent the lateralmost ridges of the dorsal field. **4.** Anterior synlophe of *H. contortus,* ventral view, showing 17 of 30 ridges at EI. Note absence of subventral ridges. **5.** Anterior synlophe of *H. placei,* ventral view, showing 19 of 34 ridges at EI. Note presence of subventral ridges. **6.** Anterior synlophe of *H. similis,* ventral view, showing 19 of 34 ridges at EI. Note presence of subventral ridges. **7.** Lateral view of *H. similis* showing one ridge of each pair of subdorsal and subventral ridges, which are present in *H. similis* and in *H. placei.*

FIGURES 8–12.   Lateral synlophe patterns and spicule tips of 3 species of *Haemonchus* (scale bars = 25 μm). **8.** Lateral synlophe of *H. contortus* showing distal ends (arrows) of more lateral of the sublateral ridges ending next to paired lateralmost ridges. **9.** Lateral synlophe of *H. similis* showing distal ends (vertical arrows) more dorsal and ventral of sublateral ridges adjacent to more lateral pair of sublateral ridges (horizontal arrows) adjacent to paired lateralmost ridges. **10.** Spicule tips of *H. contortus.* **11.** Spicule tips of *H. placei.* **12.** Spicule tips of *H. similis.*

TABLE II. Morphometrics of *Haemonchus* spp. males.*

Character	*Haemonchus contortus*	*Haemonchus placei*	*Haemonchus similis*
Number examined	23	21	24
Body length (mm)	11.0–17.0 (13.1)	10.9–18.9 (14.4)	7.3–10.1 (8.8)†
Nerve ring‡	188–326 (254)	235–319 (287)	210–244 (228)
Excretory pore‡	232–356 (285)	266–367 (321)	225–274 (246)
Cervical papillae‡	271–462 (354)	351–440 (403)	281–353 (312)
Subventral esophageal gland orifices‡	356–626 (493)	506–668 (587)	394–506 (448)
Esophagus length (mm)	1.09–1.55 (1.26)	1.31–1.74 (1.48)	0.82–1.28 (1.10)
Esophagus as percentage of body length	8.0–11.2 (9.8)	8.6–12.1 (10.4)	9.9–14.8 (12.6)
Gubernaculum length	195–255 (221)	210–270 (245)	142–198 (179)†
Spicule length	383–475 (425)	438–511 (481)	304–389 (341)†
Spicule barb length right/left	37–48 (42)/19–24 (22)	45–60 (55)/22–32 (27)	62–81 (71)†/41–65 (54)†

* Measurements (in micrometers unless noted otherwise) are ranges followed by means in parentheses.
† Measurements that differ significantly (95% confidence) from those of at least 1 of the other 2 species.
‡ Measured from anterior extremity.

TABLE III. Morphometrics of *Haemonchus* spp. females.*

Character	*Haemonchus contortus*	*Haemonchus placei*	*Haemonchus similis*
Number measured	22	21	26
Body length (mm)	14.8–27.2 (18.5)	12.5–25.5 (19.6)	10.4–17.0 (13.4)
Nerve ring†	210–394 (271)‡	225–330 (290)	165–300 (229)
Excretory pore†	205–400 (290)	281–379 (330)	188–289 (246)
Cervical papillae†	243–484 (363)	359–460 (414)	233–379 (313)
Subventral esophageal gland orifices†	435–656 (525)‡	540–728 (623)	443–564 (500)
Esophagus length (mm)†	1.15–1.66 (1.38)	1.38–1.84 (1.60)	1.09–1.42 (1.25)
Esophagus as percentage of body length	5.8–8.9 (7.6)	6.7–11.1 (8.3)	7.8–11.5 (9.5)
Vulva position (percentage)†	76.3–84.8 (82.4)	74–84 (81)	75.6–89.1 (81.7)
Anterior infundibulum length	188–394 (272)	188–461 (290)	149–319 (204)
Anterior spincter length	154–284 (222)	191–356 (244)	124–249 (184)‖**
Vestibula length	150–263 (198)	225–356 (261)	—
Vagina length	75–188 (110)	75–131 (104)#	161–300 (217)¶††
Posterior sphincter length	158–266 (227)	182–330 (245)‡	143–300 (216)‖
Posterior infundibulum length	169–341 (277)§	240–441 (306)	152–300 (205)‖
Tail length	251–530 (396)	378–720 (527)	135–278 (219)††
Phasmids (right/left)‡‡	86–235 (143)/84–249 (143)	122–239 (167)/125–239 (171)	52–93 (74)§††/60–93 (77)††

