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Cephalic Papillae of Giant Kidney Nematode *Dioctophyma renale* (Goeze, 1782) and Comparison with *Eustrongylides* spp.

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ABSTRACT: Cephalic papillae of third- and fifth-stage *Dioctophyma renale* and fourth- and fifth-stage *Eustrongylides* spp. were found to be of three kinds in addition to the amphids. In all the stages of both genera studied, six papillae were in an internal circle, six in an external circle, and eight to 10 in two lateral fields of four or five each between the internal and external circles. Amphids were closely associated with the externolateral papillae. Another porelike papilla was found between the ventroventral papillae in all but fifth-stage *D. renale*. In third-stage *D. renale*, lateral rows of somatic papillae were spatially separated from the cephalic papillae, but in fifth-stage *D. renale* and fourth- and fifth-stage *Eustrongylides*, the somatic papillae were adjacent to the cephalic papillae.

The development and life cycle of *Dioctophyma renale* have been described recently by Mace and Anderson (1975), who have confirmed the earlier work of Karmanova (1962). Eggs hatch in an aquatic oligochaete, *Lumbriculus variegatus* (Muller), develop to the third stage, and are infective to the definitive host, usually the mink, *Mustela vison* Schreber. Third-stage larvae from the oligochaete will also survive in frogs and fish, which may be important paratenic hosts (Mace and Anderson, 1975). Larvae of another dioctophymatid, *Eustrongylides*, also are found in frogs and fish, and third-stage *D. renale* are difficult to distinguish from third-stage *Eustrongylides* spp. (Mace and Anderson, 1975; Fastzkie and Crites, 1977). Fourth-stage larvae of *D. renale* are unknown. This study provides a detailed description of cephalic papillae of third-stage and adult *D. renale* with the objective of finding morphological data for identifying larval stages. Others (Crites and Jilek, 1978) are describing the cephalic papillae of larval and adult *Eustrongylides tubifex* (Nitzsch, 1819).

