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FISH PARASITOLOGY - ORIGINAL PAPER

Redescription and molecular characterization of two species of *Pauciconfibula* (Monogenea, Microcotylidae) from trachinid fishes in the Mediterranean Sea

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Abstract

Many *Pauciconfibula* spp. have a long and complicated taxonomic history. The remaining unsolved taxonomic confusion in this genus is impelled by the host range and status of *Pauciconfibula* spp. from trachinid fishes: *Pauciconfibula trachini* and *Pauciconfibula draconis*, from *Trachinus radiatus* and *Trachinus draco* (Trachinidae), respectively. *Pauciconfibula trachini* was reported on *Trachinus draco*, type host of *Pauciconfibula draconis* suggesting thus a stenoxenic specificity for the former monogenean and the occurrence of two congeneric polyopisthocotyleans on a single host. Moreover, the validity of *Pauciconfibula draconis* was repeatedly questioned by several authors, unjustified synonymy between the two species was proposed, and the delimitations between the two species remained unsolved. Original descriptions were also incomplete and poorly illustrated. In this study, we provide a detailed illustrated redescription of both species based on newly collected specimens of *Pauciconfibula trachini* and *Pauciconfibula draconis* collected from their type hosts from off three Mediterranean localities: Algeria, Tunisia, and Italy. Integrative taxonomy using COI sequences was applied to resolve the delimitation between *Pauciconfibula trachini* and *P. draconis*. This study provides the first DNA barcoding for members of this genus.

Keywords Integrative taxonomy · Barcoding · COI · *Pauciconfibula trachini* · *Pauciconfibula draconis* · *Trachinus*

Introduction

The taxonomy of monogeneans is generally based on sclerotized structures such as spines of the genital openings. *Pauciconfibula* Dillon & Hargis, 1965 is characterized by unarmed male and female genital openings (Dillon and Hargis 1965; Chisholm et al. 1991; Cantatore, Lanfranchi & Timi, 2011), which makes it a difficult case for taxonomists.

This genus includes six nominal species (Cantatore, Lanfranchi & Timi, 2011): *Pauciconfibula trachini* (Parona & Perugia, 1889) [syn.: *Microcotyle trachini* Parona & Perugia, 1889] on the starry weever *Trachinus radiatus*

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Cuvier, 1829 off the Italian coast (Parona & Perugia, 1889, 1890); *P. draconis* (Briot, 1904) [syn. : *Microcotyle trachini* Briot, 1904] on the greater weever *T. draco* Linnaeus, 1758 from the English Channel and off Scotland (Briot 1904; Nicoll 1914); *P. pogoniae* (MacCallum, 1913) on the black drum *Pogonias cromis* (Linnaeus 1766) [syn.: *M. pogoniae* MacCallum, 1913] from New York, USA (MacCallum 1913); *P. euzeti* (Ktari, 1971) [syn.: *Aspinatrium euzeti* Ktari, 1971] on the pink dentex *Dentex gibbosus* (Rafinesque, 1810) (Ktari 1971); and *P. gallieni* (Euzet & Ktari, 1971) [syn.: *A. gallieni* Euzet & Ktari, 1971] on the agujon needlefish *Tylosurus acus acus* (Lacépède, 1803) (Euzet and Ktari 1971) off the Tunisian coast. Except *P. gallieni* collected from the inner operculum of its host (Euzet and Ktari 1971), all the previously mentioned species inhabit gills of marine teleosts. In contrast, the latest species added to this genus, *P. subsolana* Chisholm, Beverley-Burton & McAlpine, 1991, was collected in the Saint John River, Canada, from the gills of the white perch *Morone americana* (Gmelin, 1789) (Chisholm et al. 1991), a Moronidae Jordan & Evermann, 1896 occurring in fresh, brackish and coastal waters.

Many *Pauciconfibula* spp. have a long and complicated taxonomic history (Table 1). First described as members of *Microcotyle* Van Beneden and Hesse, 1863 (Briot 1904; MacCallum 1913; Parona & Perugia, 1889, 1890) or of *Aspinatrium* Yamaguti, 1963 (Euzet and Ktari 1971; Ktari 1971), they have been placed in *Bradyhaptorus* Unnithan, 1971 (Unnithan 1971) then in *Pseudaspinatrium* Mamaev, 1986 (Mamaev 1986) before their inclusion in *Pauciconfibula* (Chisholm et al. 1991; Dillon and Hargis 1965).

However, the remaining unsolved taxonomic confusion in the genus *Pauciconfibula* is prompted by the host range and status of *Pauciconfibula* spp. from trachinid fishes:

1. *Pauciconfibula trachini* was first described from *Trachinus radiatus* (Bychowsky 1961; Parona 1889, 1890) then reported on a congeneric host *T. draco* (Akmirza 2004; López-Román and Guevara Pozo 1973; Radujkovic and Euzet 1989), the type host of *P. draconis*. This indicates a stenoxenic specificity for *P. trachini* and the occurrence of two congeneric polyopisthocotyleans on a single host.

2. The original description of *P. draconis* (Briot 1904) and subsequent redescriptions (Nicoll 1914) were incomplete with imprecise illustrations giving thus insufficient data for comparison. Although some authors suggested the conspecificity of *P. draconis* with *P. trachini* (Chisholm et al. 1991; Dillon and Hargis 1965; Nicoll 1914), they still listed it as a distinct species imminent additional observation (Chisholm et al. 1991; Dillon and Hargis 1965; Sproston 1946). In fact, Dillon and Hargis (1965) clearly stated "every effort should be made to redescribe of these two species and re-evaluate the validity of our actions" (Dillon and Hargis 1965). Moreover, in an unjustified action, Radujkovic and Euzet (1989) assigned their specimens collected on *T. draco* to *P. trachini* and synonymized the latest with *P. draconis* (Radujkovic and Euzet 1989).

During a parasitological survey of parasites infecting Mediterranean marine teleosts, some monogeneans were collected from the gills of two trachinids, *Trachinus radiatus*, and *Trachinus draco*. After detailed morphological study, we identified them as *Pauciconfibula trachini* and *P. draconis* (Microcotylidae). We provide here a detailed illustrated redescription of both species based on the newly collected specimens from their type-hosts. Integrative taxonomy is applied herein to resolve the delimitation between the two species.

Material and methods

Collection and sampling of fish

From March 2014 to January 2018, a total of 558 trachinid fishes belonging to *Trachinus draco* (67 specimens) and *T. radiatus* (491 specimens) were collected from four marine regions, located in the Mediterranean sea: Bay of Bizerte (37° 20′ N, 9° 53′ E), Gulf of Tunis (36° 49′ N, 10° 18′ E), Sousse (35° 50′ N, 10° 38′), and Mahdia (35° 29′ N, 11° 3′ E) along the Tunisian coast; Bouharoun (36° 37′ N, 2° 39′ E) and Algiers (28° 01′ N, 1° 39′ E) along the Algerian coast, and Gulf of Naples (off Capri Island, 40° 33′ N, 14° 13′ E), Italy. The number of examined specimens and the range of total length (TL) according to the locality are given in Table 2. Fish specimens were transferred to the laboratory shortly after capture and identified using keys (Fischer et al. 1987) and examined fresh on the day of purchase. Gill arches were removed, placed in separate Petri dishes, and observed under a microscope for the presence of monogeneans.

Monogeneans

Morphological methods

Monogeneans were removed from gills using fine dissection needles, then preserved in 70% ethanol, stained with acetic carmine, dehydrated in graded ethanol series (70, 96, and 100%), cleared in clove oil, and mounted in Canada balsam. Drawings were made with the help of a Leitz microscope equipped with a drawing tube. Drawings were scanned and redrawn on a computer with Adobe Illustrator. Measurements are in micrometers and indicated as the range followed by the mean.

