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Fasciola gigantica: surface topography of the adult tegument

T. Dangprasert[1], W. Khawsuk[1], A. Meepool[1], C. Wanichanon[1], V. Viyanant[2], E.S. Upatham[2], S. Wongratanacheevin[3] and P. Sobhon[1]*

[1]Department of Anatomy and [2]Department of Biology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand: [3]Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

Abstract

Adult *Fasciola gigantica* are leaf-shaped with tapered anterior and posterior ends and measure about 35 mm in length and 15 mm in width across the mid section. Under the scanning electron microscope its surface appears rough due to the presence of numerous spines and surface foldings. Both oral and ventral suckers have thick rims covered with transverse folds and appear spineless. On the anterior part of the ventral surface of the body, the spines are small and closely-spaced. Each spine has a serrated edge with 16 to 20 sharp points, and measures about 20 μm in width and 30 μm in height. In the mid-region the spines increase in size (up to 54 μm in width and 58 μm in height) and number, especially towards the lateral aspect of the body. Towards the posterior end the spines progressively decrease in both size and number. The tegumental surface between the spines appears highly corrugated with transverse folds alternating with grooves. At higher magnifications the surface of each fold is further increased with a meshwork of small ridges separated by variable-sized pits or slits. There are three types of sensory papillae on the surface. Types 1 and 2 are bulbous, measuring 4–6 μm in diameter at the base with nipple-like tips, and the type 2 also have short cilia. Type 3 papillae are also bulbous and of similar size but with a smooth surface. These sensory papillae usually occur in clusters, each having between 2 and 15 units depending on the region of the body. Clusters of papillae on the lateral aspect (usually types 1 and 2) and around the suckers (type 3) tend to be more numerous and larger in size. The dorsal side of the body exhibits similar surface features, but the spines and papillae appear less numerous and are smaller. Corrugation and invaginations of the surface are also less extensive than on the ventral side of the body.

Introduction

Fascioliasis is a disease that infects both domestic and wild animals, and it is one of the major tropical diseases that afflicts both the temperate and tropical regions of the world. The causative parasite in temperate regions is *Fasciola hepatica*, whilst in the tropics it is *F. gigantica*. Fascioliasis causes significant economic losses estimated at US $2000 million per annum from its effect on domestic and economic animals (Boray, 1985). The disease can also cross-infect humans, and there have been reports of increasing incidents worldwide (Chitchung *et al.*, 1992; Maurice, 1994). The prevalences of infection are as high as 30–90% in Africa, 25–90% in Indonesia (Edney & Muchlis, 1962; Soesetya, 1975; Fabiyi, 1987). In Thailand, the prevalences of *F. gigantica* in cattle and buffaloes range between 4 and 24%, with

*Author for correspondence
Fax: 662 2479880
E-mail: scpso@ mahidol.ac.th

highest incidences in the north and north-east, and the lowest in the south (Pholpark & Srikitjakara, 1989; Sukhapesna *et al.*, 1990, 1994; Sobhon *et al.*, 1998). It is clear that the disease is a major impediment to economic progress, which is exacerbated in less developed countries, particularly towards subsistence farmers who have limited resources to treat their herds.

Fascioliasis can be partially controlled by periodic treatment of the animals in endemic areas with a repertoire of drugs. Triclabendazole has been reported to be highly effective, although resistance of liver flukes to this drug has been reported (Overend & Bower, 1995). In view of the cost and possible resistance of liver fluke to the action of drugs, a better alternative would be the development of vaccines to either completely prevent the infection or arrest worm development at certain stages of the life cycle.

The tegument is the interfacing layer that helps the liver flukes to maintain their homeostasis which is essential for their survival in the hostile environment of the host. The tegument plays a key role in the absorption and exchange of nutritive and waste molecules, the regulation of ionic equilibrium between the interior of the parasite and the surrounding host fluid, and in protecting the parasites from the immune responses of the host. Understanding the structural organization of the tegument is essential in developing any rational drugs or vaccines, which might damage the parasites through their actions on the tegument. Despite few observations on the fine surface features of *F. hepatica* (Bennett 1975a,b), no studies have previously been undertaken on *F. gigantica*. In the present study, the detailed surface features of adult *F. gigantica* tegument are investigated by using scanning electron microscopy (SEM).

