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Longidorus paravineacola n. sp. (Nematoda: Longidoridae), a New Species from Arkansas[1]

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Abstract: *Longidorus paravineacola* n. sp., described herein, was found in a survey of longidorids of Arkansas. It is a parthenogenetic species characterized by its long body (6.68–9.85 mm); slightly expanded and rounded head, head width 21–27 µm; odontostyle length 95–114 µm; guide ring 28–37 µm posterior to the head end; short rounded tail, and c′ = 0.6–1.0. *Longidorus paravineacola* n. sp. is similar to the amphimictic species *L. vineacola* Sturhan & Weischer, 1964; *L. balticus* Brzeski, Peneva & Brown, 2000; *L. kuiperi* Brinkman, Loof & Barbez, 1987; and parthenogenetic species *L. crassus* Thorne, 1974, which also occurred in the type locality.

Key words: Hierarchical cluster analysis, *Longidorus paravineacola, L. vineacola,* morphology, species, SEM, taxonomy.

In a survey of longidorid species from natural (non-agricultural) sites in Arkansas in 1999–2002, five populations of an undescribed *Longidorus* species were found associated with hardwood trees along stream banks at various locations (Table 1). These populations are described herein as *L. paravineacola* n. sp., due to its close resemblance to *L. vineacola* Sturhan & Weischer, 1964.

Materials and Methods

Sampling: Soil samples, part of a survey for longidorid nematodes in Arkansas, were collected at a depth of 10–40 cm from the rhizosphere of hardwood trees growing in sandy soil on stream banks.

Nematode extraction, fixing, and mounting: Soil was suspended in water, and the suspension was poured through an 850-µm-pore sieve to remove plant debris and a 75-µm-pore sieve to extract the nematodes. Nematodes caught on the 75-µm-pore sieve were separated from soil and other debris by sucrose (specific gravity = 1.167; 568g sucrose in 1 liter water) centrifugation-flotation technique (Jenkins, 1964). Nematodes were killed and fixed by slowly adding boiling water until the volume of solution containing the nematodes was doubled; then formalin (37%) was added to make a 2% final concentration. The nematodes were processed to glycerin by a modification of Seinhorst's rapid method (1959) and permanently mounted on 25 × 75-mm microscope slides.

Morphometrics: Specimens were examined using a Nikon Optiphot II compound microscope with Nomarski interference contrast at powers up to ×1,000 magnification. Drawings were made using a Nikon drawing tube. Morphometric data were processed using Excel (Ye, 1996) and expressed as mean ± standard deviation (minimum to maximum). A population is defined herein as the same species from the same site, regardless of host.

Scanning electron microscopy (SEM): Fresh nematode specimens for SEM were fixed in Karnovsky's fixative for 2 hours after being killed by heat relaxation, washed in two changes of 0.05M cacodylate buffer (pH 7.2) for 20 minutes each, rinsed with distilled water twice, fixed with equal volumes of 0.1M cacodylate and 2% osmium for 2 hours, dehydrated in a graded ethanol series of 30%, 50%, 70%, 80%, 95%, and 100% (three changes) with 10 minutes in each solution, and then dried in hexamethyldisilazane for 5 minutes three times. The nematodes were mounted on SEM stubs using toluene-adhesive tape, sputter coated with approximately 300Å of gold, and examined with an ISI-60 SEM at 15 kv.

Hierarchical cluster analysis: The morphometric characters used were L, distance of vulva from anterior end, head width, odontostyle length, guide ring position from anterior end, esophagus length, body width, tail length, and anal body width. Hierarchical cluster analysis was performed with the JMP 4.02 (SAS Institute, Cary, NC) program. The populations (Table 1) and their measurements used for this study are listed in Tables 2 and 3. The morphometric measurements of all 131 Longidorus species are from published resources, values of which were obtained from the means of paratypes or holotype of the original species descriptions.

Systematics

Longidorus paravineacola n. sp.
(Figs. 1–3)

Measurements: See Table 2.

Description

Females (paratypes): Body spirals upon heat relaxation, tapering toward both ends, cuticle smooth as seen by light microscopy, with fine transverse striae as observed by SEM. Head region slightly expanded, hemispherical, 21–27 µm wide. Amphidial pouches deeply bilobed, extend about 75% of the distance from the anterior end to the guide ring. Odontostyle long

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TABLE 1. Population numbers, associated plants, and location of *Longidorus paravineacola* n. sp. from Arkansas.

