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# *CAIRAEANTHUS* GEN. N. (CESTODA, RHINEBOTHRIIDEA), WITH THE DESCRIPTION OF TWO NEW SPECIES FROM *DASYATIS PASTINACA* IN THE BLACK SEA AND THE SEA OF AZOV

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> Cairaeanthus gen. n. (Cestoda, Rhinebothriidea), with the Description of Two New Species from Dasyatis pastinaca in the Black Sea and the Sea of Azov. Kornyushin V. V., Polyakova T. A. - Two new species were identified among cestodes collected from Dasyatis pastinaca (Linnaeus, 1758) in the Black Sea and the Sea of Azov. Their morphology corresponds in the most characters to the descriptions of Phyllobothrium lactuca van Beneden, 1850 and P. gracilis Wedl, 1855 by L. Borcea (Borcea, 1934) and T. P. Pogorel'tseva (Pogorel'tseva, 1960) from the same host and seas. However, some morphological characters of these cestodes (namely, the marginal loculi on the pedicellate bothridia, cephalic peduncle, absence of the apical sucker and post-poral testes, genital pore opening marginally, close to posterior end of proglottid) correspond to diagnosis of Rhinebothriidea. Since the species from the Black Sea and the Sea of Azov did not fit diagnoses of any known genera currently belonging to Rhinebothriidea, the new genus, Cairaeanthus Kornyushin et Polyakova, gen. n., with two species, C. ruhnkei Kornyushin et Polyakova, sp. n. and C. healyae Kornyushin et Polyakova, sp. n., is established. Cairaeanthus gen. n. differs from Anthocephalum Linton, 1890 in absence of apical sucker, posteriorly bifid bothridia and vitelline fields interrupted by the ovary, not extending in the posterior end of the proglottid. The new genus can be distinguished from *Rhinebothrium* Linton, 1890, *Echeneibothium* van Beneden, 1850, *Rhabdotobothrium* Euzet, 1953, Rhinebothroides Mayes, Brooks et Thorson, 1981, Scalithrium Ball, Neifar et Euzet, 2003 by the absence of facial loculi on the bothridia and by numerous vitelline follicles, stopping at the anterior margin of the ovary; from Rhodobothrium Linton, 1889 - by absence of post-poral testes, and from Spongiobothrium Linton, 1889 — by numerous vitelline follicles, stopping at the level of ovary. C. ruhnkei sp. n. differs from C. healvae sp. n. in the lengths of the strobila, the cephalic peduncle and the bothridia pedicel, in size of marginal loculi and the ovary, in the number of the proglottids and the testes in the proglottid, and in the structure of genital atrium. Two new species are differentiated also from the morphologically related species Phyllobothrium pastinacae Mokhtar-Mocamouri, Zamali, 1981. Examined type specimens of *P. pastinacae* were attributed to Rhinebothiidea based on a set of morphological characters. However, they appear to differ from all known Rhinebothriidea, including the species from the genera Anthocephalum Linton, 1890 and Cairaeanthus gen. n. Taxonomic position of P.pastinacae needs further identification.

> Key words: *Cairaeanthus* gen. n., Rhinebothriidea, *Cairaeanthus ruhnkei* sp. n., *Cairaeanthus healyae* sp. n., *Phyllobothrium pastinacae, Dasyatis pastinaca,* the Mediterranean Sea, the Black Sea, the Sea of Azov.

*Cairaeanthus* gen. n. (Cestoda, Rhinebothriidea), описание двух новых видов от *Dasyatis pastinaca* из Черного и Азовского морей. Корнюшин В. В., Полякова Т. А. — В сборах цестод от *Dasyatis pastinaca* (Linnaeus, 1758) из Черного и Азовского морей идентифицировано два вида цестод, которые по большинству морфологических признаков соответствуют описаниям *Phyllobothrium lactuca* van Beneden, 1850 и *P. gracilis* Wedl, 1855 в публикациях Л. Борче (Borcea, 1934) и Т. П. Погорельцевой (1960) по материалам, собранным от этого же хозяина и в этом районе. Однако определенные морфологические признаки этих цестод (наличие краевых локул на стебельчатых ботридиях и стебля сколекса, отсутствие апикальных присосок на сколексе и поствагинальной группы семенников, наряду с расположением полового отверстия маргинально вблизи заднего края проглоттиды) соответствуют диагнозу отряда Rhinebothriidea. Поскольку цестоды от черноморских скатов не соответствует диагнозу ни одного из известных на сегодня родов отряда Rhinebothriidea, для них обоснован новый род Cairaeanthus Kornyushin et Polyakova, gen. n. с двумя новыми видами C. ruhnkei Kornyushin et Polyakova, sp. n. и C. healyae Kornyushin et Polyakova, sp. n. Новый род отличается от рода Anthocephalum Linton, 1890 отсутствием апикальных присосок на сколексе, раздвоенными в задней части ботридиями и тем, что поля желточных фолликулов прерываются на уровне яичника, не достигая заднего края проглоттиды. От Echeneibothium van Beneden, 1850, Rhinebothrium Linton, 1890, Rhabdotobothrium Euzet, 1953, Rhinebothroides Mayes, Brooks et Thorson, 1953, Scalithrium Ball, Weifar, Euzet, 2003 новый род отличается отсутствием лицевых локул на ботридиях и тем, что желточные поля достигают только переднего края яичника; от *Rhodobothrium* Linton, 1889 — отсутствием поствагинальной группы семенников, от Spongiobothrium Linton, 1889 — многочисленными желточными фолликулами, поля которых прерываются на уровне яичника. Виды *C. ruhnkei* sp. n. и *C. healvae* sp. n. отличаются друг от друга длиной стробилы, стебля сколекса и стебелька ботридий, количнеством локул на ботридиях, количеством проглоттид, семенников в проглоттиде, формой яичника, строением полового атриума. Новые виды диффернецированы также от Phyllobothrium pastinacae Mokhtar-Mocamouri et Zamali, 1981 от D. pastinaca из Средиземного моря на основании переизучения типового материала. По наличию определенных морфологических признаков эти цестоды также отнесены к отряду Rhinebothriidea. Однако они отличаются от представителей всех известных на сегодня родов Rhinebothriidea, в том числе от Anthocephalum Linton, 1890 и Cairaeanthus gen. n. Таксономический статус этих цестод требует отдельного уточнения.

Ключевые слова: *Cairaeanthus* gen. n., Rhinebothriidea, *Cairaeanthus ruhnkei* sp. n., *Cairaeanthus healyae* sp. n., *Phyllobothrium pastinacae*, *Dasyatis pastinaca*, Средиземное море, Черное море, Азовское море.

#### Introduction

Cestodes of the genus *Phyllobothrium* van Beneden, 1849 from the dasyatid stingray *Dasyatis pastinaca* (L., 1758) in the Black Sea were first reported by L. Borcea (Borcea, 1934). In his article on the cestode fauna from the Black Sea elasmobranch fishes two species were recognized as *Phyllobothrium lactuca* van Beneden, 1850 and *P. gracilis* Wedl, 1855, as new for the Black Sea. Afterwards, many researchers (e. g. Chulkova, 1939; Osmanov, 1940; Chernyshenko, 1949, 1955; Pogorel'tseva, 1952, 1960, 1964, 1970; Reshetnikova, 1955; Kurashvili, 1960; Gayevskaya et al., 1975; Mange, 1993; Miroshnichenko, 2004) reported these parasites from stingrays *D. pastinaca* and *Raja clavata* L., 1758 in the Black Sea and the Sea of Azov. After L. Borcea (Borcea, 1934) the descriptions of these species and their schematic figures were presented only in one article (Pogorel'tseva, 1960). So far as original collections of phyllobothriid of T. P. Pogorel'tseva were lost, identifications of cestodes from the Black Sea stingrays in subsequent publications were based only on the abovementioned descriptions.

