FOLIA PARASITOLOGICA (PRAHA) 32: 43—49, 1985.

TEGUMENTAL MICROTOPOGRAPHY AND ARRANGEMENT OF PAPILLAE IN ADULT PHYLLODISTOMUM FOLIUM (OLFERS, 1816) (DIGENEA: GORGODERIDAE) FROM PIKES (ESOX LUCIUS L.)

T. A. BAKKE and Z. ŽĎÁRSKÁ

Zoological Museum, University of Oslo, and Institute of Parasitology, Czechoslovak Academy of Sciences, České Budějovice

Abstract. The microtopography of *Phyllodistomum folium* specimens from the type host *Esox lucius* collected in Czechoslovakia, was studied by scanning electron microscopy. This SEM-study revealed tegumental ridges encircling the length of the flukes, especially prominent in the forebody. No spines were present. A frontal pit anterior of the oral sucker was present and a marked notch on the posterior terminal end with the opening of the excretory pore. There was observed an aciliated papilla type occurring in three size categories. A special attention was given to the arrangement of these papillae, which comprised (a) constant papillae in a constant bilateral arrangement, (b) regional concentrated, (c) randomly distributed ones. This system and its variability is described and figured. The results are compared with other investigations on Gorgoderidae species, especially *P. umblae* (Fabricius). The differences observed agains this closely analysed species, comprised: (a) new consistent papillae. (b) missing consistent papillae, (c) differences in the papilla numbers in the rows, (d) changed positions of consistent papillae.

The type species *P. folium* in genus *Phyllodistomum* Braun, 1899 was described by Olfers (1816) based on specimens recovered in pike (*Esox lucius* L.) collected in Europe (Berlin according to Braun 1899) and placed as type species in genus *Phyllodistomum* by Braun (1899). Looss (1894) redescribed the species based on specimens from ruffe (*Acerina cernua* (L.) = *Gymnocephalus cernuus* (L.)) collected in the vicinity of Leipzig. He presented a detailed redescription and a figure of the species, which replaced Olfers's (1816) original and later was used as *P. folium* in many of the main books in western Europe dealing with this species (Lühe 1909, Sprehn 1960, Dawes 1968, Yamaguti 1971).

There are however, large taxonomical problems concerning this and other species in the subfamily, which can be illustrated by three examples: Firstly, Nybelin (1926) who presented new figures of *P. folium*, placed Looss's (1894) description and classical figure of *P. folium* from *G. cernuus* as basis of a new species, *P. pseudofolium*. A species accepted among others by Markevich (1951), Pigulewskii (1953) and Bykhovskaya-Pavlovskaya et al. (1962), the two last-mentioned also presented new figures of *P. pseudofolium*.

Secondly, Dawes (1968) suggested that *P. conostomum* (Olsson, 1876), *P. acceptum* Looss, 1901, and the species erected by Nybelin (1926): *P. pseudofolium*, *P. simile*, *P. megalorchis* and *P. elongatum*, were identical with *P. folium*. He concluded that it is safe to infer from the available descriptions that *P. folium* will closely rival trematodes like *Otodistomum veliporum* in the wideness of its distribution and *Bunodera luciopercae* in the extent of its variability.

Thirdly, a study of the figures presented as *P. folium* in the literature (see Zchokke 1884, Looss 1894, Nybelin 1926, Pigulewskii 1953, Ślusarski 1958, Kozicka

1959, Kakajii 1969, Prudhoe and Bray 1982) clearly indicate that either Dawes's (1968) statement of the extreme variability is correct or which seems plausible, the earlier identified species consists of an assemblage of many species.

As earlier investigations by light microscopy (LM) (e.g. Thomas 1958) and by scanning electron microscopy (SEM) in the family (Bakke and Lien 1978, Hoole and Mitchell 1981, Bakke 1984) have revealed a taxonomical promising papillar system on adults, we feel that a microtopographical description of *P. folium* with SEM-techniques would be important in a further evaluation of the taxonomical value of the papillar system. The species *P. umblae* (Fabricius, 1780), clearly distinct from *P. folium*, as seen by LM, has been redescribed in respect to the types and arrangement of papillae by one of us (Bakke 1984). This gives for the first time the possibility of an interspecific microtopographical comparison in this genus based on the same techniques. The specimens for this study have been recovered in the Central Europe from pikes, the original type host, and compared with *P. umblae* specimens from the type host and locality (Bakke 1984).

