



Septitrema lichae n. g., n. sp. (Monogenea: Monocotylidae) from the nasal tissues of the deep-sea kitefin shark, *Dalatias licha* (Bonnaterre) (Squaliformes: Dalatiidae), off Algeria

Houda Kheddam · Leslie A. Chisholm · Fadila Tazerouti

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Abstract *Septitrema lichae* n. g., n. sp. (Monogenea: Monocotylidae: Merizocotylinae) is described from the nasal tissue of the deep-sea kitefin shark, *Dalatias licha* (Bonnaterre) (Dalatiidae) collected off Algiers, Algeria. The new genus is distinguished from the other genera in the subfamily by the number and arrangement of the loculi on the haptor having one central and seven peripheral loculi. The diagnosis of the Merizocotylinae is amended to accommodate this species and a key to the genera of the Merizocotylinae is provided. Terminology of the haptoral loculi in the

Merizocotylinae and the status of some of the genera in the subfamily are also discussed.

Introduction

The Monocotylidae is a family of monogeneans that parasitise chondrichthyans. A survey of the monogeneans from the deep-sea kitefin shark, *Dalatias licha* (Bonnaterre) was conducted between 2010 and 2012 off the coast of Algeria. This was part of a larger study to examine the parasite fauna of a diversity of shark species from the Mediterranean Sea off Algiers (see Kheddam et al., 2016). Monocotylids found on the nasal tissues of *D. licha* are described herein as a new genus and species in the Merizocotylinae. The diagnosis of the subfamily has been amended to accommodate the new genus and a key to the genera in the Merizocotylinae is given.

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H. Kheddam · F. Tazerouti
Laboratoire de Biodiversité et Environnement:
Interactions et Génomes, Faculté des Sciences
Biologiques, Université des Sciences et de la Technologie
Houari Boumediene, BP 32, El Alia Bab Ezzouar, Alger,
Algeria

H. Kheddam
Faculté des Sciences de la Nature et de la Vie, Université
Saad Dahleb-Blida, BP. 270, Route de Soumaa, Blida,
Algeria

L. A. Chisholm (✉)
Parasitology Section, South Australian Museum, North
Terrace, Adelaide, SA 5000, Australia
e-mail: leslie.chisholm@samuseum.sa.gov.au

L. A. Chisholm
Ecology and Evolutionary Biology, School of Biological
Sciences, University of Adelaide, North Terrace,
Adelaide, SA 5001, Australia

Materials and methods

Seventy specimens of the kitefin shark, *Dalatias licha* (Bonnaterre), were caught by fishermen while line fishing at four localities off Algeria: Delys (36°55'N, 3°53'E), Cap Djenet (36°43'N, 3°36'E), Bou Haroun (36°40'N, 4°40'E) and Cherchell (36°37'N, 2°11'E) between 2010 and 2012. The sharks were dissected in the laboratory shortly after capture, at which time the nasal tissues were removed and placed in Petri dishes containing filtered seawater. Live monogeneans, located using a stereomicroscope, were removed from between the nasal lamellae and were studied live unflattened or slightly flattened, between a slide and coverslip and then were fixed in 70% ethanol or Bouin-Hollande liquid. Specimens were stained with Grenacher's carmine or Semichon's carmine, dehydrated in an ethanol series, cleared in clove oil and mounted in Canada balsam. Several specimens were fixed, stained and mounted directly in Malmberg's media (Malmberg, 1970). Some nasal tissues were fixed in toto, just after necropsy, in 75% ethanol and the parasites collected were washed, stained and mounted as previously described. Preserved mounted adult specimens were examined using a Nikon compound microscope equipped with either phase contrast or DIC optics and drawings were made with the aid of a drawing tube. The drawings were scanned and redrawn on a computer using CorelDraw. Measurements were taken using a micrometer. All measurements are given in micrometres and are presented as the range followed by the mean and the number of structures measured in parentheses. Type-specimens are deposited in the Australian Helminthological Collection (AHC) at the South Australian Museum (SAMA), Adelaide, Australia.

To comply with the regulations set out in Article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of all new taxa have been submitted to ZooBank. For each new taxon, the Life Science Identifier (LSID) is reported in the taxonomic summary.