Among the cestodes collected from *D. pastinaca* in the Black Sea we identified two species corresponding to the descriptions of *P. lactuca* van Beneden, 1850 *sensu* L. Borcea (1934) and T. P. Pogorel'tseva (1960) and *P. gracilis* Wedl, 1855 *sensu* L. Borcea (1934) and T. P. Pogorel'tseva (1960) by most morphological characters. However, comparison of morphology of these cestodes with the type description of *P. lactuca* from the shark *Mustelus mustelus* (L., 1758) in the North Sea (Ruhnke, 1996a) revealed significant differences and unconformity of the cestodes from *D. pastinaca* in the Black Sea to the present diagnosis of *Phyllobothrium*. Moreover, species from the Black Sea stingrays are neither members of the family Phyllobothriidae Braun, 1900 nor members of the order Tetraphyllidea Carus, 1863. According to such morphological characters as presence of marginal loculi in pedicellate bothridia, cephalic peduncle, absence of apical suckers and post-poral testes these species was attributed to order Rhinebothriidea Healy, Caira, Jensen, Webster, Littlewood, 2009 (Healy et al., 2009). *P. gracilis* have been transferred to *Anthocephalum* Linton, 1890 (Ruhnke, 1994 b) and then to the order Rhinebothriidea (Healy et al., 2009), but species parasitizing *D. pastinaca* from the Black Sea and the Sea of Azov significantly differ from the description of this species do not completely coincide with the diagnosis of any known genera presently belonging to Rhinebothriidea.

Therefore, cestodes parasitizing *D. pastinaca* in the Black Sea and the Sea of Azov, previously identified as *P. lactuca* and *P. gracilis* (Borcea, 1934; Pogorel'tseva, 1960), are determined herein as two new species from the new genus belonging to the order Rhinebothriidea. In this paper, the descriptions of *Cairaeanthus* Kornyushin et Polyakova, gen. n. and two new species are presented.

#### Material and methods

Cestodes were collected from dasyatid stingray *D. pastinaca* captured in 1977 and during 2000–2011 in the coastal water of the Black Sea and the Sea of Azov. The spiral intestines from 112 fish, two of which were from the Sea of Azov, were examined. 437 cestodes belonging to the new genus were found in 64 stingray specimens and there were 229 mature worms among them. Worms were removed from the spiral intestine, washed

in fresh water to relax their musculature and everted cirrus, and fixed in 70° ethanol (Bychowskaya-Pavlovskaya, 1969). Cestodes were stained with acetocarmine, dehydrated in an ethanol series (70-100°), cleared in clove oil and mounted in Canada balsam (Roskin, Levinson, 1957). For comparison type specimens of *Phyllobothrium pastinacae* Mokhtar-Mocamouri, Zamali, 1981 from *D. pastinaca* from the Mediterranean Sea (holotype MNHN 90HB148 c VII and paratypes MNHN90HB149 c VII, MNHN90HB150 c VII) deposited in the Museum Nationale d'Histoire Naturelle parasitological collection (Paris, France) were investigated.

Measurements in the text are given as the range, followed in parentheses by the mean, the standard error, the number of worms examined (n) and the total number of observations if more than one observation per worm was taken (n). Measurements are reported in micrometres, unless otherwise stated. Measuring and light micrographs were made using the light microscope Zeiss Axio Imager M 1. All figures were produced in scalable vector graphics using the program Inkscape 0.48.2.-1 (Scalable Vector Graphics, 2011). Data analysis was carried out using independent t-tests and Principal Component Analysis (StatSoft Inc., 2001). The statistical analyses and their graphical representation were produced using the Statistica 6 for Windows software package.

#### Cairaeanthus Kornyushin et Polyakova, gen. n.

R h i n e b o t h r i i d e a. Mid-size, apolytic worms. Scolex tetrabothridiate. Each bothridia posteriorly bifid. Myzorhynchus absent. Bothridia pedicellate, each with numerous marginal loculi. Apical sucker absent. Cephalic peduncle present. Strobila craspedote, multiproglottided. Testes numerous, median, arranged in several layers; post-poral testes absent. Vas deferens convoluted; seminal vesicle absent. Cirrus armed with spinitriches. Genital pores opening marginally close to posterior end of proglottids, irregularly alternating. Vagina opening into genital atrium anterior to cirrus-sac. Vaginal sphincter absent. Ovary near posterior end of proglottid, median, H-shaped, tetralobed in cross-section; lobes asymmetrical; lobulated to follicular. Vitellarium follicular, numerous, lateral; consisting of 2 wide lateral bands, extending from lateral margin to median line of proglottid, surrounding excretory ducts. Vitelline fields not interrupted at level of genital atrium, becoming thin, stopping at anterior margin of ovary in mature proglottids, not extending posteriorly to ovary. Uterus ventral, reaching anterior margin of proglottid, with inner pocket-like partitioning. Parasites of Dasyatidae (Pisces).

Type species: Cairaeanthus ruhnkei Kornyushin et Polyakova, sp. n.

Other species: Cairaeanthus healyae Kornyushin et Polyakova, sp. n.

Etymology: The genus is named for Dr. Janine N. Caira, for her invaluable contributions to the investigation of tetraphyllidean and rhinebothriidean cestodes; and for the Greek *anthus* – "flower".

Differential diagnosis. *Cairaeanthus* gen. n. corresponds to the diagnosis of Rhinebothiidea (Healy et al., 2009). Among eight valid genera of this order, *Anthocephalum* most closely resembles *Cairaeanthus* in the presence of the numerous marginal loculi on the slightly pedicellate bothridia and folded bothridia, in absence of the post-poral testes, and in the marginal position of the genital pore near the posterior end of the proglot-tid. The new genus differs from *Anthocephalum* in the absence of apical sucker, in posteriorly bifid bothridia, and in vitelline fields interrupted by the ovary not extending in the posterior end of the proglottid. *Cairaeanthus* can be distinguished from other rhinoboth-riidean genera as follows: from *Echeneibothium* van Beneden, 1850; *Rhinebothrium* Linton, 1890; *Rhabdotobothrium* Euzet, 1953; *Rhinebothroides* Mayes, Brooks, Thorson, 1981; *Scalithrium* Ball, Neifar, Euzet, 2003 — by absence of facial loculi on bothridia and by numerous vitelline follicles, stopping at the anterior margin of the ovary; from *Rhodobothrium* Linton, 1889 — by absence of post-poral testes, and from *Spongiobothrium* Linton, 1889; Euzet, 1994; Ruhnke, 1994; Ball et al., 2003).

