

**Hydrolysis of Thiazolidones with Ethanolic HCl**—A mixture of 2-*o*-tolylimino-3-phenyl-4-thiazolidone (2.0 g.), ethanol (15 ml.) and concentrated HCl (6 ml.) was refluxed on a water-bath for 5 to 6 hours. After distilling off ethanol, the reaction mixture was poured into cold water and then filtered. The residue was washed with water, dried, and crystallised from ethanol, m.p. 143° C.

(Found: N, 7.20; S, 16.53.  $C_9H_7NO_2S$  requires N, 7.25; S, 16.58%.)

The above data showed that the compound was 3-phenyl-2:4-thiazolidindione as the melting point remained undepressed on admixture with 3-phenyl-2:4-thiazolidindione.

The presence of *o*-toluidine in the filtrate has been confirmed by preparation of its hydrochloride and azo- $\beta$ -naphthol derivative. Likewise the position of aryl groups in various thiazolidones have been confirmed.

**2-Arylimino-3-Phenyl-5-*p*-Tolueneazo-4-Thiazolidones.**—A solution of 2-*o*-tolylimino-3-phenyl-4-thiazolidone (0.1 mole) in glacial acetic acid was slowly added at 0° C. to a solution of *p*-toluenediazonium chloride (0.1 mole) with stirring. The mixture was kept for an hour at 0–5° and the product was crystallised, after washing, from ethanol.

Similarly 5-*p*-tolueneazo derivatives of other thiazolidones were prepared as listed in Table I. Satisfactory nitrogen analyses were obtained for these compounds.

**2-Arylimino-3-Phenyl-4-Thiazolidone Sulphones.**—2-*o*-Tolylimino-3-phenyl-4-thiazolidone (0.003 mole) dissolved in glacial acetic acid (10 ml.) was treated slowly with  $KMnO_4$  (0.003 mole) dissolved in water (40 ml.) at 0° C. After completion of the reaction, excess of  $KMnO_4$  was removed by treatment with sodium bisulphite and the sulphone was filtered, washed, dried and then crystallised from alcohol.

Similarly other 2-arylimino-3-phenyl-4-thiazolidone sulphones were prepared. Their melting points are recorded in Table I. Satisfactory sulphur analyses were obtained for these compounds.

**Fungicidal Activity Screening.**—The method employed consists of testing the fungicide at varying grades of concentration with the growth of a test fungus, *Helminthosporium ephorbiae*, parasitic on *Euphorbia geniculata*.

The experiment was performed in petri dishes in which 20 ml. of sterilized Czapek agar medium was added. The fungicide was added to the medium after autoclaving. For

each concentration of fungicide three plates were prepared and the test fungus was inoculated in the centre. Plates were incubated at 25° C.  $\pm$  2 for a week. Two controls, one containing the above quantity of the medium only and another containing the same quantity of the medium and 0.5 ml. of acetone but without the fungicide, were also prepared to compare the observation. The diameter of colonies were recorded after the lapse of a week and is presented in Table I.

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1. Surrey, A. R., *J. Am. Chem. Soc.*, 1949, **71**, 3105 and 3354.
2. — and Cutler, R. A., *Ibid.*, 1954, **76**, 578.
3. Troutman, H. D. and Long, L. M., *Ibid.*, 1948, **70**, 3436.
4. Das, N. K., Mohapatra, G. N. and Rout, M. K., *Ibid.*, 1955, **77**, 2427; *J. Sci. & Ind. Res.*, 1955, **14 B**, 448.
5. Bhargava, P. N., Miss Bhargava, K. and Kapoor, R. C., *J. Indian Chem. Soc.*, 1961, **38**, 23.
6. Dains, F. B., Irvin, K. and Harrel, C. G., *J. Am. Chem. Soc.*, 1921, **43**, 613.
7. Fry, H. S., *Ibid.*, 1913, **35**, 1539.

