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Morphological and Biological Parameters of the Knapweed Nematode, *Subanguina picridis*[1]

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Abstract: Specimens of the knapweed nematode *Subanguina picridis* (Kirjanova) Brzeski obtained from different host plants were highly variable in measurement and structure. This variability refutes the validity of six *Subanguina* species attacking plants in the Asteraceae.

Key words: *Acroptilon repens* (Russian knapweed), host specificity, *Subanguina picridis* (knapweed nematode), taxonomy, variability.

The gall forming nematode *Subanguina picridis* (Kirjanova, 1944) Brzeski, 1981, syn.: *Paranguina picridis* (Kirjanova, 1944) Kirjanova & Ivanova, 1968, has been evaluated in Canada as a potential biological control agent of Russian knapweed, *Acroptilon repens* (L.) DC., syn.: *Centaurea repens* L. The nematode has a restricted host range and damages its host. It has been released in Canada for experimental control tests of Russian knapweed (12–14).

Russian workers reported that *S. picridis* was specific to *A. repens* (5,6). However, host tests have demonstrated that the host range of *S. picridis* is not restricted to Russian knapweed but that some members of the Cynareae tribe are also hosts (12). Therefore, *S. picridis* specimens from galls on the different hosts were examined to determine if different host plants affected nematode morphology and anatomy.

Materials and Methods

Galls were obtained from *A. repens, Centaurea diffusa* Lam., *Cynara scolymus* L., and *Onopordum acanthium* L. plants inoculated with *S. picridis* (12). Galls were collected 20–40 days after their appearance and were dissected and immersed in aerated distilled water for 1 hour. Nematodes were removed from the water with a pipet and concentrated in a small volume of distilled water. They were then gently heat killed, fixed in TAF (4), and processed to pure glycerin using Seinhorst's (9) glycerol–ethanol method. Measurements of stylet length of living nematodes from *A. repens* and *C. scolymus* galls were also made. Data were analyzed by an analysis of variance with a one-way classification (10).

In addition, nematodes from *A. repens, C. diffusa, C. scolymus,* and *Cirsium flodmanii* (Rydb.) Arthur, collected and fixed similarly, were prepared for the scanning electron microscopy. Fixed specimens were dehydrated in a graded ethanol series, then transferred to amyl acetate, and critical point dried using freon. Specimens were individually placed on the adhesive surface of stubs with their heads free and coated with carbon and gold. They were examined and photographed in a Cambridge scanning electron microscope, using 20-kV electron gun potential.

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TABLE 1. Dimensions of four populations of female and male *Subanguina picridis* on different host plants (with mean and range).

Population	n	L (mm)	a	b	c	V (%)		
Female								
Acroptilon repens	10	1.43 b (1.25–1.62)	29.99 a (26.06–35.05)	8.35 ab (7.27–9.23)	17.20 a (13.89–21.27)	82.85 (80.00–88.61)		
Onopordum acanthium	10	1.65 a (1.49–1.82)	26.90 ab (21.85–30.56)	9.19 a (8.49–10.93)	17.63 ab (15.75–20.16)	85.10 (82.21–87.10)		
Cynara scolymus	10	1.40 b (1.25–1.53)	27.48 a (26.30–33.18)	7.90 b (6.64–9.01)	15.43 b (12.83–17.54)	82.90 (79.10–84.97)		
Centaurea diffusa	8	1.69 a (1.22–2.29)	23.59 b (17.78–30.89)	8.65 ab (6.86–12.12)	19.13 a (14.69–22.78)	84.22 (80.26–88.50)		
							Spicule length (μm)	Gubernaculum length (μm)
Male								
Acroptilon repens	10	1.49 b (1.20–1.98)	33.02 a (26.46–40.94)	7.62 bc (6.12–8.62)	15.82 bc (13.84–18.96)		40.50 a (36.00–48.00)	13.40 (9.01–16.51)
Onopordum acanthium	10	1.52 b (1.35–1.70)	33.26 a (27.59–37.78)	8.07 b (7.53–8.63)	16.73 b (16.08–18.42)		42.80 a (36.00–54.00)	14.30 (12.01–16.51)
Cynara scolymus	10	1.40 b (1.24–1.60)	33.97 a (29.78–37.50)	7.28 c (6.89–8.39)	14.67 c (13.03–16.19)		34.50 b (28.50–45.00)	12.80 (10.50–16.50)
Centaurea diffusa	8	1.96 a (1.73–2.26)	28.38 b (24.49–33.70)	9.49 a (8.54–10.35)	19.09 a (16.71–22.31)		43.50 a (40.50–48.00)	15.60 (15.00–16.50)

Means in the same column sharing the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple-range test.
L = body length.
a = body length ÷ greatest body width.
b = body length ÷ distance from anterior end to junction of esophagus and intestine.
c = body width ÷ tail length.
V = distance of vulva from anterior end × 100 ÷ body length.

