STUDY OF THE BODY SURFACE OF *HAPLORCHIS YOKOGAWAI* (KATSUTA, 1932) AND *H. TAICHUI* (NISHIGORI, 1924) (TREMATODA : HETEROPHYIDAE)

Tomáš Scholz[1], Oleg Ditrich[1], Milan Tuma[2] and Michal Giboda[1]

[1]Institute of Parasitology; [2]Laboratory of Electron Microscopy, Czechoslovak Academy of Sciences, České Budějovice, Czechoslovakia.

Abstract. The morphology of the surface of two heterophyid flukes, *Haplorchis yokogawai* and *H. taichui,* that can infect humans eating raw fish flesh, has been studied using scanning and transmission electron microscopy. The body surface of both species is covered with scale-like, serrated spines with the exception of areas around the oral sucker, ventrogenital opening and excretory pore. The species slightly differ by the dentation pattern of tegumental spines: *H. yokogawai* has finer and more numerous spine teeth (maximum tooth number 14-16) while *H. taichui* has more serrated spines, mostly with a lower number (up to 10-12) of somewhat more robust teeth. However, because of the variability, the shape and dentation of spines do not seem to be reliable diagnostic features enabling mutual differentiation of both species.

INTRODUCTION

People eating raw or half-raw fish can be infected by heterophyid flukes, common parasites of birds and mammals. Of the genus *Haplorchis* (Looss, 1899), *H. pumilio* (Looss, 1896), *H. taichui* (Nishigori, 1924), and *H. yokogawai* (Katsuta, 1932), have been found to infect man, particularly in South East Asia (see Velasquez, 1982 for review). The morphology of all the above mentioned species visible at the level of light microscope was thoroughly studied by Pearson (1964). However, surface morphology of these trematodes has hitherto been studied by scanning electron microscopy (SEM) only by Fujino *et al* (1989) and Srisawangwonk *et al* (1989). The former authors studied surface structures of *H. pumilio* and *H. taichui* adults, while the latter described in detail the tegumental morphology of *H. pumilio.* No data are available for *H. yokogawai,* a common parasite of wild animals in Africa and Asia (Pearson, 1964). Moreover, the paper of Fujino *et al* (1989) contains only two photomicrographs of *H. taichui* surface structures. In the present study, the surface morphology of *H. yokogawai* and *H. taichui* has been examined using SEM and transmission electron microscopic (TEM) techniques.

MATERIALS AND METHODS

Most *H. yokogawai* and *H. taichui* adults were obtained from naturally infected domestic cats. Cats were caught in the vicinity on Nam Ngum water reservoir situated 80 km north of the Laotian capital Vientiane (Ditrich *et al,* 1990). Several *H. taichui* specimens were found in the stool of a man treated with praziquantel. Some trematodes were identified by light microscope and mounted as permanent preparations. Flukes for SEM study were washed in phosphate buffer, pH 7.2, fixed in 4% paraformaldehyde, dehydrated in alcohol series and acetone, dried in CO_2 by the critical point method, gold-coated and examined in Tesla BS 300 electron microscope. Specimens for TEM study were fixed with 2.5% glutaraldehyde washed after 2 hours in cacodylate buffer with 6.8% saccharose and postfixed with 2% OsO_4. After dehydration in acetone, material was embedded in Durcupan. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in Philips EM 420 microscope.

RESULTS

The ventral body surface is covered with nu-

merous scale-like spines with the exception of small zones around the oral sucker and ventrogenital opening, and the posteriormost region surrounding the excretory pore (Figs 1-2, 4, 8-9, 12-13, 17). The density of spines, their size and shape change in relation to body region. On the whole, the spatial arrangement of spination and type of spines are rather similar in both species (Figs 1, 12). Very fine differences between *H. yokogawai* and *H. taichui* in the dentation of the distal (posterior) margin of these spines were recorded.

Haplorchis yokogawai (Figs 1-7) is characterized by the presence of slightly palmate, mostly serrated spines about 2-3 μm wide, with rather fine, short teeth. Spines lying immediately behind the oral sucker are provided with 5-9 teeth (Fig 2), while spines situated more posteriorly bear mostly 12-14, sometimes up to 16 fine teeth (Figs 3 and 5). In the middle body region, spines are somewhat narrower and armed with only 9-11 teeth (Fig 6). The posterior third of the body is covered with small, a 5- or 6-toothed spines (Fig 7).

