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MONOGENEAN PARASITES OF MARINE FISHES *GAZZA ACHLAMYS* (JORDAN & STARKS, 1917) AND *ARIOMMA INDICA* (DAY, 1871) FROM VISAKHAPATNAM COAST, SOUTH EAST COAST OF INDIA

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ABSTRACT

A survey has been conducted to collect monogenean parasites from G*azza achlamys* (Jordan & Starks, 1917) and *Ariomma indica* (Day, 1871) off Visakhapatnam coast. Altogether, four monogeneans species belong to three families (*i.e* Diclidophoridae, Gastrocotylidae and Microcotylidae) were observed. They are *Choricotyle polynemi, Upenicoloides bengalensis, Microcotylid* sp and *Pricea* sp. Out of four species two species were redescribed and one new species.

INTRODUCTION

Monogenea, commonly known as gill worms, is a class in the Phylum Platyhelminthes. Most species are ectoparasites on the gill filaments of their fish hosts, but some are ectoparasites on fins, body surfaces, in the nostrils, and buccal cavity. Others are endoparasites in the esophagus, cloaca, urinary tract, and the heart (Hendrix, 1994). In heavy infections, they can kill captive fishes and occasionally wild ones (Williams and Bunkley-Williams, 1996). Because many host species have not yet been examined for these helminths, much remains to be done to expand both geographic ranges and host records for Monogenea (Hendrix, 1994). Monogeneans are ectoparasites, the majority of which are located on gills of marine and freshwater fishes. Generally heavy infection of monogenetic parasites is rare. However, the host does suffer intensely and ultimately death results. Heavy infection is of quite frequent occurrence in fish hatcheries, small ponds and aquaria where crowding effect is prevalent.

Systematic studies on monogenea of fishes were initiated in the 18th century by Abildgaard (1794). He reported a *Microcotylic axine*. For quite some time there was lull and no information appeared on this aspect till the beginning of 19th century. During this period information on monogea started erupting from various places like Paris (Blainville, 1828) and USSR (Blanchard, 1847). Sometime in the middle of the century, came out a publication called "Historiae natualis classica" which dealt with classification.

During the 20th century a prominent work in this field was by Yamaguti (1968) who spent almost his life time to study the various parasitic worms and this include monogenetic trematodes also. His compendium "Systema helminthum" is a classic by itself. Subsequently he published a series of papers. Another intensive worker from America was Price (1962). Other prominent workers are Mamaev and Zubchenko (1984), Gerasev (1990) and Rubec (1991). In India work on monogenea was initiated in the 19th century, when Bell started his work in 1891. Several authors (*i.e*. Murugesh and Meenakshi 1995; Chisholm and Whittington, 1998; Suriano and Labriola, 1999; Al-Mathal, 2002; Chisholm *et al*., 2004; Whittington, 2005; Lackenby *et al*., 2007) made a gateway through the subject. The present work is an attempt to describe some monogenetic parasites collected from marine fishes of Visakhapatnam coast.

MATERIALS AND METHODS

The collection of, *Gazza achlamys* and *Ariomma indica* from Visakhapatnam coast have been under taken at regular intervals

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for a period of two years. The monogeneans were removed from the gills, nasal fossae and branchial cavity of host. Proper relaxation of monogenea is important because they have a firm attachment by posthaptor. Monogenean family like *Diclidophorids* is highly extensile and active at anterior region but at the hind end because of firm attachment with sucker like clamps, they were immobile. In such cases, any mechanical pulling or application of pressure would lead to damage of hooks and /or anchors leading to a great deviation from the normal taxonomical characters. Thus, chloretone sea water has been used for small-scale and mass- collecting techniques. Chloretone (Parke-Davis) empirical formula trichloro-tertiary butyl alcohol 2 gm dissolved in 500 ml of filtered sea water served the purpose. In the small-scale collection of monogenea, individual worms or gills bearing worms were immersed in the solution until complete relaxation. They pipetted out in to a suitable fixative preferably FAA. In the mass collection technique, the gills of hosts were excised and placed in a jar containing enough of solution just to cover them. The branchial material was then left in the solution for a period of 15-30 minutes depending upon the size of the parasite. The jar shaken just to favour the detachment of worms, following this sufficient quantity of fixative was added to the jar and the contents stored for latter examination. At the time of examination material was shaken, decanted in to Petri dishes and examined under a dissecting microscope. This technique appeared to work well for smaller Microcotylids as they obviate much time consuming labour and the necessity of flattening that may distort the specimens. For Gastrocotylids, it was desirable to scrap the gills with a small needle in to the relaxing solution to assure prompt collection and then examined under dissecting microscope.

