

Nematodes of Northern areas in Pakistan. *Heterodera bergeniae* sp. n. (Nematoda : Heteroderidae), a parasite of *Bergenia ciliata*

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SUMMARY

Heterodera bergeniae n.sp. parasitizing roots of a herbaceous plant, *Bergenia ciliata* (Haw.) Sternb., from Saiful Muluk, North West Frontier Province, Pakistan is described and illustrated. This new species belongs to the "goettingiana group" but it differs from all the known species of the group in having the vulval slit in a cleft of the vulval bridge and the semifenestreae well separated and surrounded by a dark-brown sclerotized basin forming a double kidney shape at top of the cone. The new species comes close to *H. carotae* with regard to the position of the vulval slit but can be distinguished from the latter species; in females, by the absence of an egg-sac, head with two annules and more anterior excretory pore; in cysts, by the surface wall pattern having regular zig-zag lines forming a coarse net-work, absence of an egg-sac, distinct fenestration and sclerotized vulval lips; in second-stage juveniles, by the cuticular annulation being coarse, especially posteriorly, stylet knobs rounded, not concave, tail with shorter hyaline portion.

RÉSUMÉ

Nématodes des régions nord du Pakistan. *Heterodera bergeniae* sp. n. (Nematoda : Heteroderidae), parasite de *Bergenia ciliata*

Description et illustrations sont données de *Heterodera bergeniae* sp. n., parasitant les racines d'une plante herbacée, *Bergenia ciliata* (Haw.) Sternb. et récolté à Saiful Muluk, North West Frontier Province, Pakistan. Cette nouvelle espèce appartient au « groupe goettingiana », mais diffère de toutes les espèces de ce groupe par la fente vulvaire située dans une dépression du pont vulvaire, et par les héli-fenestreae bien séparées et entourées par une cuvette sclérotisée brun-noir formant une structure réniforme double au sommet du cône. La nouvelle espèce est proche de *H. carotae* par la position de la fente vulvaire, mais s'en distingue par : i) les femelles dépourvues de masse d'œufs, les deux annules labiaux et le pore excréteur situé plus antérieurement; ii) les kystes ayant une ornementation superficielle composée de lignes en zig-zag formant un réseau en relief, une fenestration distincte et des lèvres vulvaires sclérotisées; iii) les juvéniles de deuxième stade présentant une annélation prononcée, en particulier à leur partie postérieure, des boutons du style arrondis (non concaves) et une partie hyaline terminale de la queue plus courte.

During the earlier survey (October 1985) of the nematode fauna (of high altitude) at about 3 500 meters above sea level in the Northern areas of Pakistan, specimens of an undescribed species of *Heterodera* (cysts, females and juveniles), were found associated with a widely spread herbaceous plant : *Bergenia ciliata* (Haw.) Sternb. (Saxifragaceae). Subsequently, in June and October 1986, more specimens of this new species were collected from the same locality but no male specimens could be found in any of the collections. This new species belonging to the "goettingiana group" is very close to *H. carotae* Jones, 1950. The new species is described and illustrated.

Cysts, white females and J2s obtained from the rhizosphere of *B. ciliata* were fixed in 3 % formaldehyde solution and mounted in glycerine (Seinhorst, 1959).

Heterodera bergeniae n. sp. (Figs 1-4)

MEASUREMENTS

White female (paratypes; n = 18) : L (excluding neck) = 0.394 mm \pm 0.043 (0.310-0.448); W = 297 μ m \pm 61.5 (192-353); neck length = 80 μ m \pm 5.8 (70-85); stylet length = 32 μ m \pm 1.4 (29-32).

Cysts : (n = 50) : Length (excluding neck) = 0.440 mm \pm 0.061 (0.360-0.520); W = 325 μ m \pm 64.7 (240-440); L/W = 1.3 \pm 0.23 (1.1-1.7).

