Third-stage juveniles of *Contracaecum* sp. (Anisakidae, Ascaridomorpha) from the round goby *Neogobius melanostomus* of the Black Sea

Natalia V. Pronkina[1] and Sergei E. Spiridonov[2]

[1]A.O. Kovalevsky Institute of Marine Biological Research, Russian Academy of Sciences, Nakhimov Avenue 2, 299011, Sevastopol, Russia
[2]A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii Prospect 33, 119071, Moscow, Russia
e-mail: natalya-pronkina@yandex.ru

Accepted for publication 25 May 2020

Summary. A long-term parasitological survey carried on the round goby, *Neogobius melanostomus*, from the Black Sea has revealed its high level of infection with anisakid juveniles. In previous studies, some of the juvenile stages of the genus *Contracaecum* were identified as *C. microcephalum* according to available identification keys based on morphology, although the latter species is considered more common in fresh-water habitats. Analysis of nucleotide sequences of LSU rDNA and ITS rDNA of *Contracaecum* juveniles from the round goby *Neogobius melanostomus* demonstrated their identity with the cryptic species *Contracaecum rudolphii* A of a species complex *Contracaecum rudolphii* Hartwich, 1964 *sensu lato*.

Key words: ascaridids, ITS rDNA, LSU rDNA, morphotype, phylogeny.

The nematodes of the genus *Contracaecum* Railliet & Henry, 1912 are common parasites of marine fish-eating birds and mammals and can also be found in fresh-water habitats. This taxon is the largest in the family Anisakidae Railliet & Henry, 1912, comprising more than 50 valid species. Adult nematodes and fourth-stage juveniles can be found in the alimentary tract of birds and mammals, while invertebrates and fishes serve as paratenic/intermediate hosts for third-stage juveniles. The identification of species with the use of the third-stage juveniles is hindered by their monotonous morphology. Often, *Contracaecum* juveniles can be identified only to the generic level as descriptions of the juvenile stages are still not available for all known *Contracaecum* species. Three species from definitive hosts in the coastal waters of the Crimean peninsula: *C. microcephalum*, *C. micropapillatum* and *C. rudolphii* (considered as *C. spiculgerum*) were reported earlier (Gaevskaya *et al.*, 1975).

The examination of the *Contracaecum* juvenile diversity in the Black Sea fishes revealed the presence of two morphotypes. One of these morphotypes was regularly found in the golden grey mullets (*Chelon auratus*) of the Black Sea, and was identified on the basis of nucleotide sequence analysis as *Contracaecum multipapillatum* (Pronkina & Spiridonov, 2018). The identification of another morphotype, which was especially common in the round goby, as *Contracaecum microcephalum* was questioned, as usually this species was reported from fresh-water habitats (Mozgovoy *et al.*, 1968; Kornyushin *et al.*, 2004).

The results of morphological and molecular study of these juveniles are presented below.

MATERIAL AND METHODS

The round gobies (439 specimens in total) were caught in 2007-2017 in shallow (0.3-1.5 m depth) waters of Karkinit Bay (offshore Lebjazhii islands – 45°52’30” N; 33°32’30” E) in the water territory of the Crimean Nature Reserve, under a permit issued by the Reserve administration. The dissection of round gobies revealed the presence of anisakid juveniles in capsules on the serosa covers and in the abdominal cavity. Parasite prevalence of 3% with parameters of the intensity of 1-2 worms per host and abundance of 0.04 worms per individual host were observed.

The nematodes recovered were fixed in 70% ethyl alcohol and mounted in glycerin-lactic acid 1:1 mixture. The middle body parts of three specimens were fixed in 95% ethanol for DNA extraction and remaining body parts were mounted to serve as a voucher specimen, 'hologenophore' (Pleijel *et al.*, 2008). The voucher specimen was deposited in the Collection of marine parasites (CMP IMBR №1267-1273.N.2p.V 1-7) of the A.O. Kovalevsky Institute of Biology of the Southern Seas, Sevastopol, IBSS collection (Dmitrieva *et al.*, 2015; http://marineparasites.org/). All the measurements are in micrometers. Line-art images were made on the basis of images obtained from an Olympus CX-41 microscope with a CAM-SC50 camera. The images were processed in Inkscape 0.48.2.-1 (2011. Scalable Vector Graphics (SVG): http://www.inkscape.org/).

Table 1. Accession numbers and related information for the *Contracaecum* sequences used in the phylogenetic analysis.