The body surface of *Haplorchis taichui* (Figs 8-19) is provided with serrated spines, with maximum width up to 3 μm. The distal margins of the spines are divided into teeth, mostly somewhat stronger (finger-like and less numerous than in *H. yokogawai.* Spines from the anteriormost body region have 5-7 teeth but then they become slightly larger and number of teeth increases, reaching up to 9-11 (exceptionally up to 12-Figs 10, 15 and 18). Behind the ventrogenital complex, the body is covered with 7- or 8-toothed spines (Fig 16). More posteriorly, the spines become finer (more tenuous) and also the number of teeth decreases (Fig 19).

The surface of a *H. taichui* specimen from cat with a partly destroyed tegument is depicted in Figs 8-11. Tegumental spines are completely visible (Figs 9 and 10) because their proximal part, normally submerged in the tegument (Fig 18) is quite denuded. TEM electrongraphs of the body surface of *H. taichui* (Fig 11) show the position of the spines in the tegument. Their base is situated closely on parabasal membrane (Figs 11 C, D). The proximal part of the spines is submerged into the tegument (Fig 11 A) and connected with the plasma membrane (Figs 11 C, D). Fig 11 B shows partly damaged tegument and the widened basal part of the spines in transverse section.

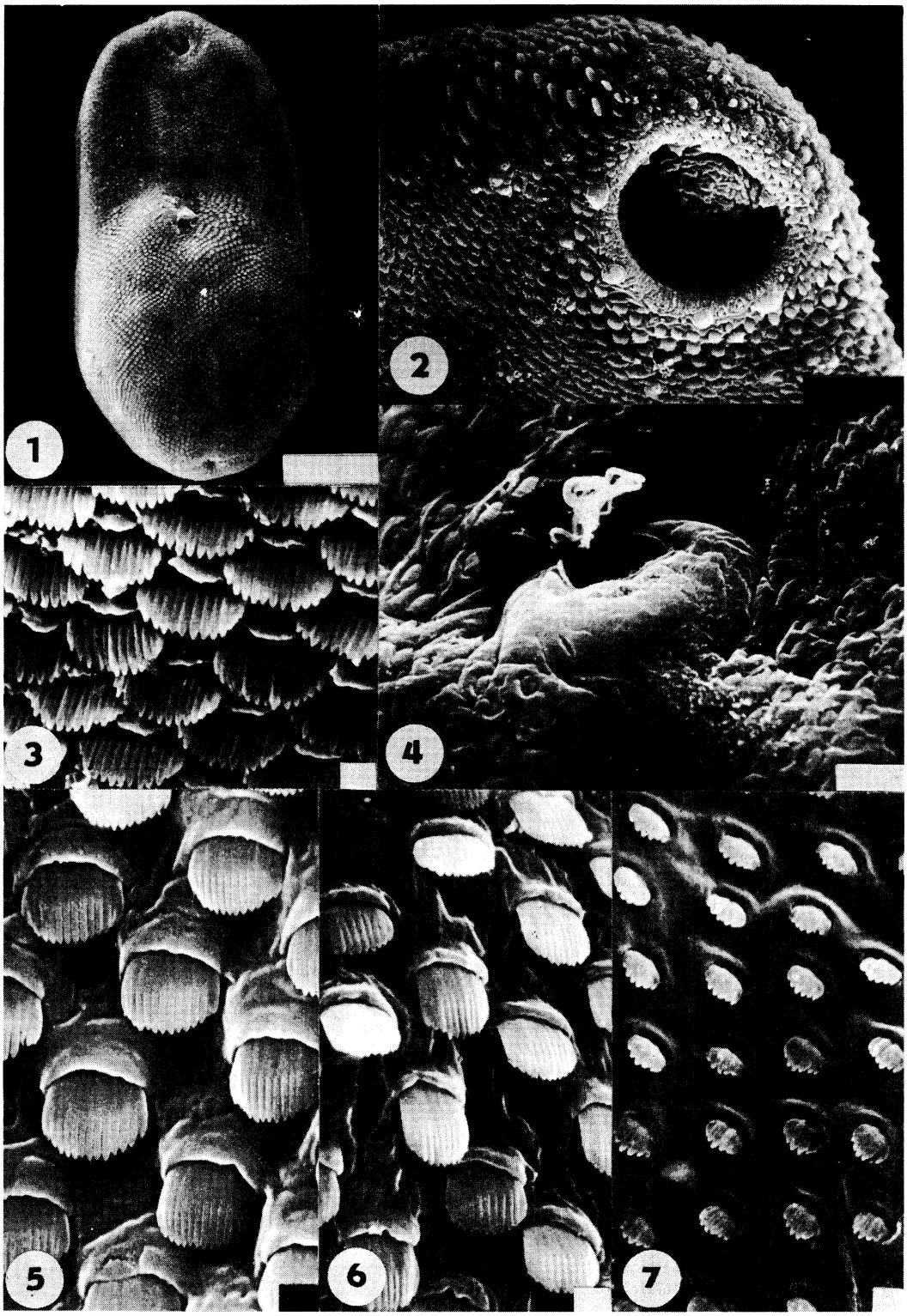
Sclerites of the ventrogenital complex (Pearson, 1964; Pearson and Ow-Yang, 1982) are not visible in normal, undamaged specimens (Figs 4 and 17). However, tegument of *H. taichui* specimens released from the definitive host (man) after treatment with praziquantel is destroyed so that crescentic (semilunar) sclerites surrounding the ventrogenital opening are clearly visible (Fig 14). Tips of large sclerites are also visible in a partly digested *H. taichui* specimen found in the gut of cat (Fig 9).

