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Article *in* Zootaxa · July 2013

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Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3691.3.7
http://zoobank.org/urn:lsid:zoobank.org:pub:A9437656-6D76-4E24-B3F9-1C4B5A74E1A9

Morphological and molecular differentiation of *Staphylocystis clydesengeri* n. sp. (Cestoda, Hymenolepididae) from the vagrant shrew, *Sorex vagrans* (Soricomorpha, Soricidae), in North America

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Abstract

Staphylocystis clydesengeri **n. sp.** is described from shrews *Sorex vagrans* in Montana and Washington, United States. It differs from the only previously known North American representative of the genus, *S. schilleri*, in having more numerous (37–42 vs 22–30) and larger (39–44 µm vs 27–30 µm) rostellar hooks. The two species also differ in several other important characters such as relative length of the cirrus pouch, position of gonads and shape of mature proglottides. Morphological differentiation of the new species from all previously known Palearctic species of *Staphylocystis* from *Sorex* is also provided. Differentiation from *Staphylocystis* parasitic in crocidurine shrews is not provided due to the high level of specificity among shrew hymenolepidids to the host genera and much greater levels of sequence divergence between *Staphylocystis* from the two groups of shrews. Molecular differentiation based on 2,800 base pair long sequences of nuclear ribosomal RNA (complete ITS region and partial 28S region), 663 base pair long sequences of mitochondrial nad1 gene and 542 base pair long sequences of mitochondrial ribosomal 16S gene strongly support the status of *Staphylocystis clydesengeri* **n. sp.** Relative utility of the DNA fragments used in this study for reliable differentiation among closely related species of mammalian hymenolepidids is discussed. Nuclear ribosomal RNA region appears to be too conserved for this purpose. Use of at least one mitochondrial gene in addition to nuclear ribosomal RNA or without it, is recommended. *Vampirolepis novosibirskiensis* Sawada & Kobayashi, 1994 is transferred to *Staphylocystis* as a junior synonym of *S. furcata* (Stieda, 1862). *Rodentolepis gnoskei* Greiman & Tkach, 2012 is transferred to *Pararodentolepis* Makarikov and Gulyaev, 2009 as a new combination *Pararodentolepis gnoskei* (Greiman & Tkach, 2012) n. comb.

Key words: *Staphylocystis clydesengeri* **n. sp.**, *Staphylocystis schilleri*, Hymenolepididae, *Sorex vagrans*, *Sorex palustris*, USA, molecular differentiation, nuclear ribosomal RNA, nad1 mitochondrial gene, 16S mitochondrial gene

Introduction

Staphylocystis Villot, 1877 is a large genus of hymenolepidid cestodes parasitic mainly in shrews, with several species reported from rodents and bats. The vast majority of the members of *Staphylocystis* parasitize crocidurine shrews in Asia, Africa and Europe. Only a few *Staphylocystis* species have been described so far from soricine shrews, all from *Sorex* spp. *Staphylocystis furcata* (Stieda, 1862) parasitizes several *Sorex* Linnaeus species and is broadly distributed throughout the Palearctic region from Western Europe to the Far East of Russia (Vaucher 1971, Genov 1984, Novikov 1995, Karpenko 2004). *Staphylocystis sibirica* (Morozov, 1957) is known from at least three *Sorex* species and is distributed in the eastern Palearctic from Lake Baikal to the Kuril Islands (Morozov 1957, Eltyshev 1975, Novikov 1995, Kornienko *et al*. 2008) and *Staphylocystis amurensis* (Karpenko, 1984) was reported from Laxmann's shrew *Sorex caecutiens* Laxmann and taiga shrew *Sorex isodon* Turov in the Khabarovskiy Kray, in the Russian Far East (Karpenko 1984, 2004).

Only one *Staphylocystis* species, namely *Staphylocystis schilleri* (Rausch & Kuns, 1950), is known from North America. It was originally described from the masked shrew *Sorex cinereus* Kerr in Wisconsin (Rausch & Kuns

Accepted by N. Dronen: 1 Jul. 2013; published: 22 Jul. 2013

1950) and subsequently reported from the same shrew species in Alaska (Voge & Rausch 1955) and the vagrant shrew *Sorex vagrans* Baird in Oregon (Locker & Rausch 1952) and Montana (Senger 1955, Kinsella, 2007). Morphological and molecular comparison of multiple specimens of *Staphylocystis* collected by us from *S. vagrans* in Montana and Washington State with specimens collected from the American water shrew *Sorex palustris* Richardson in Montana has convincingly demonstrated that these specimens represented two different *Staphylocystis* species, one of them corresponding to *S. schilleri* and the other being a new species described herein.

Materials and methods

Specimens of *Staphylocystis* were collected between 2002 and 2011 from 6 of 9 American water shrews (*S. palustris*) and 23 of 77 vagrant shrews (*S. vagrans*) collected from Pattee Canyon near Missoula, Montana and in the vicinity of Spokane, Washington, USA. Live cestodes were removed from the shrew intestines, rinsed in saline, killed with hot water and immediately fixed in 70% ethanol suitable for both morphological and molecular study. Tapeworms were stained with aqueous alum carmine or Mayer's hematoxylin, dehydrated in a graded ethanol series, cleared in clove oil (after carmine staining) or methyl salicylate (after hematoxylin staining), and mounted permanently in Damar gum. Some of the scoleces and fragments of strobilae were mounted in Berlese's medium to facilitate detailed examination of the rostellar hooks and cirrus armature. Measurements and microphotographs were taken from a Leica DM5000 automated compound microscope using LAS software (Leica Microsystems GmbH, Wetzlar, Germany). Drawings were made with the aid of a drawing tube on the same Leica microscope. All measurements are in micrometers unless stated otherwise. Measurements of the holotype are followed by the range, average values and number of measured specimens in parentheses.

Genomic DNA for molecular comparisons was extracted from 5 specimens of the new species (2 from Montana, 3 from Washington). For comparative analysis, DNA was also extracted from two specimens of *S. schilleri* collected in Montana and two specimens of *Staphylocystis furcata* collected from *Sorex araneus* Linnaeus in the Carpathian Mountains, Ukraine. DNA was extracted according to Tkach & Pawlowski (1999) or using the Zymo Genomic DNA & Concentrator kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions. A small fragment of a single adult worm was used for each DNA extraction upon preliminary morphological identification. Scoleces (from fragmented specimens) or the rest of the strobilae have been mounted on slides as vouchers and deposited in the USNPC (see Taxonomic Summary below). Voucher specimens of *Staphylocystis schilleri* from *Sorex palustris* collected in Montana were submitted to the U.S. National Parasite Collection, Beltsville, Maryland, USA (USNPC). The holotype of *Staphylocystis schilleri* (USNPC accession number 047318) was examined for comparison with our material.