When the specimens were already dead the gills of the hosts were excised and placed in a jar containing filtered sea water for a period of 15-30 minutes. The gills were shaken and allowed to settle for few more minutes. They pipetted together on a slide with a small amount of saline solution and were examined for eggs. The natural shape and size of which was checked and measured in life whenever possible. Material which was not fresh or already decomposing was discarded mostly, except to examine eggs and gross observations.

In order to fix monogenea a special technique was employed. If they fresh enough and obtained in large numbers, all of them were transferred on to a slide with a fine pipette. The excess solution was removed with blotting paper. Then the specimens were covered with a cover-glass, whose surface moistened with FAA. If the cover-glass is too large or the amount of fixative is too small for the material the pressure of the cover-glass alone may damage the parasites. For large or unusually thick material gentle to moderate pressing between the slides was necessary. It was necessary to drop fresh fixative beneath one end of the cover glass and to drain the fluid on the other slide, just to prevent desiccation. The cover-glass is released with care after 30 minutes. The best way was to immerse the slide gently in water and to wait until the cover-glass lifts from the slide. When the cover-glass was released from the slide, the worms usually remain entirely or partly attached to either the cover-glass or the slide; in such cases, flushing the slide with a pipette was helpful. Further fixation of the released worms was essential but smaller worms did not require more than three hours. Thicker specimens needed a longer time (12 hours). Fixed specimens were recovered in to embryo cups washed in water. Alumcaramine, Delafield's haematoxylin or Erlich's haematoxylin eosin are used for staining. Depending on the bulk of the material and dilution of stain 12 to 15 hours of immersion was necessary. After differentiation with acid alcohol and brief washing they are dehydrated in alcoholic grades cleared in creosote and mounted in Canadabalsom or DPX. Fast rate salt B or Catecol techniques were also employed to study the distribution of vitellaria. Specimens fixed in 70% alcohol were used for this. These techniques were effective in bringing out vitellaria in dark brown colour. Staining such preparations with alum caramine was helpful. Permanent mounts were then made after usual procedure of dehydration.

Figures were drawn with the aid of camera lucida. Measurements were made with an ocular micrometer. In case of curved structures especially for anchors and cirrus, measurements have been made across the lines i.e., from the proximal tip of the longest root to the most distal point of the curve in the descriptions of species, measurements of minimum and maximum were given. All measurements were in millimeters, unless otherwise mentioned.

RESULTS

G*azza achlamys* (Jordan & Starks, 1917) *and Ariomma indica* (Day, 1871) appears to be a good host for monogenean parasites. During the present study, altogether four monogenean species were identified. They are *Choricotyle polynemi, Upenicoloides bengalensis, Microcotylid* sp and *Pricea* sp. Detailed description of each monogenean parasite is given bellow. All measurements were in millimeters, unless otherwise mentioned.

Choricotyle polynemi

Choricotyle polynemi was observed in gills of *Ariomma indica.* These are rare and among several fishes examined only few of them were infected. Body thick reaches 2.1-2.3 in length demarcated into anterior proper body with genital organs and the posterior haptor (Fig. 1). In between there is a short connecting isthmus. The haptor 0.64-0.8 occupies one third of the body. Haptor bears four pairs of pedunculated clamps and incipient lappet at the posterior end of the body without any hooks. Anterior peduncles are short when compared to the last peduncles resulting in the final bifurcation of the body. The peduncle measures 0.18-0.21, clamps are of the open type and symmetrical 0.14-0.16 x 0.15-0.17 in diameter. Clamp sclerites are thick and sharp, clearly visible though inserted in the thick musculature forming the capsule. Each clamp has two median springs lying one above other. The basal median spring is long and conspicuous with asymmetrical arms. Second median spring short almost in the form of a simple rod without lateral arms. It stands on the top of the first median spring. There are three pairs of lateral sclerites, the first pair of sclerites is arcuate or 'C' shaped starting from the base of the clamp, bent inwards and touching the lateral arms of the first median spring. Second or the median sclerites articulate at their bases with a small protruberance given out by the first sclerite while curving inwards. Third lateral sclerite is close to the second

