# Description and SEM Observations of Dolichodorus marylandicus n. sp. with a Key to Species of Dolichodorus[1]

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*Abstract: Dolichodorus marylandicus* n. sp. is described and illustrated from grass (*Zoysia japonica*) in College Park, Maryland. Specimens have also been collected from perennial bluegrass (*Poa pratensis*) pasture at Beltsville, Maryland, and from pine (*Pinus* sp.) in North Carolina. This new species is related to *D. heterocephalus* Cobb, *D. similis* Golden, and *D. aestuarius* Chow & Taylor. Females are distinct in having a longer stylet and shorter body length than *D. aestuarius*. The excretory pore is at the level of the isthmus or slightly posterior and the tail is abruptly reduced in diameter, tapering to an acuminate terminus. The tails of *D. similis* and *D. heterocephalus* conically taper to a median point, with *D. similis* having an especially long tail. *D. marylandicus* does not possess the rounded, sclerotized accessory structures adjacent to the vulva observed in lateral views of *D. similis* and *D. heterocephalus*. SEM observations of *D. heterocephalus* and *D. marylandicus* revealed details of the head of males and females, and species difference in shape of the oral disc. *Key words:* taxonomy, morphology, *Dolichodorus,* awl nematode, new species, SEM ultrastructure, grass, *Zoysia japonica, Poa pratensis, Pinus,* pine.

The genus *Dolichodorus* was established by N. A. Cobb in 1914 from specimens obtained primarily from Silver Springs, Florida, when he described *D. heterocephalus* (6). No other species were described for more than 40 yr, until *D. obtusus* Allen in 1957 (1) and *D. similis* Golden in 1958 (10). Since then, additional species have been described and placed in this genus. However, in 1976 Andrássy (2) established *Neodolichodorus* to accommodate the species with bluntly rounded tails and four lines in the lateral field; and at the same time transferred *D. obtusus* to this new genus as the type and only species. In 1977 Siddiqi (17) shifted five other species from *Dolichodorus* to *Neodolichodorus*. Presently, there are eight *Dolichodorus* spp. associated with varied hosts including corn, celery, turf, white pine, cocoa, rubber, pepper, clove, coconut, weeds, and grasses. *Dolichodorus* spp. are commonly collected from moist habitats, but they have also been found 1.2 m deep in sandy soil lying 20–40 cm above the water table (15), in dry, shale soil at the top of a mountain (9), and in coastal sand dunes in New Zealand (5).

*Dolichodorus heterocephalus* is important in Florida agriculture (4,16). In 1975 *D. minor* Loof & Sharma was associated with galled roots of diseased cocoa and rubber trees (13), and *D. silvestris* Gillespie & Adams was found in 1962 surrounding unthrifty white pine tree roots (9). The *Dolichodorus* species described herein was found (with other associated plant-parasitic nematodes) in sparse, chlorotic stands of zoysia and perennial bluegrass. The turf stands improved in vigor after nematicide application (8).

## MATERIALS AND METHODS

Specimens used in this description were obtained from a zoysia lawn and from cultures originating from the lawn and grown on zoysia in an isolated area of a greenhouse.

Males, females, and larvae were collected

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from soil by commonly used methods, heat relaxed, and fixed in 3% formaldehyde. The procedures used in measuring, drawing, and preparing specimens were those used by Golden and Birchfield (11). Living specimens were prepared for scanning electron microscope (SEM) observation by fixing in 3% glutaraldehyde in 0.01 m phosphate buffer pH 6.7, dehydrating in ethanol, critical-point drying, and sputter coating with gold-palladium.