Approximately 3,000 base pairs of the nuclear ribosomal RNA region spanning the 3' end of the small ribosomal RNA (18S) gene, complete internal transcribed spacer 1 (ITS1) region, 5.8S gene, internal transcribed spacer 2 (ITS2) region and approximately 1500 base pairs at the 5' end of the large ribosomal RNA (28S) gene, were amplified by PCR and sequenced for inter-and intraspecific molecular comparisons. In addition, an approximately 460 base pairs long fragment of the mitochondrial 16S ribosomal gene and approximately 665 base pairs long fragment of the mitochondrial NAD(P)H dehydrogenase 1 gene (nad1) were sequenced. PCRs were run on an Eppendorf Mastercycler ep Gradient thermal cycler using several different polymerases. All PCR protocols included 40 cycles.

Forward primer ITSF (5'-CGCCCGTCGCTACTACCGATTG-3') and reverse fragment 1500RC (GACGATCGATTTGCACGTC) designed by V. Tkach were used for the nuclear ribosomal RNA amplification; annealing temperature for these reactions was set at 53ºC. Degenerate forward primer nad1f (5'-GGNTATTSTCARTNTCGTAAGGG-3') and degenerate reverse primer trnNR (5'-TTCYTGAAGTTAACAGCATCA-3') from Littlewood *et al.* (2008) were used for nad1 amplification; annealing temperature for these reactions was set at 45ºC. Forward primer Cyclo_16SF (5'-TGCCTTTTGCATCATGCT-3') and reverse primer Cyclo_16SR (5'-AATAGATAAGAACCGACCTGG-3') from Littlewood *et al.* (2008) were used for 16S amplification; annealing temperature for these reactions was set at 45ºC.

PCR products were purified using DNA Clean & Concentrator™ kit from Zymo Research or ExoSap PCR clean-up enzymatic kit from USB (now Affimetrix, Santa Clara, CA), cycle-sequenced directly using ABI

BigDye™ (Foster City, California) chemistry, alcohol-precipitated, and sequenced directly on an ABI Prism 3100™ automated capillary sequencer. Nuclear ribosomal RNA products were sequenced using the original PCR primers as well as internal forward primers m18f1 (CGTAACAAGGTTTCCGTAG), d58f (GCGGTGGATCACTCGGCTCGTG), cestl2 (5′-AAGCATATCAATAAGCGG-3′), c250f (GTCGGGTTGTTTGAGATTGC) and internal reverse primers d58r (CACGAGCCGAGTGATCCACCGC), cestl2r (CCGCTTATTGATATGCTT) and 1100r (GCGCATCACCGGCCCGTC); all primers were designed by V. Tkach. PCR primers as well as internal forward primer nad1Fb (5′- AGGTTTGARGCKTGTTTTATG -3′) and internal reverse primer nad1-470r (5′- CTYTCNGAYTCHGMATAATC-3′) designed by V. Tkach were used for sequencing of the nad1 fragment. PCR primers as well as internal forward primer 16s-460f (5′-AAGGTAGCATAATTAATTGCC-3′) and internal reverse primer 16s-585r (5′-CCGGGGTCTTTCCGTCT-3′) designed by V. Tkach were used for sequencing of the 16S fragment.

Contiguous sequences were assembled using Sequencher™ ver. 4.2 (GeneCodes Corp.) and submitted to GenBank under accession numbers KF257885–KF257889 (16S gene), KF257890–KF257895 (nad1 gene) and KF257896–KF257902 (nuclear ribosomal RNA). Sequence alignments and pairwise comparisons were done using BioEdit software, version 7.0.1 (Hall 1999). Levels of inter- and intraspecific variability were calculated as absolute numbers of variable sites and as ratio of the variable sites to the total length of the alignment.

Results

Family Hymenolepididae Ariola, 1899

Staphylocystis Villot, 1877

Staphylocystis clydesengeri n. sp.

(Figures 1–3)

Site in the host: small intestine.

Type host: *Sorex vagrans* (Baird, 1857) (Soricomorpha: Soricidae).

Type locality: Pattee Canyon, Missoula County, Montana, USA (46°, 49” N, 113°, 56” W).

Other localities: vicinity of Spokane, Washington (exact coordinates not known).

Material deposited: Holotype: USNPC 106801 (labelled *Sorex vagrans*, Pattee Canyon, Missoula County, MT, 14 July 2011, coll. J.M. Kinsella), paratypes: USNPC 106802-106808 (labelled *Sorex vagrans*, Pattee Canyon, Missoula County, MT, coll. J.M. Kinsella, different dates between May of 1998 to August 2009). Voucher specimens: USNPC 106809 (labelled *Sorex vagrans*, Pattee Canyon, Missoula County, MT, coll. J.M. Kinsella, 13 August 2011), USNPC 106810 (labelled: vicinity of Spokane, WA, 27 July 2009, coll. V. Tkach).

Etymology: This species is named in honour of Clyde Senger who was the first to note the morphological differences between *Staphylocystis schilleri* and the form described here as a new species.

Description: Based on 11 stained mounted specimens and 7 scoleces cleared in Berlese’s medium. Strobila 17.5 mm (14.4–24; 19.5; 6) mm long, with maximum width 1320 (1320–1600; 1497; 4) mm at level of gravid proglottides. Strobila consisting of 190 (138–190; 169; 6) craspedote proglottides. Scolex wider than long, 166 × 360 (166–190 × 352–430; 175 × 382; 6), clearly distinct from neck. Scolex with protracted rostellum conical, scolex with retracted rostellum slightly flattened antero-posteriorly. Suckers very muscular, thick-walled, unarmed, rounded or slightly oval, 100 × 85 (100–122 × 85–106; 109 × 97; 7). Rostellar pouch 140 × 209 (130–160 × 209–242; 143 × 232; 5), with muscular walls, its bottom reaches approximately middle of suckers. Rostellum very muscular, apex not invaginable 92 × 99 (88–96 × 99–118; 93 × 111; 7). Rostellum armed by a single crown of 37–42 (average 40; 7) hooks with strongly developed dorso-ventrally flattened guard. Hook number in holotype could not be counted precisely due to overlap of several hooks in lateral regions of crown. Hook length 42 (38–44; 41.5; 13), hook blade length 21(19–22; 21.4; 12), hook handle length 21 (19.5–23; 21.2; 12) and hook guard length 17 (16–18; 17.3; 12).

