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**TEXTUAL, TABULAR AND COMPUTER-AIDED KEYS TO SPECIES
OF THE GENUS PARAPHELENCHUS MICOLETZKY, 1922
(NEMATODA: APHELENCHIDAE)**

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Textual, tabular and computer-aided keys to species of the genus *Paraphelenchus* are given. Pathogenicity and ecology, bionomics and nematode associations with fungi are reviewed. Diagnostic characters of species are listed and discussed.

Key words: *Paraphelenchus*, Aphelenchidae, nematode-fungi associations, plant parasitic nematodes, textual key, tabular key, computer-aided identification, PICKEY, Pankhurst.

**ТЕКСТОВОЙ, ТАБЛИЧНЫЙ И КОМПЬЮТЕРНЫЙ КЛЮЧИ
К ВИДАМ РОДА PARAPHELENCHUS MICOLETZKY, 1922
(NEMATODA: APHELENCHIDAE)**

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Разработаны текстовой, табличный и компьютерный ключи к видам рода *Paraphelenchus*. Сделан обзор данных по патогенности, экологии и образу жизни видов рода, ассоциациям этих нематод с патогенными грибами. Перечислены признаки видов, проанализировано их диагностическое значение.

Ключевые слова: *Paraphelenchus*, Aphelenchidae, фитонематоды, ассоциация нематода-гриб, компьютерная идентификация, текстовый ключ, табличный ключ, PICKEY, Pankhurst.

In the Nematoda classification the genus *Paraphelenchus* belongs to the family Aphelenchidae Fuchs, 1937 (De Ley, Blaxter, 2002; Hunt, 2008) The brief:

diagnosis of the genus: Aphelenchidae, 0.4—1.1 mm long; 4—12 incisures of lateral field; pharyngeal glands enclosed in abutting basal bulb gradually continuing to isthmus, oval median bulb massive with prominent cuticular valve and three duct openings, the dorsal gland duct orifice opens just anterior to valve; the subventral gland ducts open immediately posterior to the valve. Female genital system monoprodelfic, a tube-shaped fertilization chamber, combining functions of spermatheca and crustaformera, is situated between oviduct and uterus. Vulva transversal slit-like surrounded by strong cuticular groove. Female tail subcylindroid to hemispherical, tail tip usually bears single or 1—2 pairs of mucronate papilla, sometimes devoid of mucrons and smooth. Male tail with four to five pairs of papillae; bursa alae absent or reduced to narrow bands occupying $\frac{3}{4}$ of tail length, but not surrounding a tail tip.

Paraphelenchus differs from the most close genus *Aphelenchus*, the only other genus in Aphelenchidae, in the shape of vulva (transversal slit-like vs circular in *Aphelenchus*) and the gland part of pharynx enclosed in a basal bulb (the pharyngeal glands form a dorsal lobe in *Aphelenchus*). Morphological differences of these genera were discussed by Zeidan and Geraert (1990) on example of *P. deckeri*, the species combined characters of previous diagnoses of *Paraphelenchus* and *Aphelenchus*.

Baranovskaya (1984) has published the first review of the genus with illustrated species descriptions; she gave a key to species, but with logic contradictions in characters, numbers and contents of theses of the key. Hunt (1993, 2008) developed checklists of the valid species of the *Paraphelenchus* with their synonyms. Carta et al. (2011) confirmed Hunt's last list of 23 valid species, made a detailed re-descriptions of *Paraphelenchus acontioides* and *P. intermedius*, and the morphometric table of valid *Paraphelenchus* spp. to help in the species identification. Unfortunately, because of not exact translation of the Russian species descriptions, the table includes several erroneous data. The molecular study of *P. acontioides* was carried out and the phylogenetic position of the genus according to 18S and 28S genes confirmed its grouping in one clade with *Aphelenchus*. An productive attempt to use the oviduct and the fertilization chamber structures as the diagnostic characters of species, was done (Carta et al., 2011).

These nematodes are the common pests of commercial mushrooms plantations (Goodey, 1960, 2008; Hooper, 1962; Baranovskaya, 1984; Hunt, 1993). The paraphelenchs inhabit soil and rotten plant tissues, as well as dead wood, mostly of deciduous trees. The life cycle usually includes the single mycophagous generation, but many *Paraphelenchus* spp. associated with roots and aerial parts of plants thus it is possible that the mycophagous and plant parasitic generations alternate irregularly (Ryss, 2009). Paraphelenchs may easily multiplied in vitro in cultures of fungi *Botrytis cinerea* (Ryss, Chernetskaya, 2010), *Pyrenochaeta terrestris* (Pillai, Taylor, 1967; Carta et al., 2011), *Fusarium solani*, *F. moniliforme*, *F. cf. torulosum* (Pillai, Taylor, 1967; Baynes et al., 2012). *Paraphelenchus acontioides* is associated with fungi *Fusarium solani*, *Alternaria solani*, *Rhizoctonia solani* (Pillai, Taylor 1966). Inside of tissues of cheat grass *Bromus tectorum* the mutualistic association of *Paraphelenchus acontioides* and plant endophytic fungus *Fusarium cf. torulosum* was revealed: the nematodes preferred to multiply on this strain compared with other fungi, and the relative abundance of *F. cf. torulosum* within the endophyte community was increa-

