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Sundapolystoma chalconotae n. g., n. sp. (Monogenea: Polystomatidae) from *Rana chalconota* (Schlegel) of Peninsular Malaysia

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Abstract

Sundapolystoma chalconotae. n. g., n. sp. (Polystomatidae, Polystomatinae) is proposed for a new polystomatid from the urinary bladder of *Rana chalconota* (Schlegel) in Peninsular Malaysia. This is the first species of polystomatid to be described from the amphibians of Peninsular Malaysia and the second for the Southeast Asian region. This new genus, as exemplified by *S. chalconotae*, differs from other polystomatids, and in particular *Parapolystoma* Ozaki, 1935 (*P. bulliense* (Johnston, 1912) Ozaki, 1935 and *P. johnstoni* Pichelin, 1995), in having a tubular uterus and a single diffuse testis. *P. crooki* Vande Vusse, 1976 is similar to *S. chalconotae* in having a similar type of uterus and testis, and is re-assigned as *Sundapolystoma crooki* (Vande Vusse, 1976) n. comb. *S. chalconotae* differs from *S. crooki* in having anchors with a longer outer root rather than a longer inner root and 7-8 genital spines compared to 9-13 in *S. crooki*.

Introduction

Of the 12 genera of polystomatids recorded in amphibians, only *Parapolystoma* Ozaki, 1935 has been reported from an amphibian in Southeast Asia. *P. crooki* Vande Vusse, 1976 was obtained from *Rana magna* (Ranidae) from Palawan Island, the Philippines. There are one undescribed and two other described species of *Parapolystoma* from amphibians. *P. bulliense* (Johnston, 1912) Ozaki, 1935 is from *Litoria phyllochroa* and *L. lesueurii* and *P. johnstoni* Pichelin, 1995 is from *L. nyakalensis* (see Yamaguti, 1963; Pichelin, 1995), all Australian hylid frogs The undescribed polystomatid species is from *Rana sauteri* (Ranidae) in Taiwan (Prof. D. Kok, pers. comm.).

In a recent survey of the parasites of frogs in Peninsular Malaysia, only 13 anuran species of the 155 species available (Kiew, 1984) were examined, and of these only one species, *Rana chalconota* (Ranidae), from one location was infected with a species of polystomatid. This species is new and does not fit into any of the known genera, although it is similar to *Parapolystoma crooki*. To accommodate this new species a new genus is proposed. The characteristics of the adult as well as the number of ciliated cells on the oncomiracidium of the new species are given herein. Pichelin (1995) has included the number of ciliated cells of the oncomiracidum as a subfamily characteristic, although such information is not available for all polystomatids. In this study information only on the number of ciliated cells of the oncomiracidum will be given: a detailed description of the oncomiracidium will be given elsewhere with information on the development of the species.

Materials and methods

A total of 302 amphibians belonging to 13 species were collected from two localities in Peninsular Malaysia, at the Botanical Garden (Hutan Rimba) on the University of Malaya campus, Kuala Lumpur, and at a site near the University of Malaya's Field Station in Gombak, Selangor. The species collected were *Bufo divergens, B. parvus, Kaloula pulchra, Polypedates leucomystax, Rana chalconota, R. erythraea, R. limnocharis, R. malesiana* and *R. nicrobariensis* from the two localities mentioned above, while *Bufo juxtasper, Megophrys monticola nasuta, Rana hosei* and *Staurois*

larutensis were found only around the University of Malaya's Field Station at Gombak.

The amphibians were brought back to the laboratory and kept together according to species in plastic containers with a little water. To detect the presence of monogeneans, the water was sieved through a graded series of sieves made of fine plankton netting material, and the contents from each sieving were screened under a dissecting microscope for monogenean eggs. A sieve with a mesh size 500μm was used to remove the larger debris and one with a mesh size 112μm was used to collect monogenean eggs. Eggs were left in water at room temperature (c. 25°C) in Petri dishes to hatch and enable the collection of oncomiracidia. To recover monogeneans, hosts were anaesthetised using benzocaine and the urinary bladder and kidneys examined for parasites. Some tadpoles were dissected and examined for parasitic infection.

A total of 16 adult polystomatid specimens were collected. Eleven of these specimens were used for whole-mount preparations, three for histological studies and two fixed for other studies (one each for electron-microscopical and molecular biological studies). The 11 specimens were fixed under coverslip pressure with buffered formalin for at least 24 hours, removed from the glass-slides and stored in the fixative. Prior to staining, these specimens were cleared in glycerine to study the sclerotised hard parts, such as genital spines, anchors and marginal hooks, using phase contrast microscopy. The anchors and marginal hooks were measured on flattened specimens as indicated in Figure 3 and measurements are given in micrometres (unless otherwise indicated) as means with the ranges in parentheses. These specimens were then washed in distilled water, stained with alum carmine, dehydrated in alcohol, cleared in xylene and mounted in Canada balsam. Three specimens were fixed with buffered formalin (without flattening) for 24 hours and transferred to 70% ethanol for histological sectioning in order to study the reproductive system. The parasites were embedded in paraffin wax, sectioned at 7μm and stained with Harris' haematoxylin and eosin.

