Description of *Hemicycliophora pyri* n. sp., with Observations on *Psilenchus vinciguerrae* Brzeski, 1991 and *Ditylenchus equilis* Heyns, 1964 (Nematoda: Tylenchida) from Gilgit-Baltistan, Pakistan

Sagir Hussain¹, Erum Iqbal²*, Nasira Kazi², Sher Wali Khan¹, Qamar Abbas¹ and Abdul Razaq¹

¹Department of Biological Sciences, Karakorum International University, Gilgit, Gilgit-Baltistan, Pakistan
²National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan

**ABSTRACT**

The present study provides the morphological and morphometric characterization of some plant parasitic nematode populations, recovered from agricultural fields during surveys of the districts Gilgit and Nager, Gilgit-Baltistan, Pakistan. The analysis of samples yielded a new nematode species and two new reported species belonging to the order Tylenchida as new geographical records for Pakistan. *Hemicycliophora pyri* n. sp., is characterized by the broadly rounded lip region with two indistinct annuli, closely fitting sheath with 231-247 body annuli, stylet 95-100 µm long, non-raised and non-separated labial disc and gradually tapering long tail.

*Psilenchus vinciguerrae* (Brzeski, 1991) and *Ditylenchus equilis* (Heyns, 1964) have been reported as new record species from Pakistan. These species are described and briefly re-described along with their morphometric data, photomicrographs and illustrations.

**INTRODUCTION**

In the present study, systematic surveys were conducted of different areas of Gilgit, Baltistan to identify the economically important plant parasitic nematodes that damaged the crops. As a result of some plant parasitic nematode populations, recovered from the agricultural fields of the Districts Gilgit and Nager, Gilgit-Baltistan. The analysis of samples yielded a new nematode species *Hemicycliophora pyri* n. sp., and two new reported species *Psilenchus vinciguerrae* (Brzeski, 1991) and *Ditylenchus equilis* (Heyns, 1964) belonging to order Tylenchida as new records for Pakistan.

*Hemicycliophora pyri* n. sp. belongs to the superfamilly Hemicycliophoroidea that received the common name sheath nematodes due to the presence of an additional cuticle or sheath. The superfamily comprises two families, Hemicycliophoridae and Caloosidae. The Hemicycliophoridae contain only a single genus *Hemicycliophora*, with 132 species while Caloosidae comprises two genera *Caloosia* and *Hemicaloosia* with eight and nine species, respectively. They are obligate ectoparasites of plants and inhibit moist soil and aquatic environments. Several *Hemicycliophora* species are known to damage agricultural crops in different countries (Chitambar and Subbotin, 2014).

Members of Hemicycliophoroidea are vermiform, tapering at both extremities but more so posteriorly, rounded to truncate, slightly flattened at the anterior end with an attenuated or filiform tail. Body distinctly, slightly of not recessed immediately posterior to vulva. Two to three labial annuli not modified or separated. Vulva a transverse slit over 0.5 body diameter long, lips either modified and projecting, or not modified and rounded; vagina straight or curved. The male body is generally shorter and more slender than that of female, with a rounded anterior end, offset and tapering posterior end. Spicules arcuate, semi circular, or hook shaped, cloacal lip elongated to form a penial tube. Caudal alae covering less than one third of tail. Tail longer of female.
Two species of the genus *Hemicycliophora* have been reported from Pakistan so far. Akhtar (1962) reported a known species *Hemicycliophora gracilis* Thorne, 1955 from *Agrostis* spp., for the first time from Lahore. A new species *Hemicycliophoraveechi* was described by Maqbool et al. (1986) as a new species from soil around the roots of herbaceous plants and grasses from slopes of hill around the lake of Saiful Muluk, Naran, Pakistan (Maqbool and Shahina, 2001). During the present study *Hemicycliophora pyri* n. sp., has been described from district Gilgit of Gilgit-Baltistan. Many species of the genus *Psilenchus* and *Ditylenchus* were reported earlier including new species viz., *P. khuzdarensis* (Khan et al., 2004) and *D. bilqeesae* (Khan et al., 2004; Zarina and Shahina, 2012; Shahina et al., 2019). *Psilenchus vinciguerrae* (Brzeski, 1991) and *Ditylenchus equilis* (Heyns, 1964) have been reported as new records from district Nager for the first time from Gilgit, Baltistan.