Nematode species	Host	Geographic origin	NCBI accession number	Reference
LSU rDNA				
C. eudyplulae	*Eudyptula minor*		AF226586	Nadler *et al.*, 2000
C. ogmorhini	*Arctocephalus pusillus pusillus*	South Africa	AF226582	Nadler *et al.*, 2000
C. rudolphii B	*Phalacrocorax carbo*	Policoro, Italy	AF226579	Nadler *et al.*, 2000
C. rudolphii	*Gasterosteus aculeatus*	North Sea, Germany	KT767121	Schade *et al.*, 2015
C. rudolphii	*Gasterosteus aculeatus*	North Sea, Germany	KT767120	Schade *et al.*, 2015
C. rudolphii A	*Phalacrocorax carbo*	Policoro, Italy	AF226585	Nadler *et al.*, 2000
C. septentrionalis	*Phalacrocorax carbo*	Husavik, Iceland	AF226588	Nadler *et al.*, 2000
C. septentrionalis	*Sprattus sprattus*	North Sea, Germany	KT767122	Schade *et al.*, 2015
C. micropapillatum	*Pelecanus onocrotalus*	Assuan, Egypt	AF226587	Nadler *et al.*, 2000
C. microcephalum	*Phalacrocorax pygmaeus*	Scutari lake, Yugoslavia	AF226573	Nadler *et al.*, 2000
C. multipapillatum	*Pelecanus crispus*	Psatatopi, Greece	AF226574	Nadler *et al.*, 2000
C. miroungae	*Mirounga leonina*	King George Island, Antarctica	AF226581	Nadler *et al.*, 2000
C. radiatum	*Leptonychotes weddelli*	Weddell Sea, Antarctica	AF226577	Nadler *et al.*, 2000
C. osculatum	*Sprattus sprattus*	North Sea, Germany	KT671118	Schade *et al.*, 2015
C. osculatum	*Phoca sibirica*	Lake Baikal, Russia	AF226589	Nadler *et al.*, 2000
C. osculatum B	*Phoca groenlandica*	Newfoundland, Canada	AF226580	Nadler *et al.*, 2000
ITS rDNA				
C. multipapillatum	*Chelon auratus*	Black Sea, Crymea	MH400190	Pronkina & Spiridonov, 2018
C. radiatum	Laptonychotes weddeli	South Shetlands, Antartica	AY603529	Kijewska *et al.*, 2002
C. bioccai	*Pelecanus occidentalis*	Florida, USA	JF424598	D'Amelio *et al.*, 2007
C. rudolphii C	*Phalacrocorax carbo sinensis*	Florida, USA	FJ589792	Zhang *et al.* (NCBI GanBank data)
C. rudolphii C	*Phalacrocorax carbosinensis*	Florida, USA	FJ467620	Zhang *et al.* (unpublished)
C. rudolphii	*Phalacrocorax carbosinensis*	Florida, USA	FJ589790	Zhang *et al.* (unpublished)
C. rudolphii	*Phalacrocorax aristotelis*	Sardinia, Italy	EU678869	Farjallah *et al.*, 2008
C. rudolphii	*Phalacrocorax carbo*	Poland	AY603535	Kijewska *et al.*, 2002
C. rudolphii F	*Pelecanus occidentalis*	Florida, USA	JF424597	D'Amelio *et al.*, 2007
Contracaecum sp.			AY821750	Kijewska *et al.*, 2002
C. osculatum	*Sprattus sprattus*	Baltic Sea, Denmark	KU306717	Zuo *et al.*, 2016
C. osculatum	*Sprattus sprattus*	Baltic Sea, Denmark	KU306680	Zuo *et al.*, 2016

For DNA extraction, the middle parts of three nematode specimens were rinsed in distilled water and crushed with a pestle in 1.5 ml Eppendorf tube and then processed with Wizard® SV Genomic DNA Purification Kit (Promega, Madison, USA) according to the producer's protocol. The nucleotide sequences of two loci of ribosomal repeats were amplified: LSU and ITS rDNA. Two pairs of primers were used to amplify LSU rDNA: the forward primer LSU391 (5'-AGC GGA GGA AAA GAA ACT AA-3') with the reverse one LSU390 (5'-ATC CGT GTT TCA AGA CGG G-3'), and the standard pair for D2-D3 segment of LSU rDNA: forward primer D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and reverse one D3B (5'-TCG GAA GGA ACC AGC TAC TA-3'). ITS rDNA region was amplified with primers TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3'). Annealing parameters for LSU primers were 50°C for 60 s and for ITS primers 52°C for 35 s. The obtained chromatograms were processed with Chromas 2.4.4 to obtain sequences. ITS and LSU rDNA sequences were deposited in NCBI GenBank as MT331855 and MT329686, respectively. Similar sequences were searched for in NCBI GenBank with BLAST (Altshul *et al.*, 1990). The obtained sequences were aligned with Clustal X and analysed with MEGA 7.0.14 (Kumar *et al.*, 2016).