Population number	Associated plant	Location
Long-108	Elm, osage orange, sycamore, willow	Osage Creek, Highway 412, Carroll County
Long-123	Box elder, elm, grape, maple, oak, Osage orange, red bud, sycamore	Illinois River, County Road 62 Bridge, Washington County
Long-154	Birch, sweet gum	Little Red River, South Fork, Clinton, Van Buren County
Long-216	Osage orange	Mud Creek, Old Missouri Road, Fayetteville, Washington County
Long-266	Red bud	Middle Fork of the White River, near Elkins, Washington County

and slender, odontophore base not flanged. Guide ring 5 µm wide. Nerve ring close to the odontophore base. Esophagus dorylaimoid with cylindrical esophageal bulb. Basal esophageal bulb 117–140 µm long, 22–24 µm wide in paratypes, with the normal arrangement of one dorsal gland nuclei (23–27%) of the basal esophageal bulb length and two subventral nuclei (SV1 47–58%, SV2 49–64%). Cardia conoid at the junction of the esophageal bulb and the intestine. Reproductive system amphidelphic, didelphic, with reflexed ovaries. Anterior reproductive system length 564–836 µm, posterior reproductive system length 487–1,360 µm in paratypes. Vulva a transverse slit as seen obliquely by SEM. Vagina perpendicular to body axis with thickened cuticular lining, extending to half the body width. Paratypes anterior uterus 260–335 µm long, posterior uterus 264–457 µm long. Pre-rectum 365–781 long. Tail short, bluntly rounded, less than one anal body width long. Hyaline region 10–22 µm long.

Males: Not found.

TABLE 2. Morphometrics of the holotype and paratypes of *L. paravineacola* n. sp. (Long-123).

Character	Holotype	Paratypes	Long-108	Long-154	Long-216	Long-266
n	1	25	8	1	4	2
L	8.21	8.59 ± 0.87 (7.03–9.85)	7.65 ± 0.56 (6.68–8.25)	7.31	7.64 ± 0.38 (7.10–7.95)	8.09 ± 0.66 (7.62–8.55)
a	132.6	135.7 ± 13.1 (105.2–161.3)	116.8 ± 7.9 (104.4–127.2)	116.0	127.9 ± 26.1 (101.4–159.0)	125.2 ± 6.1 (121.0–129.5)
b	18.6	18.0 ± 2.52 (11.5–25.8)	16.8 ± 1.0 (15.6–18.2)	16.8	17.8 ± 1.8 (16.6 ± 20.5)	16.9 ± 1.0 (16.2–17.6)
c	202.2	236.5 ± 36.8 (164.9–313.7)	234.7 ± 28.2 (206.2–290.7)	197.6	210.9 ± 25.5 (177.5–238.8)	224.6 ± 18.3 (211.7–237.5)
c′	0.9	0.8 ± 0.1 (0.7–1.0)	0.7 ± 0.1 (0.6–0.9)	0.8	0.9 ± 0.1 (0.8–1.0)	0.8 ± 0.0 (0.8–0.8)
G1%	7.1	7.8 ± 1.07 (6.6–10.4)	8.0 ± 2.4 (4.5–11.7)	10.9	6.7 ± 0.6 (6.1–7.4)	6.5 ± 0.0 (6.4–6.5)
G2%	7.4	8.3 ± 2.1 (6.4–15.2)	6.9 ± 2.7 (4.4–12.5)	11.1	6.8 ± 1.0 (5.7–7.9)	6.7 ± 0.6 (6.3–7.2)
V	50.4	51.6 ± 2.0 (47.7–56.1)	48.9 ± 1.6 (46.7–50.9)	49.7	48.5 ± 0.9 (47.4–49.3)	49.0 ± 0.2 (48.8–49.1)
H%	30.0	43.0 ± 7.1 (33.3–56.7)	48.8 ± 9.5 (35.0–60.0)	32.4	40.7 ± 10.1 (27.8–50.0)	38.9 ± 3.9 (36.1–41.7)
Odontostyle	107.6	105.3 ± 5.4 (100.5–113.7)	101.0 ± 3.6 (95.0–106.0)	104.0	104.5 ± 5.4 (100.0 – 112.0)	107.0 ± 4.2 (104.0 – 110.0)
Odontophore	65.0	69.5 ± 2.3 (65.0–75.1)	74.0 ± 5.7 (63.0–80.0)	59.0	63.0 ± 3.5 (60.0–66.0)	72.5 ± 6.4 (68.0–77.0)
Total stylet	172.6	174.8 ± 5.1 (168.5–184.7)	175.0 ± 7.1 (163.0–183.0)	163.0	167.5 ± 3.1 (165.0–172.0)	179.5 ± 2.1 (178.0–181.0)
Guide ring from anterior end	32.5	33.2 ± 2.0 (28.4–36.5)	32.3 ± 2.1 (29.0–35.0)	28.4	31.0 ± 1.6 (29.0–33.0)	34.5 ± 0.7 (34.0–35.0)
Head width	23.3	24.1 ± 0.8 (22.3–25.4)	25.3 ± 1.3 (23.0–27.0)	25.0	22.8 ± 1.3 (21.0–24.0)	24.5 ± 0.7 (24.0–25.0)
Body width	61.9	63.4 ± 3.4 (56.8–69.0)	65.6 ± 5.6 (58.0–76.0)	63.0	61.3 ± 10.3 (50.0–70.0)	64.5 ± 2.1 (63.0–66.0)
Tail length	40.6	36.8 ± 4.0 (30.5–44.7)	32.9 ± 3.8 (28.0–40.0)	37.0	36.5 ± 3.4 (32.0–40.0)	36.0 ± 0.0 (36.0–36.0)
ABW	44.7	48.0 ± 2.4 (42.6–50.8)	45.8 ± 2.1 (43.0–50.0)	45.0	43.0 ± 4.2 (39.0–48.0)	45.5 ± 2.1 (44.0–47.0)
Hyaline tail tip	12.2	15.8 ± 2.3 (10.2–22.3)	15.8 ± 1.8 (13.0–18.0)	12.0	14.8 ± 3.4 (10.0–18.0)	14.0 ± 1.4 (13.0–15.0)