To determine the number of ciliated cells on the oncomiracidium, live oncomiracidia hatched from the polystomatid eggs collected were killed and fixed in hot silver nitrate (0.1M) and left to develop in sunlight for 3 minutes. After washing with at least two changes of distilled water, the oncomiracidia were mounted in glycerine on slides. When not in use, the slides containing the silver-stained oncomiracidia were kept in the dark and in the cold (refrigerator) to prevent further oxidation.

Specimens of *P. bulliense* (AHC 22218; AHC 22219 and AHC 22220 from the South Australian Museum, Adelaide, Australia and GL 12109 & GL 12160 from the Queensland Museum, Australia), *P. johnstoni* (Paratype G211520 from the Queensland Museum, Australia) and *P. crooki* (Paratypes 1207-12 through to 1207-16 from the US National Parasite Collection, Maryland, USA) were examined. Also examined were the histological sections of *P. bulliense* (AHC 21442, three slides; AHC 2221, four slides from the South Australian Museum, Adelaide, Australia). Prof. D. Kok kindly lent us the polystomatid he collected from *Rana sauteri* in Taiwan.

Results

Of the 13 species of anurans examined only *Rana chalconota* from Gombak was infected with polystomatids. Sixteen adult polystomatids were collected from the urinary bladder of 12 of the 45 adult *R. chalconota* examined at this site. Different developing stages of this parasite were also recovered from the gills of *R. chalconota* tadpoles. There were no gravid forms amongst the developing forms from the gills of the tadpoles. However, the anchors and suckers of the gravid adult parasites from the urinary bladder were found to be in different stages of development (see below).

Sundapolystoma n. g.

Diagnosis

Polystomatinae. Large elongate ovoid body, c. 5 mm long. Intestinal caeca blind, with small diverticula, extend into haptor. Haptor with 3 pairs of suckers, one pair of anchors and 16 marginal hooks: hooks I & II modified, situated between anchors: hooks III to VIII of larval type, with hooks III, IV & V at base of suckers 1, 2 & 3, respectively, and hooks VI, VII & VIII at anterior margin of haptor. Testes single, diffuse, extending from pre-ovarian region posteriorly to just short of caecal extremities, also laterally into extracaecal region. Vas deferens observed close to ovary, extends antero-medially to copulatory organ (genital bulb), armed with 7-8 spines, opens into common genital pore. Ovary elongate, sinistral, in anterior third of body. Oviduct arises from posterior region of ovary, connected by genito-intestinal canal to left caecum,

receives common vitelline duct, ascends giving rise to tubular uterus. Uterus then descends reaching haptor but not beyond caeca, ascends again medially to uterine pore. Vitelline follicles reach to pharyngeal region, co-extensive with caeca; left and right vitelline ducts join to form common vitelline reservoir near ovary, with duct to oviduct. Two vaginae, antero-lateral to ovary; left and right vaginal ducts connected to respective vitelline ducts. Egg operculate, usually with developed oncomiracidium *in utero*. Adult parasitic in urinary bladder of amphibians of Southeast Asia. Type-species *Sundapolystoma chalconotae* n. sp.

Remarks

The new genus is similar to *Parapolystoma* Ozaki, 1935 (*P. bulliense, P. johnstoni* and *P. crooki*) in the morphology and locations of the ovary, uterus and genito- intestinal canal, but it differs from the type species, *P. bulliense*, in having a tubular uterus and single diffuse testis, rather than a sac-like uterus and multiple testes. These observations were confirmed by the examination of histological sections.

The name of the new polystomatid genus, *Sundapolystoma*, is derived from Sundaland, the lands of the Sunda continental shelf (Southeast Asia).

***Sundapolystoma chalconotae* n. g., n. sp.** (Adult, Figures 1-4; oncomiracidium, Figure 5)

Description

Adult 6 459 (4,900-7,868) × 1 727 (1,260-2,156); eye-spots not observed in adult, but present in oncomiracidium. Oral sucker 327 (238-406) × 239 (190-280). Pharynx pyriform, 217 (168-224) × 179 (142-202). Intestine bifurcate; caeca blind with small diverticula; left and right caeca of almost equal length, extend into haptor.