Second stage juveniles (n = 24); L = 0.396 mm \pm 0.053 (0.324-0.453); a = 18.8 \pm 2.90 (13.5-21); b = 3.1 \pm 0.30 (3.0-3.8); b' = 2.7 \pm 0.31 (2.1-3.2); c

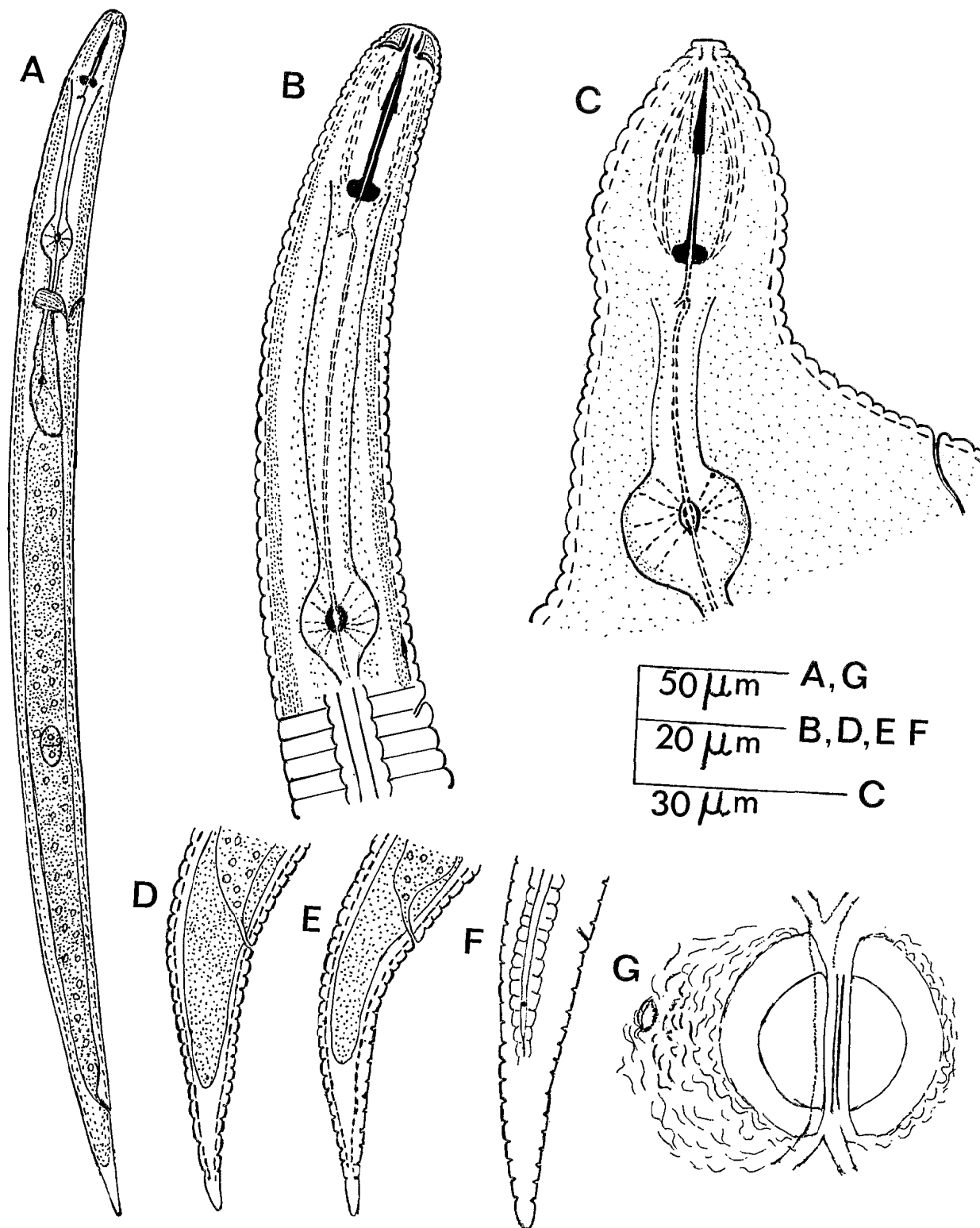


Fig. 1. *Heterodera bergeniae* n. sp. Second stage juveniles. A : Entire; B : Oesophageal region; D, E, F : Tails. Female; C : Anterior end. Cyst. G : Vulval cone top in end view.

