

# *Heterodera cynodontis* n. sp. (Nematoda : Heteroderidae) from *Cynodon dactylon* (L.) in Pakistan

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## SUMMARY

*Heterodera cynodontis* n. sp., a member of the "goettingiana group", is described and illustrated from roots of lawn grass (Burmuda grass), *Cynodon dactylon* (L.) in Karachi. This new abullate species is most closely related to *H. graminophila* Golden & Birchfield, 1972, *H. longicolla* Golden & Dickerson, 1973, *H. elachista* Ohshima, 1974 and *H. oryzae* Luc & Berdon-Brizuela, 1961 with a lemon shaped cyst, no perianal pattern, a second stage juvenile with three lateral lines, and a mean of stylet length less than 22.5  $\mu$ m. It differs from these species by having a shorter hyaline tail terminus in the second stage-juvenile and a weak and smaller underbridge in the cyst.

## RÉSUMÉ

*Heterodera cynodontis* (Nematoda : Heteroderidae) parasite de *Cynodon dactylon* (L.) au Pakistan

*Heterodera cynodontis* n. sp., appartenant au « groupe goettingiana » et parasitant les racines d'une graminée de pelouse, *Cynodon dactylon* (L.), à Karachi, est décrit et illustré. Cette nouvelle espèce, dépourvue de bullae, est proche de *H. graminophila* Golden & Birchfield, 1972, *H. longicolla* Golden & Dickerson, 1973, *H. elachista* Ohshima, 1974 et *H. oryzae* Luc & Berdon-Brizuela, 1961 par les kystes limoniformes, l'absence d'ornementation périanale, les juvéniles de deuxième stade (J2) comportant un champ latéral à trois lignes et un stylet ne dépassant pas 22,5  $\mu$ m en moyenne. *H. cynodontis* n. sp. diffère de ces espèces par la partie terminale hyaline de la queue des J2 plus courte, et un sous-pont faiblement développé chez le kyste.

In August, 1988 specimens of an undescribed cyst nematode species were found in large numbers attached to the roots of lawn grass, Burmuda grass *Cynodon dactylon* L. in the garden of a residential house in North Nazimabad, Karachi, Pakistan. A pure culture of the species was established on lawn grass in the greenhouse at the National Nematological Research Centre, University of Karachi. We herein describe this nematode as a new *Heterodera* species in the "goettingiana group".

## Materials and methods

Specimens were fixed in 3 % formaldehyde solution and mounted in glycerine (Seinhorst, 1959) on the glass slide. The cover glass was supported by the wax. Measurements were made with an ocular micrometer, and drawings were prepared with a drawing tube. Photomicrographs of cysts, females, males and juveniles were made with an automatic camera attached to a compound microscope using Nomarski's interference contrast. For the studies of the embryonic development mature females were selected. They were placed in a

drop of water on a slide and the cuticle ruptured. A cover glass was placed over the cyst and gentle pressure applied to the cover glass released eggs of different stages.

*Heterodera cynodontis* n. sp.  
(Figs 1-3)

## MEASUREMENTS\*

White female (paratypes; n = 30) : L (excluding neck) = 408.0  $\pm$  59.38 (320.0-520.0); W = 307.0  $\pm$  44.90 (224.0-376.0); neck length = 121.6  $\pm$  28.50 (80-160); stylet length = 20.8  $\pm$  0.81 (19.0-21.4).

Cyst (n = 50); length (excluding neck) = 510.0  $\pm$  45.22 (440.0-568.0); W = 412.0  $\pm$  23.01 (350.0-440.0); L/W = 1.23  $\pm$  0.62 (1.15-1.36); fenestral length = 52  $\pm$  6.32 (44-64); fenestral width = 28.3  $\pm$  4.16 (20-34); vulval bridge width = 7.2  $\pm$  1.02

\* All measurements are in micrometers unless otherwise stated.

(6.0-9.6); underbridge =  $64 \pm 3.85$  (60-70); Vulval slit =  $36.4 \pm 2.11$  (33-40); anus from posterior end =  $32.0 \pm 0.92$  (30.0-35.0).