Materials and methods

Adult specimens of *Fasciola gigantica* were collected from the bile ducts in the liver and gall bladder of cattle and water buffaloes killed at local abattoirs. Flukes were washed several times in 0.85% normal saline before being transferred into Minimum Essential Medium (MEM), with three changes. Flukes were kept in MEM supplemented with 10 μg ml^{-1} penicillin and 100 units ml^{-1} streptomycin until processed for SEM.

Entire adult worms were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer containing calcium acetate, pH 7.2, at 4°C for at least 2 h. They were washed three times with the same buffer, post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer, pH 7.2, at 4°C for 3 h, and washed in three changes of distilled water. Subsequently, they were dehydrated through increasing concentrations of ethanol, and dried in a Hitachi HCP-2 critical point drying machine using liquid carbon dioxide as a transitional medium. After drying, specimens were mounted on aluminum planchets and coated with gold in an ion-sputtering apparatus, Hitachi E-102, with a setting at 10–15 mA for 4 min. Specimens were examined in a Hitachi scanning electron microscope (SEM) S-2500 operating at 30 kV.

Results

Ventral surface

On the surface of the anterior region, spines are medium-sized and closely-spaced, each measuring about 20 μm in width and 30 μm in height and having a serrated edge with 16–20 sharp points (fig. 1E,G,H). At higher magnifications the surface of the spines, except their edges, appears highly corrugated and invaginated with small ridges and pits (fig. 1G,H). The surface area between the spines appears corrugated with transverse folds alternating with grooves (fig. 1E,G). At higher magnifications the folds are, in turn, composed of a meshwork of interlacing microfolds or small ridges separated from one another by variable-sized pits or slits (fig. 1I). In some areas there are groups of bulbous papillae, which are assumed to be sensory receptors (figs 1E–G, 2A–C). Each papilla appears as a small dome or bulb 4–6 μm in diameter at the base. The first two types of papillae, types 1 and 2, have nipple-like tips, with type 2 also having short cilia on their tips (fig. 2E,G). The third type of papillae, type 3, is fungiform in shape with a smooth top and highly pitted base (fig. 2F). On the anterior and lateral surfaces, type 1 and 2 papillae may appear singly or in a group of two to three units (fig. 1E). In contrast, both the oral and ventral suckers have thick muscular rims covered with wide transverse folds, surrounded by rows of type 3 papillae in large clusters, and pores of gland cells (fig. 1A–D).

On the surface of the middle region of the body, the spines increase in size (up to 54 μm in width and 58 μm in height) as well as number, particularly towards the edges of the body. The majority of spines, because of their large size, have blunt rather than sharp serrated edges (fig. 1F). The area between the spines appears highly corrugated with ridges separated by pits and slits. This area also contains large groups of papillae with similar characteristics to those found on the anterior region. Towards the lateral aspect of the body, the spines and clusters of papillae become very prominent in both size and number (fig. 2A–C). Each cluster of papillae is a large aggregate of 10–15 units (fig. 2C,D). Most of the papillae in the clusters are types 1 and 2 (fig. 2D,E,G).

On the surface of the posterior region of the body, the spines progressively decrease both in size and number, and they become widely separated (fig. 3A–C). The spines are usually short but still covered with highly invaginated surface (fig. 1E,F). Clusters of papillae are, however, still prominent; and each may contain as many units per group as those on the lateral aspect of the middle region (fig. 1C–E). The area between the spines also appears highly folded and invaginated, but the ridges are not as well developed as those on the anterior and middle regions. The posterior tip of the body has very few spines and appears smooth (fig. 3B).