MATERIAL AND METHODS

Specimens of *Phyllodistomum folium* were removed from the urethra/bladder of naturally infected *Esox lucius* collected by an electric fishing machine in the Mácha Lake pond system in Czechoslovakia, and washed briefly in saline. They were then killed and fixed in hot (heated to boiling) 10 % formalin, later transferred to 70 % ethyl alcohol. For scanning electron microscopical studies 10 specimens were dehydrated through an ascending series of ethyl alcohol to critical point drying with CO_2 to minimize distortion by surface tension forces. They were mounted on metal specimen stubs with silver paste, rotary coated with gold and examined with a Stereoscan Jeol 35-C, operating at 10—15 kV.

RESULTS

The body outline, location of the suckers, the "shoulders" and the posterior notch of *P. folium* are shown in Plate I-II. There are no spines. The old specimen shown in Plate I, Fig. 1, demonstrate the prominent "shoulders" situated at level of the middle of ventral sucker separating the worm in an anterior slightly tapering subcylindrical forebody (neck) and a dorsoventrally flattened, foliate, ovoid to circular, hindbody. There is an increased demarcation of the shoulders with age as seen in Plate I and a larger growth of the hindbody in relation to the forebody (allometric growth) with ontogenetical development. This implies a foreward shift of the ventral sucker. The margins of the markedly wider hindbody are smooth without special tegumental structures. There was always a prominent posterior notch present, in which the excretory pore empties. This longitudinal dorso-ventrally slit could be of variable prominence (Plate I, Figs. 1, 2), however, the most typical appearance is shown from the ventral side in Plate III, Fig. 2, and from the dorsal side in Plate VI, Fig. 3.

The oral sucker is directed anterio-ventrally with the genital pore situated in the middle between the suckers and surrounded by a slight tegumental elevation (Plate II). The whole body except for the sucker rims, the hindbody dorsally (Plate VI, Fig. 2) and the terminal parts, are covered by transverse tegumental ridges, most prominently developed in the forebody (Plate II). Within and on the rim of the oral sucker there are radially directed corrugations (Plate IV, V) as on the rim of the ventral sucker (Plate III, Fig. 1), which structurally separates the tegument covering the suckers from the body tegument. The tegument is generally thrown up into small elevations, resembling cobble-stones (Plate III—VI). The frontal pit is slightly discernible, but