Table 1 Synonyms, hosts, and localities of *Pauciconfibula* spp. in the literature. *junior synonym

Parasites	Synonyms	Type-host	Type-locality	Source
Pauciconfibula trachini (Parona & Perugia, 1889) Dillon & Hargis, 1965	*Microcotyle trachini** Parona & Perugia, 1889 *Diplasiocotyle trachini** (Parona & Perugia, 1889) Tripathi, 1954 *Aspinatrium trachini** (Parona & Perugia, 1889) Yamaguti, 1963 *Bradyhaptorus trachini** (Parona & Perugia, 1889) Unnithan, 1971	*Trachinus radiatus* (Trachinidae)	Naples, Italy, Mediterranean	(Parona & Perugia, 1889, 1890; Tripathi 1956; Unnithan 1971; Yamaguti 1963)
Pauciconfibula draconis (Briot, 1904) Dillon & Hargis, 1965	*Microcotyle draconis** (Briot, 1904) *Bradyhaptorus draconis** (Briot, 1904) Unnithan, 1971 *Pseudoaspinatrium draconis** (Briot, 1904) Mamaev, 1986	*Trachinus draco* (Trachinidae)	North Sea, North Atlantic	(Briot 1904; Dillon and Hargis 1965; Unnithan 1971) Briot (1904) ; Dillon and Hargis (1965)
Pauciconfibula pogoniae (MacCallum, 1913) Chisholm, Beverley-Burton & McAlpine, 1991	*Pseudoaspinatrium pogoniae** (MacCallum, 1913) Mamaev, 1986	*Pogonias cromis* (Linnaeus, 1766) (Sciaenidae)	New York, North Atlantic	(Chisholm et al. 1991; MacCallum 1913; Mamaev 1986)
*Aspinatrium euzeti** Ktari, 1971	*Pseudoaspinatrium euzeti** (Ktari, 1971) Mamaev, 1986 *Pauciconfibula euzeti* (Ktari, 1971) Chisholm, Beverley-Burton & McAlpine, 1991	*Dentex gibbosus* (Rafinesque, 1810) (Sparidae)	Tunisia, Mediterranean	(Chisholm et al. 1991; Ktari 1971; Mamaev 1986)
Pauciconfibula subsolana Chisholm, Beverley-Burton & McAlpine, 1991	-	*Morone americana* (Gmelin, 1789) (Moronidae)	Canada, freshwater	(Chisholm et al. 1991)
Pauciconfibula patagonensis Cantatore, Lanfranchi & Timi, 2011	-	*Congiopodus peruvianus* (Cuvier, 1829) (Congiopodidae)	Argentina, Atlantic	(Cantatore, Lanfranchi & Timi, 2011)

Molecular methods

For a complete traceability of the molecular study, special attention was given to ensure that hosts and monogeneans were labeled with respect of host-parasite relationships (Justine et al. 2013).

Specimens of *Pauciconfibula* were extracted from the same host fish, and a tissue sample from the gills of the fish was taken. The extracted monogeneans were cut in three parts using a scalpel blade. Their anterior parts (which include the genital atrium) fixed in absolute ethanol then subjected to molecular analyses; posterior parts (which include the haptor) were mounted on a slide for drawing and deposition in a museum (Ayadi et al. 2017; Bouguerche et al. 2019a; Bouguerche et al. 2019b; Bouguerche et al. 2020). Nine specimens were analyzed (Table 3). Slides were deposited in the Muséum National d'Histoire Naturelle, Paris, France (MNHN), under registration numbers MNHN HEL1421-1430.

Molecular barcoding of fish

Total genomic DNA was isolated using QIAamp DNA Mini Kit (Qiagen, Courtaboeuf, France) as per the manufacturer's instructions. The 5′ region of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified with the primers TelF1 (5′- TCGACTAATCAYAAAGAYATYGGCAC-3′) and TelR1 (5′-ACTTCTGGGTGNCCAAARAATCARAA-3′) (Dettaï et al. 2011). PCR reactions were performed in 20 μl, containing 1 ng of DNA, 1× CoralLoad PCR buffer, 3 mM MgCl2, 66 μM of each dNTP, 0.15 μM of each primer, and 0.5 units of Taq DNA polymerase (Qiagen). The amplification protocol was 4 min at 94 °C, followed by 40 cycles at 94 °C for 30 s, 48 °C for 40 sec, and 72 °C for 50 s, with a final extension at 72 °C for 7 min. PCR products were purified (Ampure XP Kit, Beckman Coulter) and sequenced in both directions on a 3730xl DNA Analyzer 96-capillary sequencer (Applied Biosystems, Foster City, USA). We used the CodonCode Aligner version 3.7.1 software (Codon Code Corporation, Dedham, MA, USA) to edit sequences, compared them to the GenBank database content with BLAST, and deposited them in GenBank under accession numbers MW484937- MW484939. Species identification was confirmed with the BOLD identification engine (Ratnasingham and Hebert 2007).

COI sequences of monogeneans

Total genomic DNA was isolated using QIAmp DNA Micro Kit (Qiagen). The specific primers JB3 (=COIASmit1) (forward 5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and JB4.5 (=COI-ASmit2) (reverse 5′-TAAAGAAAGAACATAATGAAAATG-3′) were used to amplify a fragment of 424 bp of the COI gene (Bowles et al. 1995; Littlewood et al. 1997). PCR reaction was performed in 20 μl, containing 1 ng of DNA, 5× iProof HF buffer, 0.25 mM dNTP, 0.15 μM of each primer, and 0.5 units of iProof HF DNA polymerase

Table 2 Number and range of total length (TL) of the examined hosts according to the locality

Species	Locality	Number	Range of TL (mm)
T. draco	Bay of Bizerte (Tunisia)	190	21.5–30.5
	Sousse (Tunisia)	110	15.5–28.5
	Mahdia (Tunisia)	156	19.5–29.5
	Gulf of Tunis (Tunisia)	65	15.5–23.5
	Bouharoune (Algeria)	134	18–32.5
T. radiatus	Bay of Bizerte (Tunisia)	26	19.5–34.5
	Sousse (Tunisia)	18	14.5–31.5
	Mahdia (Tunisia)	46	14.5–34.5
	Algiers (Algeria)	1	40
	Off Capri Island, Gulf of Naples (Italy)	1	31

(Biorad). Thermocycles consisted of an initial denaturation step at 94 °C for 2 min, followed by 37 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 40 s, and extension at 72 °C for 50 s. The final extension was conducted at 72 °C for 5 min. Sequences were edited with CodonCode Aligner software version 3.7.1 (CodonCode Corporation, Dedham, MA, USA), compared to the GenBank database content with BLAST, and deposited in GenBank under accession numbers MW484928- MW484936.

Trees and distances

Most sequences of Microcotylidae available in GenBank were included in the phylogenetic analysis (Table 4), with 2 *Pauciconfibula draconis* and 7 *P. trachini* sequences obtained in the present study (2 off the Algerian coast, 4 off the Tunisian coast, and 1 from Gulf of Naples, Italy). Sequences of *Allogastrocotyle bivaginalis* Nasir and Fuentes Zambrano, 1983 (Gastrocotylidae Price, 1943) and *Kuhnia scombri* (Kuhn, 1829) (Mazocraeidae Price, 1936) were used as outgroup. Phylogenetic analyses were performed in MEGA version 7 (Kumar et al. 2016) based on the best scoring. Maximum likelihood was used for the best fitting tree according to the Tamura-Nei (TN93), rates among sites were gamma distributed (G); the number of discrete gamma categories was 5. The robustness of the inferred analysis was assessed using a bootstrap procedure with 1 000 replications. Genetic distances (p-distance and Kimura-2 parameter distance (Kimura 1980) were estimated with MEGA7 and all codon positions were used.

Results

Molecular identification of fish

The provisional identification of the two fish species using morphological characteristics was confirmed by DNA barcoding approach. BLAST analysis of the COI sequences of fish species of the present study with NCBI and BOLD database showed sequence similarity values of 99.85% for *Trachinus draco* and *T. radiatus,* respectively.

Molecular characterization of monogeneans

The COI sequences of *Pauciconfibula* spp. were aligned with several microcotylid. For trees, the neighbor-joining and maximum likelihood methods led to similar topologies; we show only the later in Fig. 1. The analysis involved 28 nucleotide sequences, and there was a total of 263 positions in the final dataset.