Haptor 1,815 (1,232-2,268) × 2,235 (1,568-2,660), with 3 pairs of lateral suckers, 2 anchors and 16 marginal hooks; hooks I and II modified. Hook I longest and largest, with length 39 (36-42); hook II length 22 (21-23); hooks III, IV and V length 20 (19-20), found at bases of suckers 1, 2 and 3, respectively; hooks VI, VII and VIII length 20 (19-20), located around anterior marginal region of haptor. Anchor size and shape vary in all adult specimens obtained (Figures 3a1-3a3, 4a-4d); fully- developed anchor robust (Figure 3a), inner length 284 (231-330), outer length 337 (275-396), inner root 70 (66-110), outer

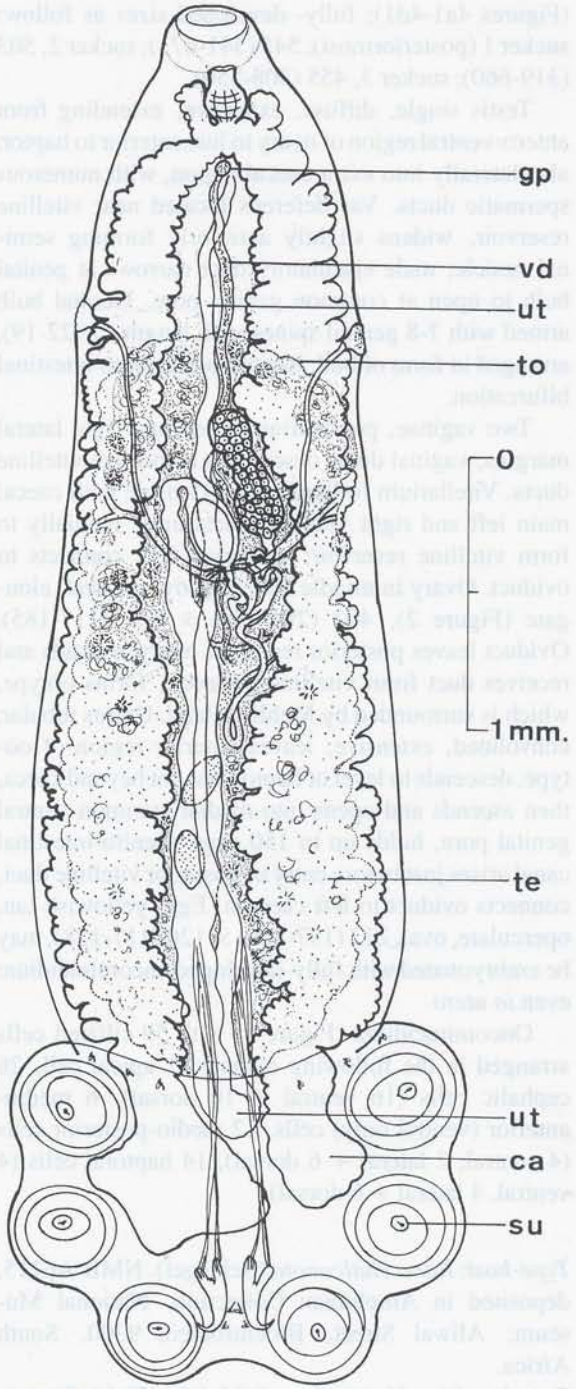

Figure 1. Composite illustration of *Sundapolystoma chalconotae* n. g., n. sp. (ventral view). *Abbreviations*: gp, genital pore; vd, vas deferens; ut, uterus; to, oultine of the extent of extensive diffuse testis; te, testis; ca, caecum; su, sucker.

root 90 (88-132), recurved point 54 (50-56). Haptoral sucker size variable, depending on age of worm (Figures 4a1-4d1); fully- developed sizes as follow: sucker 1 (posteriormost), 545 (341-671); sucker 2, 503 (319-660); sucker 3, 455 (308-550).

Testis single, diffuse, extensive, extending from antero-ventral region of ovary to just anterior to haptor, also laterally into extra-caecal region, with numerous spermatic ducts. Vas deferens located near vitelline reservoir, widens slightly anteriorly forming seminal vesicle; male ejaculatory duct narrows at genital bulb to open at common genital pore. Genital bulb armed with 7-8 genital spines with length 27 (22-19), arranged in form of bud, located posterior to intestinal bifurcation.

Two vaginae, pre-ovarian on left and right lateral margins; vaginal ducts descend to respective vitelline ducts. Vitellarium follicular, co-extensive with caeca; main left and right vitelline ducts unite medially to form vitelline reservoir; posterior duct connects to oviduct. Ovary in middle half of body, sinistral, elongate (Figure 2), 402 (294-454) × 151 (117-185). Oviduct leaves posterior region of ovary, ascends and receives duct from vitelline reservoir, forms oötype, which is surrounded by Mehlis' gland. Uterus tubular, convoluted, extensive, leaves anterior region of oötype, descends to level of haptor, but not beyond caeca, then ascends and opens into medial common ventral genital pore, holds up to 150 eggs. Genito-intestinal canal arises just before entry of posterior vitelline duct, connects oviduct to left caecum. Egg, yellowish-tan, operculate, oval, 237 (197-269) × 120 (117-128), may be embryonated with fully-developed oncomiracidium even *in utero*.

