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A redescription and new host record of *Tonkinopsis transfretanus* Lebedev, 1972 (Monogenea: Polyopisthocotylea: Bychowskicotylidae), with a description of the oncomiracidium from *Diagramma labiosum* (Haemulidae) from Heron Island, Great Barrier Reef, Australia

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Abstract

Tonkinopsis transfretanus Lebedev, 1972 is redescribed and illustrated based on new material from the gills of *Diagramma labiosum* from Heron Island, Australia. This represents a new host and locality record for this monogenean species. The generic diagnosis is revised to include details of the buccal suckers, the male copulatory organ and the common genital atrium. A description of the oncomiracidium, which is unciliated, is also included.

Introduction

The polyopisthocotylean genus *Tonkinopsis* was proposed by Lebedev (1972) for the newly described monotypic species *Tonkinopsis transfretanus* from the gills of *Plectorhinchus cinctus* collected in the Gulf of Tonkin, South China Sea. Lebedev (1972) erected the new family Calyxinellidae to accommodate the new genera *Tonkinopsis* and *Calyxinella* (now *Yamaguticotyla* Price, 1959), which were distinguished from other members of his suborder Gastrocotylinea, by the symmetrical haptor and unique male copulatory organ. Lebedev (1986) subsequently lowered the Calyxinellidae to subfamilial status, placing it, along with the Bychowskicotylinae, in the family Bychowskicotylidae Lebedev, 1969.

Specimens of a polyopisthocotylean identified as *T. transfretanus* were collected from the gills of *Diagramma labiosum* at Heron Island, Great Barrier Reef, Queensland, Australia. This constitutes a new host and locality record for the species. Characters not previously noted by Lebedev (1972) were observed and the species has been redescribed along with a description of the oncomiracidium.

Materials and methods

Specimens of *Diagramma labiosum* were collected by handline off the beach rock at Heron Island in April 1989, November–December 1992, June–July 1995 and January 1996. Fish were identified using Grant (1987) and Kuiter (1993). Live fish were killed by pithing and each gill arch was excised, placed in filtered sea-water (FSW) and examined with a stereomicroscope using incident light. Live monogeneans were removed from the gill lamellae using fine needles and transferred to dishes containing fresh FSW. Specimens were flattened and preserved beneath a coverslip in 10% buffered neutral formalin at room temperature. Parasites were either stained with Semichon's carmine or left unstained. All parasites were dehydrated in an ethanol series, cleared in xylene or cedarwood oil and mounted in Canada balsam.

Five eggs laid by a single adult parasite (in January 1996) were kept in a glass dish containing 30 ml FSW, exposed to natural illumination and incubated at a temperature of 25 °C. The eggs were examined daily for signs of hatching when the FSW was changed.

Adult specimens and oncomiracidia were examined using a compound microscope equipped with phase contrast optics and drawings were made with the aid of a drawing tube. Measurements of oncomiracidia and adults were obtained using a computerised digitising system similar to that described by Roff & Hopcroft (1986). All measurements are given in micrometres as the mean, followed in parentheses by the range and the number of structures measured. Body length, width and the dimensions of the pharynx of larvae were measured when they were compressed just enough to prevent movement, but the hooks were measured in strongly compressed specimens. The numbering of hooklets follows Llewellyn (1963).

Voucher specimens of *Tonkinopsis transfretanus* are deposited in the Queensland Museum (QM), PO Box 3300, South Brisbane, Queensland 4101, Australia and the United States National Parasite Collection (USNPC), Beltsville, Maryland 20705–2350, USA.

Bychowskicotylidae Lebedev, 1969
Calyxinellinae Lebedev, 1972

Tonkinopsis Lebedev, 1972

Revised generic diagnosis
Bychowskicotylidae. Haptor symmetrical with three pairs of clamps; posterior-most pair smallest. Hamuli and other haptoral hooklets absent. Buccal suckers with small sclerotised denticles. Pigmented eye-spots absent. Intestinal caeca unbranched; not confluent posteriorly; not extending into haptor. Genital atrium armed with 12 or 13 curved robust spines; common genital pore mid-ventral. Male copulatory organ armed with seven straight narrower spines. Testes variable in number. Seminal receptacle absent. Genito-intestinal canal leading to right intestinal caecum. Vaginal pore single, dorsomedial, unarmed. Eggs fusiform with appendage at both poles.
Type and only species: Tonkinopsis transfretanus Lebedev, 1972.