median spring and appears as if it is the lateral arm of the median spring. A pair of thick muscular pads at the base of the capsule indicates the sucker. The distal half of the capsule is occupied by the chitinous rods. Prohaptor is glandular with terminal mouth. Oral suckers with thick lips 0.43-0.043 x 0.035-0.043. Pharynx oval, oesophagus short with no rami. Caeca with extensive rami externally and less toward median line. They unite at the region of isthmus and the common branch gives off rami to each of the peduncle of the clamp.

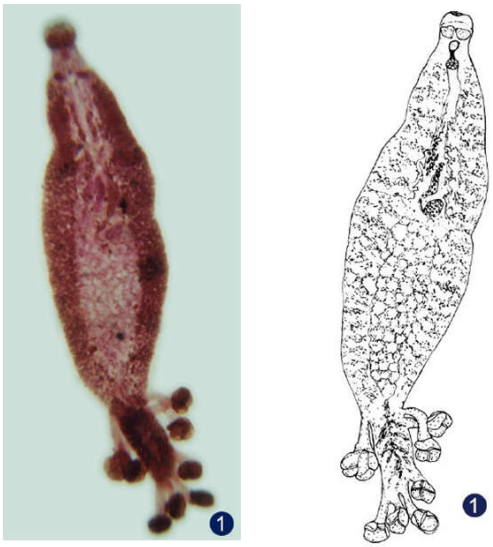

Figure 1 *Choricotyle polynemi*

Testes post-ovarian starting from the middle of the proper body. The follicles are big, spherical or sub-spherical 0.078-0.08 x 0.078-0.08 about 10-12, intercaecal extending up to isthmus and not entering into it. They are arranged in two irregular serial rows. Vas deferens narrow and opening into genital atrium, ovarian and double looped, curving over a large seminal receptacle, uterus not distinct. Genital atrium highly muscular post bifurcal 0.15-0.2 from anterior end measuring 0.03-0.4 x 0.02-0.03 with a corcnet of eight hooks. Vitellaria small, follicular co-existing with caecal branchings. Eggs fusiform, oval 0.16 x 0.043 with bipolar filaments.

Upenicoloides bengalensis

Upenicoloides bengalensis was observed in the gills of *Ariomma indica.* Body elongate, dorso-ventrally flattened with anterior end pointed and posterior end with a deep notch (Fig. 2). Total length 1.76-2.54 with maximum width 0.4-0.6 at the region of testes. Tegument thin and smooth. Posthaptor is not demarcated from the body, but continuation of it, constituting posterior one fourth of the body. It extends a length of 0.24-0.48 with maximum width 0.3-0.48 at its base. Haptor has prominent notch at its posterior end and three shallow lateral notches on each side, between the four lateral clamps. Each clamp borne on short and stumpy peduncle. The middle two clamps are bigger than the proximal and distal clamps. The average clamp size being 0.05-0.013 x 0.06-0.11. Last clamp having bigger stalk than the first three, with a length of 0.05-0.07, clamp asymmetrical and devoid of oblique striae. Lip of the capsule narrow and thin, of the two pairs of lateral sclerites outer one is long, coming beyond the mid region and stops by the lateral side. So, the articulating sclerite on the other side is short, median spring broad at its distal end dividing the clamp capsule in to two unequal parts. Cuticularization is heavy, and an incipient sucker typical of diclidophorids is present.