Materials and Methods

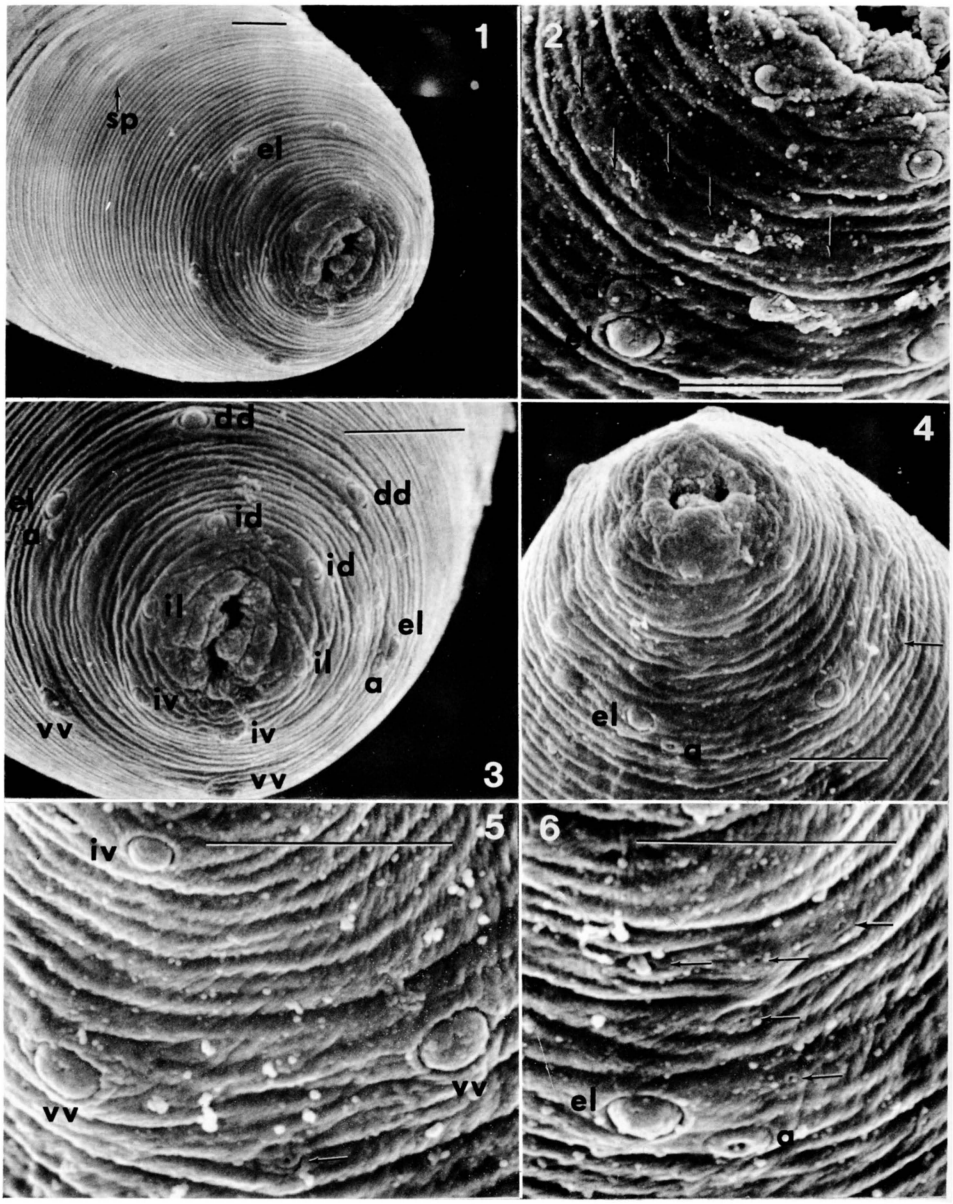
Specimens

Four fifth-stage *Dioctophyma renale* were studied. One from a dog, *Canis familiaris* Linnaeus, was collected in Washington, D.C., in 1916; and three from a mink were collected in Ontario, Canada, in 1978 by Dr. Roy Anderson, University of Guelph, Ontario, Canada. Third-stage *D. renale* from experimentally infected leopard frogs, *Rana pipiens* Schreber, were obtained from Dr. Thomas F. Mace, University of Victoria, British Columbia, Canada.

Fourth-stage *Eustrongylides* sp., ?*Eustrongylides wenrichi* Canavan, 1929 from bullfrogs, *Rana catesbeiana* Shaw, were collected in Nevada by Dr. Bert B. Babero, University of Nevada, Las Vegas, in 1971. Fifth-stage *Eustrongylides ignotus* Jägerskiöld, 1909 from a "blue heron," *Ardea* sp., were collected in Washington, D.C., in 1929.

Procedures

Terminology for papillae follows Chitwood (1950) except where indicated otherwise. Scanning electron micrographs were obtained by the methods of Madden and Tromba (1976).



Figures 1–6. ***Dioctophyma renale*** **third-stage larvae from leopard frogs. Scale bars 10 μm. 1. Anterior end showing separation between somatic papillae (sp) and externolateral cephalic papillae (el). 2. Left lateral cephalic region showing amphid (a) slightly anterior and ventral to externolateral papillae (el). Arrows indicate five small lateral field cephalic papillae. 3.** ***En face*** **view of internal and external circles of cephalic papillae and the amphids (a). The internal circle includes two internodorsals (id), two internolaterals (il), and two internoventrals (iv). The external circle includes two dorsodorsals (dd), two externolaterals (el), and two ventroventrals (vv). 4. Right lateral view of different specimen showing that the amphid (a) is sometimes posterior and ventral to the externolateral cephalic papilla (el). An arrow indicates a ventral porelike papilla slightly posterior to the external circle of cephalic papillae. 5. Ventral view of ventral porelike papilla (arrow), ventroventral papillae (vv), and an internoventral papilla (iv). 6. Right lateral view showing amphid (a), externolateral papilla (el), and five small lateral field papillae (arrows).**

Observations and Results

Three kinds of papillae, in addition to the amphids, were observed in the cephalic region. The papillae are arranged in an internal circle of six, an external circle of six, and in two lateral fields of four or five each between the two circles. Lateral somatic papillae extend posteriorly from the cephalic region to the caudal region.