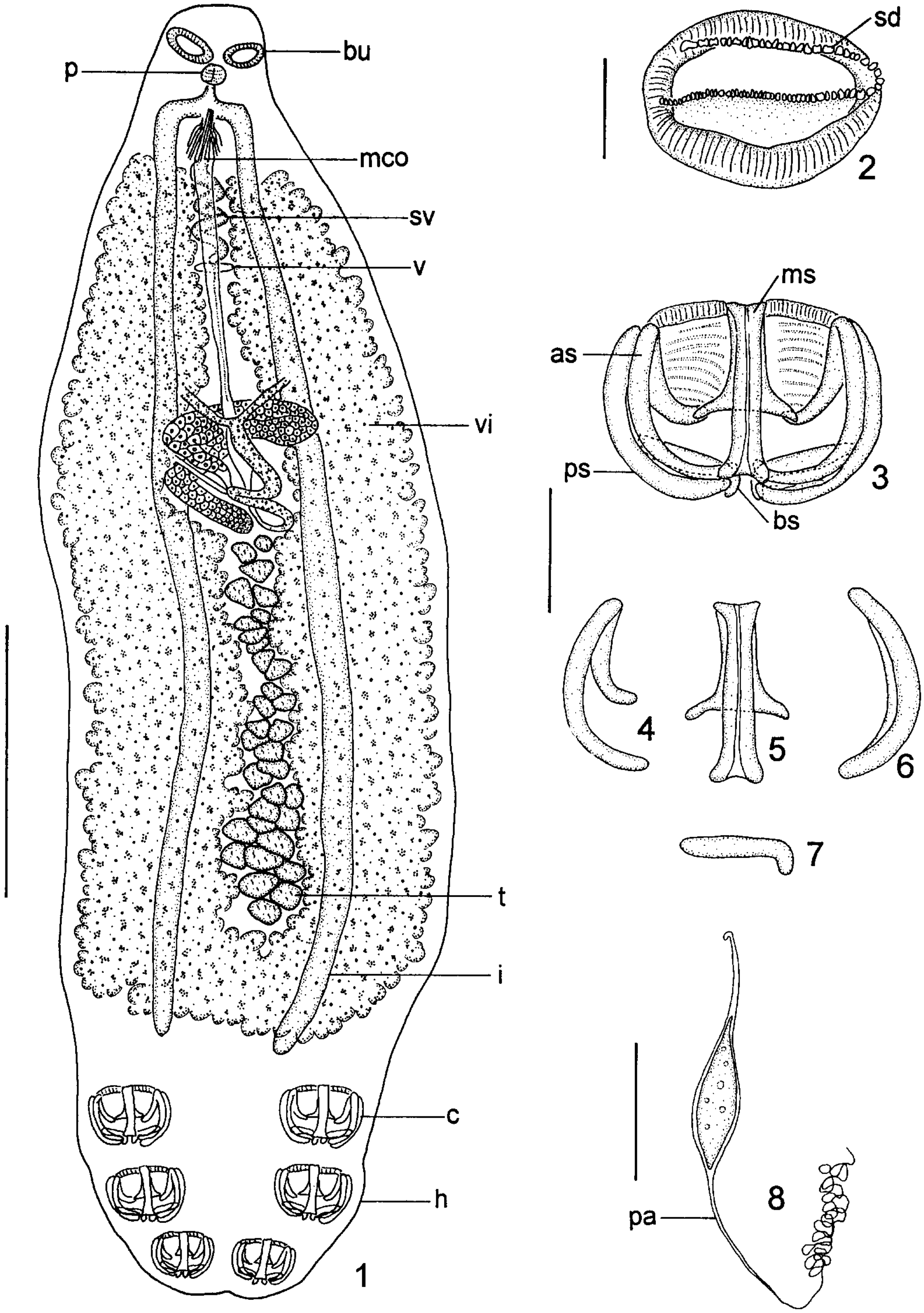
***Tonkinopsis transfretanus* Lebedev, 1972** (Figures 1–14)

