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New Species of Cyst Nematode *Heterodera pakistanensis* (Nematoda: Heteroderidae) Attacking Wheat in Pakistan[1]

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Abstract: Heterodera pakistanensis n. sp., described and illustrated from roots of common wheat (*Triticum aestivum*) from Sukkur, Sind, Pakistan, belongs to the *goettingiana* group. It is most closely related to *H. cyperi* Golden, Rau & Cobb, 1962, *H. raskii* Basnet & Jayaprakash, 1984, and *H. mothi* Khan & Husain, 1965. Second-stage juveniles (J2) can be distinguished from *H. cyperi* J2 by an areolated lateral field with four incisures and shorter stylet, whereas cysts are separated by a more elongated vulva slit and the conspicuous structure of the underbridge. It differs from *H. raskii* by having four areolated lateral lines in J2, smaller female lemon-shaped cyst, shorter fenestra length and width, conspicuous underbridge, and distinct anus with a high cuticular pattern 40–45 μm from posterior end. It also differs from *H. mothi* by the presence of four areolated lateral lines in J2 and absence of vulva denticles and bullae.

Key words: taxonomy, morphology, wheat, *Triticum aestivum.*

Little is known about the occurrence of cyst nematodes in Pakistan. Earlier Akhtar (1) reported *Heterodera schachtii* Schmidt, 1871 in association with sugarcane while Brown (3) found *Globodera tabacum* (Lownsbery & Lownsbery, 1954) Behrens, 1975 in tobacco fields. Later Maqbool (6), in a general survey of crops carried out in 1978–79 in Pakistan, identified seven species: *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 on potato; *Heterodera avenae* Wollenweber, 1924 on maize and wheat; *H. mani* Matthews, 1971 on wheat; *H. mothi* Khan & Husain, 1965 on cowpea; *H. sacchari* Luc & Merny, 1963 on sugarcane; *H. schachtii* Schmidt, 1871 on cauliflower and sugarcane; *H. vigni* Edward & Misra, 1968 on cowpea; and *H. zeae* Koshy, Swarup & Sethi, 1971 on maize and wheat.

During a March 1985 survey of wheat fields in Sukkur, Pakistan, specimens of an undescribed cyst-forming nematode were found attached to the roots of *Triticum aestivum* L. A detailed morphological study showed that it is a new species of *Heterodera* belonging to the *goettingiana* group. The name *Heterodera pakistanensis* n. sp. is proposed.

Materials and Methods

A large number of specimens obtained from soil and roots of wheat were fixed in 3% formalin and mounted in glycerine (7). Photomicrographs of cysts, vulva cones, females, and juveniles were made with an automatic camera attached to a compound microscope using an interference contrast system. Measurements of all stages were made with an ocular micrometer, and drawings were prepared with a drawing tube. All measurements are in micrometers unless otherwise stated.

Systematics

Heterodera pakistanensis n. sp. (Figs. 1–26)

Paratypes (76 females): Length (with neck) 523 (360–552); width 288 (192–336); stylet length 20 (20–22); DGO from base of stylet length 5.0 (5.0–5.6).

Holotype (female): Length (with neck) 452; width 290; stylet length 20; DGO from base of stylet 5.0.

Body white, basically lemon shaped, neck long, vulva cone protruding. Head distinctly set off from body, somewhat disc shaped, cephalic sclerotization moderate. Stylet well developed, basal knobs rounded at base, slightly directed anteriorly. Median bulb 16–17 × 16–17, valvular plates well developed. Esophageal glands in a single lobe, variable in size and shape. Excretory pore 41–44 from anterior end. Cuticular annulation in anterior region only, in a zig zag pattern, posteriorly wavy lines around vulva cone (Fig. 14). Subcrystalline layer present.

Paratypes (60 cysts): Length 560 (480–608); width 320 (280–440); L/W ratio 1.7 (1.4–2.3).

Cyst light to dark brown, lemon shaped,

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neck and vulva cone prominent. Cyst wall pattern angular, zig zag, subcuticular punctuations irregular. Vulva cone with concentric lines around vulva slit and fenestra. Vulva protruding, ambifenestrate (Fig. 18), bullae absent (Fig. 21), fenestra length 28 (25–38), fenestra width 19 (15–20), vulva slit 48 (45–51), underbridge weak, conspicuous. Anus prominent, 42 (40–45) from posterior end, surrounded by definite cuticular pattern.