Microcotylidae clustered in the same clade, distinct from the outgroup. All sequences of *Pauciconfibula trachini* and *P. draconis* generated in the present study nested within a monophyletic lineage. *Pauciconfibula trachini* from the type

Table 3 Fish, Monogeneans, and their COI sequences. To ensure full traceability and respect of host-parasite relationships, one monogenean was collected from one fish and each fish and monogenean individuals was sequenced

Fish species	Fish Id	Fish COI sequence	Monogenean species	Locality	Monogenean Id	Monogenean COI sequence	Voucher deposited in MNHN
Trachinus draco	Trdr Br1	MW484937	*Pauciconfibula draconis*	Algeria	Trdr Br1 MO-01	MW484928	HEL1421
Trachinus draco	Trdr Br2	MW484938	*Pauciconfibula draconis*	Algeria	Trdr Br1 MO-02	MW484929	HEL1422
Trachinus radiatus	TrraRamla1	MW484939	-	-	-	-	-
Trachinus radiatus	-	-	*Pauciconfibula trachini*	Algeria	TrraAl MO-01	MW484930	HEL1423
Trachinus radiatus	-	-	*Pauciconfibula trachini*	Algeria	TrraAl MO-03	MW484931	HEL1424
Trachinus radiatus	-	-	*Pauciconfibula trachini*	Italy	Trra MO-08	MW484935	HEL1425
Trachinus radiatus	-	-	*Pauciconfibula trachini*	Tunisia	Trra MO-01	MW484932	HEL1426
Trachinus radiatus	-	-	*Pauciconfibula trachini*	Tunisia	Trra MO-02	MW484933	HEL1427
Trachinus radiatus	-	-	*Pauciconfibula trachini*	Tunisia	Trra MO-04	MW484934	HEL1428
Trachinus radiatus	-	-	*Pauciconfibula trachini*	Tunisia	TraraxBrx	MW484927	HEL 1429
Trachinus radiatus	-	-	*Pauciconfibula trachini*	Tunisia	TrraRa1Pau1	MW484936	HEL1430

Table 4 Sequences used in the present molecular study

Parasite species	Host species	Origin	GenBank	Source
Pauciconfibula draconis	*Trachinus draco*	Algeria	MW484928 MW484929	Present study
Pauciconfibula trachini	*Trachinus radiatus*	Algeria	MW484930 MW484931	
		Tunisia	MW484932 MW484933 MW484934 MW484927 MW484936	
		Italy	MW484935	
Microcotyle visa Bouguerche, Gey, Justine and Tazerouti, 2019	*Pagrus caeruleostictus*	Algeria	MK275652 MK275653 MK275654	(Bouguerche et al. 2019a)
Microcotyle isyebi Bouguerche, Gey, Justine and Tazerouti, 2019	*Boops boops*	Algeria	MK317922	(Bouguerche et al. 2019b)
		Spain	MN816019	(Víllora-Montero et al. 2020)
Microcotyle algeriensis Ayadi, Gey, Justine and Tazerouti, 2017	*Scorpaena notata*	Algeria	KX926443 KX926444	(Ayadi et al. 2017)
Microcotyle sp.	*Helicolenus dactylopterus*	Algeria	KX926446 KX926447	(Ayadi et al. 2017)
Microcotyle sebastis Goto, 1894	*Sebastes schlegeli*	South Korea	NC009055 DQ412044	(Park et al. 2007)
Microcotyle erythrini van Beneden and Hesse, 1863	*Pagellus erythrinus*	France	AY009159	(Jovelin and Justine 2001)
		Spain	MN816012 MN816013	(Víllora-Montero et al. 2020)
M. whittingtoni Víllora-Montero et al., 2020	*Dentex dentex*	Spain	MN816010 MN816011	(Víllora-Montero et al. 2020)
M. caudata Goto, 1894	*Sebastes inermis*	Inland Sea	LC472527 LC472528	Kamio and Ono (unpublished data)
Microcotyle sp.	*Helicolenus dactylopterus*	Algeria	KX926446 KX926447	(Ayadi et al. 2017)
Microcotyle sp.	*Sebastiscus marmoratus*	Inland Sea	LC472526	Kamio and Ono (unpublished data)
Kuhnia scombri (Kuhn, 1829)	*Scomber japonicus*	China	KU380119	(Yan et al. 2016)
Paramicrocotyle sp.	*Pinguipes chilensis*	Chile	KJ794215	(Oliva et al. 2014)
Pedocotyle bravoi Luque-Alejos and Iannacone-Oliver, 1990	*Prolatilus jugularis*	Chile	KJ794211	(Oliva et al. 2014)
Sparicotyle chrysophryi van Beneden and Hesse, 1863	*Boops boops*	Adriatic	GQ240258	(Mladineo et al. 2009)
Sparicotyle chrysophryii	*Sparus aurata*	Adriatic	GQ240252	(Mladineo et al. 2009)
Pagellicotyle mormyri (Lorenz, 1878)	*Lithognathus mormyrus*	France	AY009160	(Jovelin and Justine 2001)
Pyragraphorus hollisae Euzet and Ktari, 1970	*Trachinotus ovatus*	France	AY009162	(Jovelin and Justine 2001)
Bivagina pagrosomi (Murray, 1931)	*Sparus aurata*	Australia	Z83003	(Littlewood et al. 1997)
Pagellicotyle mormyri	*Lithognathus mormyrus*	France	AY009160	(Jovelin and Justine 2001)

host *Trachinus radiatus* off three Mediterranean localities (Algeria, Tunisia, and Italy) clustered in a robust clade (100 bootstrap) whereas *P. draconis* from the type host *T. draco* clustered in a distinct and well-supported clade (100 bootstrap), supporting thus *P. trachini* and *P. draconis* as distinct species. The support value of other clades was generally low.

Distances were computed using p-distance and Kimura 2-parameter distance (Table 5). The differences were minor so only p-distances are commented here. Sequences of *Pauciconfibula draconis* from the type-host *Trachinus draco* off the Algerian coast were identical between them (0% intra-specific variation). Sequences of *P. trachini* from the type host *T. radiatus* off three Mediterranean localities (Algeria, Tunisia, and Italy) showed little to no variations: 0-2%, an order of magnitude inferior to the interspecific distances.

Pauciconfibula trachini (Parona & Perugia, 1889) Dillon & Hargis, 1965 (Figs. 2, 3, and 4)

Synonymes: *Microcotyle trachini* Parona & Perugia, 1889 (Parona & Perugia, 1889, 1890); *Diplasiocotyle trachini* (Parona & Perugia, 1889) Tripathi, 1956 (Tripathi 1956); *Aspinatrium trachini* (Parona & Perugia, 1889) Yamaguti, 1963 (Yamaguti 1963); *Bradyhaptorus trachini* (Parona & Perugia, 1889) Unnithan, 1971 (Unnithan 1971).

Type-host: *Trachinus radiatus* Cuvier, 1829 (Trachinidae), starry weever (Parona & Perugia, 1889, 1890).

Type-locality: off Genova, Italy, Mediterranean (Parona & Perugia, 1889, 1890).

Additional localities: off Naples, Italy, this paper; Bouharoun, off the Algerian coast, this paper; Tunisia, this paper.

Site on host: Gills.

Specimens from Algeria: Vouchers deposited in the collections of the Muséum National d'Histoire Naturelle, Paris (MNHN HEL1371 - HEL1393, MNHN HEL1410 – HEL1420).

Specimens from Tunisia: Vouchers deposited in the collections of the Muséum National d'Histoire Naturelle, Paris (MNHN HEL1394–HEL1396).

Vouchers with molecular information: posterior parts of specimens containing the haptor mounted on slide, anterior part used for molecular analysis; off the Algerian coast (MNHN HEL1423, GenBank MW484930; MNHN HEL1424, GenBank MW484931), from Italy (MNHN HEL1425, GenBank MW484935, from Tunisian waters (MNHN HEL1426, GenBank MW484932; MNHN HEL1427, GenBank MW484933; MNHN HEL1428, GenBank MW484934; MNHN HEL1429, GenBank MW484927; MNHN HEL1430, GenBank MW484936).

Description

Body elongated, slightly thinned in its anterior region (Fig. 2a). Haptor symmetrical and quadrangular, armed with 8 to 10 pairs of clamps arranged symmetrically in two parallel rows. Clamps of unequal sizes: first pair always smaller.

Clamps of *Microcotyle* type (Fig. 2d). Ventral arm of median spring *a1* elongated. Distal part of *a1* T-shaped. Ventral arm of ventral jaw *b1* arched (Fig. 4a). Dorsal arms of median spring *a2* and *a3* with two parallel rows of small pores of different shape and size. Dorsal arm of ventral jaw *b2* long. Dorsal arm of dorsal jaw *c* arched (Fig. 4b). On proximal and distal side, *a2* and *b2* connected by a muscle; *b1* and *c* surrounded by a muscle (Fig. 2d). We note the presence of numerous lateral epidermal thickenings (Fig. 4c).