TABLE 3. Morphometrics of other *Longidorus* spp. closely related to *Longidorus paravineacola* n. sp.

Character	*L. vineacola* Sturhan & Weischer, 1964	*L. vineacola* Paratype	*L. vineacola* Long-199	*L. balticus* Brzeski, Peneva & Brown, 2000 Holotype	*L. kuiperi* Brinkman, Loof & Barbez, 1987
n	18	1	4	1	20
L.	8.16 (6.90–9.20)	9.03	7.44 ± 0.83 (6.67–8.40)	7.632	7.47 ± 0.55 (6.48–8.48)
a	137 (120–149)	125.4	128.0 ± 10.5 (117.0 ± 140.0)	122	147 ± 11.6 (125–171)
b	17.6 (15.4–19.5)	21.0	17.5 ± 1.5 (16.2–19.5)	17.5	16.4 ± 1.17 (13.0–18.2)
c	213 (186–247)	225.8	224.2 ± 10.5 (213.1–238.2)	263	266 ± 19.9 (231–314)
c′	0.8*	0.7	0.8 ± 0.1 (0.8–0.9)	0.7	0.76 ± 0.68 (0.6–0.9)
G1%	9.1 (7.8–11.1)	9.4	10.1 ± 1.8 (9.0–12.7)		8.6 ± 2.15 (6–15)
G2%	8.9 (6.7–11.0)	8.0	9.8 ± 1.7 (8.4–12.1)		7.7 ± 1.42 (5–11)
V	52.2 (49.7–54.3)	50.7	52.0 ± 0.8 (51.0–52.8)	53	52.7 ± 1.45 (50–55)
H%	35.5*	35.0	29.1 ± 5.5 (23.7–35.7)	13	38*
Odontostyle	96 (90–100)	102.0	101.3 ± 2.5 (98.0–104.0)	100	106.6 ± 3.05 (101–113)
Odontophore	44 (40–50)	70.0	62.3 ± 7.6 (52.0–70.0)	65	61.4 ± 2.89 (57–67)
Total stylet	141 (136–151)	172.0	163.5 ± 10.1 (150.0–174.0)	165	168 ± 4.33 (158–175)
Guide ring from anterior end	28–35	34.0	28.5 ± 1.0 (28.0–30.0)	27	27.6 ± 1.39 (25–31)
Head width	20–23*	20.0	20.0 ± 1.4 (19.0–22.0)	20	28.6 ± 1.27 (27–31)
Body width	58.6*	72.0	58.0 ± 1.8 (56.0–60.0)	60*	48*
Tail length	42*	40.0	33.3 ± 4.3 (28.0–38.0)	27	24*
ABW	50*	54.0	40.0 ± 3.7 (35.0–44.0)	13	36.9 ± 2.49 (33–42)
Hyaline tail tip	18*	14.0	9.5 ± 0.6 (9.0–10.0)		11.9 ± 0.99 (10–14)

* Measurement from original figure.

Juveniles: Juvenile stages were not defined. They occurred in mixed populations with the similar species *L. crassus* in the type and other locations.

Type locality and habitat

Soil around box elder (*Acer negundo* L.), elm (*Ulmus americana* L.), grape (*Vitis* sp. L.), maple (*Acer* sp. L.), oak (*Quercus* sp. L.), Osage orange (*Maclura pomifera* (Raf. Schneid.), redbud (*Cercis canadensis* L.), and sycamore (*Platanus occidentalis* L.) by County Road 62 Bridge, Illinois River, Washington County, Arkansas, collected by R. T. Robbins and Weimin Ye on 15 June 1999 (population Long-123). Global positional coordinates N 36°; 01.538 minutes; W 094° 19.266 minutes.