sed by the nematode in experimental plants. The host plant growth was unaffected by nematode-fungus mutualistic association (Baynes et al., 2012)

The anhydrobiosis of *Paraphelenchus* sp. was detected in the desert soil in Nevada, USA; the dormant specimens had coiled body shape and they belonged to different stages of individual development (Freckman et al., 1977). I did extraction of *Paraphelenchus pseudoparietinus*, *P. zae* и *Paraphelenchus* sp. from dry stems of *Avena sativa* и *Triticum vulgare* in Chelyabinsk region (unpublished data), as well as *P. myceliophthorus* from dry wood of *Betula pendula* (Ryss, Chernetskaya, 2010). Neher et al. (1999) advanced the enclosure hypothesis that during desiccation, soil generates pockets of moisture enclosing *Paraphelenchus* sp. and other nematodes and their food in relatively high concentrations creating patches of activity separated by larger areas of inactivity. In field experiments it was demonstrated that *Paraphelenchus* spp. preferred relatively low temperatures and mean humidity (Bakonyi, Nagy, 2000), and together with other fungal-feeding nematodes they may be used as bioindicators of the composting process maturity (Steel et al., 2013).

Paraphelenchus spp. are detected in all continents (Baranovskaya, 1984). They are found even in Antarctica (Ryss, 2013). Besides of fungi, they are associated with plants of the following families: Araliaceae, Cucurbitaceae, Dioscoreaceae, Fabaceae, Poaceae, Rosaceae, Solanaceae (data are collected from original species descriptions).

According to Hunt (2008) checklist, the genus *Paraphelenchus* includes 23 species (valid names only): *Paraphelenchus pseudoparietinus* Micoletzky, 1922 (type species); *P. acontoides* Taylor & Pillai, 1967; *P. alii* Fortuner, 1985; *P. amblyurus* Steiner, 1934; *P. basili* Das, 1960; *P. batavicus* Filipjev, 1934; *P. crenatus* Das & Singh, 1968; *P. deckeri* (Zeidan, Geraert, 1992) Andrassy, 2007; *P. fidicaudatus* Eroshenko, 1966; *P. goodeyi* Tandon & Singh, 1970; *P. heterolineatus* Haque, 1967; *P. intermedius* Thorne & Malek, 1968; *P. myceliophthorus* J. B. Goodey, 1958; *P. obscurus* Muchina, 1988; *P. octolineatus* Schavrov, 1968; *P. orientalis* Muchina, 1988; *P. paramonovi* Haque, 1967; *P. porrectus* Eroshenko, 1966; *P. sacchari* Husain & Khan, 1967; *P. tritici* Baranovskaya, 1958; *P. ussuriensis* Eroshenko, 1966; *P. zae* Romaniko, 1968; *P. zicsii* Andrassy, 1989.

The goal of this paper is to give the efficient keys to species, mostly based on the published species descriptions.

MATERIAL AND METHODS

Besides of the literature data, the author collected and studied four species: *P. pseudoparietinus* (2013, Russia, Leningrad region, *Phaseolus vulgaris*); *P. myceliophthorus* (2006, Russia, Karelia, Chupa, White Sea Biological Station of St. Petersburg State University, *Betula pendula*, dead wood); *P. tritici* (2011, Russia, Moscow region, *Triticum vulgare*); *P. zae* (2011, Russia, Chelyabinsk region, *Zea mays*).

Characters and their states are collected from original descriptions as was done before for *Bursaphelenchus* spp. (Ryss et al., 2005). The morphometric characters are quantified into the efficient set of states using the algorithm described in Ryss, 2008.