TABLE 2. Dimensions of eggs from gall cavities and from within the uterus of females of four populations of *Subanguina picridis* on different host plants (with mean and range).

Population	n	Length (μm)	Width (μm)
Gall cavity			
Acroptilon repens	10	71.6 b (56.3–85.5)	53.1 a (42.8–60.3)
Onopordum acanthium	10	79.7 ab (67.5–94.5)	44.8 b (38.3–54.0)
Cynara scolymus	10	81.9 a (72.0–96.8)	36.5 c (33.8–45.0)
Centaurea diffusa	10	73.6 ab (60.8–87.8)	39.9 c (27.0–45.0)
Uterus			
Acroptilon repens	10	70.9 b (60.8–76.5)	39.4 a (31.5–45.0)
Onopordum acanthium	10	78.1 a (69.8–83.3)	36.5 ab (31.5–40.5)
Cynara scolymus	5	84.6 a (76.5–101.3)	35.1 b (31.5–38.3)
Centaurea diffusa	No eggs observed		

Means in the same column sharing the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple-range test.

RESULTS

Dimensions of *S. picridis* from different hosts varied considerably (Tables 1, 2). Length of females from *A. repens* was ($P \leq 0.05$) shorter than specimens from *O. acanthium* and *C. diffusa* but not different from *C. scolymus* (Table 1). Significant ($P \leq 0.05$) differences occurred among the populations in the values of a, b, and c. Males from *C. diffusa* galls differed ($P \leq 0.05$) from males from galls on other host plants in the measurements of L, a, b, and c, and spicules of males from galls on *C. scolymus* were shorter ($P \leq 0.05$) than spicules of males from other plants.

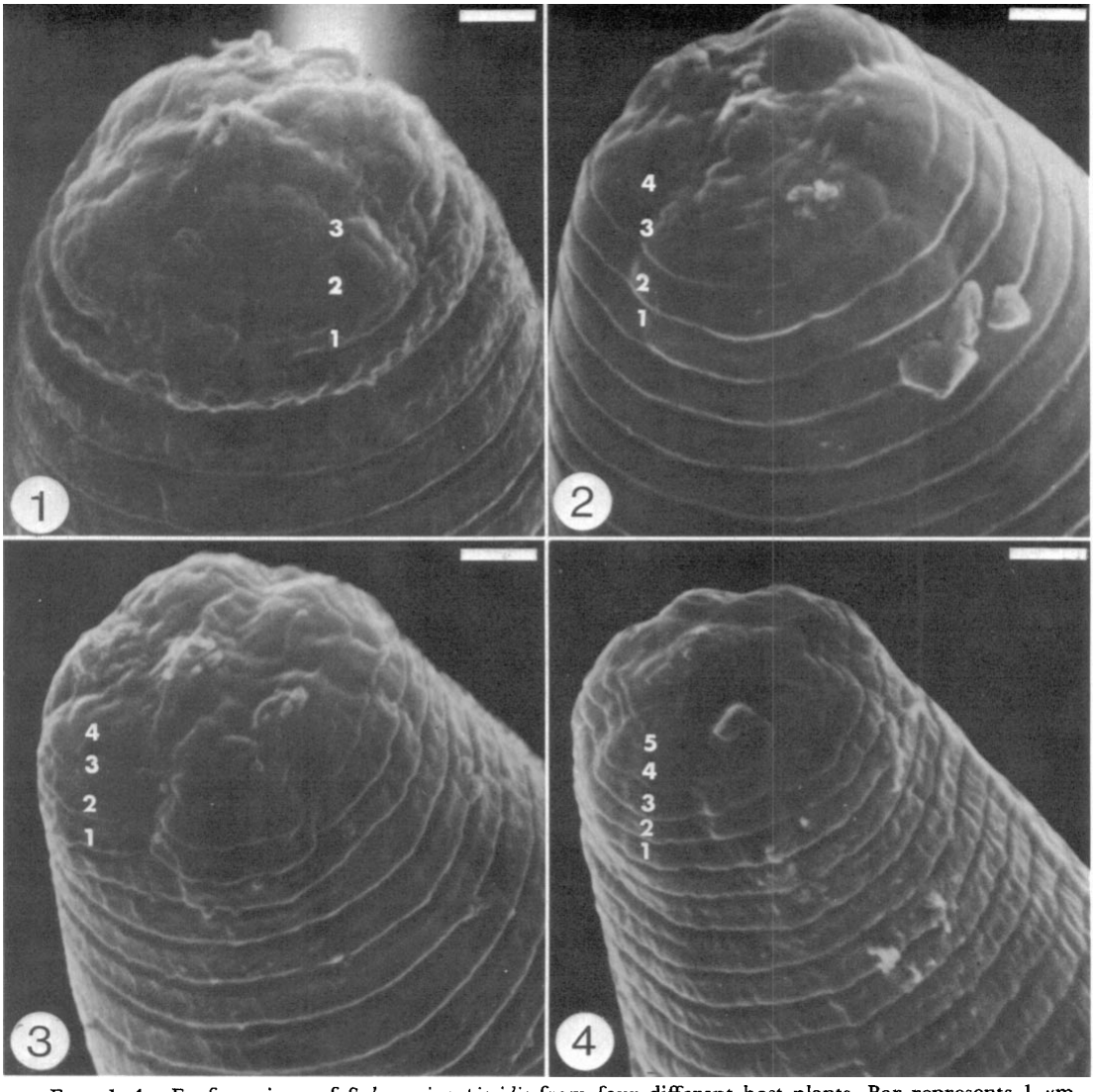
The stylets in some fixed specimens were obscure, so comparisons of stylets among populations could not be made. Most of the visible stylets in preserved specimens were 8–10 μm long. However, stylets of living adults from *A. repens* and *C. scolymus* galls were 12 μm long.