Type specimens

Holotype female (slide T-568t) deposited in Nematology Laboratory Collection, USDA, ARS, Beltsville, Maryland. Two paratype females are deposited as follows: Department of Nematology, University of California, Riverside; Department of Nematology, University of California, Davis; CABI Bioscience, UK Centre, Surrey, UK; Department of Nematology, Agricultural University, Wageningen, the Netherlands; and Institute of Parasitology Collection, Moscow, Russia. The remaining paratypes deposited in the Nematology Laboratory Collection, USDA, ARS, Beltsville, Maryland.

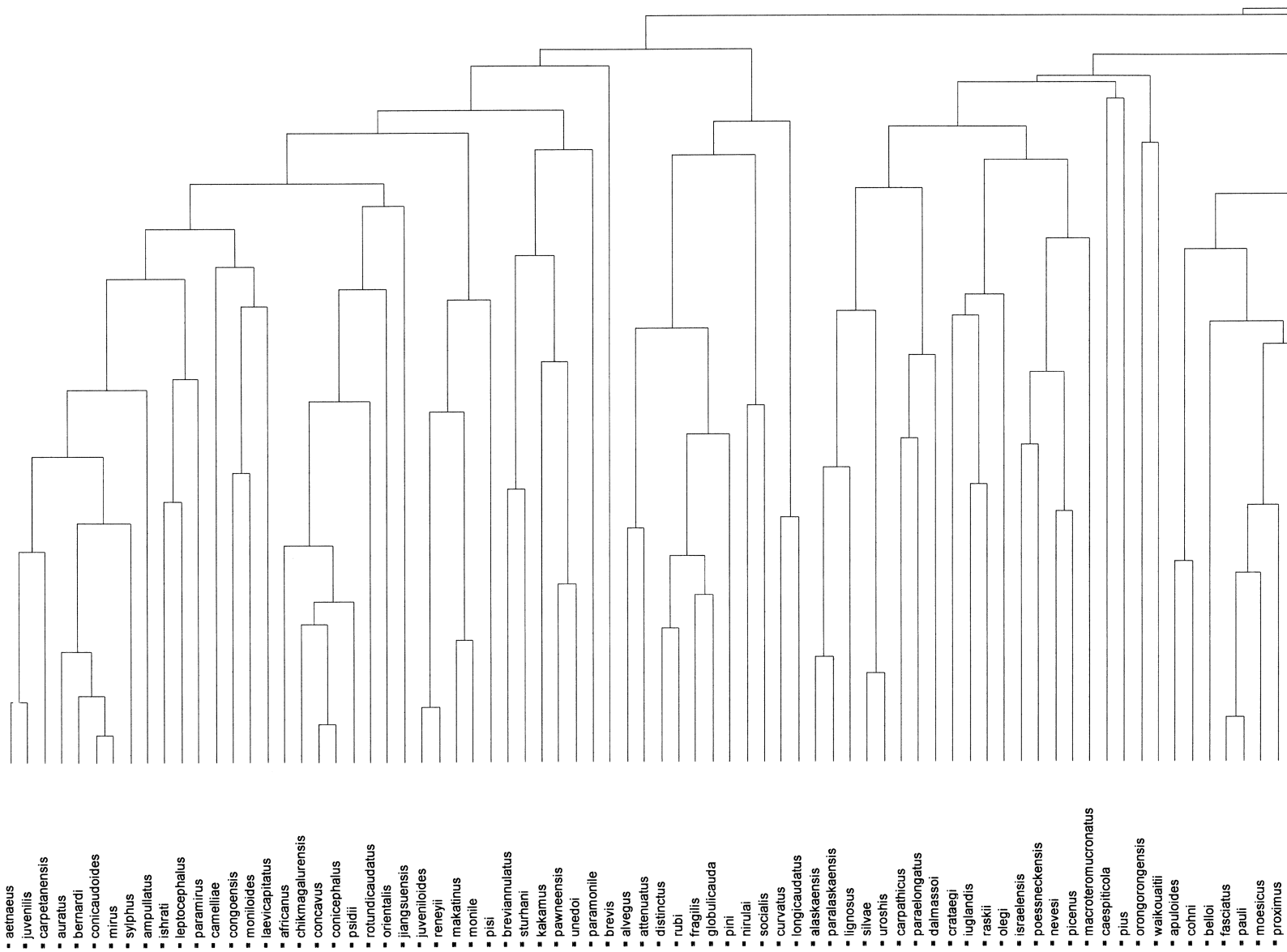
