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Myctolaimellus robiniae n. sp. (Diplogasterida: Cylindrocorporidae) from Larval Cavities of the Locust Borer, *Megacyllene robiniae* Forster

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Abstract: A new nematode species of the family Cylindrocorporidae and the genus *Myctolaimellus* from subcortical cavities made by the locust borer (*Megacyllene robiniae* Forster) in black locust (*Robinia pseudoacacia* L.) is described. Males of the new species have a length of 700 to 1,050 µm; a bursate tail, peloderan with nine pairs of rays; and knobbed, curved spicules with tips bending gently into a hook. The distinctive gubernaculum is half the length of the spicules, deeply grooved longitudinally along both its dorsal and ventral surfaces, and has a spoon-shaped end. Females have a length of 830 to 1,340 µm, an amphidelphic reproductive tract with long ovaries crossing each other to extend beyond the equatorial vulva, and a gradually tapering tail.

Key words: Appalachian, black locust, Cerambycidae, Coleoptera, locust borer, *Megacyllene robiniae,* morphology, *Myctolaimellus robiniae,* nematode, *Robinia pseudoacacia,* species, systematics, taxonomy, technique.

The genus *Cylindrocorpus* was established to include those species formerly placed in the genus *Cylindrogaster* Goodey, 1927 (Goodey, 1939). Simultaneously, the family Cylindrocorporidae was proposed to replace Cylindrogasteridae Chitwood 1933 and included the genera *Cylindrocorpus, Goodeyus, Myctolaimus,* and *Longibucca* (Goodey, 1939). *Protocylindrocorpus* Rühm, 1959 was later added as a genus by Paramonov (Kinn, 1984; Rühm, 1959). In 1984, Andrássy regrouped the family into four genera based on the female reproductive system (monodelphic or amphidelphic) and the male bursa (peloderan or leptoderan). Thus, the genera *Longibucca* and *Cylindrocorpus* were deleted and a new genus, *Myctolaimellus,* was created for amphidelphic females and peloderan males while the genus *Goodeyus,* including *G. ulmi* (Goodey, 1930, 1939), was reserved for prodelphic females and peloderan males. Males of both *Myctolaimus* and *Protocylindrocorpus* have leptoderan tails; however, *Protocylindrocorpus* females are prodelphic whereas *Myctolaimus* females are amphidelphic.

The majority of nematodes in the family Cylindrocorporidae are saprobes, often found in symbiotic relationships with insects. The habitats of the species comprising the family Cylindrocorporidae are widely varied. *Myctolaimus erectus* (Massey, 1960) was found associated with scolytid bark beetles in American elm; *M. macrolaima* Goodey, 1938 (Massey, 1960) in rotting plant tissue; *M. curzii* (Goodey, 1935) in rotting bitter cassava; *M. coprophaga* (Goodey, 1927) free-living in rat feces; *M. longistoma* (Stefañski, 1922) in manure and under tree bark; and *M. rifflei* (Massey and Hinds, 1970) in aspen cankers.

A diverse guild of nematodes was discovered while investigating the invertebrate associates of locust borer larvae (*Megacyllene robiniae* Forster) in larval cavities of black locust trees, *Robinia pseudoacacia* L. Subcortical cavities are excavated by borer larvae in late April-early May and become enlarged, filled with borer frass, and soaked with sap. This medium is attractive to a variety of sap-feeding insects including beetles, dipterans, and ants, which probably phoretically transport nematodes. This paper describes the morphology and morphometrics of a new

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species of *Myctolaimellus* flourishing in cavities inflicted on black locust trees by the locust borer.

Materials and Methods

Over the summers of 1999 and 2000, starting in June and continuing until late August, nematodes were collected in western Maryland from black locust trees bearing evidence of locust borer attack using detection methodology described by Harman and Harman (1990). Nematodes were extracted with cold water from wound fragments of black locust tissue using the Baermann method. Observations and measurements were made of heated, temporary wet mounts using differential interference contrast (transmitted light Nomarski) phase-contrast, as well as bright-field light microscopy (Olympus IX50/IX70 inverted system microscope). Several high-resolution (1500X) images of living specimens were obtained by Image-Pro and pieced together using Adobe Photoshop 5.5. Nematodes were fixed in hot glycerin-formaldehyde after identification and examination. Permanent mounts were made in glycerin after transferring the nematodes to glycerin through ethanol using the Seinhorst method (Seinhorst, 1959). All measurements are in micrometers (μm) unless otherwise stated.