Among the other cestode taxa, parasitizing elasmobranches, representatives of the family Phyllobothriidae from the order Tetraphyllidea are similar to *Cairaeanthus*. The new genus resembles *Phyllobothrium* (Ruhnke, 1996 a) in the presence of posteriorly bifid bothridia, but differs from it in the presence of the marginal loculi in the pedicellate bothridia and the absence of the neck, the apical sucker, the glandular apical organ, the vaginal sphincter and the post-poral testes. Moreover, cestodes of the new genus are apoly-

tic vs anapolytic worms in *Phyllobothrium* spp. By absence of apical sucker *Cairaeanthus* gen. n. differs also from the following phyllobothriidean genera: Anthobothrium van Beneden, 1850; Orygmatobothrium Diesing, 1863; Monorygma Diesing, 1863; Dinobothrium van Beneden, 1889: Crossobothrium Linton, 1889: Calvptrobothrium Monticelli, 1893: Marsupiobothrium Yamaguti, 1952; Gastrolecithus Yamaguti, 1952; Chimaerocestos Williams, Bray, 1984; *Clistobothrium* Dailey, Vogelbein, 1990; *Paraorygmatobothrium* Ruhnke, 1994; *Anindobothrium* Marques, Brook, Lasso, 2001; *Orectolobicestus* Ruhnke, Caira, Carpenter, 2006 (Yamaguti, 1959; Euzet, 1994; Ruhnke, 1994, 1996b; Marques et al., 2001: Ruhnke et al., 2006: Ruhnke, Caira, 2009). The new genus can be distinguished from the genera Thysanocephalum Linton, 1889; Myzocephalus Shipley, Hornell, 1906; Myzophyllobothrium Shipley, Hornell, 1906; Pithophorus Southwell, 1925; Scyphophyllidium Woodland, 1927; Caulobothrium Baer, 1948; Glyphobothrium Williams, Campbell, 1977; Duplicibothium Williams, Campbell, 1978 by the absence of post-poral testes. From Trilocularia Olsson, 1867; Tritaphros Loennberg, 1889; Carpobothrium Shipley, Hornell, 1906; Pseudanthobothrium Baer, 1956; Pentaloculum Alexander, 1963; Phormobothrium Alexander, 1963; Zvxibothrium Hayden, Campbell, 1981 new genus can be differentiated by the presence of marginal loculi, and from *Clydonobothrium* Euzet, 1959 – by the absence of the myzorhynchus (Euzet, 1994; Yamaguti, 1959; Randhawa et al., 2008).

### *Cairaeanthus ruhnkei* Kornyushin et Polyakova, sp. n. (fig. 1–8)

Type host: Dasyatis pastinaca (L., 1758) (Dasyatidae).

Type locality: Karantinnaya Bay, Sevastopol, the Black Sea (44°36.41'N, 33°29.54'E).

Additional localities: the Black Sea (off Sevastopol, Streletskaya and Martynova Bay; off the cape Sarych and Phoros; Karkinitskiy Bay; the Black Sea Biosphere Reserve, Yagorlytskiy Bay; water area of Karadag Nature Reserve; Kerch Channel) and the Sea of Azov (Obitochny Bay).

Site of infection: spiral intestine.

Type specimens: holotype C. 186.002.32 (06.11.2003; 1349/157; spiral valve N 17; 1 specimen; Karantinnaya Bay, Sevastopol, the Black Sea; IBSS, DEP; coll. T. A. Polyakova) and paratypes C. 177.002.19 (11.09.2003; 1154/146; spiral valve N 8; 1 specimen; Streletskaya Bay, Sevastopol, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 178.002.20 (11.09.2003; 1154/147; spiral valve N 18; 1 specimen; Streleckava bay, Sevastopol, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 179.002.21 (11.09.2003; 1154/148; spiral valve N 15; 1 specimen; Streleckaya Bay, Sevastopol, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 199.002.47 (29.09.2005; 2297/165; spiral valve N 11; 1 specimen; Karantinnaya Bay, Sevastopol, the Black Sea; IBSS; Department Ecological Parasitology; coll. T. A. Polyakova), C. 206.002.67 (29.09.2005; 2297/166; spiral valve N 14; 1 specimen; Karantinnaya Bay, Sevastopol, the Black Sea; IBSS, DEP; coll. T. A. Polyakova) ex D. pastinaca from the Black Sea deposited in the collection of the Department of Ecological Parasitology, Institute of Biology of the Southern Seas NASU, Sevastopol. Additional paratypes CP 35.1 and CP 35.2 (04.07.1977; mk 12; Black Sea, Biosphere Reserve, Yagorlytskiy Bay; Institute of Zoology; coll. V. V. Kornyushin) ex D. pastinaca from the Black Sea are in the collection of the Department of Parasitology, I. I. Schmalhausen Institute of Zoology, NASU, Kyiv.

Etymology: This species is named for Dr Timothy R. Ruhnke, for his contribution to the study of phyllobothriidean cestodes.

Description. Based on holotype, 5 paratypes and 24 specimens from *D. pastinaca* in the Black Sea and 6 specimens from the same host in the Sea of Azov.

Worms mid-size, craspedote, apolytic,  $56-255 \text{ mm} (123 \pm 8.3; n = 36) \log_3 300-2200 (957 \pm 57; n = 36; n = 72)$  wide. Proglottids  $495-1803 (981 \pm 48; n = 36)$  in number. Scolex large,  $800-3130 (1472 \pm 76; n = 33) \log_1 1100-2800 (1832 \pm 71; n = 33)$  wide, tetrabothridiate (fig. 1, 5). Myzorhynchus absent. Apical sucker absent. Bothridia  $110-1500 (836 \pm 50; n = 33; n = 34) \log_2 200-1500 (622 \pm 40; n = 30; n = 30; n = 30; n = 30) \log_2 200-1500 (620 \pm 100) \log_2 200 (600 \pm 100$ 



Fig. 1–4. *Cairaeanthus ruhnkei* ex *Dasyatis pastinaca* from the Black Sea: 1 - scolex, paratype C. 178.002.20; 2 - immature proglottid, holotype C. 186.002.23; 3 - cirrus, holotype C. 186.002.23; 4 - mature proglottid, holotype C. 186.002.23. Scale bars:  $1 - 300 \,\mu\text{m}$ ;  $2 - 100 \,\mu\text{m}$ ;  $3, 4 - 200 \,\mu\text{m}$ .

Рис. 1–4. *Cairaeanthus ruhnkei* от *Dasyatis pastinaca* из Черного моря: 1 — сколекс, паратип С178.002.20, 2 — неполовозрелая проглоттида, голотип С. 186.002.23; 3 — циррус, голотип С. 186.002.23; 4 — половозрелая проглоттида, голотип С. 186.002.23. Масштабная линейка: 1 — 300 мкм; 2 — 100 мкм; 3, 4 — 200 мкм.

51) wide; posteriorly bifid; with numerous marginal loculi, each 50-100 ( $59 \pm 3$ ; n = 31) long, 30-100 ( $55 \pm 3$ ; n = 31) wide; with short and wide pedicel, 100-500 ( $279 \pm 19$ ; n = 32; n = 37) long, 100-500 ( $242 \pm 18$ ; n = 32; n = 34) wide (fig. 6). Cepalic peduncle indistinct, short, 200-680 ( $460 \pm 22$ ; n = 36) long, 280-900 ( $546 \pm 23$ ; n = 36) wide. Lateral excretory ducts 10-70 ( $35 \pm 2$ ; n = 36) in diameter.