Type-host: Plectorhinchus cinctus (Temminck & Schlegel) (Haemulidae).
Type-locality: Gulf of Tonkin, off Northern Vietnam, South China Sea.
Additional host: Diagramma labiosum (Thunberg) (Haemulidae).
Additional locality: Heron Island, Great Barrier Reef, Queensland, Australia (23° 27′S, 151° 55′E).
Site: Gills.
Infection details: For 1989: 2 fish examined (fork length 53.5 and 59.5 cm), both infected, one with 6 and one with 10 parasites. For 1992: 3 fish examined (34–49 cm), one infected with 3 parasites. For 1995: 4 fish examined (fork length 40–69 cm), 2 infected with 2 parasites each. For 1996: 2 fish examined (fork length 57 and 47 cm), one infected with one parasite.
Specimens examined: Twenty-two voucher specimens: 11 specimens in the QM G213188-213198; 11 specimens in the USNPC 86358.

Redescription

Bychowskicotylidae, Calyxinellinae. Measurements based on 9 flattened adult specimens. Total body length including haptor 2,632 (1,936–3,246, n = 9); maximum body width at level of ovary 629 (339–919, n = 9) (Figure 1). Haptor 509 (397–619, n = 8) in maximum width; not clearly delineated from rest of body. Three pairs of clamps present: posterior-most pair (pair I) smallest, 90 (76–99, n = 9) wide; pair II, 114 (102–129, n = 9) wide; pair III, 105 (73–118, n = 9) wide. Sclerites of clamps (Figure 3) consisting of one median U-shaped piece bifurcated at each end (Figure 5), 2 curved lateral pieces (Figure 4), 2 broader pieces external and posterior to curved lateral sclerites (Figure 6), and 2 basal accessory pieces (Figure 7). Inner walls of clamp structure with muscular ribs (Figure 3).

Buccal suckers 71 (51–81, n = 18) wide; armed with small sclerotised denticles (Figure 2). Pharynx 41 (31–49, n = 9) long, 35 (32–48, n = 9) wide. Oesophagus without diverticula; intestine bifurcate; caeca unbranched, extending into posterior body region but not into haptor; not confluent posteriorly. Testes irregular in shape, 17–34 in number (number estimated because vitellarium obscures some testes). Vas deferens not observed from testes to level of transverse vitelline ducts; anterior to transverse vitelline duct, vas deferens dorsal to uterus, widening to form coiled seminal vesicle, then narrowing to enter male copulatory organ posteriorly. Common genital pore ventral, immediately posterior to intestinal bifurcation, 243 (155–355, n = 9) from anterior end. Walls of common genital atrium armed with 12–13 robust hook-like spines (Figure 9), 64 (52–84, n = 17) long. Male copulatory organ (Figure 9) tubular, armed with 7 long slender spines 103 (81–117, n = 18) long. Ovary dorsal, long and tubular; shape as in Figure 10. Genito-intestinal canal arising from common vitelline duct dorsally and



Figures 1–8. Tonkinopsis transfretanus Lebedev, 1972. 1. Whole animal, ventral view. Composite drawing from nine flattened specimens. 2. Buccal sucker showing sclerotised denticles. 3. Complete haptoral clamp. 4. Antero-lateral clamp sclerite. 5. Median clamp sclerite. 6. Postero-lateral clamp sclerite. 7. Basal accessory clamp sclerite. 8. Egg. *Abbreviations:* as, antero-lateral clamp sclerite; bs, basal accessory clamp sclerite; bu, buccal sucker; c, clamp; h, haptor; i, intestine; mco, male copulatory organ; ms, median clamp sclerite; p, pharynx; pa, polar appendage; ps, postero-lateral clamp sclerite; sd, sclerotised denticle; sv, seminal vesicle; t, testes; v, vaginal pore; vi, vitellarium. *Scale-bars:* 1, 500 μm; 2, 30 μm; 3–7, 40 μm; 8, 200 μm.

