FIRST RECORD AND DESCRIPTION OF *CATATROPIS INDICUS* SRIVASTAVA 1935 (DIGENEA: NOTOCOTYLIDAE), IN AUSTRALIA

MAREE KOCH

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Each life cycle stage, except the miracidium, of *Catatropis indicus* Srivastava 1935 is described in Australia for the first time. The life cycle was completed experimentally by feeding metacercariae from naturally infected snails, *Gabbia australis* (Bithyniidae), to domestic ducks, *Carrina moschata*. Metacercariae obtained by infecting laboratory-bred snails (*Gabbia australis*) with eggs in faeces of wild *Anas superciliosa*, (Pacific Black duck) were also fed to domestic ducks. Adult trematodes were found in the intestinal caecae of the ducks. Chickens and rats could not be infected. Eggs are operculate with two polar filaments. Sporocysts attach to the lower outer wall of the oesophagus and intestine of *G. australis*. Mother rediae are found on the intestine. Daughter rediae and cercariae develop in the gonads of *G. australis*. Metacercariae remain viable for four months. Comparison with all other known species of *Catatropis* shows that this species resembles most closely *Catatropis indicus* from India in all morphological and life cycle aspects. Slight differences in size can be attributed to geographical variation. ❐ *Notocotylidae, life cycles, Bithyniidae, Catatropis indicus*.

Maree Koch, School of Biological, Biomedical & Molecular Sciences, University of New England, Armidale 2351, Australia (e-mail: mkoch@pobox.une.edu.au); 5 January 2002.

Research in Australia on the digenean family Notocotylidae is limited to work done by Nicoll (1914), Johnston (1928), Smith & Hickman (1983), and Cribb (1991). The family contains 3 principal genera, mainly found in birds with aquatic affinities. Adult *Catatropis* is characterised by ventral glands consisting of a uniform median ridge and, in most species, lateral papillae (Yamaguti, 1971), whereas closely related *Notocotylus* has 3 rows of ventral individual papillae (Cribb, 1991). *Paramonostonum* has no papillae or glandular ridges ventrally (Yamaguti, 1971). *Catatropis indicus* Srivastava, 1935 has been recorded from Kuala Lumpur, Malaysia (Rohde & Onn, 1968) and India (Tandon & Roy, 1996). In this paper, each stage of the life cycle of this species is described from Australia for the first time and its taxonomic relationships are discussed.

METHODS

Snails, *Gabbia australis* (Bithyniidae), were collected from dams and water courses in the New England region of New South Wales, and dissected to study intramolluscan stages of the trematode life cycle. Three sets of concurrent life cycle experiments were conducted. In the first, 10 chickens (*Gallus gallus domesticus*) aged 2 weeks, and 9 ducklings (*Carrina moschata*) aged 10 days, and 10 rats aged 4 weeks, were each fed live snails with 20 metacercariae (aged 14 days) attached to the shells. Animals were autopsied at 17, 23, 39 and 40 days post infection.

In the second experiment, the viability of metacercariae over time was tested. Metacercarie gathered from the same sites as experiment one, were housed in the laboratory under simulated natural conditions. At age 4, 8, 16, 20, 24 and 28 weeks, the metacercariae were fed to 6 ducklings (*C. moschata*) aged 10 days. Each duck received 20 metacercariae and were autopsied at 23 days post infection. In the third experiment, fresh faeces obtained from wild ducks, *Anas superciliosa*, at the sites in experiments 1 and 2, were screened for trematode eggs which were then fed to aquaria of laboratory-bred parasite-free adult snails of *Physa*, *Glyptophysa*, *Austropeplea* and *G. australis*. Metacercariae thus produced were fed at age 14 days, to 4 ducks (*C. moschata*) aged 4 weeks. The ducks were autopsied at 23 days post infection.

All trematode specimens were studied in vivo and then fixed in 10% hot formalin, stained with Grenacher's carmine alum or acetocarmine, dehydrated in an alcohol series, and mounted in Canada balsam. Drawings were done using a camera lucida and body parts were measured with a calibrated eyepiece graticule.