**CERCARIA MELANOCRUCIFERA, A  
NEW MAGNACERCOUS CERCARIA  
(OPISTHORCHIOIDEA) FROM THE  
MARINE GASTROPOD, TURRITELLA  
ATTENUATA REEVE, 1897, FROM THE  
BAY OF BENGAL, MADRAS**

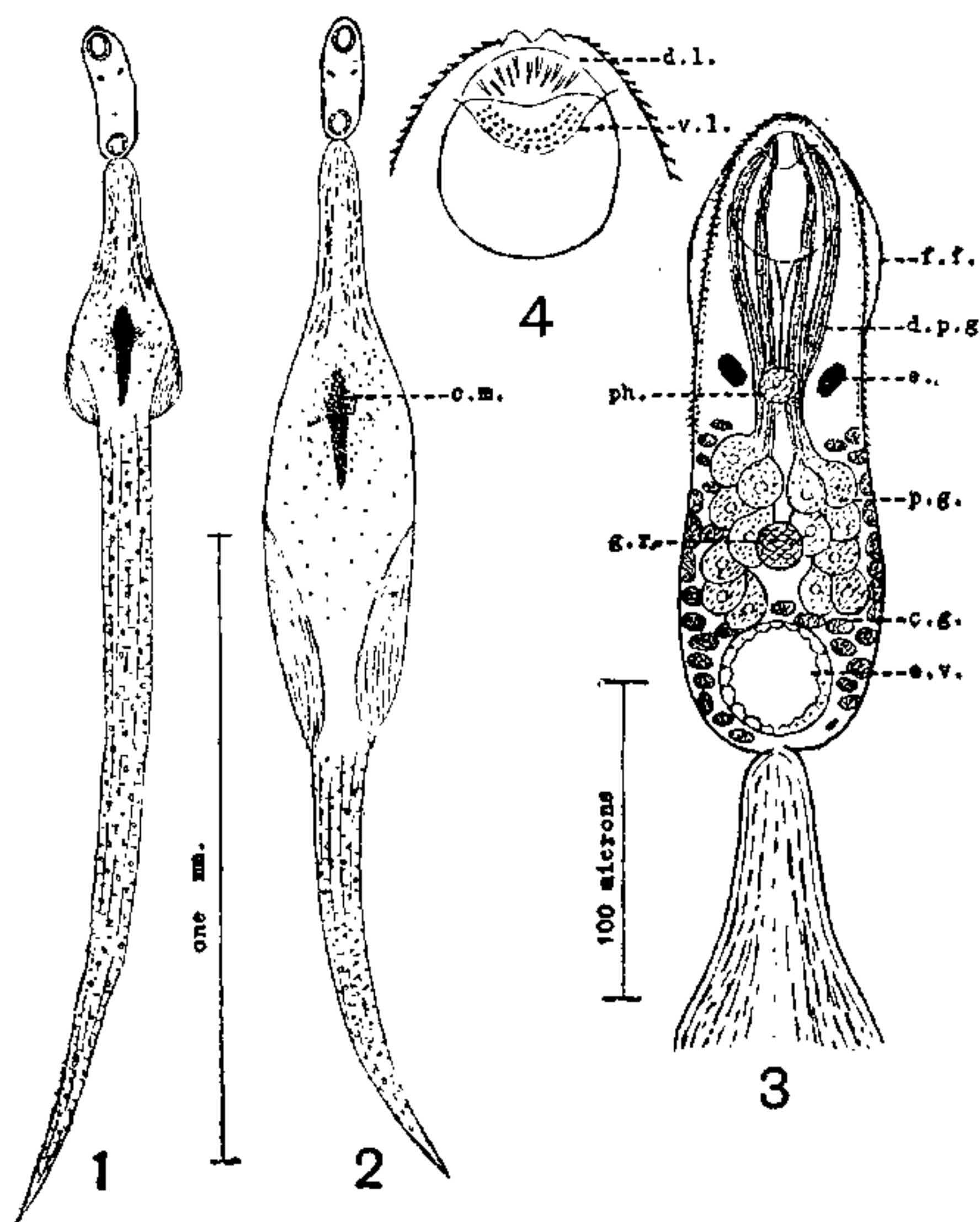
*Magnacercous cercariae* possessing enormously enlarged and distinctively pigmented tails, devoid of a finfold encountered in other opisthorchioid cercariae, have been reported only by a few workers. Four species of such cercariae, *C. caribbea* XVI-XIX were obtained from *Cerithium algicola*, *C. variabile*, and *Territella exoleata*, in Puerto Rico.<sup>1</sup> All cercariae of the Opisthorchioidea described up to 1960 were grouped together in a key,<sup>2</sup> which included *C. caribbea* XIV-XIX from Puerto Rico, and *C. purpuracaudata* Miller 1925, and *C. equitator* Sinitzin, 1911, from the Black Sea and Washington respectively. In the world list of marine cercariae,<sup>3</sup> thirteen species were mentioned as belonging to the family Heterophyidae Odhner 1914, and three of unknown affinity. Of the former group, seven species were