DISCUSSION

The present study revealed close similarity of surface morphology of *H. yokogawai* and *H. taichui.* Our results support the assumption of Fujino *et al* (1989) of the presence of rather uniform type of spines on the body surface of all heterophyid trematodes (Koie, 1977; Rees, 1979; Lee *et al,* 1984). Nevertheless, considering the data of Fujino *et al* (1989) and Srisawangwonk *et al* (1989) as well as our results, minute differences in dentation of tegumental spines among the three species of the genus *Haplorchis* exist.

H. pumilio is typified by distinctly pectinated spines with sharp, very long (finger-like) teeth. The maximum number of teeth is 7-9 (Fujino *et al,* 1989; Srisawangwonk *et al,* 1989). *H. taichui* bears spines rather similar in shape to those of *H. pumilio,* but they are serrated rather than pectinated with shorter teeth. Moreover, tooth number is slightly higher (maximum 10-12) (Fujino *et al,* 1989; our results). The species *H. yokogawai* differs slightly by possession of mostly serrated spines with relatively short, rarely finger-like teeth. Besides, the maximum number of spine teeth is somewhat higher than in *H. pumilio* and *H. taichui* and reaches up to 14-16. However, because of great variability of spine shape and dentation pattern, the above characters should be considered as only auxiliary. Moreover, our SEM study of the body surface of *Stellantchasmus falcatus* Onji et Nishio, 1916, other member of the subfamily Haplorchiinae (unpublished data), revealed that some tegumental spines are morphologically similar to those of *H. yokogawai* while others resemble by their shape and number of teeth on their distal margins those of *H. taichui.*

