Taxonomy, evolution and phylogeny of the genus *Radopholus* (didelphic species) according to morphological data, with a key to species (Nematoda: Tylenchida)

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Ryss, A.Y. 2003. Taxonomy, evolution and phylogeny of the genus *Radopholus* (didelphic species) according to morphological data, with a key to species (Nematoda: Tylenchida). *Zoosystematica Rossica*, **11**(2), 2002: 243-256.

Present taxonomic status and synonymy of the didelphic species of the genus *Radopholus* Thorne are considered. A monoentry polytomous key to *Radopholus* is presented, as well as the rows of characters and a matrix of species and characters which were used for the phylogenetic analysis in the PAUP package. Phylogenetic tree of the genus includes four main species groups (clusters); all the species of the Indo-Asian origin (not inhabiting Australia and Oceania) form a separate monophyletic branch. Basing on the frequency analysis of the character states among the species of the genus, the conclusions on the main morphological adaptations of *Radopholus* are drawn.

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At present, there are two different views on the taxonomy and species composition of *Radopholus*. The first point of view (Siddiqi, 1986, 2000; Ryss, 1988) is that the genus includes only didelphic species (the type species: *Radopholus similis* Cobb). In females of didephic species, the genital system consists of two equal-sized opposite branches (Fig. 9.0). The close genus *Radopholoides* de Guiran (the type species: *Radopholoides littoralis* de Guiran) is characterized by the monodelphic (prodelphic) females. Posterior genital branch in monodelphic females is reduced and non-functional. According to the second view (Fortuner et al., 1987-1988), both didephic and monodelphic species are considered in the genus *Radopholus*. The latter opinion based on the absence of other significant differences between *Radopholus* and *Radopholoides* except the alternative: didelphic or monodelphic genital system in females. The author of the present paper shares the first point of view.

There are three pairs of genera in the family Pratylenchidae (*Zygotylenchus–Pratylenchus*, *Pratylenchoides–Apratylenchoides*, *Radopholus–Radopholoides*), in which the main difference is the number of female genital branches. The character is very convenient for classification. If one considers the genital system structure as non-significant for the generic division, it is to be applied to the family Pratylenchidae on the whole. However, this standpoint suggests the comprehensive revision of the family involving new characters. Because of this, here is accepted a more conservative view on *Radopholus* and *Radophoides* as separate genera. Reduction of the posterior genital branch is a secondary feature; therefore, this view cannot influence conclusions on phylogenetic relations between didelphic (i.e., the most primitive) species.

Material and the basic information sources

The collections of the following institutions were used in research: Zoological Institute, Russian Academy of Sciences; Institute for Nematology and Vertebrates, Münster, Germany (German National Collection of Nematodes); Manaaki Whenua – Landcare Research, Auckland, New Zealand (Collection of Nematodes of New Zealand and Australia).

Material for investigation was loaned also by Mr. L. Waeyenberge and Dr. M. Moens (Centrum Landbowkundig Onderzoek, Merelbeke, Belgium). The database of characters of the *Radopholus* species in MS Excel, MS Access and in the dbf format (Ryss, 1997a, 1997b, 1998, 2001) as well as the numerous literature sources cited in the list of species below, have been used to create the identification key and study the phylogenetic relations among the species of the genus.

List of didelphic species of the genus *Radopholus* (including synonymy)

Radopholus Thorne, 1949

Thorne, 1949: 53. – *Neoradopholus* Khan & Shakil, 1973: 16-17.

Type species: *Tylenchus similis* Cobb, 1893, by original designation.

Radopholus similis (Cobb, 1893). Cobb, 1893: 301, pl. VII (*Tylenchus*); Goodey, 1932: 112-115, figs 41-42 (*Angullulina*); Micoletzky, 1922: 543 [*Tylenchus* (*Chitinotylenchus*)]; Filipjev, 1934: 142 [*Tylenchus* (*Tylenchorhynchus*)]; Filipjev, 1936a: 543 (*Rotylenchus*); Thorne, 1949: 53-54, fig. 4 (a-f). – *granulosus* Cobb, 1893: 300-301 (*Tylenchus*); Goodey, 1932: 166 (*Angullulina*); Filipjev, 1934: 152 [*Tylenchus* (*Bitylenchus*)]; Filipjev, 1936b: 81 (*Tetylenchus*). – *acutocaudatus* Zimmerman, 1898: 42-43, fig. 12 (*Tylenchus*). – *biformis* Cobb, 1909: 63-66 (*Tylenchus*). – *citrophilus* Huettel et al., 1984: 32-34, tables 1-2; Siddiqi, 1986: 300 (*similis* ssp. *citrophilus*).

Radopholus brevicaudatus Colbran, 1970. Colbran, 1970: 443, fig. 3 (f-k).

Radopholus bridgei Siddiqi & Hahn, 1995. Siddiqi & Hahn, 1995: fig. 1 (a-j).

Radopholus capitatus Colbran, 1970. Colbran, 1970: 454-455, fig. 10 (g-l).

Radopholus citri Machon & Bridge, 1996. Machon & Bridge, 1996: 128-130, fig. 1, tables 1-3.

Radopholus clarus Colbran, 1970. Colbran, 1970: 451, fig. 8.

Radopholus colbrani Kumar, 1980. Kumar, 1980: 43-46, fig. 1 (a-h).

Radopholus crenatus Colbran, 1970. Colbran, 1970: 439-441, fig. 2.

Radopholus inaequalis Sauer, 1958. Sauer, 1958: 100-103, fig. 2; Khan & Shakil, 1973: 16-17 (*Neoradopholus*).

Radopholus inanis Colbran, 1970. Colbran, 1970: 449-451, fig. 7.

Radopholus intermedius Colbran, 1970. Colbran, 1970: 441-443, fig. 3 (a-e).

Radopholus kahikateae Ryss & Wouts, 1997. Ryss & Wouts, 1997: 4-9, fig. 3.

Radopholus magniglans Sher, 1968. Sher, 1968: 229, fig. 4.

Radopholus megadorus Colbran, 1970. Colbran, 1970: 447-449, fig. 6.

Radopholus musicola Stanton, Mundo-Ocampo, Baldwin & Kaplan, 2001. – Stanton et al., 2001: 690-696, figs 1-3, tab. 1.

Radopholus nativus Sher, 1968. – Sher, 1968: 233, fig. 6 (a-f). – *ferax* Colbran, 1970: 445-447, fig. 5. – *neosimilis* (non Sauer, 1958): Siddiqi, 1986: 300.

Radopholus nelsonensis Ryss & Wouts, 1997. Ryss & Wouts, 1997: 2-4, figs 1-2.