Immature proglottids rectangular, 20–200 (117  $\pm$  7; n = 36; n = 48) long, 280–1550 (688  $\pm$  35; n = 36; n = 49) wide (fig. 2). Mature proglottids trapezoid, almost square

130-630 (315 ± 18; n = 36; n = 41) long, 550-1760 (1166 ± 45; n = 36; n = 39) wide. Terminal proglottids 330-2200 (1027 ± 86; n = 35; n = 40) long, 630-2200 (1207 ± 46; n = 36; n = 39) wide (figs 4, 8). Mature proglottid with 51-98 (83 ± 2; n = 36; n = 43) testes. Testes round, 30-130 (57 ± 3; n = 36; n = 41) in diameter, median, arranged in two layers; post-poral testes absent (fig. 2, 4, 8). Cirrus-sac pyriform or spherical, 50-300 (204 ± 12; n = 36; n = 38) long, 40-270 (158 ± 10; n = 36) wide (fig. 3, 7). Cirrus armed with spinitriches. Total length of everted cirrus 800-850 (833 ± 17; n = 3), of inverted 220-750 (482 ± 23; n = 34; n = 44) (fig. 4). Vas deferens 10-100 (61 ± 5;



Fig. 5–8. Photomicrographs of *Cairaeanthus ruhnkei* ex *Dasyatis pastinaca* from the Black Sea: 5 – scolex, paratype C178.002.20; 6 – marginal locules, paratype C178.002.20; 7 – cirrus, holotype C186.002.23; 8 mature proglottid, holotype C186.002.23. Scale bars: 5 – 300  $\mu$ m; 6 – 50  $\mu$ m; 7, 8 – 200  $\mu$ m.

Рис. 5–8. Микрофотография *Cairaeanthus ruhnkei* от *Dasyatis pastinaca* из Черного моря: 5 — сколекс, паратип С. 178.002.20, 6 — краевые локулы, паратип С. 178.002.20; 7 — циррус, голотип С. 186.002.23; 8 — половозрелая проглоттида, голотип С. 186.002.23. Масштабные линейки: 5 — 300 мкм; 6 — 50 мкм; 7, 8 — 200 мкм.

n = 36; n = 38) in diameter, coiled, extending anteriorly to cirrus-sac, displaced to poral side in mature proglottids; seminal vesicle absent. Genital pores lateral,  $15-57 \% (32 \pm$ 2; n = 36; n = 44) of proglottid length from posterior end, irregularly alternating. Vagina extending from Mehlis' gland anteriorly, initially median, strongly wriggle, then lateral to cirrus-sac in mature proglottids, opening anterior to cirrus-sac in genital atrium; vaginal sphincter absent. Genital atrium simple, tubular,  $50-300 (110 \pm 9; n = 36) \log_{10}$  $30-200 (91 \pm 7; n = 36)$  wide (fig. 3, 7). Ovary near posterior end of proglottids, median, H-shaped, tetralobed in cross-section; lobes asymmetrical. In mature proglottids, ovary lobes surrounding excretory ducts, extending almost to lateral margin of proglottid, separating vitellarium from posterior end of proglottid. Aporal ovary lobe 100–650 (268  $\pm$ 27; n = 36) long, 110-700 (329  $\pm$  21; n = 36) wide, poral lobe 100-600 (250  $\pm$  25; n = 36) long, 100-600 (312  $\pm 20$ ; n = 36) wide (fig. 4, 8). Ovicapt at posterior margin of isthmus, large, spherical or oviform, 50-200 ( $84 \pm 5.4$ ; n = 36; n = 39) in diameter. Mehlis' gland 20-200 (61 ± 8; n = 32) wide, invisible in some proglottids. Uterus ventral to vagina, extending from isthmus to median line of proglottid, not reaching its anterior end (fig. 4, 8). Vitellarium follicular; follicles numerous, 10-90 (43 ± 4; n = 36) in diameter in mature proglottids (fig. 2, 4, 8); in 2 lateral fields, 100-720 (246 ± 19: n = 36; n = 39) wide, each consisting of one dorsal and one ventral row of follicles, between which excretory ducts passes: extending from anterior end of proglottid to anterior margin of ovary, not interrupted at level of genital atrium, becoming thin, not extending posteriorly to ovary. Eggs oval, 25-40 ( $36 \pm 3$ ; n = 4; n = 6) long, 20 wide (n = 4).

Remarks. Absence of apical sucker is considered as a main differential character of the new genus. For the proof of this fact, the living specimens of *Cairaeanthus* were studied. So far as the marginal loculi of *C. ruhnkei* are small and the apical suckers may be only slightly larger than the marginal loculi, that makes their recognition difficult, especially on fixed material (e. g. in many species of *Anthocephalum*), movements of bothridia in the living specimens were examined. Additional structures on the bothridia were not found in both alive and fixed specimens.

Differential diagnosis. *Cairaeanthus ruhnkei* sp. n. differs from all known species of the closely related genus *Anthocephalum*, namely *A. gracile* (Wedl, 1855), *A. centru-rum* Ruhnke, 1994, *A. alicae* Ruhnke, 1994, *A. cairae* Ruhnke, 1994, *A. duszynskii* Ruhnke, 1994, *A. kingae* Ruhnke, Seaman, 2009, *A. michaeli* Ruhnke, Seaman, 2009, *A. lukei* Ruhnke, Seaman, 2009 and *A. currani* Ruhnke, Seaman, 2009 in a number of characters. In addition to differences mentioned above in the generic diagnosis, *C. ruhnkei* can be distinguished from them by larger total length (56–255 mm vs 40–50 mm, 11–20 mm, 4–9 mm, 8–14 mm, 18–31 mm, 8.6–13 mm, 5.7–16.3 mm, 7.9–17.2 mm, 6.6–14.4 mm, respectively) and by more proglottids per worm (495–1803 vs 500–600, 35–65, 9–15, 80–110, 120–160, 33–50, 23–41, 28–56, 35–70, respectively). Moreover, *C. ruhnkei* differs from *A. gracile* in smaller number of testes (51–98 vs 100–130) and from *A. cairae*, *A. kingae*, *A. michaeli*, *A. lukei* and *A. currani*, on the contrary, in larger number of testes (51–98 vs 28–52, 30–37, 30–49, 32–48, 37–50 respectively).

# Cairaeanthus healyae Kornyushin et Polyakova, sp. n. (fig. 9-17)

Type host: Dasyatis pastinaca (L., 1758) (сем. Dasyatidae).

Type locality: Martynova Bay, Sevastopol, Black Sea (44°37.08'N, 33°30.28'E). Additional localities: the Black Sea (off Sevastopoloff; the cape Sarych and Phoros; Karkinitskiy Bay, of the Black Sea Biosphere Reserve, Yagorlytskiy Bay).

Site of infection: spiral intestine.