For SEM observations, live specimens were

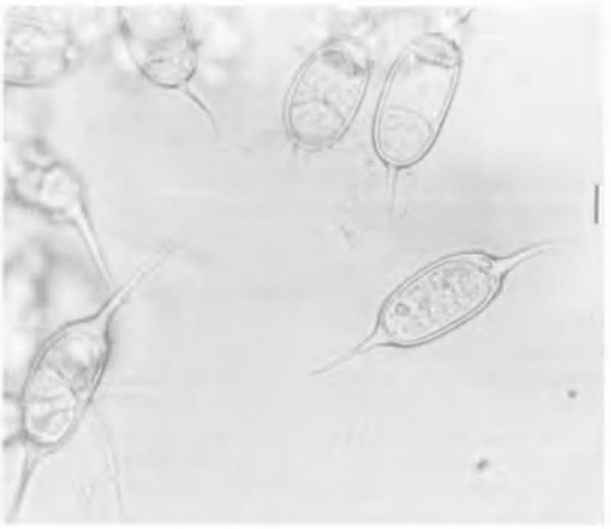


FIG. 1. Eggs (live) of *C. indicus*. Scale: 0.01mm.

fixed in 10% hot formalin, buffer washed in 0.1m PO_4 (pH 2.2), post-fixed in 1.0% OSO_4 in a 0.1M PO_4 buffer (pH 2.2), and dehydrated through an ascending alcohol series. The worms were then critical point dried and sputter-coated with gold. Specimens were examined with a scanning electron microscope at an accelerating voltage of 15kV.

Representatives of the specimens studied have been deposited with the Queensland Museum, Brisbane (QMG217660–217663). Measurements are quoted in millimetres and where ranges are shown, the mean is noted in parentheses.

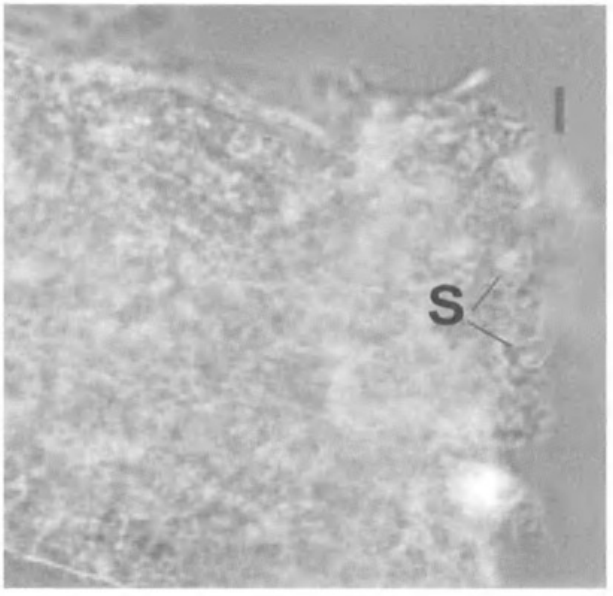


FIG. 2. Sporocysts (live) of *C. indicus* attached to buccal mass of *G. australis*. Scale: 0.1mm.

RESULTS

EXPERIMENT 1. 160 adult worms were recovered from 8 of the 9 ducks from the first experiment, and all were located in the intestinal caecae, initially in the proximal sections and moving toward the distal sections with increasing days post infection. No worms were recovered from the chickens or rats.

