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Differentiation of Fourth and Early Fifth Stages of *Parascaris equorum* (Goeze, 1782) Nematoda: Ascaridoidea

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ABSTRACT: The morphology of *Parascaris equorum* of horses was studied; light and scanning electron microscopy were used. Late fourth-stage larvae have narrow, rectangular lips bearing few, large, triangular, irregularly spaced denticles and the lips are not markedly set off from the body. The cuticle of the late fourth stage is completely transversely striated, with incomplete longitudinal ridges creating a brickwork pattern; longitudinal alae are present. Total body lengths of fourth-stage larvae range from 10 to 32 mm. A single molting fourth-stage male was found to be 33 mm long. Early fifth-stage nematodes have wide, trilobate lips with a deep transverse groove on their medial surface; the lips bear many small regularly spaced denticles and are set off from the body by a deep postlabial constriction. The fifth-stage cuticle is finely striated, without markings; longitudinal alae are lacking. Early fifth stages are 33.3 to 85.0 mm long.

The recent increase in the number of horses has been followed by a resurgence of interest in controlling their helminth parasites. Testing the efficacy of anthelmintics requires that helminths be identified to taxon and to stage of development. Although newly developed keys are available for adult helminths (Lichtenfels, 1975), identification standards for their larvae are lacking. This report describes anatomical characters that can be used to differentiate fourth-stage larvae and adults of *Parascaris equorum* (Goeze, 1782), the large intestinal roundworm of horses.

Apparently, the morphology and morphometrics of late fourth-stage larvae and early fifth stages of *P. equorum* have not been studied. More data have been published on the larval development of *Ascaris suum* Goeze, 1782 of swine (Roberts, 1934; Douvres et al., 1969), but reliable morphometrics of the late developmental stages of this nematode are also lacking (F. G. Tromba, personal communication).

Materials and Methods

About 130 fourth-stage larvae, 1 molting fourth-stage larva, and 35 early fifth stages of *P. equorum*, collected from natural infections of yearling, mixed breed horses in Kansas were available for study. Specimens were kindly provided by Dr. Danny D. Cox of Bayvet Division, Cutter Labs., Inc., Shawnee, Kansas. Other specimens studied were obtained from the U.S. National Parasite Collection. The nematodes were cleared for study in a solution of phenol alcohol (80% melted phenol crystals, 20% absolute alcohol) in temporary wet mounts. Photomicrographs were prepared with the aid of a 35-mm camera mounted on a microscope, equipped with an interference contrast attachment, and with a 35-mm camera mounted on a dissecting microscope. Scanning electron micrographs were prepared according to the methods of Madden and Tromba (1976). In describing the cuticle, the terms striation and annule have been used as defined by Chitwood and Chitwood (1950).