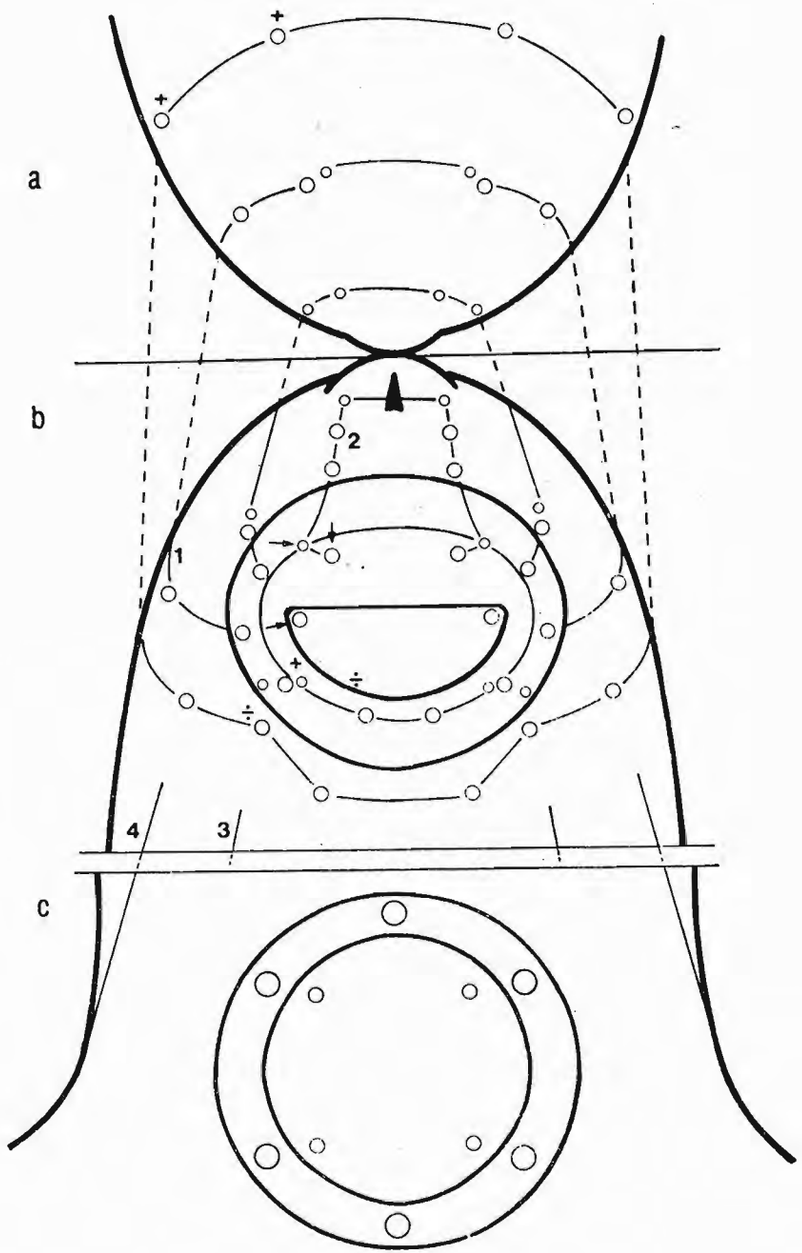


Fig. 1. The papillary arrangement on the anterior end of *P. folium*. The circles indicate the approximate position of the consistent papillae which occur, as shown, in three size groups, on the, anterior dorsal end (a), anterior ventral end (b) and ventral sucker (c) (For arrows, lines, numbers plus and minus sign, see text).

without an anterior tegumental elevation. The frontal pit is, however, always limited laterally by a papilla on each side (Plate IV).

Papillae, irregularly or regularly distributed, depending on body region, can be found over the whole surface. The only papilla type observed by SEM, the "button papillae", are unciliated with a approximately smooth surface. This "button type", may generally occur from randomly to consistent, i.e. in specific constant positions and arrangements on the body and suckers. They can be divided into three classes based on relative sizes. The largest "button papillae" (1) occur only on the ventral sucker rim in a number of six (Plate III, Fig. 1); the second in size (2) are the most common and occur in large numbers from random to consistent in a distinctly bilateral arrangement on the body and on the oral sucker (Plate II—VI); the third and smallest type (3) are found in a distinct bilateral pattern within the ventral sucker (based on position they correspond to the "domed papillae" of Hoole and Mitchell (1981) and Bakke (1984)), and on the oral sucker and anterior dorsal side (see Plate IV, Plate VI, Fig. 1).

On the hindbody all papillae are randomly distributed. Anterior ventral on the forebody between the suckers, there is a longitudinal arrangement of papillae in two rows with a restricted, but slightly variable number. However, four prominent consistent papillae of size two are typically present in each row (Plate II, Fig. 3 — large arrows). Laterally there are an arrangement of six similar papillae, which as shown in Plate II, Figs. 3, 4 (small arrows) extends to the posterior level of ventral sucker.

On the ventral sucker rim six large consistent, papillae (size 1) are arranged with specific position and pattern (Plate III, Fig. 1 — black arrows), the same as four consistent papillae (size 3) within the ventral sucker (Plate III, Fig. 1 — white arrows). Sixteen consistent papillae have a specific position in a bilateral arrangement around the oral sucker, 6 posteriorly (size 2) and 10 (6 of size 2, and 4 of size 3) from laterally to anteriorly (Plate IV, Fig. 1 — black arrows). On the oral sucker rim (black arrows) and within the sucker (white arrows) 20 consistent papillae have a regular position in a bilateral arrangement, 8 posteriorly (4 of size 2, and 4 of size 3) and 12 (10 of size 2, and 2 of size 3) laterally to anteriorly (Plate IV, Fig. 2, see arrows). Plate V demonstrates the slight variability in positions of these papillae and the observed tendency of coalescence of the size 2 and 3 papillae situated in the positions marked with white arrows.