Systematics

Myctolaimellus robiniae n. sp.
(Figs. 1–3)

Description

Cuticle thin, with fine transverse and longitudinal striations. In both sexes, the body tapers increasingly anterior to the esophagus and, in the female, posterior to the anus (Figs. 1C; 3A). The mouth is surrounded by six forward-pointing conical lips, each possessing one tiny papilla (Figs. 1A; 3B). Cheilorhabdions are distinct. The stoma is long, narrow, and flexible. The esophagus, typical of the genus, is composed of a muscular, cylindrically shaped fused metacorpus and procorpus, slightly broader posteriorly; an isthmus without prominent musculature; and a glandular terminal bulb lacking a valve apparatus (Figs. 1A,B; 3B). The nerve ring, difficult to discern, encircles the isthmus near the excretory pore and is located slightly anterior to the terminal bulb (Fig. 3B).

Holotype (female in glycerin): L = 980; a = 22.27; b = 5.93; c = 8.52; v = 49.50%.

Female: Measurements of 27 mature females are listed in Table 1. The vulva, a slightly protuberant transverse slit, is equatorially located. The amphidelphic reproductive tract, located to the right of the intestine, is reflexed, with long ovaries crossing each other to extend beyond the region of the vulva (Figs. 1E,F,G; 3A). The branches of the reproductive tract are similar in length (the average length of the anterior and posterior branches is 248.9 and 241.7 μm, respectively) and lie dorsal to the intestine; however, in the region of the vulva, the reproductive tract expands ventrally, forcing the intestine in this region to a more dorsal position (Figs. 1E; 3A). The oocytes are arranged in a single row, and eggs possess a rough shell (Figs. 1H; 3A). A single egg may be present in both uteri simultaneously (Fig. 1D). The female tail is sharp and tapers to a slender point (Figs. 1C; 3A).

Allotype (male in glycerin): L = 890; spicules = 31; gubernaculum = 15; a = 22.25; b = 5.74; c = 30.70.

Male: Measurements of 25 mature males are listed in Table 1. The testis, located left of and slightly ventral to the intestine, is single with the anterior portion slightly reflexed (Figs. 2C,D; 3D). The curved spicules are paired, stubby, and possess both lateral and ventral flexures. Though they broaden somewhat anteriorly into a ventral prominence wider than the open manubrium, the spicule points are slim, rounded, and bent slightly into a hook (Figs. 2H; 3C). The gubernaculum is almost half the length of the spicules and grooved along both its dorsal and ventral surfaces, though more deeply ventrally, and has a rounded, spoon-shaped end (Fig. 2F). The peloderan tail has an open bursa supported by nine pairs of rays,

