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Sphaeronema sasseri n. sp. (Tylenchulidae), a Nematode Parasitic on Fraser Fir and Red Spruce[1]

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Abstract: Sphaeronema sasseri n. sp. is described from Fraser fir and red spruce on Mount Mitchell in North Carolina. Females are distinguished from other species in the genus by body shape, occurrence of body annulations, stylet morphology, head shape, and by several morphometric characters. The nematodes occur in colonies surrounding the bases of lateral and feeder roots, and the infected tissues show a general breakdown of the cortex and bark. The roots appear to be severely damaged by high populations of nematodes. This parasite may be important in the etiology of the slow decline of spruce and fir that has occurred in recent years in the southern Appalachian Mountains.

Key words: taxonomy, scanning electron microscopy, spruce decline, fir decline.

A new species of *Sphaeronema* Raski and Sher, 1952 was found in association with declining stands of red spruce (*Picea rubens* Sarg.) and Fraser fir (*Abies fraseri* (Pursh) Poir.) on North Carolina's Mount Mitchell, the highest peak in eastern North America (elevation 2,037 m). Declining trees are characterized by varying degrees of defoliation and decreased growth rates. Increment cores revealed that all trees above 1,935 m elevation have experienced a reduction in growth increment since the early 1960s (1). Decline and dieback symptoms of red spruce and Fraser fir are similar to those of declining trees in parts of the northeastern United States and in central Europe (1,7,10).

The etiology of the red spruce and Fraser fir decline syndrome is not understood. Several suggested causes include long-term climatic changes, natural stand dynamics, pest infestations, disease, gaseous pollutants, drought, direct and indirect effects of trace-metal accumulation in forest soils, and direct and indirect effects of acid deposition (7). The possible role of the new species of *Sphaeronema* remains to be elucidated.

Roots and soil samples collected from Mount Mitchell by plant pathologists at North Carolina State University in a survey of ectomycorrhizae of red spruce and Fraser fir were examined for plant-parasitic nematodes. When root segments were placed in water for microscopic inspection, numerous juvenile nematodes were dislodged from beneath layers of dead cortical root tissue. Upon closer examination, eggs enveloped in a gelatinous matrix and small, swollen sedentary females were discovered. Additional morphological studies indicated that the nematode was an undescribed species of *Sphaeronema,* which is described herein as *S. sasseri* n. sp.

This paper gives the first report of a *Sphaeronema* sp. in North Carolina or in the Appalachian Mountains. Previous findings in North America include *S. californicum* Raski and Sher on California laurel (*Umbellularia californica* Nutt.) and *Arctostaphylos* sp. (9) in California, *S. whittoni* Sledge and Christie on sweetgum (*Liquidambar styraciflua* L.) in Florida (12), and an undescribed species on *Picea* sp. in Oregon (11).

Materials and Methods

Specimens were obtained from infected roots of Fraser fir (*Abies fraseri*) and red spruce (*Picea rubens*) collected in the type locality, Mount Mitchell, North Carolina. Eggs, second-stage juveniles, males, and females were mounted in physiological saline solution and immediately examined, measured, sketched, and photographed. Line drawings were made with a camera lucida, and photographs were taken with a bright field light microscope. Type specimens were prepared according to Eisenback (2).

