

Diplectanum squamatum n. sp. (Monogenea: Diplectanidae) from the gills of *Cynoscion guatucupa* (Sciaenidae) in Southwest Atlantic waters

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Abstract

Diplectanum squamatum n. sp. is described from the gills of the sciaenid *Cynoscion guatucupa* collected off Mar del Plata, Argentina. The new species is distinguished from other species of the genus by the morphology of the hamuli and the male copulatory organ, and presence of scales on the haptor and body. This is the first species of *Diplectanum* to be reported from southwest Atlantic waters and, with the possible exception of *D. cayennense* Euzet & Durette-Desset, 1974, the only species from the Atlantic coast of South America.

Introduction

Members of the Diplectanidae Bychowsky, 1957 parasitise the gills of teleosts of the order Perciformes and have a world-wide distribution (Oliver, 1993). Species of Diplectanum Diesing, 1858, which are characterised by the presence of a dorsal and a ventral squamodisc, three bars and two pairs of hamuli on the haptor, and a sclerotised tubular copulatory organ, have been recorded from both marine and freshwater fishes (Oliver, 1987, 1993). There have, however, been no reports of their occurrence on marine fish in South American Atlantic waters (Kohn & Paiva, 2000; Santos & Carbonell, 2000), although species of the related Pseudorhabdosynochus Yamaguti, 1958 (see Santos, Buchmann & Gibson, 2000) are present. During a survey of sciaenids off Mar del Plata, Argentina, a new species of Diplectanum was encountered; this is described below.

Materials and methods

Hosts, *Cynoscion guatucupa* (Cuvier in Cuvier & Valenciennes), were caught using trawl-nets and landed at the port of Mar del Plata. The worms, collected alive from the gills, were fixed in 70% ethanol. Some worms were stained in Gomori's trichrome or Mayer's paracarmine, differentiated in acid-alcohol, dehydrated in an ethanol series, cleared in beechwood creosote and mounted in Canada balsam. Others were examined in Hoyer's medium as temporary mounts. Drawings were made with the aid of a drawing tube. Measurements are presented in micrometres. The methodology for the measurements of the hamuli follows Oliver (1968).

For scanning electron microscopy (SEM), specimens were post-fixed for 1h in a solution of 1% osmium tetroxide in 0.1 M cacodylate buffer, dehydrated through a graded ethanol series, critical-point dried and sputter-coated to a thickness of 20–40 nm with gold. They were examined using a JSM-8500 scanning electron microscope at an accelerating voltage of 15 kV. Type and voucher specimens are deposited in the helminthological collections of the Insitute Oswaldo Cruz, Brazil (CHIOC) and The Natural History Museum, London (BMNH).

Diplectanum squamatum n. sp.

Type-host: Cynoscion guatucupa (Cuvier in Cuvier & Valenciennes) (Sciaenidae).

Type-locality: Off Mar del Plata (38°08′S, 57°32′W), Argentina.

Site: Gills.

Type-specimens: Holotype CHIOC 34538 a; paratypes CHIOC 34538 b-d; BMNH 2001.5.2.1-3; vouchers BMNH 2001.5.2.4-40.

Prevalence: 87/87 fish; 100%.

Mean intensity (range) [data from 20 fish]: 80.8 (15–360).

Description (Figures 1–13)

[Based on 20 whole-mounts]. Diplectanidae. Body slender; total length 435–750; width 82–172 at ovarian level (Figure 1). Anterior region bears 3 pairs of head organs, 2 pairs of eye-spots (anterior pair smaller) and small number of irregularly distributed pigment granules. Much of body covered with imbricate anteriorly directed scales between pharyngeal level and haptor; scales not visible in Hoyer preparations and clearly seen on whole-mounts only laterally along body but readily visible in SEM micrographs (Figures 3–5); largest (length 3–5) scales cover posterior half of body (Figure 3); from mid-body scales gradually diminish in size anteriorly, disappearing at level of pharynx.

Haptor wider than body, $53-90 \times 135-240$, with dorsal and ventral squamodiscs, 2 pairs of lateral hamuli, 3 bars and 14 marginal hooks. Scales cover parts of lateral areas of haptor (Figures 4–5). Squamodiscs round, overlie distal peduncle, 75–90 in diameter, with 23–29 concentric rows of elements which are associated with imbricate scales visible using SEM; scales smaller than those on posterior body; first 10 or so open rows of elements semi-circular; posterior rows (c. 13) transverse (Figures 2, 4–6). Dorsal hamuli with long, stout, deep root and poorly developed superficial root; A: 41–50, B: 46–48, C: 23–28 and D: 19–21 (Figures 7–8)¹. Ventral hamuli with long deep, superficial roots: A: 46–54, B: 41– 51, C: 37–41 and D: 10–17 (Figure 9). Dorsal bars

¹As the material was fixed unflattened in alcohol, certain details of the internal anatomy are difficult to determine and some measurement ranges, in cases where structures are not lying flat, may be slightly wider than normal.