reported to have been assigned to the family by this writer himself (independent of the list by Dunagan<sup>2</sup>), and six others, *C. caribbea* XVI-XIX from Puerto Rico, *Cercaria komiya* and *Cercaria nigrocaudata* from brackish water hosts in Tokyo Bay.<sup>4</sup> *Cercaria caribbea* LXXI from *Cerithium variable* in Jamaica was added to the known fauna by Cable.<sup>5</sup> No cercaria of this category or description has been reported from India yet.

This account of a magnacercous cercaria is based on a study of a large number of specimens obtained from the hepatopancreas of one of two hundred individuals of *Turritella attenuata* dredged from the shore in Madras and dissected for the purpose.

**Specific diagnosis.**—Magnacercous cercaria, of the Opisthorchioid Group, with proximal region of tail inflated a little behind the base and not abruptly enlarged; distal region slender and tapering to a point. The enlarged portion showed a variable shape, the commoner one as a short pear-shaped structure (Fig. 1) and the other longer and spindle-shaped (Fig. 2). Body 0.170-0.200 mm. long, widening behind the middle, and 0.060-0.080 mm. in maximum width. Tail 0.900-1.470 mm. long and 0.210 mm. in maximum width, with reddish-brown longitudinal pigmented streaks at the base, and a black cruciform marking in the dilated and vacuolated region, discernible more clearly in live specimens. The postero-lateral corners of the enlargement are equipped with muscle fibres in hemispherical patches. The small posterior part of the tail has a width of 0.051-0.110 mm. Oral sucker 0.022-0.032 mm. in diameter, eye spots dark and rectangular. Body spination (Fig. 3) confined to anterior half. A delicate narrow finfold present on each side in the anterior third of the body. Subterminal mouth; dorsal lip (Fig. 4) of oral sucker with two alternating rows of spines, and ventral lip with three rows of short spines in semi-circular arrangement; prepharynx narrow and straight, ending in globular embryonic pharynx, 0.007-0.0095 × 0.008-0.0115 mm.; rest of the digestive system not evident. Ventral sucker undeveloped. Penetration glands, seven on each side, posterior to eye spots; ducts in two bundles on each side, three in the outer and four in the inner, opening anterior to the mouth in crypts. Cystogenous glands, numerous filling the parenchyma behind the eye spots. Genital rudiment a mass of cells midway between pharynx and posterior end of the body. Excretory vesicle spherical 0.027-

0.034 mm. in diameter; ducts and flame cells not observed.



FIGS. 1-4. *Cercaria melanocrucifera* n.sp. Fig. 1. Entire, with pear-shaped inflation of tail. Fig. 2. Entire, fusiform inflation of tail. Fig. 3. Head, to show organization, especially fin-fold on body, penetration glands, cystogenous glands, spherical excretory vesicle, and spination. Fig. 4. Oral sucker, with dorsal and ventral lips bearing spines. *c.g.*, cystogenous glands; *c.m.*, cruciform marking; *d.l.*, dorsal lip; *d.p.g.*, ducts of penetration glands; *e.*, eye; *e.v.*, excretory vesicle; *f.f.*, fin-fold; *g.r.*, genital rudiment; *p.g.*, penetration glands; *ph.*, pharynx; *v.l.*, ventral lip.

**Redia**, simple, with reddish-brown horizontal patches in the body, 0.620-1.180 mm. long and 0.125-0.190 mm. broad, sometimes swollen irregularly.

**Discussion.**—Species of *Turritella* have been known to harbour larval trematodes belonging to the Rhodometopa<sup>6,7</sup> and the Opisthorchioid<sup>1</sup> Groups. On a comparison with all the known magnacercous cercariae, the present one is found to resemble *C. caribbea* XVII Cable, 1956, to the greatest degree, but is distinctive in the presence of finfolds on the body and a black cruciform marking in the dilated region of the tail. It is accordingly designated as *Cercaria melanocrucifera* n.sp.