Anterior dorsally 14 papillae (8 of size 2, and 6 of size 3) have a constant position and arrangement in three sectors comprising 4, 6 and 4 papillae (Plate VI, Fig. 1 — black arrows). Posterior dorsally the papillae occur evenly distributed.

Generally, the body papillae seem to be more frequent ventrally, and with a regional concentration at the anterior end of *P. folium* and on the suckers. Ventrally and laterally on the forebody most of the papillae in *P. folium* seem to occur in a specific arangement (Plate II, IV, V), on the hindbody in contrast, they are always randomly distributed (Plate I, III, Fig. 2).

There are three types of papilla distribution: 1. random, 2. concentrated in particular regions, 3. consistent with fixed location and numbers in a bilateral symmetrical arrangement. The position and arrangement of the papillae of type 3 distribution on the forebody with the ventral sucker are indicated diagrammatically and summarized in Fig. 1. The circles indicate the constant papillae of the three size types, their arrangement (see lines) and approximate position (some atypical cases may be found with missing or extra papillae).

DISCUSSION

There is a obvious need for a redescription of *P. folium* by LM-microscopy, on specimens from pikes (preferably from the Berlin area) and an analysis of the geographical variability of this species in pikes and other functional host species. Our material is determined on basis of light microscopical comparisons with earlier descriptions (Pigulewskii 1953, Ślusarski 1958).

Few electron microscopical investigations have been performed on the tegument of gorgoderids. Studies of the microstructure of the outer surfaces by SEM are restricted to those of Nadakavukaren and Nollen (1975) (on *Gorgoderina attenuata* (Stafford, 1902)), Hoole and Mitchell (1981) (on *G. vitelliloba* (Olsson, 1876)), Bakke and Lien (1978) and Bakke (1984) (on *Phyllodistomum umblae* (Fabricius, 1780) (syn. *P. conostomum* (Olsson, 1876)). Differences in the papillar system are earlier found between *P. umblae* and *P. simile* Nybelin, 1926 (see Thomas 1958), *P. coregoni* Dechtiar, 1966 (see Dechtiar 1966), *G. attenuata* (see Nadakavukaren and Nollen 1975), and *G. vitelliloba* (see Hoole and Mitchell (1981)). Dechtiar (1966) specifically indicates that *P. coregoni* differs from 10 listed species in the genus in number and arrangement of papillae on the suckers and (or) on the surfaces and margins of the body. Nevertheless, there is a need for a reinvestigation of the species earlier only LM-investigated to make them comparable with the present data revealed by SEM.

The present *P. folium*-results compared with *P. umblae* reveal that the hindbody outline is more round to ovoid/foliate than in *P. umblae* and accordingly the shoulders more distinct. The frontal pit, however, is more prominent in *P. umblae*; the tegumental topography with ridges, their distribution and orientation much of the same, except that dorsally on the hindbody the transverse tegumental corrugations in *P. folium* seem less developed than in *P. umblae*.

As for the types of papillae, no clear evidence for a ciliate and rosette type as in *G. vitelliloba* and *P. umblae* (Hoole and Mitchell 1981, Bakke 1984) was found in *P. folium* adults. However, the papillae within the ventral sucker (of size 3) may represent the "domed type" based on the position. The "button type" of *P. folium* demonstrated a size variability, as in the adults of the other species. However, in *P. folium* they could be grouped into three size classes: 1. large (arranged on ventral sucker rim), 2. medium (the most frequently observed, randomly or consistantly arranged on the whole body), and 3. small (arranged on the anterior end around the sucker and frontal pit, and within the ventral sucker).