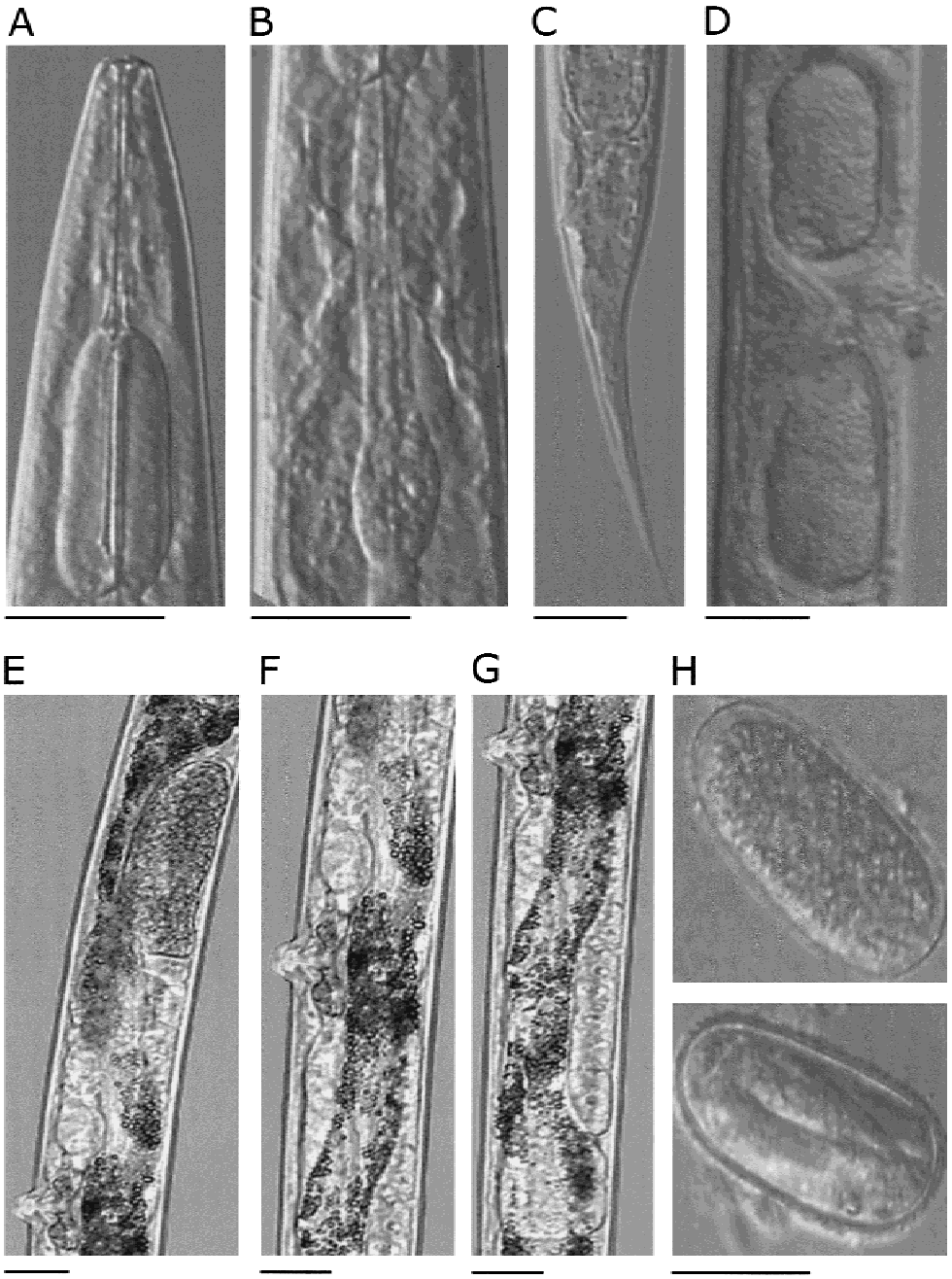

FIG. 1. Photomicrographs of *Myctolaimellus robiniae* n. sp. females. A) Anterior view showing fused procorpus and metacorpus, Nomarski. B) Anterior view showing isthmus and basal bulb, Nomarski. C) Lateral view showing rectum, anus, and tail, Nomarski. D) Uterus with eggs, Nomarski. E) Lateral view of anterior portion of amphidelphic reproductive tract, bright-field. F) Uterus lacking eggs and vulva, bright-field. G) Lateral view of posterior portion of amphidelphic reproductive tract, bright-field. H) External views of rough-coated eggs, one with an unhatched larva, Nomarski. Scale bars equal 25 µm.

three pre-anal and six post-anal, in a 1-2-2-4 ratio (Figs. 2E,F,G; 3C). The most anterior of the rays is unpaired and directed ventrally. The second and third as well as the fourth and fifth rays are paired. In each of the pairs, the first ray is directed ventrally and the second ray directed laterally. Furthermore, the fourth and fifth rays are di-