leading to right intestinal caecum (Figure 10). Oviduct descending dorsal to common vitelline duct. Oötype dorsal to common vitelline duct and ventral to ovary (Figure 10). Mehlis' gland not observed. Uterus dorsal to transverse vitelline ducts. Vaginal pore dorsomedial, 584 (494–755, n = 6) from anterior end (Figure 1); path of vagina not observed. Seminal receptacle absent. Vitellarium follicular, distributed from level of posterior portion of male copulatory organ to posterior end of body proper; not entering haptor. Transverse vitelline ducts arising at level of oötype, joining medially to form common vitelline duct.

Eggs 201 (193–207, n = 3) long (excluding appendages), 59 (38–70, n = 3) wide (measurements based on free eggs laid by adults); with 2 polar appendages (Figure 8); due to coiling, lengths of polar appendages could not be measured accurately.

Incubation and hatching of eggs

Eggs laid by adults were incubated in FSW at 25 °C. The oncomiracidia appeared to be fully embryonated inside the eggs 12 to 14 days after laying, but they failed to hatch spontaneously. Hatching was induced after 16 days by placing eggs in FSW on a glass slide and applying slight coverslip pressure. The operculum appeared to be weakened and became detached from the egg and the uncilated oncomiradium was released from the egg capsule. Most oncomiracidia attached the anterior end to the glass slide, contracted the body and pulled themselves free of the egg.

Description of oncomiracidium

Observations and measurements based on 5 oncomiracidia; description incomplete. Larvae approximately 204 (151–277, n = 4) long , 81 (74–88, n = 4) in maximum width. Haptor distinctly bilobed posteriorly (Figure 11). Larvae unciliated. Eyes absent. Prominent lipid droplets throughout body proper and haptor (Figure 11). Pharynx 26 (23–31, n = 5) long; 23 (20–26, n = 5) wide. Mouth and oesophagus present. Gut bilobed; containing numerous lipid droplets.

Numerous anterior gland duct openings observed but gland-cells, ducts and openings difficult to see and count. One gland-cell on each side of body containing granular secretion anterolateral to pharynx (Figure 11); single duct from each gland-cell running anteriorly, opening at swollen duct endings medially at anterior extremity of head. At least one gland-cell containing needle-like secretion on either side of body at level of pharynx (Figure 11); single duct from each gland-cell running anteriorly to open lateral to duct openings containing granular secretion. Additional gland-cells near margin of body just posterior to pharynx; glands appear to contain needle-like secretion but this requires confirmation. Ducts from these glands running anteriorly and may branch to open at least at 2 places on either side of lateral margin of head (Figure 11).

Three pairs of flame-cells observed: 2 pairs anterior to pharynx and one pair in haptor just anterior to hamuli (Figure 11). Excretory bladders located on either side of body at level of pharynx (Figure 11).

Haptor with one pair of hamuli 55 (53–56, n = 10) long (Figure 12), located between hooklet pairs II and III; strongly recurved blade extending into each haptoral lobe (Figure 11). One pair of hinged hooks (Figure 13) (modified hooklet pair I) 64 (62–65, n = 10) long; domus absent; shaft and blade extending into each haptoral lobe as illustrated (Figure 11). Five pairs of hooklets (pairs II–VI) each of similar shape, with domus present (Figure 14), 21 (20–23, n = 15) long, arranged as in Figure 11.

Discussion

Tonkinopsis transfretanus was originally described from the gills of *Plectorhinchus cinctus* from the Gulf of Tonkin off Northern Vietnam, South China Sea. Type-material of *T. transfretanus* could not be examined due to difficulties in shipping specimens from Vladivostok. This study is, therefore, based on a comparison with the original description (Lebedev, 1972). Our specimens are similar to those illustrated by Lebedev (1972) and the morphometric data obtained for specimens from *Diagramma labiosum* from Heron Island fall within the ranges quoted for specimens from *P. cinctus* from the South China Sea. Subsequent correspondence with Dr Lebedev has confirmed the identity of the specimens from Heron Island as *T. transfretanus*.