Radopholus neosimilis Sauer, 1958. Sauer, 1958: 103-106, fig. 3; Khan & Shakil, 1973: 16-17 (*Neoradopholus*).

Radopholus rectus Colbran, 1970. Colbran, 1970: 439, fig. 1.

Radopholus rotundisemenus Sher, 1968. Sher, 1968: 231, fig. 5 (g-l).

Radopholus serratus Colbran, 1970. Colbran, 1970: 445, fig. 4.

Radopholus trilineatus Sher, 1968. Sher, 1968: 229-231, fig. 5 (a-f).

Radopholus vacuus Colbran, 1970. Colbran, 1970: 454, fig. 9.

Radopholus vangundyi Sher, 1968. Sher, 1968: 227, fig. 3.

Radopholus vertexplanus Sher, 1968. Sher, 1968: 231-233, fig. 5 (m-r).

Radopholus sp. n., population from Tasmania. This undescribed new species is also included in the investigation. It differs from the other *Radopholus* species but was not described as a new species in the earlier paper (Ryss & Wouts, 1997), because Dr. Wouts was not sure in the species status of the Tasmanian population. I include the population here as a separate taxon (both in the matrix and the phylogenetic study).

Achlysiella williamsi Siddiqi, 1964. Siddiqi, 1964: 207-208, fig. 7 (*Radopholus*); Hunt et al., 1989: 401-407, figs 1-2 (*Achlysiella*).

A. williamsi was originally described as *Radopholus williamsi*; this species has all morphological and biological features of the genus *Radopholus*. The only difference is that the inseminated female gradually becomes obese inside plant roots. The species is included in the analysis because of the evident phylogenetic closeness to *Radopholus*.

Species transferred to other genera of the family Pratylenchidae

Radopholus megalobatus (Bernard, 1984) Ryss, 1988, now *Pratylenchoides megalobatus* Bernard, 1984 (the name used by Bernard in the original description of the species).

Radopholus nigeriensis Sher, 1968, now *Zygradus nigeriensis* (Sher, 1968) Siddiqi, 1991.

Nomen nudum

Radopholus paludosus Whitlock, 1957

Species inquirenda

Radopholus cavenessi Egunjobi, 1968

During the last years several keys to *Radopholus* were published, both traditional ones (Ryss, 1988; Ryss & Wouts, 1997) and computerized polytomous (Ryss, 1997a, 1997b). Here below a monoentry dichotomous key is given.

Key to the didelphic species of the genus *Radopholus* (mainly to females)

1. Spermatheca empty 2
– Spermatheca filled with sperm 3
2. Cephalic region dome-shaped . . . . **R. nativus**
– Cephalic region flattened . . . . **R. vertexplanus**
3. Spermatheca filled with round sperm cells 4
– Spermatheca filled with rod-like sperm cells 12
4. Tail terminus annulated 5
– Tail terminus smooth 10
5. One stylet knob significantly larger than others . . . . **R. citri**
– All stylet knobs equal in size 6
6. Lateral field with 5 or 6 incisures . . . . **R. rotundisemenus**
– Lateral field with 4 incisures 7
7. Tail terminus broadly rounded . . . . **R. rectus**
– Tail terminus narrow to pointed 8
8. Male spicule 20 µm or longer . . . . **R. kahikatea**
– Male spicule 16 µm or shorter 9

9. Tail 75 µm or longer . . . . **R. colbrani**
– Tail 45 µm or shorter . . . . **R. serratus**
10. Anterior spermatheca significantly larger than posterior one . . . . **R. intermedius**
– Anterior and posterior spermathecae of equal size 11
11. Cephalic region with 2 annuli; lateral field between phasmid and tail tip with 3 incisures . . . . . . **R. vacuus**
– Cephalic region with 3 annuli; lateral field between phasmid and tail tip with 4 incisures . . . . . . **R. inanis**
12. Anterior spermatheca significantly larger than posterior one . . . . **R. magniglans**
– Anterior and posterior spermathecae of equal size 13
13. One stylet knob significantly larger than others . . 14
– All stylet knobs equal-sized 15
14. Lateral field between phasmid and tail tip with 4 incisures; postrectal sac present . . . . **R. inaequalis**
– Lateral field between phasmid and tail tip with 3 incisures; postrectal sac absent . . . .**R. citri**
15. Tail tip smooth 16
– Tail tip annulated 19
16. Central band of lateral field distinctly narrower than outer ones 17
– All bands of the lateral field equal in width 18
17. Tail tip rounded; tail length 31 µm or less (1.5 stylet lengths or less), index *b* 5.2 or less . . . . **R. brevicaudatus**
– Tail tip narrowly conical to spike-like; tail length 57 µm or more (3.7 stylet lengths or more), index *b* 6.9 or more . . . . **R. musicola**
18. Body length 500 µm and more; stylet 17 µm and longer; *c′* = 3.0 or more; egg-laying female worm-like . . . . **R. similis**
– Body length 450 µm or less; stylet 16 µm or shorter; *c′* = 2.7 or less; adult female becoming swollen at egg-laying stage . . . . **Achlysiella williamsi**
19. Lateral field with 3 incisures . . . . **R. trilineatus**
– Lateral field with 4 incisures or more 20
20. Central band of lateral field distinctly narrower than outer ones 21
– All bands of the lateral field equal in width 22
21. Tail broadly rounded; cephalic region smooth, without distinct annuli . . . . **R. megadorus**
– Tail narrow, conical; cephalic region with 3-4 distinct annuli . . . . **R. bridgei**
22. Cephalic region smooth . . . . **R. crenatus**
– Cephalic region annulated 23
23. Cephalic region with 2 annuli . . . . **R. capitatus**
– Cephalic region with 3 annuli or more. 24
24. Cephalic region flattened . . . . **R. neosimilis**
– Cephalic region dome-shaped 25
25. Tail broad, cylindrical 26
– Tail conical 28
26. Lateral field with 3 incisures between phasmid and tail tip . . . . **R. nativus**
– Lateral field with 4 incisures between phasmid and tail tip 27
27. Ratio of tail length to anal body width 2.7 or more; body of egg-laying female slender . . . . **R. clarus**
– Ratio of tail length to anal body width 2.7 or more; body of egg-laying female thick, slightly swollen . . . . **R. nelsoni**
28. Lateral field with 4 incisures between phasmid and tail tip . . . . **R. vangundyi**
– Lateral field with 3 incisures between phasmid and tail tip . . . . **R. similis**

Evolutionary changes within the family Pratylenchidae are the adaptations to parasitism inside plant roots: the body length is decreased, the stylet is shortened and the cephalic region becomes more flattened (Paramonov, 1970; Siddiqi, 1980). Here below the rows of character states are given according to the general tendencies of specialization to the parasitism.