Etymology

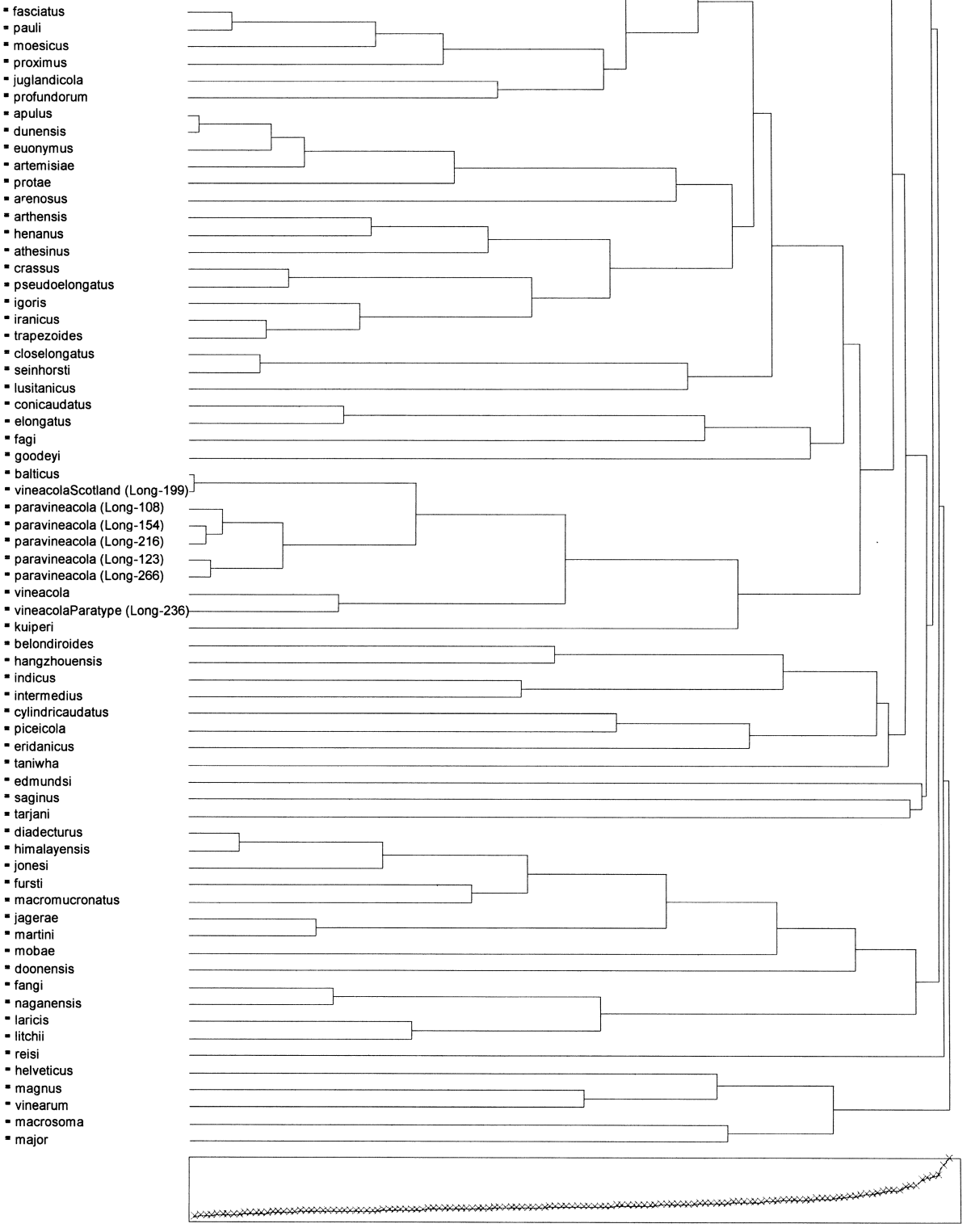
The prefix "para" means "near" and refers to the close morphological relationship with *L. vineacola* Sturhan & Weischer, 1964.

Diagnosis

Longidorus paravineacola n. sp. is a parthenogenetic species. It is characterized by its body length (6.68–9.85 mm), rounded slightly expanded head, head width 21–27 µm, odontostyle 95–114 µm, guide ring 28–37 µm

FIG. 4. A portion of a hierarchical cluster dendrogram (average method) of 131 species of *Longidorus* showing five populations of *L. paravineacola* n. sp. and closely related species.