Family Monocotylidae Taschenberg, 1879

Subfamily Merizocotylinae Johnston & Tieg, 1922

Amended diagnosis

With characters of the Monocotylidae (*sensu* Chisholm et al., 1995). Haptor with one central

loculus (absent or replaced by central depression in species of *Mycteronastes* Kearn & Beverley-Burton, 1990 and in *Empruthotrema longipenis* Kritsky, Bullard, Ruiz & Warren, 2017) and four to seven peripheral loculi (*sensu* Kritsky et al., 2017). Haptor rarely with three loculi (*Triloculotrema* Kearn, 1993) or numerous randomly distributed loculi (*Cathariotrema* Johnston & Tieg, 1922). Interperipheral loculi present or absent. Marginal loculi present or absent, when present, interhamular marginal loculi one to four. Marginal valve of haptor absent. Fourteen marginal hooklets present. Eye-spots present or absent. Three prominent gland duct openings containing needle-like secretion present on either side of anterior end. Testis single. Male copulatory organ sclerotised (except in *Mycteronastes undulatae* Kearn & Beverley-Burton, 1990 and *M. caalusi* Kritsky, Bullard, Bakenhaster, Scharer & Poulakis, 2017). Ovary not lobed at blind end. Descending limb of oötype present or absent. Two vaginae present, walls of vaginae not sclerotised. Vaginal pores and common genital pore lacking spines. Parasites of Elasmobranchii and Holocephali.

Type-genus: *Merizocotyle* Cerfontaine, 1894.

Additional genera: *Cathariotrema* Johnston & Tieg, 1922; *Empruthotrema* Johnston & Tieg, 1922; *Holocephalocotyle* Derouiche, Neifar, Gey, Justine & Tazerouti, 2019; *Mycteronastes* Kearn & Beverley-Burton, 1990; *Squalotrema* Kearn & Green, 1983; *Septitrema* n. g. Kheddad, Chisholm & Tazerouti, 2020; *Thaumatocotyle* Odhner, 1910; *Triloculotrema* Kearn, 1993.

Septitrema n. g.

Diagnosis

Haptor divided by septa into one central loculus and seven peripheral loculi. Marginal loculi and interperipheral loculi absent. Single hamulus associated with each posterolateral radial septum. Fourteen (seven pairs) of hooklets in margin of haptor. Pairs 1 and 2 associated with posteriormost loculus, pairs 3, 4, 5 and 6 associated with the posterolateral loculi and pair 7 associated anterolateral loculi. Eye-spots not observed. Distal portion of sclerotised male copulatory organ curved. Ejaculatory bulb present; two internal chambers absent. Common genital pore and vaginal pores unarmed. Parasites of the nasal tissue of the Dalatiidae.

Type-species: Septitrema lichae n. g., n. sp.

ZooBank registration: The Life Science Identifier (LSID) for *Septitrema* n. g. is urn:lsid:zoobank.org:act:6F6F14E3-A825-414C-8068-84BFBA6CEC5B.

Etymology: The genus name *Septitrema* is derived from the word septa meaning seven to denote the number of peripheral loculi on the haptor.

***Septitrema lichae* n. sp.**

Type-host: Dalatias licha (Bonnaterre) (Squaliformes: Dalatiidae).

Type-locality: Delys (36°55'N, 3°53'E), Algerian coast.

Other localities: Off Cap Djenet (36°43'N, 3°36'E), Bou Haroun (36°40'N, 4°40'E), Cherchell (36°37'N, 2°11'E), Algerian coast of the Mediterranean.

Type-specimens: Holotype (AHC 36727); and 24 paratypes (AHC 36728–36751).

Site on host: Nasal tissue, attachment by hamuli piercing tissue.

Prevalence and intensity: 23 of 70 animals (33%) infected with mean intensity of 1.23 worms.

Zoobank registration: The Life Science Identifier (LSID) for *Septitrema lichae* n. sp. is urn:lsid:zoobank.org:act:96B62214-B216-4607-83B7-0B4909A68DE3.

Etymology: Professor Louis Euzet proposed the new species name “lichae”, when the worms were first collected, which relates to the host species. We have used this name to honour his original choice.