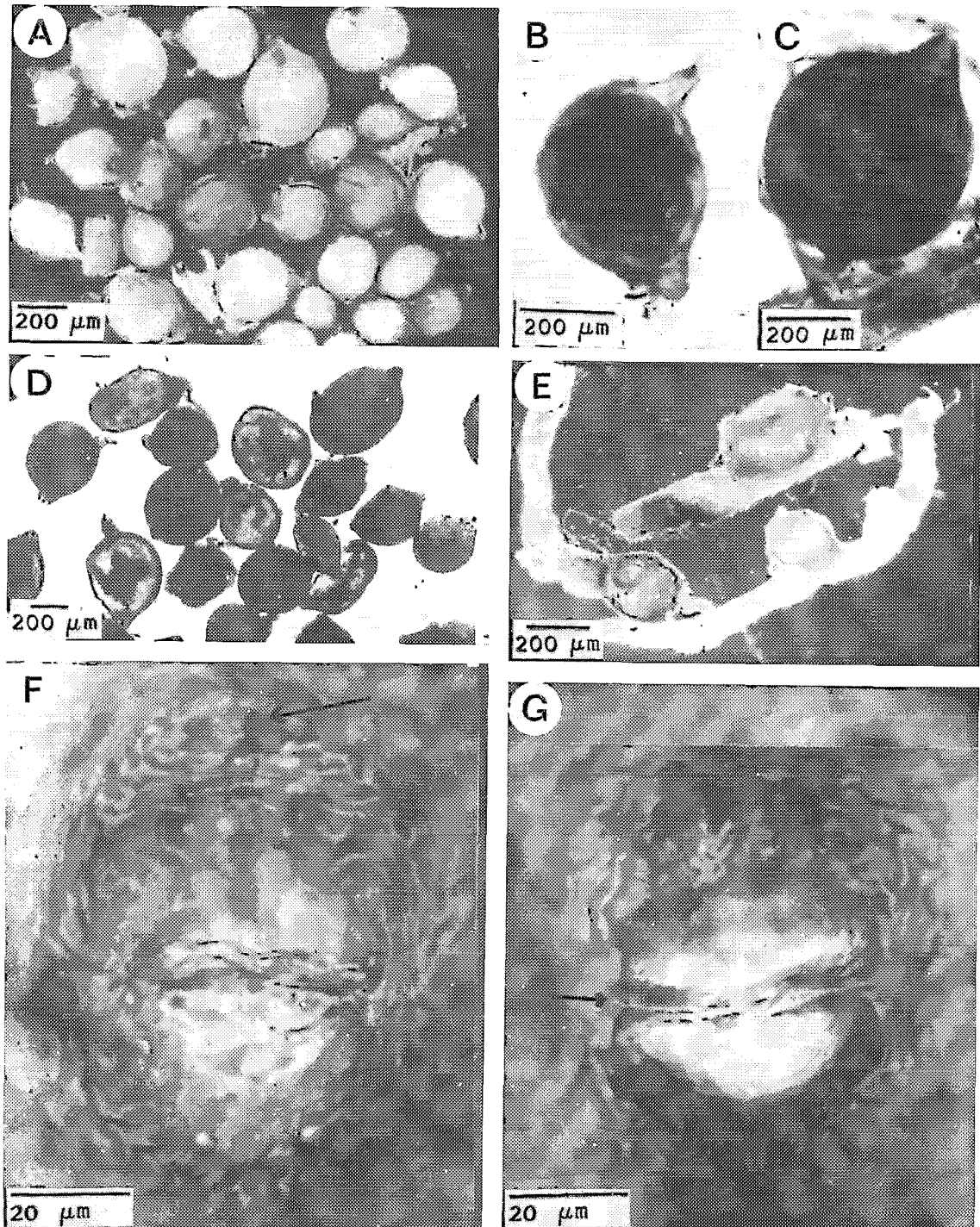


Fig. 2. *Heterodera bergeniae* n. sp. A : White females; B : Whole female; C : Mature cyst attached to the root; D : Mature cysts; E : Newly formed cyst still attached to the root; F : Cyst cone (arrow indicates anus); G : Cyst cone (arrow indicates vulval slit).