*Male* (n = 20); L =  $784.5 \pm 43.87$  (708.0-824.0); a =  $38.24 \pm 3.183$  (32.63-44.13); b =  $5.22 \pm 0.39$  (4.6-5.9); c =  $150.3 \pm 32.15$  (126.0-201.6); c' =  $0.48 \pm .07$  (0.35-0.58); stylet =  $22.4 \pm 0.8$  (20.8-23.2); outlet of dorsal esophageal gland from base of stylet =  $3.36 \pm 1.32$  (2.4-4.0); spicules =  $25.6 \pm 2.7$  (22.4-28.0); gubernaculum =  $7.2 \pm 0.56$  (6.4-8.0).

*Second stage juveniles* (n = 25); L =  $345.38 \pm 15.09$  (317.0-374.0); a =  $17.7 \pm 0.92$  (16.5-19.4); b =  $2.42 \pm 0.19$  (2.1-2.8); c =  $7.5 \pm 0.29$  (7.2-8.0); c' =  $3.7 \pm 0.41$  (3.3-4.6); stylet =  $20.0 \pm 1.41$  (19.2-20.8); tail length =  $45.7 \pm 2.46$  (40.8-48.0); tail hyaline portion =  $18.8 \pm 0.91$  (16.0-20.0); tail length/hyaline portion of tail =  $2.42 \pm 0.20$  (2.12-2.68).

*Eggs* (n = 50); length =  $92.8 \pm 2.53$  (88-96); width =  $38.8 \pm 1.41$  (36-40); L/W ratio :  $2.3 \pm 0.11$  (2.2-2.5).

*Holotype* (female); L (excluding neck) = 370.0; W = 270.0; neck length = 96.0; stylet length = 20.8.

#### DESCRIPTION

*White female* : Body pearly white, lemon shaped with protruding neck and vulva, neck expanding rapidly. Cuticle thick,  $4.5 \pm 0.25$  (3.5-5.0) at mid body with zig-zag pattern, and subcuticular punctations present. Cuticle annulated in anterior region and concentric wavy lines present around vulval cone. Head distinctly offset with a single disc-shaped annule. Cephalic sclerotization weak. Stylet delicate, with basal knobs sloping posteriorly. Outlet of dorsal esophageal gland  $4 \pm 0.81$  (3.2-5.0) from base of stylet. Median esophageal bulb prominent,  $20.8 \pm 0.10$  (19.0-21.0) long and  $17.6 \pm 0.12$  (16.0-18.0) wide, with strongly developed valve plates. Esophageal glands appearing as a single lobe. Excretory pore distinct, located at position of the gland lobe. Vulva conspicuous, protruding posteriorly. Egg sac absent. Anus small, conspicuous.

*Cyst* : Cysts light to dark brown, lemon shaped, with protruding neck and vulva. Cuticular zig-zag pattern conspicuous, irregularly arranged subcuticular punctation present. Egg sac absent. Cone top abullate, ambifenestrate; fenestral length  $52 \pm 6.32$  (44-64), fenestral width  $28.3 \pm 4.16$  (20-34). Semifenestrae symmetrical, separated by a wide vulval bridge  $7.2 \pm 1.02$  (6.0-9.6) encircled by a wide sclerotized dark-brown basin  $8.6 \pm 0.86$  (8.0-10.0) wide; underbridge weak but prominent, bifurcate at the ends,  $64 \pm 3.85$  (60-70) long. Vulval slit averaging about  $36.4 \pm 2.11$  (33-40) in length. Anus small, conspicuous, without surrounding

pattern and situated about  $32.0 \pm 0.92$  (30.0-35.0) from posterior end.