Third-stage *Dioctophyma renale* larvae (Figs. 1–6)

The six papillae of the internal circle are buttonlike, almost round, without projecting spines, and with a central porelike depression. They are symmetrically arranged in an oval ring around the dorsoventrally elongated mouth. Papillae of the external circle are similar in shape to those of the internal circle but are twice as large. The amphids are similar in size to the papillae of the internal circle but have a larger central pore. The amphids usually are located slightly anterior and ventral to the externolateral papillae (Figs. 1–3), but they may also be located posterior to the externolateral papillae (Figs. 4, 6). Small porelike papillae are located in two irregular and asymmetrical lateral cephalic fields of five each between the internal and external circles (Figs. 2, 6). Lateral somatic papillae (Fig. 1) are located in two lateral rows in the cervical region and extend posteriorly to the caudal region. The somatic papillae are separated from the cephalic papillae by a space of 40–60 μm in third-stage larvae. The somatic papillae are morphologically similar to the papillae of the internal circle.

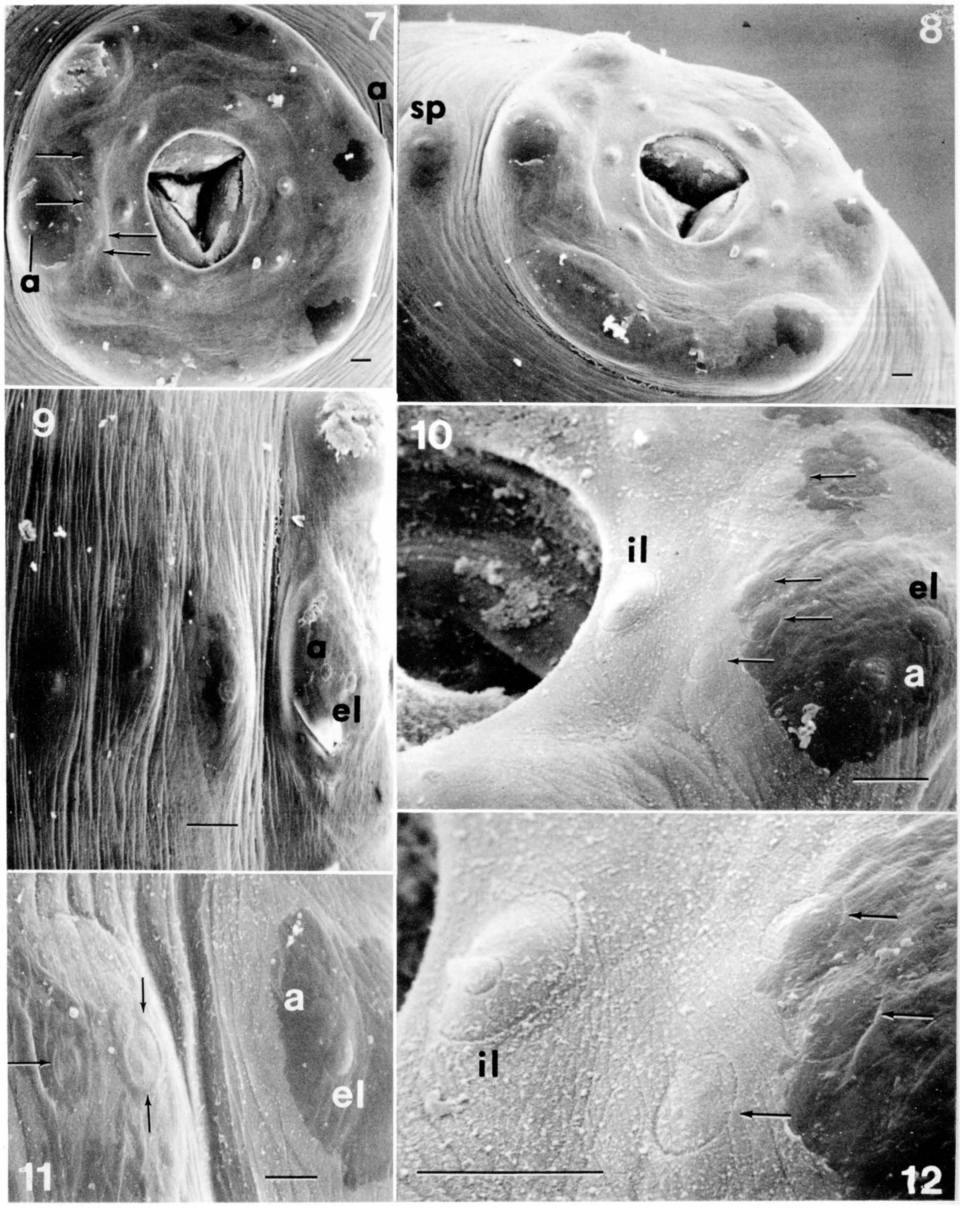
A single porelike papilla is located just posterior to the external circle in what is believed to be a ventral position (Figs. 4, 5). There is no corresponding papilla on the opposite side.