Consequently, the opinion of Srisawangwonk *et al* (1989) that surface structures may be used as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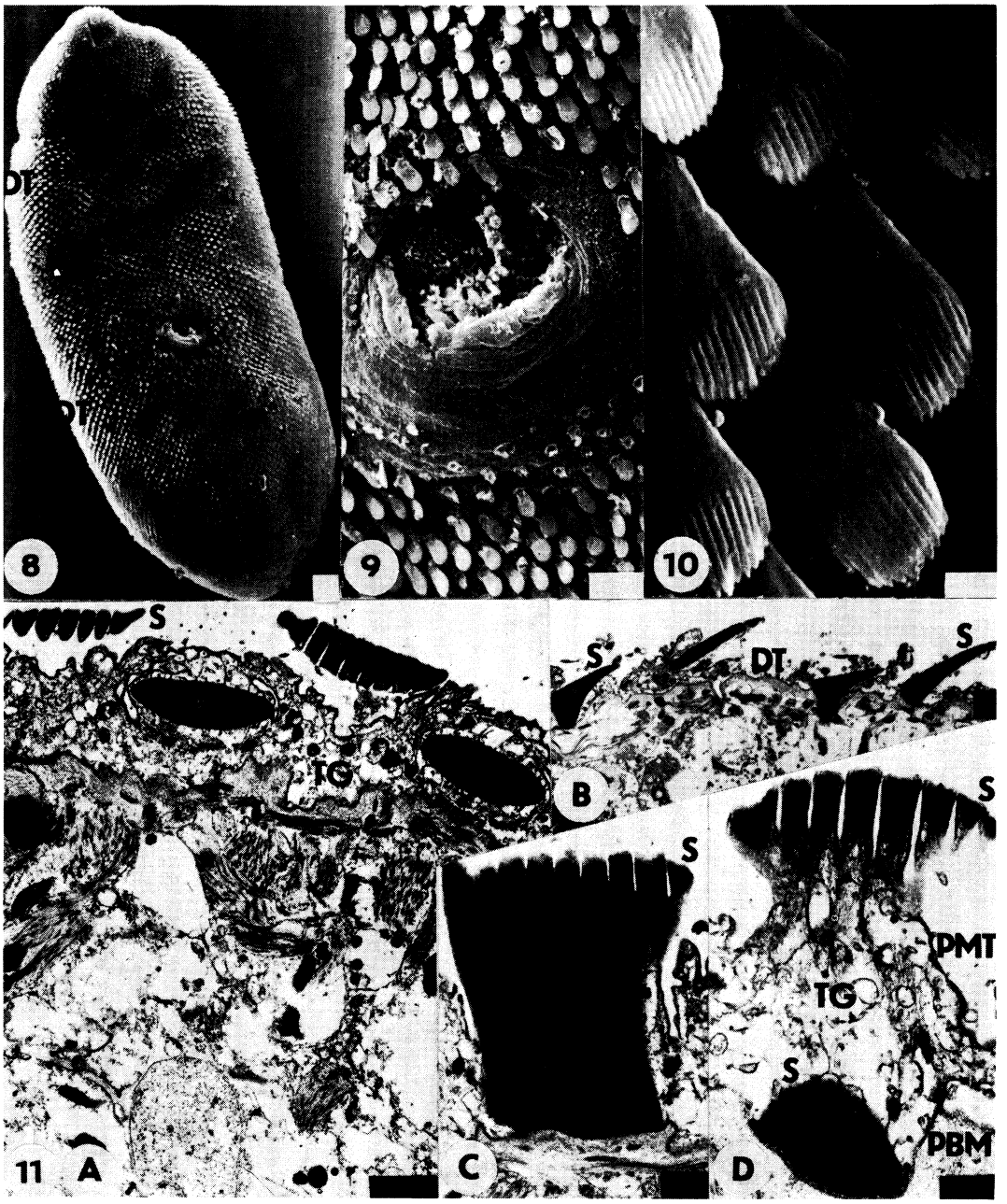

Fig 1—*Haplorchis yokogawai* - total view, ventral side. Scale 50 μm.
Fig 2—*H. yokogawai* - detail of oral sucker surrounded with papillae. Scale 10 μm.
Fig 3, 5, 6 and 7—*H. yokogawai* - scale-like tegumental spines. Scales 1 μm.
Fig 3 and 5—Anterior third of body, spines with 12-14 teeth.
Fig 6—Middle third of body, spines with 10-11 teeth.
Fig 7—Posteriormost part of body, spines with 5-6 teeth.
Fig 4—*H. yokogawai* - opening of ventrogenital complex with spermatozoa. Scale 5 μm.
Fig 8—*Haplorchis taichui* - ventral view of a specimen from cat with partly destroyed tegument (DT). Scale 5 μm.
Fig 9—*H. taichui* - ventrogenital opening, tegument completely missing. Scale 10 μm.
Fig 10—*H. taichui* - tegumental spines in anterior body half, tegument completely lacking. Scale 1 μm.
Fig 11—*Haplorchis taichui* - TEM photographs of surface with spines. A - transverse section, tegument (TG) complete. Scale 1 um. B - sagittal section, tegument strongly destroyed (DT). Scale 1 um. C, D - longitudinal section. Parabasal membrane (PBM) and plasmatic membrane of tegument (PMT) visible. Scale 0.5 μm.