Second-stage juveniles, males, females,

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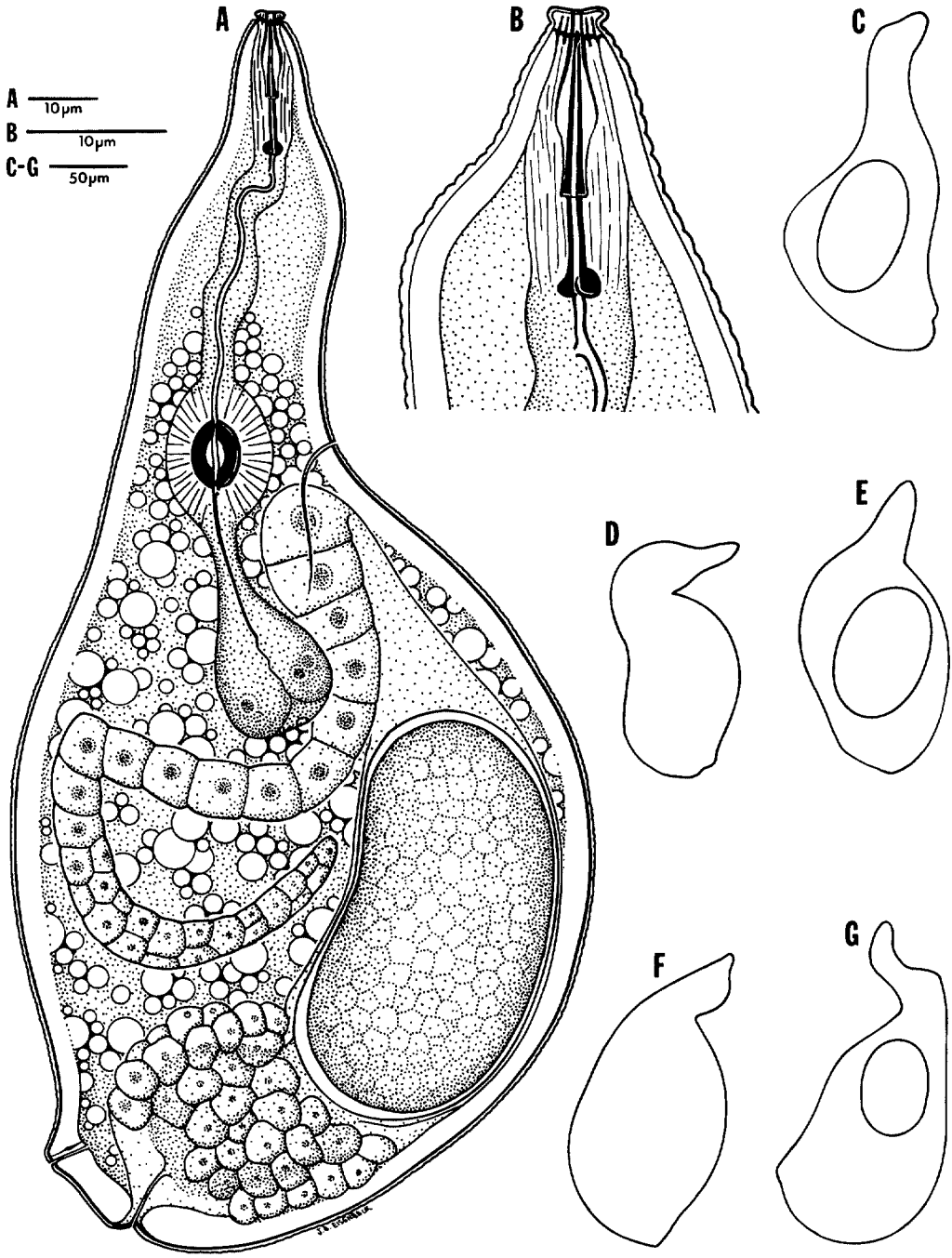

FIG. 1. Drawings of females of *Sphaeronema sasseri* n. sp. (lateral). A) Whole female. B) Anterior portion. C–G) Outlines of whole specimens, some with a developing egg.