Type specimens: holotype C. 137.001.163 (10.07.2002; 982/139; spiral valve N 8; 1 specimen; Martynova Bay, Sevastopol, the Black Sea; IBSS, DEP; coll. T. A. Polyakova) and paratypes C. 26.001.27 (21.07.2001; 87/30a; spiral valve N 11; 1 specimen; Martynova Bay, Sevastopol, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 48.001.54 (28.12.2001; 186/45; spiral valve N 10; 1 specimen; cape Phoros, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 49.001.55 (28.12.2001; 186/46; spiral valve N 10; 1 specimen; cape Phoros, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 49.001.55 (28.12.2001; 186/46; spiral valve N 10; 1 specimen; cape Phoros, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 49.001.55 (28.12.2001; 186/46; spiral valve N 10; 1 specimen; cape Phoros, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 49.001.55 (28.12.2001; 186/46; spiral valve N 10; 1 specimen; cape Phoros, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 49.001.55 (28.12.2001; 186/46; spiral valve N 10; 1 specimen; cape Phoros, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 49.001.55 (28.12.2001; 186/46; spiral valve N 10; 1 specimen; cape Phoros, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 49.001.55 (28.12.2001; 186/46; spiral valve N 10; 1 specimen; cape Phoros, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 49.001.55 (28.12.2001; 186/46; spiral valve N 10; 1 specimen; cape Phoros, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 49.001.55 (28.12.2001; 186/46; spiral valve N 10; 1 specimen; cape Phoros, the Black Sea; IBSS, Phoros, the Black Sea; Phoros, the Bl

imen; cape Phoros, the Black Sea; IBSS, DEP; coll. T. A. Polyakova), C. 59.001.65 (28.12.2001; 186/57; spiral valve N 10; 1 specimen; cape Phoros, the Black Sea; IBSS, DEP; coll. T. A. Polyakova) ex *D. pastinaca* from the Black Sea deposited in the collection of the Department of Ecological Parasitology, Institute of Biology of the Southern Seas NASU, Sevastopol. Additional paratypes CP 36.1 (04.07.1977; mk 12; Black Sea, Biosphere Reserve, Yagorlytskiy Bay; Institute of Zoology; coll. V. V. Kornyushin) ex



Fig. 9–12. *Cairaeanthus healyae* ex *Dasyatis pastinaca* from the Black Sea: 9 – scolex, paratype C. 26.001.27; 10 immature proglottid, holotype C. 137.001.163; 11 – inverted cirrus, holotype C. 137.001.163; 12 – mature proglottid, holotype C. 137.001.163 (vitelline follicles in poral side proglottides have been omitted). Scale bars:  $9 - 300 \mu m$ ; 10, 12 – 200  $\mu m$ ; 11 50  $\mu m$ .

Рис. 9–12. *Cairaeanthus healyae* от *Dasyatis pastinaca* из Черного моря: 9 — сколекс, паратип С. 26.001.27; 10 — неполовозрелая проглоттида, голотип С. 137.001.163; 11 — инвагинированный циррус, голотип С. 137.001.163; 12 — половозрелая проглоттида, голотип С. 137.001.163 (желточные фолликулы на поральной стороне проглоттиды были опущены). Масштабная линейка: 9 — 300 мкм; 10, 12 — 200 мкм; 11 — 50 мкм.

*D. pastinaca* from the Black Sea are in the collection of the Department of Parasitology, I. I. Schmalhausen Institute of Zoology, NASU, Kyiv.

Etymology: This species is named for Dr Claire J. Healy, for her contribution to the study of tetraphyllidean and rhinobothriidean tapeworms.

Description. Based on holotype, 4 paratypes and 26 specimens from *D. pastinaca* from the Black Sea.

Worms mid-size, craspedote, apolytic,  $108-315 \text{ mm} (188 \pm 8.8; n = 31) \log_2 200-1700 (687 \pm 49; n = 31; n = 62)$  wide. Proglottids  $1003-2395 (1450 \pm 74; n = 31)$  in number. Scolex large,  $720-3000 (1650 \pm 75; n = 31; n = 34) \log_1 (1250-3000 (2133 \pm 67; n = 31; n = 35))$  wide, tetrabothridiate (figs 9, 13). Myzorhynchus absent. Bothridia  $600-1620 (1002 \pm 42; n = 30; n = 33) \log_2 260-1440 (631 \pm 42; n = 30; n = 54)$  wide; posteriorly bifid; with numerous marginal loculi, each  $10-30 (25 \pm 1; n = 31; n = 51) \log_1 10-30 (24 \pm 1; n = 31; n = 51)$  wide; with pedicel  $180-500 (318 \pm 14; n = 31; n = 38) \log_1 130-540 (244 \pm 14; n = 31; n = 41)$  wide (fig. 14). Apical sucker absent. Cephalic peduncle very long,  $2.2-11.7 \text{ mm} (8.1 \pm 0.3; n = 31)$  in length,  $200-750 (465 \pm 20; n = 31; n = 43)$  wide. Lateral excretory ducts  $10-90 (31 \pm 2.2; n = 31; n = 43)$  in diameter.

Immature proglottids square, 30-380 ( $130 \pm 8$ ; n = 31; n = 71) long, 230-950 ( $516 \pm 100$ ) 19: n = 31; n = 70) wide, initially wider than long (fig. 10), becoming longer than wide with maturity. Mature proglottids trapezoid, 230-1170 (445 ± 28; n = 31; n = 38) long,  $400-1500 (918 \pm 39; n = 31; n = 41)$  wide. Terminal proglottids,  $520-2100 (1024 \pm 39)$ 63; n = 31; n = 50) long, 320-1700 ( $859 \pm 44$ ; n = 31; n = 51) wide, greatly elongate, sometimes oval, tapered to posterior end (fig. 17). Testes numerous, 103-157 ( $127 \pm 2$ ; n = 31; n = 51) in number (fig. 10, 12, 17), median, arranged in two layers, round, 20-80  $(46 \pm 2.3; n = 31; n = 44)$  in diameter; post-poral testes absent. Cirrus-sac big, oval, 50-280 (148 ± 8.5; n = 31; n = 46) long, 50-450 (169 ± 13; n = 31; n = 40) wide (fig. 11, 15, 16). Cirrus armed with spinitriches. Total length of everted cirrus 850-1350 (1052  $\pm$ 92; n = 5), of inverted 260–1030 (427  $\pm$  20; n = 30; n = 53). Vas deferens 10–180 (63  $\pm$ 5; n = 31; n = 43) in diameter, coiled, extending anteriorly to cirrus-sac; median, slightly displaced to poral side in mature proglottids; seminal vesicle absent. Genital pores lateral, 20-50 % ( $30 \pm 1.2$ ; n = 31; n = 47) of proglottid length from posterior end, irregularly alternating. Vagina extending from Mehlis' gland to genital atrium, initially median, straightened, then lateral to cirrus-sac in mature proglottids, opening anterior to cirrus-sac in genital atrium; wall of distal end of vagina dense, glandular, with inside folds almost closing vaginal duct; vaginal sphincter absent. Genital atrium 40-210 (98 ± 9; n = 31; n = 33 long, 30-180 (71 ± 5; n = 31; n = 35) wide; its proximal part near to opening of cirrus-sac covered by numerous spinitriches (fig. 11, 15, 16). Ovary near posterior end of proglottids, median, H-shaped, tetralobed in cross-section; lobes asymmetrical. In mature proglottids, ovary lobes surrounding excretory ducts, extending almost to lateral margin of proglottid, separating vitellarium from posterior end of proglottid. Aporal ovary lobe 100–640 (197  $\pm$  20; n = 31; n = 38) long, 110–500 (262  $\pm$  13; n = 31; n = 38) wide, poral ovary lobe 100–550 (166  $\pm$  16; n = 31; n = 38) long, 110–440 (248  $\pm$ 13; n = 31; n = 38) wide. Ovicapt 50–200 (94 ± 5; n = 31; n = 34) in diameter. Mehlis' gland cariniform, fabiform or spherical, 10-60 (28 ± 2; n = 29) wide. Uterus ventral to vagina, extending from isthmus to median line of proglottid, reaching its anterior end (figs 12, 17). Vitellarium follicular; follicles numerous, 10-90 (43 ± 3; n = 31; n = 38) in diameter in mature proglottids; in 2 lateral fields, 90-400 (173 ± 11; n = 31; n = 37) wide, each consisting of one dorsal and one ventral row of follicles, between which excretory ducts passes; extending from anterior end of proglottid to anterior margin of ovary, not interrupted at level of genital atrium, becoming thin, not extending posteriorly to ovary. Eggs oval, 40 long, 20 wide (n = 1).