Dorsal surface

Generally, the anterior and middle regions of the dorsal surface exhibit similar features to those of the ventral surface, but they tend to have smaller-sized spines and fewer papillae (fig. 3G,H). Spines on the

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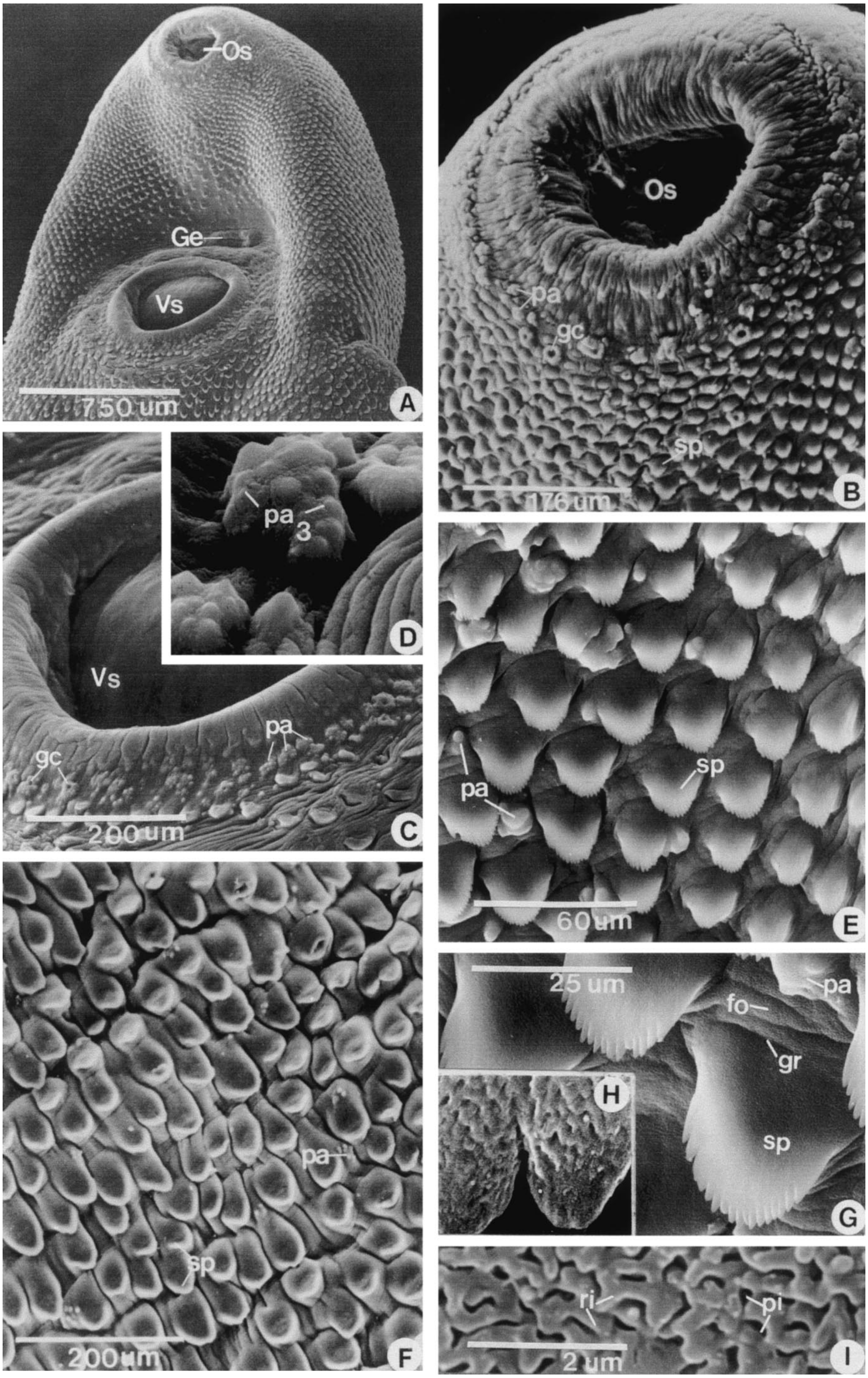

Fig. 1. A–D. The anterior ventral surface of adult *Fasciola gigantica*, with oral (Os) and ventral suckers (Vs) surrounded by rows of ventral papillae (pa) with papilla cluster (pa_3) in D, flat spines (sp), pores of glands (gc) and opening of genital canal (Ge). E and F. The anterior and middle surface regions, respectively, with closely-spaced spines (sp) and clusters of papillae (pa). G and H. Serrated spines with highly corrugated surface. Between the spines the surface appears as a series of alternating folds (fo) and grooves (gr). I. Surface of fold with small ridges (ri) separated by pits and slits (pi).