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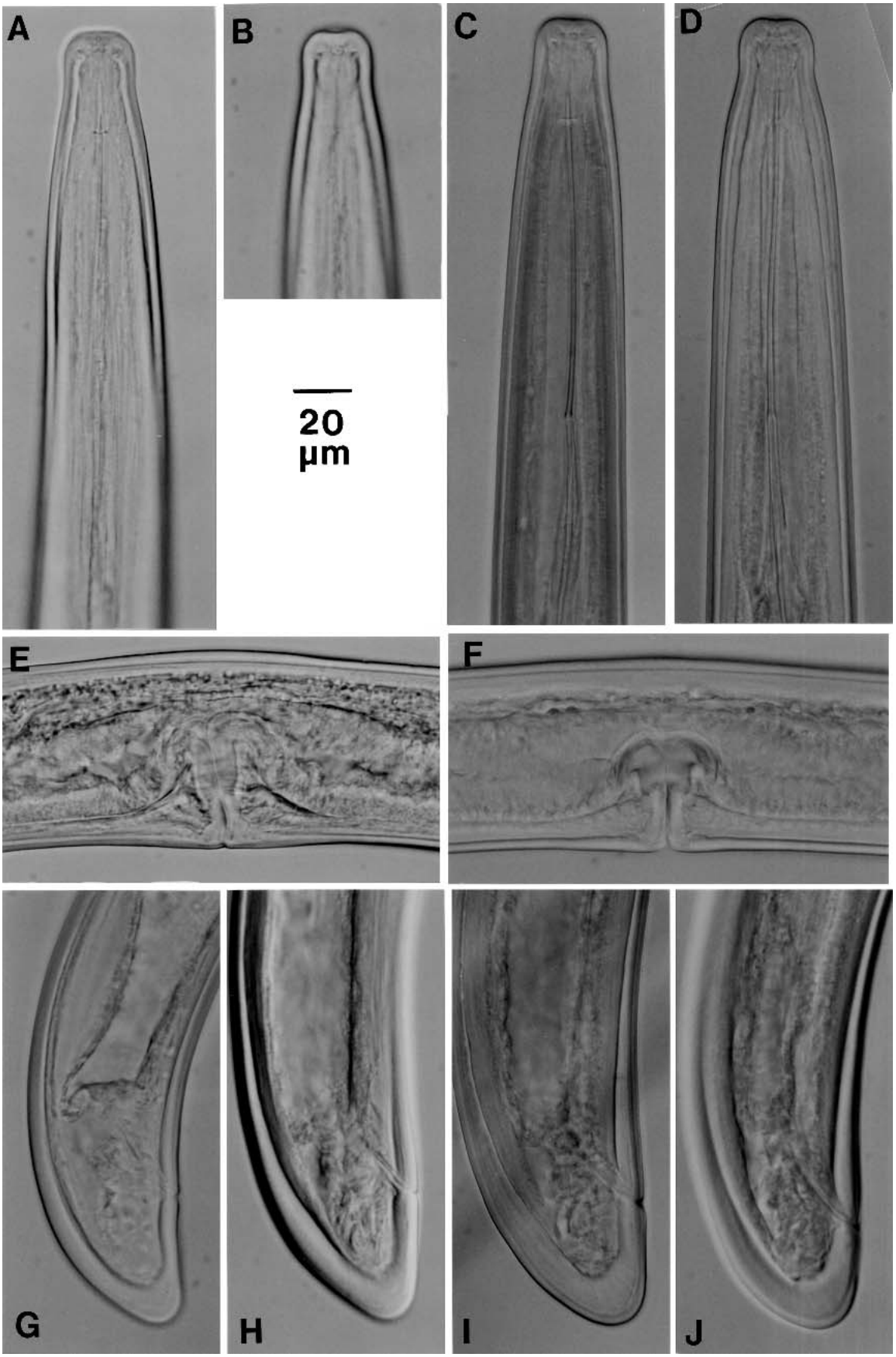

FIG. 1. A–J) Photographs of female *Longidorus paravineacola* n. sp. A, B, E, G) Holotype. A) Female head region. B) Amphid region. C–D) Paratype female head regions. E–F) Vulval region. G–J) Variations in female tail shape.