TEXTUAL KEY TO SPECIES OF THE GENUS *PARAPHELENCHUS*

1. Rectum strongly curved, its length more than 3 anal body diameters, cephalic region offset, very high *P. porrectus*.
- Rectum straight, its length not more than 3 anal body diameters, cephalic region continuous or, if offset, low 2.
2. Lateral field at vulval region with 10 or more incisures 3.
- Lateral field at vulval region with 8 or less incisures 5.
3. Postvulval uterine sac longer than 60 % of vulva-anus distance *P. batavicus*.
- Postvulval uterine sac shorter than 50 % of vulva-anus distance 4.
4. Tail cylindroid and devoid of mucro, stylet 15 μm or longer . . . *P. deckeri*.
- Tail conically rounded, with short and thin ventral mucro, stylet 14 μm or shorter *P. pseudoparietinus*.
5. Tail tip furca-shaped: thick mucrons are on ventral and dorsal sides
. *P. fidicaudatus*.
- Tail tip not furca-shaped: devoid of mucrons, or with single mucron, or with a pair of mucrons on ventral side. 6.
6. Tail tip with single mucron 7.
- Tail tip smooth or with a pair of mucrons 15.
7. Lateral field at vulval region with 4 incisures 8.
- Lateral field at vulval region with 6—8 incisures 10.
8. Index a more than 30, postvulval uterine sac 2.5 of the vulval diameter or longer *P. sacchari*.
- Index a less than 30, postvulval uterine sac 2.0 of the vulval diameter or shorter 9
9. Mucro conical with broad base, ventral; rectum length 2 anal diameters *P. crenatus*.
- Mucro needle-like, central; rectum length 1 anal diameter *P. basili*.
10. Lateral field at vulval region with 8 incisures 11.
- Lateral field at vulval region with 6 incisures 12.
11. Postvulval uterine sac less than 50 % of vulva-anus distance, excretory pore at nerve ring, stylet knobs distinct *P. acontioides*.
- Postvulval uterine sac longer than 50 % of vulva-anus distance, excretory pore posterior to nerve ring, stylet knobs absent *P. heterolineatus*.
12. Tail short, c'-value 1.5 *P. paramonovi*.
- Tail longer, c' value more than 2 13.
13. Excretory pore at anterior border of nerve ring, cephalic region offset
. *P. amblyurus*.
- Excretory pore posterior to nerve ring, cephalic region continuous 14.
14. Tail conical, c = 22 or less, stylet 14 μm or less *P. alii*.
- Tail subcylindroid, c = 23 or more, stylet more than 15 μm
. *P. intermedius*.
15. Tail tip with prominent ventral pair of thick conical mucrons 16.
- Tail tip devoid of papilla or with a pair of pore-like papilla 17.
16. Stylet 12 μm , rectum 1 anal body diameter, head offset *P. goodeyi*.
- Stylet 15 μm or more, rectum 2 anal body diameters, head continuous
. *P. myceliophthorus*.
17. Lateral field with 8 incisures at vulval region 18.
- Lateral field with 6 incisures at vulval region 19.

18. Tail cylindroid, slightly curved ventrally, c'-value 2 or more, excretory pore anterior to nerve ring *P. octolineatus*.
 -- Tail hemispherical, annulated at tip, c'-value 1.5 or less, excretory pore posterior to nerve ring *P. orientalis*.
 19. Tail tip with 3 humps, c'-value 4 or more, rectum more than 3 anal diameters long *P. zicsii*.
 -- Tail tip smooth, c'-value 2.5 or less, rectum less than 3 anal diameters long 20.
 20. Postvulval uterine sac less than 35 % of vulva-anus distance *P. ussuriensis*.
 -- Postvulval uterine sac more than 40 % of the vulva-anus distance 21.
 21. Stylet 16 μm or longer *P. zaeae*.
 -- Stylet 13 μm or shorter 22.
 22. Postvulval uterine sac less than 50 % of vulva-anus distance, excretory pore at posterior border of nerve ring, c'-value 1.7 or less *P. obscurus*.
 -- Postvulval uterine sac more than 50 % of vulva-anus distance, excretory pore far posterior to nerve ring, c'-value 2 or more *P. tritici*.

**LIST OF CHARACTERS USED FOR THE TABULAR KEY
OF PARAPHELENCHUS**

Character 1. Number of incisures in lateral field at vulval region:

- 1: 4 incisures;
- 2: 6 incisures;
- 3: 8 incisures;
- 4: 10 or more incisures.

Character 2. Tail tip:

- 1: Tail tip with single central mucron;
- 2: Mucron single, ventral;
- 3: Tail tip furca-shaped in lateral view: mucrons form ventral and dorsal processes;
- 4: Mucron absent, tail tip smooth.

Character 3. Excretory pore position:

- 1: Anterior to nerve ring;
- 2: At nerve ring;
- 3: Posterior to nerve ring;
- 4: Far posterior to nerve ring.

Character 4. c'-value of female:

- 1: 2 or more;
- 2: 1.5 or less.