*Male* : Males numerous; body cylindrical elongate, usually dorsally curved and gradually tapering at each end. Cuticle distinctly annulated, annulations 1.6-2.4 apart at mid-body. Lateral field about 1/4 body width with slight areolation appearing sometimes as three lines but in many cases as four with the two inner lines much closer than the outer ones, and outer lines weakly crenate. Head broadly hemispherical with four or five annules,  $8.0 \pm 0.8$  (7.2-9.6) long and  $4 \pm 0.6$  (3.2-5.0) wide, slightly set off. Cephalic sclerotization heavy. Stylet well developed with slightly anteriorly directed knobs. Procorpus a slender tube ending in a distinct median bulb, with prominent valve plates. Esophageal glands as a single lobe, with three nuclei, overlapping the anterior region of the intestine. Excretory pore, with prominent duct,  $119.1 \pm 2.85$  (116-124) from anterior end. Hemizonid two or three annules anterior to the excretory pore. Testis single, outstretched  $415.0 \pm 29.14$  (360.0-452.0) long. Spicule arcuate, and finely bidentate. Gubernaculum simple, cloacal opening protruding. Phasmids not observed.

*Second stage juvenile* : Body vermiform, tapering at both extremities but much more so posteriorly. Cuticle with distinct annulation, 1.6-2.4  $\mu$ m wide at mid body. Lateral lines areolated and composed of three lines. Head slightly offset,  $9.2 \pm 0.43$  (8.8-9.6)  $\mu$ m wide and  $3.5 \pm 0.43$  (3.2-4.0)  $\mu$ m high, with three annules. Cephalic framework heavily sclerotized. Stylet well developed with prominent, anteriorly directed knobs. Dorsal esophageal gland duct opens  $5.6 \pm 0.8$  (4.8-6.4) behind the base of the stylet. Median esophageal bulb ovoid with strong valvular apparatus; esophageal glands elongate and overlapping the intestine laterally with three prominent nuclei. Excretory pore  $76.6 \pm 4.25$  (70.0-80.0)  $\mu$ m from anterior end. Hemizonid more than two annules wide, 2-3 annules anterior to the excretory pore. Genital primordium two celled,  $8.0 \pm 1.02$  (6.0-9.0)  $\mu$ m long,  $4.0 \pm 0.82$  (3.2-5.6)  $\mu$ m wide,  $179.2 \pm 9.59$  (169.0-188.0)  $\mu$ m from the anterior end. Tail gradually tapering, conoid with rounded terminus; hyaline terminal part unusually short. Phasmid very small,  $32 \pm 1.02$  (30-35)  $\mu$ m anterior to tail tip.

*Eggs* : Embryonic development basically the same as in other species of *Heterodera*. One celled eggs with large number of embryonic granules and prominent nucleus at centre. Two celled eggs divided transversely at right angles to longitudinal axis with blastomeres equal in size. Two celled eggs divide further transversely to form three and four celled eggs. Some eggs with complete embryonic development and first stage juvenile present. Numerous egg with completely development and spear beginning to form in second stage juvenile.