ROWS OF MORPHOLOGICAL CHARACTERS USED IN PHYLOGENETIC ANALYSIS WITH NOTES ON THEIR EVOLUTION (Figs 1-21)

Character 1. Number of cephalic annuli:

0, six annuli;
1, five annuli;
2, four annuli;
3, three annuli;
4, two annuli;
5, cephalic region smooth, devoid of annulation.

Cephalic region of the primitive Tylenchida (families Psilenchidae, Tylenchidae, Belonolaimidae, Dolichodoridae) is high. Within Pratylenchidae the cephalic region lowers. It leads to decrease in number of the cephalic annuli (see comments to character 12).

Character 2. Number of incisures in lateral field (oligomerization):

0, four;
1, three.

Incisures of the lateral field border the bands of lateral field. The initial structure of lateral field in the order Tylenchida and the subclass Secernentea generally includes three bands and four incisures (Lorenzen, 1981; Ryss, 1993). Within *Radopholus* both decrease in number of incisures to three as well as increase to six, took place. Because of this, the character changes are divided here in two rows, the oligomerization (character 2) and polymerization (character 3).

Character 3. Number of incisures in lateral field (polymerization):

0, four;
1, five;
2, six.

See comments to character 2.

Character 4. Number of lateral field incisures between phasmid and tail terminus:

0, four;
1, three.

Character 5. Central band of lateral field:

0, equal to outer bands in width;
1, narrower than outer bands.

In lower Tylenchida (e.g., family Tylenchidae) the lateral field bands do not differ in width.

Character 6. Postrectal sac:
0, absent;
1, present.

The postrectal sac is a unique feature of *Radopholus*; its presence is considered here as a secondary state.

Character 7. Cephalic region:
0, dome-shaped;
1, flattened.

See comments to character 1.

Character 8. Sperm cells in spermatheca:
0, round;
1, rod-like;
2, absent.

Rod-like shape of sperm cells is the unique feature of *Radopholus* considered here as an advanced state. In amphimictic species sperm is usually present in the female genital tube inside a special sac-like structure, the spermatheca. Spermatheca makes possible the permanent egg production in spite of rare copulations (usually, males are rare in the genus). In parthenogenetic species, the spermatheca is empty (state 3).

Character 9. Anterior spermatheca:
0, equal-sized to posterior one;
1, distinctly larger than posterior one.

The primary state for Nematoda in general is the female genital tube consisting of two branches of equal size. Reduction of the posterior genital tube took place independently in different taxa of Nematoda (Lorenzen, 1981). Decrease of the posterior spermatheca is a part of the reduction process.

Character 10. Tail terminus sculpture:
0, annulated;
1, smooth.

Species of *Pratylenchoides* and *Amplimerlinius* (the most primitive taxa in the suborder Hoplolaimina) have annulated terminus with thick hyaline part. Annulation increases the adhesion to substratum. Transition to parasitism inside roots leads to decrease of the role of tail in the movement, which leads to the reduction of tail and its terminus sculpture (state 2).

Character 11. Terminus shape:
0, mucronate;
1, narrow to acute;
2, rounded;
3, broadly rounded.

Primitive taxa of Tylenchida have the filiform tail (Paramonov, 1970). Transformation to the rounded tail terminus is evidently an advanced feature.

Character 12. Stylet length:
0, 27 µm or more;
1, 25-26 µm;
2, 23-24 µm;
3, 21-22 µm;
4, 19.5-20 µm;
5, 18-19 µm;
6, 16-17 µm;
7, 14-15 µm;
8, 13 µm or less.

Decrease in length of the stylet and increase in size of the oesophageal glands is a consequence of the enforcement of the biochemical influence and decrease of mechanical damage during feeding of specialized plant parasites (Maggenti, 1971; Paramonov, 1970).

Character 13. Body length:
0, 841 µm or more;
1, 781-840 µm;
2, 721-780 µm;
3, 661-720 µm;
4, 601-660 µm;
5, 531-600 µm;
6, 471-530 µm;
7, 411-470 µm;
8, 410 µm or less.

Shortening of the body is typical feature of the specialization to parasitism (Dogiel, 1962) including the parasitism in plant roots (Ryss, 1987, 1988).

Character 14. Index *b* (ratio of body length to length of oesophagus till oesophago-intestinal valve):
0, 9.6-10.3;
1, 8.7-9.5;
2, 7.8-8.6;
3, 6.9-7.7;
4, 6.1-6.8;
5, 5.2-6.0;
6, 5.1 or less.

The index *b* increases during the postembryonic growth of pratylenchids (Ryss, 1984) because the growth occurs mainly due to the elongation of trophico-genital part (term by Paramonov, 1962, 1970) of the body. Shortening of the body caused by the specialization to parasitism leads to suppression of the postembryonic growth and decrease of the *b*-value in the specialized taxa (Ryss, 1987).

Character 15. Index *c* (ratio of body length to tail length):
0, 10 or less;
1, 11-12;
2, 13-15;
3, 16-18;
4, 19-21;

5, 22-23;
6, 24-26;
7, 27-29;
8, 30 or more.

Reduction of the tail (see comments to Character 9) leads to increase in the *c*-values.

Character 16. Index *c′* (ratio of tail length to anal body width):

0, 5.8 or more;
1, 5.3-5.7;
2, 4.7-5.2;
3, 4.1-4.6;
4, 3.6-4.0;
5, 3.0-3.5;
6, 2.4-2.9;
7, 1.9-2.3;
8, 1.8 or less.

Reduction of tail: see comments to Character 9.

Character 17. Length of hyaline part of tail:

0, 25 µm or more;
1, 23-24 µm;
2, 20-22 µm;
3, 17-19 µm;
4, 14-16 µm;
5, 11-13 µm;
6, 8-10 µm;
7, 5-7 µm;
8, 4 µm or less.

Hyaline part of tail is reduced. It is a part of the tail reduction caused by the specialization to parasitism (see Character 9).

Character 18. Length of tail:

0, 92 µm or more;
1, 83-91 µm;
2, 75-82 µm;
3, 66-74 µm;
4, 57-65 µm;
5, 48-56 µm;
6, 40-47 µm;
7, 31-39 µm;
8, 30 µm or less.

Reduction of tail: see comments to Character 9.

Character 19. Ratio of tail length to stylet length:

0, 5.1 or more;
1, 4.6-5.0;
2, 4.0-4.5;
3, 3.5-3.9;
4, 3.0-3.4;
5, 2.5-2.9;
6, 1.9-2.4;
7, 1.4-1.8;
8, 1.3 or less.

Reduction of tail: see comments to Character 9.