## SPECIES DESCRIPTION

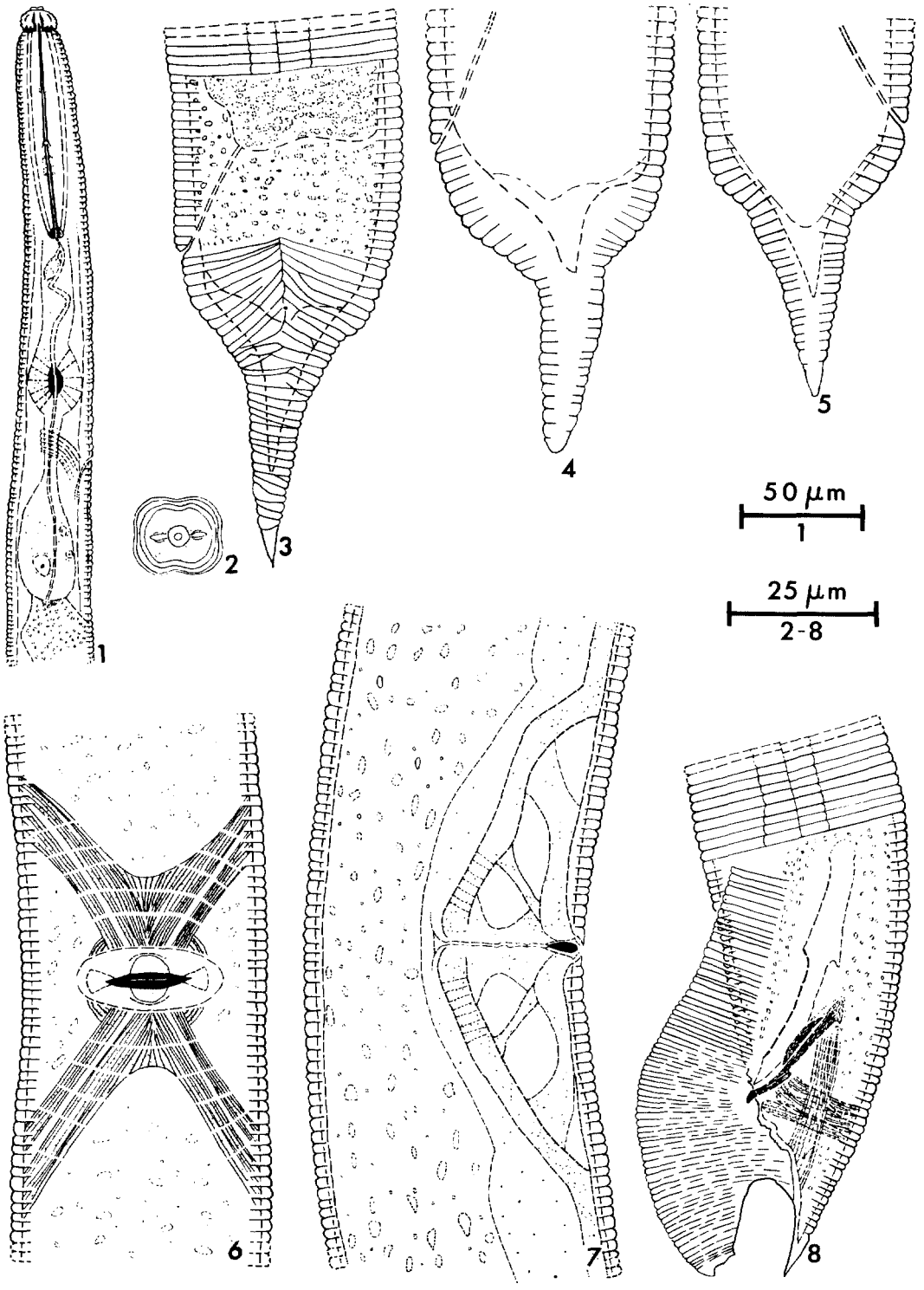
*Dolichodorus marylandicus* n. sp.

*Females* (30): Length 1646–2489 μm (average 2120.2 μm, standard deviation [SD] 245.7 μm); width 39.2–54.6 μm (45.3 μm, SD 4.0); a = 36–54 (45.5, SD 5.0); b = 6.0–10.1 (8.5, SD 0.8); c = 30.2–51.4 (38.8, SD 4.9); V = 50–61 (55, SD 5.0); total stylet 83.5–92.4 μm (88.8 μm, SD 3.0); stylet conus 46.6–51.3 μm (49.3 μm, SD 1.9); dorsal esophageal gland orifice (DGO) 3.8–5.8 μm (4.9 μm, SD 0.8) from base of stylet; center of median bulb 134.8–154.8 μm (147.9 μm, SD 8.7) from anterior end; excretory pore 156.6–193.9 μm (180.9 μm, SD 11.0) from anterior end; anal body width 29.9–41.8 μm (36.6 μm, SD 2.9).