Oncomiracidium (Figure 5) with 59 ciliated cells arranged in the following manner: 1 apical cell, 26 cephalic cells (16 ventral + 10 dorsal), 6 medio-anterior (ventral only) cells, 12 medio-posterior cells (4 ventral, 2 lateral + 6 dorsal), 14 haptoral cells (4 ventral, 4 lateral + 6 dorsal).

Type-host: *Rana chalconota* (Schlegel). NMB A6115, deposited in Amphibian Collection, National Museum, Aliwal Street, Bloemfontein 9300, South Africa.
Type-locality: University of Malaya Field Station, Gombak, Selangor.
No. of host species infected: 12 of 45 examined.
Site of larvae: Gills of tadpoles.
Site of adults: Urinary bladder.
No. of adult parasites obtained (measured): 16 (11).
Type-material: Holotype (BMNH no.2000.11.29.1) and two paratypes (BMNH no. 2000.11.29.2-3) in the Parasitic Worms Collection at The Natural History Museum, London. Four paratypes (NMBP246-249) in Parasitic Worm Collection, National Museum, Aliwal Street, Bloemfontein, South Africa and four paratypes in the collection of Lim L.H.S. Histological sections deposited in Parasitic Worm Collection, National Museum, Aliwal Street, Bloemfontein, South Africa.

Remarks

This species is similar to the *Parapolystoma* spp. (*P. bulliense, P. johnstoni* and *P. crooki*) in having blind caeca, a uterus extending into the haptor but not beyond the caeca, the presence of two anchors and the arrangement of the 16 marginal hooks. Small diverticula on the caeca are observed in the present species and *P. crooki*. The marginal hooks of both the present species and *P. crooki* persist in the adult (see Vande Vusse, 1976), but there are supposedly no trace of the marginal hooks in the majority of adult *P. bulliense* (see Johnston, 1912) and in *P. johnstoni* the hooks were 'not clearly visible' (Pichelin, 1995). However, marginal hooks were observed in all the type-specimens of *Parapolystoma* spp. examined (using a long working distance objective and differential interference contrast). The arrangements of the anchors, suckers and 16 marginal hooks, as described above, are similar to those in *Parapolystoma* spp. (see Pichelin, 1995) and are probably characteristics of the family Polystomatidae.

This new species and the three *Parapolystoma* species possess a similar type of female reproductive system. However, *P. bulliense* and *P. johnstoni* have a sac-like uterus and multiple testes, while the present species and *P. crooki* possess a tubular uterus and a single, diffuse testis (Johnston, 1912; Vande Vusse, 1976; Prudhoe & Bray, 1982; Pichelin, 1995; present study). The present species differs from *P. crooki* in having anchors shorter than the diameter of the first pair of suckers, 7-8 genital spines (*vs* 11-12 genital spines in *P. crooki*) and larger eggs [237 (197-269) × 120 (117-128) *vs* 142 (132-156) × 70 (63-84)]. The egg dimensions for *P. crooki* were incorrectly given in original description and were re-measured from paratypes examined.

The oncomiracidium of *S. chalconotae* n. g., n. sp. is morphologically similar to that of *P. bulliense*, with