Character 20. Male spicules:

0, 25 µm or more;
1, 23-24 µm;
2, 22 µm;
3, 20-21 µm;
4, 19 µm;
5, 17-18 µm;
6, 16 µm;
7, 15 µm or shorter;
8, male unknown.

The body shortening leads to decrease in size of organs, including the copulative ones (states 0-7). Evolutionary loss of males, i.e. transition to parthenogenesis, is evidently a secondary feature (state 8).

Character 21. Stylet knobs:

0, all knobs of equal size;
1, one knob larger then others.

Unequal stylet knobs are very rare in tylenchs. It is evidently an advanced state.

Pratylenchoides magnicauda is included here as an outgroup for the phylogenetical analysis. It is a primitive species of Pratylenchidae with morphological variability studied in detail (Ryss & Sturhan, 1994). Because of this, two additional characters are given below.

Character 22. Deirid:

0, present;
1, absent.

Deirid is a cervical papilla of large phylogenetic importance for the class Nematoda as a whole (Lorenzen, 1981). Evolutionary loss of the deirid in the order Tylenchida was analyzed by Ryss (1993).

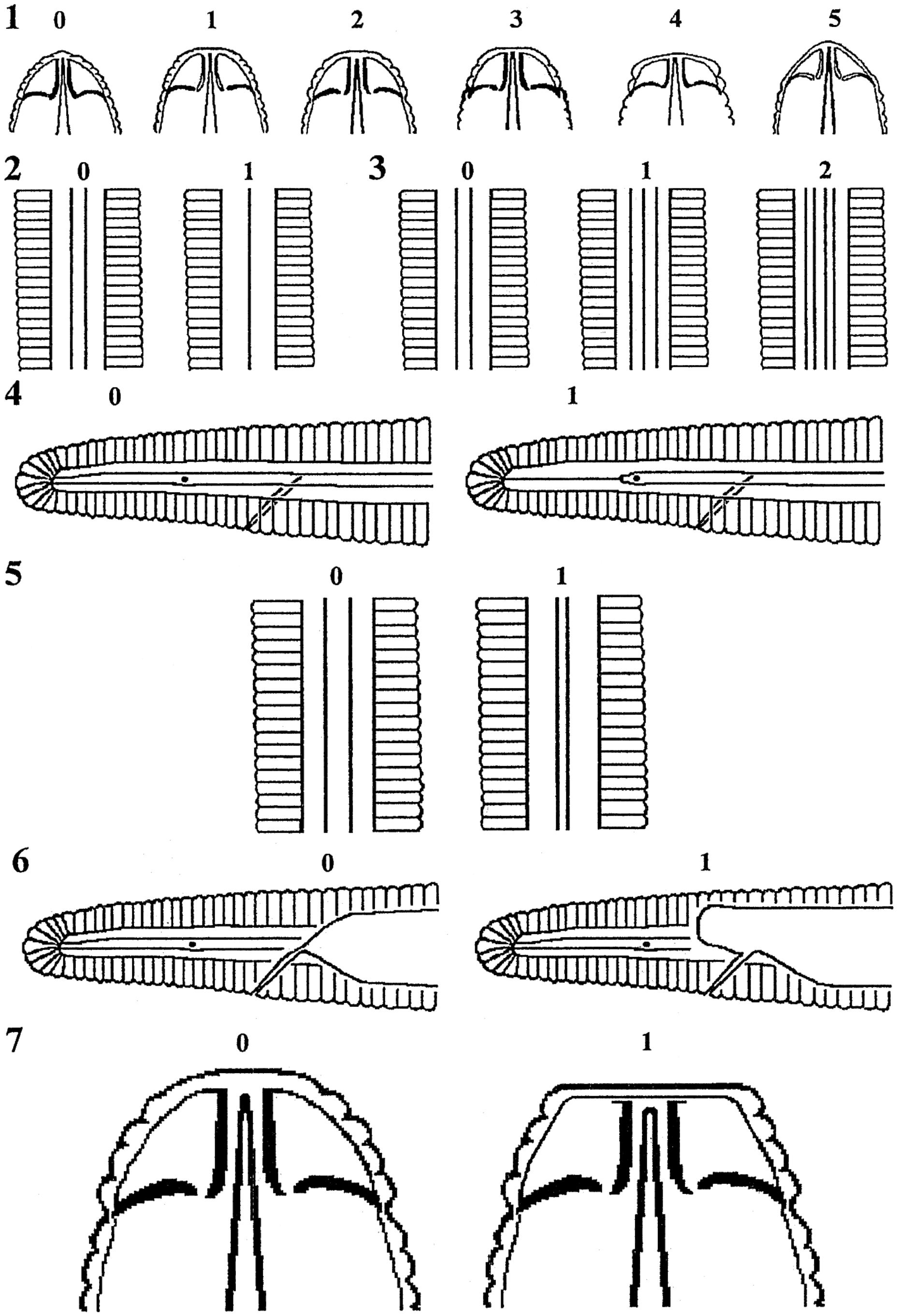
Character 23. Glands of oesophagus:

0, glands in compact body, cardium present;
1, glands in a dorsal lobe, cardium absent.