Figure 1. Diplectanum squamatum n. sp. Holotype, entire body. *Abbreviations*: apr, anterior prostatic reservoir; gp, genital pore; pb, proximal bulb; ppr, posterior prostatic reservoir; sr, seminal receptacle. *Scale-bar*: 50 μ m.



Figure 2. Diplectanum squamatum n. sp. Micrograph of squamodisc; Hoyer preparation. *Scale-bar*: 50 µm.

53–71² long, lateral (Figure 11). Ventral bar 94–133² long, with transverse groove, constricted median portion and narrow, curved lateral extremities (Figure 12). Marginal hooks 10–13 long (Figure 10).

Mouth ventral, sub-terminal; pharynx subspherical, $32-45 \log \times 30-45$ wide; oesophagus short to apparently absent; caeca simple, blind.

Genital pore mid-ventral near mid-body. Testis post-ovarian, entire, inter-caecal. Copulatory organ 128–178 long, in form of slender sclerotised tube with curved tip (Figure 13); proximal bulb small, indistinct. Anterior prostatic reservoir not always conspicuous; posterior prostatic reservoir with hooked anterior cone, adjacent to middle of copulatory organ.

Ovary equatorial, inter-caecal. Oviduct encircles right caecum, linked to Mehlis' gland and vitelline ducts, all at same level. Vagina small, sinistral, forms slightly sclerotised duct connected to seminal receptacle, opens at level of or just posterior to genital pore. Uterus inter-caecal, anterior to level of vagina, appears to open via genital pore¹. Vitellarium follicular; fields extend posteriorly from level of pharynx in 2 bilateral bands into post-testicular region of body. Eggs not observed.

Etymology: The specific name reflects the imbricate scales which cover much of the body and haptor.



Figure 3. SEM micrograph of *Diplectanum squamatum* n. sp. Posterior half of body showing larger scales. *Scale-bar:* $50 \mu m$.

Discussion

Species of Diplectanum that parasitise marine sciaenid fishes include: D. aculeatum Parona & Perugia, 1889; D. banyulense Oliver, 1968; D. belengeri (Chauhan, 1945) Chauhan, 1953; D. bilobatum Hargis, 1955; D. bocqueti Oliver, 1980; D. cayennense Euzet & Durette-Desset, 19743; D. chabaudi Oliver, 1980; D. dollfusi Oliver, 1980; D. elongatum Obiekezie, 1988; D. fujianensis Zhang, Liu & Ding, 1995; D. furcelamellosum Zhang, Liu, Ding & Chen, 2000; D. glandulosum Williams, 1989; D. grassei Oliver, 1974; D. jamestownense Obiekezie, 1988; D. labourgi Oliver, 1974; D. maculatum Tripathi, 1957; D. melvillei Oliver & Paperna, 1984; D. minutum Tripathi, 1957; D. orissai (Gupta & Krishna, 1979) Oliver, 1987; D. nagibinae Oliver & Paperna, 1984; D. oliveri Williams, 1989; D. sciaenae van Beneden & Hesse, 1863; D. simile Bychowsky, 1957; D. tangzhongzhangi Zhang, Liu & Ding, 1995; and D. umbrinum Tripathi, 1957. Five other species have been recorded from freshwater sciaenid fishes, all

 $^{^{2}}$ A small number of anomalous measurements were obtained – one dorsal bar measurement was 90 and two ventral bar measurements 158 and 180 μ m.

³Euzet & Durette-Desset (1974) indicated that this parasite is marine, but, according to Keith et al. (2000), *Plagioscion auratus* is a freshwater host which also occurs in estuaries, lagoons and brackish seawater – it is possible, therefore, that this may not be a marine parasite. Kritsky & Thatcher (1984) described four species of *Diplectanum* from *Plagioscion* spp. in freshwater in Brazil.



Figures 4–5. SEM micrographs of Diplectanum squamatum n. sp. 4. Ventral view of hindbody showing scales covering its surface, squamodisc and lateral sides of haptor. 5. Haptor showing scales and both dorsal and ventral hamuli. Scale-bars: 10 μ m.