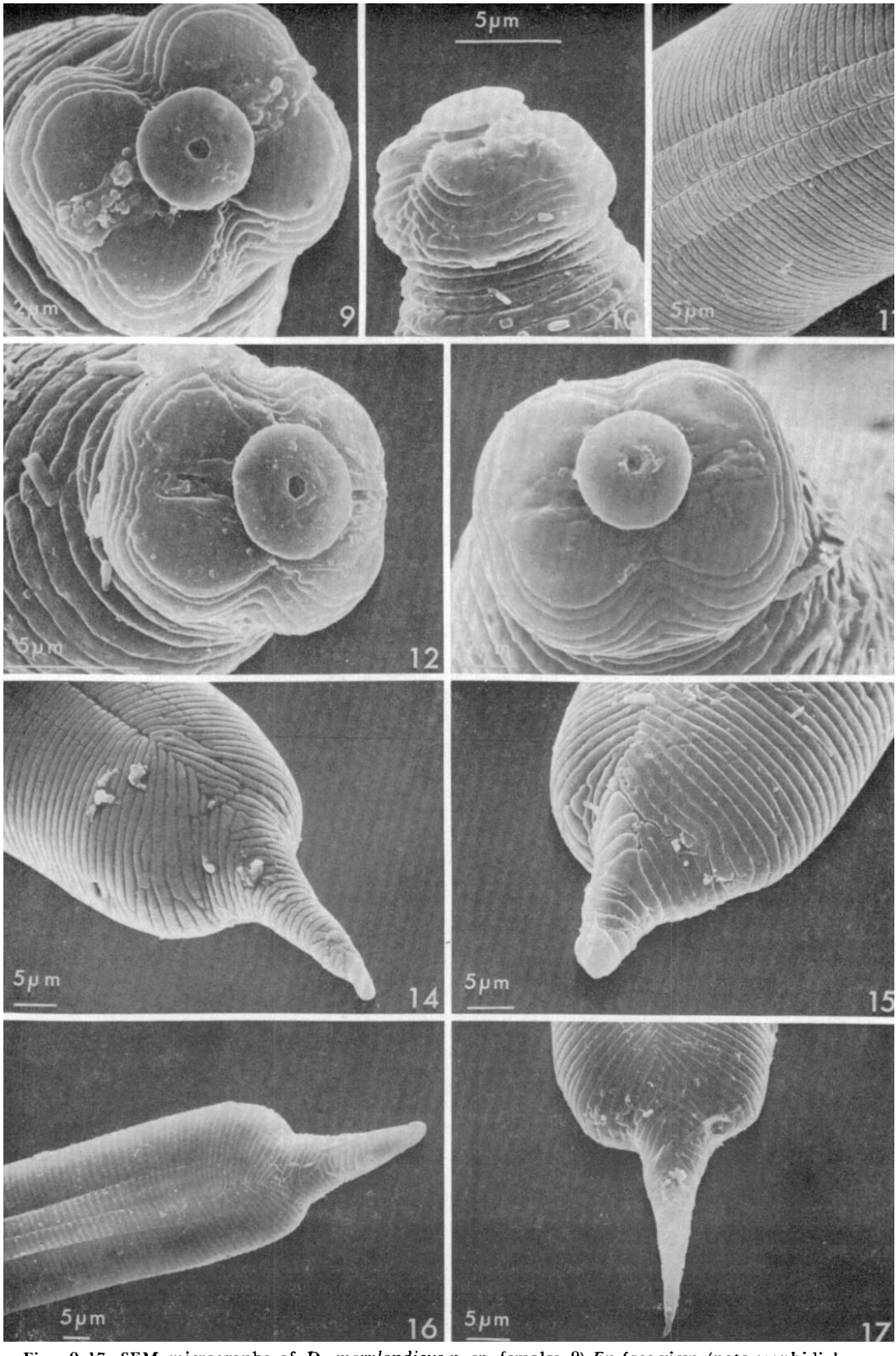
*Holotype* (female): Length 2038.4 μm; 40.1 μm; a = 50.8; b = 8.8; c = 34.8; V = 52; stylet 86.7 μm; stylet conus 47.5 μm; DGO 4.4 μm from base of stylet; center of median bulb 137.2 μm from anterior end; excretory pore 181.4 μm from anterior end; phasmids 46.6 μm from tail terminus; anal body width 35.6 μm.