There are specific differences in the arrangement of the consistent papillae between *P. folium* and all the other species investigated. The difference against *P. umblae*, the best analysed species, (see Bakke 1984), comprises irrespective of papillae types: 1. Missing papillae on *P. folium* (two bilaterally arranged within the oral sucker on the "posterior lip"; two bilaterally arranged posterior of the oral sucker (see Fig. 1, positions marked with —); 2. Extra papillae in regular arrangement on *P. folium* (two on the posterior rim of the oral sucker, four dorsally as a sector posterior of the two described for *P. umblae* (see Fig. 1, positions marked with +); 3. Differences in the row-system as earlier described for *P. umblae*: (row 1) no variability observed (papillae number 2+2 in *P. folium*); (row 2) no variability observed (papillae number 3+3 in *P. folium*); (row 3) lower restricted number in *P. folium* and the presence of 4+4 consistent and prominent papillae with regular appearance; (row 4) a row specific of *P. folium* which comprises papillae in a restricted number with 6+6 consistent and prominent papillae appearing laterally on the forebody (a tendency for an anterior lateral concentration without constant ones was traceable in *P. umblae*);

4. Changes in positions of the consistent papillae on *P. folium* (the arrows in Fig. 1 indicate those papillae whose positions seem most clearly changed in relation to *P. umblae*, and the direction of the "displacement". There are no principal differences in the arrangements of the consistent papillae (see lines, Fig. 1) around and on the oral sucker between these species. The arrangement of the remarkably consistent papillae observed on the ventral sucker is similar to that of *P. umblae*. Generally, there was a more restricted number of the randomly scattered body papillae in *P. folium* compared to *P. umblae* from Arctic char, *Salvelinus alpinus* (L.) (Bakke 1984).

There is clearly a need for a closer analysis of other *Phyllodistomum* species and of other gorgoderids. There is also of importance to study the anatomical and functional properties of the papillae in adults for a sound evaluation of their taxonomical and classificational value.

Acknowledgement. We are most grateful to Dr. T. Barnard at the Electron Microscopical Unit for Biological Sciences University of Oslo for providing the working facilities with SEM and we particularly thank Ing. E. Braaten for the important technical assistance. We also appreciate the help given by Dr. F. Moravec in collecting the parasitized hosts and are indebted to Mr. R. Jacquet for technical help and to The Norwegian Royal Ministry of Foreign Affairs for a travelling grant under the cultural exchange program between Norway and Czechoslovakia concerning specialists.

МИКРОТОПОГРАФИЯ ТЕГУМЕНТА И РАСПРЕДЕЛЕНИЕ ПАПИЛЛ У МАРИТЫ ТРЕМАТОДЫ *PHYLLODISTOMUM FOLIUM* (OLFERS, 1816) (DIGENEA: GORGODERIDAE) ОТ ЩУКИ (*ESOX LUCIUS* L.)

Т. А. Бакке и З. Ждярска

Резюме. Микротопографию тегумента трематоды *Phyllodistomum folium* от щуки *Esox lucius* из Чехословакии изучали при помощи сканирующей электронной микроскопии. Обнаружены тегументальные складки, окружающие тело трематоды, особенно выступающие в передней части тела. Шипы не встречались. Перед ротовой присоской обнаружено фронтальное углубление и вырезка на заднем конце, с отверстием экскреторной поры. Обнаружены папиллы без ресничек, встречающиеся в трех разных размерах. Внимание уделяется особенно распределению этих папилл, которые включают а) постоянные папиллы в константном билатеральном распределении, б) регионально концентрированные папиллы, и в) беспорядочно распределенные папиллы. Дано описание и рисунки этой системы и ее изменчивость. Результаты сравниваются с результатами, полученными при изучении видов семейства Gorgoderidae, особенно *P. umblae* (Fabricius). Выявлены следующие различия: а) новые консистентные папиллы, б) отсутствующие консистентные папиллы, в) различия в количестве папилл в рядах, г) измененные положения консистентных папилл.

REFERENCES

BAKKE T. A., A redescription of adult *Phyllodistomum umblae* (Fabricius, 1780) (Digenea; Gorgoderidae) from *Salvelinus alpinus* (L.) in Norway. Zool. Scripta (13: 89—99), 1984.