The discovery of *T. transfretanus* on *Diagramma labiosum* at Heron Island represents both a new host and locality record for this monogenean. It is of interest that this species of parasite is found over such a wide geographical range and from another host species since, on present evidence, the Bychowskicotylidae appear to be host specific (see Lebedev, 1986). This current finding also represents the first Australian record of a gastrocotylid from the Haemulidae. The majority of gastrocotylids recorded from Australia have been found on members of the Scombridae

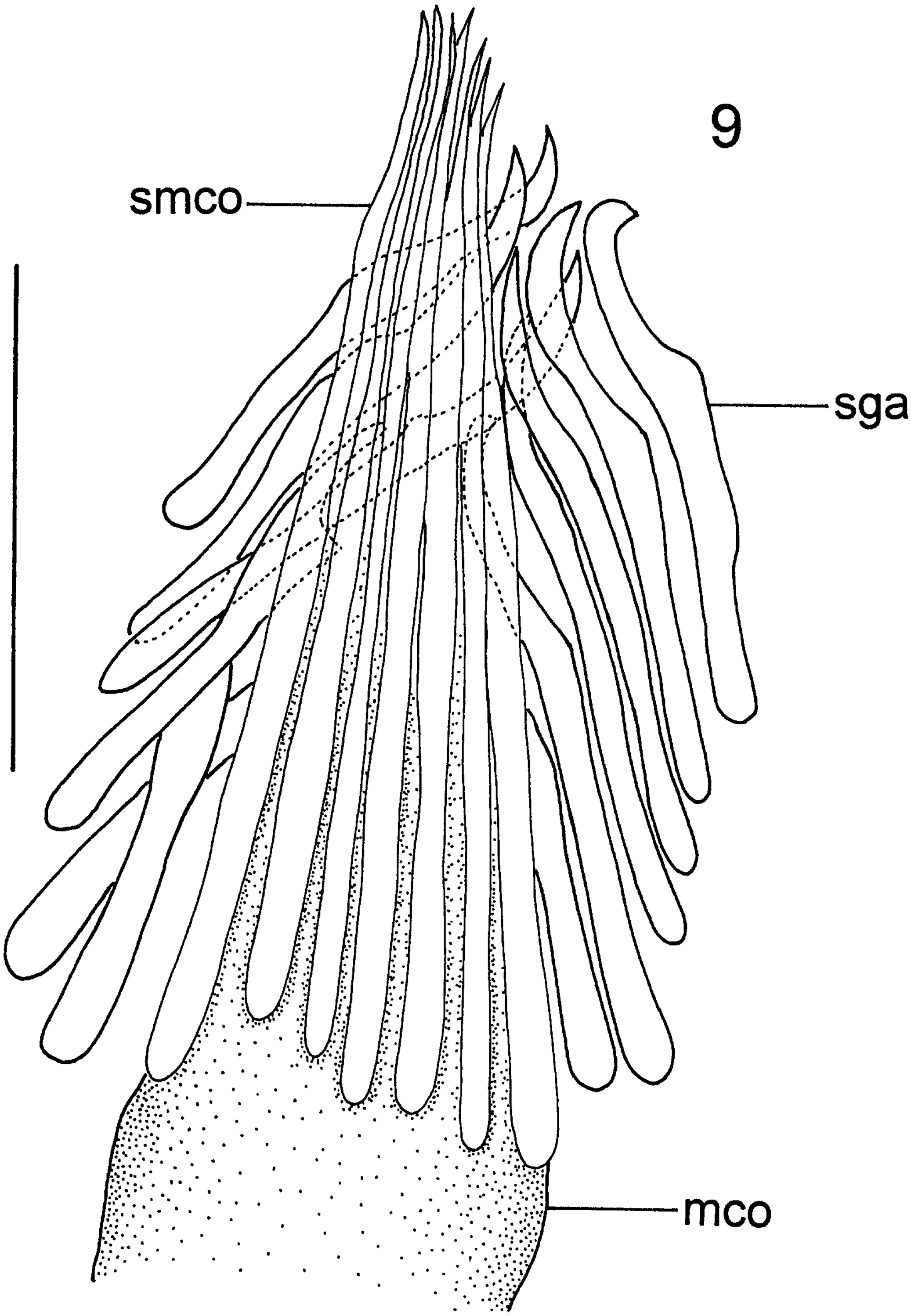


Figure 9. Male copulatory organ of *Tonkinopsis transfretanus*. *Abbreviations:* mco, male copulatory organ; smco, spines of male copulatory organ; sga, spines of genital atrium. *Scale-bar*: 50 μm.