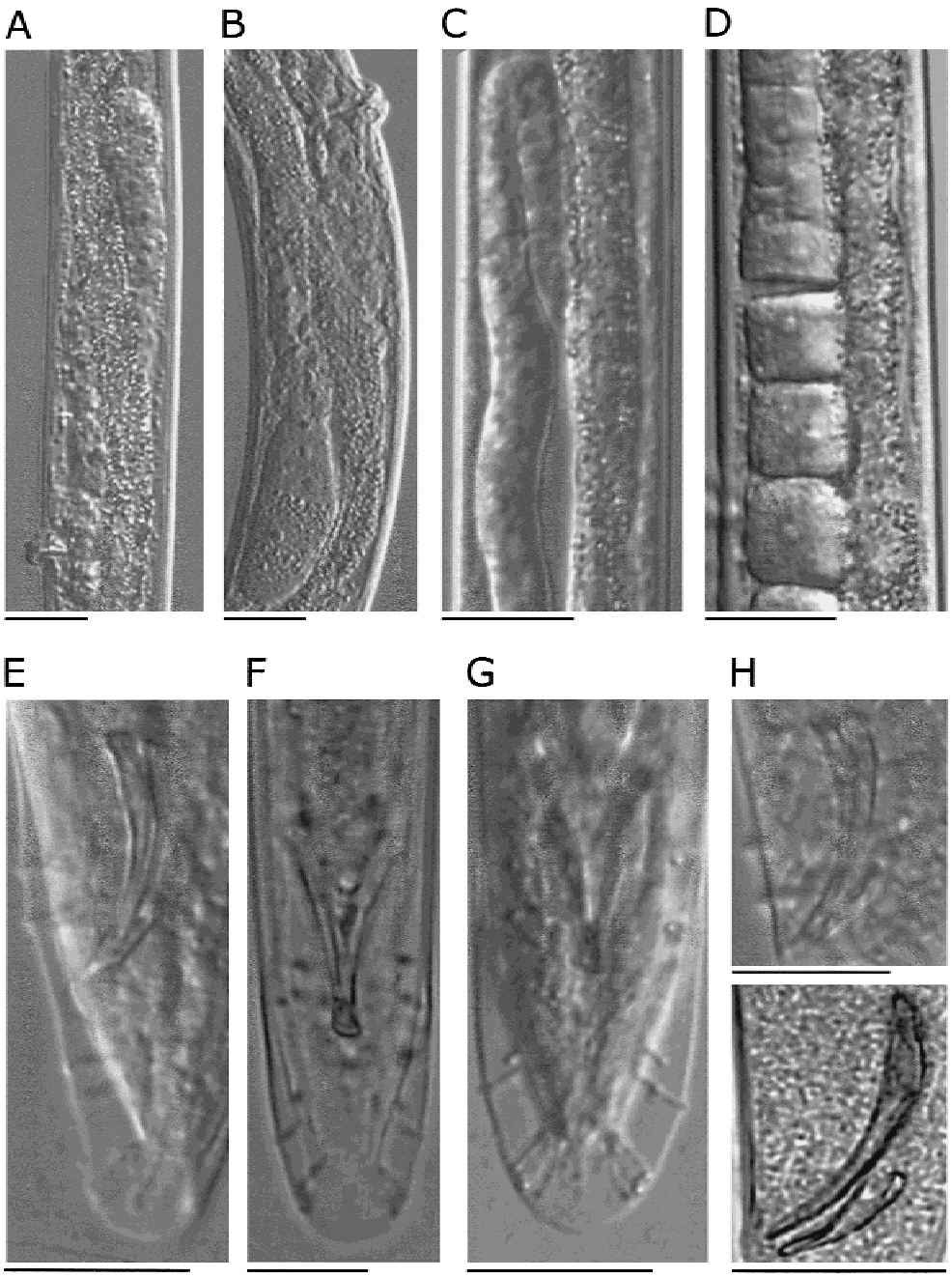

FIG. 2. Photomicrographs of *Myctolaimellus robiniae* n. sp. A) Lateral view of anterior portion of amphidelphic reproductive tract, Nomarski. B) Lateral view of posterior portion of amphidelphic reproductive tract, Nomarski. C) Anterior portion of testis, Nomarski. D) Posterior portion of testis, Nomarski. E) Lateral view of bursa, Nomarski. F) Ventral view of bursa with rays, bright-field (note spoon-shaped end of gubernaculum). G) Ventral view of bursa with rays, Nomarski. H) Two lateral views of paired spicules and gubernaculum, Nomarski, bright-field. Scale bars equal 25 µm.

rected more posteriorly than the second and third. The sixth, seventh, eighth, and ninth rays are fused in a common base at their roots. All of these are directed posteriolaterally; however, while the sixth, seventh, and eighth rays are parallel, the ninth ray is directed more acutely toward the tip of the peloderan tail (Figs. 2E,F,G; 3C).