* Measurements (in micrometers unless noted otherwise) are ranges followed by means in parentheses.
† Measured from anterior extremity.
‡ n = 20.
§ n = 21.
‖ n = 22.
n = 19.
¶ n = 24.
** Measurement includes vestibula length.
†† Measurements that differ significantly (95% confidence) from at least 1 of the other 2 species.
‡‡ Distance from distal tip of tail.

there are only 15 ridges dorsally and 15 ventrally (total 30) (Figs. 4, 19). This number was constant in both males and females in all lots that were studied, but minor variations were frequently observed due to either the presence of short extra ridges in atypical locations (Figs. 6, 7) or crossovers where one ridge reversed positions with an adjacent ridge.

Two of the 4 additional ridges present in *H. similis* and *H. placei* are illustrated as dot–dash lines in Figures 5 and 6. They are situated 1 on each side of the 3 ventralmost ridges and are herein referred to as subventral ridges. There are also 2 similar "subdorsal" ridges in an equivalent position, subdorsally in these 2 species. The subventral and subdorsal ridges usually extend anteriorly only to a level equal to the middle third of the esophagus but more rarely as far anteriorly as the cervical papillae or excretory pore. They are continuous for most of their length but may be interrupted near their anterior and posterior ends. *Haemonchus placei* differs from *H. similis* in that the subventral and subdorsal ridges of the former extend posteriorly for 4–5 mm and end
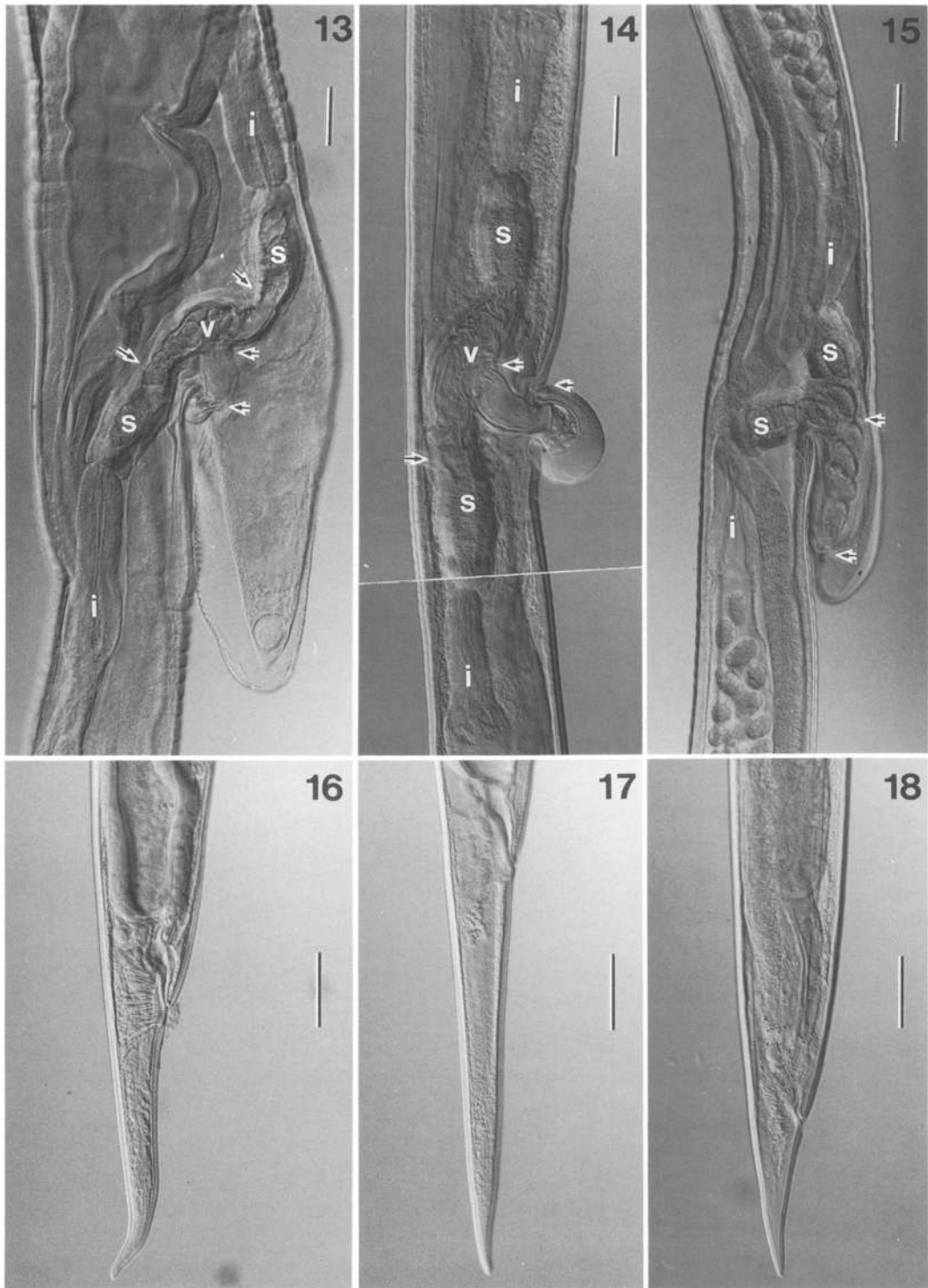
FIGURES 13–18.   Paired, opposed ovejectors and tails of females of 3 species of *Haemonchus* (i, infundibula; s, sphincters; v, vestibule; arrows, both ends of vagina and at junction of sphincters and vestibule) (scale bars = 100 μm). Note that variation in the anatomy of vulval lobes within species is high and is not a reliable character for identifying species. **13.** Ovejectors of *H. contortus* with large lobe over vulva. **14.** Ovejectors of *H.*