Remarks. In the living specimen of *C. healyae* bothridia are very mobile and resemble the movement of the octopus feelers. The specimens of *C. healyae* are very delicate in comparison with the scolex and the strobila of *C. ruhnkei*. Spinitriches were found in



Fig. 13–17. Photomicrographs of *Cairaeanthus healyae* ex *Dasyatis pastinaca* from the Black Sea: 13 – scolex, paratype C. 26.001.27; 14 – marginal locules, paratype C. 26.001.27; 15 – inverted cirrus, holotype C. 137.001.163; 16 – everted cirrus, holotype C. 137.001.163; 17 – mature proglottid, holotype C. 137.001.163. Scale bars: 13 – 300  $\mu$ m; 14, 15 – 50  $\mu$ m; 16, 17 – 200  $\mu$ m; A – vagina; B – spinitriches of genital atrium; C – cirrus; D – cirrus-sac.

Рис. 13–17. Микрофотография *Cairaeanthus healyae* от *Dasyatis pastinaca* из Черного моря: 13 — сколекс, паратип С. 26.001.27; 14 — краевые локулы, паратип С. 26.001.27; 15 — инвагинированный циррус, голотип С. 137.001.163; 16 — эвагинированный циррус, голотип С. 137.001.163; 16 — эвагинированный циррус, голотип С. 137.001.163; 17 — половозрелая проглоттида, голотип С. 137.001.163. Масштабная линейка: 13 — 300 мкж; 14, 15 — 50 мкж; 16, 17 — 200 мкж; А — вагина; В — спинитрихии полового атриума; С — циррус; D — бурса цирруса.

the genital atrium of *C. healyae* near opening of cirrus-sac, but their shape was not good examined in the present work. However, size of these spinitriches exceeds the size of the spinitriches of the cirrus. In immature proglottids the spinitriches of the genital atrium are located in its central part on equal distance from the cirrus-sac and the vagina (fig. 15, B) looking as «muff».

Differential diagnosis: *Cairaeanthus healyae* sp. n. significantly differs from *C. ruhnkei* sp. n. in longer strobila (108-315 mm vs 56-255 mm), smaller sizes of marginal loculi ( $10-30 \times 10-30 \text{ vs } 50-100 \times 30-100$ ) larger length of cephalic peduncle (2.2-11.7

loculi ( $10-30 \times 10-30 \ vs \ 50-100 \times 30-100$ ), larger length of cephalic peduncle (2.2-11.7 mm vs 200-680), smaller diameter of testes ( $20-80 \ vs \ 30-130$ ) and smaller width of vitelline fields ( $90-400 \ vs \ 100-720$ ), number of testes ( $103-157 \ vs \ 51-98$ ) and proglot-tids ( $1003-2395 \ vs \ 495-1803$ ). Additionally, the wall of the genital atrium is armed by the spinitriches in *C. healyae*, but is not in *C. ruhnkei*.

In addition to characters mentioned in generic diagnosis, *C. healyae* can be distinguished from all known *Anthocephalum* spp. by larger total length (108–315 mm vs 40–50 mm in *A. gracile*, 11–20 mm in *A. centrurum*, 4–9 mm in *A. alicae*, 8–14 mm in *A. cairae*, 18–31 mm in *A. duszynskii*, 8.6–13 mm in *A. kingae*, 5.7–16.3 mm in *A. michaeli*, 7.9–17.2 mm in *A. lukei*, 6.6–14.4 mm in *A. currani*), more proglottids per worm (1003–2395 vs 500–600, 35–65, 9–15, 80–110, 120–160, 33–50, 23–41, 28–56, 35–70, respectively) and by larger number of the testes (103–157 vs 100–130, 35–75, 30–67, 28–52, 35–71, 30–37, 30–49, 32–48, 37–50, respectively).

# Morphometric analysis of Cairaeanthus ruhnkei sp. n. and C. healyae sp. n.

The choice of diagnostic characters for species determination is one of the principal questions in generic taxonomy. In order to deicide which characters and which differences are adequate for the species differentiation, it is necessary to have information on the range of intraspecific variability. For this purpose 38 characters (36 measured and 2 discrete) from 67 specimen of *Cairaeanthus* spp. were analysed using t-tests for independent variables (table 1) and Principal Component Analysis (PCA).



Fig. 18. PCA plot of the contributions made by 24 characters to the first two factors calculated from 66 specimens of two species of *Cairaeanthus* spp. ex. *Dasyatis pastinaca* from the Black Sea and the Sea of Azov. Separated are 14 characters making the greatest contribution to separation of this species: 1 - number of proglottids; 2 - length of cephalic peduncle; 3 - number of testes; 4 - length of mature proglottids; 5 - length of pedicels bothridia; 6 - length of strobila; 7 - width of scolex; 8 - length of bothridia; 9 - length of cirrus-sac; 10 - length of mature proglottids; 12 - width of mature proglottids; 13 - length of marginal loculi; 14 - width of marginal loculi.

Рис. 18. РСА график факторных нагрузок 26 признаков, использованных для дифференциации 66 экземпляров *Cairaeanthus* spp. от *Dasyatis pastinaca* из Чёрного и Азовского морей. Выделены 14 признаков, делающих наибольший вклад в разделение видов: 1 — количество проглоттид; 2 — длина головного стебелька; 3 — количество семенников; 4 — длина половозрелых проглоттид; 5 — длина стебелька ботридия; 6 — длина стробилы; 7 — ширина сколекса; 8 — длина ботридий; 9 — длина бурсы цирруса; 10 — длина яичника; 11 — ширина половозрелых проглоттид; 12 — ширина стробилы; 13 — длина краевых локул; 14 — ширина краевых локул. *C. ruhnkei* significantly differs from *C. healyi* in 26 of the 38 investigated characters. These different characters describe size of strobila, marginal loculi, cephalic peduncle, immature and mature proglottids and ovary, as well as width of scolex, terminal proglottids, genital atrium, vitelline fields, Mehlis' gland, length of bothridia and pedicel, cirrus-sac, diameter of excretory ducts and testes, number of testes and proglottids (table 1). These 26 characters were also analysed by PCA, they were reduced to two principal components (i. e. Factors) which explained more than 50 % of the overall variance, and

Table 1. Morphological characters of	Cairaeanthus	ruhnkei sp. 1	1. and	C. healya	e sp. n.	. ex	Dasyatis	pastinaca
from the Black Sea and the Sea of Azor	V							