Second-stage juveniles (85): Length 446 (412–452); a = 24 (24–27); b = 6.4 (6.0–8.8); b′ = 2.4 (2.3–2.8); c = 8.3 (6.0–9.2); stylet length 16.8 (16.8–18); DGO from base of stylet 6.4 (5.6–6.8); tail length 70 (68–72); hyaline portion of tail 32 (32–35) long.

Body cylindrical, slightly curved, tapering slightly anteriorly, markedly posteriorly. Cuticle finely annulated, annules 1.6 wide at midbody. Head 3.6 high, 6.4 wide, continuous with three distinct annules. Cephalic framework moderate. Stylet well developed, basal knobs rounded, slightly anteriorly directed. Median bulb ovoid, valvular apparatus well developed. Excretory pore 90–98 from anterior end. Hemizonid two annules long, 4–6 annules anterior to excretory pore. Lateral field, with four lines areolated. Tail long, tapering to rounded terminus. Anus distinct. Phasmid near middle of tail.

Paratypes (14 males): Length 983 (947–1,281); a = 32 (28–37); b = 3.8 (3.2–4.8); b′ = 7.8 (7.5–9.2); c = 149 (138–165); stylet length 23.4 (21.4–24.4); DGO from base of stylet 5.6 (5.6–6.6); spicule length 36 (30–36); gubernaculum length 8.8 (8.0–10.4).

Allotype (male): Length, 1.201; a = 32; b = 3.8; b′ = 9.0; c = 149; stylet length 22.0; DGO from base of stylet 6.0; spicule length 36; gubernaculum length 10.4.

Body cylindrical tapering gradually at both extremities. Annules distinct, 1.6–2.0 wide at midbody. Head continuous with three annules, cephalic framework heavily sclerotized. Stylet strong with anteriorly directed basal knobs. Median bulb with distinct valvular apparatus. Excretory pore 128 (121–132) from anterior end. Hemizonid prominent, three annules long, six annules anterior to the excretory pore. Lateral field areolated with four incisures. Spicules slightly arcuate. Tail short, rounded. Phasmid near terminus.

Eggs (60): Length 116 (112–130); width 48 (40–48); L/W ratio 2.5 (2.4–2.8). Egg shell hyaline without surface markings, juveniles forming five folds.

Type specimens: Holotype (female) collected in March 1985 from wheat in Sukkar, Sind, Pakistan. Slide No. NNRC 40/55-56, deposited in the National Nematological Research Centre (NNRC) Nematode Collection, University of Karachi, Karachi, Pakistan. Allotype (male). Slide No. NNRC 40/57-59, same data and collection as holotype. Paratypes (76 females, 14 males, 60 cysts, 85 J2, 60 eggs) deposited in the NNRC Nematode Collection, University of Karachi, Karachi, Pakistan. Cysts, females, and J2 deposited in United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

Type host and locality: Roots of wheat (*Triticum aestivum* L.), Sukkur, Sind, Pakistan.

Diagnosis: Heterodera pakistanensis n. sp. belongs to the *goettingiana* group (cyst lemon shaped, abullate, and ambifenestrate). It is most closely related to *H. cyperi* Golden, Rau & Cobb, 1962 (4), *H. raskii* Basnet & Jayaprakash, 1984 (2), and *H. mothi* Khan & Husain, 1965 (5) because of the presence of cuticular pattern around the anus. It differs from *H. cyperi* by having four areolated lateral lines and shorter stylet in J2, longer vulva slit and conspicuous underbridge. (In *H. cyperi* three lateral lines, stylet length 19.2–20.7, vulva slit 30–35,