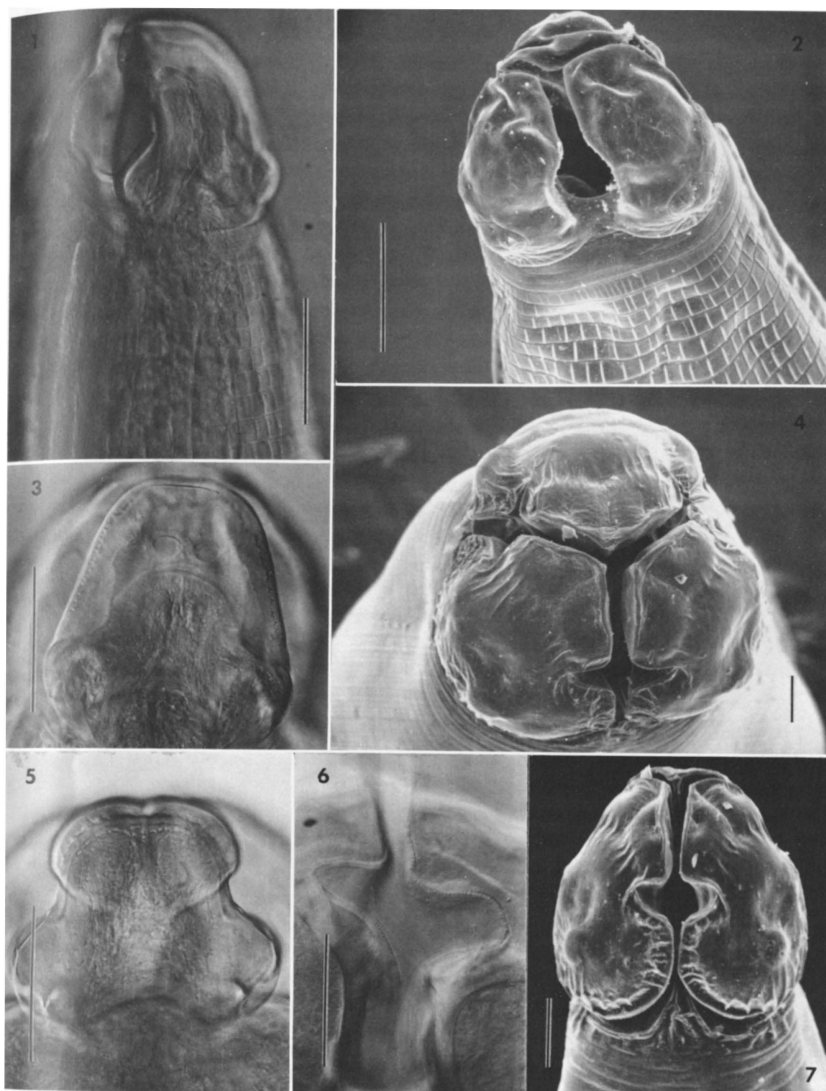
Table 1. Body measurements of fourth-stage larvae, molting fourth-stage larva, and early fifth stages of *Parascaris equorum* collected from natural infections of yearling, mixed breed horses.*

Anatomical feature	Fourth-stage larvae		Molting fourth-stage larva		Early fifth stages	
	Males	Females	Male	Males	Females	
Total length	12.60–25.00 (20.00)	10.00–32.00 (22.68)	33.00	39.00–65.00 (54.80)	33.30–85.00 (66.63)	
Width (maximum)	0.46– 0.91 (0.71)	0.51– 1.04 (0.78)	0.96	0.95– 1.93 (1.40)	1.13– 2.37 (1.79)	
Esophagus†	1.50– 2.70 (2.18)	1.40– 2.90 (2.37)	2.90	3.80– 5.70 (4.90)	3.50– 5.90 (5.05)	
Excretory pore†	0.46– 0.75 (0.62)	0.47– 0.79 (0.67)	0.71	0.91– 1.47 (1.17)	0.75– 1.49 (1.33)	
Spicule length	—	—	0.42	0.62– 1.37 (1.04)	—	
Vulva†	—	4.20–11.90 (8.10)	—	—	11.70–26.70 (21.05)	
Tail length	0.27– 0.38 (0.33)	0.37– 0.56 (0.49)	0.38	0.47– 0.71 (0.58)	0.66– 1.29 (1.07)	
Width of annule‡	0.034–0.055 (0.045)	0.021–0.063 (0.045)	0.063	0.003–0.005 (0.004)	0.003–0.005 (0.004)	