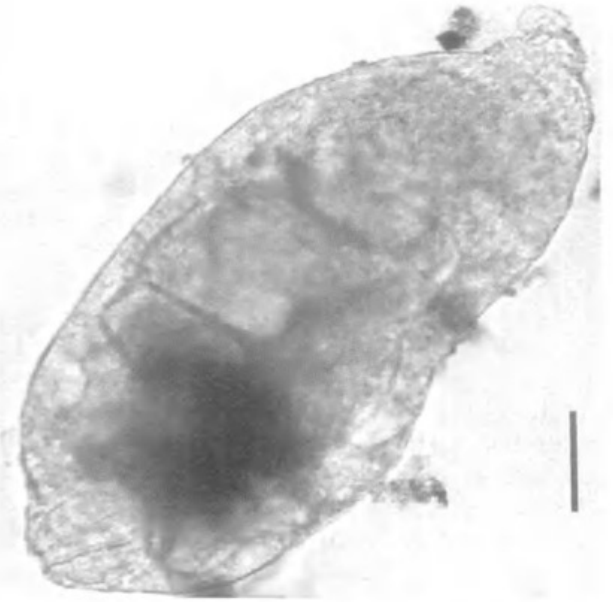


FIG. 3. Mother redia (live) of *C. indicus*. Scale: 0.1mm.

DESCRIPTIONS

Eggs. The eggs are oval, operculate, and have 2 polar filaments, one at each pole. They were apparent in the faeces of infected ducks at ~17 days post infection. Eggs collected from the faeces of infected ducks were incubated at room temperature in filtered pond water and daily observations made. No miracidia were observed to hatch from the eggs. Serial sectioning through the uterus of an adult worm was done at positions proximal, medial and distal to the ovary. A small degree of miracidial development and increase in egg filament length was observed between the proximal and distal egg stages. (Fig.1).

Egg length: 0.0147–0.027mm (0.025); width: 0.010–0.030mm (0.028), n=12. Filament length 0.029–0.132mm (0.081), n=12.

Sporocysts. Sporocysts were attached to the external surfaces of the lower oesophagus and intestine of *G. australis*. They contained multiple

FIG. 4. Daughter redia (fixed) of *C. indicus*. Scale: 0.1mm.

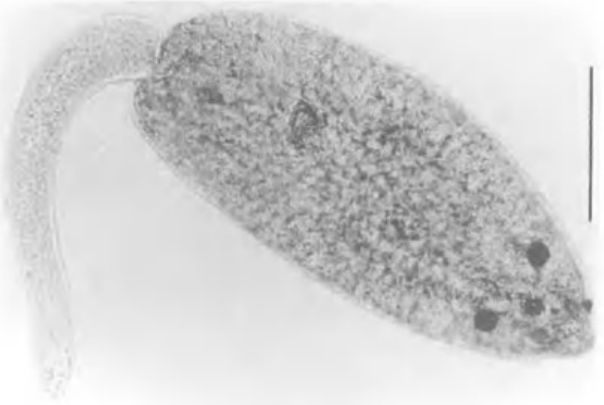


FIG. 5. Cercaria (fixed) of *C. indicus*. Scale: 0.1 mm.

developing mother rediae and germ balls. Sporocysts and mother rediae were found only in spring (September–October), suggesting a seasonal occurrence of these life cycle stages. (Fig. 2). L:0.16–0.18mm (0.17); W:0.09–0.10mm (0.095), n=4.

Mother Rediae. Mother rediae were found attached by fine white threads of tissue to the external surface of the intestine of *G. australis*. Germ balls and developing daughter rediae were contained within the body posterior to the short caecum. A posterior caudal appendage was demonstrated in vivo. (Fig. 3). L:0.59–0.62mm (0.61); W:0.20–0.23mm (0.21), n=4.

Daughter Rediae. Daughter rediae were found within, and attached externally by fine white tissue to, the gonads of *G. australis*. They could be distinguished from mother rediae by their brown pigmentation and distinctive embryonic cercariae. A muscular pharynx was visible anteriorly, leading into a short caecum. A birth pore was demonstrated in vivo. (Fig. 4).

L:0.392–0.539mm (0.443); W:0.098–0.176 mm (0.125), n=12.

Cercariae. Free cercariae were found in the same tissues in which the daughter rediae occurred. Cercariae had 2 lateral eyespots and 1 diffuse median eyespot. Two excretory trunks of the stenostomate type with the main ducts united across the anterior part of the body, opened into the posterior excretory bladder.