Description

[Based on 15 whole-mounted specimens; Figs. 1, 2.] Adult 1,280–2,700 (1,880, n = 9) long, 380–800 (503, n = 9) wide at level of ovary (Fig. 1A). Haptor oval, 180–490 (366, n = 10) long, 220–530 (426, n = 10) wide. Haptor with 1 central and 7 peripheral loculi (Fig. 1A). Hamulus (Figs. 1A, 1C) 110–270 (215, n = 11) long, associated with the posterolateral septa. Fourteen hooklets (Fig. 1D) 19–25 (21, n = 6) long, distributed in margin of haptor as illustrated (Fig. 1A). Mouth ventral, subterminal. Three anterolateral gland-duct openings containing what appears to be needle-like secretion open on each side of ventrolateral margin of anterior extremity (Fig. 1A); glands producing and ducts carrying these secretions as

illustrated (Fig. 1A). One pair of gland ducts containing what appears to be granular secretion open medially on the anterior extremity (Fig. 1A); glands producing these secretions not observed. Eye-spots not observed. Pharynx 110–240 (157, n = 8) long, 80–190 (128, n = 8) wide; pharyngeal glands not seen. Intestinal caeca 2, lacking diverticula, terminating blindly in prehaptoral region. Testis single. Vas deferens arises from left side of testis, runs anteriorly and inflates to form seminal vesicle (Figs. 1A, 2). Seminal vesicle then narrows slightly and enters posterior part of ejaculatory bulb. Ejaculatory bulb 68–103 (85, n = 9) long, 44–85 (68, n = 9) wide; internal chambers absent. Male accessory glands not observed. Male copulatory organ sclerotised, 60–80 (71, n = 6) long, distal end curved (Figs. 1A, B, 2). Ovary encircles right intestinal caecum dorsoventrally. Vaginal pores unarmed, open ventrally as illustrated (Figs. 1A, 2), common vaginal duct enters oval seminal receptacle dorsally. Vitellarium extends from level of posterior part of pharynx to posterior portion of body proper. Transverse vitelline ducts following path as illustrated (Figs. 1A, 2). Common vitelline duct and duct from seminal receptacle joining to oviduct not seen. Oviduct runs posteriorly and then turns anteriorly entering oötype posteriorly. Oötype muscular opening at unarmed, median common genital pore (Figs. 1A, 2). Mehlis' gland not observed. Eggs not observed.

Key to the genera of the Merizocotylineae

- | | | |
|----|--|-------------------------|
| 1a | Haptor with numerous unevenly distributed loculi; large hamuli | <i>Cathariotrema</i> |
| 1b | Hamuli present or absent | 2 |
| 2a | Hamuli absent | <i>Empruhotrema</i> |
| 2b | Hamuli present | 3 |
| 3a | Marginal ring of haptoral loculi absent | 4 |
| 3b | Marginal ring of haptoral loculi present | 7 |
| 4a | Haptor with three loculi | <i>Triloculotrema</i> |
| 4b | Haptor with central loculus and five or seven peripheral loculi | 5 |
| 5a | Haptor with central loculus and seven peripheral loculi | <i>Septitrema</i> n. g. |
| 5b | Haptor with central loculus and five peripheral loculi; interperipheral loculi present or absent | 6 |