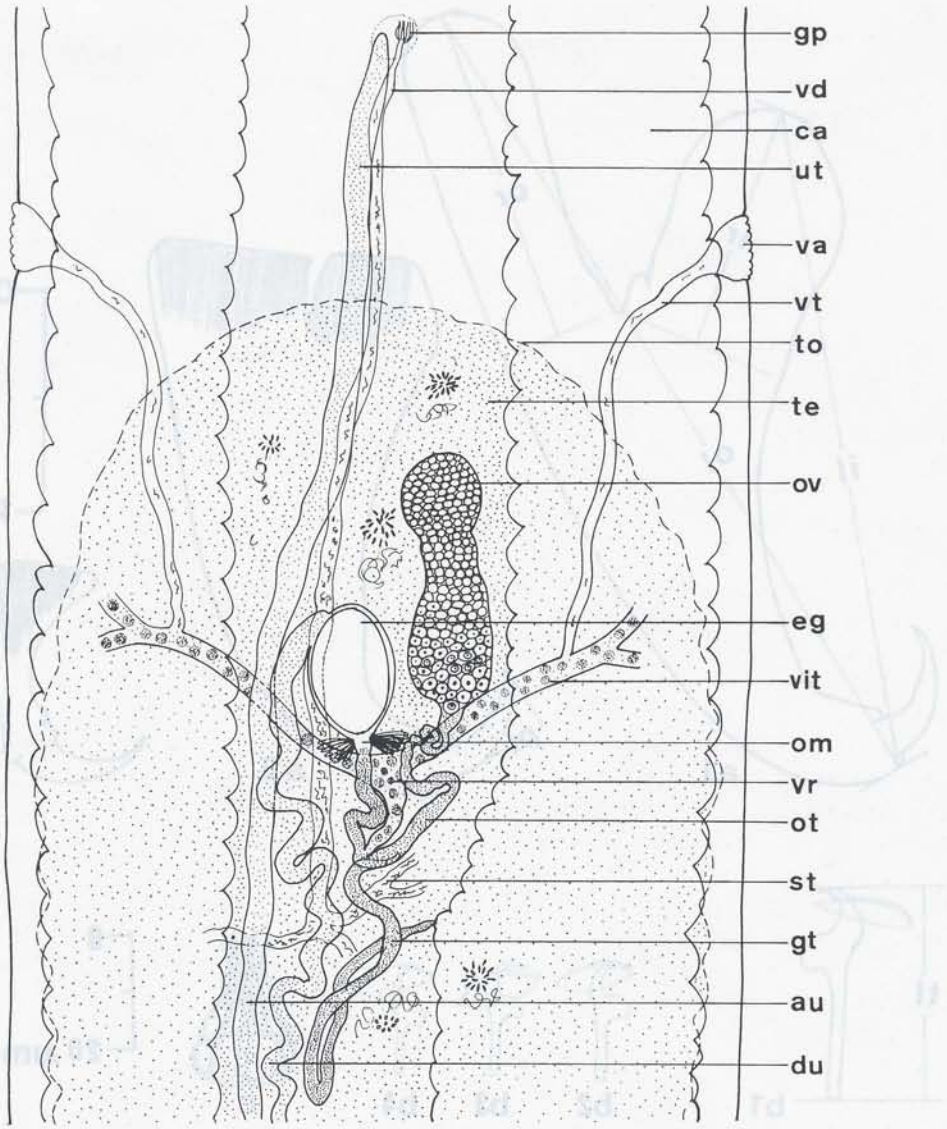

Figure 2. Composite illustration of the reproductive systems of *Sundapolystoma chalconotae* n. g., n. sp. (ventral view). *Abbreviations*: gb, genital bulb; vd, vas deferens; ca, caecum; ut, uterus; va, vagina; vt, vaginal duct; to, outline of the extent of extensive diffuse testis (te); ov, ovary; eg, egg; vit, vitelline duct; om, oõtype (with Mehlis' gland); vr, vitelline reservoir; ot, oviduct; st, spermatic duct; gt, genito-intestinal duct; au, ascending limb of uterus; du, descending limb of uterus.

59 ciliated cells arranged in a similar manner, while *P. crooki* has an oncomiracidium with 55 ciliated cells (see Vande Vusse, 1976). However, the number of ciliated cells on *S. crooki* has to be verified.

The species is named *Sundapolystoma chalconotae* n. g., n. sp., reflecting the specific name of the host, *Rana chalconota*.

As already noted, *P. crooki* differs from other members of *Parapolystoma* (*P. bulliense* and *P. johnstoni*) and is similar to *S. chalconotae* n. sp. in having a single diffuse testis and tubular uterus rather than multiple testes and a sac-like uterus. Consequently, *P. crooki* is re-assigned as *Sundapolystoma crooki* (Vande Vusse, 1976) n. comb. We have also examined a single specimen of a polystomatid *from Rana* sauteri in Taiwan and found it to be morphologically similar to *S. chalconotae* and *S. crooki* and probably also belongs to *Sundapolystoma*.