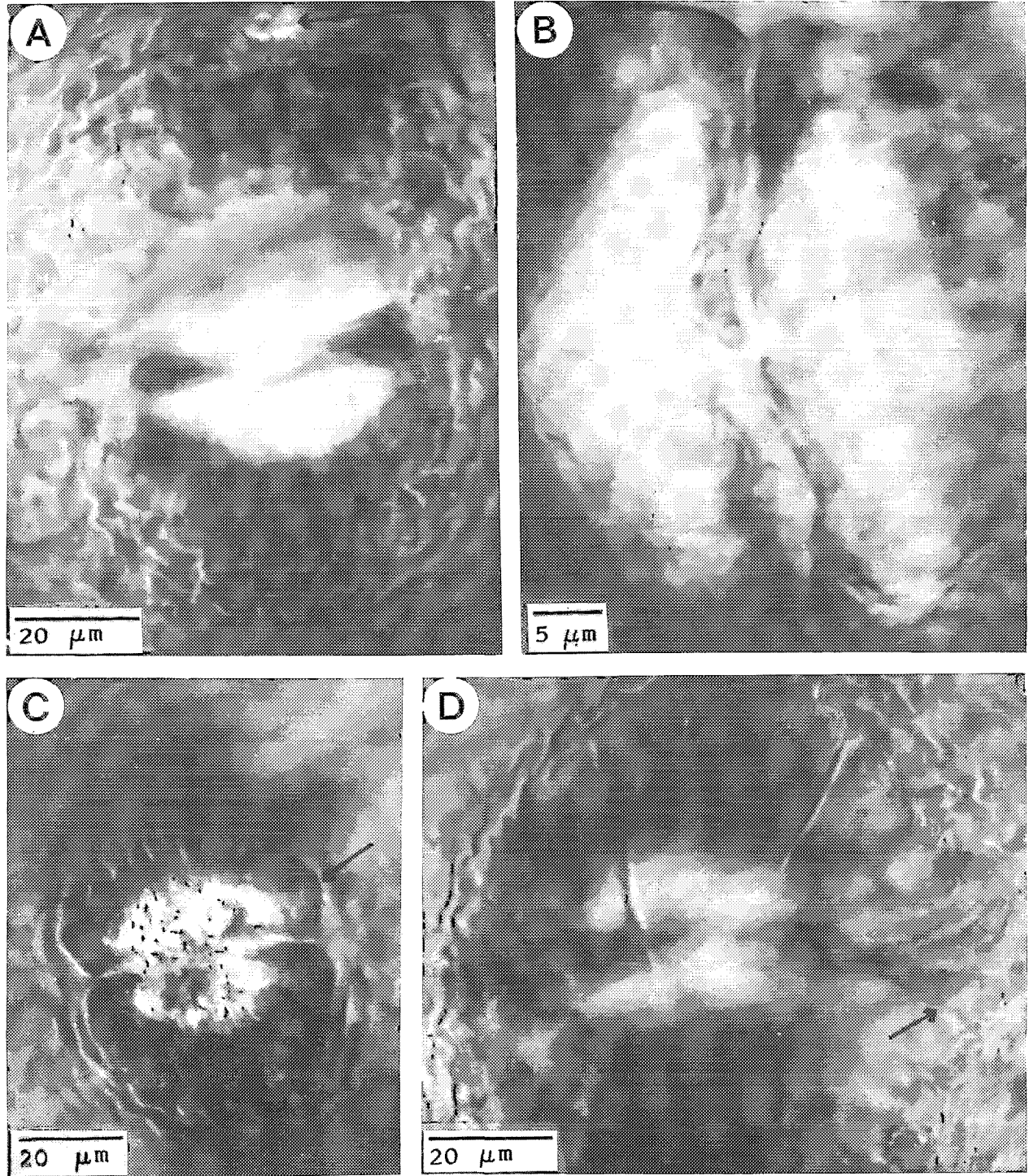


Fig. 3. *Heterodera bergeniae* n. sp. A, B : Underbridges; C : Fenestrae (arrow indicates edge of the basin); D : Underbridge (arrow indicates furcate edge).