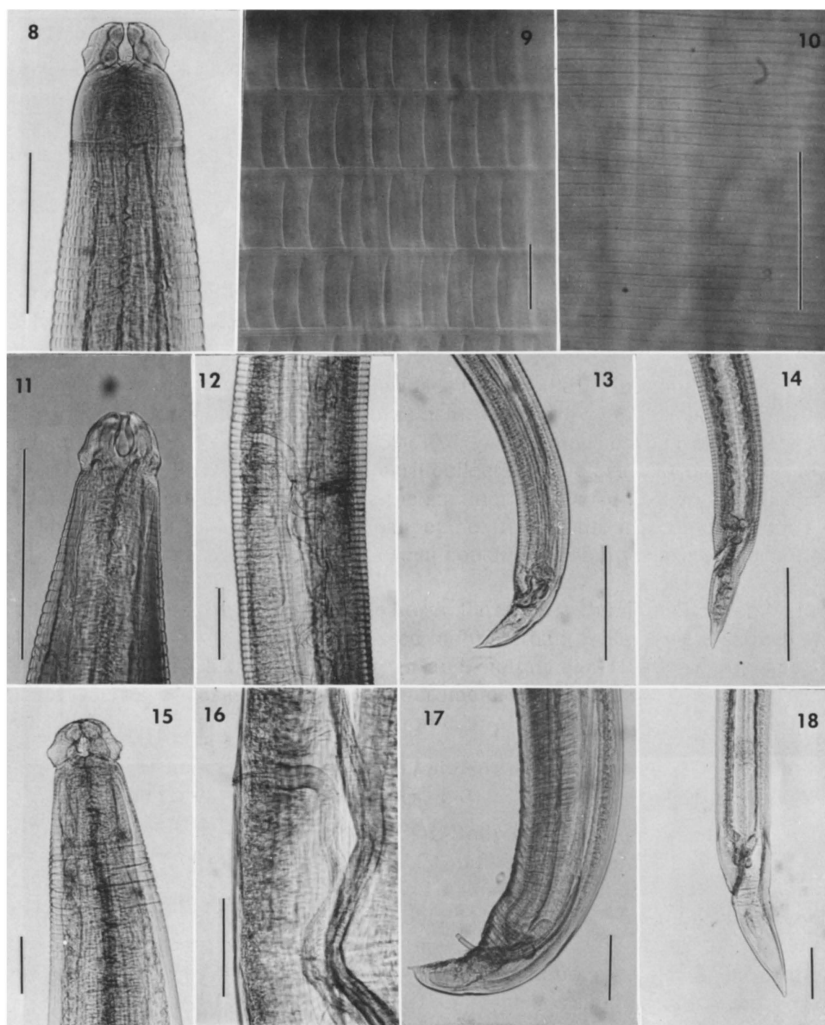
* Ranges (and averages) in mm for 10 specimens except for a molting fourth-stage male in which only a single specimen was available.

† Structures were measured from the anterior end.

‡ Annule was measured in area at base of esophagus.



Figures 1-7. *Parascaris equorum*, photomicrographs of late fourth-stage larvae, molting fourth-stage larva, and early fifth stages, showing labial and cuticular morphology. Scale bars 100 μ m. 1. Late fourth stage, cephalic region, showing transverse cuticular striae with incomplete longitudinal ridges, longitudinal ala, and subventral lip denticles. 2. Late fourth stage, similar to Figure 1, scanning electron micrograph (SEM). 3. Late fourth stage, dorsal lip showing large, irregularly spaced denticles. 4. Early fifth stage, *en face* view, SEM, showing shamrocklike lips. 5. Dorsal lip characteristic of fifth stage in the molting fourth-stage male. 6. Early fifth stage, showing small regularly spaced denticles on subventral lip margins. 7. Early fifth stage, same specimen as in Figure 4, subventral view, SEM, showing small interlabia.



Figures 8–18. *Parascaris equorum*, photomicrographs of molting fourth-stage larva, late fourth-stage larvae and early fifth stages, continued. Scale bars 500 μm in Figures 8, 11–18; scale bars 50 μm in Figures 9–10. 8. Molting fourth-stage male subventral view, showing anterior end emerged from fourth-stage cuticle. 9. Cuticle (near base of esophagus) of molting fourth-stage male, showing “brickwork” pattern of complete transverse striae with incomplete longitudinal ridges. 10. Cuticle of early fifth-stage male (near base of esophagus) showing fine transverse striae. 11. Late fourth-stage larva, cephalic region, subventral view. 12. Late fourth-stage female larva showing prepatent vulva (vagina and branching uterus) beneath fourth-stage cuticle. 13. Short, ventrally curved tail of late fourth-stage male. 14. Long, straight tail of late fourth-stage female. 15. Early fifth stage, cephalic region, subventral view, showing atypical wrinkling of the cuticle. 16. Early fifth stage, patent vulva. 17. Early fifth stage, male tail. 18. Early fifth stage, female tail.