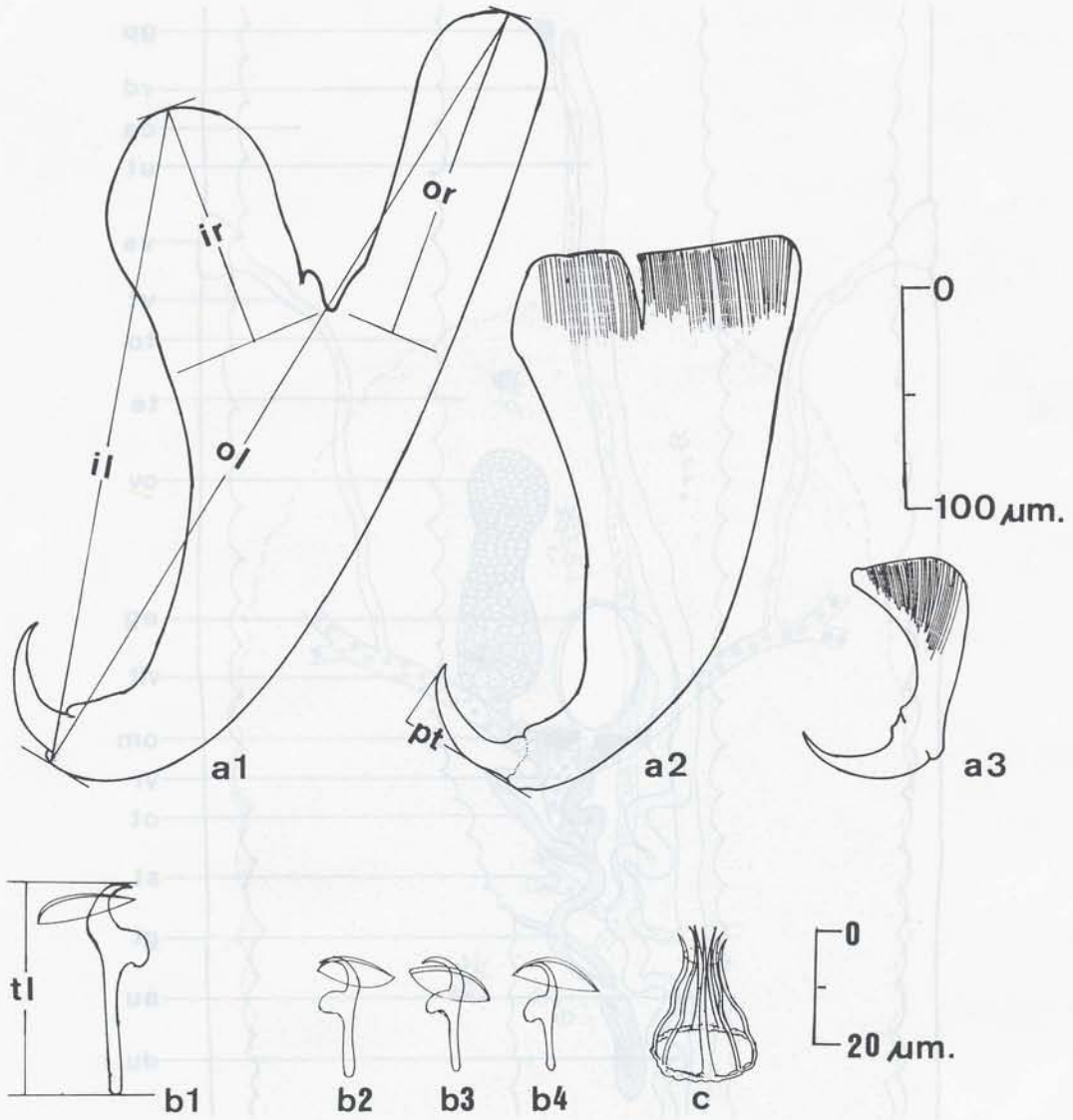

Figure 3. Sclerotised parts of adult *Sundapolystoma chalconotae* n. g., n. sp.: a1. developed anchor; a2:-a3. developing anchors of adult worm; b1. hook 1; b2. hook 2; b3-b4. hooks 3-8; c. genital spines. *Abbreviations*: il, inner length; ol, outer length; ir, inner root; or, outer root; pt, point; tl, total length. *Scale-bar*: a, for a1-a3.

Discussion

There are currently two described and probably one undescribed species of *Sundapolystoma* and two species of *Parapolystoma* from amphibians in Southeast Asia (including Taiwan) and Australia, respectively. It is premature to draw any conclusion concerning the distribution patterns of the two genera, because of the paucity of records from both Southeast Asia and Australia. In this study only one host species (*Rana chalconota*), of the 13 anuran species examined, was found to be infected. Similarly in the Philippines only one (*R. magna*) of the examined 11 species harboured polystomatids (see Vande Vusse, 1976). This low occurrence could be due to limited research effort and also an over-dispersed distribution pattern, as indicated by the absence of *S. chalconotae* from the *R. chalconota* from the campus of the University of Malaya and its presence on the same host species in Gombak.