*Description of females:* Body long and cylindrical, slightly arcuate. Lip region prominent, markedly offset from body (Fig. 1), bearing seven very fine annules (Figs. 10, 13). Lip region *en face* with four lobes and with an offset oral disc (Figs. 2, 9, 12, 13). Anterior portion of head with strong cephalic framework and massive basal plate. Observations of the smooth elongate lips with a transmitted-light microscope revealed curved lines in the lateral sectors which could not be definitely identified. However, SEM micrographs revealed the presence of wide (0.8 μm) amphid openings 2.9–3.3 μm long. The shape and width of the openings may be partially determined by a raised area (labial rib) on each side of the opening (Figs. 12, 13, 18, 21, 22). Cavities or clefts appear under the oral disc in the dorsal and ventral sectors. Stylet with conus longer than shaft and with slightly pointed knobs sloping posteriorly (Fig. 1). Guides for both the stylet shaft and extension very faint. Esophagus typical from the genus with the nerve ring encircling the basal portion of the isthmus. Excretory pore clearly visible and located slightly anterior to the basal bulb but occasionally located from the level of the upper half of the basal bulb to the anterior of the isthmus. Hemizonid 17.4–26.1 μm and hemizonion 23.2–34.5 μm below excretory pore, respectively. Anterior cephalids opposite, 4–5 annules posterior to constriction at lip region. Vulva a transverse slit (Fig. 6) without sclerotized accessory pieces observed in lateral views of *D. heterocephalus* and *D. similis*. Vagina strongly sclerotized near vulva, with serrated opening and with well-developed, convoluted cuticular lining extending to almost half the body width (Fig. 7). Amphidelphic, with oöcytes in several rows in germinal zone. Proximal end of oviduct with conspicuous spermatheca. Cuticle finely striated with areolated lateral field having three lines, occupying about 1/5 body width (Fig. 3). Center line more prominent than outer lines and extending from a point adjacent to anterior 1/3 of stylet to a deeply-cut line just anterior to the phasmid after which the line becomes uneven. Outer lines not as distinct, extending from the level of the procorpus to a point just anterior to the level of the anus, and appearing more faint posterior to the vulva. Phasmids opposite, pore-like, averaging 46.2 μm from tail tip in a cleft formed by center line in lateral field (Fig. 3). Tail 54.9 μm (50.6–63.8), abruptly tapering to an acuminate, often spikate, terminus with more reduction in diameter ventrally than dorsally or laterally. Tail shape generally appearing as in Figs. 3, 4, 5, 14, 17 but also occurring as in Figs. 15, 16. Juveniles with tails conically tapering to a long median point.