near the middle of the length of the synlophe (about one-fourth of the length of the nematode from the anterior end), but in *H. similis* the subventrals and subdorsals extend to the end of the synlophe, which is near midbody.

The synlophe of experimentally produced hybrids (Lichtenfels et al., 1986) from matings of *H. contortus* and *H. placei* in sheep have an intermediate pattern of subventral ridges. When the 2 subventral and 2 subdorsal ridges were present they were much shorter than in *H. placei* and usually the subdorsals and subventrals were absent on either the left or right side.

A single lot of specimens identified as *H. contortus* (No. 70404) had traces of subdorsal and subventral ridges, but they were short (usually less than 1 mm in length) and were not present in all 4 usual locations.

In the anterior half of the body of all 3 species, 4 pairs of ridges (illustrated as dashed lines in Figs. 4–7) occur parallel to the lateralmost ridges (and are herein named the sublateral ridges). Posteriorly in all 3 species the sublateral ridges disappear at 2 different levels, 2 pairs (1 pair on the left and 1 pair on the right) at about the middle of the length of the synlophe and the second 2 pairs more or less near the end of the synlophe. Because the pairs of sublateral ridges may not end posteriorly in a completely symmetrical pattern (left pair may extend slightly longer than corresponding pair on the right, and/or ventral of pair may extend further than dorsal of pair) and because the ridges frequently end by becoming discontinuous, it is difficult to find a perfectly symmetrical cross section showing the standard number of sublateral ridges. In *H. contortus* the number of ridges is reduced from 30 to 26 (see 25 ridges in Fig. 20) 3–4 mm from the anterior end and from 26 to 22 5–6 mm from the anterior end and 2–3 mm anterior to the end of the synlophe (Figs. 21, 22). *Haemonchus placei* loses the first 2 pairs of sublateral ridges 3–4 mm from the anterior end, reducing the number from 34 (Fig. 23) to 30 (Fig. 24); loses the 2 subdorsals and 2 subventrals 4–6 mm from the anterior end, reducing the number to 26 (see 25 ridges in Fig. 25); and loses the second 2 pairs of sublaterals 0.1–0.5 mm anterior to the end of the synlophe