Character 5. Ratio: Postuterine sac length to vulva-anus distance:

- 1: Less than 33 %;
- 2: 33—50 %;
- 3: More than 50 %.

Tabular key to species of the genus *Paraphelenchus*

Species/Characters	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12	C13
<i>P. acontioides</i>	3	2	3	1	(12)	2	1	1	2	2	2	2	1
<i>P. alii</i>	2	2	3	1	1	2	1	1	2	2	1	1	2
<i>P. amblyurus</i>	2	2	1	1	2	1	2	2	1	2	2	1	2
<i>P. basili</i>	1	1	3	1	1	2	2	1	1	2	2	1	2
<i>P. batavicus</i>	1	4	2	1	3	3	2	1	1	1	3	1	2
<i>P. crenatus</i>	1	2	3	1	1	2	1	2	2	2	2	1	2
<i>P. deckeri</i>	4	4	3	1	2	1	2	2	2	1	2	2	2
<i>P. fidicaudatus</i>	3	3	2	1	2	2	1	2	1	1	2	2	2
<i>P. goodeyi</i>	2	2	2	1	3	3	1	1	1	3	2	1	2
<i>P. heterolineatus</i>	3	2	3	1	2	2	1	1	2	3	2	2	2
<i>P. intermedius</i>	(12)	2	3	1	2	1	1	2	2	2	2	2	2
<i>P. myceliophthorus</i>	2	2	3	1	3	1	3	2	2	2	3	1	2
<i>P. obscurus</i>	2	4	2	2	2	2	2	2	2	(12)	2	1	2
<i>P. octolineatus</i>	3	4	1	1	3	2	2	1	2	2	3	1	1
<i>P. orientalis</i>	3	4	3	2	2	2	3	1	2	2	2	1	2
<i>P. paramonovi</i>	2	1	4	2	2	2	1	1	1	3	1	1	2
<i>P. porrectus</i>	3	1	3	1	2	2	1	3	1	2	2	2	2
<i>P. pseudoparietinus</i>	4	2	2	1	2	2	1	1	2	2	2	1	1
<i>P. sacchari</i>	1	1	4	1	2	2	1	1	1	(12)	3	1	2
<i>P. tritici</i>	2	4	4	1	3	3	2	1	1	2	2	1	2
<i>P. ussuriensis</i>	2	4	1	1	1	2	2	2	1	(12)	2	2	2
<i>P. zae</i>	2	4	1	1	3	1	2	1	1	2	3	1	2
<i>P. zicsii</i>	2	4	3	1	2	2	2	2	2	1	2	1	2

Note. Columns C01—C13 are the characters from 1 to 13. The numbers of characters and character states in cells correspond to those listed in the List of characters. Sets of states for polymorphic characters are given in brackets.

Character 6. Stylet length:

1: Longer than 16 μm ;

2: 13—16 μm ;

3: Shorter than 13 μm .

Character 7. Tail shape:

1: Conoid;

2: Cylindroid;

3: Rounded: length is almost equal to anal body diameter.

Character 8. Rectum:

1: 1—1.5 of anal body diameter, straight;

2: 2—3 of anal body diameter, straight;

3: More than 3 of anal body diameters, arched.

Character 9. Cephalic region:

- 1: Offset;
- 2: Continuous.

Character 10. a-value of female:

- 1: More than 34;
- 2: 23—34;
- 3: Less than 23.

Character 11. Ratio: Postuterine sac length to vulval body diameter:

- 1: Less than 1.7 vulval diameters;
- 2: 1.7—3 vulval diameters;
- 3: More than 3 vulval diameters.

Character 12. Sperm in female genital system:

- 1: Present;
- 2 : Absent.

Character 13. Stylet knobs:

- 1: Distinct;
- 2: Absent or indistinct.

**PICTORIAL MULTI-ENTRY POLYTOMOUS KEY TO THE
GENUS *PARAPHELENCHUS* IN THE SOFTWARE PICKEY FOR WIN**

The taxonomic identification software PICKEY8 WIN2x (Dianov, Lobanov, 1997, 2005) was already used to construct the dialogue pictorial keys of different nematode genera (Ryss, 1997; Ryss et al., 2008). To reach the identification for the minimum number of steps, the built-in algorithm automatically selects the efficient characters dividing the set of species into approximately equal (in numbers) species groups; at each step the characters are ranged according to their diagnostic value in the screen (figure, *A*). It is the Pankhurst (1978, 1991) approach for the computer-aided identification, which is an alternative to the Linnean keys (as the text key above); the latter are based on the selection of the unique species characters, thus splitting off one species at each identification step, providing the reliable but long identification with maximum number of steps.