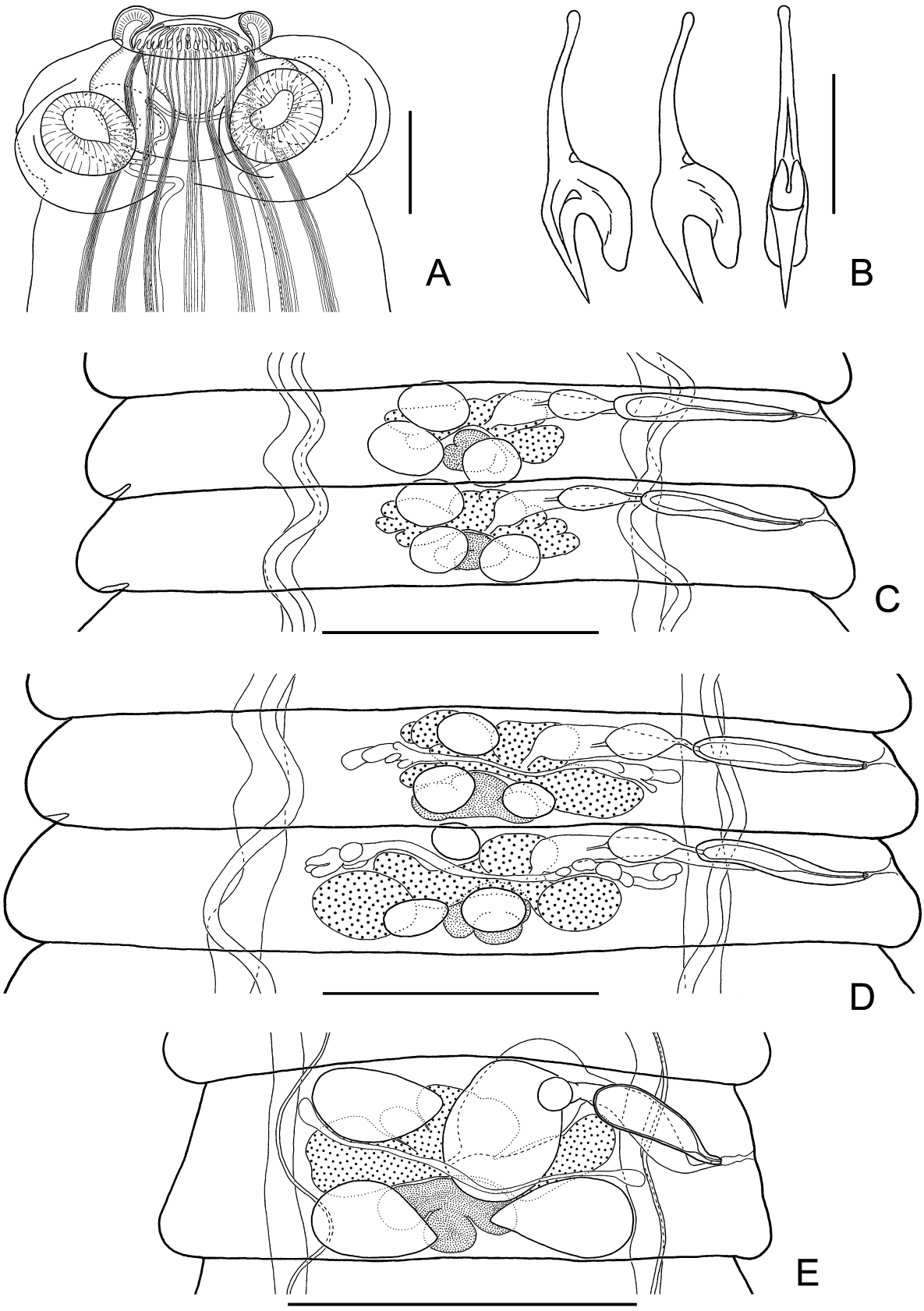

FIGURE. 1. *Staphylocystis clydesengeri* **n. sp. A.** Holotype, dorso-ventral view of scolex. **B.** Paratype, rostellar hooks in profile and view from posterior surface showing enlarged hook guard. **C.** Holotype, male mature proglottis. **D.** Holotype, hermaphroditic mature proglottis. **E.** Hermaphroditic mature proglottis of *Staphylocystis schilleri* from *Sorex palustris*. (scale bars: A = 100 µm; B = 20 µm; C–E = 250 µm).

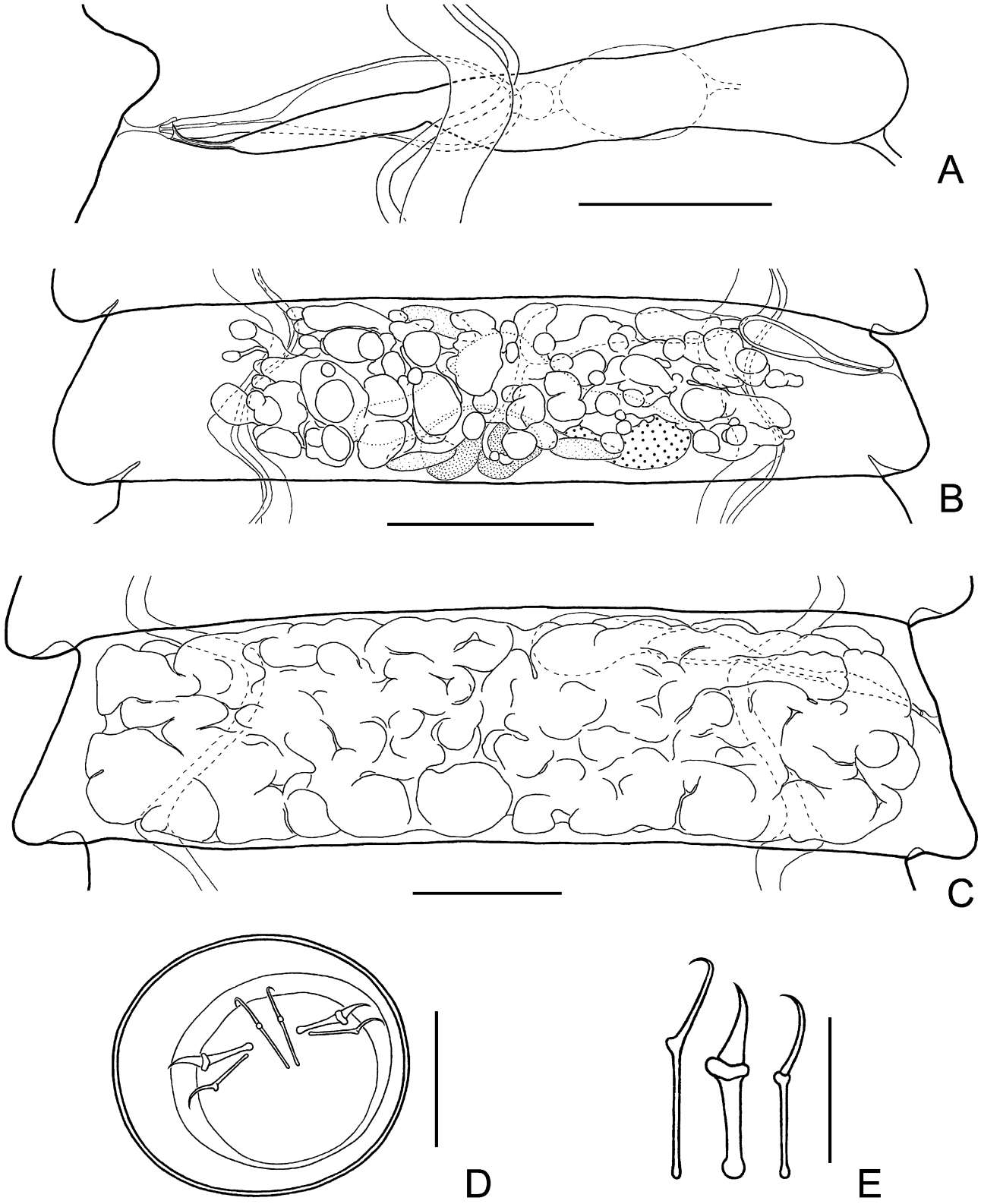

FIGURE. 2. *Staphylocystis clydesengeri* **n. sp. A.** Paratype, genital ducts. **B.** Holotype, pregravid proglottis, showing uterus development. **C.** Paratype, gravid proglottis. **D.** Paratype, egg. **E.** Paratype, embryonic hooks (from left to right: median, antero-lateral, postero-lateral) (scale bars: A = 100; B, C = 250 µm; D = 20 µm; E = 10 µm).