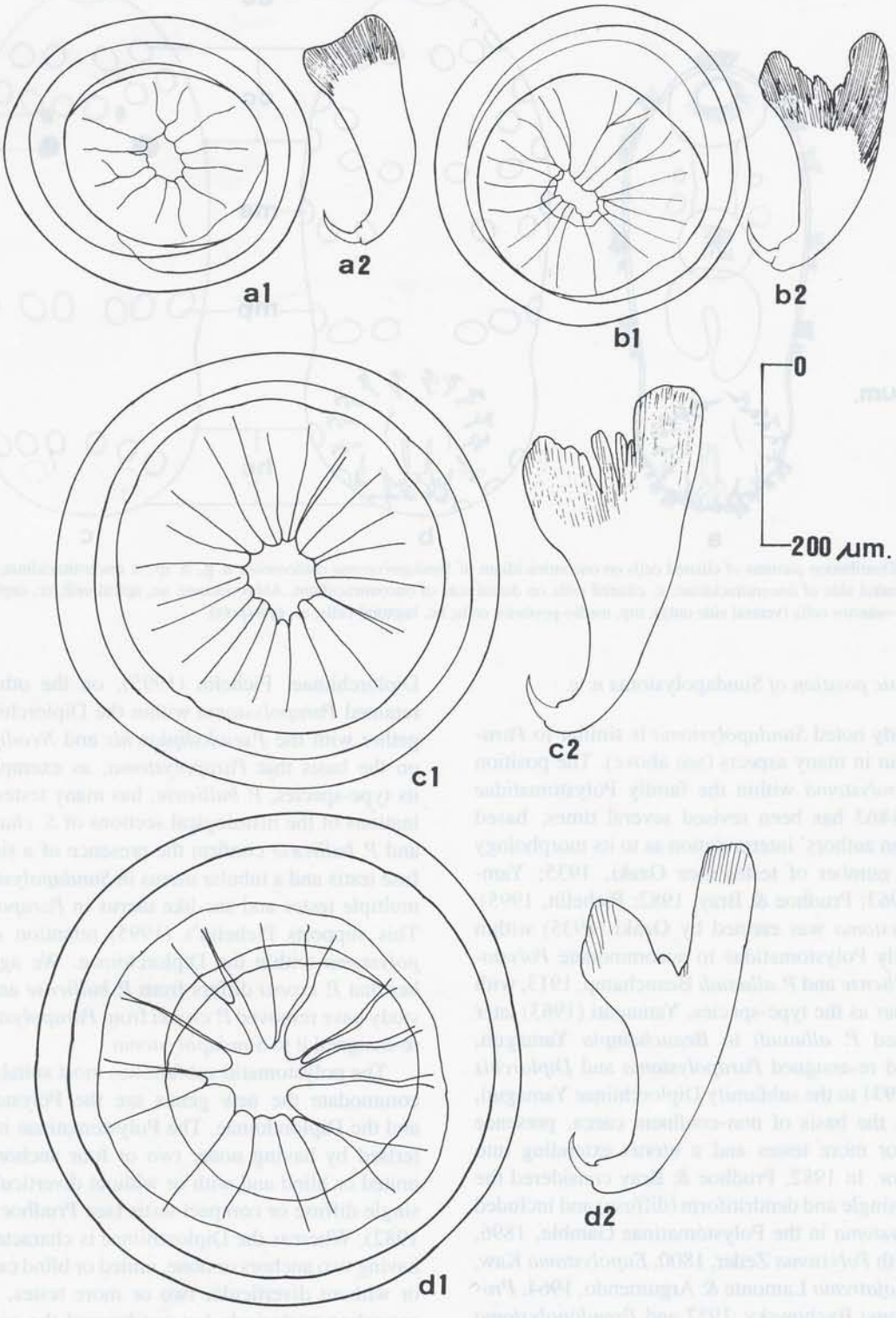

Figure 4. Suckers and anchors of gravid adult *Sundapolystoma chalconotae* n. g., n. sp. at different developmental stages: a1-d1. developing stages of sucker 1; a2-d2. developing stages of anchor.