RESULTS

Contracaecum sp. juveniles (Fig. 1)

Dissected from the intermediate host, a round goby *Neogobius melanostomus* (Pallas, 1814) (Gobiidae).

Locality. Karkinit Bay (45°52'30" N; 33°32'30" E).

Localisation in host. Inside capsules of serosa including intestinal mesentery and in the abdominal cavity.

Material. 1 hologenophore, 7 voucher specimens (CMP IMBR №1267-1273.N.2p.V 1-7).

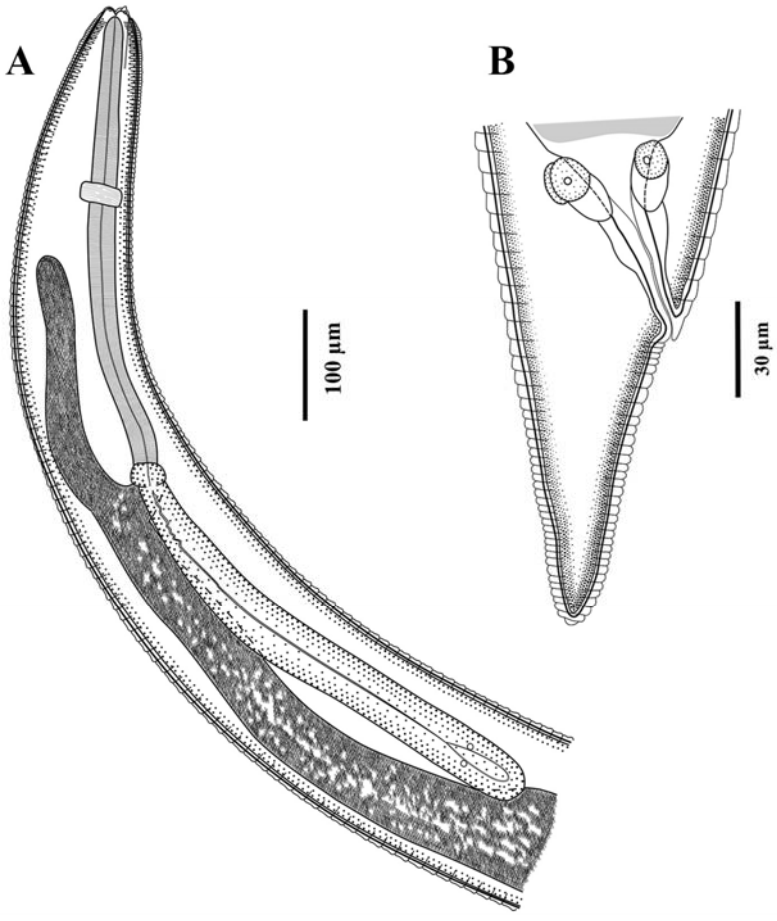

Fig. 1. Morphology of *Contracaecum* juveniles from the Black Sea *Neogobius melanostomus*. A – anterior end, laterally; B – tail end, laterally.

Molecular characterisation. Two nucleotide sequences of approximately 660 and 760 bp length were obtained for LSU rDNA of the studied *Contracaecum* juveniles with primers LSU391-LSU390 and D2A-D3B. These two sequences were completely identical in overlapping 415-420 bp. The sequence obtained with primers LSU391-LSU390 was completely identical to the sequence of *Contracaecum rudolphii* A (AF226585). This sequence was obtained from adult nematodes obtained after dissection of the cormorant *Phalacrocorax carbo* in Policoro, Italy (Table 1). Several sequences of *C. rudolphii* and unidentified *Contracaecum* sp. and also the sequence of *C. eudyptulae* from Australia (AF226586) differed from the studied one in a single nucleotide while the nucleotide differences with all remaining species of the genus were 36-67 bp.

Partial LSU rDNA sequence of the studied juveniles obtained with primers D2A-D3B was found to be identical with that of *C. rudolphii* 'A' (AF226585) and *C. rudolphii* '371_N' (KT767121). The last sequence was obtained from juveniles found in marine fishes in the German sector of the North Sea (Table 1). *Contracaecum* sequence for *C. rudolphii* 178N, KT767120 from the North Sea differed in 1 bp, and a set of other *Contracaecum* sequences (*C. rudolphii* 'B', *C. ogmorhini*, *C. eudyptulae*) differed in 2 bp. All remaining *Contracaecum* spp. differed in 44-71 bp. The sequences of four species, *C. rudolphii*, *C. eudyptula, C. ogmorhini* and *C. septentrionalis*, constituted a separate clade with strong support (Fig. 2).