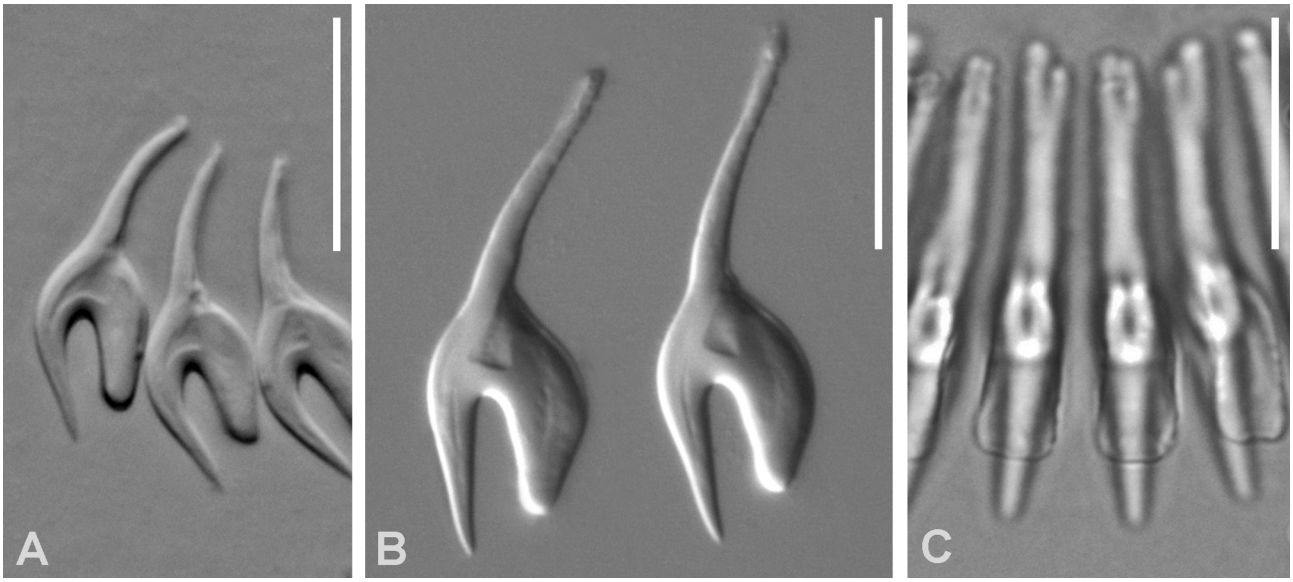

FIGURE. 3. Rostellar hooks of *S. schilleri* and *S. clydesengeri* **n. sp. A.** *Staphylocystis schilleri* from *Sorex palustris* collected near Missoula, Montana. **B, C.** Paratype of *S. clydesengeri* **n. sp.** Note that all photographs are taken at the same magnification. (scale bars: A–C = 20 µm).

Osmoregulatory canals penetrate through rostellar pouch wall. Neck narrower than scolex. Ventral osmoregulatory canals 30 (28–40; 32; 7) at level of hermaphroditic proglottides, transverse anastomoses not observed. Dorsal osmoregulatory canals thin, 12 (9–12; 11.5; 7) at level of hermaphroditic proglottides, usually situated directly above ventral canals. Genital pores unilateral, dextral, genital ducts pass dorsally to both ventral and dorsal longitudinal osmoregulatory canals. Development of proglottides gradual, male and female gonads developing at approximately same rate. All segment in the strobila transversely elongated, but the length:width ratio increases from young to gravid proglottides.

Mature proglottides 110 × 782 (87–130 × 782–911; 110 × 846; 5), trapezoid (Fig. 3D). Testes relatively small, three, spherical or somewhat elongated, situated in a relatively tight triangle, one poral and two antiporal. Poral testis 51 × 57 (50–58 × 57–102; 54 × 90; 5), middle testis 51 × 67 (50–58 × 67–103; 54 × 92; 5), aporal testis 48 × 57 (48–66 × 57 – 110; 56 × 89; 5). Cirrus pouch 148 × 38 (135–168 × 37–47; 151 × 41; 9) in hermaphroditic proglottides, normally only reaching or somewhat overlapping poral osmoregulatory canals, but not crossing them. Genital atrium simple, infundibular, deep, opens laterally approximately at level of border between first and second thirds of lateral proglottis margin. Evaginated cirrus not observed. Internal seminal vesicle elongate, 103 × 33 (91–103 × 33–42; 96 × 36; 8), occupying more than half of cirrus pouch length. External seminal vesicle ovoid, 90 × 38 (90–130 × 38–66; 106 × 50).

Ovary 90–296 (72–95 × 272–360; 83 × 318; 5) wide, median, usually consisting of three large lobes, sometimes with secondary smaller lobes, ventral to male genital organs, occupying half to two-thirds of median field, usually overlapping testes. Vitellarium irregularly shaped, sometimes lobed 47 × 82 (40–61 × 82–132; 52 × 107; 7), postovarian, median. Copulatory part of vagina not clearly distinct from seminal receptacle; ventral to cirrus pouch. Seminal receptacle elongated, 250 long (178–260 × 54–80 × 63; 5), was not filled with sperm in mature hermaphroditic proglottides of holotype, but was filled in gravid proglottides. In majority of other specimens seminal receptacle was filled in mature hermaphroditic proglottides.

Uterus first appears as transversely elongated sac, not extending beyond osmoregulatory canals, situated dorsally to other organs. With proglottis development, uterus grows and forms numerous lateral pockets and dorso-ventral diverticula. Testes and vitellarium persist in postmature proglottides; cirrus pouch and vagina persist in gravid proglottides. Gravid proglottides 340 × 1286 (340–420 × 1286–1490; 382 × 1395). Fully developed uterus labyrinthine, extending into both lateral fields, saccate; walls of gravid uterus usually deeply folded or invaginated sometimes creating impression of being perforated. Uterus contains numerous small eggs. Eggs 40–44 × 32–39 (42 × 35; 8), spherical or subspherical; embryophore thin, without polar filaments, close to surface of oncosphere; oncosphere 22–28 × 18–24 (25 × 22; 8). Median embryonic hooks 16, antero-lateral and postero-lateral hooks

14.5–15. Antero-lateral embryonic hooks much more robust than slender postero-lateral and median hooks.