On the basis that "the excretory vesicle of the Galactosominae is sac-shaped or tubular, rounded anteriorly, without a suggestion of a bifurcation and, hence, more likely to be derived from the spherical vesicle possessed by the four species of magnacercous larvae than

from the vesicles of other types of opisthorchioid cercariæ", these larvæ were assigned to the sub-family Galactosominæ, Heterophyidæ. This relationship with the Heterophyidæ has been adopted in the later studies of Cable and other workers.<sup>3,5,8,9</sup> Encysted metacercariæ of *Galactosomum spinetum* were obtained<sup>9</sup> from the visceral adipose tissue of the fish *Hyporhamphus unifasciatus* in Florida, following exposure to magnacercous cercariæ. From these morphological and experimental evidences, it has been suggested<sup>5</sup> that "adults of magnacercous larvæ probably are heterophyids which belong to the genus *Galactosomum* and are common parasites of shore birds". It is of interest that four species of *Galactosomum*, three of them as adults in the Sea Gull, *Larus argentatus*, and one as a juvenile in the crab, *Matuta victor*, have been described from the Madras Coast.<sup>10,11</sup> From an ecological standpoint, the cercaria now described may be expected to be the larva of any of the above-mentioned species or a related one. The life-history of *Galactosomum* may, therefore, be understood to include a magnacercous cercaria from the gastropod encysting in fish and reaching maturity in ichthyophagous birds, occasionally utilizing a crab or other crustaceans as accessory or paratenic hosts.<sup>10</sup> Since the evidence for the identity of the magnacercous cercariæ with the Heterophyidæ is nevertheless slender, experimental studies are needed for decisive taxonomic inferences.

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April 11, 1968.

1. Cable, R. M., *N.Y. Acad. Sci.*, 1956, **16**, 490.
2. Dunagan, T. T., *Proc. Helm. Soc. Wash.*, 1960, **27**, 44.
3. Holliman, R. B., *Tulane Studies Zool.*, 1961, **9**, 74.
4. Ito, J., *Jap. J. Med. Sci. and Biol.*, 1956, **9**, 235.
5. Cable, R. M., *Zeitschr. f. Parasit.*, 1963, **23**, 429.
6. Rothschild, M., *Parasitology*, 1935, **27**, 152.
7. Hutton, R. F., *J. Mar. Biol. Assoc.*, 1955, **34**, 249.
8. La Rue, C. R., *Exper. Parasit.*, 1957, **6**, 306.

9. Sogandares-Bernal, F. and Hutton, R. F., *Proc. Helm. Soc. Wash.*, 1960, **27**, 75.
10. Anantaraman, S., *Ph.D. Thesis* (Zool.), University of Madras, 1961.
11. —, *J. Mar. Biol. Assoc. India*, 1963, **5**, 1.

#### A STUDY OF GROWTH RINGS IN OTOLITHS OF FISH BY MICRORADIOGRAPHY

OTOLITHS of fishes are used for the determination of their age by many conventional methods (Trout, 1958). Ehrenberg and White (1957), described microradiography and its uses in industrial radiography. An attempt is made here to study the growth rings in otoliths of fishes by microradiographic technique. This technique avoids the cumbersome procedures of grinding and polishing the otoliths for good resolution of rings. Otoliths of *Pomadasys hasta* (Bleeker), which forms a good trawl fishery along the Bombay coast, were taken for investigation.

Otoliths were washed in water and dried. The mounting of the sample was done in the dark room. They were placed at the centre of the lead frame over a black paper. A double-coated film "F" type, folded in a black paper was kept over it. Another black paper was placed on it so that the film remained in position over the specimen. This arrangement was made tight by keeping a lead sheet measuring 4" × 4" × ½" over it with an adjustable screw. The mounted specimen was placed on a stand 13 cm. away from the X-ray tube. The specimen was exposed to X-rays of 18 kV with 10 mA. current for 10 minutes. (35 kV X-rays with 60 mA. for 6 seconds gave result with less contrast and kV higher than 35 did not give satisfactory results). After the exposure, the film was removed to the dark room and developed. The negative was enlarged to the desired size.

The differential concentrations of the materials in the otoliths make a pattern of image on the radiographic plate according to the concentration in a particular area. When exposed to X-rays, there will be more absorption of X-rays in the opaque areas resulting in less incident radiation on the film and less darkening of the radiographic plate. In the hyaline areas (less dense) as there will be less absorption of the incident X-rays, more X-rays fall on the film making it more dark. As the plate is the positive of the radiograph, the hyaline areas are seen white and the opaque areas dark (Fig. 1, A). A photograph (Fig. 1 B) of the