Anterior extremity rounded. Prohaptoral suckers paired, septate, with numerous minute spines. Pharynx median, voluminous, (Fig. 2b) Caeca with numerous lateral and axial diverticula; caeca obscured by vitellarium in most body parts.

Testes numerous, in intercæcal field of posterior third. Vas deferens wide, conspicuous, running forward along body midline to genital atrium. Genital atrium middorsal, muscular and unarmed (Fig. 2b).

Ovary pre-testicular begins on the right side of the body. It makes a handle and passes on the left by a thin elbow (Fig. 3). It goes up on the midline, makes a second loop to return to the right, where it widens before emerging into the oviduct. Oviduct dorsal, connecting common vitelline duct and uterus. Uterus descending and then ascending, running forward along body midline dorsal to vas deferens.

Vitellarium follicular, co-extensive with caeca, extending from level of genital atrium to the haptor. Posterior extremities of vitelline fields symmetrical often joined. Vitelloducts Y-shaped with very long lateral branches. Dorsal transverse vitelloducts fused at level of ovary. Common vitelline duct median and dorsal to ovary, fairly long. Eggs fusiform with two polar filaments: anterior filament long and curled; posterior filament short and thick (Fig. 2c).

Pauciconfibula draconis **(Briot, 1904) Dillon & Hargis, 1965 (Figs. 5, 6, and 7)**

Synonymes: *Microcotyle draconis* Briot, 1904 (Briot 1904); *Bradyhaptorus draconis* (Briot, 1904) Unnithan, 1971 (Unnithan 1971); *Pseudoaspinatrium draconis* (Briot, 1904) Mamaev, 1986 (Mamaev 1986).

Type-host: *Trachinus draco*, Linnaeus 1758 (Trachinidae), greater weever (Briot 1904).

Type-locality: English Channel, North Sea, Atlantic (Briot 1904).

Additional localities: Spain (López-Román and Guevara Pozo 1973); Montenegro (Radujkovic and Euzet 1989); Turkey (Akmirza 2004); off the Algerian coast, Bouharoun, this paper.

Site on host: Gills.

Specimens from Algeria: Vouchers deposited in the collections of the Muséum National d'Histoire Naturelle, Paris (MNHN HEL1397 – HEL1409).

Vouchers with molecular information posterior parts of specimens containing the haptor mounted on slide, anterior part used for molecular analysis; off the Algerian coast (MNHN HEL1421, GenBank MW484928, MNHN HEL1422, GenBank MW484929).

Description

Body stocky (Fig. 5a). Haptor quadrangular armed with 8 to 10 pairs of clamps arranged in two symmetrical rows; clamps rows spaced apart; clamps size regularly decreasing anteroposteriorly. Clamps *Microcotyle* type (Fig. 5e), disposition of sclerites is identical to that of *Pauciconfibula trachini* (Fig. 7a, b). We also note the presence of numerous lateral epidermal thickenings (Fig. 7c).

Prohaptoral suckers paired, globular, opening laterally and surrounded by numerous papillae (Fig. b). Dorsally, cavity of each buccal organ is divided by transverse partition in two unequal compartments. Pharynx bulky. Intestinal caeca with numerous lateral diverticula.

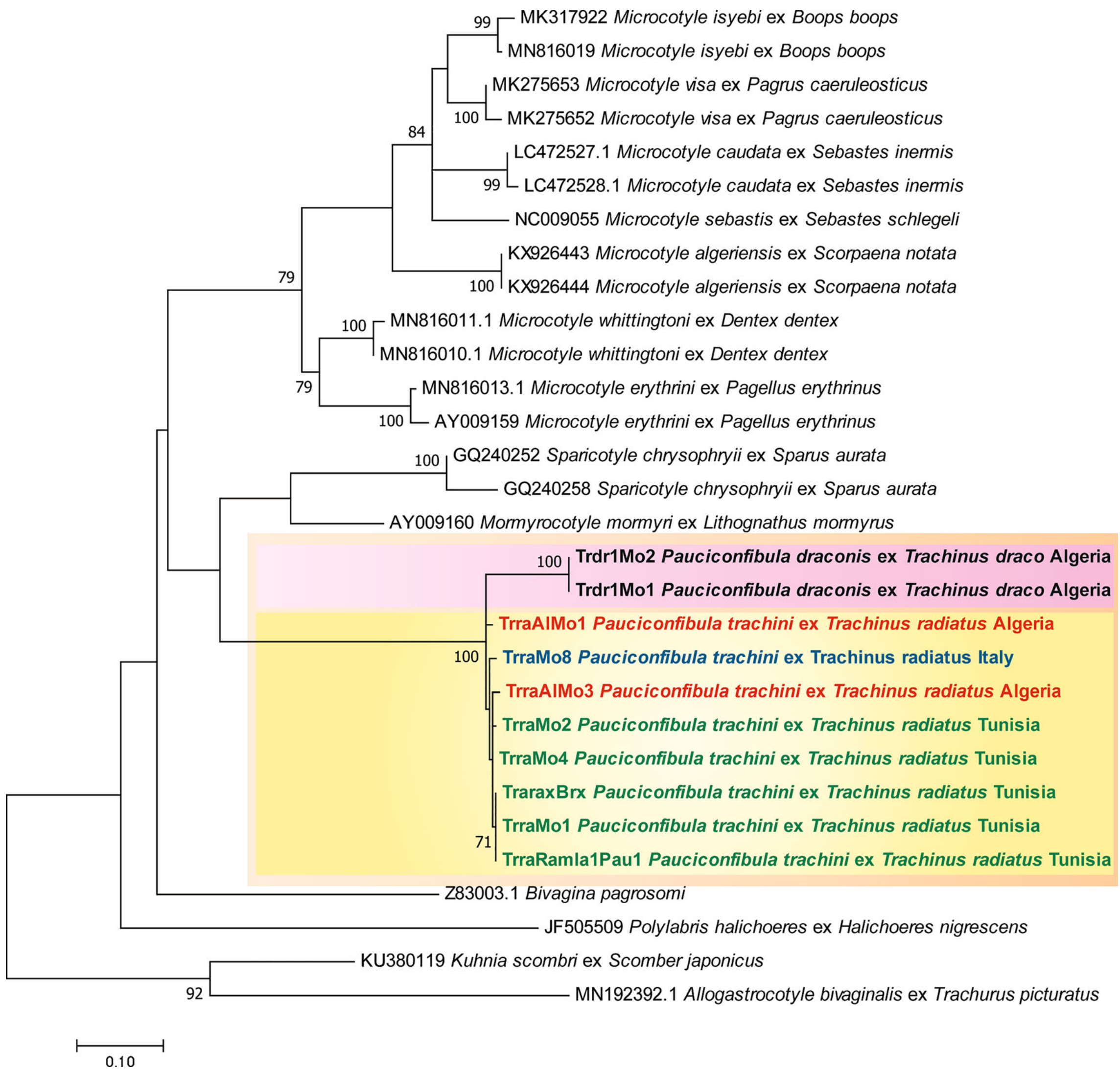


Fig. 1 Molecular phylogenetic analysis by the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model [1] of COI sequences of monogeneans. Bootstraps percentages (1000 replicates) are indicated next to the branches when significant. There was a total of 263 positions in the final dataset. The Neighbor-Joining tree had similar topology and is not represented

Testes variously shaped and sized, occupying the entire intercaecal field of posterior quarter of body. Vas deferens dorsal, broad, and conspicuous, running forward along body midline to genital atrium. Genital atrium anterior, midventral (Fig. 5c).

Ovary pre-testicular, oblong, and large. Ovary begins on left side of body by an elongated mass (Fig. 6). It goes up to the left then passes to the right and dorsally descends to the immature part. It ascends, then passes again to the left where it widens before emerging into oviduct. Genito-intestinal canal detaching from oviduct and abutting ventrally in left intestinal caecum. Uterus dorsal, running forward along body midline to genital atrium.

Vitellarium follicular, surrounding laterally intestinal caeca; vitellarium extending from level of genital atrium to the haptor. Posterior extremities of vitelline fields symmetrical, fused posteriorly. Vitelloducts Y-shaped with very long lateral branches. Dorsal transverse vitelloducts fused at ovary. Common vitelline duct median and dorsal to ovary, long. Eggs oval and elongated, with two polar filaments: posterior filament shorter and ends in a claw (Fig. 5d).