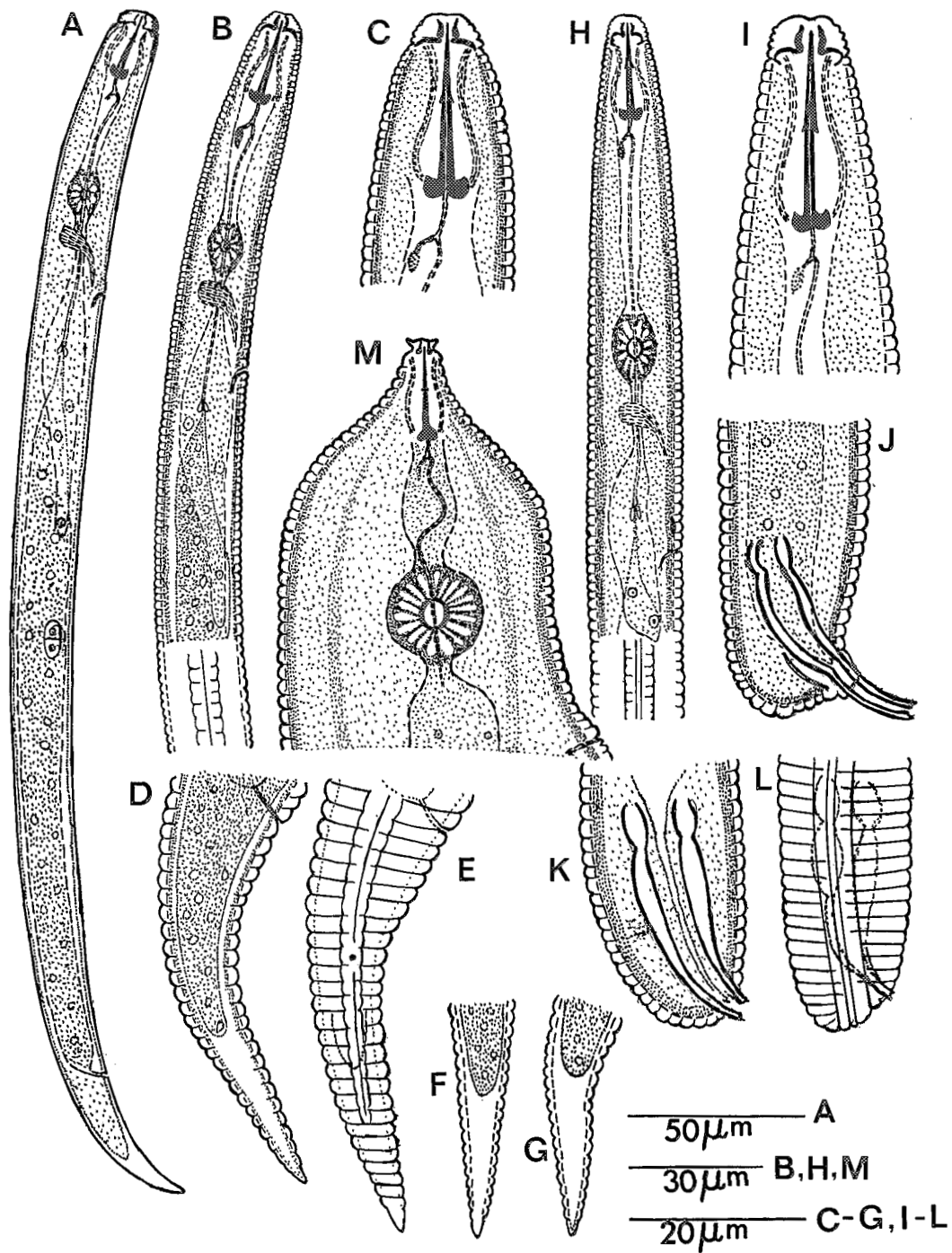


Fig. 1. *Heterodera cynodontis* n. sp. — A-G Second stage juveniles. A : Entire; B : Oesophageal region; C : Head; D, E : Tails; F, G : Tail terminus. — H-L Male. H : Oesophageal region; I : Head; J, K, L : Tails. — M Female, anterior region.