—, LIEN L., The tegumental surface of *Phyllodistomum conostomum* (Olsson, 1876) (Digenea), revealed by scanning electron microscopy. Int. J. Parasitol. 8: 155—161, 1978.

BRAUN M. G. C. C., Über einige wenig bekannte resp. neue Trematoden. Verh. Deutsch. Zool. Gesellsch. 2: 44—52, 1892.

—, Über *Clinostomum* Leidy. Zool. Anz. 22: 484—493, 1899.

BYKHOVSKAYA-PAVLOVSKAYA I. E., et al., Key to Parasites of freshwater Fish of the USSR, Leningrad, 1962. Jerusalem IPST, 1964. (Transl. from Russian.)

DAWES B., The Trematoda with special reference to British and other European forms. University Press, Cambridge, 649 pp., 1968.

DECHTIAR A., A new species of *Phyllodistomum* (Trematoda): Gorgoderidae) from *Coregonus clupeaformis* (Mitchill) from Lake of the Woods (Ontario). Can. J. Zool. 44: 135—140, 1966.

HOOLE D., MITCHELL J. B., Ultrastructural observations on the sensory papillae of juvenile and adult *Gorgoderina vitelliloba* (Trematoda: Gorgoderidae). Int. J. Parasitol. 11: 411—417, 1981.

KAKAJII V. L., Studies of helminth parasites of Indian fishes. Part III. Some trematode parasites of fresh water fishes of Uttar. Ind. J. Helminthol. 21: 49—80, 1969.

KOZICKA J., Parasites of fishes of Lake Druzno. Part VIII. Acta Parasit. Polon. 7: 1—72, 1959.

LOOSS A., Die Distomen unserer Fische und Frösche. Stuttgart, Verlag von Erwin Nägele, 296 pp., 1894.

LÜHE M., Parasitische Plattwürmer. I. Trematodes. Die Süsswasserfauna Deutschlands, Heft 17, G. Fischer Verlag, Jena, 217 pp., 1909.

MARKEVICH A. P., Parasitic fauna of freshwater fish of the Ukrainian SSR., Kiev 1951. Jerusalem, IPST, 1963. (Transl. from Russian.)

NADAKAVUKAREN J. J., NOLLEN P. M., A scanning electron microscopic investigation of the outer surfaces of *Gorgoderina attenuata*. Int. J. Parasitol. 5: 591—595, 1975.

NYBELIN O., Zur Helminthenfauna der Süsswasserfische Schwedens. I. Phyllodistomen. Göteborg. Kungl. Vetensksamh. Handl. 31 (3): 1—29, 1926.

PIGULEWSKII S. W., Family Gorgoderidae Looss, 1901. In: K. I. Skryabin et al. (Eds.) Trematodes of animals and man. 8: 253—615, 1953. Izdat. Akad. nauk SSSR, Moskva, 1964. (Transl. from Russian.)

PRUDHOE S., BRAY R. A., Platyhelminth parasites of the Amphibia. British Museum (Nat. Hist.), Oxford University Press, 217 pp., 1982.

ŚLUSARSKI W., The adult Digenea from Salmonidae of the basin of the Vistula and of the South Baltic. Acta parasit. Polon. 6: 247—460, 1958.

SPREHN C., Trematoda und Cestoidea. Die Tierwelt Mitteleuropas. Verlag Quelle und Meyer, Leipzig, 197 pp., 1960.