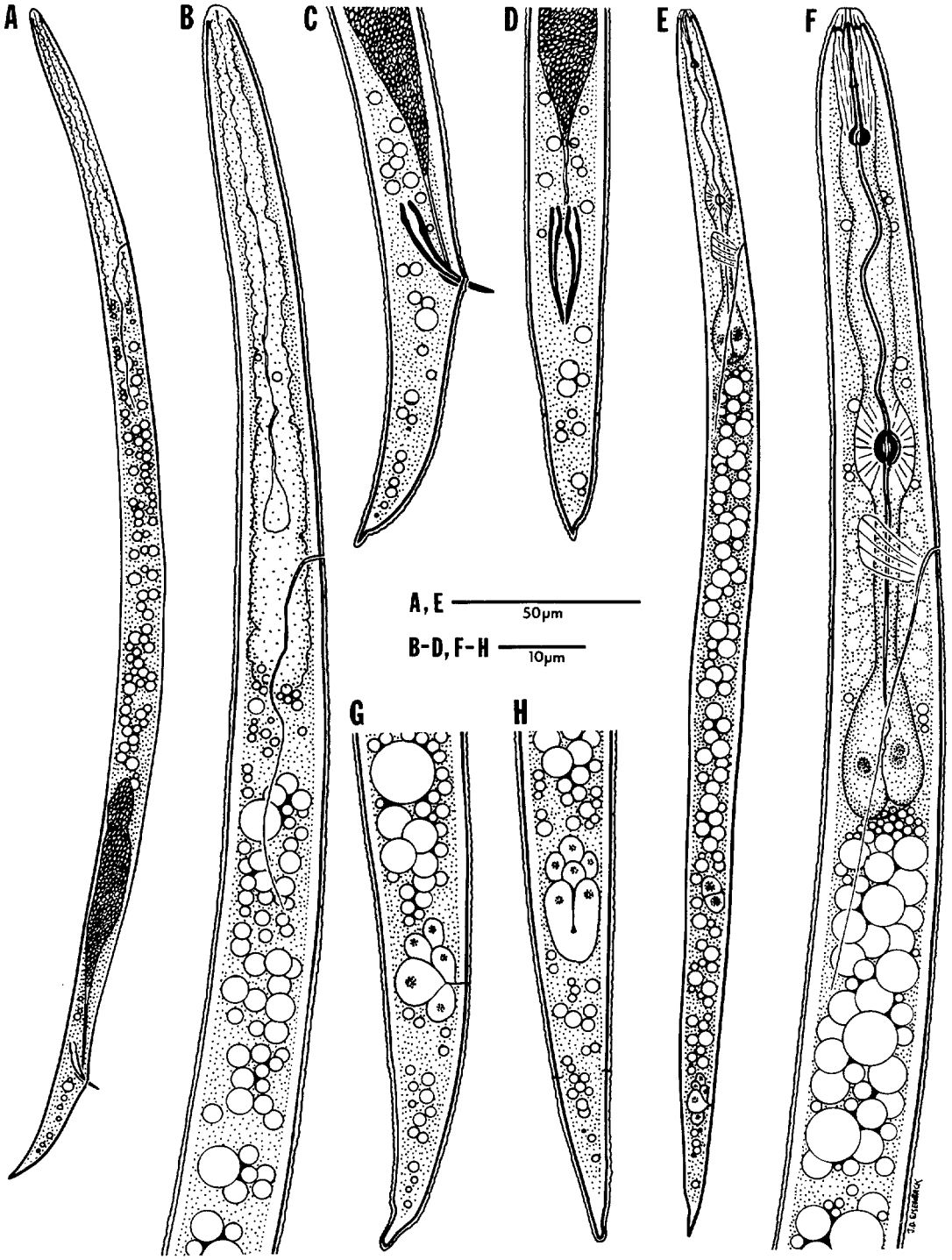

FIG. 2. Drawings of males and second-stage juveniles of *Sphaeronema sasseri* n. sp. A) Whole male (lateral). B) Anterior portion of male (lateral). C) Male tail (lateral). D) Male tail (ventral). E) Whole second-stage juvenile (lateral). F) Anterior portion of second-stage juvenile (lateral). G) Second-stage juvenile tail (lateral). H) Tail of second-stage juvenile (ventral).

and stylets of juveniles and females were prepared for scanning electron microscopy (SEM) as previously described (3,4). At least 30 specimens each of juveniles, males, and females were examined with a JEOL T200 SEM operating at 25 kV.

Species Description

Sphaeronema sasseri n. sp.
(Figs. 1–4)

Females

Measurements of 30 females in physiological saline are listed in Table 1.

Measurements (holotype in glycerin): Body length 213 μm; body width 98 μm; excretory pore to head end 72 μm; stylet length 20.4 μm; stylet knob width 3.6 μm; dorsal esophageal gland orifice (DEGO) to stylet base 3.2 μm; and anus to vulva distance 10.1 μm. Female as in general description.

Description: Body translucent white, subspherical to lemon-shaped with protruding vulva, elongate neck of variable shape due to influence of cellular structure of root. Head cap set off from neck, first body annule smaller in diameter than head cap. Cuticle finely annulated, 7–9 μm thick. Excretory pore located near level of median bulb. Tail reduced to slight posterior protuberance, often marked with a mucron. Cephalic framework weak, hexaradiate; vestibule and extension moderately sclerotized. Stylet delicate; cone nearly twice as long as shaft; knobs small, rounded, separate, set off from shaft. Distance of DEGO to stylet base relatively short, 2.4–4.0 (3.2) μm; ampulla indistinct. Procorpus elongate, cylindrical; median bulb rounded to slightly elongate, muscular, cuticularized triradiate lumen lining prominent. Subventral esophageal gland orifices located immediately posterior to enlarged lumen lining of median bulb. Ampullae of subventral glands distinct. Nerve ring and hemizonid not observed. Isthmus slender, distinct, often slightly curved anteriorly; enlarged distinct basal bulb extends into anterior portion of swollen body cavity. Basal bulb with one large dorsal esophageal gland nucleus and nucleolus and two smaller subventral gland nuclei and their nucleoli. Esophago–intestinal junction indistinct. Intestine obscure. Anus pore-like. Phasmids extremely small, pore-like, located midway between tail tip and anus. Ovary single, convoluted around esophagus and intestine, terminating in a large multicellular uterus. Vulva large, protruding, subterminal.