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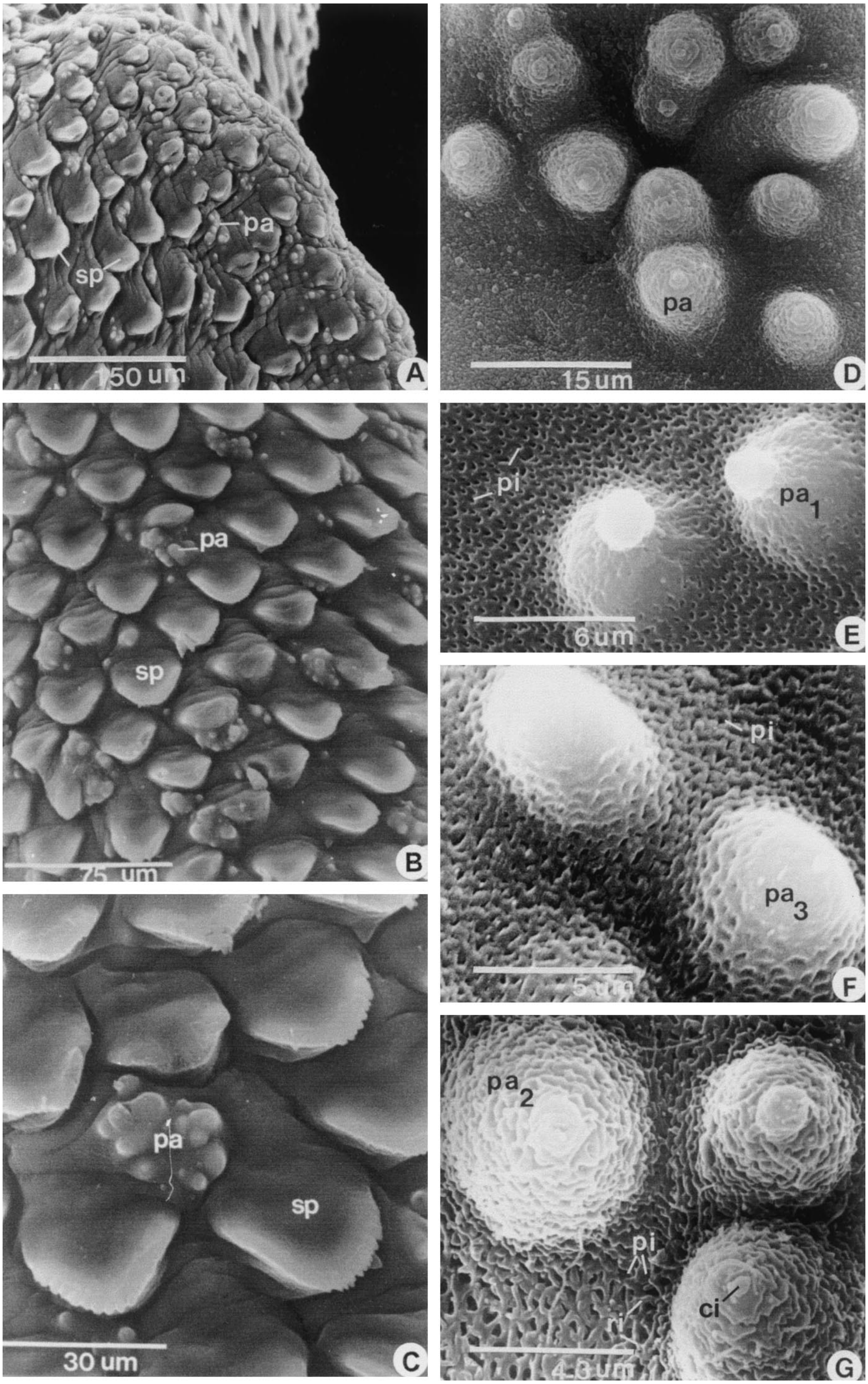

Fig. 2. A–C. The anterior-lateral (in A) and middle-lateral (in B) regions of the ventral surface of *Fasciola gigantica*, with numerous flattened and highly serrated spines (sp), and large clusters of papillae (pa). D. A large cluster of papillae. E. Type 1 papillae (pa1) with nipple-like tips. F. Type 3 papillae (pa_3) fungiform in shape, with smooth surface. G. Type 2 papillae (pa_2) with short cilia (ci) on the nipple-like tips.