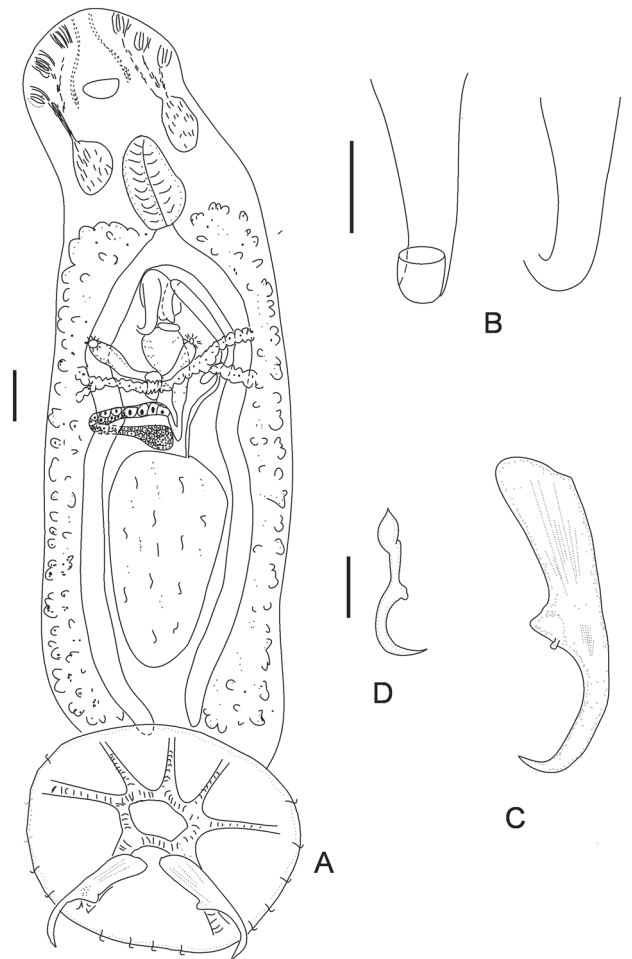


Fig. 1 *Septitrema lichae* n. g., n. sp. A, Whole adult worm, ventral view; B, Sclerotised male copulatory organ showing curved distal end; C, Hamulus; D, Marginal hooklet. Scale-bars: A, C, 100 μ m; B, 30 μ m; D, 10 μ m

- 6a Haptor with central loculus, five peripheral loculi and seven interperipheral loculi *Holocephalocotyle*
- 6b Haptor with central loculus and five peripheral loculi; interperipheral loculi absent *Squalotrema*
- 7a Haptor with central loculus, four peripheral loculi, 12 marginal loculi and one interhamular marginal loculus *Thaumatocotyle*
- 7b Haptor without this arrangement of loculi 8
- 8a Haptor lacking distinct central loculus (depression may be present); five peripheral loculi, 17 marginal loculi and one interhamular marginal loculus *Mycteronastes*
- 8b Haptor with one central loculus, six or seven peripheral loculi, 17 marginal loculi and one or four interhamular marginal loculi (except for *M. urolophi* which has four peripheral loculi, 11 marginal loculi and one interhamular marginal loculus) *Merizocotyle*

Discussion

Septitrema lichae n. g., n. sp. is best accommodated in the Merizocotylineae because it has three prominent anterolateral gland-duct openings containing needle-like secretion that open on each side of the ventrolateral margin of anterior end, two vaginae and a single

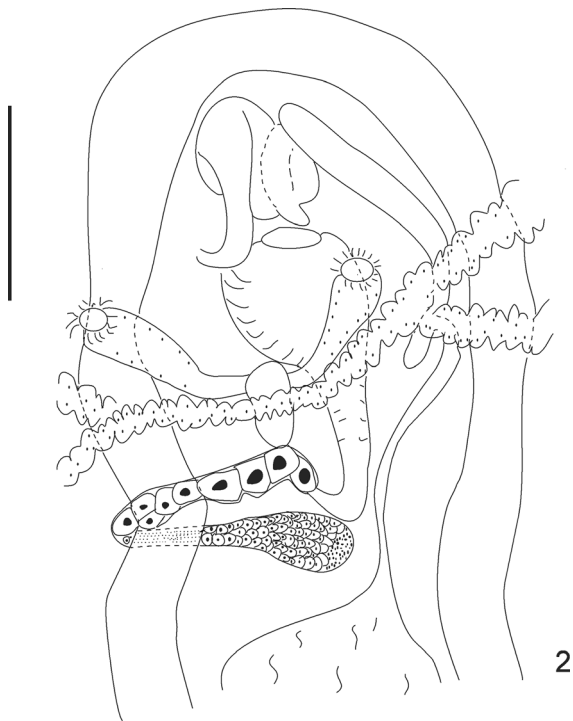


Fig. 2 Reproductive system of *Septitrema lichae* n. g., n. sp., ventral view. Scale-bar: 100 μ m

testis. *Septitrema* can be easily distinguished from the other genera in the subfamily by the haptor which has one central and seven peripheral loculi. With the erection of *Septitrema* there are now nine genera in the Merizocotylineae.