		Black Sea and Sea of Azov	Black Sea	
Characters		X ±	SE*	T-test
		Cairaeanthus ruhnkei sp. n. $(n = 36)$	Cairaeanthus healyae sp. n. $(n = 30)$	1 1001
Strobila	Length, mm	$123.0 \pm 8.3$	$182.2 \pm 9.6$	4.7**
	Width	$960 \pm 29$	677 ± 31	-6.7
Scolex	Length	$1447 \pm 72$	$1642 \pm 83$	1.8
	Width	$1789 \pm 70$	$2096 \pm 75$	3.0
Bothridia	Length	$839 \pm 47$	986 ± 42	2,3
	Width	$639 \pm 34$	$622 \pm 24$	-0.4
Pedicels bothridia	Length	$273 \pm 17$	357 ± 19	3.3
	Width	$251 \pm 20$	$247 \pm 15$	0.1
Marginal loculi	Length	$57 \pm 2$	$25 \pm 1.1$	-11.6
	Width	$52 \pm 2$	$24 \pm 1.1$	-12.2
Cephalic peduncle	Length	$460 \pm 22$	8117 ± 354	23.7
	Width	$558 \pm 26$	459 ± 21	-2.9
Excretory ducts	Diameter	$35 \pm 2$	$28 \pm 2$	-2.3
Immature proglottids	Length	$121 \pm 8$	$165 \pm 9,4$	3.5
	Width	$703 \pm 35$	557 ± 23	-3.6
Mature proglottids	Length	$331 \pm 19$	$447 \pm 26$	3.7
	Width	$1198 \pm 44$	$867 \pm 45$	-5.2
Terminal proglottids	Length	$1071 \pm 95$	$1105 \pm 81$	0.3
	Width	$1194 \pm 50$	796 ± 52	-5.5
Position of genital pores, %		$32 \pm 2$	$31 \pm 1.2$	-0.2
Number of testes		$86 \pm 2$	$126 \pm 2.1$	13.03
Testes	Diameter	$62 \pm 4$	47 ± 3	-2.9
Vas deferens	Diameter	$61 \pm 5$	$60 \pm 5$	-0.13
Cirrus-sac	Length	$204 \pm 13$	$159 \pm 10$	-2.8
	Width	$156 \pm 11$	$176 \pm 17$	1.03
Cirrus	Length	$526 \pm 27$	$549 \pm 48$	-1.3
Genital atrium	Length	109 ± 9	93 ± 9	-1.2
	Width	91 ± 7	$68 \pm 5$	-2.6
Ovary	Length	$255 \pm 28$	$138 \pm 16$	-2.8
	Width	$343 \pm 22$	$237 \pm 14$	-4.3
Ovicapt	Diameter	$86 \pm 6$	92 ± 5	0.8
Mehlis' gland	Width	$61 \pm 8$	$28 \pm 2$	-3.7
Vitellaruim filds	Width	$241 \pm 17$	$164 \pm 10$	-3.7
Vitelline follicles	Diameter	$43 \pm 4$	42 ± 3	0.1
Eggs	Length	34	40	34.2
	Width	20	20	20.0
Number of proglottids		$981 \pm 48$	$1447 \pm 69$	5.7

Таблица. 1 Морфологические признаки *Cairaeanthus ruhnkei* sp. n. и *C. healyae* sp. n. от *Dasyatis pastinaca* из Черного и Азовского морей

\* X – mean,  $\mu$ m;  $\pm$  SE – standard error,  $\mu$ m; T-test – independent t-test.

\*\* T-test: significant differences at the  $p \le 0.05$ 



Fig. 19. PCA plot of the scores of the first two factors calculated from 14 characters for 67 specimens belonging to two species of *Cairaeanthus* spp. ex. *Dasyatis pastinaca* from the Black Sea and the Sea of Azov ( $\rightarrow$  direction of increasing characters separating the specimens).

Рис. 19. РСА график расположения 67 экземпляров *Cairaeanthus* spp. от *Dasyatis pastinaca* из Черного и Азовского морей в системе координат первых двух факторов, вычисленных на основе 14 морфологических признаков (— направление увеличения значения признаков).

14 characters, which made the greatest contributions for this Factors were chosen (fig. 18). These were the total number of proglottids and testes, the length of cephalic peduncle, mature proglottids, bothridium pedicel, strobila, bothridia, cirrus sac, ovary and marginal loculi, width of scolex, mature proglottids, strobila and marginal loculi.

As a result of PCA done on metrical data for 14 selected characters, 67 measured specimens were clearly divided on the PCA plots based on their scores in the first plane of the PCA (explaining 60 % of the overall variance) into two groups corresponding to each species (fig. 19). The number of proglottids and testes and the length of cephalic peduncle contributed most to separation of *C. ruhnkei* sp. n. from *C. healyae* sp. n. by Factor 1, explaining 44 % of the overall variance (fig. 19). The size of marginal loculi, the width of strobila and mature proglottids made the greatest contributions for Factor 2, however, explained only 16 % of the overall variance. Thus, using the methods of the multivariate statistics permits successful differentiation of morphologically related congeneric cestodes.

# Discussion

New species, *C. healyae* and *C. ruhnkei*, resemble *Phyllobothrium pastinacae* Mokhtar-Mocamouri, Zamali, 1981 (Mokhtar-Mocamouri, Zamali, 1981) in the following characters: bothridia posteriorly bifid; myzorhynchus absent; bothridia pedicellate, cephalic peduncle present; post-poral testes absent; genital pore opening marginally, close to posterior end of proglottid; vitelline follicles numerous, extending from anterior end of proglottid to ovary, interrupted at level of ovary, not extending posteriorly to it. Studied material on *Phyllobothrium pastinacae* (MNHN 90HB148 c VII; MNHN 90HB149 c VII; MNHN 90HB150 c VII) included two total specimens and one detached terminal proglottid. Since the authors of *P. pastinacae* did not specify the holotype specimen, we supposed that the specimen from the slide MNHN 90HB148 c VII, which was represented on fig. 1 in Mokhtar-Mocamouri, Zamali (1981: p. 377) as the holotype. Cestodes from the both slides differed from *Cairaeanthus ruhnkei* sp. n. and *C. healyae* sp. n. by

the presence of the apical sucker and by the facial loculi in the specimen from MNHN 90HB148 c VII.

F. Mokhtar-Mocamouri and Z. Zamali assigned *P. pastinacae* to the genus *Phyllobothrium* based on the shape of the scolex, presence of 4 pedicel bothridia, which are posteriorly bifid and have the marginal loculi, of long cephalic peduncle and absence of the post-poral testes in the proglottids (Mokhtar-Mocamouri, Zamali, 1981: p. 379). However, mentioned morphological characters, except for posteriorly bifid bothridia, do not correspond to the present diagnosis of the genus *Phyllobothrium* (Ruhnke, 1996 a).



Fig. 20–23. Photomicrographs of *Phyllobothrium pastinacae* (MNHN 90HB148 с VII): 20 — scolex; 21 — facial locules; 22 — inverted cirrus; 23 — mature proglottid. Scale bars: 20 — 500 µm; 21 — 100 µm; 22, 23 — 200 µm. Puc. 20–23. Микрофотография *Phyllobothrium pastinacae* (MNHN 90HB148 с VII): 20 — сколекс; 21 — лицевые локулы; 22 — инвагинированный циррус; 23 — половозрелая проглоттида (желточные фолликулы на поральной стороне проглоттиды были опущены). Масштабная линейка: 20 — 500 мкж; 21 — 100 мкж; 22, 23 — 200 мкм.