Fifth-stage (adult) *Dioctophyma renale* (Figs. 7–12)

The six papillae of the internal circle are located on a narrow, flat, shelflike rim around the round mouth. The papillae are moundlike, have an oval base delimited by a ring-shaped depression in the cuticle, and are topped by a digitiform tip (Fig. 12). The six papillae of the external circle are found on large, domeshaped elevations that surround the shelflike rim around the mouth. Each elevation bears a single, round, buttonlike papilla delimited by a ring-shaped depression in the cuticle. The amphids are slightly smaller than the papillae of the external circle and are located either anterior or posterior and dorsal or ventral to the externolateral papillae (Figs. 7–11). Eight small papillae are located between the internal and external circles in two irregular lateral fields of four each (Figs. 7, 10). Lateral somatic papillae extend posteriorly from the externolateral papillae. The anteriormost somatic papillae are sometimes paired (Fig. 11), but the more posterior ones are single.

Fourth-stage *Eustrongylides* sp. (Figs. 13, 14)

The six papillae of the internal circle each have a large, flat, round base delimited by a cuticular depression, and a long, tapering, spinelike central process (Figs. 13, 14). The six papillae of the external circle have an umbonate shape with a large, round base and a short, blunt central process with an apical, porelike depression (Fig. 14). The amphids are slightly ventral to and at about the same level as the externolateral papillae (Figs. 13, 14). The amphids are smaller than



Figures 7–12. *Dioctophyma renale* **adults from kidney of mink. Scale bars 20 μm. 7.** ***En face*** **view of internal circle of six papillae on shelflike rim around mouth, amphids (a) posterior to the papillae of the external circle, and four right lateral field cephalic papillae (arrows) between the internal and external circles. 8. Anteroventral view of specimen shown in Figure 7 showing anteriormost somatic papilla (sp) on left side and the dome-shaped elevations that bear the papillae of the external circle. 9. Right lateral cephalic region of specimen in Figures 7 and 8 showing amphid (a), externolateral papilla (el), and three somatic papillae. 10. Left cephalic region showing internolateral papilla (il), four lateral field cephalic papillae (arrows), amphid (a), and externolateral papilla (el). 11. Right side of specimen in Figure 10 showing amphid (a) slightly dorsal and posterior to externolateral papilla (el), and double (double arrows) and single (single arrow) somatic papillae. 12. Higher magnification of specimen in Figures 10 and 11 showing internolateral papilla (il) with digitiform tip and three of the four lateral field cephalic papillae (arrows).**

either the papillae of the external circle or the lateral somatic papillae (Figs. 13, 14). There are four small, round or oval papillae in each of two lateral cephalic fields between the internal and external circles (Figs. 13, 14), and a single ventral porelike papilla is located between the ventroventral papillae (Fig. 14).