Dimensions of eggs from galls on different hosts also varied. Eggs from a gall on *A. repens* were shorter ($P \leq 0.05$) than those from a *C. scolymus* gall and wider than those from *O. acanthium, C. scolymus,* or *C. diffusa* galls (Table 2). Eggs within the uterus of females from *A. repens* galls were shorter ($P \leq 0.05$) than eggs in females from *O. acanthium* and *C. scolymus* galls. Eggs in females from galls on *A. repens* were also wider than those in females from galls on *C. scolymus.*

The number of lip annuli on specimens of *S. picridis* from *A. repens* galls varied from three to five, whereas specimens from galls on the other plants had four or five lip annuli (Figs. 1–4). Such differences have been used to separate species of *Subanguina,* but populations of *S. picridis* exhibited similar variability when reared on different hosts.

DISCUSSION

Prior to Brzeski's (2) taxonomic treatment of Anguinidae, *Paranguina* contained six species (10). Species were distinguished by host range and morphology (6). Morphological characters used were the number of head annuli, position of excretory pore, width of stylet base, stylet length, spicule and egg dimensions, and cauda morphology. For those *Subanguina* species that parasitize members of the Cynareae tribe of the Asteraceae family, most dimensions of the different species overlap (6,8). The report by Ahmed et al. (1) of *Anguina microlaenae* (Fawcett, 1938) Steiner, 1940 causing leaf and stem galls on *Centaurea rigida* Willd. and *Cousinia stenocephala* Boiss., both of the Cynareae tribe



FIGS. 1–4. En face views of *Subanguina picridis* from four different host plants. Bar represents 1 μm. Numbers indicate the number of lip annuli. 1) Adult from a gall on *Acroptilon repens*. 2) Juvenile from a gall on *Centaurea diffusa*. 3) Adult from a gall on *Cynara scolymus*. 4) Adult from a gall on *Cirsium flodmanii*.

of the Asteraceae family, in Iraq is probably incorrect because the nematode was identified using a key that did not include *Paranguina* species.

Because a species is a dynamic group of individuals, variability within natural populations is to be expected. Populations that are morphologically different are not necessarily taxonomically different (3). In this study, differences were shown to exist among populations of *S. picridis* in galls from different host plants. The degree of variation in morphological characters of *S. picridis* suggests that the six species of *Subanguina* that form leaf and stem galls on plants of the Cynareae tribe are a single species. Possibly host races have developed on the various host plants in the Soviet Union. Sturhan (11) suggested that the probability of new distinct races forming under natural conditions is low and that races may develop in geographical isolation or may evolve in ecological or physiological isolation within a host plant.

The species of *Subanguina* on the Cynareae host plants are not geographically isolated. They are all found in the same area of the southern Soviet Union (O. V.

Kovalev, pers. comm.). *Subanguina picridis* is widespread in the river valleys of Tadzhikistan, and four of the other species of *Subanguina* on Cynareae host plants occur in the same geographical area. *Subanguina chartolepidis,* reported in Armenia, is geographically isolated from Tadzhikistan, but *S. picridis* also occurs in the same region of Armenia (8).

Populations of *Subanguina* are isolated within galls on their host plants, and the opportunity for host race development may be enhanced. However, Meagher and Brown (7), found no tendency of cereal cyst nematode (*Heterodera avenae* Filipjev, 1934) populations to increase and develop biological races in five successive plantings of moderately resistant hosts. The galls on the different Cynareae host plants caused by *Subanguina* species described by Kirjanova and Ivanova (6) were rather small leaf and stem galls that caused little damage to their host plants and appeared similar to those observed on different hosts of *S. picridis* (12).

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