Encystment. Cercariae were observed exiting the snails via the mantle cavity. With their tails raised over the top of their bodies, vigorous side-to-side movement instigated a forward swimming direction. Various snail shells were examined for 10 – 20 minutes prior to an encystment site being chosen, with preference shown for the operculum and inner shell lip. Cercariae manually dissected from snails encysted on walls or bases of cavity blocks after a period of free swimming ranging from ten seconds to one minute. During encystment, the cercariae attached themselves to the substrate via the adhesive organs on two caudal appendages, and using anterior to posterior body undulations, exuded cyst walls from central dorsal cytogenous glands for 30 seconds. The tail then detached as the cercariae performed 3 to 5 rotations (360°) anticlockwise prior to settling in position within the cyst. (Fig. 5). Body length:0.333–0.382mm (0.355). Tail length:0.353–0.392mm (0.372), n=20.

Metacercariae. Metacercariae were hemispherical in side view, with the attached portion being flat and the free surface convex. There appeared to be two layers of cyst wall.

Cyst diameter:0.157–0.162mm (0.159), n=20. (Figs 6, 7).

Adult. Body elongate and flat, and rounded posteriorly, lacking spines or scales. Adults concave ventrally in vivo. Ventral glands arranged in one continuous median ridge and two lateral rows of 10 – 12 single glands which run from slightly anterior to the seminal vesicle to just posterior to the ovary. Ventral papillae with two or more splits in surface. Intestinal caecae terminate posterior to the ovary, with the excretory bladder between the testes. Ovary medial, anterior to the excretory bladder and twin-lobed. Mehlis' gland immediately anterior to the ovary. Excretory vesicle posterior to ovary. Vitellaria extend in two lateral groups from the middle of the body to anterior end of the testes. Testes symmetrical and placed posteriorly on both sides of the ovary. Uterus strongly coiled between the cirrus pouch and the Mehlis' gland, intercaecal and overlapping the caeca on both

FIG 6. Metacercaria (fixed) of *C. indicus*. Scale: 0.1mm.

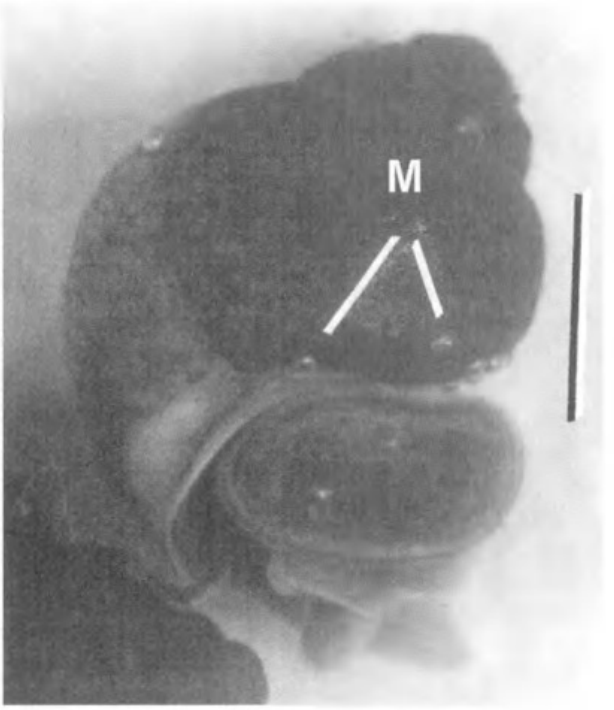


FIG 7. Metacercariae attached to shell of *G. australis* (live). Scale: 1.0mm.

sides. External seminal vesicle posterior to cirrus pouch and strongly coiled. Cirrus pouch containing seminal vesicle and ejaculatory duct. Common genital pore ventral and immediately posterior to oral sucker. Tegument surrounding the genital pore and the ventral surface between papillae covered with many uniformly raised tegumental processes with rounded tips. Excretory pore opens dorsally approximately level with the posterior margin of the testes. SEM of the dorsal surface shows no obvious sensory or tegumentary specialisations. Tegument surrounding the oral aperture studded with scattered rounded uniciliate papillae, probably sensory. The rim of the oral sucker with radially orientated folds. (Figs 8 - 10). Measurements of adults removed from the ducks and fixed in 10 % hot formalin are detailed in Table 1.