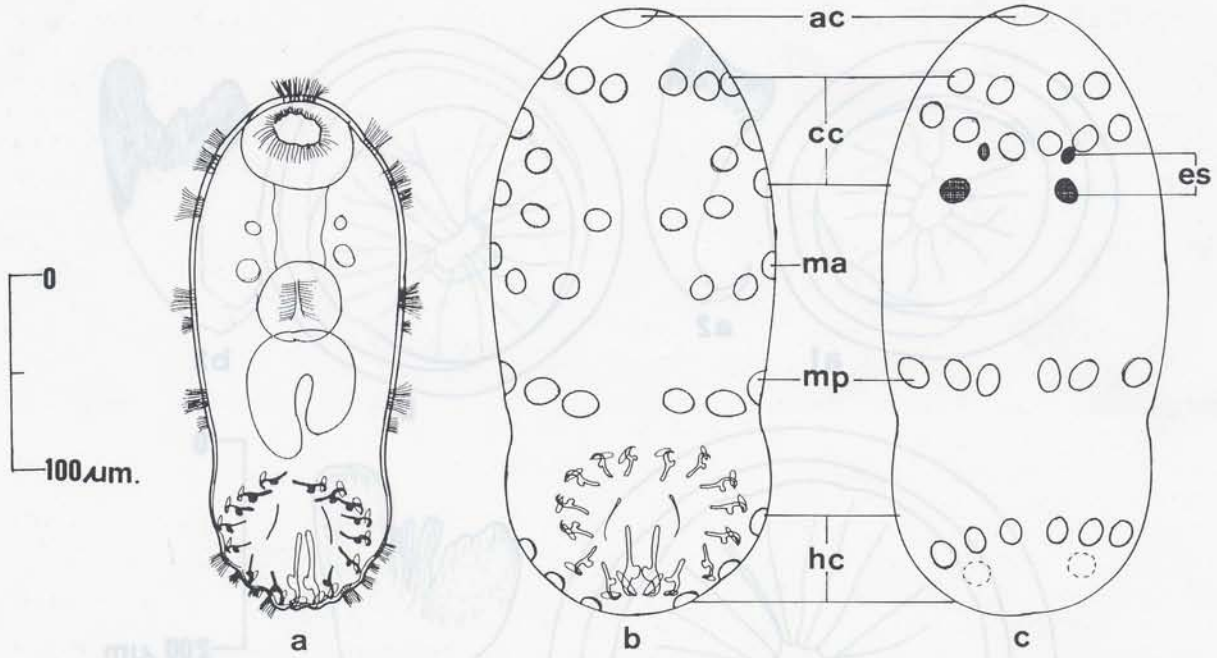

Figure 5. Distribution patterns of ciliated cells on oncomiracidium of *Sundapolystoma chalconotae* n. g., n. sp.: a. oncomiracidium; b. ciliated cells on ventral side of oncomiracidium; c. ciliated cells on dorsal side of oncomiracidium. *Abbreviations*: ac, apical cell; cc, cephalic cells; ma, medio-anterior cells (ventral side only); mp, medio-posterior cells; hc, haptoral cells; es, eye-spots).

Taxonomic position of Sundapolystoma *n. g.*

As already noted *Sundapolystoma* is similar to *Parapolystoma* in many aspects (see above). The position of *Parapolystoma* within the family Polystomatidae Carus, 1863 has been revised several times, based mainly on authors' interpretation as to its morphology and the number of testes (see Ozaki, 1935; Yamaguti, 1963; Prudhoe & Bray, 1982; Pichelin, 1995). *Parapolystoma* was erected by Ozaki (1935) within the family Polystomatidae to accommodate *Polystomum bulliense* and *P. alluaudi* Beauchamp, 1913, with the former as the type-species. Yamaguti (1963) later transferred *P. alluaudi* to *Beauchampia* Yamaguti, 1963 and re-assigned *Parapolystoma* and *Diplorchis* Ozaki, 1931 to the subfamily Diplorchiinae Yamaguti, 1963 on the basis of non-confluent caeca, presence of two or more testes and a uterus extending into the haptor. In 1982, Prudhoe & Bray considered the testis as single and dendritiform (diffuse) and included *Parapolystoma* in the Polystomatinae Gamble, 1896, along with *Polystoma* Zeder, 1800, *Eupolystoma* Kaw, 1950, *Riojatrema* Lamonte & Argumendo, 1964, *Protopolystoma* Bychowsky, 1957 and *Pseudopolystoma* Yamaguti, 1963 and placed *Pseudodiplorchis* Yamaguti, 1963 and *Neodiplorchis* Yamaguti, 1963 in the Diplorchiinae. Pichelin (1995), on the other hand, retained *Parapolystoma* within the Diplorchiinae, together with the *Pseudodiplorchis* and *Neodiplorchis*, on the basis that *Parapolystoma*, as exemplified by its type-species, *P. bulliense*, has many testes. Examinations of the histological sections of *S. chalconotae* and *P. bulliense* confirm the presence of a single diffuse testis and a tubular uterus in *Sundapolystoma* and multiple testes and sac-like uterus in *Parapolystoma*. This supports Pichelin's (1995) retention of *Parapolystoma* within the Diplorchiinae. We agree with her that *P. crooki* differs from *P. bulliense* and in this study have removed *P. crooki* from *Parapolystoma* and re-assigned it to *Sundapolystoma*.

The polystomatid subfamilies most suitable to accommodate the new genus are the Polystomatinae and the Diplorchiinae. The Polystomatinae is characterised by having none, two or four anchors, caeca united or blind and with or without diverticula, and a single diffuse or compact testis (see Prudhoe & Bray, 1982). Whereas the Diplorchiinae is characterised by having two anchors or none, united or blind caeca with or without diverticula, two or more testes, a uterus extending posteriorly but not beyond the caeca, and an oncomiracidium with 59 ciliated cells (see Pichelin, 1995). Although *Sundapolystoma* n. g. has also

a uterus, which extends close to the caecal extremities and an oncomiracidium with 59 ciliated cells, it differs mainly from *Parapolystoma* in having a single diffuse testis rather than multiple testes. Based on the presence of a single testis, *Sundapolystoma* is here included within the subfamily Polystomatinae. The two genera, *Parapolystoma* (Diplorchiinae) and *Sundapolystoma* n. g. (Polystomatinae), share many characteristics, such as an oncomiracidium with 59 ciliated cells, the situation of the female reproductive system, blind caeca and the arrangement of haptoral suckers, marginal hooks and anchors, but these are probably diagnostic characters for the family.

Sundapolystoma n. g. differs from all other polystomatines in a combination of characters. The presence of blind caeca of equal length distinguishes it from all other polystomatid genera except *Protopolystoma, Pseudopolystoma, Polystomoides* Ward, 1917, *Polystomoidella* Price, 1939 and *Neopolystoma* Price, 1939. The single pair of anchors of *Sundapolystoma* differentiates it from *Protopolystoma* and *Polystomoides*, which have two pairs and *Pseudopolystoma* and *Neopolystoma* which lack anchors. *Sundapolystoma* has genital spines, which *Polystomoidella* does not.

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