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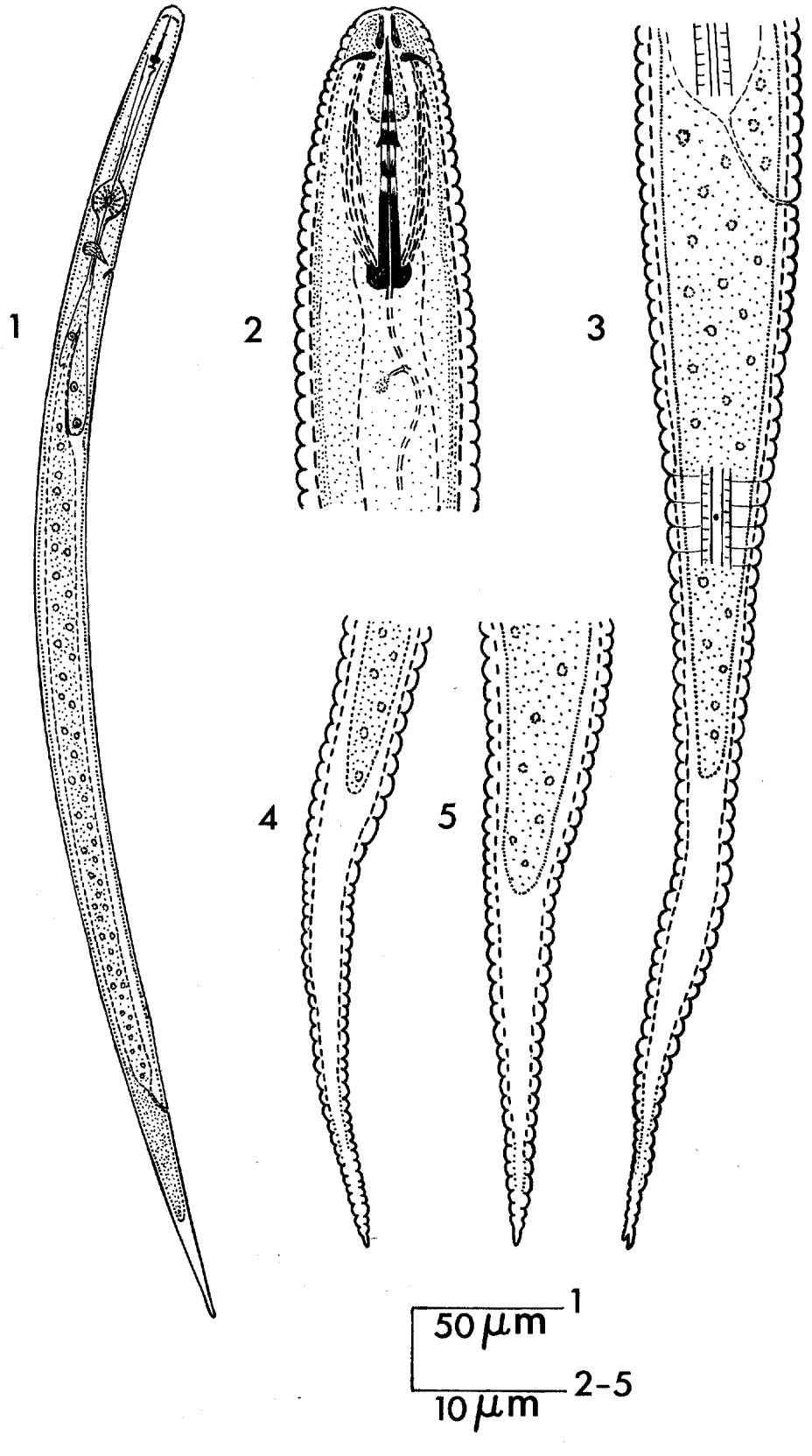
FIGS. 1–5. *Heterodera pakistanensis* n. sp. second-stage juveniles. 1) Whole body. 2) Anterior region. 3) Posterior region. 4, 5) Tail.

FIGS. 6–8. *Heterodera pakistanensis* n. sp. 6) Male anterior region. 7) Male tail. 8) Female esophageal region.