Comparison

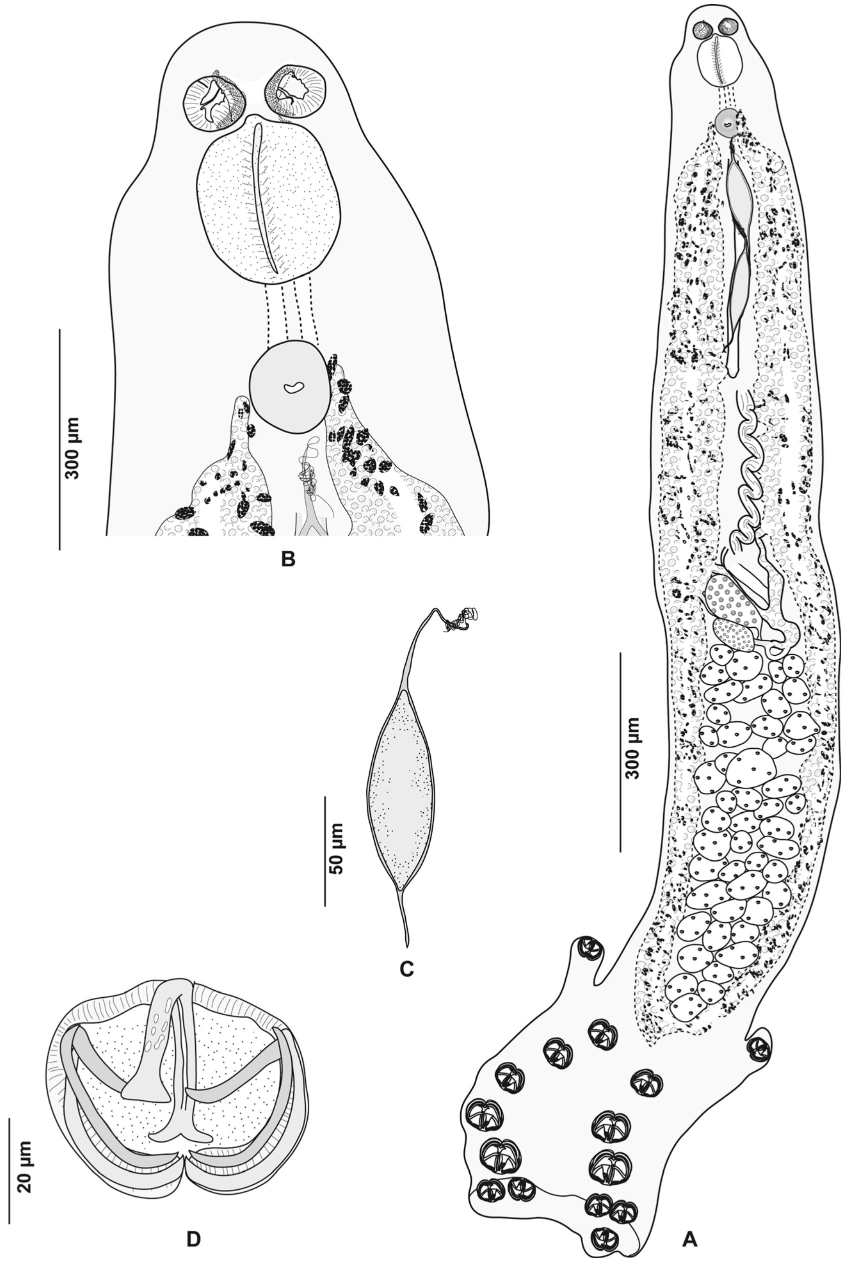
In the present study, we compared measurements and counts of *Pauciconfibula trachini* from its type host *Trachinus radiatus*, off two Mediterranean localities, Tunisia and Algeria: *P. trachini* from Algeria and Tunisia are identical, with an overlap in morphometric data (Table 6).

Morphometric data of *Pauciconfibula trachini* off Algeria and Tunisia were within the range of the original description (Parona & Perugia, 1889, 1890) [in the following section,

Table 5 Estimate of evolutionary divergence between sequences. There was a total of 263 positions in the dataset. Distances are p-distances, shown as percentages

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Microcotyle whittingtoni ex *Dentex dentex*																													
Microcotyle whittingtoni ex *Dentex dentex*	1																												
Microcotyle erythrini ex *Pagellus erythrinus*	12	11																											
Microcotyle erythrini ex *Pagellus erythrinus*	13	12	2																										
Microcotyle caudata ex *Sebastes*	15	15	17	18																									
Microcotyle caudata ex *Sebastes inermis*	14	14	16	17	1																								
Microcotyle isyebi ex *Boops boops*	14	14	15	14	11	11																							
Microcotyle isyebi ex *Boops boops*	15	15	14	14	11	11	2																						
Microcotyle visa ex *Pagrus caeruleosticus*	12	12	13	13	9	9	8	7																					
Microcotyle visa ex *Pagrus caeruleosticus*	12	12	13	14	10	10	10	9	2																				
Microcotyle algeriensis ex *Scorpaena notata*	17	16	17	17	14	14	13	13	13	14																			
Microcotyle algeriensis ex *Scorpaena notata*	17	16	17	17	14	14	13	13	13	14	0																		
Microcotyle sebastis ex *Sebastes schlegeli*	16	16	14	17	10	10	10	8	10	11	13	13																	
Polylabris halichoeres ex *Halichoeres nigrescens*	21	21	23	24	24	24	23	23	22	22	23	23	23																
Mormyrocotyle mormyri ex *Lithognathus mormyrus*	19	20	20	20	18	17	21	20	18	19	19	19	15	22															
Sparicotyle chrysophryii ex *Sparus aurata*	21	21	19	19	23	24	24	24	24	24	23	23	21	24	16														
Sparicotyle chrysophryii ex *Boops boops*	22	22	20	20	24	24	24	24	24	24	24	24	22	26	18	5													
Kuhnia scombri ex *Scomber japonicus*	26	26	25	25	26	26	26	27	27	28	27	27	26	24	21	27	28												
***Pauciconfibula draconis* ex *T. draco* Algeria**	23	22	22	22	25	24	23	23	24	24	22	22	22	27	22	25	27	28											
***Pauciconfibula draconis* ex *T. draco* Algeria**	23	22	22	22	25	24	23	23	24	24	22	22	22	27	22	25	27	28	**0**										
***Pauciconfibula trachini* ex *T. radiatus* Algeria**	23	23	21	22	24	23	25	25	25	27	23	23	22	27	21	24	26	27	**8**	**8**									
***Pauciconfibula trachini* ex *T. radiatus* Algeria**	24	23	21	22	23	23	24	24	25	26	22	22	22	27	20	24	26	26	**8**	**8**	**2**								
***Pauciconfibula trachini* ex *T. radiatus* Tunisia**	24	23	22	22	24	22	24	24	25	26	22	22	22	27	21	24	27	25	**8**	**8**	**1**	**2**							
***Pauciconfibula trachini* ex *T. radiatus* Tunisia**	24	23	22	22	24	22	24	24	25	26	22	22	22	27	21	24	27	25	**8**	**8**	**1**	**2**	**0**						
***Pauciconfibula trachini* ex *T. radiatus* Tunisia**	24	23	22	22	24	22	24	24	25	26	22	22	22	27	21	24	27	26	**8**	**8**	**1**	**2**	**1**	**1**					
***Pauciconfibula trachini* ex *T. radiatus* Tunisia**	23	23	21	21	24	22	24	24	25	26	22	22	22	27	20	24	26	26	**8**	**8**	**1**	**2**	**0**	**0**	**0**				
***Pauciconfibula trachini* ex *T. radiatus* Italy**	23	22	21	21	22	22	24	23	24	25	22	22	21	27	20	23	25	26	**9**	**9**	**2**	**2**	**2**	**2**	**2**	**1**			
***Pauciconfibula trachini* ex *T. radiatus* Tunisia**	24	23	22	22	24	22	24	24	25	26	22	22	22	27	21	24	27	25	**8**	**8**	**1**	**2**	**0**	**0**	**1**	**0**	**2**		
Allogastrocotyle bivaginalis	27	27	29	28	26	26	23	23	25	25	28	28	27	29	26	30	30	21	29	29	29	28	28	28	28	28	29	28	
Bivagina pagrosomi	19	19	22	23	22	22	20	21	21	22	20	20	22	24	22	20	22	25	24	24	24	24	24	24	24	24	24	24	26

Fig. 2 *Pauciconfibula trachini* from *Trachinus radiatus*. **A** Whole body, MNHN HEL1412. **B** Anterior extremity showing relative positions of prohaptoral suckers, pharynx, and male copulatory organ, MNHN HEL1412. **C** Egg. **D** Clamp (dorsal view), MNHN HEL1411



compared measurements are giving as follow: from Tunisia, from Algeria vs. off type locality]; especially with regard of body width (900 (700–1100), 541 (364– 825) vs. 500); prohaptoral suckers and pharynx size; clamps number (8–10, 6–10 pairs vs. 8 –10) and size; testes number (39–53, 34–58 vs. 30), and eggs size (257 (250–270) ×79 (62–97), 261 (240 − 285) ×75 (70 − 80) vs. 280 × 80). We noted than specimens from off the Italian coast were remarkably larger (4100 (3700–4800), 4082 (2244 − 5595) vs. 9000–11000).