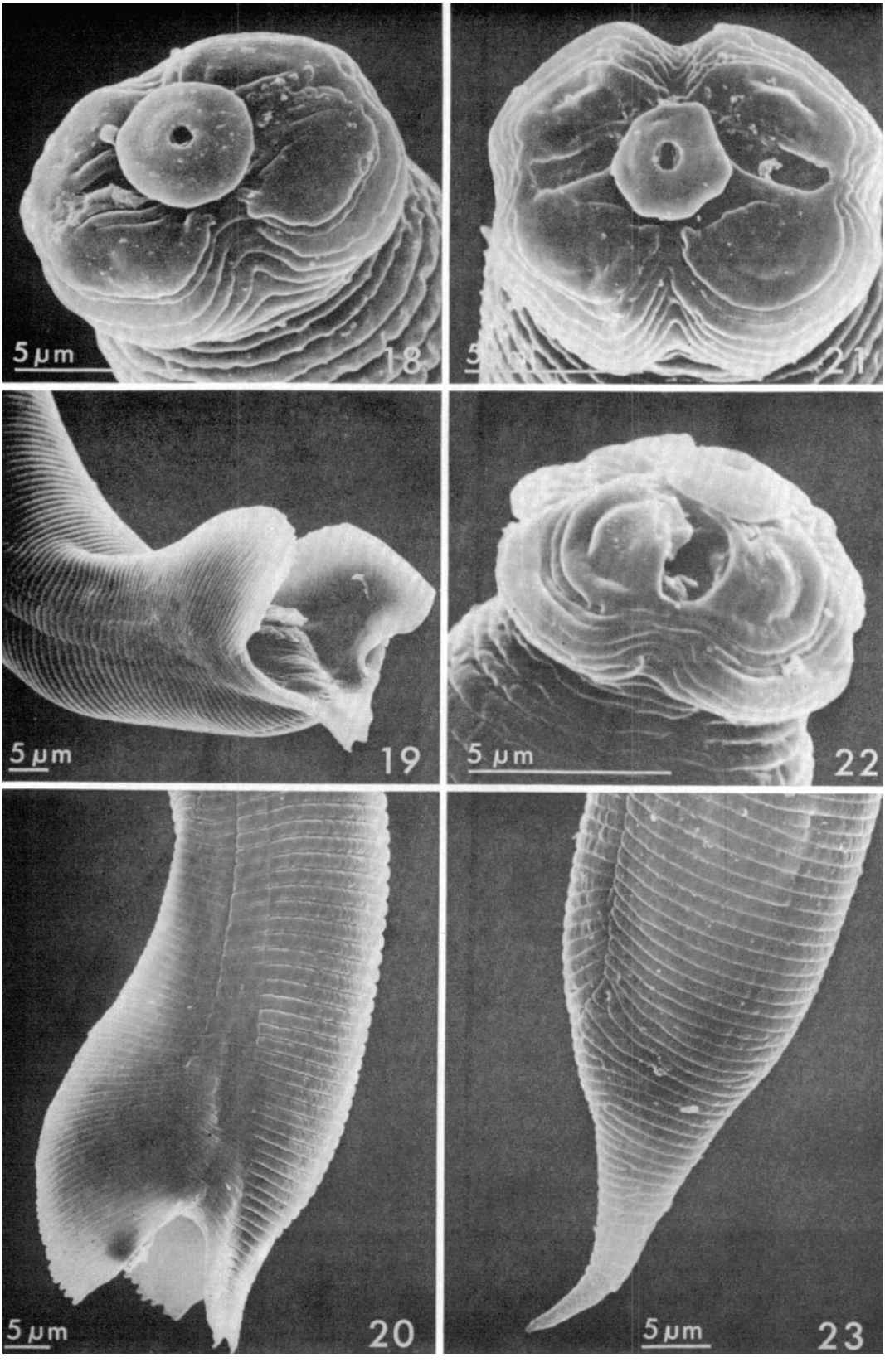
*Males* (25): Length 1685.6–2114.1 μm (1950.4 μm, SD 130.3); width 35.5–44.4 μm (42.3 μm, SD 3.3); a = 41.1–55.8 (46.3, SD 4.2); b = 6.7–9.4 (8.3, SD 0.6); c = 47.1–




Figs. 1–8. Drawings of *Dolichodorus marylandicus* n. sp. 1) Anterior region of female (note location of excretory pore and massive basal plate of sclerotized labial framework). 2) *En face* view of head. 3–5) Tail shapes of females (note reduction in tail diameter and location of anus). 6) Ventral view of vulva. 7) Lateral view of vulva (note lack of sclerotized accessory pieces adjacent to vulva, the strongly sclerotized distal vagina, and convoluted proximal vagina). 8) Male tail, lateral view (note sclerotized structure between gubernaculum and spicule).