in males and 0.7–2.2 mm from the end of the synlophe in females, reducing the number of ridges to 22 (Fig. 26). In *H. similis* the number of ridges is reduced by 4 twice: from 34 (Fig. 27) to 30 near the middle of the synlophe (3–4 mm from the anterior end) (Figs. 28, 29) and then to 26 (Fig. 30) 2–3 mm anterior to the end of the synlophe in females and near the end of the synlophe in males.

In both *H. placei* and *H. contortus,* the sublateral ridges next to the lateral most ridges end posteriorly first (Fig. 8), before the more dorsal and ventral of the sublaterals end, but the situation is reversed in *H. similis* (Fig. 9).

Key to species

The characters described above were used to construct an identification key to both males and females of the 3 species of *Haemonchus* parasitic in domesticated ruminants. Hybrids between *H. contortus* × *H. placei* have characteristics that are intermediate between the 2 species (Lichtenfels, unpubl. data). For a discussion of the use of spicule lengths to identify species, see Lichtenfels et al. (1988).

1a. Male specimens 2
1b. Female specimens 4
2a. Spicule length about 300–400 μm (Fig. 1) and barbs of spicules near distal ends relatively distant from distal tips (right 62–81, left 41–65; about 20 and 15% of spicule lengths, respectively) and right and left barbs in nearly symmetrical positions (Figs. 2, 12) .... *Haemonchus similis*
2b. Spicule lengths about 400–500 μm (Fig. 1); and barbs of spicules near distal ends relatively close to tips with right barb about twice as far from tip (37–60 μm) as the left barb (19–32 μm) (about 11 and 5% of spicule lengths, respectively) (Figs. 2, 10, 11) .. 3
3a. Mean spicule length of 10 specimens greater than 450 μm; or, synlophe with subventral and subdorsal ridges (Fig. 5) that begin in region of esophagus and extend posteriorly 3–5 mm .... *Haemonchus placei*
3b. Mean spicule length (of 10 or more random specimens) significantly less than 450 μm; or synlophe without subventral or subdorsal