Fifth-stage *Eustrongylides ignotus* (Figs. 15–18)

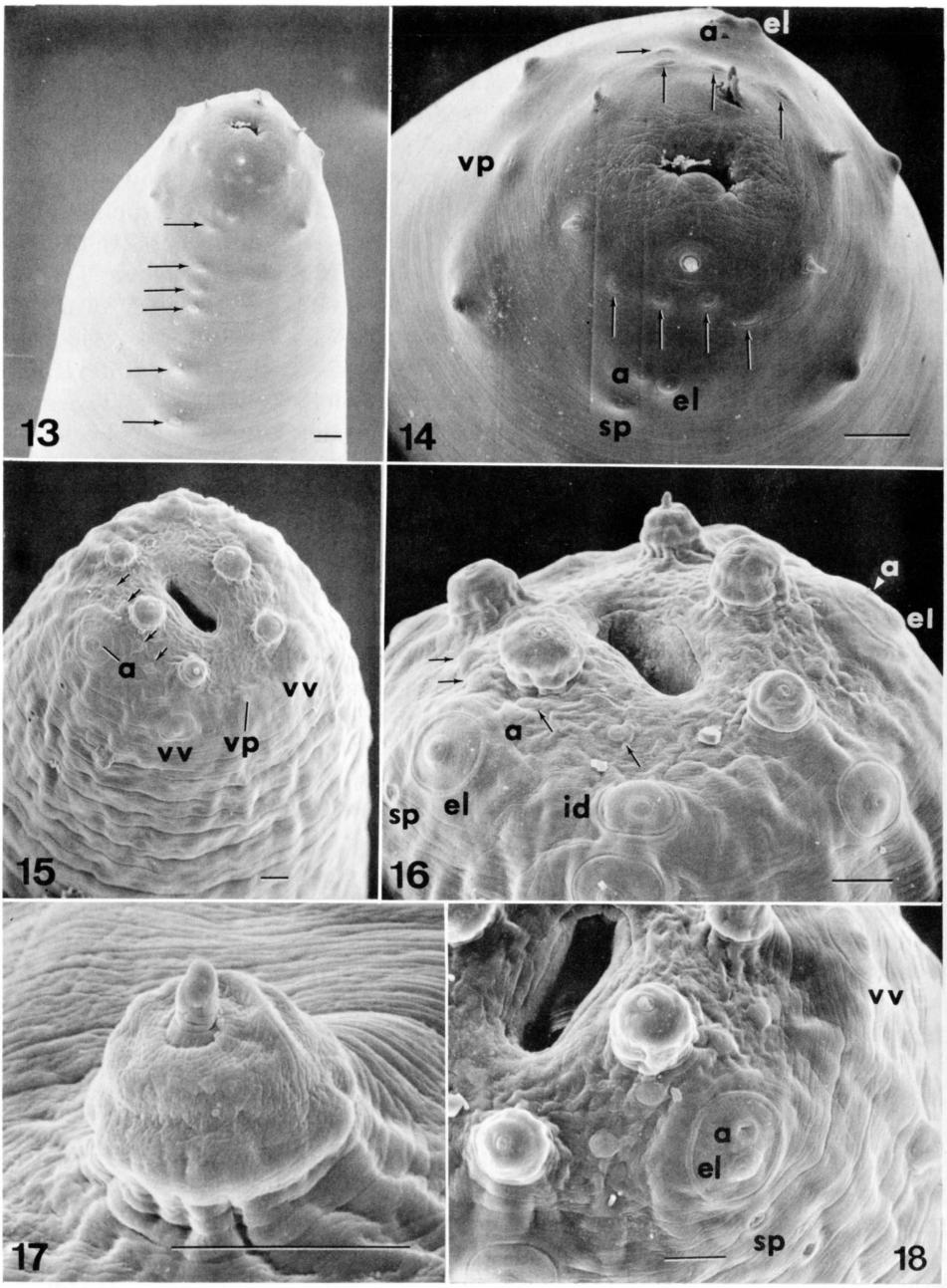
Each of the six papillae of the internal circle has a raised base and an apical digitiform process (Fig. 17) which is frequently missing in fixed specimens. The six papillae of the external circle have broad, low, convex, circular bases delimited by a depression in the cuticle. Small, buttonlike elevations with a central porelike depression are located in the centers of the circular bases. The amphid on the right side (Fig. 18) is located on the base of the right externolateral papilla and is porelike. The left amphid, however, is apparently anterior to the base of the left externolateral papilla and indistinguishable from the small lateral cephalic papillae between the internal and external circles (Fig. 16). A single porelike papilla is located ventrally, slightly anterior to the outer circle of papillae.