Remarks: Morphological differentiation. The only other *Staphylocystis* species previously reported in North America is *S. schilleri* originally described from *Sorex cinereus* in Wisconsin (Rausch & Kuns 1950). The two species share general morphological characteristics such as the hook shape, overall scolex and strobila anatomy including triangular arrangement of testes and gravid uterus expanding into lateral fields of proglottides. But *S. clydesengeri* **n. sp.** differs from *S. schilleri* in several significant morphological features. The two species can be most easily distinguished based on the size and number of their rostellar hooks. *Staphylocystis clydesengeri* has 37–42 (average 40) hooks 39–44 µm (average 40.7 µm) long while *S. schilleri* has 22 hooks 27–30 µm long according to the original description. In our material of *S. schilleri* from Montana the hook number was higher (28–30, average 29). We could not count hooks in the holotype of *S. schilleri* very precisely due to their dense arrangement in the crown, but it looked like the number 22 is correct or at least very close to the real situation. In our experience, counting rostellar hooks in the laterally positioned crowns of *Staphylocystis* on total mounts usually results in underestimations of their numbers comparing with the crowns of the same species from the same sample cleared and apically oriented (or squished) in Berlese's medium. The cirrus pouch in mature (hermaphroditic) proglottides of *S. schilleri* distinctly crosses the poral ventral osmoregulatory canal. In *S. clydesengeri* the cirrus pouch only barely reaches or, rarely, slightly crosses the poral ventral osmoregulatory canal. Gonads in *S. clydesengeri* occupy the central part of the middle field of the proglottis and do not reach the canals on either side. In fully mature proglottides of *S. schilleri* gonads normally fill the middle field of the proglottis entirely and reach or even overlap osmoregulatory canals on either side of the proglottis. Mature proglottides in heat-killed relaxed specimens of the two species distinctly differ in shape. In the new species proglottides are relatively shorter than in *S. schilleri* (Fig. 1 D, E). Finally, the two species differ in the early development of the uterus and its extent in gravid proglottides. In the new species the early uterus has numerous pockets and chambers from the very beginning while in *S. schilleri* the uterus at is initially formed as a two-winged saccular structure. The fully developed uterus in the new species extends well into the lateral fields while in *S. schilleri* it is confined to the median field of the proglottides and may only somewhat overlap the osmoregulatory canals on either side.

Staphylocystis clydesengeri clearly differs from all Palearctic species parasitic in *Sorex*. While there are multiple characters separating these species, the rostellar hook number and size are discriminative enough, therefore we are not providing other differentiating features. The new species has 37–42 hooks 39–44 µm long while *S. furcata* has 23–30 hooks 23–27 µm long (Vaucher 1971, Genov 1984), *S. sibirica* has 28–30 hooks 32–35 µm long (Morozov 1957, Gulyaev & Shakhmatova 1990) and *S. amurensis* has 34 hooks 19–22 µm long (Karpenko 1984, 2004).

Vampirolepis novosibirskiensis Sawada & Kobayashi, 1994 described from *Sorex araneus*, *Sorex caecutiens* and *Sorex minutus* in the Novosibirsk region and Altai Krai, Russian Federation (Sawada & Kobayashi 1994), clearly belongs to *Staphylocystis*. By the combination of morphological characteristics such as rostellar hook shape, size and number, organization of mature segments and other features, it is morphologically indistinguishable from *Staphylocystis furcata*. Therefore, we consider *V. novosibirskiensis* a junior synonym of *S. furcata*.

We do not provide here differentiation of the new species from *Staphylocystis* spp. parasitic in crocidurine shrews because of the very high level of specificity among shrew hymenolepidids to the host genus (see the detailed discussion below). These two groups of shrews do not share their cestodes even when they occur in the same habitat, let alone on different continents. In the absence of any crocidurine shrews in North America we are certain that our specimens described herein as a new species cannot belong to one of the numerous *Staphylocystis* species known from *Crocidura* and related genera. In addition, *Staphylocystis* from the two groups of shrews deminstrate a high level of genetic divergence (see the Molecular differentiation below).

Molecular differentiation. The aligned trimmed sequenced rRNA fragments from 5 specimens of *S. clydesengeri* (sequence length 2856 base pairs), one *S. schilleri* (2866 base pairs) and one *S. furcata* (2862 base pairs) comprised a short portion of the 18S gene, complete ITS1 spacer, complete 5.8S gene, complete ITS2 spacer, and partial 28S gene. Due to introduced gaps the length of pairwise alignments between the three species varied from 2862 to 2872 base pairs. No intraspecific variability was detected among 4 replicates of *S. clydesengeri*. The two North American species *S. clydesengeri* and *S. schilleri* differed in only 3 bases while both these species differed from the Palaearctic *S. furcata* in 7 bases.

Sequences of mitochondrial genes nad1 and 16S provided further convincing evidence of the status of *S. clydesengeri* as a new species. The nad1 alignment of 4 sequences of *S. clydesengeri* (2 from Montana and 2 from

Washington), one *S. schilleri* and one *S. furcata* was 663 bases long and had no gaps. Among 4 sequences of the new species only one specimen from Montana had a single base substitution. Results of pairwise comparisons are presented in Table 1. *Staphylocystis clydesengeri* and *S. schilleri* differed in 33 bases while the two North American species differed from *S. furcata* in 45–49 positions (Table 1).

TABLE 1. Number of variable sites with (%) based on pairwise comparison of 663 base-pair long fragment of mitochondrial nad1 gene (above diagonal), and number of variable sites with (%) based on pairwise comparison of 542 base-pair long fragment of mitochondrial 16S gene (below diagonal).

Cestode species	*S. clydesengeri* **n. sp.**	*S. schilleri*	*S. furcata*
S. clydesengeri **n. sp.**	--------	33 (5.0%)	49 (7.4%)
S. schilleri	11 (2.4%)	--------	45 (6.8%)
S. furcata	16 (3.5%)	11 (2.4%)	---------

The 16S alignment of 3 sequences of *S. clydesengeri* (2 from Montana and one from Washington), one *S. schilleri* and one *S. furcata* was 452 bases long and had no gaps. No intraspecific variability was detected in 16S sequences of 4 sequenced specimens of *S. clydesengeri*. Results of pairwise comparisons are presented in Table 1. *Staphylocystis clydesengeri* and *S. schilleri* differed in 11 bases while the two North American species differed from *S. furcata* in 11–16 positions (Table 1). Thus, molecular data strongly support the status of *S. clydesengeri* as a new species.

We do not provide a detailed molecular differentiation between *S. clydesengeri* and members of the genus known from crocidurine shrews because the new species shows much greater levels of interspecific sequence divergence from *Staphylocystis* parasitic in crocidurine shrews than from *Staphylocystis* parasitic in *Sorex*. For instance, the approximately 1400 base pair long sequence of the nuclear ribosomal 28S gene of *S. clydesengeri* obtained in the present study was identical to that of *S. furcata* collected in the Ukraine. In the same DNA region, *S. clydesengeri* had 41 nucleotide differences from *Staphylocystis brusatae* (Vaucher, 1971) collected from lesser white-toothed shrews *Crocidura suaveolens* Pallas in the Ukraine (GenBank JQ260805) and in 29 nucleotides from *Staphylocystis* sp. collected from Asian house shrews *Suncus murinus* Linnaeus in Thailand (V. Tkach, unpublished data). This provides additional evidence of the significant differences between *Staphylocystis* from soricine and crocidurine shrews.