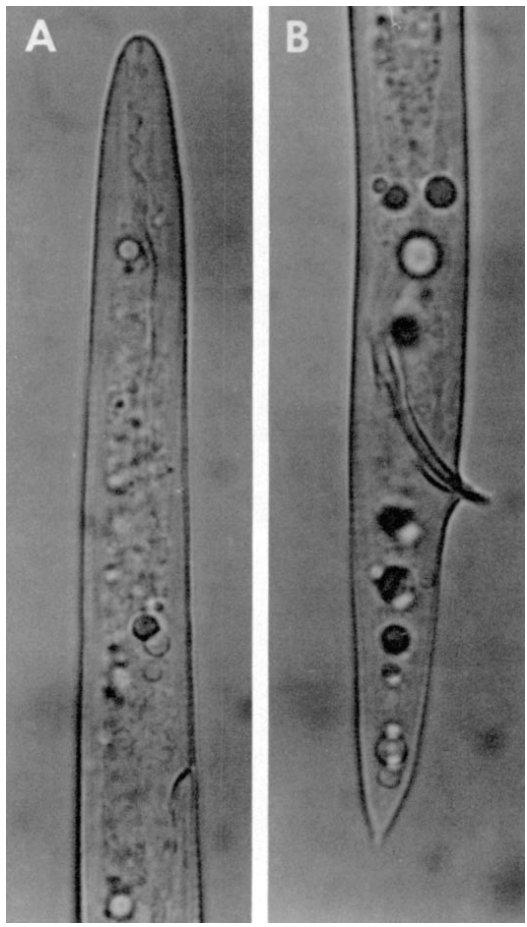

Fig. 3. Light micrographs of *Sphaeronema sasseri* n. sp. A) Male head. B) Male tail.

Males

Measurements of 30 males in physiological saline are listed in Table 2.

Description: Body translucent white, vermiform, tapering at both ends; cuticle marked by narrow annulation; lateral lines not observed. Head cap completely fused with head region, lips and head annulations absent. Excretory pore located anterior to esophago–intestinal junction. Tail conoid with rounded terminus, often slightly curved dorsally, usually marked with a mucron. Cephalic framework re-