FIGS. 9–15. Photomicrographs of *Heterodera pakistanensis* n. sp. Valvulated females. 9) White females. 10, 11) Females attached to roots. 12, 13) Esophageal region. 12) Head, stylet with basal knobs. 13) Valvulated median bulb. 14) Ridge pattern on vulva cone. 15) Cuticular pattern on neck region.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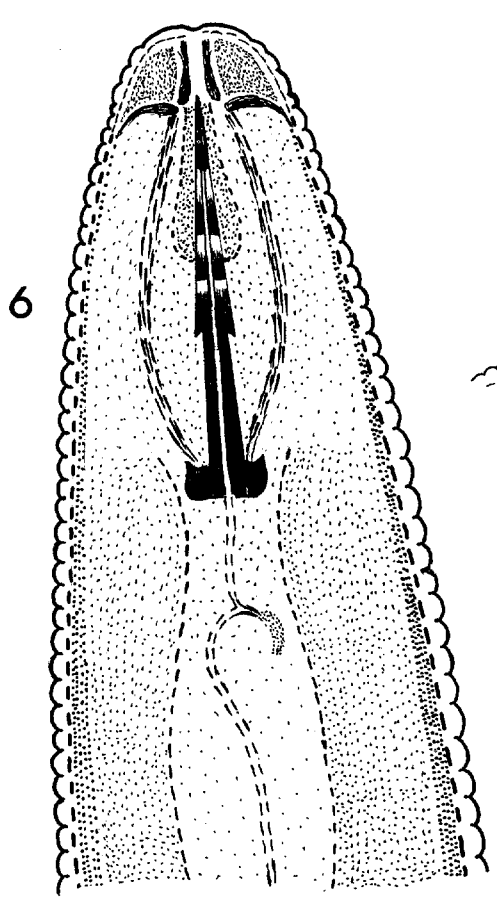
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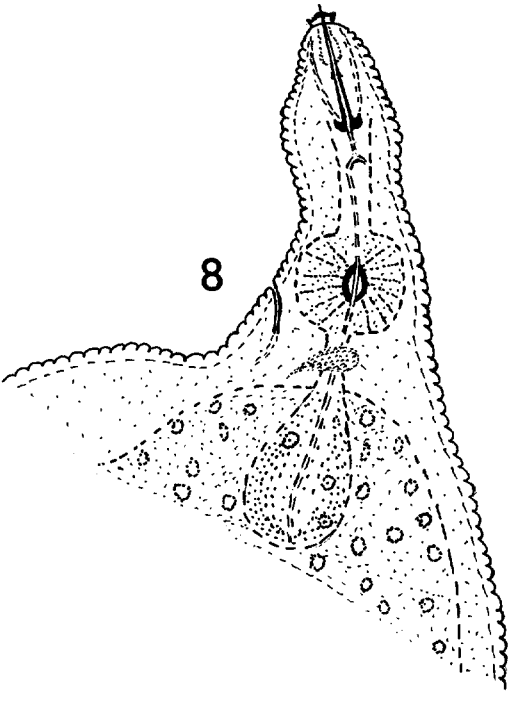
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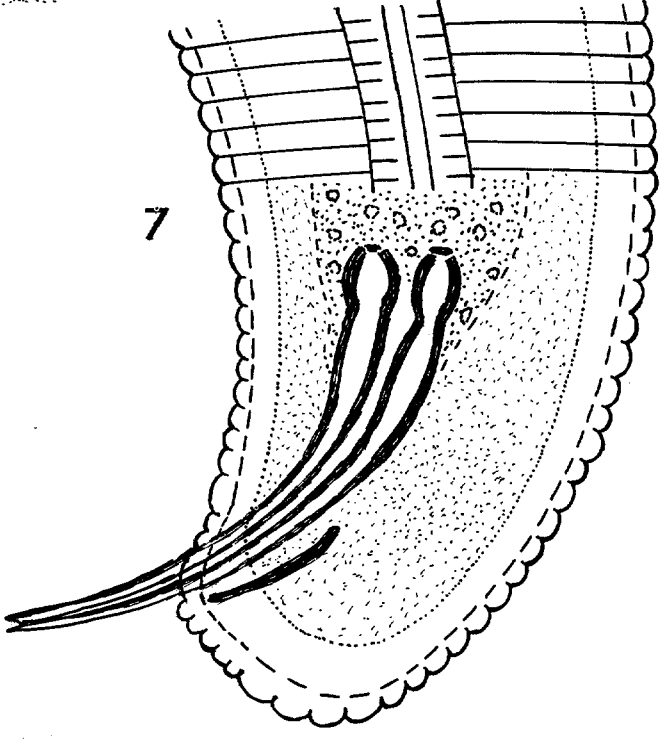
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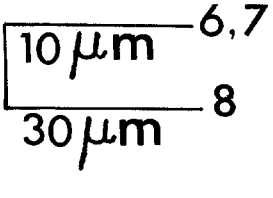