Discussion

Currently available information clearly indicates that *Staphylocystis* is a composite, non-monophyletic taxon that needs a thorough revision using multiple sources of both morphological and molecular data (Haukisalmi *et al.* 2010, Greiman and Tkach 2012). For instance, Greiman and Tkach (2012) recently described a new species of hymenolepidid with an armed rostellum, *Rodentolepis gnoskei* Greiman & Tkach, 2012, from crocidurine shrews in Malawi. Traditionally, cestodes with similar morphology from shrews were included in *Staphylocystis*. However, *R. gnoskei* was provisionally placed in the genus *Rodentolepis* Spassky, 1954 because in the molecular phylogenetic analysis it clustered together with *Rodentolepis fraterna* (Stiles, 1906), a cosmopolitan parasite of rodents. Greiman & Tkach (2012) were unaware of the publication by Makarikov and Gulyaev (2009) who established a new genus *Pararodentolepis* Makarikov and Gulyaev, 2009 that included *P. fraterna* (Stiles, 1906) Makarikov and Gulyaev, 2009 as a new combination. *Rodentolepis gnoskei* is fully morphologically consistent with the diagnosis of *Pararodentolepis* in having typical fraternoid hooks, sinistral genital pores, testes situated in one row and embryophore with polar filaments. Therefore, we transfer this species into *Pararodentolepis* as new combination *Pararodentolepis gnoskei* (Greiman and Tkach, 2012) n. comb.

Sorex vagrans has the second most diverse helminth fauna of all shrew species studied so far in North America. With 26 helminth species (2 digeneans, 15 cestodes and 9 nematodes) it is second to only *Sorex cinereus* which is known as the host of 32 helminth species (Kinsella & Tkach 2009, Gulyaev *et al.* 2007, 2010). *Staphylocystis clydesengeri* is the 16th cestode species reported from *S. vagrans*. The diversity of parasitic worms in *S. cinereus* and *S. vagrans* can at least partly be explained by the very broad distribution area of both species. These species have also been a subject of more studies than other shrews in North America.

Senger (1955) tentatively identified cestodes from *S. vagrans* from Flathead, Lake, and Sanders Counties, Montana as *S. schilleri* but pointed out that these cestodes had 34 to 45 hooks 37 to 47 microns long. It seems highly likely that these specimens were *S. clydesengeri*. The only other western records of *S. schilleri* were by Locker & Rausch (1952) from *S. vagrans* in Oregon and from *S. cinereus* in the Katmai National Park, Alaska. No morphological data were provided in these publications so the presence of *S. schilleri* in Oregon and Alaska needs to be verified.

Our specimens of *S. clydesengeri* from Washington were immature, therefore we cannot adequately compare other morphological features between the two localities. Hook size and number in the new species did not differ substantially in specimens collected in Montana and Washington except for a single specimen from Washington that had 37 hooks 38 µm in length. It should be noticed, however, that the majority of other morphological features in the new species varied rather substantially. In our opinion, the absolute size of testes, ovary and vitellarium are of little use for differentiation among species of *Staphylocystis* because the size of these organs varies greatly among progottides in the same strobila and changes quite dramatically depending on the level of strobila contraction. The absolute length of the cirrus pouch which is more stable, should also be used with caution. At the same time, the relative size of the ovary compared to the width of mature hermaphroditic proglottides seems to be a good character as well as the relative length of the cirrus pouch and its position in relation to the osmoregulatory canals.

According to numerous studies in different parts of the Palearctic, species of *Staphylocystis* demonstrate rather strict specificity to genera of their hosts (Vaucher 1971, Genov 1984, Binkiene *et al*. 2011, V. Tkach, unpublished data). For instance, species parasitic in shrews of the genus *Crocidura* Wagler and other crocidurines do not occur in shrews of the genus *Sorex* and vice versa; and cestodes found in *Neomys* Kaup are not shared with those normally found in *Sorex* and vice versa. One of the authors (V. Tkach) studied shrew helminths in numerous localities in the Ukraine where members of *Crocidura* and *Sorex* or *Neomys* and *Sorex* occurred symbiotopically and were sometimes caught in the same pitfall traps. In more than 20 years of research in the region he did not see a single case of hymenolepidid cestode (including *Staphylocystis*) sharing between any two genera of shrews. In one of the localities in vicinities of Kiev each of 11 examined *Crocidura suaveolens* was infected with *Staphylocystis brusatae* and several among more than 60 examined individuals of *Sorex araneus* were infected with *Staphylocystis furcata*. No cross-infection with any cestode between the two shrew species was detected.

It seems that the this pattern of strict specificity of shrew hymenolepidids to host genera persists even within subfamilies of shrews. As mentioned above, the two soricine genera *Neomys* and *Sorex* do not normally share their cestodes. A similar pattern was observed between crocidurine genera. *Diplomesodon* Brandt is crocidurine genus which includes only one species *Diplomesodon pulchellum* Lichtenstein (piebald shrew) distributed in Central Asia and sharing habitats with *Crocidura* (another species was recently described by Cheke 2011 based on an old manuscript not supported by any material). Helminthological studies of *D. pulchellum* have demonstrated that all cestodes of this shrew, including two species of *Staphylocystis*, were new and apparently specific to *Diplomesodon* (Tkach & Velikanov 1990, 1991; Velikanov & Tkach 1990). This example is particularly interesting because the molecular phylogenetic analysis by Dubey *et al*. (2008) has placed *Diplomesodon* as a long-branch clade among Asian *Crocidura*. Unfortunately, no molecular data are currently available for any of the cestodes of *Diplomesodon* to verify their phylogenetic position in relation to that of *Staphylocystis* parasitic in *Crocidura*.

The host associations of *Staphylocystis* in North America follow the same pattern (Kinsella & Tkach 2009, our unpublished data). Despite a very large overlap in distribution between *Sorex* spp. and *Blarina* spp., species of *Staphylocystis* have been found only in members of *Sorex* (Kinsella & Tkach 2009; present study). There are several additional genera of shrews in North America, e.g., *Cryptotis* Pomel and *Notiosorex* Coues, all of them overlapping with *Sorex* in their distribution; however, their helminth fauna has not been studied so far. Based on the current knowledge on the host specificity of shrew cestodes we predict that *Staphylocystis* is unlikely to be found in North America in members of any shrew genera other than *Sorex*.

With the description of *S. clydesengeri*, members of *Staphylocystis* are now known from Alaska, Washington, Montana and Wisconsin. They have not been found anywhere else in the United States, although there have been a number of reports of cestodes from *Sorex* spp. in more southern and eastern parts of the country (Kinsella & Tkach 2009).