Fig. 24–28. Photomicrographs of *Phyllobothrium pastinacae* (24, 25, 26, 27 – MNHN 90HB149 c VII, 28 – MNHN 90HB150 c VII): 24 – scolex; 25 – marginal loculi; 26 – apical sucker; 27 – mature proglottid; 28 – gravid proglottid. Scale bars: 24 – 400  $\mu$ m; 25, 26, 27 – 200  $\mu$ m; 28 – 100  $\mu$ m.

Рис. 24–28. Микрофотография *Phyllobothrium pastinacae* (24, 25, 26, 27 — MNHN 90HB149 с VII, 28 — MNHN 90HB150 с VII): 24 — сколекс; 25 — краевые локулы; 26 — апикальные присоски; 27 — половозрелая проглоттида; 28 — зрелая проглоттида. Масштабная линейка: 24 — 400 мкм; 25, 26, 27 — 200 мкм; 28 — 100 мкм.

New morphological characters were found during the reinvestigation of the type material of *P. pastinacae*. On the bothridia of the holotype specimen numerous facial loculi are present, and the paratype specimen has marginal loculi; in all type specimens of *P. pastinacae* the vaginal sphincter is absent and the vitelline fields stop at the anterior margin of the ovary, not reaching the posterior end of the proglottid. These facts confirm that *P. pastinacae* is not a representative of *Phyllobothrium*.

Moreover, the presence of facial loculi on bothridia in one of the two examined type specimens of *P. pastinacae* distinguishes it from the following phyllobothrideans:

Anthobothrium van Beneden, 1850; Monorygma Diesing, 1863; Orygmatobothrium Diesing, 1863; Dinobothrium van Beneden, 1889; Thysanocephalum Linton, 1889; Crossobothrium Linton, 1889; Calyptrobothrium Monticelli, 1893; Carpobothrium Shipley, Hornell, 1906; Myzocephalus Shipley, Hornell, 1906; Myzophyllobothrium Shipley, Honell, 1906; Pithophorus Southwell, 1925; Scyphophyllidium Woodland, 1927; Gastrolecithus Yamaguti, 1952; Marsupiobothrium Yamaguti, 1952; Pseudanthobothrium Baer, 1956; Clydonobothrium Euzet, 1959; Clistobothrium Dailey, Vogelbein, 1990; Paraorygmatobothrium Ruhnke, 1994; Orectolobicestus Ruhnke, Caira, Carpenter, 2006 (Yamaguti, 1959; Euzet, 1994; Ruhnke, 1994, 1996 a, b; Ruhnkeetal., 2006; Marquesetal., 2001; Randhawaetal., 2008). The presence of the apical sucker on the scolex of the both type specimens of P. pastinacae distinguishes them from Trilocularia Olsson, 1867; Tritaphros Lonnberg, 1889; Pentaloculum Alexander, 1963; Phormobothrium Alexander, 1963; Zyxibothrium Hayden, Campbell, 1981 and the absence of post-poral testes — from Caulobothrium Baer, 1948; Glyphobothrium Williams, Campbell, 1977; Duplicibothium Williams, Campbell, 1978; Chimaerocestos Williams, Bray, 1984 and Anindobothrium Marques, Brook, Lasso, 2001 (Euzet, 1994).

Finally, type specimens of *P. pastinacae* clearly differ in the scolex shape, absence of armaments of the scolex and post-poral testes, and also in the position of the genital pore near the posterior end of the proglottid from the following taxa of the order Tetraphyllidea: Onchobothriidae Braun, 1900; Disculicipitidae Joyeux, Baer, 1936; Prosobothriidae Baer, Euzet, 1955; Litobothriidae Dailey, 1969; Dioecotaeniidae Schmidt, 1969; Cathetocephalidae Dailey, Overstreet, 1973; Chimaerocestidae Williams, Bray, 1984 (Euzet, 1994).

Thus, the cestodes in the type material of *P. pastinacae* belong neither to the genus *Phyllobothrium* nor to the order Tetraphyllidea.

The two whole type specimens of *P. pastinacae* should be assigned to the order Rhinebothiidea (Healy et al., 2009) based on the presence of the apical sucker on the pedicel bothridia, of the facial loculi in one specimen and the marginal loculi in the other, by the presence of cephalic peduncle and also by the absence of post-poral testes and by the distal position of the genital pore near the posterior end of proglottid.

Moreover, the two specimens from the slides examined differ from each other by some substantial qualitative characters (e. g., presence of the marginal, or the facial loculi) (fig. 20-28) and a number of metrical characters, and apparently belong to two separate species of Rhinebothriidea.

Reinvestigation of the type material of *Phyllobothrium pastinacae*, which has some similarity with *Cairaeanthus* spp., showed that these specimens do not belong to *Phyllobothrium* and the order Tetraphyllidea, but are the members of the order Rhinebothriidea. However, the quality of available slides was found to be insufficient for the finally decision on the taxonomic belonging of these cestodes. Additional material from the same sea and host is required for more detailed description of these species and identification of their generic status. It might be even supposed that these species and *Cairaeanthus* spp. may be members of different genera from one family, but for confirmation of this assumption further study is necessary.

During the recent years, the interest of helminthologists to the lowest cestodes parasitizing elasmobranches, namely tetraphyllideans, lecanicephalideans, trypanorhyncha etc., has taken a remarkable start. At present, a tendency to the restriction of orders, families and genera with the separation of new or the restoration of "old" taxa presents in the works (Ruhnke, 1994 b, 1996 a–b; Ruhnke, Seaman, 2009; Ruhnke et al., 2006; Ruhnke, Carpenter, 2008). The results of the molecular phylogenetic analysis of rhinobothriines confirmed previous hypotheses on their independence from Tetraphyllidea and proved monophyly of the order Rhinebothriidea (Healy et al., 2009). Eight valid genera, previously the members of Tetraphyllidea, were included in the restored order Rhinebothriidea in the new combination. In connection with this revision the taxonomic status of most cestode species from elasmobranches in the Black Sea and the Sea of Azov needs to be defined more precisely. Before the present study, cestodes from stingrays *D. pastinaca* and *R. clavata* from the Black Sea and the Sea of Azov have been identified as *Phyllobothrium lactuca* and *P. gracilis* (Borcea, 1934; Pogorelceva, 1960; Gaevskaya et al., 1975). We showed that those were representatives of the order Rhinebothriidea and belonged to a new genus *Cairaeanthus*. The order Rhinebothriidea thus comprises nine genera now.It should be noted that in the previous articles (Borcea, 1934; Pogorelceva, 1960; Gaevskaya et al., 1975) *Phyllobothrium lactuca* and *P. gracilis* in the Black Sea and the Sea of Azov were reported from both stingray species occurring in these seas. However, we found both species of *Cairaeanthus* only in *D. pastinaca*, although cestodes of *R. clavata* were examined too. The latter fish species was parasitized by four species of cestodes: *Grillotia erinaceus* (van Beneden, 1858), *Acanthobothrium dujardini* (van Beneden, 1850), *Echeneibothrium variabile* (van Beneden, 1850), and *Echinobothrium typus* (van Beneden, 1849).

Thus, *D. pastinaca* in the Black Sea off Crimea is parasitized by *C. ruhnkei* and *C. healyae*, and in the Sea of Azov — only by *C. ruhnkei*. These species have been found only in these seas and only in this host so far. Moreover, in the Black Sea the genus *Phyllobothrium* s. str., is currently represented by one species, *Phyllobothrium squali* (Yamaguti, 1952) parasitizing *Squalus acanthias* L., 1758 (Vasileva et al., 2002).

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