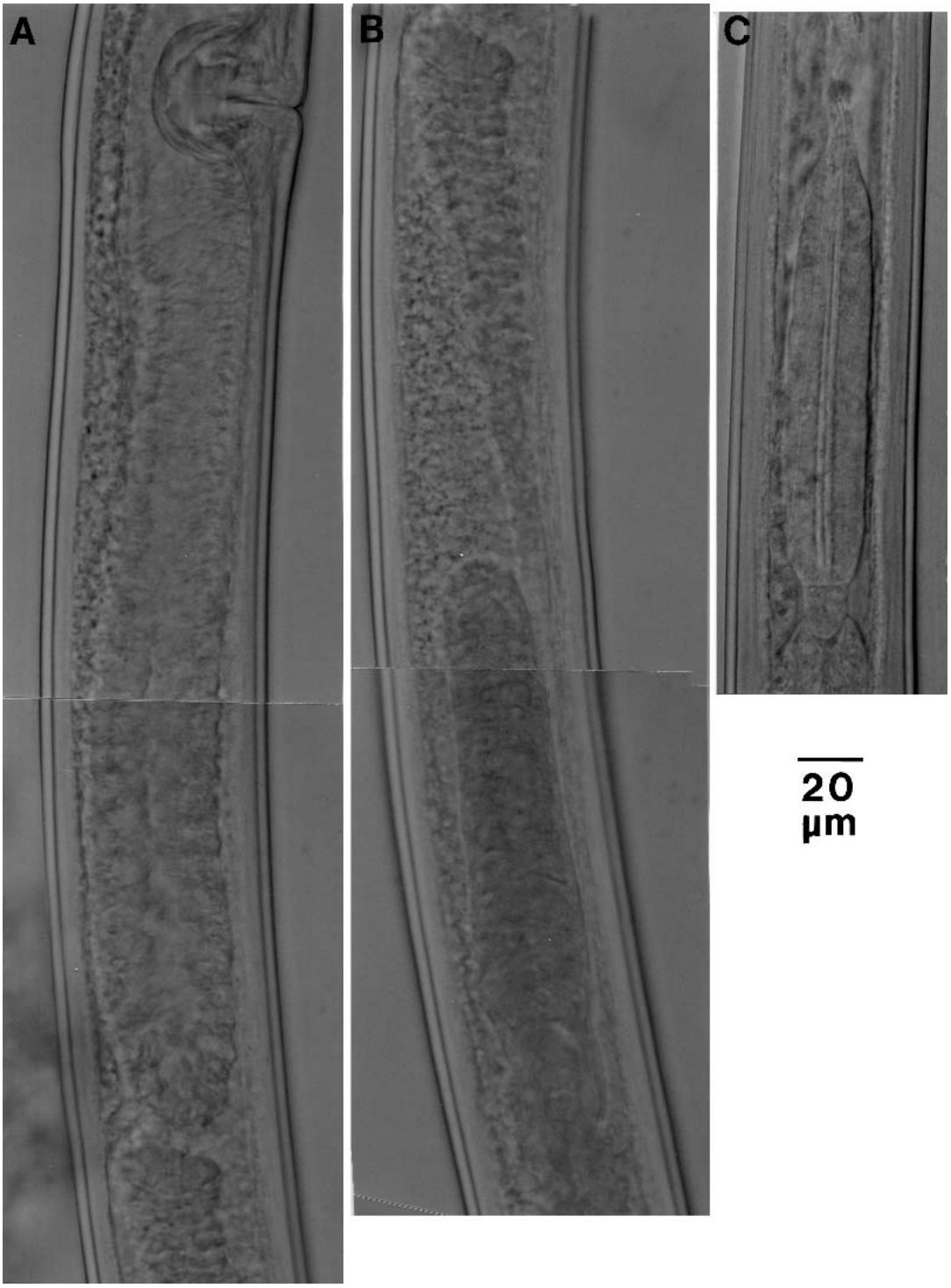

FIG. 2. A–C) Photographs of female *Longidorus paravineacola* n. sp. A) Vulval region to sphincter between uterus and oviduct. B) Oviduct to reflex of ovary. C) Basal esophageal bulb.

posterior to the anterior end, short rounded tail, c′ = 0.6–1.0. The code for identifying the new species according to the polytomous key of Chen et al. (1997) is: A34-B45-C23-D3-E2-F345-G234-H1-I1.

Relationships

Longidorus paravineacola n. sp. is similar to *L. vineacola* Sturhan & Weischer, 1964 described from Germany; *L. balticus* Brzeski, Peneva & Brown, 2000 described from Poland; *L. kuiperi* Brinkman, Loof & Barbez, 1987 described from the Netherlands; and superficially to *L. crassus* Thorne, 1974 described from South Dakota and Iowa that occurred in some of the samples as a mixture. It can be distinguished from *L. vineacola* by its wider head, 21–27 vs. 20–23 µm in *L. vineacola,* and parthenogenetic vs. amphimictic reproduction (Tables 2, 3). Populations of *L. vineacola* from Germany and the United Kingdom have abundant males. *Longidorus para-*