This pattern may reflect the evolutionary and geographic expansion of the genus *Staphylocystis* in the New World. The distribution of *Staphylocystis* in North America roughly spans the region from eastern Beringia to south

of the former Cordilleran and Laurentide ice sheets of the Quaternary and may be consistent with a complex history of geographic expansion and isolation that has potentially influenced diversification of these cestodes in the Nearctic (e.g., Hoberg *et al*. 2012). These historical associations may be apparent even in the context of relatively poor sampling and insufficient knowledge of cestodes among species of *Sorex* and other shrews across much of the North American continent (Kinsella and Tkach 2009). The low diversity of *Staphylocystis* on the continent may suggest that the genus could have reached North America later than some other genera (e.g., *Staphylocystoides* Yamaguti, 1959, *Lineolepis* Spasskii, 1959) that have diversified more significantly in the New World (Kinsella & Tkach 2009).

This study provided an opportunity to compare the relative utility of some of the DNA regions commonly used in systematic and taxonomic studies. The sequence data obtained in this study as well as previously published information on mammalian hymenolepidids (e.g., Haukisalmi *et al.* 2010, Greiman & Tkach 2012) and unpublished sequences deposited in the GenBank, we may conclude that nuclear ribosomal genes traditionally used for phylogenetic purposes and successfully used for species differentiation in many groups of parasitic flatworms (see Nolan & Cribb 2005, Olson & Tkach 2005) are too conserved for reliable differentiation among members of some hymenolepidid genera parasitic in mammals. Our data on *Staphylocystis* from *Sorex* corroborate the similar conclusion made by Greiman & Tkach (2012) regarding the members of the closely related genus *Pararodentolepis*. The latter authors have demonstrated lack of sequence variability in the 1400 bp long fragment of nuclear large ribosomal subunit gene in *P. gnoskei* (Greiman & Tkach, 2012) n. comb. parasitic in African shrews and *P. fraterna*, a cosmopolitan parasite of rodents. Thus, even a relatively low level of sequence divergence in the nuclear ribosomal gene sequences is likely to be meaningful for species differentiation in this hymenolepidid lineage assuming that the sequences are of high quality. In the present study, approximately 2,800 base pair long sequences of the nuclear ribosomal ITS and 28S regions obtained from two North American *Staphylocyctis* species and *S. furcata* collected in Europe at least 8,000 kilometers away, differed in only 7 nucleotides or 0.25% of the alignment length.

In contrast, much shorter fragments of the two mitochondrial genes used in this study in addition to the nuclear ribosomal RNA, efficiently discriminated between the two North American species and *S. furcata* from Europe with levels of interspecific variability between the three species varying from 5.0–7.4% in the nad1 gene and 2.4–3.5% in the 16S gene. In both genes, the divergence between the two North American species was somewhat lower than their differences with the Palearctic *S. furcata* (Table 1). The differences between *S. schilleri* and *S. clydesengeri* are particularly meaningful taking into account that the two species were collected from the same locality. At the same time, sequences of *S. clydesengeri* collected in Montana and Washington showed no intraspecific variability in nuclear ribosomal RNA region and the mitochondrial 16S gene, and had only a single base mutation in the nad1 gene. Thus, it is recommended that at least one mitochondrial gene be used in addition to ribosomal genes (or without them) for differentiation among closely related species of mammalian hymenolepidids. It should be noted, however, that the divergence threshold has to be established in each hymenolepidid separately because the mutation rates are likely to be at least somewhat different in each group of these tapeworms due to the differences in their generation time, fecundity, biogeographic history, geographic distribution, co-evolution with their hosts and other factors. Whenever possible, sequence comparison should be complemented with phylogenetic analysis to test the monophyly of the lineage in question as the primary diagnostic criterion for outlining a taxon.

Acknowledgments

We are grateful to Christina Brewer for her help with obtaining specimens of *Staphylocystis* from Washington state. Eric Hoberg and Patricia Pillit (U.S. National Parasite Collection, Beltsville, MD) kindly arranged the loan of the holotype of *Staphylocystis schilleri*. We sincerely thank Boyko Georgiev (Central Laboratory of General Ecology, Sofia, Bulgaria) and Eric Hoberg for their useful comments on our manuscript. This study was funded in part by the National Science Foundation grants DEB 0818696 and 0818823.

References

Binkienė, R., Kontrimavichus, V. & Hoberg, E.P. (2011) Overview of the cestode fauna of European shrews of the genus *Sorex* with comments on the fauna in *Neomys* and *Crocidura* and an exploration of historical processes in post-glacial Europe. *Helminthologia*, 48, 207–228.
http://dx.doi.org/10.2478/s11687-011-0031-5

Cheke A. (2011) Sonnerat's shrew - evidence for a new and possibly extinct species in an early 19th century manuscript (Mammalia: Soricidae). *Journal of the Bombay Natural History Society*, 108, 95–97.

Dubey, S., Salamin, N., Ruedi, M., Barrière, P., Colyn M. & Vogel, P. (2008) Biogeographic origin and radiation of the Old World crocidurine shrews (Mammalia: Soricidae) inferred from mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution*, 48, 953–963.
http://dx.doi.org/10.1016/j.ympev.2008.07.002

Eltyshev, Y.A. (1975) [Helminth fauna of mammals in the Barguzin basin and its geographical analysis. I. Systematic survey of helminths]. *In: Parasitic organisms of north-east of Asia.* Publishing house of the Far-Eastern scientific center, Vladivostok, pp. 135–167. (In Russian)

Genov, T. (1984) [*Helminths of insectivores and rodents in Bulgaria*]. Izdatelstvo na Bulgarskata Akademiya na Naukite, Sofia, 348 pp. (In Bulgarian).

Greiman, S.E. & Tkach. V.V. (2012) Description and phylogenetic relationships of *Rodentolepis gnoskei* n. sp. (Cyclophyllidea: Hymenolepididae) from a shrew *Suncus varilla minor* in Malawi. *Parasitology International*, 61, 343–350.
http://dx.doi.org/10.1016/j.parint.2012.01.003

Gulyaev, V.D., Dokuchaev, N.E. & Kornienko, S.A. (2007) [The cestodes of the genus *Staphylocystoides* Yamaguti, 1959 (Cestoda, Hymenolepididae) in shrews of Beringia]. *Vestnik Severo-Vostochnogo Nauchnogo Tsentra Dal'ne-Vostochnogo Otdeleniya RAN, issue 4*, 75–84. (in Russian)

Gulyaev, V.D., Dokuchaev, N.E., & Lykova, K.A. (2010) [Description of *Spasskylepis rauschi* sp. n. (Cestoda, Hymenolepidae) from shrews *Sorex* in Alaska]. *Vestnik Severo-Vostochnogo Nauchnogo Tsentra Dal'ne-Vostochnogo Otdeleniya RAN*, issue 2, 75–84. (in Russian)

Gulyaev, V.D. & Shakhmatova, V.I. (1990) [On the morphology of cestode *Staphylocystis sibirica* (Morozov, 1957) (Hymenolepididae)]. *In:* G. S. Zolotarenko (Ed.), *Taxonomy of insects and helminths.* Nauka, Novosibirsk, pp. 8–11 (In Russian)

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.