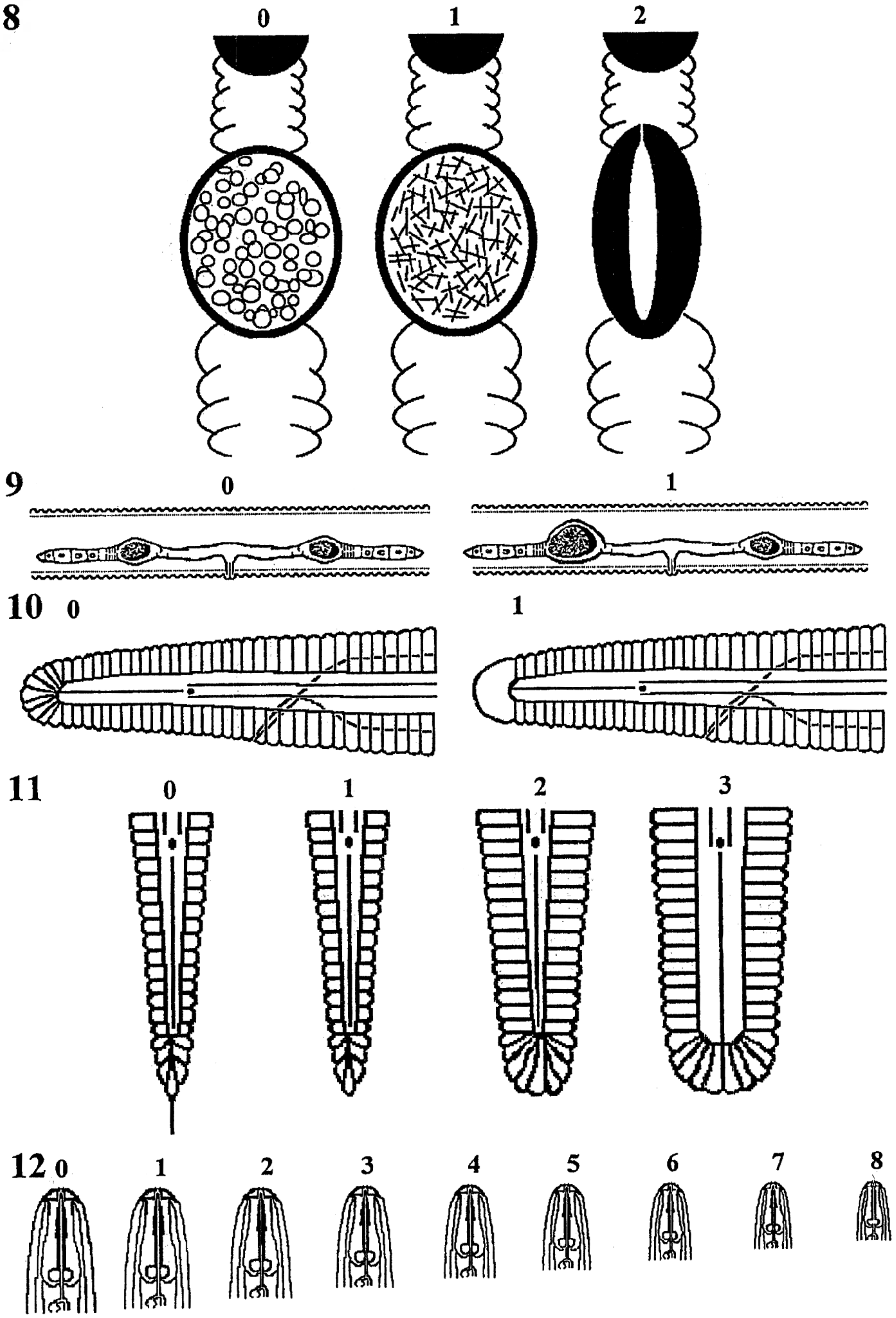
Reduction of cardial sphincter and development of the gland lobe situated in the body cavity is an adaptation to parasitic feeding on plant root cells. Glands produce secretions to dissolve plant cells. The volume of glands increases in specialized parasites. In primitive forms (*Tylenchorhynchus*, many species of *Pratylenchoides*), three oesophageal glands are situated at the same level and attached to the cardium. In the specialized plant parasitic taxa, enlarged bodies of glands have not enough space for such arrangement in narrow nematode body. Because of this, the cardium is reduced, and glands are situated in a row, one after another (*Radopholus*; see Ryss, 1993).

Table. Matrix of characters of the *Radopholus* species. LATNAM column contains species names, columns C1-C21, the characters from 1 to 21. Numbers of characters and character states in cells correspond to those listed in the rows of characters. Sets of character states for polymorphic characters are given in brackets.

LATNAM	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23
brevicaudatus	3	0	0	1	1	(01)	0	1	0	1	2	4	(78)	(56)	(123)	(567)	(67)	(78)	(78)	8	0	1	1
bridgei	(23)	0	0	1	1	0	0	1	0	0	1	(567)	(3456)	(345)	0	2	8	(2345)	(1234)	(56)	0	1	1
capitatus	4	0	0	0	0	(01)	0	1	0	0	1	(45)	(567)	(456)	(234)	(567)	(56)	(78)	7	8	0	1	1
citri	(12)	0	0	1	0	0	0	(01)	0	0	2	(456)	(1234)	(23)	(123)	(456)	(67)	(456)	(45)	(567)	1	1	1
clarus	2	0	0	0	0	0	0	1	0	0	3	(45)	(345)	(345)	(123)	(456)	6	(567)	(56)	(56)	0	1	1
colbrani	5	0	0	1	0	0	0	0	0	0	(12)	(78)	(345)	(234)	0	0	7	(012)	0	(67)	0	1	1
crenatus	6	0	0	1	0	0	0	1	0	0	3	(45)	(4567)	(34)	(123)	(56)	6	(67)	(67)	(567)	0	1	1
inaequalis	3	0	(12)	0	0	1	0	1	0	0	2	(23)	(23456)	(0123456)	(23)	(678)	(67)	(567)	(67)	(0123)	1	1	1
inanis	3	0	0	0	0	0	1	0	0	1	2	7	(56)	(45)	(12)	(456)	8	(67)	5	(567)	0	1	1
intermedius	3	0	0	0	0	0	1	0	1	1	2	8	(678)	(456)	(12)	(3456)	8	7	(45)	8	0	1	1
kahikatea	(0123)	0	0	(01)	0	0	0	0	0	0	1	(12345)	(12345)	(23456)	(01234)	(234567)	(0123456)	(234567)	(3456)	(123)	0	1	1
magniglans	(34)	0	0	1	1	1	0	1	1	(01)	2	(2345)	(567)	(456)	(123)	6	(567)	(01234567)	(67)	8	0	1	1
megadorus	5	0	0	1	1	0	0	1	0	0	3	(34)	(456)	(345)	2	(56)	(56)	(56)	6	5	0	1	1
musicola	(34)	0	0	1	1	0	0	1	0	1	(01)	(34567)	(234)	(123)	(01)	(01234)	(4567)	(1234)	(23)	(234567	0	1	1
nativus	(123)	0	0	1	0	0	0	(12)	0	0	(123)	(23456)	(234567)	(3456)	(123)	(34567)	(45678)	(45678)	(5678)	(012345	0	1	1
nelsonensis	(123)	0	0	0	0	0	0	1	0	0	3	(012)	(23456)	(456)	(5678)	8	(67)	8	8	(345)	0	1	1
neosimilis	(23)	0	0	0	0	0	1	1	0	0	2	(56)	(2345)	(2345)	(123)	(567)	(78)	(567)	(56)	(1234)	0	1	1
rectus	(23)	0	0	1	0	0	0	0	0	0	3	(56)	(4567)	(45)	(23)	(67)	(78)	7	6	(5678)	0	1	1
rotundisemenus	3	0	(12)	1	0	(01)	0	0	0	0	(23)	(345)	(45)	(345)	(23)	(456)	(567)	(567)	(56)	8	0	1	1
serratus	3	0	0	1	0	0	0	0	0	0	1	(678)	(678)	(345)	(12)	(45)	(67)	(67)	(56)	(67)	0	1	1
similis	(0123)	0	0	1	0	(01)	0	1	0	(01)	(012)	(456)	(0123456)	(123456)	(012)	(0123456)	(0123456)	(01234)	(01234)	(2345)	0	1	1
sp.n.(Tasmania)	(012)	0	0	0	0	0	0	1	0	0	(12)	(23)	(456)	(56)	2	6	(67)	(67)	(67)	8	0	1	1
trilineatus	4	1	0	1	0	1	0	1	0	0	2	(345)	(456)	(45)	(23)	(567)	7	(567)	(56)	8	0	1	1
vacuus	4	0	0	1	0	0	(01)	(01)	0	1	(23)	(78)	(678)	(456)	(123)	(3456)	8	(678)	(456)	(67)	0	1	1
vangundyi	(23)	0	0	0	0	1	0	1	0	0	(12)	(56)	(4567)	(234)	(123)	(56)	(67)	(67)	6	(567)	0	1	1
vertexplanus	(34)	0	0	1	0	0	1	2	0	0	(12)	(67)	(567)	(456)	(12)	(3456)	(567)	(567)	(45)	8	0	1	1
A_williamsi	(23)	(01)	0	1	0	0	0	1	0	1	2	(678)	(78)	(456)	(123)	(567)	(67)	(78)	6	(34567)	0	1	1
P_magnicauda	0	0	2	0	0	0	0	0	0	0	3	(01)	(01234)	(78)	(2345)	(5678)	(3456)	(4567)	7	0	0	0	0