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placei with vulva anterior to protruding knob. **15.** Ovejectors of *H. similis* with small vestibule between sphincters and the long vagina extending to the vulva near the distal end of the long lobe. **16.** Tail of *H. contortus* female. (Note: dorsal curvature of tail is of no significance.) **17.** Tail of *H. placei* female. **18.** Tail of *H. similis* female.
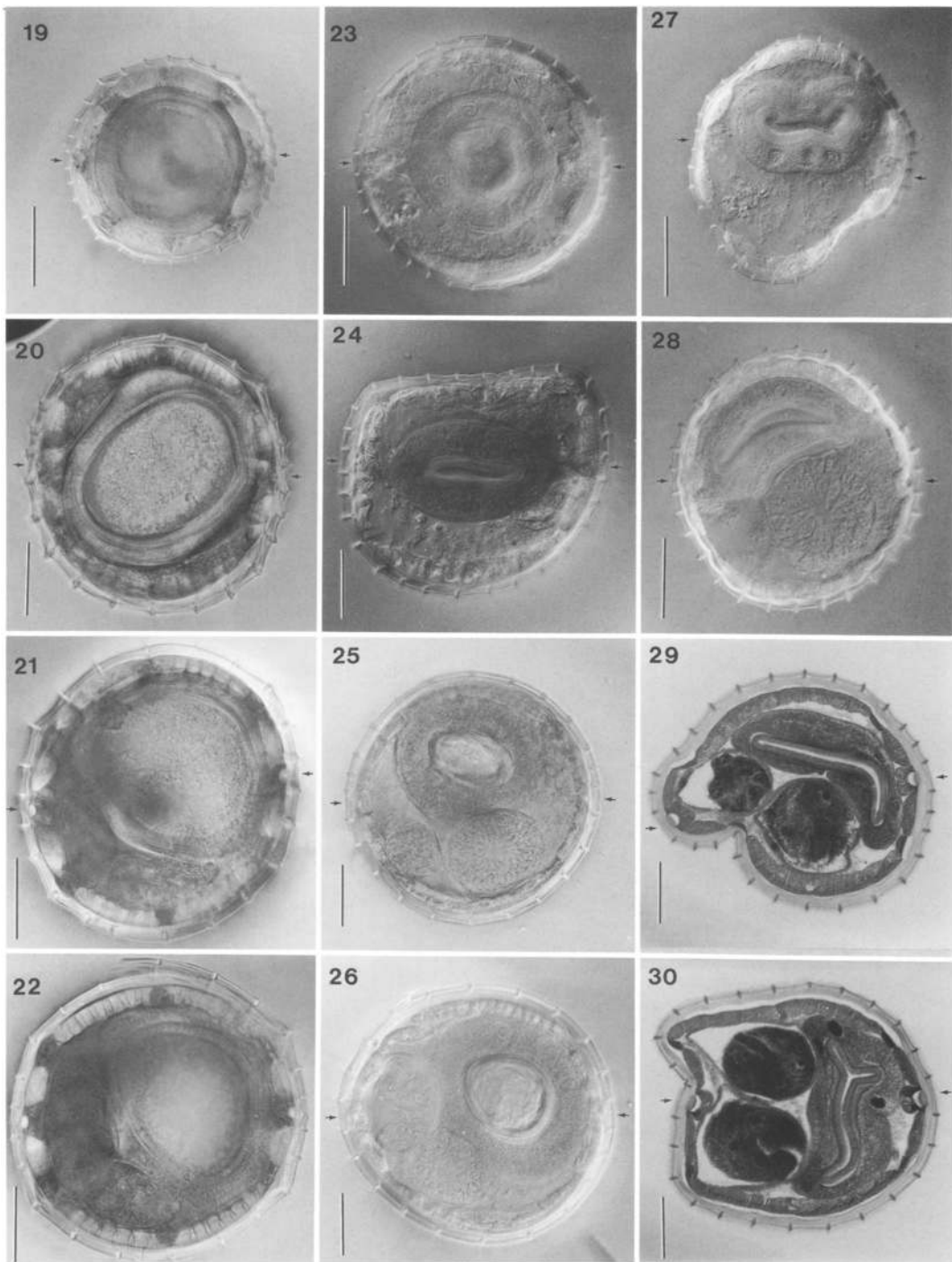
FIGURES 19–30.   Cross sections of female specimens of 3 species of *Haemonchus* at 4 comparable body locations to show the number and structure of the ridges of the synlophe (arrows at lateral lines) (scale bars = 50 μm). **19.** *H. contortus,* at esophageal–intestinal (EI) junction, showing 30 ridges. **20.** *Haemonchus contortus,* 4 mm from anterior end, showing 25 ridges. **21.** *Haemonchus contortus,* 6 mm from anterior end, showing 22 ridges. **22.** *Haemonchus contortus,* near midbody, showing 22 ridges. **23.** *Haemonchus placei,* at EI, showing 34 ridges. **24.** *Haemonchus placei,* 4 mm from anterior end, showing 30 ridges. **25.** *Haemonchus placei,* 6 mm from

ridges (Fig. 4) or with only a trace of 1 or 2 of them .... *Haemonchus contortus*