Figs. 9–17. SEM micrographs of *D. marylandicus* n. sp. females. 9) *En face* view (note amphidial secretion covering amphid openings). 10) Labial region with offset oral disc. 11) Lateral field just anterior to vulva. 12–13) View of cephalic region (note amphidial opening in Fig. 12). 14–17) Variation in tail shape.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64.8 (56.2, SD 5.1); total stylet 82.9–92.4 μm (87.3 μm, SD 3.1); stylet conus 45.8–51.6 μm (48.6 μm, SD 1.8); DGO 3.7–5.8 μm (4.7 μm, SD 0.5) from base of stylet; center of median bulb 134.8–152.8 μm (143.1 μm, SD 6.7) from anterior end; spicules 41.4–51.0 μm (45.6 μm, SD 2.0); gubernaculum 24.4–29.0 μm (27.0 μm, SD 1.1); tail 30.1–39.4 μm (34.9 μm, SD 2.6); phasmids 17.4–23.7 μm (19.2 μm, SD 2.1) from tail terminus; anal body width 22.0–28.2 μm (25.1 μm, SD 1.9); excretory pore 157.5–207.9 μm (182.0 μm, SD 13.5) from anterior end.

*Allotype* (male): Length 1920.8 μm; a = 49.0; b = 8.9; c = 57.5; total stylet 83.2 μm; stylet conus 46.2 μm; DGO 5.1 μm from base of stylet; center of median bulb 139.2 μm from anterior end; spicules 41.6 μm, gubernaculum 24.4 μm; tail 33.4 μm; phasmids 19.4 μm from tail terminus; anal body width 22.0 μm; excretory pore 160.3 μm from anterior end.

*Description of males:* Body similar to female in head shape (Fig. 18), annulation, and location of excretory pore; but slightly smaller, with posterior slightly hooked ventrally, and with tail terminus bifurcate (Figs. 19, 20). Lateral field about 1/4 of body width; with three lines beginning anteriorly as for the female and extending, areolated, to the posterior portion of the body. One outer line fades near the proximal portion of the caudal alae and the other two lines extend to a level adjacent to the middle of the spicules or beyond. Caudal alae typical for the genus, with small projection on each distal portion and small, pore-like phasmids located about half the distance from anus to tail tip (Fig. 8). Testis outstretched with globular sperm in seminal vesicle. In lateral view, gubernaculum slightly curved with proximal part enlarged, often forming a hook. Gubernaculum with accessory structure interposed with adjacent spicule (Fig. 8).

*Holotype* (female): Collected in June 1971 by A. Morgan Golden at College Park, Maryland, and subsequently grown on zoysia and perennial bluegrass in an isolated greenhouse area. Slide T-325t, USDA Nematode Collection (USDANC), Beltsville, Maryland, USA.

*Allotype* (male): Slide T-326t. Same data as holotype. USDANC, Beltsville, Maryland, USA.

*Paratypes* (males, females, juveniles): USDANC, Beltsville, Maryland, USA; Department of Plant Pathology and Physiology, Clemson University, Clemson, South Carolina, USA; Nematology Department, Rothamsted Experimental Station, Harpenden, Herts, England; Laboratoire des Vers, Muséum National d'Histoire Naturelle, Paris, France; Institute voor Dierkunde, Laboratorium voor Morfologie en Systematiek der Dieren, Ledeganckst, 35, B-900, Gent, Belgium; and Laboratory voor Nematologie, Binnehaven 15, Wageningen, Netherlands.

*Type host and locality:* Soil around roots of *Zoysia japonica* in a lawn in College Park, Maryland, USA.

*Diagnosis: Dolichodorus marylandicus* n. sp. is related to *D. heterocephalus* Cobb, 1914 (6), *D. aestuarius* Chow & Taylor, 1979 (3), and *D. similis* Golden, 1958 (10). It differs from these species by, among other characters, tail shape and lack of rounded sclerotized structures (18) adjacent to the strongly sclerotized distal portion of the vagina. It also differs from *D. similis* by the location of the excretory pore. Characters useful in distinguishing species within the genus are provided in the key given below.

*Distribution: D. marylandicus* has also been found in soil around roots of perennial bluegrass (*Poa pratensis*) pasture in Maryland and from pine (*Pinus* sp.) in North Carolina.