EXPERIMENT 2. Metacercarial viability was demonstrated to extend from 4 weeks to 16 weeks post-encystment only. 20 adult worms were consistently produced from each age bracket of viable metacercariae.

EXPERIMENT 3. Intramolluscan stages of *C. indicus* were found in 3 locations in the New England Tablelands. The eggs of *C. indicus* were retrieved from faeces of *A. superciliosa* at each of

TABLE 1. Measurements of adult worms (*C. indicus*) fixed in 10% hot formalin (mm), n = 20.

Age of Infection	17 Days	23 Days	39 Days
Length	1.98 - 2.20 (2.09)	2.45 - 2.70 (2.58)	2.70 - 3.40 (2.95)
Max. breadth	0.753 - 0.784 (0.769)	0.88 - 1.07 (0.975)	0.88 - 1.16 (1.01)
Oral Sucker length	0.125 - 0.157 (0.141)	0.11 - 0.167 (0.138)	0.094 – 0.172 (0.133)
max. breadth	0.125 - 0.157 (0.141)	0.157 - 0.167 (0.162)	0.125 – 0.147 (0.136)
Testes length	0.408 - 0.44 (0.424)	0.50 (0.50)	0.50 - 0.69 (0.574)
max. breadth	0.19 - 0.22 (0.205)	0.19 - 0.22 (0.205)	0.25 - 0.28 (0.26)
Ovary length	0.125 - 0.19 (0.041)	0.157 - 0.177 (0.167)	0.174 - 0.28 (0.225)
max. breadth	0.125 - 0.157 (0.016)	0.16 - 0.22 (0.19)	0.19 - 0.22 (0.20)
Cirrus Pouch length	0.063 – 0.063 (0.063)	0.014 – 0.05(0.045)	0.343 - 0.941 (0.642)
max. breadth	0.44 - 0.502 (0.471)	0.561 – 0.60 (0.582)	0.721 - 0.91 (0.837)
Vitellaria length	0.47 - 0.502 (0.486)	0.60 (0.60)	0.753 - 0.972 (0.857)

FIG. 8. Adult (fixed) of *C. indicus*. Scale: 0.1mm.

these sites. Adult worms were recovered from the intestinal caecae of all 4 experimental ducks and were identical both in morphology and location within the host, to those adult worms recovered in experiment 1. In the laboratory, intra-molluscan stages of the parasite could only be established in *G. australis* snails.

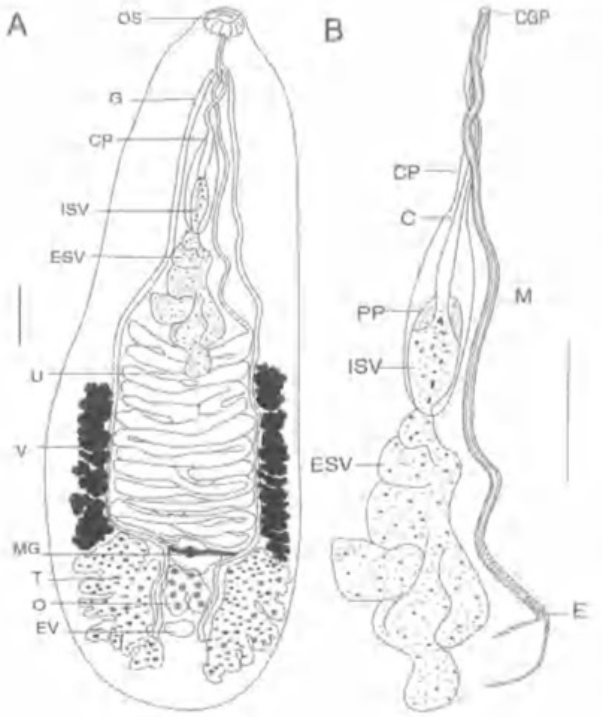


FIG. 9. Schematic drawings of *C. indicus*. A, Adult – internal anatomy. B, Terminal genitalia. C, cirrus; CGP, common genital pore; CP, cirrus pouch; E, eggs; ESV, external seminal vesicle; EV, excretory vesicle; G, gut; ISV, internal seminal vesicle; M, metraterm; MG, Mehlis' gland; O, ovary; OS, oral sucker; T, testes; U, uterus; V, vitelline follicles. Scale lines: 0.1mm.