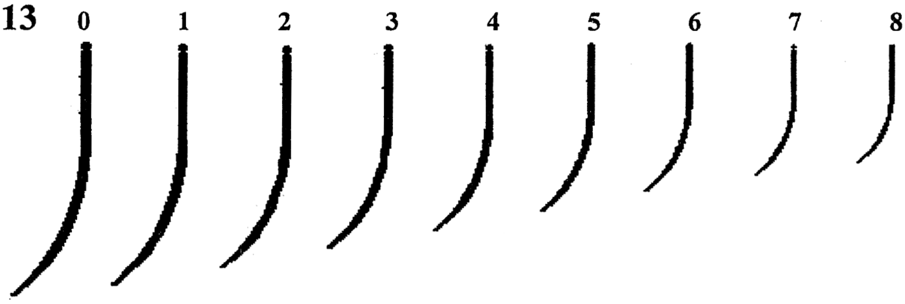


Figs 1-21. Rows of morphological characters of the genus *Radopholus*. Numbers of characters (1-21, to the left, bold font) and numbers of their states (0-9, along rows, standard font) correspond to the numbers of characters and character states given in the list of rows of characters. Illustrations to characters 16, 18 and 19 are joined into one set.

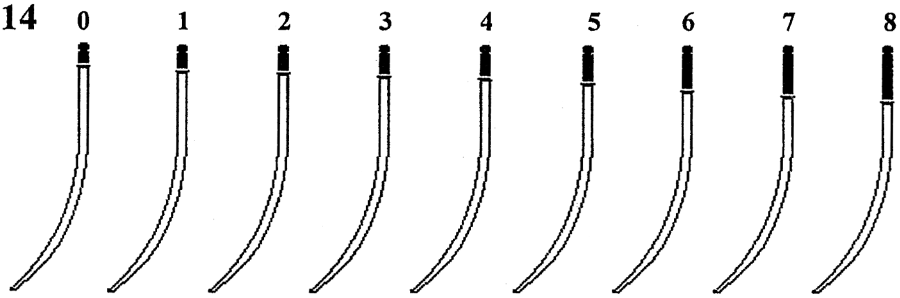


Figs 1-21. *Continued*

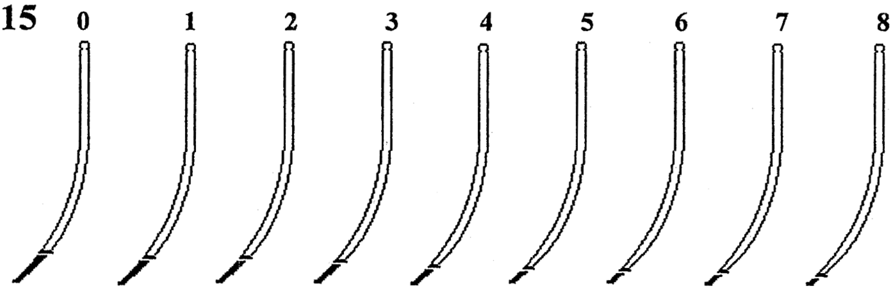
13



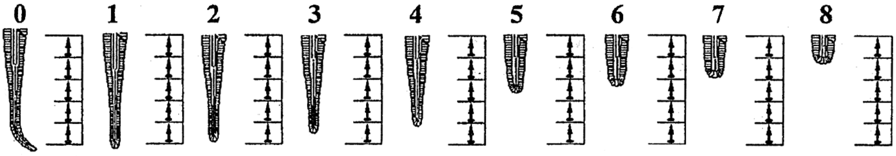
14



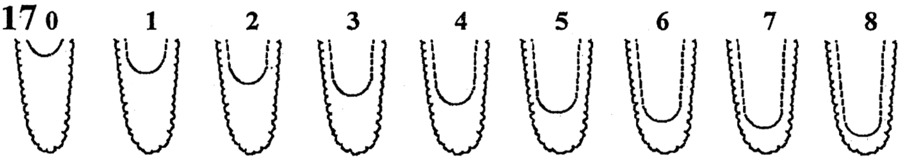
15



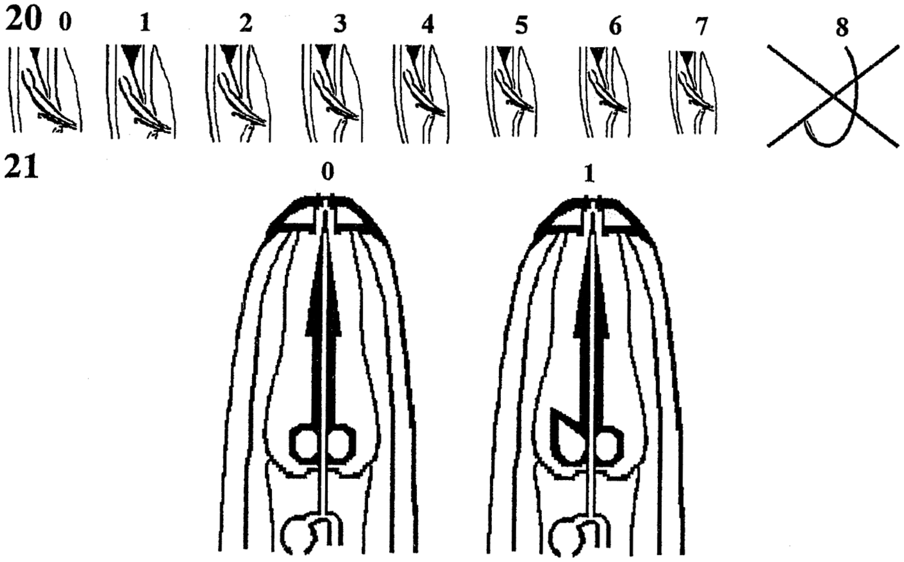
16,18,19



17



Figs 1-21. ***Continued***



Figs 1-21. *Continued*

Phylogenetic tree (Figs 22, 23)