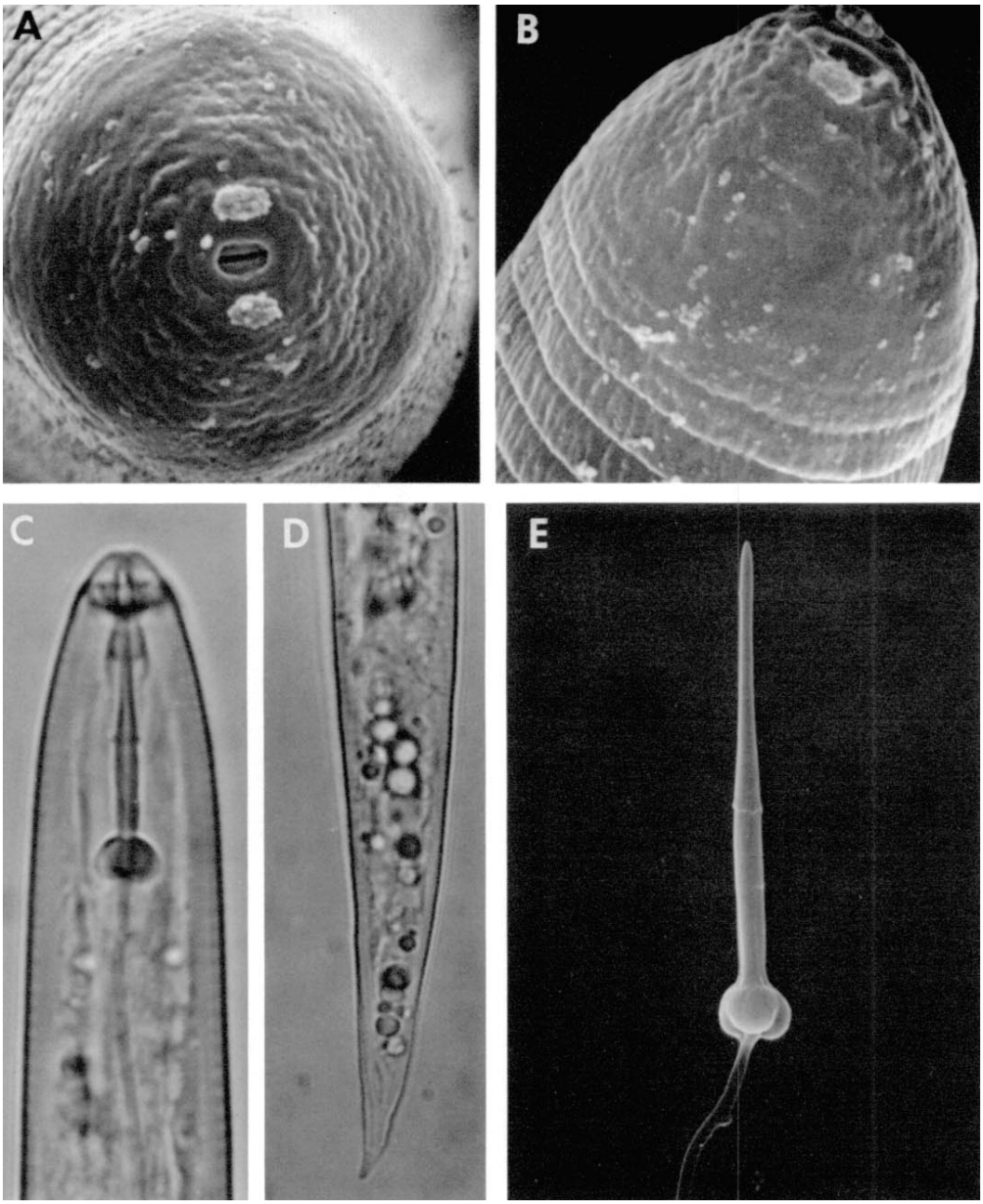

FIG. 4. Micrographs of second-stage juvenile of *Sphaeronema sasseri* n. sp. A) SEM of face view. B) SEM of lateral view. C) Light micrograph of head (lateral). D) Light micrograph of tail (lateral). E) SEM of excised stylet.

duced or absent, stylet absent, esophagus degenerate. Nerve ring and hemizonid not observed. Intestine filled with globules. Testis single, directed anteriorly, filled with many small, rounded to elongate sperm. Spicules long, slender, slightly curved ventrally; gubernaculum simple, slightly curved ventrally; bursa absent. Phasmids small, pore-like; located midway between cloacal opening and tail tip.

Second-stage juveniles

Measurements of 30 second-stage juveniles (J2) in physiological saline are listed in Table 3.

Measurements (allotype in glycerin): Body

TABLE 1. Measurements of 30 females of *Sphaeronema sasseri* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variation (%)
Linear (μm)					
Body length	190.0–310.0	235.7	5.75	3.2	13.4
Body width	57.9–124.6	89.3	2.92	16.0	17.9
Excretory pore to head end	43.9–101.7	69.7	2.46	13.5	19.3
Stylet length	15.1–20.0	17.7	0.20	1.1	6.2
Stylet knob width	3.2–4.5	3.7	0.06	0.3	9.3
DEGO	2.4–4.0	3.2	0.07	0.4	11.4
Anus to vulval distance	8.9–13.8	11.3	0.23	1.3	11.4
Ratios					
a	0.18–0.40	0.27	0.01	0.1	20.8

length 418 μm; body width 16 μm; tail length 41 μm; excretory pore to head end 76 μm; stylet length 16.4 μm; stylet knob width 3.6 μm; DEGO to stylet base 3.4 μm; esophagus length 116 μm. Juvenile as in general description.