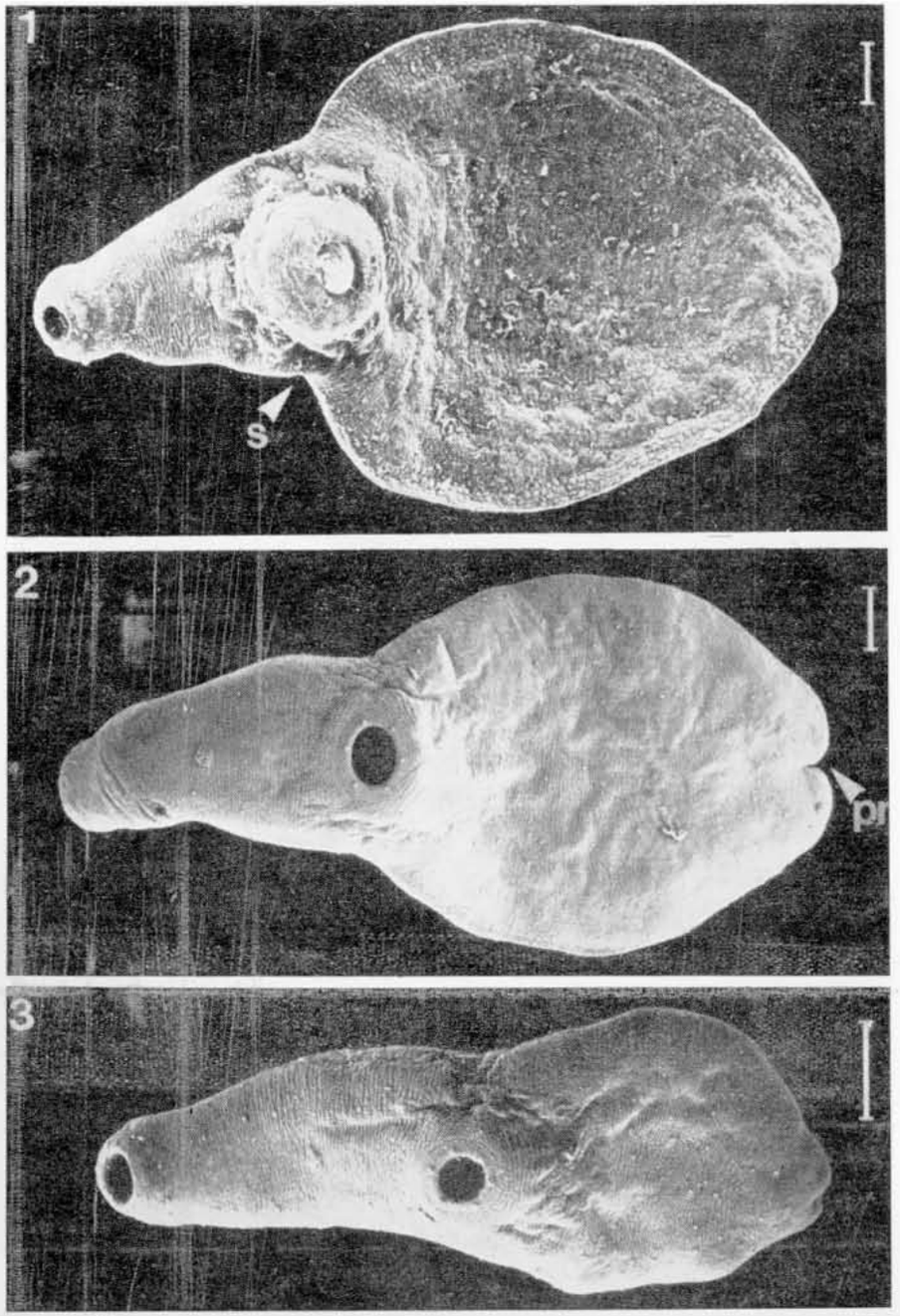
THOMAS J. D., Studies on the structure, life history and ecology of the trematode *Phyllodistomum simile* Nybelin, 1926 (Gorgoderidae: Gorgoderinae) from the urinary bladder of brown trout, *Salmo trutta* L. Proc. zool. Soc. London 130: 397—435, 1958.

YAMAGUTI S., Synopsis of digenetic trematodes of vertebrates. Vol. I. Keigaku Publ. Co., Tokyo, 1971.