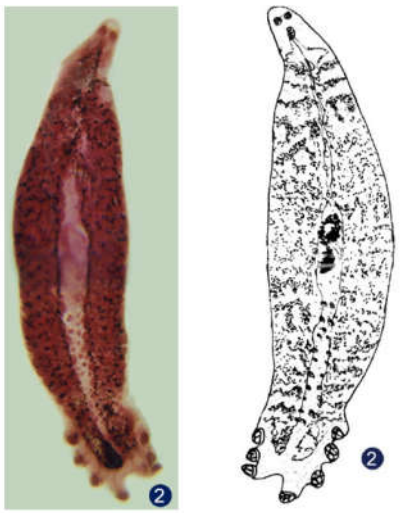

Figure 2 *Upenicoloides bengalensis*

Prohaptor is narrow, anterior to oral suckers with scattered cephalic glands. Mouth wide and crescentic. Oral suckers spherical, highly muscular with thick lips lying close together in the buccal region. It measures 0.047-0.054 x 0.043-0.057. Pharynx median oval 0.071 x 0.05-0.058 with anterior triangular knob looking like prepharynx. Thick bands of muscular striations arise from oral suckers and run towards posterior side. Oesophagus short with slight ramifications deviding into two caeca just anterior to the genital atrium. Caeca thick with rami extensive externally but less towards median line. Caeca while entering the haptor do not divide repeatedly but end as lobular thick structures extending up to the posterior end of the haptor and loaded with clumps of haematin.

Testes 12-15 post-ovarian at distance of 1.0-1.58 from anterior end. The follicles are big and spherical arranged in two regular alternating rows in the intercaecal space without entering the haptor. Vas deferens thin and narrow originates from the anterior region of testes, passing beneath the ovary and opening in to the male genital pore. It is surrounded by highly muscular genital atrium at the place of oesophageal bifurcation. The genital atrium spherical 0.03-0.046 x 0.03-0.05 with six hooks. These hooks sharp and bent towards the centre of the opening. Ovary pre-testicular, distinguishable into bulky distal part and a long tube-like proximal part, curving round the median seminal receptacle. Ova of different sizes, with the distal end of large oocytes. Oviduct thin and narrow originates from the posterior corner of the distal ovary, runs posterior wards parallel to the male genital duct and opens into a spherical ootype. Uterus sinuos and open into genital atrium. Eggs oval and bipolar. Vitellaria in the form of big follicles in the lateral fields from the level of intestinal bifurcation to the distal end of caeca. Transverse and median vitalline ducts in the form of 'Y' ventral to ovary. Vegina absent.

Pricea sp

This monogenean was collected from the gills of *Gazza achlamys.* Body cylindrical, opisthohaptor symmetrical, clamps numerous, pedunculated, in two marginal rows (Fig. 3). Length

of the body 3.3-5.7 x 0.7-1.2 prohaptor with a pair of oral suckers 0.08-0.15x 0.05-0.09, septate and conspicuous gland duct opening into it. Pharynx 0.05-0.07 x 0.04-0.058 spherical and high glandular. Oesophagus short without ramifications and divides into two ceaca with extensive diverticulae both median and lateral, without extending into the haptor.

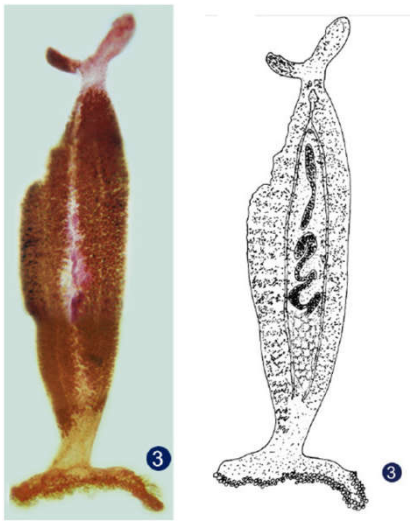

Figure 3 *Pricea* sp

Posthaptor lies at right angles to the body with a stalk-like peduncle. The haptoral row 1.37-3.2 in length with 2-3 rows of small pedunculated gastrocotylid clamps 52-60 pairs with 5 to 7 ribs and a pair of anchors on the terminal lappet. Each clamp measures 0.04-0.058 x 0.058-0.089.

Testes 20-30 post ovarian arranged into irregular rows occupying the intercaecal area not entering into the haptor. Cirrus armed with 12-14 spines. Ovary pretesticular, uterus median and opening into unarmed genital atrium. Vagina is in the form of a cup shaped sucker at a distance of 0.6-0.9 from anterior end. The rim of the cup is thick with sclerotized lines. At its posterior end are two pairs of vaginal hooks of which middle pair of hooks longer 0.04-0.054 with their inner ends serrated and expanded. The two larger hooks run parallel for half of their distance, and then they are set apart facing with their inner serrated side. The outer most hooks are small 0.02-0.023 having the same structure, but it appears that these two are united distally by musculature appearing as a collar for the longer hooks.