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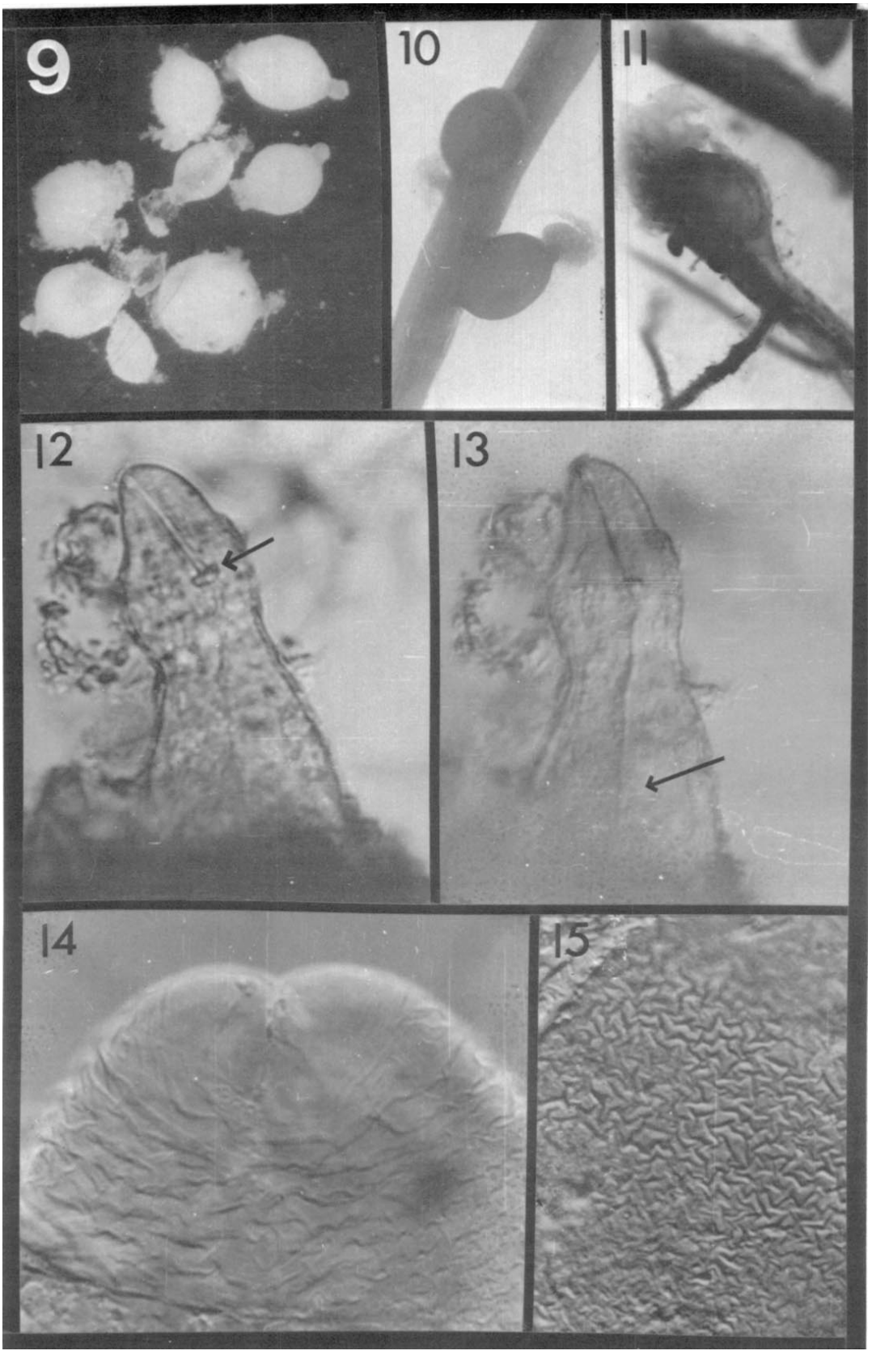
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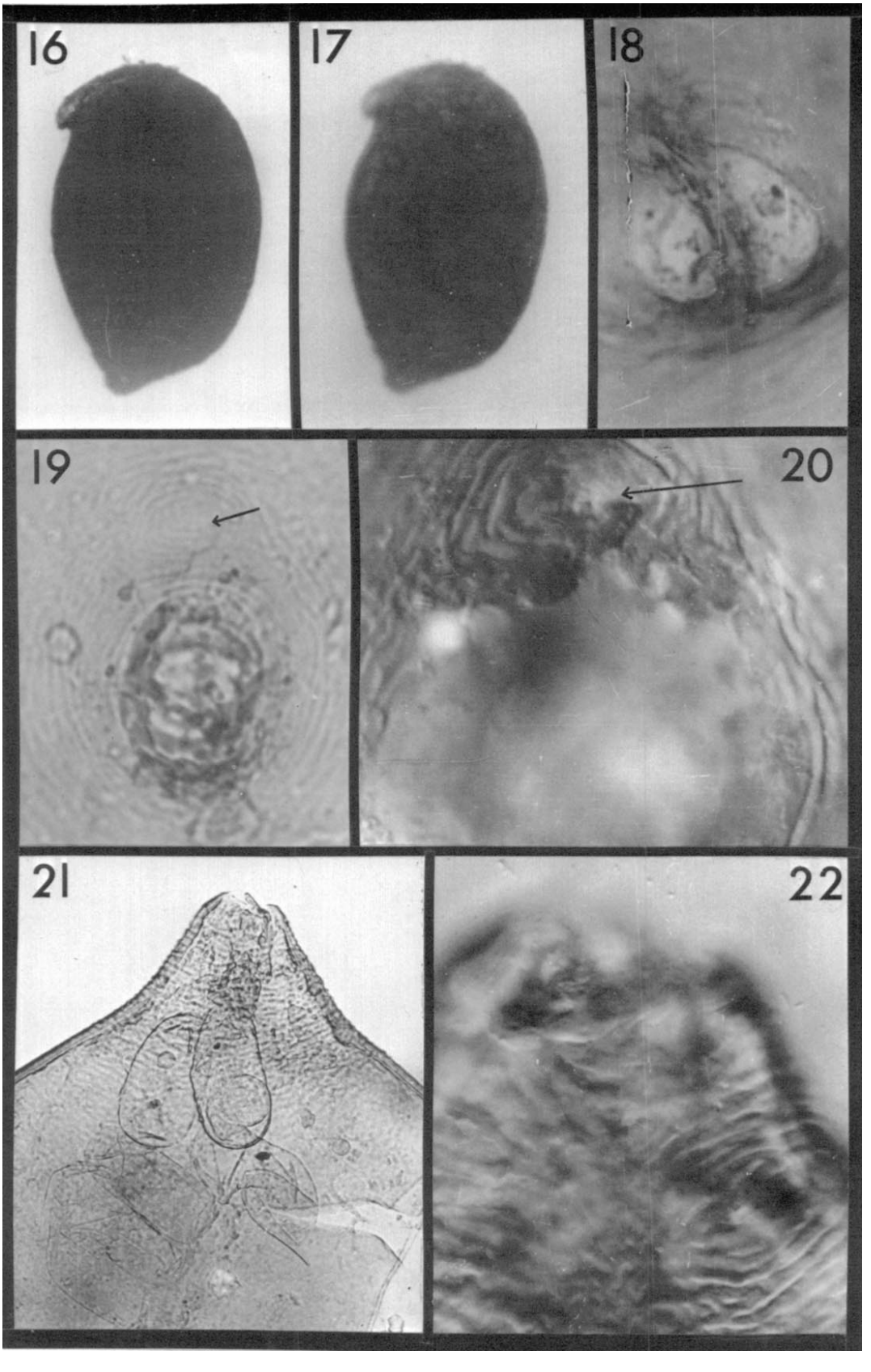
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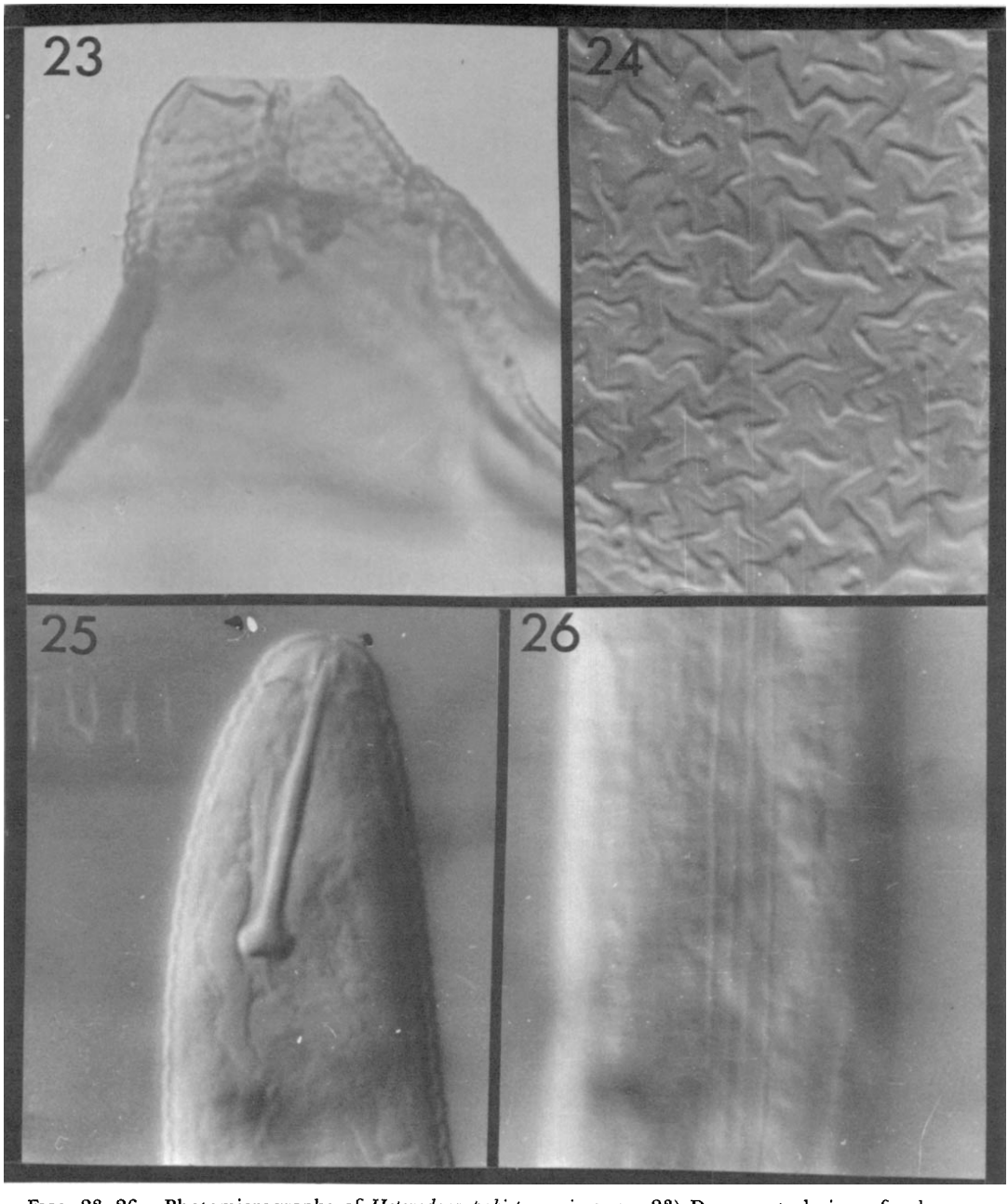