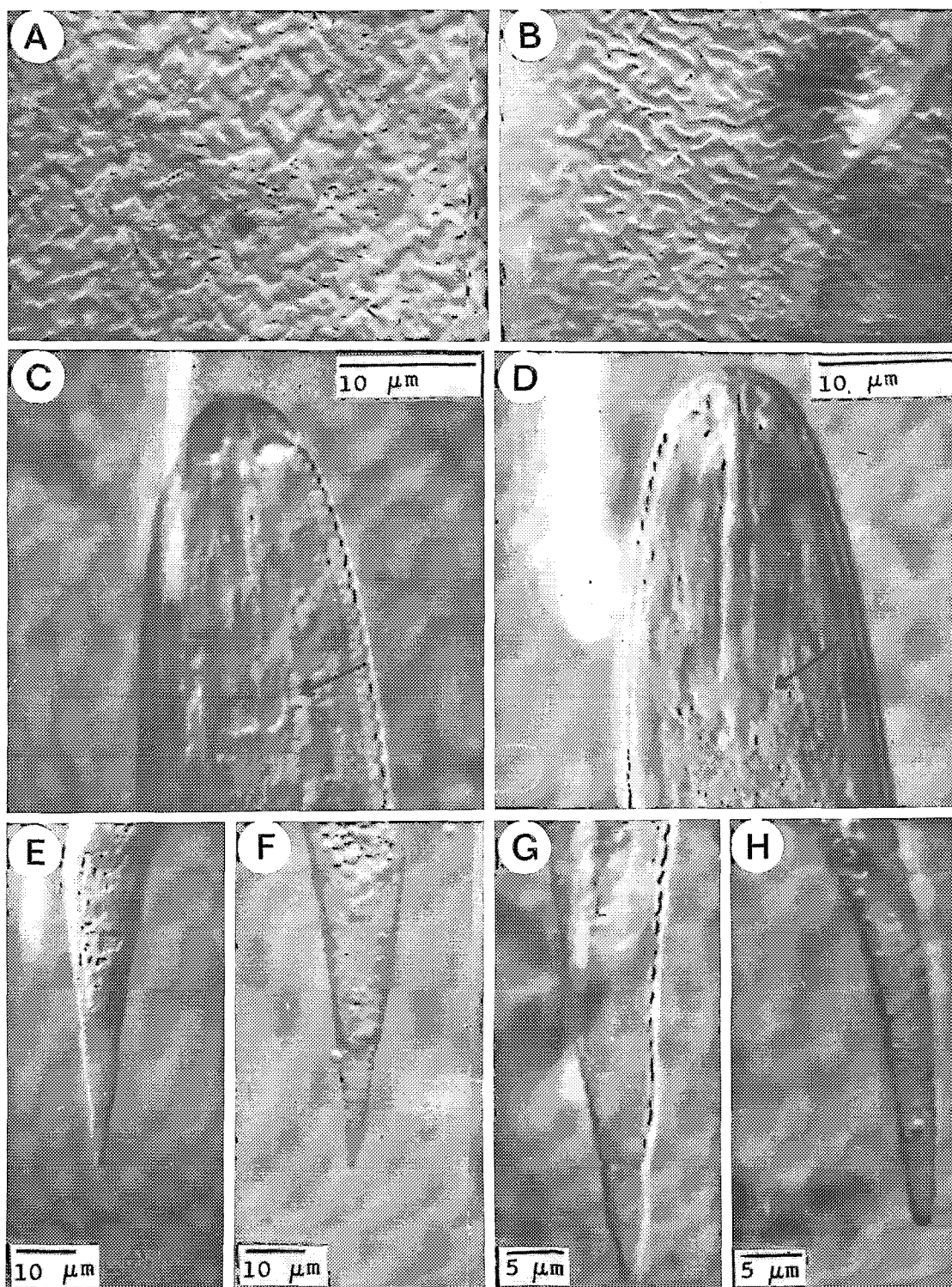


Fig. 4. *Heterodera bergeniae* n. sp. A, B : Superficial cuticular pattern of cyst at mid-body; D : Stylet of J2; G, H : Tails of J2. *Heterodera carotae* Jones, 1950; C : Stylet of J2; E, F : Tails of J2.