Measurements and counts of *Pauciconfibula draconis* agreed with the original description (Briot 1904) and the subsequent redescription (Nicoll 1914), in regard of body length (2428 (1652 –2944 vs. 5000) and clamps number (11–15 vs. 5–11).

Pauciconfibula draconis differs from *P. trachini* off the Algerian coast by having a smaller pharynx (64 − 122 × 61 − 114 vs. 137 − 190 × 130 − 178) and a smaller genital atrium (44 − 81 × 48 − 65 vs. 58 – 105 × 63 – 105); by length of anterior (40 − 46 vs. 47– 90), median (50–71 vs. 64– 102) and posterior (40 − 54 vs. 61– 85) clamps; by having more clamps (11–15 pairs vs. 6–10 pairs) and fewer testes (20–28 vs. 34–58).

Discussion

In the present study, we compared measurements and counts of *Pauciconfibula trachini* from their type host *Trachinus*

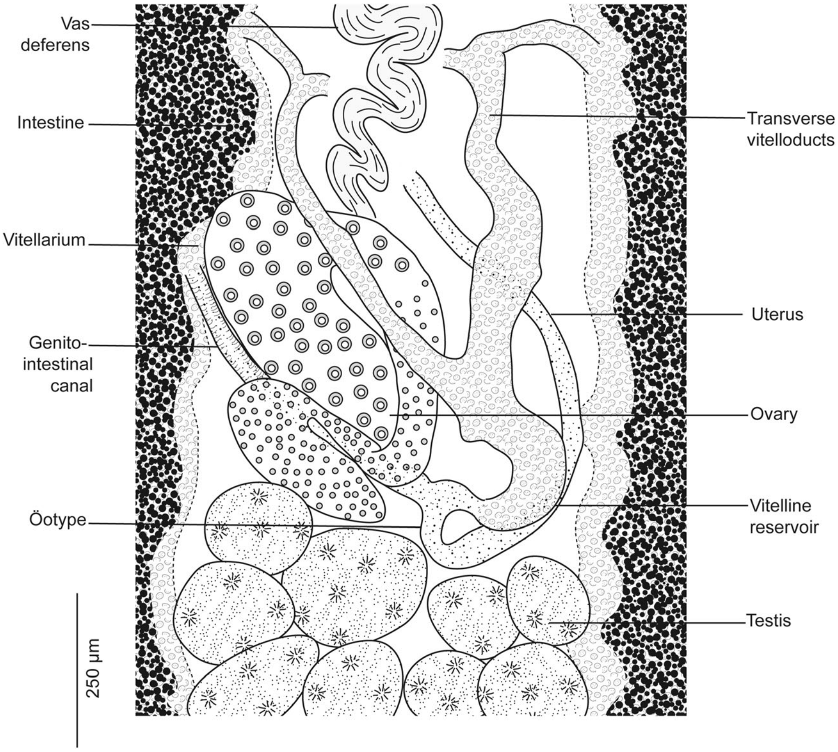


Fig. 3 *Pauciconfibula trachini* from *Trachinus radiatus*. Detail of anatomy of reproductive organs in the region of ovary, MNHN HEL1412

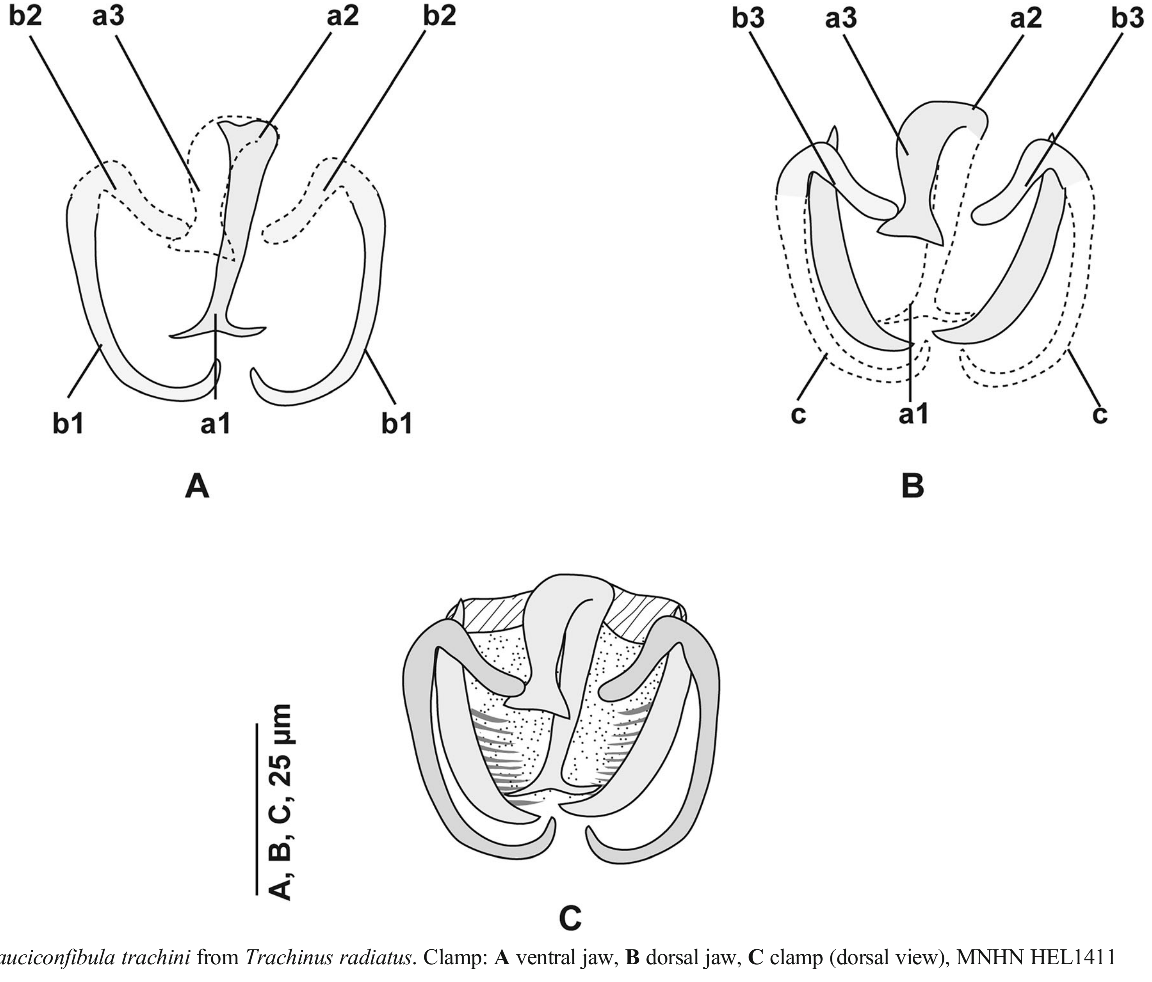


Fig. 4 *Pauciconfibula trachini* from *Trachinus radiatus*. Clamp: **A** ventral jaw, **B** dorsal jaw, **C** clamp (dorsal view), MNHN HEL1411