Discussion

Previous interpretations of cephalic papillae of Dioctophymatoidea differ from that presented above. Chitwood (1950) considered as cephalic papillae only the six papillae of the internal circle, the six large papillae of the external circle, and four of the eight small lateral field cephalic papillae. Thus, in Chitwood's system, the external circle had six large and four small papillae, an arrangement that agrees with that of some other aphasmidian nematodes, the Enoploidea (Chitwood, 1950). Chitwood considered the lateralmost two of the four small papillae between the internal and external circles to be somatic papillae extending anteriorly from the lateral areas.

Another interpretation of the papillae of the anterior region of nematodes is that of de Coninck (1942, 1965). In de Coninck's system are an inner circle of six labial papillae, an outer circle of six labial papillae, and two bilateral groups of two cephalic bristles or papillae. Hyman (1951) adopted de Coninck's system and noted that the number of bristles (=papillae) in the cephalic circle is often augmented by doubling or by the forward migration of body (=somatic) papillae. In the present study, the eight to 10 small lateral papillae between the internal and external circles were found to be of uniform morphology. Furthermore, in third-stage larvae of *D. renale,* a considerable distance separates the cephalic papillae from the lateral rows of somatic papillae in the cervical region. We have, therefore, labeled as cephalic papillae all small papillae located in lateral fields between the two circles of large labial papillae. Our observations appear to support de Coninck's system of hexaradiate labial and bilateral cephalic papillae. We interpret the cephalic papillae of *Dioctophyma* and *Eustrongylides* to represent an inner circle of six labial papillae, an outer circle of six labial papillae, and two bilateral groups of four or five cephalic papillae.

In a light micropscopy study of third-stage larval *D. renale,* Mace and Anderson (1975) placed the amphid midway between the internal and external circles of labial papillae and showed the externolateral papillae as double. We believe that what they labelled as amphids were some of the eight to 10 small cephalic papillae between the circles and that the ventral partners of their double exter-



Figures 13–18. 13, 14. *Eustrongylides* sp. fourth-stage larva from bullfrogs. Scale bars 20 μm. 13. Anterolateral view of anterior end showing cephalic papillae and somatic papillae (arrows). 14. Higher magnification of specimen in Figure 13 showing six spinelike papillae of the internal circle, six umbonate papillae of the external circle, two amphids (a), the anteriormost somatic papilla (sp), an unpaired ventral papilla (vp), and four small lateral field cephalic papillae on each side between the internal and external circles (arrows) (el = externolateral papillae). 15–18. *Eustrongylides ignotus* adult from a "blue heron." Scale bars 20 μm. 15. Ventrolateral view of anterior end showing a dorsoventrally elongated mouth surrounded by an internal circle of six raised papillae, a ventral porelike papilla (vp) between the