Haukisalmi, V., Hardman, L.M., Foronda, P., Feliu, C., Laakkonen, J., Niemimaa, J., Lehtonen, J.T. & Henttonen, H. (2010) Systematic relationships of hymenolepidid cestodes of rodents and shrews inferred from sequences of 28S ribosomal RNA. *Zoologica Scripta*, 39, 6, 631–641.
http://dx.doi.org/10.1111/j.1463-6409.2010.00444.x

Hoberg, E.P, Galbreath, K.E, Cook, J.A., Kutz, S.J. & Polley, L. (2012) Northern host-parasite assemblages: History and biogeography on the borderlands of episodic climate and environmental transition. *Advances in Parasitology*, 79, 1–97.

Karpenko, S.V. (1984) [Two new species of hymenolepidids (Cestoda: Hymenolepididae) from the shrews of the Khabarovsk krai*]. In: Izvestija Sibirskogo otdelenija Akademii Nauk SSSR, serija biologicheskih nauk, vypusk 3*, Nauka, Novosibirsk, pp. 117–124.

Karpenko, S.V. (2004) *Helminths of shrews in the Khabarovsk krai. Materials of the All-Russian scientific-practical conference dedicated to the 65th anniversary of the Khabarovsk krai.* Khabarovsk, pp. 80–86.

Kinsella, J.M. (2007) Helminths of the vagrant shrew, *Sorex vagrans*, from western Montana, USA. *Acta Parasitologica*, 52, 151–155.
http://dx.doi.org/10.2478/s11686-007-0021-4

Kinsella, J.M. & Tkach, V.V. (2009) Checklist of helminth parasites of Soricomorpha (=Insectivora) of North America north of Mexico. *Zootaxa*, 1969, 36–58.

Kornienko, S.A., Zubova, O. A., Gulyaev, V. D. & Dokuchaev, N. E.. (2008) [Cestodes of shrews on Kunashir Island]. *In*: K.V. Galaktionov & A.A. Dobrovolskij (Eds.), *Proceedings of the IV Congress of the Russian Society of Parasitologists, Russian Academy of Sciences, Vol. 2*, Lema, St. Petersburg, pp. 75–77. (in Russian)

Littlewood, D.T.J., Waeschenbach, A. & Nikolov, P. N. (2008). In search of mitochondrial markers for resolving the phylogeny of cyclophyllidean tapeworms (Platyhelminthes, Cestoda)—a test study with Davaineidae. *Acta Parasitologica*, 53, 133–144.
http://dx.doi.org/10.2478/s11686-008-0029-4

Locker, B. & Rausch, R. (1952) Some cestodes from Oregon shrews, with descriptions of four new species of *Hymenolepis* Weinland, 1858. *Journal of the Washington Academy of Sciences*, 42, 26–31.

Makarikov, A.A. & Gulyaev, V.D. (2009). [*Pararodentolepis* gen. n., - a new cestode genus from rodents and the description of *P. sinistra* sp. n. (Cyclophyllidea: Hymenolepididae).] *Parazitologiya*, 43, 454–459. (in Russian)

Morozov, Y.F. (1957) [Three new hymenolepidids from pygmy shrew]. *Uchenie zapiski Gor'kovskogo Gosuderstvennogo Pedagogicheskogo Instituta*, 19, 35–42. (in Russian)

Novikov, M.V. (1995) Cestodes of shrews (Insectivora, Soricidae) from the Magadan region, north-east Siberia.' *Acta Parasitologica*, 40, 37–42

Rausch R. & Kuns, M.L. (1950) Studies on some North American shrew cestodes. *Journal of Parasitology*, 36, 433–438. http://dx.doi.org/10.2307/3273168

Nolan, M.J. & T.H. Cribb. (2005) The use and implications of ribosomal DNA sequencing for the discrimination of digenean species. *Advances in Parasitology*, 60, 101–163. http://dx.doi.org/10.1016/s0065-308x(05)60002-4

Olson, P.D. & Tkach, V.V. (2005) Advances and trends in the molecular systematics of the parasitic platyhelminthes. *Advances in Parasitology*, 60, 165–243. http://dx.doi.org/10.1016/s0065-308x(05)60003-6

Sawada, I. & Kobayashi, S. (1994) Cestode parasites of some micromammalians (Insectivora) from the adjacent area of Akademgorodok City, Southern Central Siberia and Northern Teletskoye Lake, Altai Region, Russia. *Proceedings of the Japanese Society of Systematic Zoology*, 52, 14–33.

Senger, C.M. (1955) Observations on cestodes of the genus *Hymenolepis* in North American shrews. *Journal of Parasitology*, 41, 167–170. http://dx.doi.org/10.2307/3273786

Spassky, A.A. (1954) [Classification of Hymenolepididae from mammals]. *Trudy Gel'mintologicheskoj Laboratorii Akademii Nauk*, 7, 120–167. (in Russian)

Spasskii, A.A. (1959) [A more precise definition of the types of relative positions of the genitalia in the Hymenolepididae]. *Zoologicheskii Zhurnal*, 38, 31–37. (in Russian)

Stieda, L. (1862) Ein Beitrag zur Kenntniss der Tànien. *Archiv für Naturgeschichte, Berlin*, 1, 200–209.

Stiles, C.W. (1906) Illustrated key to the cestode parasites of man. *Bulletin of the Hygienic Laboratory of the U.S. Public Health and Marine-Hospital Service*, 25, 1–104.

Tkach, V. & Pawlowski, J. (1999) A new method of DNA extraction from the ethanol-fixed parasitic worms. *Acta Parasitologica*, 44, 147–148.

Tkach, V.V. & Velikanov, V.P. (1990) [A new cestode species (Cestoda, Hymenolepididae) from desert shrew]. *In*: *Novosti faunistiki i sistematiki*, Naukova dumka, Kiev, pp. 7–10 (in Russian).

Tkach, V.V. & Velikanov, V.P. (1991) *Pseudhymenolepis turkestanica* sp. n. (Cestoda: Hymenolepididae), a new cestode from shrews. *Annales de Parasitologie Humaine et Comparée*, 66, 54–56.

Vaucher, C. (1971) Les cestodes parasites des Soricidae d'Europe. Etude anatomique, révision taxonomique et biologie. *Revue Suisse de Zoologie*, 78,1–113.

Velikanov, V.P. & Tkach, V.V. (1993) [New cestode species (Cestoda, Hymenolepididae) from desert shrew]. *Vestnik zoologii*, N 5, 3–11 (in Russian).

Villot, F.C.A. (1877) Classification du règne animal. *Revue des Sciences Naturelles, Montpellier*, 47, No. 6.

Voge, M. & Rausch, R. (1955) Occurrence and distribution of hymenolepidid cestodes in shrews. *Journal of Parasitology*, 41, 566–574. http://dx.doi.org/10.2307/3274136

Yamaguti, S. (1959) *Systema Helminthum, Vol. 2. The Cestodes of Vertebrates*. Interscience Publishers, Inc., New York, 860 pp.