Partial 798 bp long ITS rDNA sequence of the *Contracaecum* juveniles from the round goby was obtained and found to be completely identical to the sequences of *C. rudolphii* deposited under accession numbers EU678869 and AY603535 from Sardinia, Italy and Poland, respectively (Table 1). These three sequences formed the strongly supported subclade in the clade consisting of several *C. rudolphii* sequences (Fig. 3). The latter ones have only weak or modest support.

Morphological characterisation. Body length 2755-3522, maximum diameter 166-247; width at nerve ring level 81-109, width at anal level 53-65. Body surface annulated. Excretory pore located at anterior end. Cephalic end with three protruding cuticular projections and dorsal tooth. Nerve ring near pharynx middle, 18-28 long and 43-50 wide, situated in 133-164 from apex. Ventriculus globular, 32-53 wide and 19-44 long. Ventricular appendix 299-440 long and 33-45 wide. Intestinal appendix 155-238 long and 39-50 wide (length ratio of ventricular to intestinal appendix 1.5:1). Genital primordium not discernible. Prominent rectal glandular cells. Conical tail, 81-101 long.

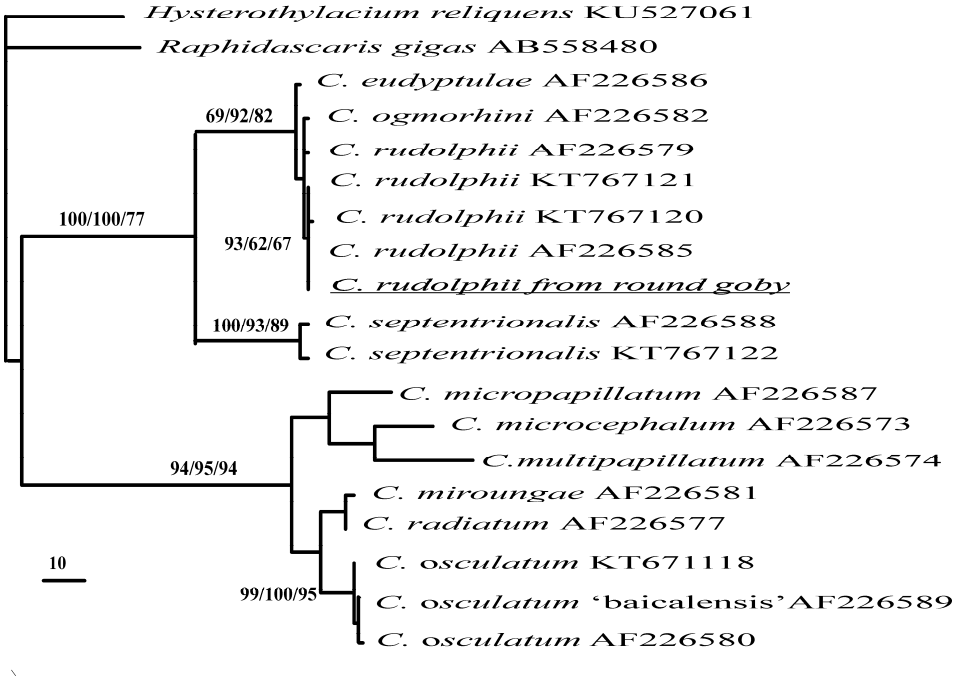

Fig. 2. Phylogenetic relationships of *Contracaecum* juveniles from the Black Sea *Neogobius melanostomus*, as inferred from the analysis of LSU rDNA. Bootstrap support near nodes in the format MP/NJ/ML.