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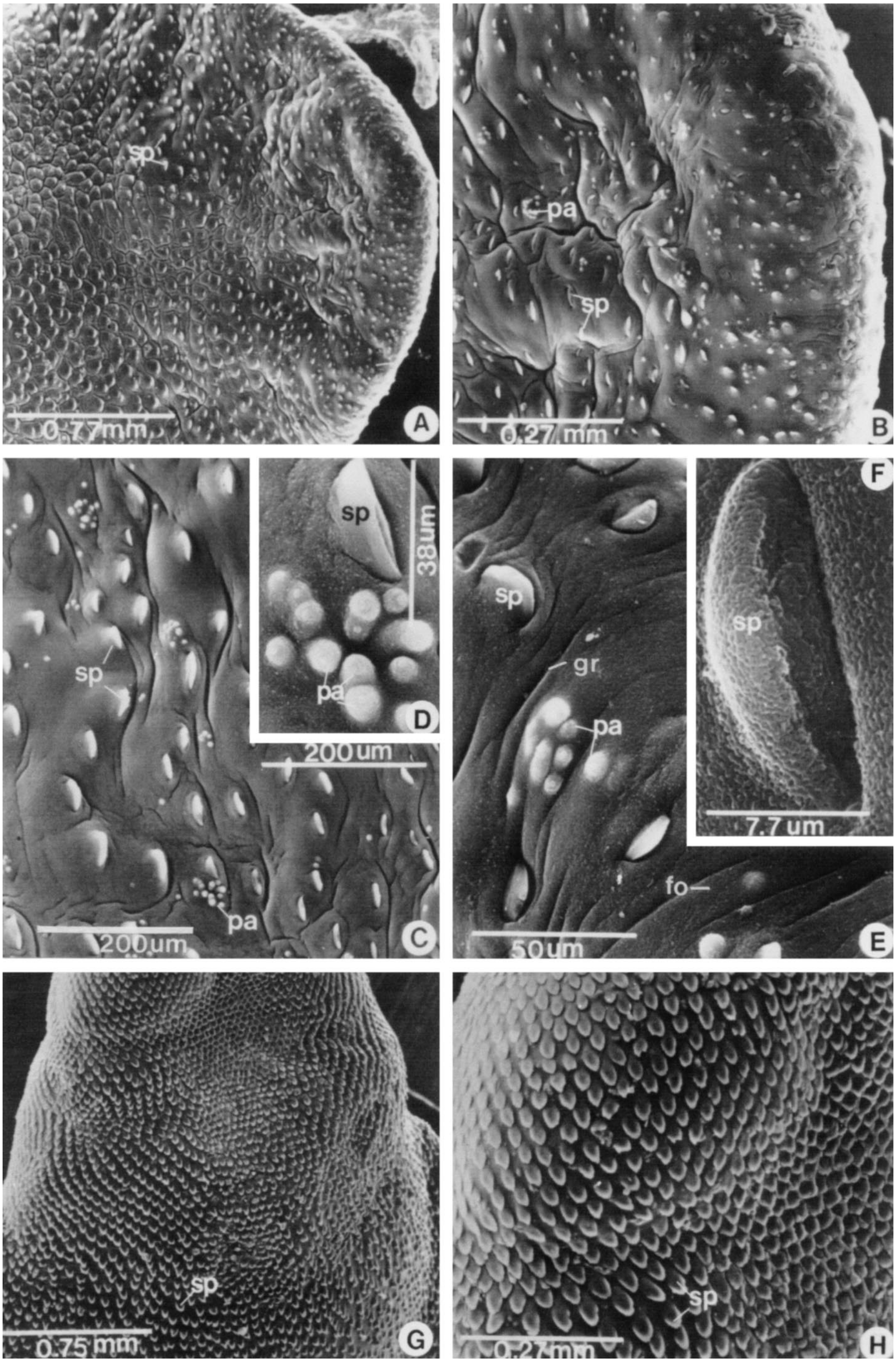