The matrix of characters (Table) has been imported into a file of the Nexus format. Peculiarity of the matrix is the presence of multistate sets of characters for the majority of species (the multistate sets are placed in brackets). All characters (considered to be of equal weight) are coded as listed above. Characters 1-21 are coded as "ordered", characters 22 and 23 as "irreversible". *Pratylenchoides magnicauda* has been coded as an outgroup. Then all possible parsimonious trees (9323; length = 612) were generated using the heuristic search in the PAUP package. A consensus tree was calculated using 50% Majority Rule (Fig. 22). The Strict Consensus mode is used to check the stability of clusters.

The Consensus tree of 50% Majority Rule has the following parameters: Consistency index (CI) = 0.9183; Homoplasy index (HI) = 0.8448; CI excluding uninformative characters = 0.9178; HI excluding uninformative characters = 0.0822; Retention index (RI) = 0.7537; rescaled consistency index (RC) = 0.6921.

The most primitive species are *R. nelsoni*, *R. inaequalis* and *R. kahikatea*. Among other species, the following clusters and species groups may be distinguished (apomorphic changes for each group are indicated in brackets):

1. Cluster **similis** (Chr. 14: 5 → 3; 16: 6 → 5; 18: 7 → 6; node 46):

1.1. Subcluster **similis**: *R. similis*, *R. bridgei*, *R. musicola*, *R. colbrani*, *R. citri*, *R. nativus, R. neosimilis*, *R. vangundyi* (Chr. 12: 4 → 6; 15: 2 → 1; 17: 6 → 7; node 43).

Within the subcluster, the species group of *R. similis*, *R. bridgei*, *R. musicola*, *R. colbrani*, and *R. citri* (Chr. 19: 5 → 4; node 41) is the most interesting one, because the members of this group are all the species of the genus distributed outside of Australasia (see discussion below).The first four species of the group are especially close (Chr. 11: 2 → 1; 15: 1 → 0; 16: 4 → 2; 18: 4 → 2; 19: 4 → 2; node 40).

1.2. Subcluster **crenatus**: *R. clarus*, *R. crenatus*, *R. megadorus*. (Chr. 11: 2 → 3; node 45).

2. Cluster **vacuus.** Cluster includes 2 subclusters and the following species: *R. capitatus*, *R. rotundisemenus*, *R.* sp. n. from Tasmania (Chr. 13: 4 → 5; 20: 5 → 8; node 37):

2.1. Subcluster **vacuus**: *R. vacuus, R. inanis, R. intermedius, R. vertexplanus, R. serratus, R. rectus* and *Achlysiella williamsi* (Chr. 12: 4 → 6; 20: 8 → 7; node 34). The presence of the last species among species of *Radopholus* may be explained by the fact that the only minute biological difference is known between this species and *Radopholus*: the morphology of mature female corresponds to *Radopholus*, only inseminated females become obese inside plant roots.

2.2. Subcluster **trilineatus**: *R. trilineatus*, *R. brevicaudatus*, *R. magniglans* (Chr. 6: 0 → 1; 8: 0 → 1; node 30).

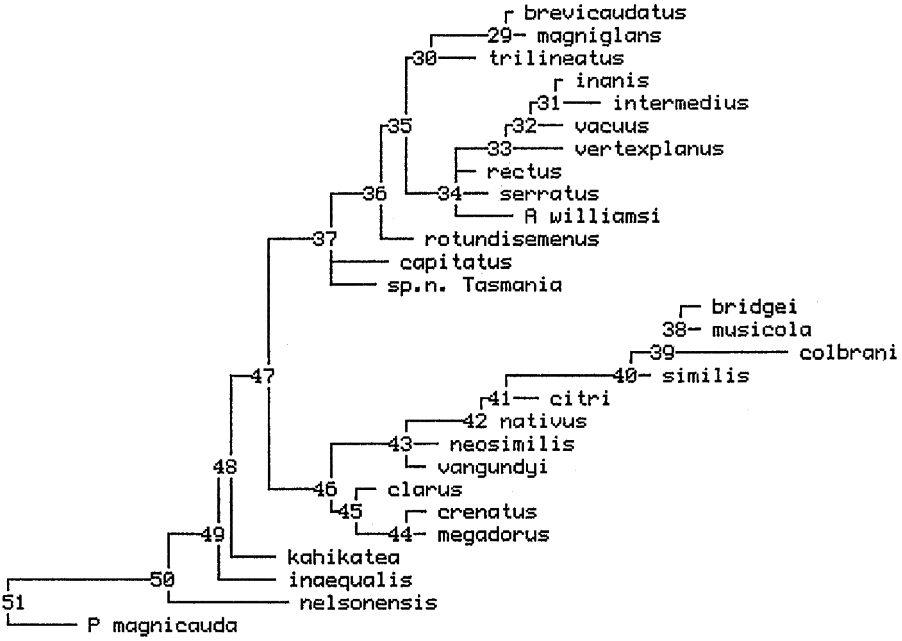


Fig. 22. Phylogram of didelphic species of the genus *Radopholus*. Nodes are numerated from final tree terminals (species).

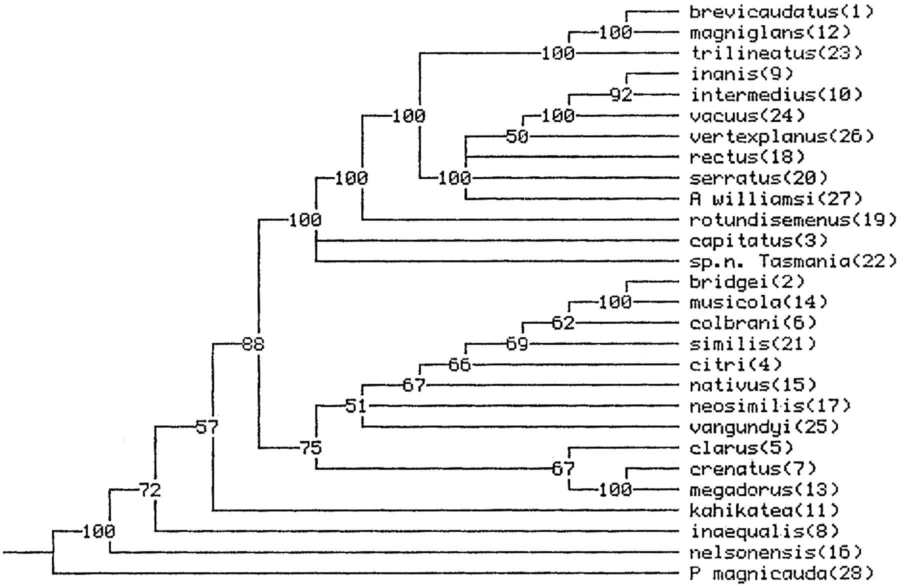


Fig. 23. Cladogram of didelphic species of the genus *Radopholus*. Numbers in brackets correspond to numbers of terminals (species); numbers on branches correspond to group frequencies of nodes in a sample 9323 trees (all possible parsimonious trees).