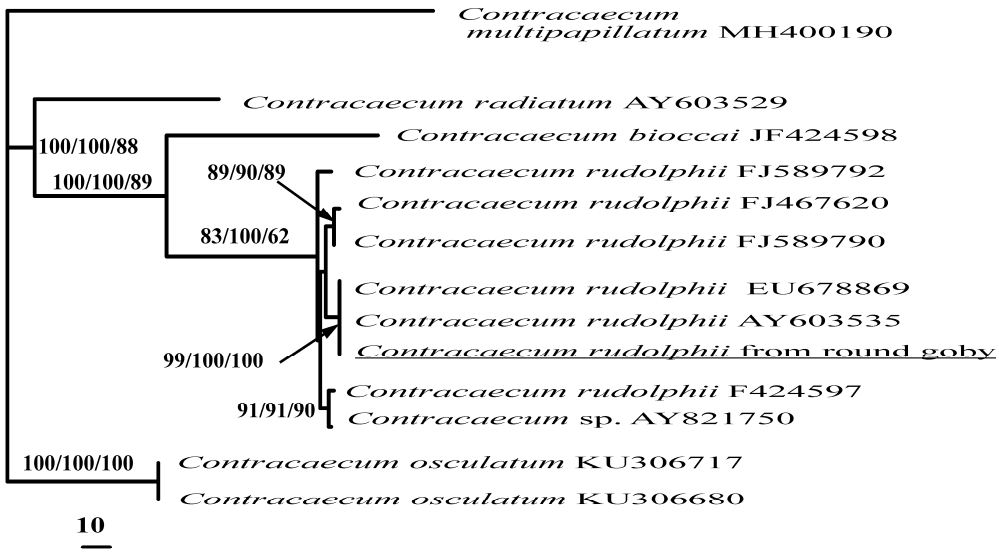

Fig. 3. Phylogenetic relationships of *Contracaecum* juveniles from the Black Sea *Neogobius melanostomus*, as inferred from the analysis of ITS rDNA. Bootstrap support near nodes in the format MP/NJ/ML.

DISCUSSION

The obtained sequences of the *Contracaecum* sp. juveniles from the round goby were found to be identical with the LSU and ITS rDNA sequences of *C. rudolphii* deposited in NCBI GenBank. Morphological features of these juveniles are also similar to those of *C. rudolphii* (Moravec, 1994). Therefore, we can identify these juveniles as *C. rudolphii*.

Anisakid nematodes are quite widespread as parasites of marine and fresh-water animals and even considered as 'emerging zoonoses' (McCarthy & Moore, 2000). Knowledge of the local anisakid diversity and the identification of these parasites are crucial for the proper understanding of parasite burden in ecosystems. It is especially important for the anisakid genus *Contracaecum*, as up to 50 nominal species of this genus are known and it has been recently demonstrated that some species in fact represent complexes of several cryptic species (D'Amelio *et al.*, 2007). It is also important to understand the level of specificity of *Contracaecum* parasitism.

The life cycle of *Contracaecum rudolphii* was only studied in experimental conditions and the list of possible intermediate hosts was also compiled after experimental inoculations (Mozgovoy *et al.*, 1968; Moravec, 2009). Juvenile stages of anisakids identified as *C. rudolphii* were reported from fish hosts, but such findings were not confirmed with molecular data. The identifications of *C. rudolphii* based on both morphological and molecular data were obtained for brackish-water fishes *Neogobius melanostomus* (Szostakowska & Fagerholm, 2007) in Poland and *Dicentrarchus labrax*, *Anguilla anguilla*, *Atherina boyeri*, *Aphanius fasciatus* in Italy (Mattiucci *et al.*, 2002; 2020).

Several forms comprising the species complex *Contracaecum rudolphii sensu lato* were reported exclusively from shags and cormorants (*Phalacrocorax* spp.). Five potential definitive hosts of *C. rudolphii s.l.* are common in the Black and Azov seas: great cormorant (*P. carbo*), European shag or common shag (*P. aristotelis*), pygmy cormorant (*P. pygmaeus*), great white pelican (*Pelecanus onocrotalus*) and Dalmatian pelican (*P. crispus*) (Beskorovajnyj, 2012). The latter species is quite rare in the area where great cormorant is considered as an invasive species, first reported at the Crimean peninsula only in the 1970s. For *C. rudolphii*, the prevalence of 35% and the parasite intensity range 1-295 nematodes per host in the common shag *P. aristotelis* were reported (Kornyushin *et al.*, 2004). Comparative data for different Crimean *Phalacrocorax* species are absent and at this stage we can presume that all three species can serve as hosts for *C. rudolphii* A.

The ITS rDNA sequence of *C. rudolphii* from the Crimean round goby was 100% identical to that

of specimens identified as *C. rudolphii* A from the same fish host, collected in the Gdansk area in Poland (Kijewska *et al.*, 2002). As indicated above, the LSU rDNA sequence amplified with primers LSU391-LSU390 was identical to the sequence of *C. rudolphii* A (AF226585) from Italy. Definitive hosts for these nematodes were not defined in Poland, but the host for the adult nematode specimens from Italy was *Phalacrocorax carbo*. It was proven that this bird species was a host for all other *C. rudolphii* A specimens (Shamsi *et al.*, 2009). Initially, it was presumed that *C. rudolphii* A can be found only in cormorants inhabiting marine and brackish habitats and *C. rudolphii* B distribution was limited to fresh-water habitats (Mattiucci *et al.*, 2002). Later, Szostakowska & Fagerholm (2007; 2012) demonstrated the presence of both these anisakid species in marine, brackish and fresh-water habitats of northern Poland. Some level of habitat specificity was only found in the distribution of juvenile stages: *C. rudolphii* B was only found in fresh-water fishes, whereas *C. rudolphii* A was common in both fresh-water and saltwater fishes.