Fig. 3. A–C. The posterior part of the ventral surface and the tip of *Fasciola gigantica* with short, flattened, widely-spaced spines (sp) and clusters of papillae (pa). D. Large cluster of papillae. E. Large cluster of papillae and surface folds (fo) and grooves (gr). F. Spine with highly corrugated surface. G and H. The anterior dorsal surface with numerous spines but fewer papillae.

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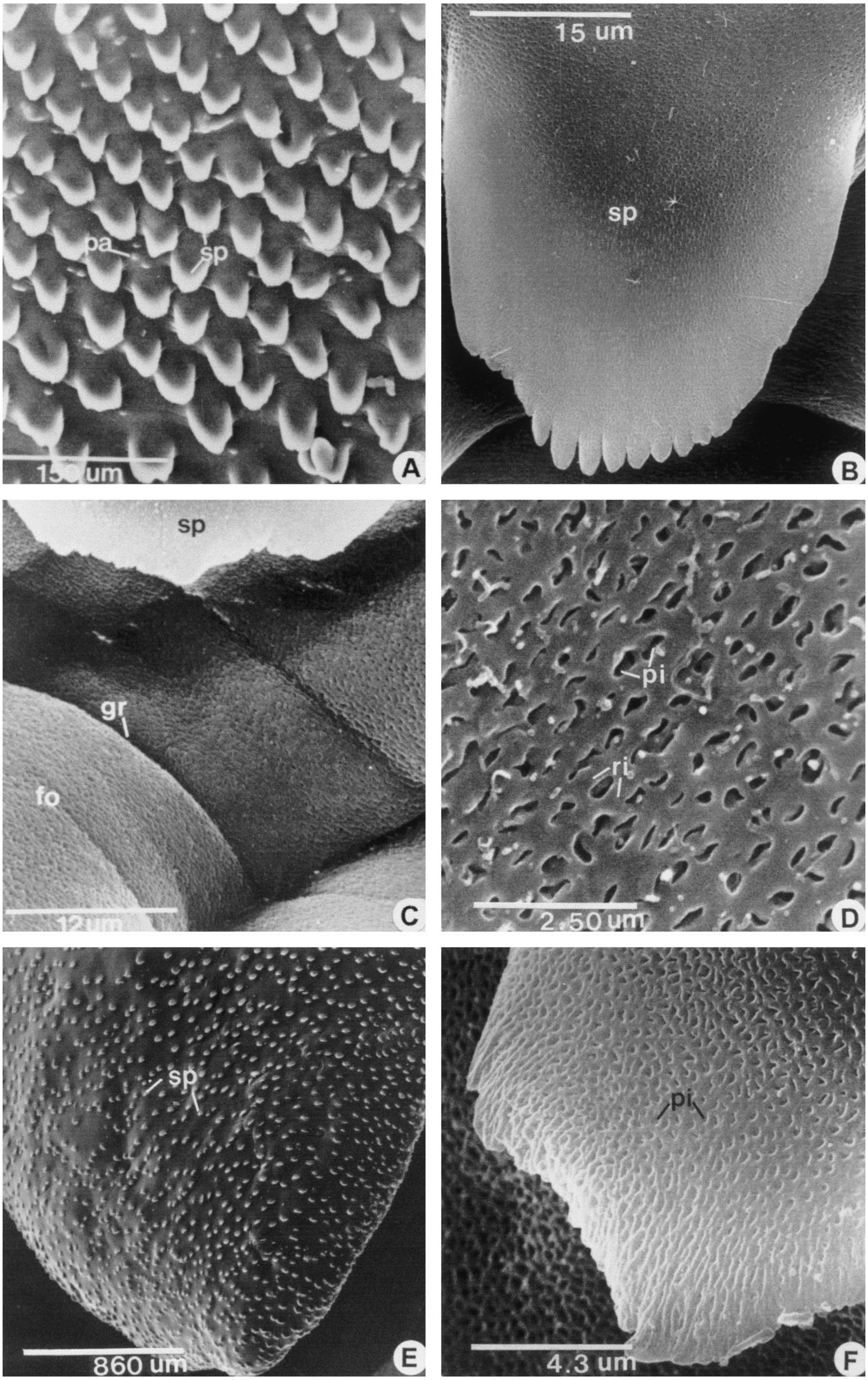