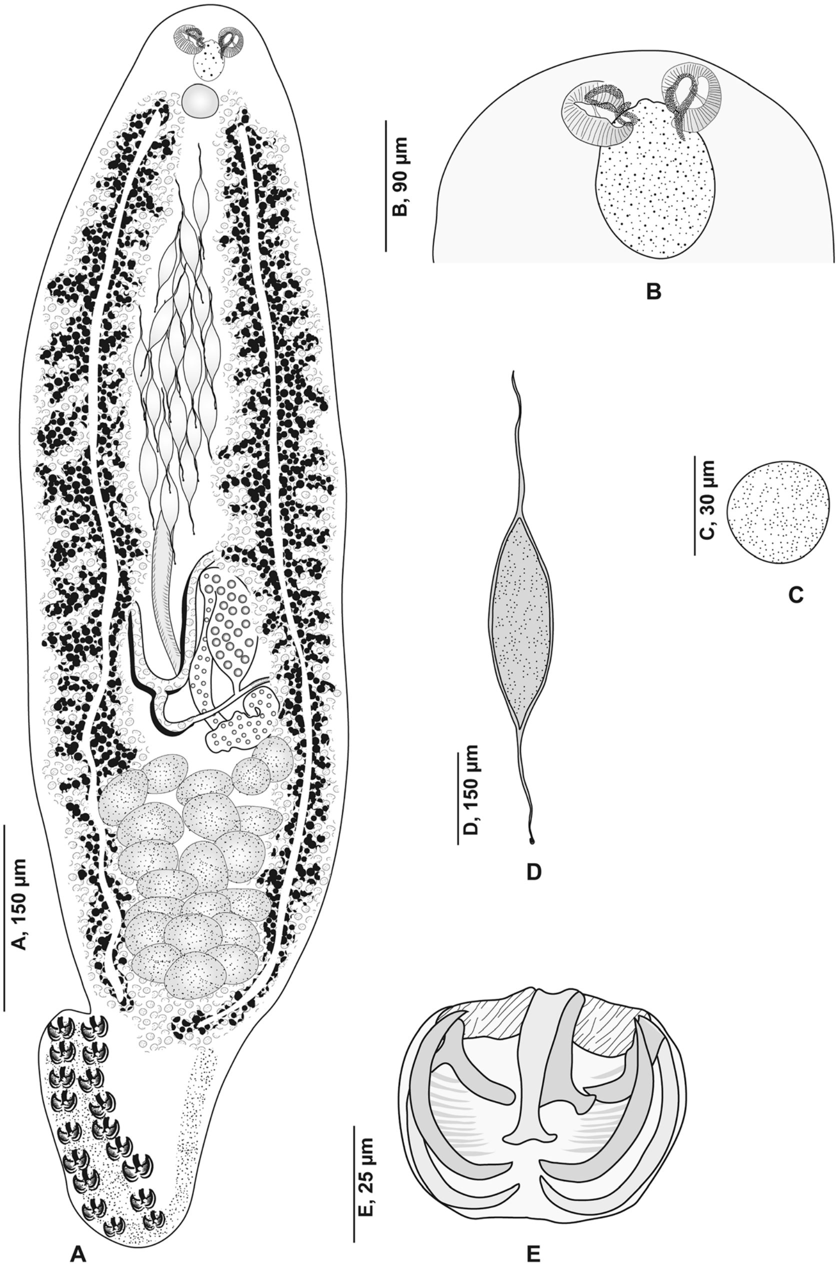


Fig. 5 *Pauciconfibula draconis* from *Trachinus draco*. **A** Whole body, MNHN HEL1404. **B** Anterior extremity showing relative positions of prohaptoral suckers and pharynx, MNHN HEL1404. **C** Genital atrium, MNHN HEL1409. **D** Egg, MNHN HEL1408. **E** Clamp, MNHN HEL1409 (ventral view), MNHN HELxxx

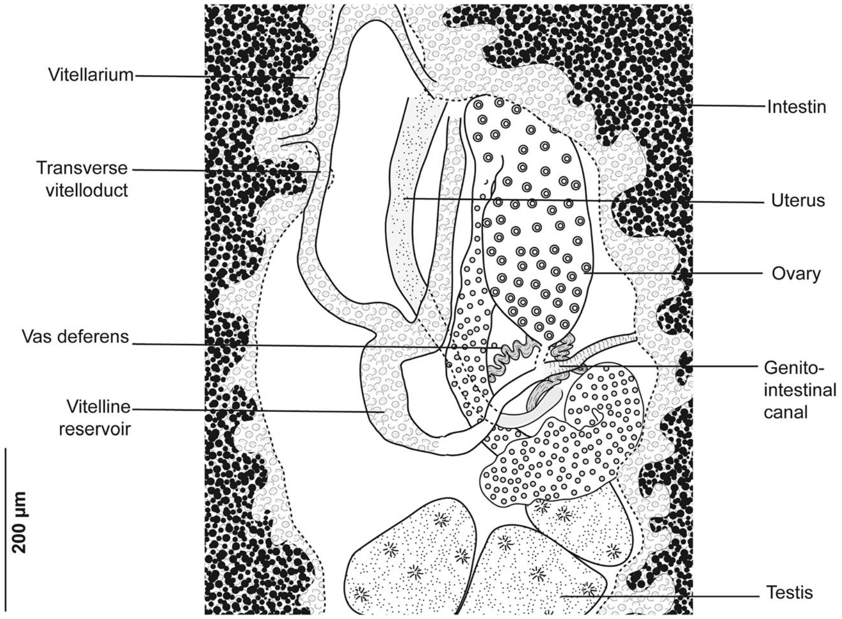
radiatus, off two Mediterranean localities, Tunisia and Algeria, and there was an apparent overlap. Specimens described in the original description were larger. However, it is well known that body length is the least reliable feature for species delimitation in monogeneans (Rohde and Watson 1985).

COI sequences of *Pauciconfibula trachini* off the three Mediterranean localities, Algeria, Tunisia, and Italy differed by only 0−2%. Differences lower than 4% in COI sequences are commonly read as intraspecific variations (Aquaro et al. 2012; Hansen et al. 2007; Vanhove et al. 2013; Ward et al. 2005). Differences ranging from 0.2−5.6% in COI sequences of polyopisthocotylean monogeneans were reported as intraspecific divergence (Bouguerche et al. 2019a). We thus consider that all specimens belong to the same species.

Pauciconfibula draconis was distinguished from *P. trachini* off the Algerian coast by the size of pharynx, genital atrium, clamps; number of clamps and testes. They differed also by their body shape, with *P. draconis* having an evident stocky appearance. However, we note that the number of studied specimens was low, and measurements and counts overlapped.

Most importantly, COI sequences of *Pauciconfibula trachini* from *Trachinus radiatus* differed from those of *P. draconis* from *T. draco* by 8−9%, a divergence higher than 4% threshold in COI sequences of Monogenea (Aquaro et al.

Fig. 6 *Pauciconfibula draconis* from *Trachinus draco*. Detail of anatomy of reproductive organs in the region of ovary, MNHN HEL1404.



2012) and other reported intraspecific variations (Bouguerche et al. 2019a). The 8−9 % difference reported here do not fall within the intraspecific range and thus we consider that *P. trachini* from *T. radiatus* and *P. draconis* from *T. draco* are regarded as different and distinct species. We thus confirm, for the first time with molecular evidence, delimitation