**MATERIALS AND METHODS**

Soil samples were collected from different agricultural fields of districts Gilgit and Nager, Gilgit-Baltistan. Nematodes were extracted from soil samples by using Cobb sieving and decanting technique (Cobb, 1918), followed by modified Baermann funnel method (Baermann, 1917). Nematodes were then gently heat killed, fixed in TAF (Tri-ethanol-amine Formaldehyde) solution (Courtney et al., 1955). From TAF, nematodes were transferred to glycerine (Seinhorst, 1959) and subsequently mounted on microscopic slides for identification (Siddiqi, 2000). Measurements, including Demanian indices (de Man, 1880) and other ratios, were taken with the compound microscope Nikon Eclipse E400, equipped with a drawing tube (Camera Lucida) attached to it for illustrations. Photomicrographs were made with a Nikon DS, Film camera, attached to the same microscope.

*Hemicycliophora pyri* n.sp.  
*(Fig. 1, Table I)*

**Description**

**Female**

Body straight or very slightly curved ventrad. Cuticular sheath closely fitting, sheath annules slightly flattened than body annules. Lip region rounded with two annuli. Labial area with open amphids, labial disc not raised and merged with the lip annules. Labial frame work weakly sclerotized. Stylet slender, slightly curved dorsad. Metenchium 80-88µm long and telenchium 12-17µm long. Stylet knobs well-sloped anteriorly with a distinct cavity posteriorly. Oesophagus typical of the genus. Median oesophageal bulb amalgamated with procorpus 14-18µm wide with a distinct valve. Isthmus short encircled by nerve ring. Nerve ring 144-150µm from anterior end. Hemzonid not clearly seen. Excretory pore situated from two to four annuli posterior to base of oesophagus. Vulval lips elongated. Vulval sheath one to two annuli long, vagina thick walled. Gonad prodelphic, spermatheca round to oval, mostly filled with small round sperm cells. Oocytes arranged in a single row. Anus indistinct. Body posterior to vulva gradually narrows to about last quarter. Tail narrows uniformly, ending in a narrow rounded or sometimes slightly wider rounded terminus, annuli on last distinct and smaller than on rest of body.

**Male**

Not found.

**Type habitat and locality**

The specimens were collected from soil round the roots of apple (*Pyrus malus* L.) and cherry (*Prunus avium*...
L.) from district Gilgit (village Danyour), Gigit-Baltistan, Pakistan.

Table I. Morphometrics of Hemicycliophora pyri n.sp. (Measurement in µm except L).