4a. Synlophe without subventral or subdorsal ridges (Fig. 4) or with only a trace of 1 or 2 of them .... *Haemonchus contortus*

4b. Synlophe with subventral and subdorsal ridges (Figs. 5, 6) that extend posteriorly from the midlength of the esophagus for several millimeters or more 5

5a. Tail short (135–278 μm) and conical (Fig. 18); vulva usually in vulval lobe distant from body wall (Fig. 15); subventral and subdorsal ridges extend to posterior end of synlophe .... *Haemonchus similis*

5b. Tail long (378–720 μm) and almost filiform (Fig. 17); vulva flush with body wall (Fig. 14); subventral and subdorsal ridges extend posteriorly for about one-half the length of the synlophe .... *Haemonchus placei*

DISCUSSION

This report presents newly recognized characteristics of the 3 common large stomach worms of ruminants of North America that make it possible, for the first time, to identify individual intact, whole adult specimens to species. The characters useful for identifying individuals can be determined in fixed and cleared, living or freshly thawed frozen specimens at 400× magnification. Previously only populations (not individuals) of *H. contortus* and *H. placei* could be identified by studying the anatomy of at least 10 specimens to determine the percentage of the body bearing the synlophe (Lichtenfels et al., 1986), mean spicule lengths (Lichtenfels et al., 1988), or the mean distance from the left spicule barb to the distal tip (Roberts et al., 1954; Herlich et al., 1958). Previously described methods for identifying individual specimens include cytological methods (Bremner, 1955) to determine the presence or absence of a swollen chromosome in *H. placei*, and DNA polymerase chain reaction primers to amplify variable DNA spacer regions within the ribosomal DNA repeat for which diagnostic bands can be visualized by agarose gel electrophoresis (Zarlenga et al., 1993).

Synlophe

The distribution of surface cuticular ridges has been used previously by Lichtenfels et al. (1986) to distinguish populations of *H. contortus* from *H. placei*. The earlier studies (Lichtenfels et al., 1986) described only differences in the proportion of the body length bearing ridges to the unridged cuticle (the total body length in males and prevulvar length in females), but not in number and pattern of distribution of ridges in the synlophe. Gibbons (1979), who did not recognize *H. placei*, reported 32 ridges for *H. similis* and 22–30 for *H. contortus* but did not describe the pattern of distribution of the ridges. In the present study we described 30–22 ridges on *H. contortus*, 34–22 on *H. placei*, and 34–26 on *H. similis*. The more detailed description of the synlophe of *H. contortus*, *H. placei*, and *H. similis* reported herein was possible only because populations of *H. placei* and *H. contortus* could be identified with confidence using the earlier characterization of the synlophe of these species (Lichtenfels et al., 1986).

The presence (in numerous populations from a very wide geographic region, Table I) of a pair of subventral and a pair of subdorsal ridges in *H. placei* and *H. similis* and the absence of these ridges in *H. contortus* (with 1 exception in which traces of subdorsal and subventral ridges were found) provided a useful character for identifying *H. contortus*. The principal value of this new synlophe character is in separating individual specimens of either sex of the similar species *H. contortus* and *H. placei*, but the difference in length of the subventral and subdorsal ridges between *H. placei* and *H. similis* is also useful as a supplementary character for separating females of these species.

In the present (Table I) as well as in previous investigations, the synlophe characteristics were found to be stable irrespective of the host species (Lichtenfels, 1974, 1977; Lichtenfels and Pilitt, 1983, 1991; Beveridge and Durette-Desset, 1992). Also, specimens of the Conder et al. (1990) strain of *H. contortus* (No. 70404, Table I), which has traces of subventral and subdorsal ridges, had

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anterior end, showing 25 ridges. **26.** *Haemonchus placei*, slightly anterior to midbody, showing 22 ridges. **27.** *Haemonchus similis*, at EI, showing 34 ridges. **28.** *Haemonchus similis*, 4 mm from anterior end, showing 30 ridges. **29.** *Haemonchus similis*, 6 mm from anterior end, showing 30 ridges. **30.** *Haemonchus similis*, near midbody, showing 26 ridges.