= 9.7 ± 0.49 (8.9-10.3); c' = 4 ± 0.2 (3.5-4); stylet = $24 \mu\text{m} \pm 0.93$ (22.4-24.8); tail length = $36 \mu\text{m} \pm 3.3$ (30-38); tail hyaline portion = $16 \mu\text{m} \pm 1.7$ (16-20); tail length/hyaline portion of tail = 1.3 ± 0.27 (1.0-1.9).

Eggs (n = 30) : Length $97 \mu\text{m} \pm 5.8$ (92-108); width = $56 \mu\text{m} \pm 2.5$ (53-59); L/W ratio = 1.6 ± 0.2 (1.5-2.0).

Holotype (female) : L (excluding neck) = 0.402 mm; W = $285 \mu\text{m}$; neck length = $81 \mu\text{m}$; stylet length = $29 \mu\text{m}$.

DESCRIPTION

White female : Round to lemon-shaped with distinctly protruding neck and vulval cone. Egg sac absent. Head bearing two annules continuous with body. Cephalic sclerotization weak. Stylet well developed, basal knobs rounded, anteriorly flattened. Dorsal cesophageal gland opening $4-5 \mu\text{m}$ behind base of stylet knobs. Median cesophageal bulb rounded. Excretory pore at level of median bulb or $70 \mu\text{m}$ (65-70) from anterior end. Cuticular annulation only in anterior region.

Cyst : Light to dark-brown, basically round to lemon-shaped with protruding neck and vulval cone. Cyst surface wall-pattern with irregular zig-zag lines forming a coarse network, without punctations. Subcrystalline layer present on young cysts but is readily lost. Egg-sac absent. Bullae absent. Underbridge about $96 \mu\text{m}$ (88-96) long and $12 \mu\text{m}$ (10-12) wide, vagina wall thin, weak, but conspicuous. Vulval slit $48 \mu\text{m}$ (44-48) long, occurs in a cleft of the vulval bridge on the top of the cone. Fenestration distinct, ambifenestrate; fenestral length $50 \mu\text{m}$ (50-55). Semifenestrae symmetrical, separated by a well developed vulval bridge, $6.4 \mu\text{m}$ (5-7) wide. Semifenestra length $24 \mu\text{m}$ (20-24), width $42 \mu\text{m}$ (40-48), encircled by a wide sclerotized dark-brown basin. In older cysts the vulval cone may appear circumfenestrate. Anus small but distinct, without surrounding pattern located $24 \mu\text{m}$ (22-26) from fenestra.

Second-stage juvenile : Body slightly curved, tapering slightly anteriorly but markedly posteriorly. Cuticular annulation of body coarse, $1.6-2.0 \mu\text{m}$ at mid body, in posterior region annules very large and prominent $2.0-2.5 \mu\text{m}$ wide. Lateral fields with four incisions with crenate margin, areolated posteriorly. Head continuous, $4.5 \mu\text{m}$ (4-5) high and $8.8 \mu\text{m}$ (8-9) wide with 3-4 annules. Cephalic framework heavily sclerotized. Stylet strong, stylet knobs $1.6 \mu\text{m}$ (1.5-2.0) high and $4 \mu\text{m}$ (3.5-4.5) wide, rounded. Dorsal cesophageal gland opening situated $4 \mu\text{m}$ (3-4) behind stylet base. Median bulb rounded, well developed, with strong valvular apparatus. Cesophageal glands elongated, covering the intestine dorsoventrally, nuclei not seen. Nerving situated in middle of isthmus. Excretory pore $96 \mu\text{m}$ (95-118) from anterior end, hemizonid two annules long, two to

four annules anterior to the excretory pore. Genital primordium consisting of two cells, situated at about 60 % (56-62) of body length from anterior end. Tail conoid tapering to a rounded terminus, with coarse annulation. Phasmid small, near anterior to middle of tail at about 1/3 tail length, $8-14 \mu\text{m}$ or 1.0-1.2 times anal body width from anus.