| Morphological characters | Holotype female | Paratype females (n=15) Mean ±SD (range) |
|--------------------------|----------------|------------------------------------------|
| L                        | 0.99           | 1.02±0.05 (0.90-1.12)                     |
| a                        | 21.5           | 21.01±0.94 (19.8-23.0)                    |
| b                        | 5.8            | 5.9±0.50 (5.1-6.8)                        |
| c                        | 12.6           | 10.35±2.12 (10.7-12.6)                    |
| c’                       | 3.1            | 3.28±0.29 (3.0-4.1)                       |
| V%                       | 85             | 83.05±2.00 (80.1-85.7)                    |
| O                         | 7.5            | 7.5±1.09 (6-9.3)                          |
| DGO                       | 6.5            | 7.3±1.10 (6-9)                            |
| Stylet length            | 98             | 97.83±2.03 (95-100)                       |
| Metenchium length        | 84             | 83.58±3.01 (80-88)                        |
| Telenchium length        | 15.5           | 15.58±1.32 (12-17)                        |
| Stylet knob width        | 6.5            | 7.07±0.82 (6-8)                           |
| Stylet knob height       | 4.5            | 4.54±0.44 (4-5)                           |
| Excretory pore from head end | 178         | 185.90±5.93 (177-196)                     |
| Width at mid body        | 49.2           | 49.27±4.15 (45-58)                        |
| Lip region width         | 16.6           | 16.61±1.07 (15-18)                        |
| Lip region height        | 5.8            | 6.07±0.82 (5-7)                           |
| Annules width            | 5.5            | 5.58±0.49 (5-6)                           |
| Tail length              | 94.6           | 94.61±7.61 (80-108)                       |
| Oesophagus length        | 176.8          | 176.81±3.32 (172-182)                     |
| Vulva-anus distance      | 55             | 58.33±3.59 (54-66)                        |
| R                         | 232            | 238.54±10.44 (231-247)                    |
| Rst                       | 26             | 26.0±1.23 (24-28)                         |
| Roes                     | 44.5           | 44.58±1.97 (41-47)                        |
| Rex                      | 47             | 47.45±1.37 (44-49)                        |
| RV                       | 45             | 44.6±5.37 (36-49)                         |
| R Van                    | 18.1           | 18.1±1.58 (15-20)                         |
| Ran                      | 22             | 24.3±2.3 (21-29)                          |
| VL/VB                    | 3.5            | 3.52±0.33 (3.1-4.0)                       |
| St % L                   | 9.4            | 9.45±0.66 (8.4-11.0)                      |
| Vulval width             | 44.5           | 46.2±2.18 (44-50)                         |

Type material
Holotype slide no NNRC 126/1 and paratypes slide Nos 126/2-6 (14 female) deposited in the National Nematode Collection of NNRC University of Karachi, Karachi 75270, Pakistan. Slide no 126/7 (one female) deposited in the British Nematode Collection at the Food and Environmental Research Agency, Sand Hutton York, England.

Diagnosis and relationship
Hemicycliophora pyri n. sp., is characterized by the presence of broadly rounded, continuous lip region with two indistinct annuli, closely fitting sheath with 231-247 body annuli, stylet 95-100µm long non raised and non-separated labial disc and long gradually tapering tails.

According to the dicotomus key proposed by Chitambar and Subbotin (2014), the new species comes close to H. subaolice. Jairajpuri and Bagri (1973) in body and stylet length and number of body annules (R), but differs from it in labial disc which is not elevated vs elevated; lateral field without anastomoses vs lateral field, marked with anastomoses and there are no delicate scratches outside the lateral field annuli vs outside later field without lateral field annuli may be marked with numerous delicate scratches. It also differs from it by slightly higher number of Rst and Rvan (24-28 vs 19-24 and 15-20 vs 10), respectively.

Psilenchus vinciguerrae Brzeski, 1991
(Fig. 2, Table II)

Fig. 2. Psilenchus vinciguerrae. A, Female whole body; B, Male whole body; C, Vulvar region; D, Head region; E, Oesophageal region; F, Male tail; G, Female tail.
Table II. Morphometric data of *Psilenchus vinciguerrae* and *Ditylenchus equilis*. Measurements (except where indicated) are in µm and in the form mean ± SD (range).