Currently, *Contracaecum rudolphii sensu lato* represents a complex of genetically close species and not all these cryptic species have full scientific descriptions. These separate species are still treated as operational taxonomic units (OTU) designated with Latin binomial for species complex and letter code, as: *C. rudolphii* A and B (D'Amelio *et al.*, 1990; Cianchi *et al.*, 1992; Mattiucci *et al.*, 2002; Li *at al.*, 2005, Zhu *et al.*, 2007; Farjallah *et al.*, 2008) distributed mainly in Europe; *C. rudolphii* C and F (D'Amelio *et al.*, 2007; 2012) found in Atlantic coastal waters of North America; and *C. rudolphii* D and E (Shamsi *et al.*, 2009) from Australia. Morphometric differences between specimens of some these OTU are quite pronounced (Garbin *et al.*, 2013), but it is the common opinion that for *e.g. C. rudolphii* A and *C. rudolphii* B "there are no definitive diagnostic morphological characters for their specific identification" (Li *et al.*, 2005). With a deficit of diagnostic features for adult nematodes, there is no wonder that juvenile stages of *Contracaecum* from the Black Sea were not identified correctly. An analysis of nucleotide data proved its decisive value in anisakid identification worldwide. Further studies in *Contracaecum* diversity in the Black Sea and the Sea of Azov area are needed.

ACKNOWLEDGEMENTS

This work is a part of the state-supported studies of the Black Sea biota in the A.O. Kovalevsky Institute of Biology of the Southern Seas, Russian Academy of Sciences (project no. AAAA-A18-118020890074-2). The molecular work was supported by the RSF grant to S.E.S. (project no. 19-74-20147). We thank Dr Paolo Merella (Dipartimento di Medicina Veterinaria Universita' di Sassari, Italy) for his support in the editorial preparation of this paper.

REFERENCES

ALTSCHUL, S.F., GISH, W., MILLER, W., MYERS, E.W. & LIPMAN, D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403-410. DOI: 10.1016/S0022-2836(05)80360-2

BESKOROVAJNYJ, M.M. 2012. [*Birds of the Crimean Peninsula*]. Ukraine, Biznes Inform. 336 pp. (in Russian).

CIANCHI, R., ORECCHIA. P., BERLAND, B., PAGGI, L., D'AMELIO, S., MATTIUCCI, S., NASCETTI, G. & BULLINI, L. 1992. Genetic studies on some *Contracaecum* species, parasites of fish-eating birds. In: *Abstracts of the VIth European Multicolloquium of Parasitology*. p. 127. The Hague, The Netherlands, EFP.

D'AMELIO, S., NASCETTI, G., MATTIUCCI, S., CIANCHI, R., ORECCHIA, P., PAGGI, L., BERLAND, B. & BULLINI, L. 1990. Ricerche electroforetiche su alcune specie del genere *Contracaecum*, parassiti di uccelli ittiofagi (Ascaridida: Anisakidae). *Parassitologia* 32 (Supplementary 1): 77.

D'AMELIO, S., BARROS, N.B., INGROSSO, S., FAUQUIER, D.A., RUSSO, R. & PAGGI, L. 2007. Genetic characterization of members of the genus *Contracaecum* (Nematoda: Anisakidae) from fish-eating birds from west-central Florida, USA, with evidence of new species. *Parasitology* 134: 1041-1051. DOI: 10.1017/S003118200700251X

D'AMELIO, S., CAVALLERO, S., DRONEN, N.O., BARROS, N.B. & PAGGI, L. 2012. Two new species of *Contracaecum* Railliet & Henry, 1912 (Nematoda: Anisakidae), *C. fagerholmi* n. sp. and *C. rudolphii* F from the brown pelican *Pelecanus occidentalis* in the northern Gulf of Mexico. *Systematic Parasitology* 81: 1-16. DOI: 10.1007/s11230-011-9323-x

DMITRIEVA, E.V., LYAKH, A.M., KORNYYCHUK, YU.M., POLYAKOVA, T.A., POPYUK, M.P., YURAKHNO, V.M. & PRONKINA, N.V. 2015. *IMBR Collection of Marine Parasites. Collection of Marine Parasites Maintained by the Institute of Marine Biological Research*. URL: http://www.marineparasites.org/ (accessed: November 26, 2016).