Fig. 4. A. The middle region of the dorsal surface of *Fasciola gigantica*, with flat serrated spines (sp) close together. B. Spine C. Surface between the spines, highly corrugated with folds (fo) and grooves (gr). D. Surface of fold, with flat ridges (ri) separated by pits and slits (pi). E. The posterior dorsal surface, with short widely separated spines. F. Spine with highly invaginated surface and unserrated edge.

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anterior and middle regions are still serrated (fig. 4A,B), whilst those located towards the lateral and posterior regions are smaller and not well serrated. The surface area between the spines appears highly convoluted with folds and grooves (fig. 4C), but ridges on each fold tend to be flattened when compared with those on the anterior and middle regions of the ventral surface (fig. 4D).

The posterior region of the dorsal surface possesses fewer, smaller, and widely-spaced spines (fig. 4E). Each spine is short and unserrated, but still covered with a highly invaginated surface (fig. 4F). The area between the spines is invaginated with large pits, whilst the ridges are not well developed. Unlike the ventral surface, the posterior end of the dorsal surface has only a small number of papillae.

Discussion

The most remarkable features of the surface topography of fully mature *F. gigantica* are the presence of a highly corrugated surface which consists of series of alternating grooves and folds, and the presence of spines. The surface of each fold is increased further by a meshwork of microfolds or ridges separated by pits and slits. This increased surface area could enhance the effectiveness of absorption and exchange of materials by the tegument, a characteristic which is also observed in other trematodes, such as *F. hepatica* (Bennett, 1975b), *Opisthorchis viverrini* (Apinhasmit *et al.*, 1993), and the schistosomes (Hockley, 1973; Hockley & McLaren, 1973, 1977; Jinxin & Yixun, 1981; Sobhon & Upatham, 1990). The development of ridges and pits varies in different regions of the worm surface. The ventral and lateral surfaces tend to have more complex ridges and pits than the dorsal surface, and the anterior and middle regions also tend to have more developed ridges and pits than the posterior region. This suggests different degrees of absorptive capacities in various regions of the tegument, a characteristic which is similar to other helminths (Bennett, 1975b; Hockley & McLaren, 1977; Sobhon & Upatham, 1990; Apinhasmit *et al.*, 1993). Another distinguishing surface feature is the presence of numerous spines covering all parts of the body's surface. We have not yet examined the surface of metacercaria and juvenile stages of this species, but juveniles of *F. hepatica* possess large serrated spines on their entire surface (Bennett, 1975a,b), while those of *O. viverrini* possess serrated spines on the anterior and single-pointed spines on the posterior parts of the body (Scholz *et al.*, 1992; Apinhasmit *et al.*, 1993). These spines may facilitate the movement of juvenile flukes during their migration to the biliary system of the liver. Upon reaching the liver, mature *O. viverrini* become spineless (Apinhasmit *et al.*, 1993), and the loss of spines may be due to the decreased movement of the fluke in the bile ducts. In contrast, adults of *F. gigantica* and *F. hepatica* still retain spines on the surface which implies that they may have more movement in the bile ducts of the liver. Their more active movement in the biliary tree of the liver may explain why they cause more severe fibrosis of the bile ducts in comparison with *O. viverrini*. However, for most of the time adult *F. gigantica* probably remain attached to the wall of the bile ducts using their relatively large and muscular ventral suckers, which are surrounded by smooth fungiform (type 3) papillae which could act as pressure receptors. On the other hand, type 1 and 2 papillae, which are distributed elsewhere on the surface of the bodies, may act as tactile receptors.

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