| Morphological characters | *P. vinciguerrae* | *D. equilis* |
|--------------------------|------------------|--------------|
|                         | Female (n=5)     | Male (n=5)   | Female (n=8) |
| L (mm)                   | 0.89 ± 0.10      | 0.79 ± 0.07  | 0.61 ± 0.09  |
|                          | (0.69-0.98)      | (0.69-0.88)  | (0.54-0.84)  |
| a                        | 49.23 ± 1.56     | 42.94 ± 2.67 | 32.38 ± 2.81 |
|                          | (46.6-51.63)     | (39.15-46.2) | (28.77-37.6) |
| b                        | 6.77 ± 0.49      | 6.11 ± 0.29  | 5.19 ± 0.67  |
|                          | (6.02-7.56)      | (5.72-6.50)  | (4.21-6.39)  |
| c                        | 8.04 ± 0.29      | 6.90 ± 0.65  | 12.19 ± 1.51 |
|                          | (7.94-8.76)      | (6.50-8.05)  | (10.37-14.06) |
| c'                       | 8.81 ± 1.05      | 9.23 ± 1.09  | 4.0 ± 0.61   |
|                          | (7.46-10.10)     | (7.81-10.86) | (2.85-4.72)  |
| V %                      | 49.18 ± 0.57     | -             | 83.15 ± 2.82 |
|                          | (48.72-50.16)    | (81.06-90)   |              |
| Stylet                   | 12.8 ± 1.16      | 12.75 ± 0.82 | 8.12 ± 1.53  |
|                          | (11-14)          | (12-14)      | (7.0-12.0)   |
| Excretory pore           | 97.8 ± 6.46      | 96 ± 10.08   | 86.87 ± 12.38 |
|                          | (86-104)         | (79-109)     | (78.0-112.0) |
| Oesophagus               | 132.4 ± 11.05    | -             | 119.37 ± 11.93 |
|                          | (116-148)        | (101-132)    |              |
| Tail length              | 130.4 ± 192      | -             | 50.12 ± 5.10 |
|                          | (122-188)        | (122-188)    | (40.0-60.0)  |
| Maximum body width       | 18.2 ± 1.72      | 18.5 ± 2.17  | 12.75 ± 2.22 |
|                          | (18-20)          | (15-21)      | (11.0-18.0)  |
| Anal body width          | 12.4 ± 2.24      | -             | 19.12 ± 2.36 |
|                          | (10-15)          | (15.0-24.0)  |              |
| Spicule                  | -                | 18.25 ± 1.78 |              |
|                          | (16-20)          | (16-20)      |              |
| Gubernaculum             | -                | 6.75 ± 0.43  |              |
|                          | (6.0-7.0)        | (6.0-7.0)    |              |

**Morphological characterization**

**Female**

After killing with the gentle heat, the body slightly to strongly ventrally curved and open c-shaped. The head continuous, 2-4 µm high. Body has small annules less than 0.1 µm wide but below the anus 0.2-2.5 µm. Lateral field with four lines and 1/2 µm of the total body. Stylet 11-14 µm in length without knobs and lumen pass through median bulb towards basal bulb. Amphids aperture invisible. The length of procrpus 70-80 µm from the head and the length from middle of the median bulb to base of basal bulb 54-60 µm. Isthmus 30-32 µm. The nerve ring 90-110 µm from the anterior region. The distance of excretory pore 86-104 µm from anterior end at the upper side of basal bulb. Basal bulb is flask like 20-22 µm long and oval in shape. Hemizonid anterior to excretory pore. Ovary didelphic with vulval flaps at position of 48-50%. Spermaticca filled and 12-14 µm long filled with sperms. Phasmids below the anus. Tail elongate filiform and 122-188 µm in length.

**Male**

Males are similar to females and slightly smaller in body. Spicules ventrally curved and 16-20 µm in length. Gubernaculum below the specula with 6-7 µm long.

**Remarks**

This species is reported for the first time from Pakistan. Specimens of *Psilenchus vinciguerrae* were collected from the soil around the roots of potato from district Nager (village Hoper and Jaffarabad). Measurement and morphology of this population fit with the original description given by Brzeski, 1991. But the only difference was in body width (a= 37-43 vs 46-51 µm).

**Ditylenchus equilis** Heyns, 1964

(Fig. 3, Table II)

Fig. 3. *Ditylenchus equilis*. A, Female whole body; B, Reproductive region; C, Oesophageal region; D, Anterior region; E, Anus with tail terminus; F, Lateral lines.
Morphological characterization

Female

After killing by gentle heat body slightly arcuate or opens “C” shaped but tail portion carved. Head 2-3µm high and 5-6µm wide, continuous with body. Body with small annuli 0.5-1µm at the vulval position. Stylet 8-12µm long, shaft 5-6 and conus 4-5µm long. DGO near the base of stylet at 1-2µm. Nerve ring 70µm from the head end. The length of isthmus 65-70µm. The length of median bulb 20-24µm and width 4-7µm, oval elongate in shape. Hemizonid near the excretory pore. Excretory pore 78-112µm from anterior end.