Egg : Shell hyaline without any markings, juveniles forming five folds.

Male : Unknown.

TYPE SPECIMENS

Holotype (female) : deposited in the NNRC National Nematode Collection, University of Karachi, Karachi, Pakistan.

Paratypes : 12 females, 125 cysts, 200 juveniles and 50 eggs; deposited in the NNRC (National Nematode Collection), University of Karachi, Karachi, Pakistan. 25 cysts, 6 females and 25 juveniles deposited in United States Department of Agriculture (USDA) Nematode Collection, Beltsville, Maryland, USA.

TYPE HOST AND LOCALITY

Specimens collected from soil and roots of *Bergenia ciliata* (Haw.) Sternb. (Saxifragaceae) in October 1985 and June and October 1986 at a height of 3 500 meters above sea level from the slopes of Malika Parbat surrounding the lake of Saiful Muluk, North West Frontier Province, Pakistan.

DIAGNOSIS AND RELATIONSHIPS

Heterodera bergeniae n. sp. belongs to the « *goettigiana* group » owing to the presence of lemon-shaped, abullate and ambifenestrate cysts but *H. bergeniae* differs from all the known species of the group by having the vulval slit in a cleft of vulval bridge and semifenestrae well separated having dark brown sclerotized basin forming a double kidney shape at the top of the cone. It comes close to *H. carotae* Jones, 1950 with regard to the position of the vulval slit but can be distinguished from the latter by the following characters : in females, egg-sac absent, head with two annules and more anterior excretory pore; in cysts, surface wall pattern with regular zig-zag lines forming a coarse net-work, egg-sac absent, fenestration distinct, ambifenestrate, vulval lips sclerotized, anus opening very large and prominent but without any cuticular pattern around it; in second-stage juveniles, cuticular annulation coarse, especially posteriorly, stylet knobs round not concave, median bulb rounded and well developed, tail and hyaline portion of tail shorter. (In *H. carotae*, females have large egg-sac, head with single annule, excretory pore $81-119 \mu\text{m}$ from

Table 1
Comparative measurements of *Heterodera carotae* and *H. bergeniae* n. sp.

Characters	<i>Heterodera carotae</i> Jones, 1950 (from Mathews, 1975)	<i>H. bergeniae</i> n. sp.
<i>Cyst</i>		
Surface wall pattern	Irregular zig-zag lines forming a close net-work	Regular zig-zag lines forming a coarse network
Egg-sac	Present, large	Absent
Fenestration	Indistinct, ambifenestrated	Distinct, ambifenestrated
Basin	Absent	Prominent dark-brown sclerotized
Vulval slit	Occurring in recessed cleft on the top of cone, partially open	Occurring in the cleft of vulval bridge, broadly open
Vulval lip	Un-sclerotized	Sclerotized
Anus	Small, distinct, surrounded by a cuticular pattern	Large, prominent, without cuticular pattern
<i>Female</i>		
Egg-sac	Present, large	Absent
Head	Single annule	Two annules
Excretory pore from anterior end	81-119 μm	65-70 μm
<i>Second stage juvenile</i>		
Cuticular annulation	Fine	Coarse especially in posterior portion
Stylet knobs	Concave anterior face	Rounded not concave
Median bulb	Oval, poorly developed	Rounded, well developed
Tail length	51.8 μm (43.5-58.6)	36 \pm 3.3 μm (30-38)
Hyaline portion	28.3 (20.3-31.8)	16 \pm 1.7 (16-20)

anterior end; cysts have surface wall pattern of irregular zig-zag lines forming a close net-work, egg-sac present, fenestration indistinct ambifenestrated, vulval lips un-sclerotized and anus opening small but conspicuous surrounded by cuticular pattern. In second stage juveniles, cuticular annulation is fine, stylet knobs have concave anterior face, median bulb is oval and poorly developed, tail length 43.5-58.6 μm , hyaline portion of tail 20.3-31.8 μm . The detailed comparison is given in Tab. 1.

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