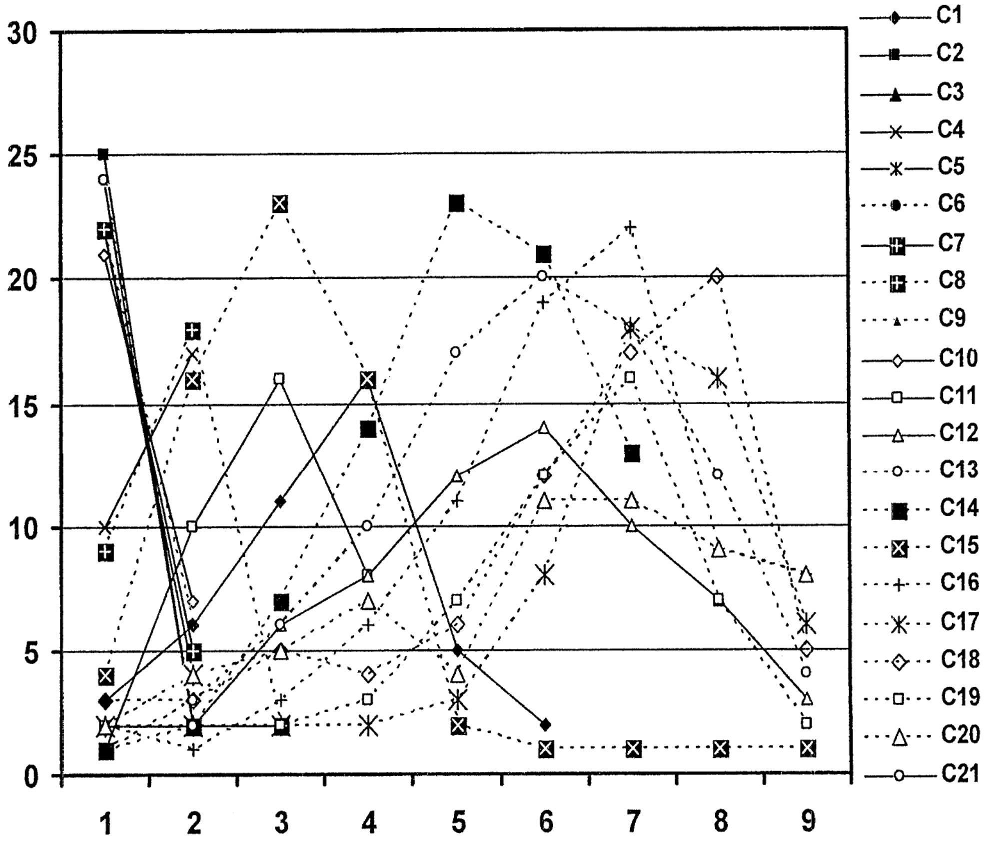


Fig. 24. State frequencies within the rows of characters in the genus *Radopholus*. Ordinate: number of species with corresponding character state; abscissa: values 0-9 identical to the character states in the rows of characters. Styles of graphics lines are shown to the right in the diagram legend (in rectangular frame); C13, C14 … are characters 13, 14, etc., respectively, where the character numbers correspond to those in the list of characters.

The important conclusion is that the species characterized by the origin and distribution outside of Australasia on agricultural crops form the monophyletic group. In particular, *R. similis* is worldwide in subtropics and tropics on agricultural plants; *R. colbrani* is found in India (Chikmagalur region) on *Coffea arabica* crop at the coffee research station (Kumar, 1980), *R. citri* and *R. bridgei*, in Indonesia (Java) on *Citrus* sp. rootstock and *Curcuma zedoaria,* respectively (Siddiqi & Hahn, 1995; Machon & Bridge, 1996); *R. musicola* is found in Australia (Northern Territory), and also during a survey of banana crops in roots of *Musa acuminata* cv. Williams planted at Berrimah Research Station, Darwin (Stanton et al., 2001).

The genus *Radopholus* seems to be of the Australasian origin (Sher, 1968; Stanton et al., 2001). It means that historically the splitting of the genus and expansion of the monophyletic group outside the Australasia took place only once. Possibly, it was caused by the splitting out and the movement of the Indian subcontinent towards the Asian continent.

Notes on evolution

As it may be concluded from the diagram of frequency pattern of the different character states within the character rows (Fig. 24), some character rows within the genus display the peaks of frequency distinctly shifted to the advanced states. It means that the majority of the *Radopholus* species feature the most advanced states of characters. It may be suggested that the evolutionary changes in such characters may have an adaptive significance because of which most species of *Radopholus* as well as the genus as a whole are successful plant parasite taxa. These characters have numbers 13, 14, 16-20 in the "Rows of morphological characters" list given above. Possible adaptive features are as follows: shortening of the body (Chr. 13), elongation of the oesophagus and its glandular part (Chr. 14), shortening of the tail (Chr. 16,18,19) and reduction of its hyaline part (Chr. 16), and reduction of the male copulative organs (Chr. 20). Possibly, all these changes are adaptations to the migratory endoparasitism in plant roots, where miniaturization of the body and its parts and elongation of the oesophagus and its glands responsible for the parasitic feeding may arise in parallel phylogenetic lines.

Acknowledgements

Author thanks the following colleagues for help: Dr. W.M. Wouts (Landcare Research, Auckland, New Zealand), Dr. D. Sturhan (Institute for Nematology and Vertebrates, Mьnster, Germany), Mr. L. Waeyenberge and Dr. M. Moens (Centrum Landbowkundig Onderzoek, Merelbeke, Belgium) for loaning the nematode materials; Drs. I.M. Kerzhner, S.J. Tsalolikhin, A.O. Averyanov and A.V. Bochkov (Zoological Institute, St.Petersburg) for discussions on taxonomy and phylogeny, Mr. Wim Bert (Gent University, Belgium) for kind help with the literature.

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Received 17 September 2002