Figures 6–13. Diplectanum squamatum n. sp. 6. Detail of rows of elements of squamodisc. 7. Detail of measurements used for hamuli. 8. Dorsal hamulus. 9. Ventral hamulus. 10. Marginal hook. 11. Dorsal lateral bar. 12. Ventral transverse bar. 13. Male copulatory organ. *Scale-bar*: $6,8-13, 50 \mu$ m.

from *Plagioscion* spp. in Brazil: *D. decorum* Kritsky & Thatcher, 1984; *D. gymnopeus* Kritsky & Thatcher, 1984; *D. hilum* Kritsky & Thatcher, 1984; *D. pescadae* Kritsky & Thatcher, 1984; and *D. piscinarius* Kritsky & Thatcher, 1984.

Of these species, only D. bocqueti, D. chabaudi, D. gymnopeus, D. maculatum and D. oliveri have a copulatory organ as large as or close to the size of that in D. squamatum n. sp. (i.e. >120 μ m). Apart from host and geographical distribution, the new species can be differentiated from D. bocqueti by the size and form of the squamodisc (75–90 μ m with 23–29 rows of elements in the new species versus 120–160 μ m with 26-32 rows) and copulatory organ (128-178 versus 90–123 μ m, respectively), and from D. chabaudi, D. maculatum and D. oliveri by the size and form of the squamodisc (respectively, 98–136 μ m with 25–39 rows, 38–53 x 68–68 μ m with 16 rows, and 166– 189 μ m with c. 40 rows) and the size of the ventral hamuli (A: 46–54 µm, D: 10–17 µm versus A: 54–77, D: 23-35; A: 57-72, D: no data; and A: 67-77, D: 30–32 for the three species, respectively). D maculatum also apparently lacks eye-spots and has a tri-lobed ovary (Tripathi, 1957). In the case of D. gymnopeus, from an Amazonian freshwater fish, it can be differentiated on the basis of its smaller copulatory organ (94-108) and the shape of the vagina, which has a large terminal pouch enclosing the seminal receptacle.

Diplectanum spp. from non-sciaenid host groups, with a copulatory organ larger than 100 μ m, include D. aequans (Wagener, 1857), D. blairense Gupta & Khanna, 1974, D. jaculator Mizelle & Kritsky, 1969, D. megacirrus (Maillard & Vala, 1980), D. paralatesi Nagibina, 1976 and D. veropolinemi Nagibina, 1976. D. blairense (= D. flagritubus Nagibina, 1976^4) and D. paralatesi can be readily differentiated by the small number of rows of elements on the squamodisc (11– 14 and 12–13, respectively); D. jaculator presents a different form of vagina with sclerotised spines and an ornate copulatory organ with an accessory piece; D. megacirrus can be distinguished by the shape and smaller size of the haptor, hamuli and squamodiscs; D. aequans has a larger squamodisc which is not round and elements with a distinctive shape (see Oliver, 1968, 1987); while D. veropolynemi possesses a pair of distinct digitiform processes which support the inner pair of marginal hooks.

D. squamatum n. sp. is not unique amongst species of Diplectanum in possessing scales on the haptor and body: this feature also occurs in D. aequans, D. aculeatum and D. sciaenae (see Oliver, 1987), and in D. megacirrus and D. spinosum (Maillard & Vala, 1980) (see Maillard & Vala, 1980), although the latter species have a different shape and pattern of distribution. As both D. aculeatum and D. sciaenae also occur on sciaenids, it is likely that are closely related to the new species. Since they are often difficult to see in LM preparations and are not visible in Hoyer preparations, it is very possible that body scales may also be found in other species. This highlights a problem of relying entirely on media with a low refractive index, as these will tend to clarify certain features but may obscure others (cf. Figures 2 & 4). Scales are also manifest in species of Pseudorhabdosynochus (as Cycloplectanum Oliver, 1968⁵) (see Beverley-Burton & Suriano, 1981; Santos et al., 2000) and Rhabdosynochus Mizelle & Blatz, 1941 (see Kritsky, Boeger & Robaldo, 2001). It is intriguing that scales occur on the body of a range of diplectanids included in different subfamilies by Oliver (1987), which may indicate that some of the subfamilies comprising the Diplectanidae may be unnatural.

The geographical distribution of *Cynoscion guatucupa*, the host of the new species, ranges in the southwestern Atlantic between Rio de Janeiro and Argentina. This is the first species of *Diplectanum* to be reported from southwest Atlantic waters and, apart perhaps from *D. cayennense*³, the only species from the Atlantic coast of South America.

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⁴This synonym was made by Hayward (1997), although Oliver (1987) considered the two species distinct.

 $^{^{5}}$ We are sceptical about the synonymy of these two genera: see the discussion in Santos et al. (2000).

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