FARJALLAH, S., MERELLA, P., INGROSSO, S., ROTTA, A., SLIMANE, B.B., GARIPPA, G., SAID, K. & BUSI, M. 2008. Molecular evidence for the occurrence of *Contracaecum rudolphii* A (Nematoda: Anisakidae) in shag *Phalacrocorax aristotelis* (Linnaeus) (Aves: Phalacrocoracidae) from Sardinia (western

Mediterranean Sea). *Parasitology International* 57: 437-440. DOI: 10.1016/j.parint.2008.05.003

GAEVSKAYA, A.V, GUSEV, A.V., DELYAMURE, S.L., DONETS, Z.S., ISKOVA, N.I., KORNYUSHIN, V.V., KOVALEVA, A.A., MARGARITOV, N.M., MARKEVICH, A.P., MORDVINOVA, T.N., NAIDENOVA, N.N., NIKOLAEVA, V.M., PARUKHIN, A.M., POGORELTSEVA, T.P, SMOGORZHEVSKAYA, L.A., SOLONCHENKO, A.I., SHTEIN, G.A. & SHULMAN, S.S. 1975. [*Key to the Parasites of Vertebrata of the Black and Azov Seas*]. USSR, Naukova Dumka. 552 pp. (in Russian).

GARBIN, L.E., MATTIUCCI, S., PAOLETTI, M., DIAZ, J.I., NASCETTI, G. & NAVONE, G.T. 2013. Molecular identification and larval morphological description of *Contracaecum pelagicum* (Nematoda: Anisakidae) from the anchovy *Engraulis anchoita* (Engraulidae) and fish-eating birds from the Argentine North Patagonian Sea. *Parasitology International* 62: 309-319. DOI: 10.1016/j.parint.2013.03.001

KIJEWSKA, A., ROKICKI, J., SITKO, J. & WEGRZYN, G. 2002. Ascaridoidea: a simple DNA assay for identification of 11 species infecting marine and freshwater fish, mammals, and fish-eating birds. *Experimental Parasitology* 101: 35-39. DOI: 10.1016/s0014-4894(02)00031-0

KORNYUSHIN, V.V., SMOGORZHEVSKAYA, L.A. & ISKOVA, N.I. 2004. [Circulation of helminths in colonies of Pelicaniformes and Ciconiiformes in the south of Ukraine]. *Branta: Sbornik Nauchnykh Trudov Azovo-Chernomorskoj Ornitologicheskoy Stancii* 7: 241-277 (in Russian).

KUMAR, S., STECHER, G. & TAMURA, K. 2016. MEGA7: molecular evolutionary genetics analysis. Version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870-1874. DOI: 10.1093/molbev/msw054

LI, A.-X., D'AMELIO, S., PAGGI, L., FANG, H.E., GASSER, R.B., LUN, Z.-R., ABOLLO, E, TURCGETTO, M. & ZHU, X.-Q. 2005. Genetic evidence for the existence of sibling species within *Contracaecum rudolphii* (Hartwich, 1964) and the validity of *Contracaecum septentrionale* (Kreis, 1955) (Nematoda: Anisakidae). *Parasitology Research* 96: 361-366. DOI: 10.1007/s00436-005-1366-y

MATTIUCCI, S., TURCHETTO, M., BRAGANTINI, F. & NASCETTI, G. 2002. On the occurrence of the sibling species of *Contracaecum rudolphii* complex (Nematoda: Anisakidae) in cormorants (*Phalacrocorax carbo sinensis*) from Venice and Caorle lagoons: genetic markers and ecological studies. *Parassitologia* 44 (Supplementary 1): 105.

MATTIUCCI, S., SBARAGLIA, G.L., PALOMBA, M., FILIPPI, S., PAOLETTI, M., CIPRIANI, P. & NASCETTI, G. 2020. Genetic identification and insights into the ecology of *Contracaecum rudolphii* A and *C. rudolphii* B (Nematoda: Anisakidae) from cormorants and fish of aquatic ecosystems of Central Italy. *Parasitology Research* 119: 1243-1257. DOI: 10.1007/s00436-020-06658-8

MCCARTHY, J. & MOORE, T.A. 2000. Emerging helminth zoonoses. *International Journal for Parasitology* 30: 1351-1360. DOI: 10.1016/S0020-7519(00)00122-3

MORAVEC, F. 1994. *Parasitic Nematodes of Freshwater Fishes of Europe*. The Netherlands, Academia and Kluwer Academic Publishers. 473 pp.

MORAVEC, F. 2009. Experimental studies on the development of *Contracaecum rudolphii* (Nematoda: Anisakidae) in copepod and fish paratenic hosts. *Folia Parasitologica* 56: 185-193. DOI: 10.14411/fp.2009.023

MOZGOVOY, A.A., SHAKHMATOVA, V.I. & SEMENOVA, M.K. 1968. [Life cycle of *Contracaecum spiculigerum* (Ascaridata: Anisakidae), a parasite of domestic and game birds]. *Trudy Gel'mintologicheskoi Laboratorii AN SSSR* 19: 129-136 (in Russian).