After selection of a character (an illustration of a character appears in the right side of the screen, Figure, *A*), a user select the appropriate illustrated character state in the second screen (figure, *B*), thus decreasing the number of species in the current set, and finishing the step of identification. After several steps, a user reaches an identification of species with its scrolled description and illustration (figure, *C*), which may be printed as the PDF file (figure, *E*). At each step it is possible to look at the protocol of identification, to check characters and their states used in the identification path (figure, *D*). The number of states of the morphometric character have to be minimized to the calculated statistic aggregations, because the artificial increase of the states may lead to erroneous increase of diagnostic value of such character. To quantify the morphometric characters states it is necessary to use the special algorithm (Ryss, 2008).

a

b

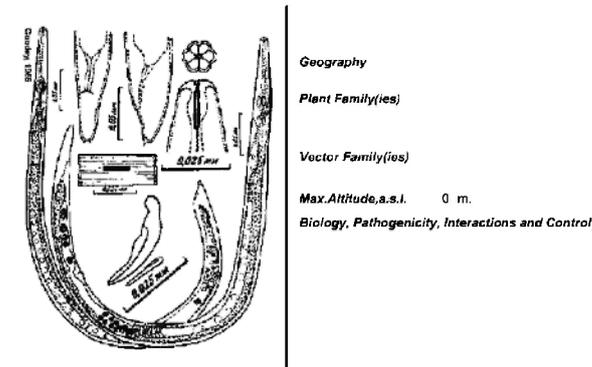
c

d

Protocol of the Identification	
Step	Used Character
1	Number of incisures in lateral field at vulva
2	Mucro position
3	Ratio: Postuterine sac length to vulval body dia
4	Value of female

e

P. mycellophorus



Geography
Plant Family(ies)
Vector Family(ies)
Max. Altitude, a.s.l. 0 m.
Biology, Pathogenicity, Interactions and Control

P. mycellophorus J.B. Goodey, 1958

Stylet 16 μm; L= 699-790 μm; a= 22-34; b= 4.1-6.6; c= 13-24; c'= 1; V= 71-78; PUS/VD= 4; PUS/VA= 66%.

Cephalic region continuous, low. Stylet knobs indistinct. Deirids at excretory pore. Median bulb oval. Excretory pore at and posterior to nerve ring.

Female. Lateral field 6 incisures at vulva and 4 incisures at anus. Oocytes in 1 or 2 rows. Sperm in female genital tube: rounded large in postuterine sac and in anterior genital tube. Anal flap. Rectum 2 ana body diameters. Tail short conoid-cylindroid. Tail tip broadly rounded with a pair of ventral mucros.

Male male spicules along arc = 28 μm; gubernaculum = ?; Male tail papillae: P2 preanal at mid-spicule, P3 - at mid-tail, P4 - near tail tip, subventral; P5 absent.

Distribution: UK England West Chillington, West Sussex (type location), Russia: Moscow region, Far East Central Asia: Uzbekistan, Kyrgyzstan

Plant Host:

Fungus: Agaricus hortensis, fungal compost

REFERENCE

Goodey, J.B. 1958. *Paraphelenchus mycellophorus* n. sp. (Nematoda: Aphelenchidae). *Nematologica*, 3: 1-5.

Identification with the *Paraphelenchus* computerized key in the PICKEY8 for WIN program.

a — first screen with the characters ranged according to diagnostic values, right: illustration of a selected character; *b* — second screen: a selection of a character state; *c* — final screen of identification with a scrolled species description and its illustration; *d* — protocol of identification steps; *e* — species description printed by the software as a PDF file.

The *Paraphelenchus* key has the following parameters: 23 species, 24 morphological characters (14 qualitative and 10 morphometric ones) and 2 non-morphological characters (the distribution regions and hosts). Number of states for morphological characters vary from 2 to 4. Mean identification path varies from 3 to 4 steps (figure, *D*). For analytical purposes, the first step (screen) of identification is the most important (figure, *A*). The sequence of the morphological characters at the first step, in the decreasing Pankhurst's diagnostic value, is as follows: tail tip mucron shape; c-value of female; number of incisures of lateral field at vulval region; b-value of female; a-value of female; sperm shape in female genital system; mucron position; excretory pore position; V-value of female; male spicule length along arc; body length; stylet length; ratio: post-uterine sac length to vulva-anus distance; ratio: post-uterine sac length to vulval body diameter; tail tip shape (except mucron); tail shape; ratio: rectum to anal body diameter; c'-value of female; cephalic region shape; stylet knobs (basal swellings); median bulb shape; tail curvature; tail post-anal contraction; male tail papilla pattern.

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