Description: Body translucent white, vermiform, tapering at both ends; cuticle marked by narrow annulations; lateral lines not observed. Head cap completely fused with head region, lips and head annulations absent. In SEM (Fig. 4A) stoma slit-like, prestoma ovoid to hexagonal. Pore-like openings of six inner labial sensilla located within prestoma, often obscured. Amphidial openings slit-like, located slightly posterior to lateral edges of prestoma; usually obscured by amphidial exudate. Lips absent, head region smooth. Body annulations distinct. In light microscopy (Fig. 4C), head region less heavily sclerotized and smaller in diameter than body wall, set off from body annulation. Cephalic framework well developed, hexaradiate; vestibule and extension prominent. Stylet strongly developed; cone longer than shaft and knobs together; shaft cylindrical, slightly constricted near its junction with knobs; knobs smooth, rounded, set off from shaft. Distance of DEGO to stylet base 2.8–3.6 (3.2) μm; ampulla indistinct. Procorpus elongate, cylindrical; median bulb slightly elongate, muscular, cuticularized triradiate lumen lining prom-

TABLE 2. Measurements of 30 males of *Sphaeronema sasseri* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variation (%)
Linear (μm)					
Body length	317.7–437.9	391.6	4.50	24.7	6.3
Body width	10.3–14.9	11.8	0.18	1.0	8.4
Tail length	27.6–38.1	32.5	0.52	2.9	8.8
Excretory pore to head end	54.5–72.5	62.3	0.68	3.7	6.0
Spicule length	16.9–19.9	18.1	0.14	0.8	4.3
Gubernaculum length	4.5–5.7	4.9	0.05	0.3	5.8
Testis length	94.3–140.6	109.6	2.02	11.1	10.1
Ratios					
a	26.8–37.2	33.4	0.45	2.5	7.3
c	10.7–14.2	12.1	0.15	0.8	7.0
Percentages					
T (%)	23.6–34.3	28.0	0.50	2.7	9.8

TABLE 3. Measurements of 30 second-stage juveniles of *Sphaeronema sasseri* n. sp.

Character	Range	Mean	Standard error of mean	Standard deviation	Coefficient of variation (%)
Linear (μm)					
Body length	356.0–448.6	420.1	3.75	20.5	4.9
Body width	13.6–15.2	14.9	0.08	0.5	3.4
Tail length	32.6–45.7	39.3	0.59	3.2	8.2
Excretory pore to head end	62.3–75.7	70.7	0.55	3.0	4.3
Stylet length	14.2–17.0	16.0	0.13	0.7	4.4
Stylet knob width	3.0–4.1	3.5	0.04	0.2	3.4
DEGO	2.8–3.6	3.2	0.05	0.3	8.6
Esophagus length	100.5–127.4	110.6	5.55	1.0	5.0
Ratios					
a	25.5–31.3	29.0	0.25	1.4	4.8
b	3.2–4.1	3.8	0.04	0.2	5.3
c	9.5–12.4	10.7	0.15	0.8	7.6
d	3.3–4.8	3.8	0.07	0.4	10.1

inent. Subventral esophageal gland orifices located immediately posterior to enlarged lumen lining of median bulb. Ampullae of subventral glands distinct. Isthmus slender, distinct, elongate, cylindrical. Distinct basal bulb with one large dorsal esophageal gland nucleus and nucleolus, and two smaller subventral gland nuclei and nucleoli. Esophago–intestinal junction distinct. Intestine filled with many large globules. Anus pore-like, obscure; rectum surrounded by 6–12 lobe-like cells. Nerve ring and excretory pore located near anterior portion of isthmus; hemizonid not observed. Phasmids small, pore-like, located midway between anus and tail tip. Tail conoid with rounded terminus, often slightly curved dorsally, sometimes marked with a mucron.

Eggs

Measurements (30 eggs in physiological saline): Length 520.7–816.1 μm (mean 743.6 μm, standard error of mean 10.9, standard deviation 59.7, coefficient of variation 8.0%); width 318.6–509.1 μm (mean 441.6 μm, SE 9.9, SD 54.0, COV 12.2%). Length/width ratio = 1.2–2.3 (mean 1.7, SE 4.0, SD 22.1, COV 12.9%).

Description: Translucent white, oblong; contained within a gelatinous matrix.