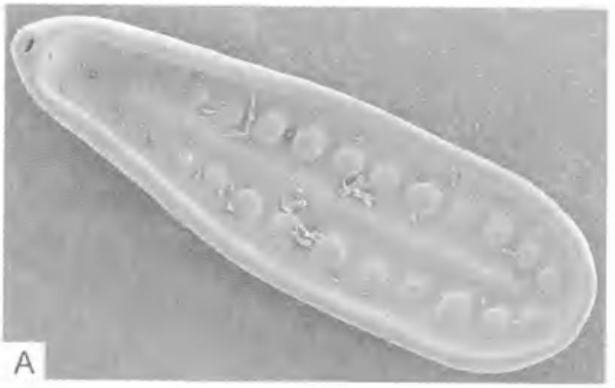
DISCUSSION

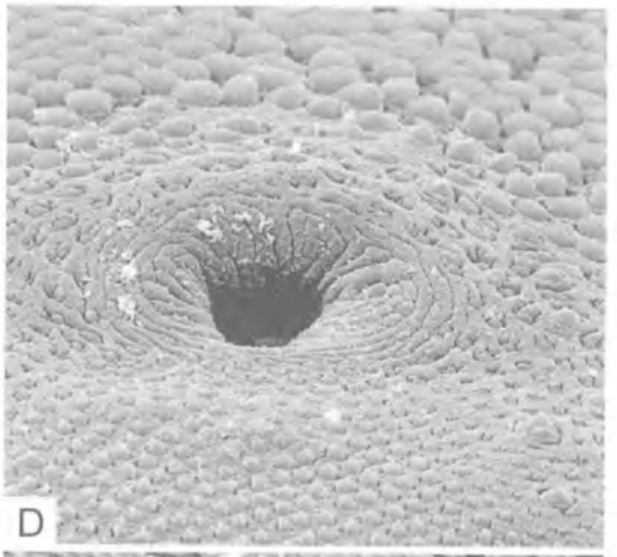
SEM work on this species confirmed a uniform median ridge with lateral papillae, thus placing it in *Catatropis* (Notocotylidae, Digenea). The Australian species differs from others in the same genus in the following aspects. *Catatropis nicolli* (Cribb, 1991) does not have lateral ventral papillae, and its definitive host is a mammal, the water rat *Hydromys chrysogaster*. The description of *C. lagunae* from France by Bayssade-Dufour et al., (1996), details 2 significant differences between it and the Australian species. There are fewer lateral papillae in *C. lagunae* (6–9 only), and a voluminous external seminal vesicle was demonstrated using TEM techniques. Although the general anatomy and morphology of my specimens are similar to *C. verrucosa* from Bulgaria (Kanev et al., 1994), it cannot be placed in the same species. This description of *C. verrucosa* is of a much larger species with tegumental spines and its life cycle was completed experimentally in chickens.

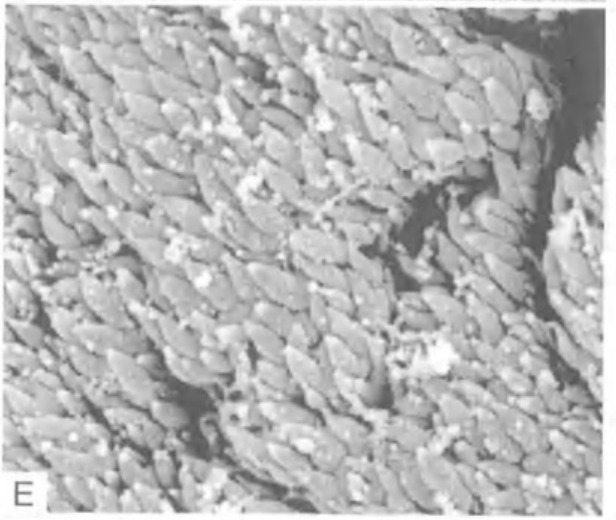
Rohde & Onn (1968) described *C. indicus* Srivastava 1935 from Kuala Lumpur as possessing tegumental spines. They could successfully infect both chickens and ducks. All life cycle stages are slightly larger in size than those of my species, and both the number of lateral papillae (12–13) and the location of sporocysts on the buccal mass and oesophagus in their snail host are different to the characteristics described in my experiments.