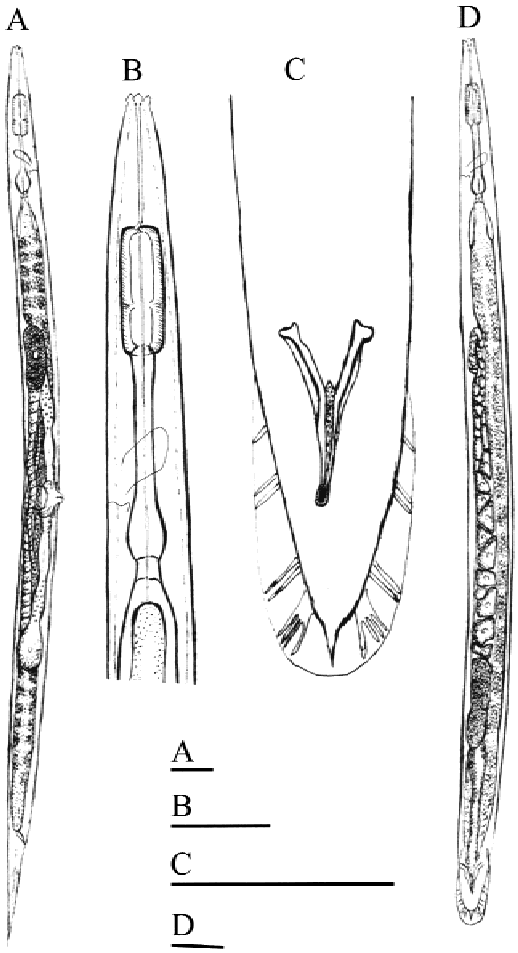

FIG. 3. *Myctolaimellus robiniae* n. sp. A) Full view of female. B) Anterior view of fused procorpus and metacorpus. C) Male tail. D) Full view of male. Scale bars equal 50 µm.

Type host and locality

From the subcortical cavities of locust borer (*M. robiniae*) infected black locust trees, *R. pseudoacacia,* Frostburg State University campus, Savage Mountain, and Garrett County. All sites in western Maryland, USA.

Bionomics

Saprophage and locust borer associate (Larson, 1998).

Type specimens

Holotype: Female collected July 1999 in western Maryland, USA. Slide no. T-541t, deposited in the U.S. Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland.

Allotype: Same collection data as holotype. Slide no. T-542t, deposited in USDANC, Beltsville, Maryland.

Paratypes: Four males, four females, and a larval stage deposited in USDANC, Beltsville, Maryland; numerous paratypes, deposited in Frostburg State University Nematode Collection, Frostburg, Maryland.

Diagnosis

Females of *M. robiniae* n. sp. are characterized by an expansive reproductive tract extending nearly to the esophagus anteriorly and the anus posteriorly, an equatorial vulva, and long ovaries overlapping each other. A sharply spiked tail terminus extending to the margin of a bluntly rounded bursa characterizes males. Also distinctive, the four posterior-most bursal rays are fused at their roots, and the spicules are short and stubby.

Relationships

The only other species in the genus *Myctolaimellus* is *M. walkeri,* an ectophoretic associate of a cerambycid beetle, *Lagochirus araneiformis* (L.), found in St. Lucia, West Indies, described by Hunt (1980) as *Cylindrocorpus* and later changed to *Myctolaimellus* by Andrássy (1984). *Myctolaimellus walkeri* is, on average, smaller than *M. robiniae* n. sp., with an average length of 750 µm in the male and 890 µm in the female. The vulva position (v = 53.5%) of *M. walkeri* is slightly more posterior than the equatorial vulva (v = 49.5%) observed in *M. robiniae* n. sp. Additionally, the female reproductive tract is less expansive than observed in *M. robiniae* and, unlike *M. robiniae,* the ovaries do not extend past the vulva. The male bursa of *M. walkeri* is conical, ending in a blunt, almost-square tip extending well beyond the tip of the tail. Furthermore, all bursal rays are described as being separate. These characteristics differ from the male morphology observed in *M. robiniae* described previously.