FIGS. 23–26. Photomicrographs of *Heterodera pakistanensis* n. sp. 23) Dorsoventral view of vulva cone showing underbridge. 24) External cuticular pattern of cyst at midbody. 25) Second-stage juvenile (J2) head and stylet. 26) J2 lateral field.

underbridge simple.) It can be distinguished from *H. raskii* by the four areolated lateral lines in J2, smaller female, lemon-shaped cyst, shorter fenestral length and width, underbridge conspicuous, anus distinct with high cuticular pattern 40–45 from posterior end. (In *H. raskii* three lateral lines in J2, female length 575–700,

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FIGS. 16–22. Photomicrographs of *Heterodera pakistanensis* n. sp. cysts. 16, 17) Whole cysts with posterior protuberance. 18) Fenestra and vulva slit. 19) Fenestral area and anal pattern. 20) Enlarged photo of anal fenestra. 21) Posterior portion of cyst. 22) Vulva cone with distinct anus.

width 210–290, cyst elongate ovoid, fenestral length 48–54, width 40–45, underbridge circumanal, cuticular pattern 50–66 from posterior end.) It differs from *H. mothi* by having four areolated lateral lines in J2 and absence of vulva denticles and bullae. (In *H. mothi,* three lateral lines in J2, vulva denticles and bullae present.)

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