similar synlophe characteristics whether raised in lambs or gerbils, *Meriones unguiculatus*, although the nematodes from gerbils were stunted (reduced in size and development of the reproductive system). These results are consistent with a previous test of the stability of synlophe characteristics (Lichtenfels, 1974) in experimental and naturally occurring populations of *Nippostrongylus braziliensis* Travassos, 1914. From these studies, it appears that host effects, even when causing severe stunting of growth and development of the nematode, do not affect the number and pattern of distribution of the ridges of the synlophe.

The presence of subventral and subdorsal ridges in *H. placei* and their absence in *H. contortus* was a consistent feature in all monospecific populations studied (Table I) with one exception. The Conder strain of *H. contortus* from lambs (Coll. No. 70404, Table I) and gerbils (Conder et al., 1990) had a synlophe with short, asymmetrical subventrals and subdorsals very similar to that found in experimentally produced hybrids. The possibility that the Conder strain (Conder et al., 1990) represents a hybrid was investigated. However, the reproductive vigor of this strain (Conder et al., 1990) contrasts with the extremely low reproductive potential of hybrids between *H. contortus* and *H. placei* reported by Le Jambre (1983a, 1983b). The Conder strain was identified as *H. contortus* by a DNA probe (Zarlenga et al., 1993) specific for that species.

Spicule morphology

The relatively long distance from the barbs to the distal tips of the spicules in *H. similis*, especially in the left spicule, is an easily observed character for distinguishing this species from *H. contortus* and *H. placei*. Gibbons (1979) showed that the relative positions of the spicule barbs are quite variable among the species of the genus *Haemonchus*. Thus, because of the low variability within species of this character and the large differences among species, it is a useful character for identifying males to species, especially *H. similis*.

Lichtenfels et al. (1988) showed that spicule lengths provided the quickest and easiest character to use for separating most populations of *H. contortus* and *H. placei*. Although there is considerable overlap in the range of spicule lengths of *H. contortus* and *H. placei*, Lichtenfels et al. (1988) found that small samples (10–12

specimens) from individual hosts were relatively homogeneous, and the means had small standard errors, and were usually distributed above 450 μm for *H. placei* and below 450 μm for *H. contortus*. Spicule length and barb position can be used to identify *H. similis*. Thus, a study of the synlophe to determine the presence or absence of subdorsal or subventral ridges is necessary only to separate a few populations of *H. placei* or *H. contortus* with unusual spicules such as in stunted specimens, specimens in mixed infections, or hybrids; or, to confirm the identity of some female *H. contortus* or *H. placei*.

Female tail length

The conical shape and short tail length of *H. similis* females are especially useful for separating the 2 species commonly found in cattle (*H. placei* and *H. similis*) in regions where they are sympatric. In the U.S.A., *H. similis* has been found only in the southern states. The shape and length of the female tail can be observed with low magnification and in combination with vulva position, living females of *H. similis* can be identified in a dish with the aid of a dissection microscope. *Haemonchus contortus* females have tails of intermediate lengths between those of *H. similis* and *H. placei* so the presence or absence of subventral or subdorsal ridges must be determined if the presence of *H. contortus* is suspected.

This newly recognized character of the synlophe greatly enhances the feasibility of genetic studies on these important pathogenic nematodes of sheep and cattle. Prior to this study only populations could be identified to species on the basis of the study of at least 10 specimens. Now, theoretically, individuals can be identified alive and then returned to a host and mated with another identified individual. With this new potential of identifying individual specimens to species, studies might be designed to shed some light on many mysteries of *Haemonchus* of sheep and cattle. For example, does one species do better in some hosts or environmental conditions? How important are gene flow and selection pressure in maintaining the specific distinctness of these closely related species under sympatric conditions? In mixed grazing conditions what happens to the nematode species composition, population levels, and effects on the hosts? The prospects for research on these nematodes are exciting indeed.

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