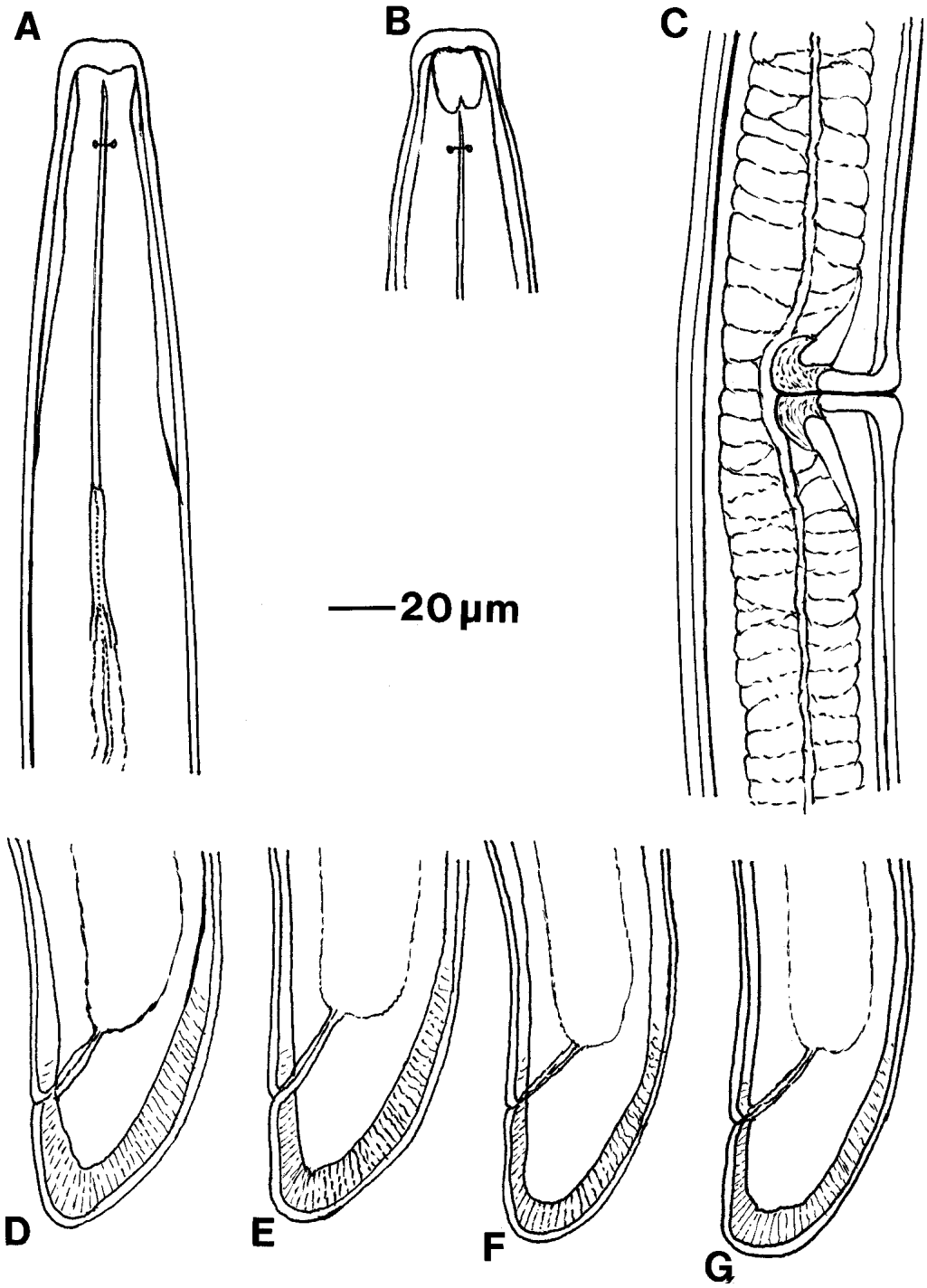

FIG. 3. Drawings of *L. paravineacola* n. sp. female paratypes. A) Anterior region. B) Amphid region. C) Vulval region. D–G) Variations in tail shape.

vineacola n. sp. differs from *L. balticus* by its wider head (21–27 mm vs. 20 µm), more posterior guide ring (28–37 vs. 27 µm), and parthenogenetic reproduction vs. amphimictic reproduction. *Longidorus paravineacola* n. sp. differs from *L. kuiperi* by its more posterior guide ring (28–37 vs. 25–31 µm), narrower head (21–27 vs. 27–31 µm), and parthenogenetic reproduction vs. amphimictic reproduction. *Longidorus paravineacola* n. sp. can be distinguished from *L. crassus* (Arkansas populations) by its longer body (6.68–9.85 vs. 3.35–7.67 mm) and wider head (21–27 vs. 15–23 µm). Phylogenetic analysis based on 18S gene DNA sequencing revealed that *L. paravineacola* n. sp., *L. crassus* from Arkansas, and *L. vineacola* from Scotland are different species (unpubl. data).

Distribution

Five populations of *L. paravineacola* were found associated with hardwood trees on stream banks in sandy soil at five locations in Arkansas (Table 1).

Hierachical cluster analysis

The dendrogram obtained from the hierachical cluster analysis by the Average method (by JMP software) from all 131 published Longidorus species, and the five populations of this species demonstrated that *L. kuiperi, L. balticus, L. vineacola* paratypes, and *L. vineacola* from Scotland (courtesy of D. Brown) are in the same cluster with five populations of *L. paravineacola* (Fig. 4). One paratype of *L. vineacola,* borrowed from D. Sturhan and measured by us, is in the same cluster with the 18 paratypes based on means measured by Sturhan and Weischer (1964) but separated with *L. vineacola* from Scotland. This indicates that *L. vineacola* has a high degree of variability. *Longidorus balticus* has high similarity with *L. vineacola* from Scotland based on this dendrogram.

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