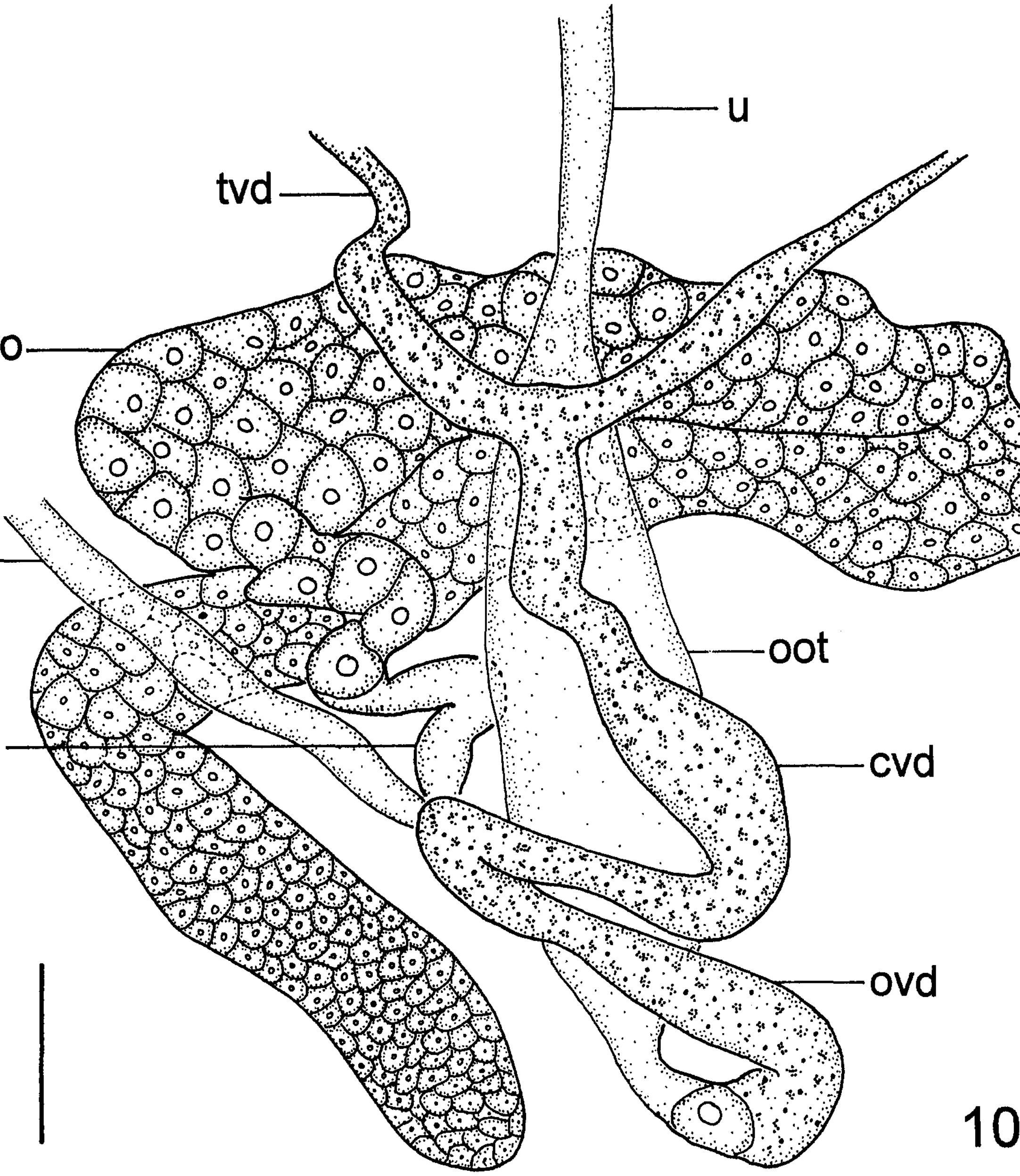