## Key to Genera of Dolichodorinae

1. Stylet very strong, much less than 60 μm; female tail greatly attenuated ..... *Brachydorus* De Guiran & Germani, 1968 (7).

Figs. 18–23. SEM micrographs of *D. marylandicus* n. sp. males and *D. heterocephalus* females. 18) Cephalic region of *D. marylandicus* n. sp. 19–20) Caudal area of *D. marylandicus* n. sp. 21–22) *D. heterocephalus* cephalic region (note large amphidial opening and asymmetrical oral disc). 23) Caudal area of *D. heterocephalus*.

Stylet greater than 60 μm; female tail not greatly attenuated ..... 2
2. Female tail conically pointed; lateral fields each with three incisures ..... *Dolichodorus* Cobb, 1914 (6).
Female tail bluntly rounded, lateral fields each with four incisures ..... *Neodolichodorus* Andrássy, 1976 (2) (= *Plesiodorus* Siddiqi, 1976 (17,18).

Key to Species of *Dolichodorus* Cobb, 1914

1. Excretory pore opposite, or anterior to median bulb ..... 2
Excretory pore posterior to median bulb ..... 5
2. Excretory pore opposite procorpus or base of stylet ..... *D. pulvinus* Khan, Seshadri, Weischer, & Mathen, 1971 (12).
Excretory pore opposite median bulb .. 3
3. Female T/ABW 3; body less than 2 mm long ..... *D. nigeriensis* Luc & Caveness, 1963 (14).
Female T/ABW 2 or less; body greater than 2 mm long ..... 4
4. Lip region deeply offset, cushion-shaped or flattened; anterior cuticle appearing as rectangular plates; stylet length less than 120 μm ..... *D. profundus* Luc, 1960 (15).
Lip region offset but rounded; anterior cuticle not marked by rectangular plates; stylet length greater than 130 μm ..... *D. silvestris* Gillespie & Adams, 1962 (9).
5. Excretory pore posterior to nerve ring and at a level with the middle of the basal bulb .. *D. similis* Golden, 1958 (10).
Excretory pore at level of isthmus ..... 6
6. Body length less than 2 mm long ..... *D. minor* Loof & Sharma, 1975 (13).
Body length greater than 2 mm ..... 7
7. Stylet length 62–76 μm, unusually short in relation to a body length of 2.50–2.87 mm ..... *D. aestuarius* Chow & Taylor, 1978 (3).
Stylet 83 μm or more ..... 8
8. Stylet 99 μm or more, tail smoothly tapering to irregularly conoid, acute terminus (Fig. 23) ..... *D. heterocephalus* Cobb, 1914 (6).
Stylet 83–92 μm, tail short, abruptly reduced in diameter, acuminate terminus often spikate ..... *D. marylandicus* n. sp.

*Observations on the identity of D. heterocephalus:* The male in the original description of *D. heterocephalus* was reported by Cobb to have come from Douglas Lake, Michigan (6). Examination of his original pencil drawings indicate that a male from Silver Springs, Florida, and one from Michigan were drawn. Measurements included in his publication are of the Michigan male, but the drawings are of the male from Florida.

Thorne reported stylet lengths of 83–95 μm for females of *D. heterocephalus* (19). The stylet lengths of collections of *D. heterocephalus* gathered for this study and of the drawing of the female in the type description (6) were at least 99 μm. In addition, Loof & Sharma reported stylet measurements of at least 99 μm for this species (13).

The accessory piece, later called the gubernaculum, pictured in Cobb's drawing of the male *D. heterocephalus* is composed of two sclerotized parts. There is the narrow, long, distally recurved portion shown in drawings of the different species. Also present is a shorter piece, observed in lateral views of *D. heterocephalus* and *D. marylandicus* n. sp., between the gubernaculum proper and the adjacent spicule (Fig. 8). This portion has not been pictured in drawings of the gubernaculum and spicula since Cobb's 1914 article (6). All species of *Dolichodorus* should be examined for the presence of this character, and if it is uniformly present, the generic description should be amended.

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