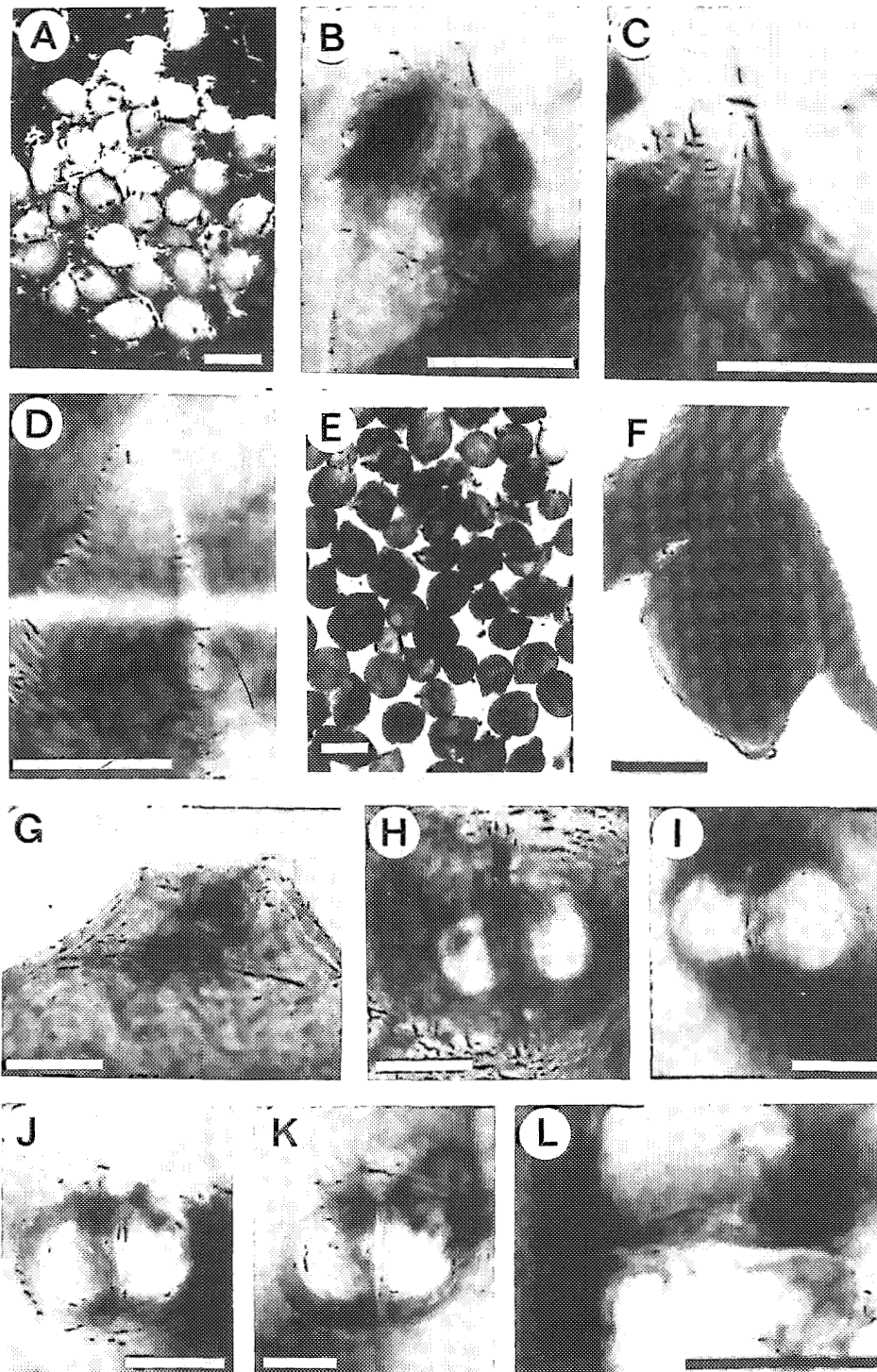


Fig. 2. *Heterodera cynodontis* n. sp. — A-D Female. A : White females; B : Oesophageal region; C : Anterior region showing single head annule and stylet; D : Cuticular annulation and single head annule. — E-L Cyst. E : Mature cysts; F : Cyst attached to the roots; G : Lateral view of vulva-cone showing underbridge; H : Underbridge showing bifurcate ends; I : Vulval cone showing ambifenestriae; J : Fenestrae indicating edge of basin; K : Fenestrae showing vulval slit; L : Vulval slit and vulval bridge, magnified (Scale bar : A, E = 400  $\mu$ m; B = 50  $\mu$ m; C-D, G-L = 25  $\mu$ m; F = 200  $\mu$ m).