Ovary monodelphic. Vulva posterior 81-90%. The distance from vulva to anus 60-62µm. Lateral field with four incisures, 1/3 of the total body length, the maximum body width 15-24µm. The length of post uterine sac 12-14µm. The length of rectum 1µm. Tail length 40-60µm.

Male

Not found.

Remarks

This species is reported for the first time from Pakistan. Specimens of Ditylenchus equalis were collected from soil around the roots of pear district Nager (village Hoper). Measurement and morphology of this population fit with the original description given by Heyns (1964).

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Akhtar, S.A., 1962. Free-living nematodes inhabiting Lahore soils. Agric. Pak., 13: 64-80.

Baermann, G., 1917. Eine einfache Method zur Affindung von Ankylostomum- (Nematoden)-larven in Erdproben. Geneesk. Tijdschrift. Nederl. Vereenig., 57: 131-137.

Brzeski, M.W., 1991. Psilenclus vinceguerrae n. sp. (Nematoda: Tylenchidae). Nematologica, 37: 1-7. https://doi.org/10.1163/187529291X00015

Chitambar, J.J., and Subbotin, S.A., 2014. Systematics of the sheath nematodes of the superfamily Hemicycliophoroidea. In: Nematology monographs and perspectives (eds. D.J. Hunt and R.N. Perry), 10, (Series Editors), Brill, Leiden, Boston, pp. 732. https://doi.org/10.1163/9789004187894

Cobb, N.A., 1918. Estimating the nema population of soil. Agric. Tech. Circ. Dept. Agric., I: 48.

Courtney, W.D., Polley, D. and Miller, V.L., 1955. TAF, an improved fixative in nematodes technique. Pl. Dis. Rep., 39: 570-571.

de Man, J.G., 1880. Die einheimischen, frei in der reinen Erde und im süssen Wasserlebenden Nematoden. Vorläufiger Bericht und descriptive systematischer Theil. Tijdsch. Nederl. Dierk. Vereenig., 5: 1-104.

Heyns, J., 1964. Aphelenchoides helices n. sp. and Ditylenchus equalis n. sp. two new soil inhabiting nematodes. South Afr. J. agric. Sci., 7: 147-150.

Khan, A., Batool, S.K., and Khatoon, N., 2004. Two new species of Tylenceidea, Psilenclus khuzdarnsis n. sp. and Ditylenchus bilqeesae n. sp. (Nemaoda: Tylenceidea). Proc. Parasitol., 38: 81-87.

Jairajpuri, M.S. and Baqri, Q.H., 1973. Four new species of Tylenceida. Nematodes of high altitudes in India. Nematologica, 19-30.

Maqbool, M.A. and Shagina, F., 2001. Systematic and distribution: Biodiversity of nematode fauna in Pakistan. National Nematological Research Centre, University of Karachi, Karachi-75270 Pakistan, pp. 179.

Maqbool, M.A., Shagina, F. and Zarina, B., 1986. Two new species of Hemicycliophorinae Skarbilovich, 1959 (Nematoda: Criconematidae) from Pakistan. Pak. J. Nematol., 4: 43-49.

Seinhorst, J.W., 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica, 4: 67-69. https://doi.org/10.1163/187529259X00381

Shagina, F., Nasira, K., Firoza, K. and Erum, Y.I., 2019. Overview of the nematode fauna of Pakistan. Pak. J. Nematol., 37:171-243. https://doi.org/10.18681/pjn.v37.i02.p171-243

Siddiqi, M.R., 2000. Tylenceida: Parasites of plant and insect, 2nd Edition. CABI Publication, Wallingford, UK, pp. 833.

Thorne, G., 1955. Fifteen new species of the genus Hemicycliophora with an emended description of H. typicae man (Tylenceida: Criconematidae). Proc. helminthol. Soc. Washington, 22: 1-16.

Zarina, B., and Shagina, F., 2012. Annotated bibliography on nematology in Pakistan. 2nd Edition. National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan, pp. 850.