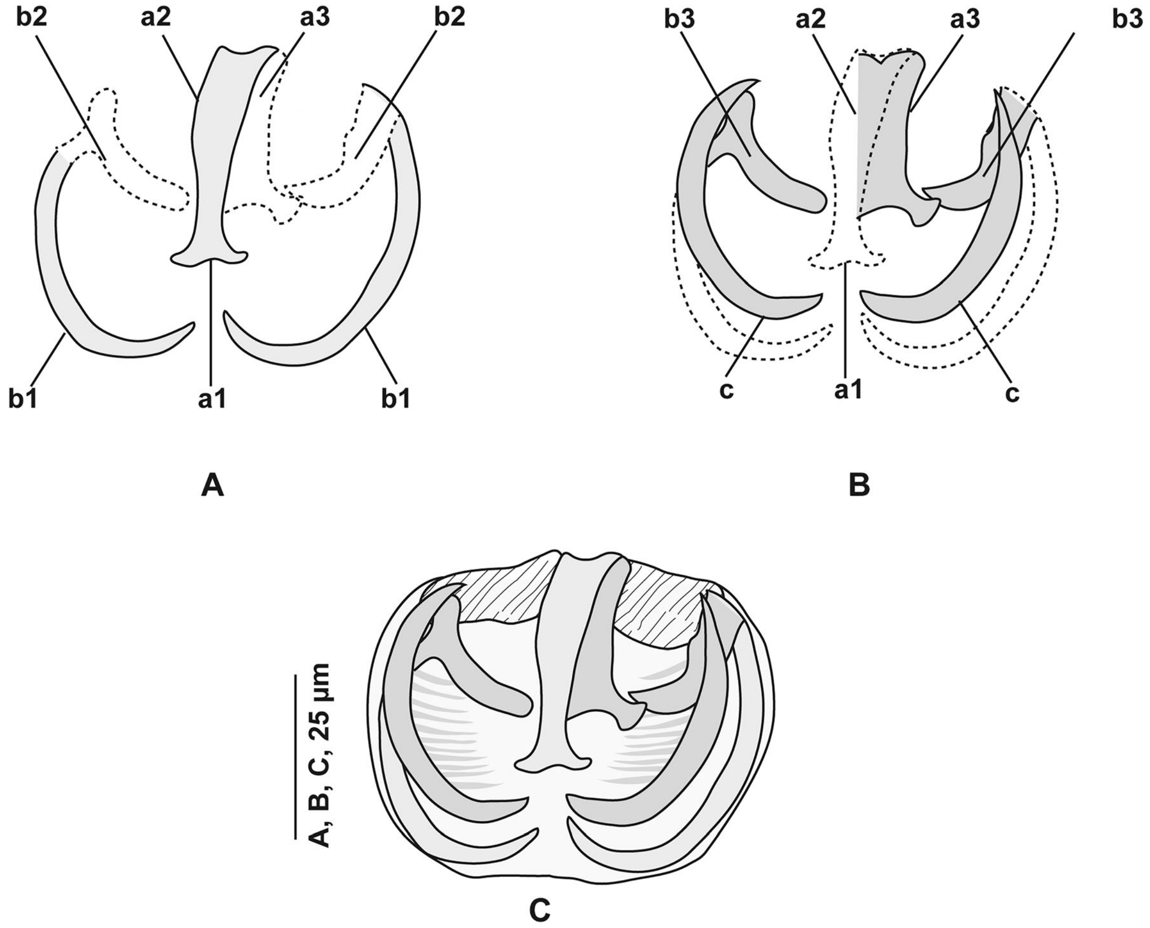


Fig. 7 *Pauciconfibula draconis* from *Trachinus draco*. Clamp: **A** dorsal jaw, **B** ventral jaw, **C** Clamp (dorsal view), MNHN HEL1409.

Table 6 Measurements of *Pauciconfibula trachini* from various localities.-: not stated. *Diameter.

Parasites	*Pauciconfibula trachini*			*Pauciconfibula draconis*						
Hosts	*Trachinus radiatus*			*Trachinus draco*						
Previous identification	*Microcotyle trachini*	/	/	*Microcotyle draconis*			*Aspinatrium trachini*			/
Locality	Italy, Mediterranean	Algeria, Mediterranean	Tunisia, Mediterranean	English Channel, Atlantic		Scotland, Atlantic	Spain, Mediterranean	Montenegro, Mediterranean	Aegean Sea, Mediterranean	Algeria, Mediterranean
Number of specimens	-	11, mature	16, mature	> 30	2, immature	1, mature	4	2	9	10, mature
Source	(Parona and Perugia 1889; 1890)	Present study	Present study	(Briot 1904)	(Nicoll 1914)		(López-Román and Guevara Pozo 1973)	(Radujkovic and Euzet 1989)	(Akmirza 2004)	Present study
Body length	(9000 – 11000)	3205 (1010 – 4465)	3102 (1120 – 3796)	5000	-	-	3680 – 4300	2800 – 4000	2700 – 4100	2428 (1652 – 2944)
Haptor length	-	783 (343 – 1190)	900 (800 – 1100)	-	-	-	1000 – 1300			582 (343 – 790)
Total length	-	4082 (2244 – 5595)	4100 (3700 – 4800)	-	-	-				3355 (2244 – 6621)
Body width	500	541 (364 – 825)	900 (700 – 1100)	1300	-	-	860 – 1030	650	620 – 790	793 (364 – 915)
Prohaptoral suckers length	56 *	63 (41 – 73)	64 (60 – 68) *	-	-	-	63 – 77	53 *		49 (35 – 58)
Prohaptoral suckers width		67 (46 – 77)		-	-	-	63 – 69			50 (32 – 60)
Pharynx length	98 *	157 (137 – 190)	139 (120 – 160) *	-	-	-	88 – 99 *	-	-	100 (64 – 122)
Pharynx width		146 (130 – 178)		-				-	-	97 (61 – 114)
Clamps number (of pairs)	(8 – 10)	8 (6 – 10)	8 (7 – 10)	5	7	11	13 – 16	8 – 10	9 – 13	13 (11 – 15)
Clamp length	Largest: >126* Smallest :42	64 (47 – 90) 90 (64 – 102) 78 (61 – 85)	112 (100 – 120)	-	-	-	83 – 100	80 – 98		42 (40 – 46) 57 (50 – 71) 49 (40 – 54)
Clamp width		61 (40 – 84) 80 (66 – 93) 68 (47 – 80)	81 70 – 95	-	-	-	70 – 90	67 – 72	90	38 (35 – 40) 42 (35 – 48) 41 (35 – 54)
Genital atrium length	-	82 (58 – 105)	-	-	-	-		-	-	60 (44 – 81)
Genital atrium width	-	84 (63 – 105)	-	-	-	-		-	-	56 (48 – 65)
Number of testes	30	47 (34 – 58)	48 (39 – 53)	-	-		50	50 – 55	50 – 55	23 (20 – 28)
Distance genital atrium–anterior end	-	360 (290 – 430)	466 (410 – 520)	-	-	-	300 – 400	-	-	277 (210 – 365)
Egg length	80	261 (240 – 285)	257 (250 – 270)	-	-	-	280 – 316	-	-	260 (223 – 311)
Egg width	28	75 (70 – 80)	79 (62 – 97)	-	-	-	89 – 116	-	-	68 (57 – 85)

between the two species and the validity of *P. draconis*. The synonymy between *P. trachini* and *P. draconis* (Radujkovic and Euzet 1989) was unjustified.

The remaining puzzling situation regards the host range of *Pauciconfibula trachini* (Table 7). The mention of this monogenean on *Trachinus draco* (Akmirza 2004; López-Román and Guevara Pozo 1973; Radujkovic and Euzet 1989), type host of *P. draconis* advocate a stenoxenic specificity for *P. trachini*, and the occurrence of two congeneric polyopisthocotyleans, on a single host.

However, our careful comparison of specimens of *Pauciconfibula* assigned to *P. trachini*; and collected from *Trachinus draco* off the Aegean Sea (Akmirza 2004), Montenegro (Radujkovic and Euzet 1989), and Spain (López-Román and Guevara Pozo 1973) with the microtylid assigned to this host *P. draconis* (Briot 1904; Nicoll 1914) showed an overlap in all measurements and counts (Table 6). Most interestingly, the stocky body appearance of *Pauciconfibula* cf. *trachini* reported on *T. draco* (Akmirza 2004; López-Román and Guevara Pozo 1973; Radujkovic and Euzet 1989) matched that of *P. draconis* (*P. trachini* being rather more elongated). We suggest that previous mentions of *P. trachini* on *T. draco* (Akmirza 2004; López-Román and Guevara Pozo 1973; Radujkovic and Euzet 1989) were in fact misidentifications of *P. draconis*. Hence, new host records and considerations of generalist polyopisthocotyleans are to be considered with caution and should be based imperatively on integrative taxonomy.

Table 7 Previous records of *Pauciconfibula trachini* (Parona & Perugia, 1889) and *Pauciconfibula draconis* (Briot, 1904). Note that all previous localities of *P. trachini* are from the Mediterranean

Hosts/localities	Sources
Pauciconfibula trachini	
Trachinus radiatus (type host)	
Italy	(Bychowsky 1961; Parona 1889, 1890
Algeria	Present study
Tunisia	Present study
Italy	Present study
Trachinus draco	
Spain	(López-Román and Guevara Pozo 1973)
Montenegro	(Radujkovic and Euzet 1989)
Aegean Sea	(Akmirza 2004)
Trachinus araneus	
Turkey	(Akmirza 2013)
Tunisia	(Azizi et al. 2017)
Pauciconfibula draconis	
Trachinus draco (type host)	
English Channel, North Atlantic	(Briot 1904)
Scotland, North Atlantic	(Nicoll 1914)
Algeria, Mediterranean	Present study

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Declarations

Competing interests The authors declare no competing interests.

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