Microcotyle n. sp

Observed in gills of *Gazza achlamys*. Numerous specimens obtained from nearly 500 hosts examined. Measurements based on six specimens. Body elongate, fusiform, the anterior part in front of genital atrium narrowed considerably appearing distinct from the rest of the body, which is expanded reaching 2.3-3.6 long x 0.7-1.0 wide at the testicular region (Fig. 4). Prohaptor rounded anteriorly with a pair of spherical anterior suckers 0.03 x 0.039 in diameter, postero-lateral in position and armed by conical tooth like structures on its rims. Cephalic glands occur in clusters lateral to pharynx but less anterior to the buccal suckers. Posterior is small, narrow and elongated cotylophore in the form of 'V' 0.4-0.7 long x 0.3-0.5 wide armed with 13-20 clamps in two nearly equal ventro-lateral rows. Clamps similar in structure but show slight variation in size, middle ones largest, anterior and posterior smaller, average clamp size being 0.03-0.04 x 0.04-0.06.

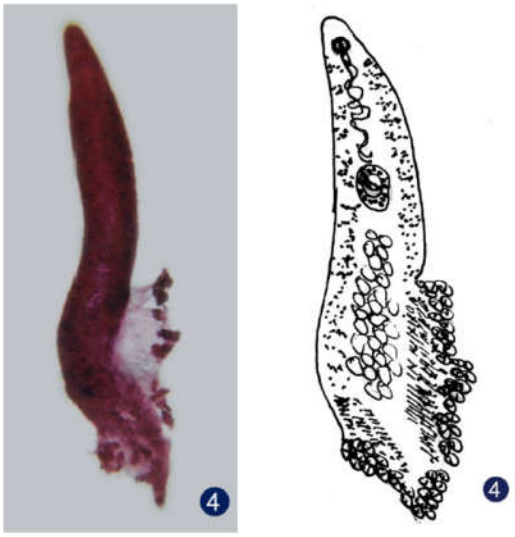

Figure 4 *Microcotyle* n. sp

Clamps are typically microcotylid type with the dorsal, ventral and basal sclerites enclosing the median piece, and it has two short widely divergent lips constituting the clamp capsule which is thickened with fibrous tissue. In young specimens posthaptor contains in addition to 13 clamps, two pairs of anchors situated at the posterior end. These anchors may be lost in the larger specimens. Mouth sub-terminal, ventral, and almost circular. Pre-oral glands conspicuous, pharynx big, ovoid 0.04-0.058 x 0.03-0.05 with a double row of fine teeth in the middle. Oesophagus wide, lacking diverticula and bifurcating in to caecae.

Testes follicular, rounded, 20-40 in number are massed together in the intercaecal field posterior to ovarian complex and entering in to haptor. Vas deference extending anteriorly dorsal to uterus enlarged and more conspicuously coiled before entering the genital atrium. Cirrus absent. The genital atrium at a distance of 0.14-0.18 from anterior end, approximately rectangular with the corners rounded and the posterior margin concave at the middle measuring 0.14-0.17 x 0.14-0.19. the inner diameter is 0.105-0.117 x 0.105-0.47 encircled by a thick layer of radial muscle fibers and covered inside with numerous 'Y' shaped spines which are up to 150-200. These spines are 0.012 long but markedly reduced on the ventral surface covering the pre-atrial bulb.

Ovary is irregularly looped lies just anterior to the middle of the body with the distal end on the right side of the median line. The ducts genitor-intestinalis and Mehli's gland complex are clear. The 'Y' shaped vitelline reservoir lies ventral to the ovary. Vaginal pore simple, unarmed, posterior to genital atrium at distance of 0.56-0.57 from anterior end. The vitelline follicles surrounding the caeca extend posteriorward beyond the middle of the cotylophore. The large, spindle shaped eggs measure 0.23 x 0.12 have a slender pointed process about 0.117 long at one pole and along filament at the other.

DISCUSSION

Choricotyle polynemi is conspecific with the type described by Mamave, (1972) excepting few details of minor importance like the length ratio of haptor to the total body. In the present specimen from Visakhapatnam, length of haptor is only 1/3 of the total body. Usually there are ten testicular follicles arranged in two irregular rows. It is new host recorded.