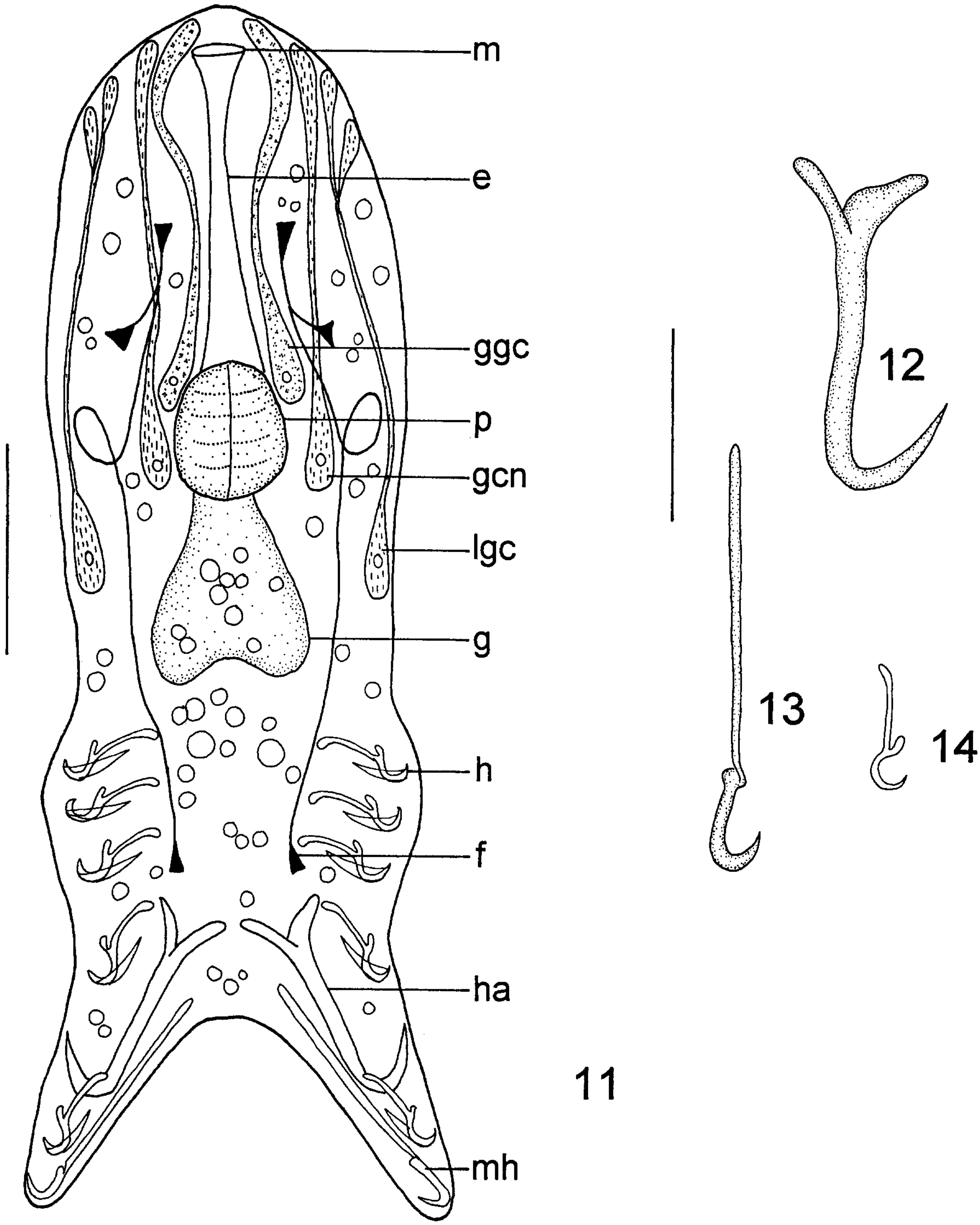