nolaterals are actually the amphids. This interpretation is supported by the different morphology of these porelike papillae in third-stage larvae. In adult *D. renale,* only their smaller size helps in identifying the amphids. In *Eustrongylides* spp., the identification of the amphids is especially difficult, and we relied somewhat on a position equivalent to that found in *D. renale* because all other labial and cephalic papillae have equivalent positions. Chitwood (1950), however, indicated that the amphids of dioctophymatids are posterior to the externolaterals. However, Gibson and McKiel (1972) figured amphids anterior to papillae of the outer circle in *Eustrongylides* sp. from a muskrat. Panesar and Beaver (1979) were unable to recognize amphids in fourth-stage larvae of *Eustrongylides wenrichi,* although they described the externolateral papillae as double. Fastzkie and Crites (1977) also were unable to recognize amphids in adult *Eustrongylides tubifex* (Nitzsch, 1819). Jones (1978) described the cephalic papillae of *Eustrongylides acrochordi* Jones, 1978 without distinguishing amphids. Additional studies are needed to determine conclusively which of the labial and cephalic papillae are the amphids.

The single, small, porelike papilla found between the ventroventral papillae of third-stage larval *D. renale* and fourth-stage larval and adult *Eustrongylides* spp. could not be found in the limited material of adult *D. renale.* However, Karmanova (1968) and Roman (1965) illustrated this papilla in a drawing by M. Rather of an adult *D. renale.* Jones (1978) figured a papilla between the ventroventral papillae and another between the dorsodorsal papillae in *E. acrochordi.* Despite a careful search, a dorsal papilla was not found in the present study. The position of the ventral papilla was confirmed only in fourth-stage *Eustrongylides* sp. by relating the papilla position to that of the large ventral nerve cord and the glandular subventral sections of the esophagus. The function of this porelike papilla is unknown.

Some evidence shows that the morphology of cephalic papillae may be determined by their position on the face of the nematode. In Figures 15–17, the left internodorsal papilla is displaced toward the external circle and resembles the papillae of the external circle more than it resembles other internal papillae. The right amphid (Fig. 18), on the base of the right externolateral papilla, is different morphologically from the left amphid (Fig. 16), which is displaced anteriorly. The left amphid is morphologically similar to the four adjacent small lateral field cephalic papillae.

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ventroventral papillae (vv) of the external circle, an amphid (a) slightly anterior and ventral to the externolateral papilla, somatic papillae posterior to the externolateral papilla, and four right lateral field cephalic papillae (arrows) between the two circles. 16. Higher magnification of the specimen in Figure 15 viewed anterodorsally, showing asymmetrical arrangement of amphids, with the left amphid (black a) apparently grouped with the four left lateral field cephalic papillae (arrows) rather than on the base of the externolateral papilla (el) like the right amphid (white a). The left internodorsal papilla (id) is also asymmetrically arranged, being closer to the external circle than the others (sp = somatic papilla). 17. Higher magnification of the right internoventral papilla showing apical digitiform process that is frequently missing in fixed specimens. 18. Higher magnification of right lateral region showing amphid (a) on the base of the externolateral papilla (el), four small lateral field cephalic papillae between the papillae of the internal and external circles, and somatic papillae (sp) posterior to the externolateral papilla (vv = ventrolateral papilla).

Determination of the usefulness of labial cephalic papillae for separating larvae of *D. renale* and *Eustrongylides* spp. must await the publication of studies of third-stage *Eustrongylides,* but the usefulness of the small lateral field cephalic papillae must be questioned because of the difficulty in observing them with the light micropscope. However, Crites and Jilek (1978) described the larger cephalic papillae of the internal circle of third-stage *Eustrongylides tubifex* as having a "cone-shaped, almost spine-like central projection." Thus, the papillae of the internal circle appear to be useful in separating third-stage larvae of *D. renale* and *E. tubifex.*

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