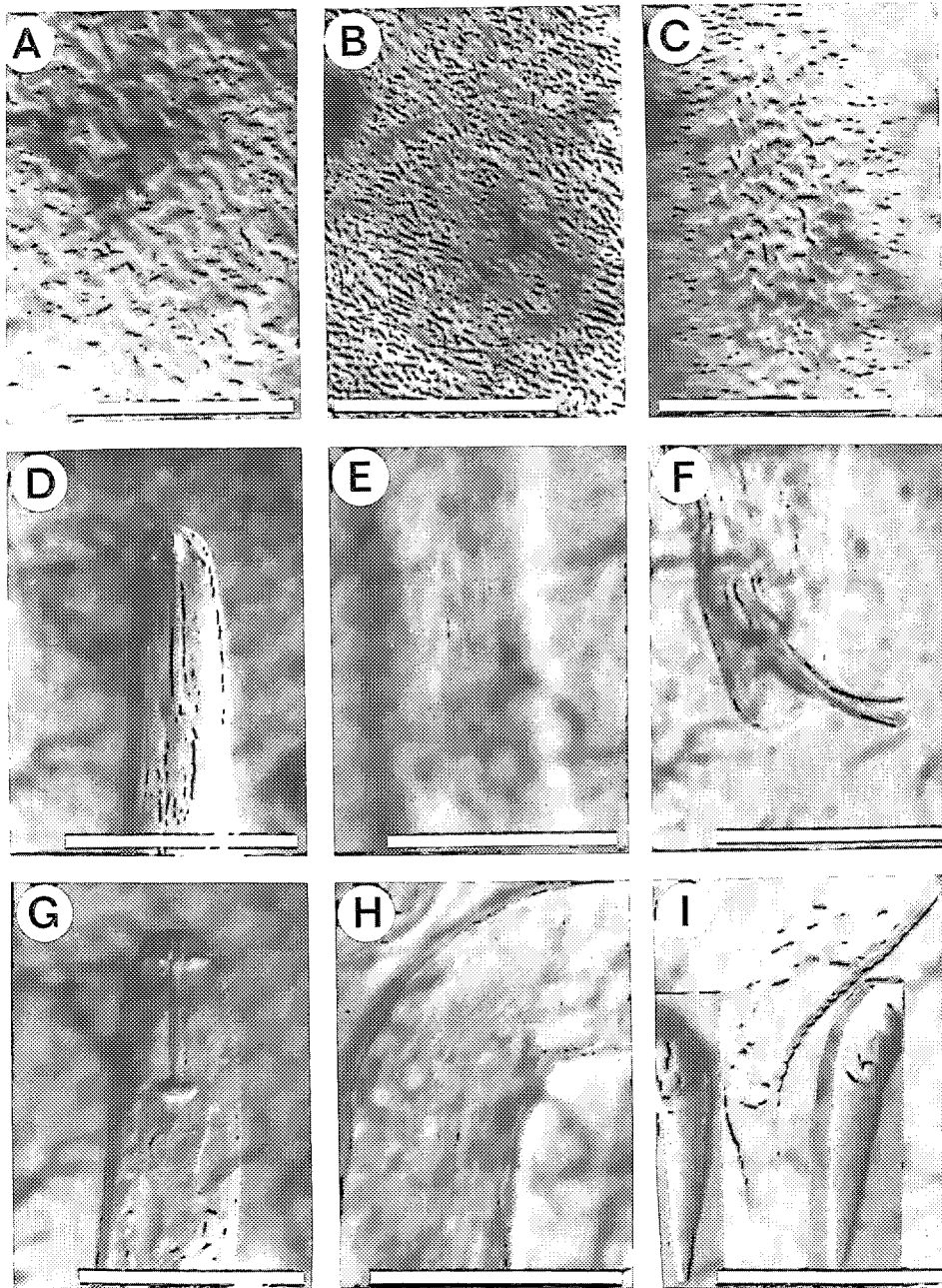


Fig. 3. *Heterodera cynodontis* n. sp. — A, B, C Cyst wall; A : Zig-zag lines at mid body; B : Subcuticular punctation; C : Zig-zag lines and subcuticular punctation. — D, E, F Male. D : Anterior region; E : Four lateral lines; F : Posterior region. — G, H, I Second stage juveniles. G : Anterior region; H : Three lateral lines; I : Tail and hyaline portion (Scale bars = 30  $\mu$ m).