Figure 10. Female reproductive system, ventral view of *Tonkinopsis transfretanus*. *Abbreviations:* cvd, common vitelline duct; gic, genito-intestinal canal; o, ovary; od, oviduct; oot, oötype; ovd, ovovitelline duct; tvd, transverse vitelline duct; u, uterus. *Scale-bar:* 50 μm.

(see Lester & Sewell, 1989). There are currently 17 genera and approximately 150 species of haemulids found worldwide (see Nelson, 1994) with four genera and 21 species being described from Australian waters (Jeff Johnson, pers. comm.). All members of the Bychowskicotylidae, including *Tonkinopsis*, have been recorded from the gills of haemulids (see Lebedev, 1986). Surveys of the Haemulidae of Australia would be useful to determine whether other gastrocotylids are present and whether they are species specific.

Lebedev (1972) originally listed *Plectorhinchus* in the Pomadasyidae, but this family is now considered synonymous with the Haemulidae (see Grant, 1987;



Figures 11–14. Oncomiracidium of *Tonkinopsis transfretanus*. 11. Whole animal. Composite drawing from five live specimens. 12. Hamulus. 13. Modified hooklet I. 14. Hooklet VI. *Abbreviations:* e, oesophagus; f, flame-cell; g, gut; gcn, anterior gland-cell with needle-like secretion; ggc, anterior gland-cell with granular secretion; h, hooklet VI; ha, hamulus; lgc, lateral gland-cell with needle-like secretion; m, mouth; mh, modified hooklet I; p, pharynx. *Scale-bars:* 11, 50 μm; 12–14, 30 μm.

Nelson, 1994). It should also be noted that the correct type-host citation for *T. transfretanus* is *Plectorhinchus cinctus* (Temminck & Schlegel, 1844). *Plectorhinchus* is often mis-spelled as *Plectorhynchus* and the authority date was incorrectly cited as 1843 by Lebedev (1972, 1986).

The buccal suckers of *T. transfretanus* possess a row of small sclerotised denticles, a character not noted in the original description. Although buccal denticles are found in many polyopisthocotyleans (e.g. in the microcotylids; see Dillon, Hargis & Harrises, 1983), they have not been described for other members of the Bychowskicotylidae and appear to be unique for the genus. However, type-material of other species in the family should be examined to verify this conclusion.

Lebedev (1972) stated that the male copulatory organ of *T. transfretanus* was armed with 20 spines, ranging from 55 to 92 μm in length, with the central long straight hooks being associated with the male copulatory organ. We observed that total spine number varied between 19 and 20 spines with seven longer (81–117 μm), straight, slender spines arming the male copulatory organ and 12 or 13 shorter (52–84 μm), robust, curved spines associated with the wall of the common genital atrium (Figure 10). Lebedev (1972) suggested that these curved spines may aid either in holding the parasites together during mating or by widening the vagina during copulation.

The eggs, which were not described by Lebedev (1972), are fusiform in shape and have an appendage at both poles. Although the larvae appeared to be fully embryonated 12 to 14 days after laying, they did not hatch spontaneously after incubation for 16 days. However, they could be extracted from the eggs readily upon slight coverslip pressure. The larvae of *T. transfretanus* are similar to the larvae described by Ktari (1969) for *Microcotyle salpae* Parona & Perugia, 1890. The larvae of *M. salpae* are also unciliated, the eyes are absent, the complement of hooks is identical to *T. transfretanus* and the haptor has two distinct lobes. Ktari (1969) noted that, although the eggs *of M. salpae* appeared to be fully developed 10 days after being laid, they did not hatch spontaneously. Ktari (1969) induced hatching by adding aquarium water to the eggs in which the host had been kept for at least 48 hours. He also demonstrated that egg hatching occurred, but to a lesser extent, when they were mechanically disturbed. Thus, Ktari (1969) concluded that a chemical factor was the most important trigger for hatching, but that mechanical disturbance also played a role. Further investigation is required to determine the hatching stimulus or stimuli for *T. transfretanus*.

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