Chisholm et al. (1995) synonymised *Mycteronastes* and *Thaumatocotyle* with *Merizocotyle* based on results of their morphological phylogeny. However, Chisholm et al. (2001) suggested that these genera could be resurrected in the future when their analysis based on 28S rDNA showed *Merizocotyle* to be paraphyletic. Indeed, *Thaumatocotyle* was considered valid by Neifar et al. (2000), de Buron & Euzet (2005) and Marie & Justine (2006) and recently, *Mycteronastes* was resurrected by Kritsky et al. (2017). Kritsky et al. (2017) pointed out that the loculi characters Chisholm et al. (1995) chose for their analysis were too generalised and probably masked some potential synapomorphies for the genera in question. As such Kritsky et al. (2017) also introduced new nomenclature for the marginal loculi present on the haptor of Merizocotylineae species in the genera *Empruthotrema*, *Merizocotyle*, *Mycteronastes* and

Thaumatocotyle. They termed the marginal loculi located between hamuli, “interhamular loculi” to help identify homologs of marginal loculi. The number of “interhamular loculi” can vary in number from one to four. Recently Derouiche et al. (2019) amended the diagnosis of the Merizocotylineae to accommodate their new genus *Holocephalocotyle*. They defined the subfamily, in part, as having “one to four interhamular loculi”. This is incorrect because interhamular loculi, by definition, are absent in *Holocephalocotyle*, *Septitrema* n. g., *Squalotrema* and *Triloculotrema* which do not have marginal loculi. We have renamed the marginal loculi between the hamuli as “interhamular marginal loculi” to avoid future confusion. We have amended the diagnosis of the Merizocotylineae to accommodate the locular arrangement in *Septitrema* n. g. and to refine the marginal loculus terminology of Kritsky et al. (2017).

With the description of *Septitrema lichae* n. g., n. sp., there are now 40 species considered valid in the Merizocotylineae. The marginal valve of the haptor is absent in members of the subfamily (see Chisholm et al., 1995) and the haptor of most merizocotylineae has a marginal ring of loculi (or numerous randomly distributed loculi as seen in *Cathariotrema selachii* (MacCallum, 1916) Johnston & Tieg, 1922). Chisholm et al. (1995) postulated that when the marginal valve has been secondarily lost, the marginal loculi may serve to make the muscular rim of the haptor thinner thereby providing an effective seal. For members of the Merizocotylineae genera where the marginal ring of loculi is absent (*Holocephalocotyle*, *Septitrema* n. g., *Squalotrema* and *Triloculotrema*), it is possible that the seal is less efficient, and a supplementary method of attachment is necessary. Indeed, we observed that *Septitrema lichae* n. g., n. sp. attaches firmly to *D. licha* by piercing the nasal tissue with the sharp points of the hamuli. Boudaya & Neifar (2016) also observed this mode of attachment in *Triloculotrema euzeti* Boudaya & Neifar, 2016 and concluded that this was effective and did not rely on the presence of loculi. Other species of the Merizocotylineae should be examined to determine if hamuli are the primary mode of attachment in species lacking marginal loculi.

The present study was part of a large-scale survey conducted between 2009 and 2015 (see Kheddam et al., 2016) to investigate the diversity of sharks and their monogenean/cestode parasites in Mediterranean

Sea off Algiers, Algeria. A total of 765 specimens of sharks representing ten species were surveyed (see Kheddad et al., 2016) and only four monogenean species were found. These included *Hexabothrium appendiculatum* (Kuhn, 1829) von Nordmann, 1840 from *Scyliorhinus canicula* (Linnaeus), *Protocotyle grisea* (Cerfontaine, 1899) Euzet & Maillard, 1974 from *Hexanchus griseus* (Bonnaterre), *Squalonchocotyle euzeti* Kheddad, Justine, & Tazerouti, 2016 from *D. licha* and *Septitrema licha* n. g., n. sp. described here from *D. licha* (see Kheddad et al., 2016; Kheddad unpublished data). Tazerouti (2007) examined 388 rays representing 15 species from the same four localities off Algiers that we sampled in the present study and found 22 monogenean species. Whittington & Chisholm (2003) showed that monogenean species diversity is generally higher on rays than sharks. They provided evolutionary and biological reasons why this may be the case, but also proposed that this observation may simply be due to sampling bias and lack of negative data reporting. Examining sharks for parasites can present various collecting and handling difficulties and therefore they are certainly less targeted in surveys. However, finding only four monogenean species on the 765 sharks examined during the large-scale survey compared to the 22 monogenean species found on half the number of rays collected in the same region (Tazerouti, 2007) suggests that in this case, the low diversity may be due to evolutionary and/or biological factors. More large-scale surveys of sharks and their parasites are required to test this further.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guidelines on the care and use of laboratory animals.

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