Unnithan (1966) erected a genus *Upenicola* with contrasting characters *i.e* the clamps are sessile and the number of testes are limited to only eleven, the name of the genus was derived from the host. It was also found that the incipent suckers of the Diclidophorid type of clamps are not seen in *Upenicoloides bengalensis.* Clamps show more asymmetry due to the unequal lengths of the lateral sclerites consisting the clamp. This genus was monotypic consisting of only one from *U. upenoides*. Mamaev (1972) redescribed the above from with short peduncles and different number of testes. Mamaev included a new form into the genus *Upenicola* as *U. capheni* resembling the new species in all significances, but he was not furnished all the structural aspects. It is now considered necessary to erect a new genus *Upenicoloides* with combines the characters of both the genera *Diclidophora* and *Upenicola*. The type specimen *Upenicoloides bengalensis* having clamps with short peduncles, testes 12-15 not intruding into haptor, genital hooks are six. The present specimen recovered from *Ariomma indica*, closely resembles with *Upenicoloides bengalensis*. So, it is a new host recorded.

The Pricea sp collected form the hosts (*Gazza achlamys)* are similar with some characters to *P. multae.* Chauhan (1945) reiterating the facts given by Hargis (1959) and Lebedev (1971). They considered that all the 6 species *Pricea tetracanthum*, *P. triacantha*, *P. armata*, *P. minuta*, *P. robusta* and *P. melanae*, erected by Ramlingam (1952) are only infraspecific variations based on the characters of immature forms and show they nearly synonimised all the six species of *Pricea* to *P. multae* of Chauhan (1945). Body hooks and the second pair of anchors described as specific characters in *P. tetracantha* are found in immature forms only. So, it is desirable to synonymise all his species to *P. multae*. The number of testes and the number of clamps gradually increase with the maturity of the form and the specimens collected from *G. achlamys*. So, it is a new host recorded.

The genus *Microcotyle* is large one containing a variety of species. The genus was erected by Beneden and Hesse (1863) with *M. donavini* as type collected from *Labrus donavini*. Yamaguti (1963) listed 68 species from all over the world both from fresh and marine waters. Unnithan (1971) mentioned 90 species with all the recent inclusions. But to avoid complications most of the species in this genus have been pulled into new genera erected by him. Mainly speciation is based on the shape of the clamps, size of pharynx relative to oral sucker, presence or absence of diverticula in the oesophagus, entry of crura into haptoral zone and their unequal development terminalis, shape and number of species associated with male terminalia, and number and extent of testes. However, some of the species are so inadequately described that to put them into any of the new genera erected recently is a difficult task.

Three microcotylid forms have been described from India by Tripathi (1959) as *M. leiognathi*, *M. madrasi* and *M. pamae* from Chilka Lake and Hoogly River. This new form does not resemble any of the Indian species described so far, instead it resembles the Japanese from *M. cepolae*, Yamaguti, 1937 and *M. donavini* Beneden and Hesse, 1863.

Dillon and Hargis (1965) removed five species of *Microcotyle* has presented by Yamaguti (1963), *M. gonostomi* (Sandars, 1945); *M. draconis* (Briot, 1904); *M. pagrosomi* (Murray, 1931); *M. brevis*, *M. nemadactylus*, *M. neozealancus* and *M. debueni*. *M. Polymixiae*, (Parukhin and Roitman 1970); *M. hemia triospinalis* from Seriola species (lopez-Roman and Guevaru Pozo 1973).

The new form differs from these considerably in the nature of the haptore, genital atrium and the clamp. So, it is felt necessary to erect a new species to accommodate the present form from the host *Gazza achlamys*.

CONCLUSION

G*azza achlamys* (Jordan & Starks, 1917) *and Ariomma indica* (Day, 1871) appears to be a good host for monogenean parasites. In the present study on *Gazza achlamys and Ariomma indica* four species of monogeneans, from the family Gastrocotylidae, Microcotylidae and Diclidophoridae have been obtained. Out of four species two species were redescribed and one new species. These monogenean parasites inhabit the gills, skin, nasal fossae and branchial cavity of the host. During the present study, the monogenean parasites were observed in the gill of *Gazza achlamys* and *Ariomma indica*

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