Results

Range of measurements (and averages) of fourth-stage larvae, molting fourth-stage larva, and early fifth stages of *P. equorum* are given in Table 1. Specimens have been deposited in the National Parasite Collection as USDA Parasite Collection Nos. 67115, 67116, and 67117 for fourth stage, molting fourth stage, and early fifth stage, respectively.

Fourth-stage larvae (Figs. 1–3, 9, 11–14)

Lips narrow, rectangularly truncate (Fig. 3), not noticeably set off by constriction from rest of body (Fig. 11). Interlabia absent. Longitudinal alae present, extending from base of lips (Figs. 1–2) to phasmidial openings in tail. Denticles on lip margins few, large, widely and irregularly spaced (Fig. 3). Cuticle coarsely transversely striated; annules 0.021–0.063 mm wide, with incomplete longitudinal ridges present on annules; appearing in surface view as a brickwork pattern (Figs. 1, 2, 9).

Molting fourth-stage larva (Figs. 5, 8)

A single male specimen was available for study. The anterior end of the specimen has emerged from the fourth-stage cuticle. Lips are characteristic of the fifth stage and a small part of the body posterior to the lips has the fine striation of the fifth-stage cuticle. The rest of the body is enclosed within the fourth-stage cuticle (Fig. 8).

Early fifth stages (Figs. 4, 6–7, 10, 15–18)

Lips large, shamrocklike (Fig. 4), with deep transverse groove on medial surface (Figs. 4, 6–7); set off from rest of body by deep postlabial constriction (Fig. 15), giving body shouldered appearance. Small interlabia present (Fig. 7). Longitudinal alae absent. Many, small, fine denticles on lip margins (Fig. 6), closely and regularly spaced. Cuticle very finely striated; annules narrow (0.003–0.005 mm) without markings (Fig. 10). In some adult specimens the cuticle may appear wrinkled in various parts of the body. This atypical wrinkling (Fig. 15), possibly due to fixation, should not be confused with the coarse annulation of the fourth stage.

Discussion

The most useful characters for distinguishing late fourth-stage larvae and early fifth stages are the labial and the cuticular morphology. The size and shape of the lips and the size, shape, and spacing of the denticles around the lip margins are markedly different between the stages (Figs. 1–7). The fourth-stage cuticle is marked by longitudinal alae and complete transverse striation; the annules are wide with incomplete longitudinal ridges (Figs. 1, 2, 9) that appear in surface view as a brickwork pattern. Douvres et al. (1969) first used the term "brickwork" in describing the fourth-stage larval cuticle of *A. suum*. The cuticle of the fifth-stage *P. equorum* is finely striated, the annules narrow, without markings (Fig. 10) or alae. Hinz (1963) reported larger specimens (130–220 mm long) to have annules 0.009–0.010 mm wide.

Additional characters for separation of both stages and sexes include the lo-

cation of the vulva, the length and shape of the tail, and the total body lengths (Table 1). The prepatent vulva is slightly anterior to midbody and can be seen beneath the cuticle of the late fourth-stage larva (Fig. 12). The tail of the fourth-stage male larva is short (Fig. 13) and sharply curved ventrally; caudal alae are absent. Developing spicules can be seen in late fourth-stage male larvae. The patent vulva in the fifth-stage female is located in the anterior third of the body (Fig. 16). The tail of the early fifth-stage male (Fig. 17) is shorter than the female (Fig. 18) and is characterized by two spicules of equal length; gubernaculum and caudal alae are absent.

The fourth-stage larvae in the present study were 10 to 32 mm long and probably represent middle and late phases of fourth-stage development. Douvres et al. (1969) reported *A. suum* in an early phase of fourth-stage development to be 1.94 to 2.45 mm long. We have observed no overlap or gap in body length between the stages; however, body lengths can be expected to overlap. Douvres et al. (1969) described nine developmental phases of *A. suum* in swine based on morphological features and noted that total body measurements of late third-stage, third molt, and early fourth-stage larvae frequently overlap; therefore, body lengths are unreliable to separate these developmental stages. The morphological characters presented here permit workers to separate late developmental stages of *P. equorum*. The most useful differentiating characters are the labial and the cuticular morphology.

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