The morphology of the female reproductive tract in *G. ulmi* may be variable—either prodelphic or amphidelphic—depending

TABLE 1. Morphometric data in µm of *Myctolaimellus robiniae* n. sp. in wet mounts.

Morphological characters	Adult nematodes					
	Males (n = 25)			Females (n = 27)		
	Mean	SD	Range	Mean	SD	Range
Lengths						
Body	916.9	108.5	700.0–1,050.0	1,139.3	148.5	830.0–1,340.0
Stoma	42.1	3.3	37.5–45.5	46.3	2.2	45.0–49.0
Corpus	47.9	4.5	43–55	47.4	3.2	45.0–53.0
Spicules	32.0	1.7	30.0–35.0	—	—	—
Gubernaculum	16.6	1.0	15.0–18.0	—	—	—
Reproductive tract						
Anterior branch	—	—	—	248.9	33.6	180.0–290.0
Posterior branch	—	—	—	241.7	43.3	170.0–300.0
Widths						
Lip area	9.2	1.0	7.5–11.0	9.6	0.9	8.0–11.0
Stoma	2.1	0.2	2.0–2.5	2.1	0.2	2.0–2.5
Corpus	16.5	1.4	14.0–18.7	18.1	1.8	16.0–20.0
Basal bulb	15.2	2.2	12.0–18.0	15.4	1.7	12.0–18.0
Mid body	43.9	5.9	33.0–60.0	53.3	5.9	37.5–60.0
Anus	28.9	4.6	25.0–33.0	29.4	4.3	25.0–35.0
Ratios						
v	—	—	—	49.8	0.7	45.0–52.0
a	22.6	2.6	18.4–26.6	21.6	2.2	18.0–26.2
b	5.5	0.5	4.1–6.2	6.6	0.7	5.0–7.2
c	29.3	3.5	26.5–36.7	8.8	1.0	7.3–9.7

on food supply (De Ley, pers. comm.); thus, a distinction between this nematode and *M. robiniae* n. sp. must also be made. Comparison of *M. robiniae* with Andrássy's description of *G. ulmi* indicates that *M. robiniae* is slightly larger than *G. ulmi*. Additionally, the vulva position of *G. ulmi* (v = 56 to 59%) is more posterior than the equatorial vulva of *M. robiniae* (v = 45.0 to 52.0%). The spicules of *G. ulmi* with a length ranging from 42 to 52 µm (average of 48) are longer and more slender than those observed in *M. robiniae,* with a length ranging from 30 to 35 µm (average of 32 and SD of 1.7). Additionally, in the males of *G. ulmi,* the ratio of body length to esophagus length is greater (6 to 9) than in *M. robiniae* (4.1 to 6.2).

Remarks

The systematics for this family are often inconsistent. Andrássy's 1984 classification, in addition to being relatively recent, seemed the most logical. The only other reference to nematodes associated with locust borers is by Forschler and Nordin (1988), who explore the comparative pathogenicity of certain entomogenous nematodes to various pest hardwood borers, including the locust borer, *Megacyllene robiniae*.

Nematodes were neither retrieved from old wounds from previous years nor observed in larval cavities until June. Cavity fauna prominence diminished as the borer larvae matured and pupated and cavities began drying and sealing due to normal wound healing by the tree.

DISCUSSION

The nematode population, described herein, is collected from locust borer larval subcortical cavities in western Maryland. It is presented as a new species of the family Cylindrocorporidae and genus *Myctolaimellus*. While little is known of its biology, it was probably phoretically transported to this niche by one of the many invertebrates frequenting the site. During the course of the study, numerous borer larvae, ants, nitidulid larvae, and flies collected from the site were examined for the presence of nematodes; however, no nematodes were found.

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