NADLER, S.A., D'AMELIO, S., FAGERHOLM, H.P., BERLAND, B. & PAGGI, L. 2000. Phylogenetic relationships among species of *Contracaecum* Railliet & Henry, 1912 and *Phocascaris* Host, 1932 (Nematoda: Ascaridoidea) based on nuclear rDNA sequence data. *Parasitology* 121: 455-463. DOI: 10.1017/s0031182099006423

PLEIJEL, R., JONDELIUS, U., NORLINDER, E., NYGEREN, A., OXELMAN, B., SCHANDER, C., SUNDBERG, P. & THOLLESSON, M. 2008. Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution* 48: 369-371. DOI: 10.1016/j.ympev.2008.03.024

PRONKINA, N.V. & SPIRIDONOV, S.E. 2018. Morphological and molecular characterisation of anisakid juveniles from the Golden grey mullet of the Black Sea. *Russian Journal* of *Nematology* 26: 87-92. DOI: 10.24411/0869-6918-2018-10008

SCHADE, F.M., RAUPACH, M.J. & MATHIAS WEGNER, K. 2016. Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual temperature fluctuations. *Journal of Sea Research* 113: 73-84. DOI: 10.1016/ j.seares.2015.09.002

SHAMSI, S., NORMAN, R., GASSER, R. & BEVERIDGE, I. 2009. Genetic and morphological evidences for the existence of sibling species within *Contracaecum rudolphii* (Hartwich, 1964) (Nematoda: Anisakidae) in Australia. *Parasitology Research* 105: 529-538. DOI: 10.1007/s00436-009-1424-y

SZOSTAKOWSKA, B. & FAGERHOLM, H.P. 2007. Molecular identification of two strains of third-stage larvae of *Contracaecum rudolphii sensu lato* (Nematoda: Anisakidae) from fish in Poland. *Journal of Parasitology* 93: 961-964. DOI: 10.1645/GE-1100R.1

SZOSTAKOWSKA, B. & FAGERHOLM, H.P. 2012. Coexistence and genetic variability of *Contracaecum rudolphii* A and *Contracaecum rudolphii* B (Nematoda: Anisakidae) in cormorants, *Phalacrocorax carbo sinensis*, in the Baltic region. *Journal of Parasitology* 98: 472-478. DOI: 10.1645/ge-2884.1

ZHU, X.Q., D'AMELIO, S., GASSER, R.B., YANG, T.B., PAGGI, L., HE, F., LIN, R.Q., SONG, H.Q., AI, L., & LI, A.X. 2007. Practical PCR tools for the delineation of *Contracaecum rudolphii* A and *Contracaecum rudolphii* B (Ascaridoidea: Anisakidae) using genetic markers in nuclear ribosomal DNA. *Molecular and Cellular Probes* 21: 97-102. DOI: 10.1016/j.mcp.2006.08.004

ZUO, S., HUWER, B., BAHLOOL, Q., AL-JUBURY, A., CHRISTENSEN, N.D., KORBUT, R., KANIA, P. & BUCHMANN, K. 2016. Host size-dependent anisakid infection in Baltic cod *Gadus morhua* associated with differential food preferences. *Diseases of Aquatic Organisms* 120: 69-75. DOI: 10.3354/dao03002

URL: http://www.inkscape.org/ (accessed: January 20, 20202).

Н.В. Пронькина и С.Э. Спиридонов. Личинки третьей стадии *Contracaecum* sp. от черноморского бычка *Neogobius melanostomus*.

Резюме. В процессе многолетних исследований зараженности рыб Черного моря личинками нематод сем. Anisakidae, часть обнаруживаемых личинок третьей стадии нематод рода *Contracaecum* определяли в соответствии с их морфологическими особенностями как *Contracaecum microcephalum*. Такое видовое определение вызывало сомнения, поскольку имеются указания на циркуляцию представителей этого вида в пресноводных биотопах (Kornyushin *et al.*, 2004). Анализ нуклеотидных последовательностей двух участков рибосомальных повторов (LSU rDNA и ITS rDNA) неполовозрелых нематод рода *Contracaecum*, паразитирующих в Черном море у бычка кругляка *Neogobius melanostomus*, показал их принадлежность к криптическому виду *Contracaecum rudolphii* 'A' из комплекса видов *Contracaecum rudolphii* Hartwich, 1964 *sensu lato*.