TYPE SPECIMENS

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

*Paratypes* : 30 females, 100 cysts, 25 males, 250 juveniles and 100 eggs; deposited in the NNRC National Nematode Collection, University of Karachi, Karachi, Pakistan. 25 cysts, 15 females, 5 males and 25 juveniles deposited in the United States Department of Agriculture (USDA) Nematode Collection, Beltsville, Maryland, USA.

TYPE HOST AND LOCALITY

Specimens collected from soil and roots of *Cynodon dactylon* (L.) in August, 1988 from the North Nazimabad, Karachi, Pakistan.

DIAGNOSIS AND RELATIONSHIP

*Heterodera cynodontis* n.sp. belongs to the "goettingiana group" (bullae rarely present, underbridge either absent, slender or strongly developed vulva cone ambifenestrate or bifenestrate). On the basis of a combination of characteristics, *H. cynodontis* n.sp. can be separated from all the species of the "goettingiana group" by lemon shaped cyst, mean L/W ratio 1.3, perianal pattern absent, weak, smaller underbridge, second stage juvenile with three incisures in the lateral fields, mean stylet length much less than 22.7, smaller hyaline tail terminus length.

*H. cynodontis* n.sp. comes close to *H. graminophila* Golden & Birchfield, 1972, *H. longicolla* Golden & Dickerson, 1973, *H. elachista* Ohshima, 1974 and *H. oryzae* Luc & Berdon-Brizuela, 1961, with the lemon shaped cyst, mean L/W ratio 1.3, perianal pattern absent, lateral field of the second stage juvenile with three incisures. It can be distinguished from *H. graminophila* by the smaller stylet of the female, the smaller fenestral width and shorter underbridge and vulval slit of the cyst, the shorter body and spicule of the male and the shorter body, stylet, tail and hyaline tail of the second stage juvenile (in *H. graminophila* : female stylet = 21.3-23.8; fenestral width = 43-50; underbridge = 115-140; vulval slit = 40-52; male = 850-1,110; spicules = 31-36; second stage juvenile = 360-460; J2 stylet = 21.8-23.5; J2 tail = 57-67; hyaline tail = 25-38). *H. cynodontis* n.sp. differs from

*H. longicolla* by the smaller L/W ratio and single head annule of the female, the presence of an underbridge, narrower fenestral and shorter vulval slit and a strongly developed vulval bridge in the cyst and in the male; greater "a", smaller "c" and longer stylet in the male and the longer stylet and smaller hyaline tail of the juvenile (in *H. longicolla* : female cyst L/W ratio = 1.5-2.7; underbridge absent; fenestral width = 40; vulval slit = 45; vulval bridge weak; male a = 23-32; c = 215-1580; male stylet = 17.9-20.2; juvenile stylet = 16.8-18.5; hyaline tail = 20-33). It can be separated from *H. elachista* females by the absence of an egg sac; from cysts by the absence of bullae, the longer fenestra and semifenestra and the shorter underbridge; from the males by the smaller body length and anteriorly directed stylet knobs and from the juvenile by the shorter hyaline tail (in *H. elachista* : gelatinous egg-sac with eggs, and bullae present; fenestrae 25-37; semifenestrae = 9-17; underbridge = 75-90; male L = 820-940; stylet knobs sloping posteriorly; J2 hyaline tail = 26-36). *H. cynodontis* n.sp. can be distinguished from *H. oryzae* by the shorter stylet of the female, the absence of bullae, the longer fenestrae and shorter vulval slit and underbridge; the smaller stylet of the male with the stylet knobs directed anteriorly and the shorter juvenile with tail and hyaline, tail without elongate conical terminus (in *H. oryzae* : female stylet : 28-30, bullae present; fenestral length = 40-55; vulval slit = 42-50; underbridge = 110-150; male stylet = 23-25 with knobs directing posteriorly; J2, L = 370-507; J2 tail = 67-69; hyaline tail = 35-45 with elongated terminus and pointed extremity).

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