Srivastava (1935) described only adult specimens removed from the intestinal caecae of the Indian fowl, *Gallus bankiva murghi*. Life cycle comparisons cannot be drawn with his original classification. However, Srivastava stated the ratio of the length to the maximum breadth of the body of his specimens as 4:1. Adult worms from my experiments and those described by Tandon & Roy (1996), also from India, exhibit a body length to maximum breadth ratio of 2:1, suggesting that Srivastava's specimens may have been more mature.

Catatropis indicus from India (Tandon & Roy, 1996) differs from the Australian species only slightly in length (~0.01–0.2mm) in some life cycle stages. Geographical variability can be responsible for this, and, as there are no significant morphological and life cycle differences, I cannot justify the a new species for the Australian specimens. Thus, I tentatively place my specimens in *C. indicus* (Tandon & Roy, 1996). Slight morphological variations amongst







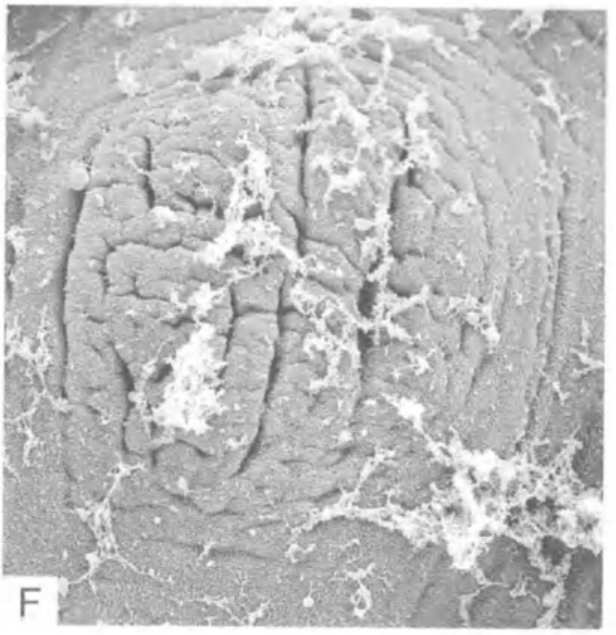


FIG. 10. SEMs of adult worm (*C. indicus*). A, adult – ventral (* 55); B, adult – dorsal (* 55); C, oral sucker showing arrangement of papillae (* 800). D, gonopore posterior to oral sucker (ventral surface) (* 2 000). E, papillae on ventral surface (* 10 000). F, close-up of surface of ventral papillae (* 850).

members of a species can be expected. rDNA sequencing of adult worms from my experiments currently in progress in conjunction with Littlewood & Olsen (2001), will hopefully enable taxonomic placement of this species and provide insight into the evolutionary steps taken by the trematode in its journey to cross continents.

As *G. australis* is a member of the Bithyniidae, (Beesley et al., 1998) and the snail host of *C. indicus* in Malaysia and India (*Bithynia siamensis*) also belongs to this family (Fretter & Graham,1962), the trematode appears to be specific to different species of different genera belonging to the same family. The range of *G. australis* extends throughout central and western NSW and into the NT (Ponder, et al., 2000), concurrent with the range of *A. superciliosa* (Simpson & Day, 1993). Future sampling will confirm the range of *C. indicus* in this country and the depth of its final host specificity. Other ducks similar in physiology and habitat useage may also be vulnerable (e.g. *Biziura lobata*, *A. gibberifron*, *Aythya australis*).

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