Diagnosis

Sphaeronema sasseri n. sp. is morphologically distinct from all other described species in the genus. The shape of the female body, morphology of the head, and occurrence of fine annulations on the body clearly distinguish it from *S. californicum* (9), *S. whittoni* (12), and *S. minutissimum* Goodey (5). Morphologically, *S. sasseri* n. sp. is most similar to *S. rumicis* Kirjanova (8), but several major differences occur. In *S. rumicis,* the distance of the excretory pore to the anterior end is 80.5–133.0 (110.1) μm, compared with 43.9–101.7 (69.7) μm in *S. sasseri* n. sp.; the distance from the vulva to anus is 35–91 (64.6) μm, compared with 8.9–13.8 (11.3) μm in *S. sasseri* n. sp.; the distance of the DEGO to the base of the stylet is 7.2–9.0 (8.5) μm, compared with 2.4–4.0 (3.2) μm in *S. sasseri* n. sp.; and the stylet length is 20–27 (23.7) μm, compared with 15.1–20.0 (17.7) μm in *S. sasseri* n. sp.

Differences between the two species also exist in the morphology and morphometrics of the second-stage juveniles. The stylet of *S. rumicis* is long, 19.8–23.4 (21.4) μm, and the knobs have a posterior protuberance; but *S. sasseri* n. sp. has a short stylet, 14.2–17.0 (16) μm, and smooth, rounded knobs (Fig. 4E). The body of *S. rumicis* is longer, 416–540 (504) μm, and wider, 16–18 (17.4) μm, than *S. sasseri* n. sp. which is 356–449 (420) μm long and 13.6–15.2 (14.9) μm wide. Also the tail of *S. rumicis* is longer, 57.6–91 (75) μm, than *S. sasseri* n. sp., 32.6–45.7 (39.3) μm, which affects the "c" ratio.

Biology: Sphaeronema sasseri n. sp. occurs in colonies at the bases of small lateral and feeder roots and ectomycorrhizae. Generally, eggs are deposited into a communal gelatinous matrix produced by several (3–10) females, lying within close proximity. As many as 100 eggs may be deposited in each egg mass. Numerous juveniles and males may also occur in each gelatinous matrix which is transparent at first but darkens with age.

Often several groups of females encircle the base of a single root. Feeder roots and ectomycorrhizae with large colonies of nematodes surrounding them are usually stunted or dead. Colonies are often located underneath sloughed layers of cortical cells and are difficult to detect unless roots are thoroughly inspected microscopically. Few specimens, if any, are recovered from soil. The nematodes generally remain with the root tissues, and juveniles and males move rather slowly when dissected from the host plant.

Sphaeronema sasseri n. sp. has been found parasitizing roots of red spruce and Fraser fir at elevations of 1,700–2,036 m above sea level. The majority of samples taken from trees in various stages of decline growing above 1,800 m were infected. The roots appeared to be severely damaged by the nematodes.

Mount Mitchell receives more than 175 cm of precipitation annually. Mean temperatures range from −3 to 15 C (6). Thus, the nematode appears to be cold tolerant.

Holotype (female): Isolated from infected roots of Fraser fir (*Abies fraseri*) collected from the type locality. Slide T-388 t, USDA Nematode Collection (USDANC), Beltsville, Maryland.

Allotype (second-stage juvenile): Same data as holotype. Slide T-389 t, USDANC, Beltsville, Maryland, USA.

Paratypes (females, males, second-stage juveniles): Same data as holotype. USDANC, Beltsville, Maryland. University of California, Davis Nematode Collection (USCDNC), Davis, California.

Type host and locality: Roots of Fraser fir (*Abies fraseri*) on Mount Mitchell, North Carolina.

Discussion

Sphaeronema sasseri n. sp. is difficult to detect because the adult females are sedentary and minute and the males and infective juveniles remain within the egg masses or migrate slowly to nearby root tissues. Few occur free in soil, thus are rarely detected in soil samples. This species is detected only by thorough microscopic examination of infected root tissues. As suggested for other members of the genus (9), *S. sasseri* n. sp. may be widely distributed and may attack other hosts.

The association between *Sphaeronema sasseri* n. sp. and declining fir and spruce trees on Mount Mitchell is clear; however, the nematode's role in the etiology of the decline of the trees is not known. The nematode may be an injurious parasite, and roots may die as a direct result of the nematode attack, but secondary pathogens and other stress factors may also be involved. The extent of the damage is not known, and it will be necessary to collect data from many geographical locations before final conclusions about the effect of this nematode on the decline of fir and spruce trees can be made. However, further investigations are warranted.

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