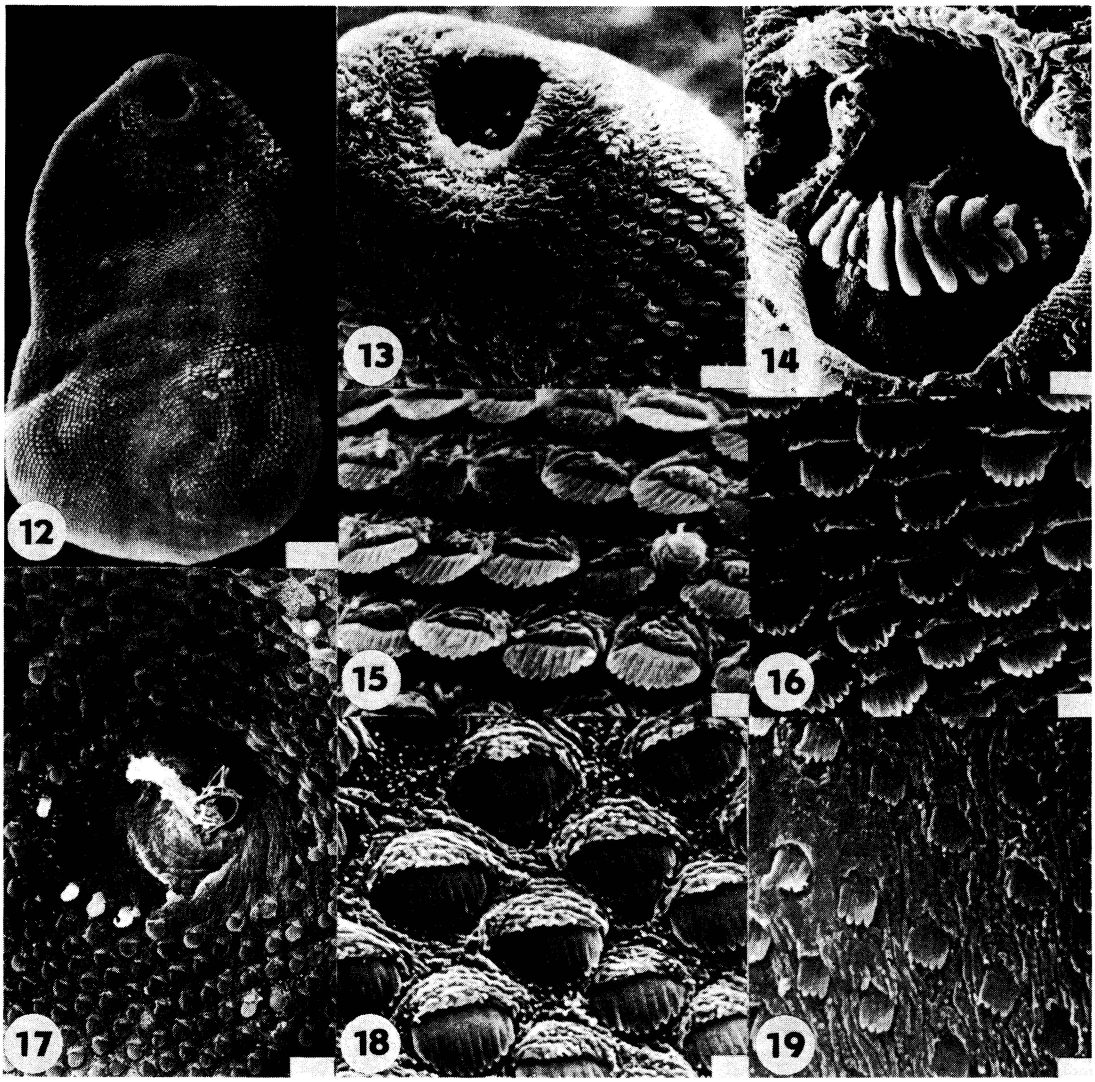

Fig 12—*Haplorchis taichui* - total view, ventral side. Scale 20 μm.
Fig 13—*H. taichui* - anterior body part with oral sucker. Scale 10 μm.
Fig 14—*H. taichui* - ventrogenital opening with semilunar (crescentic) sclerites. A damaged specimen obtained from man after treatment with praziquantel. Scale 5 μm.
Fig 15, 16, 18 and 19—*H. taichui* - scale-like tegumental spines. Scale 1 μm.
Fig 15—Anteriormost body part. Spines with 9-10 teeth.
Fig 16—Middle body region. Spines with 7-8 teeth.
Fig 17—*H. taichui* - spination of region around ventrogenital complex. Scale 5 μm.
Fig 18—Anterior body third. Spines with 10-11 teeth.
Fig 19—Posteriormost part of body. Spines with 4-6 teeth.

additional morphological characters for specific identification of some heterophyid flukes has not been supported.

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