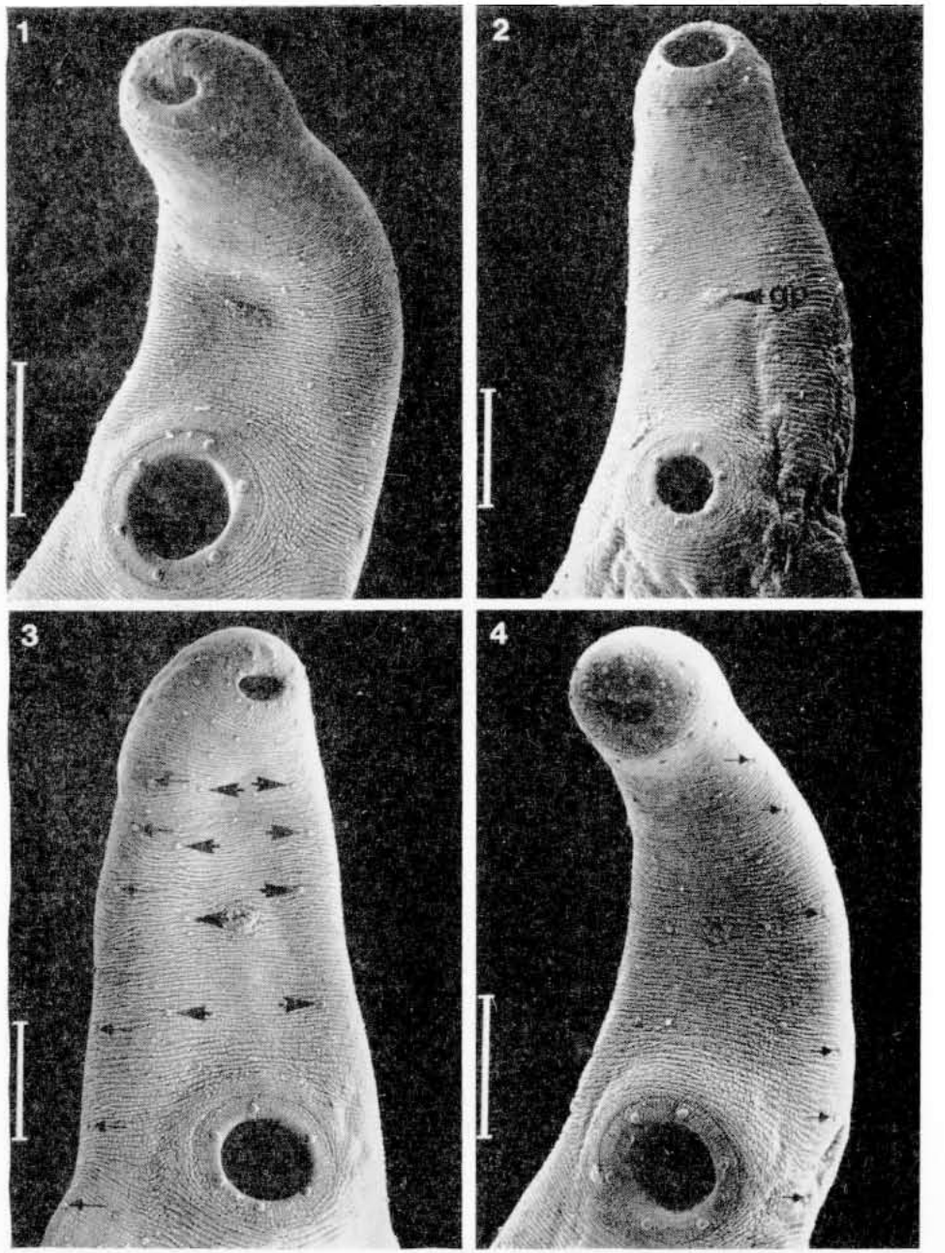
ZCHOKKE F., Recherches sur l'organisation et la distribution zoologique des vers parasites des poissons d'eau douce. Arch. de Biol. 5 (2): 153—241, 1884.

Received 28 March 1984.

T. A. B., Zoological Museum,
University of Oslo,
N-0562 Oslo 5, Norway



Three specimens of *P. folium* showing the body outline with prominent shoulders (s) and the posterior notch (pn); **Fig. 1.** Old mature specimen. **Fig. 2.** Mature specimen. **Fig. 3.** Young premature specimen (Scale bars 100 μm).



Four specimens of *P. folium* demonstrating the anterior ventral side with the genital pore (gp). Large arrows point to the four ventral pairs of consistent papillae, small arrows indicate the six lateral pairs of consistent papillae (Scale bars 100 μm).

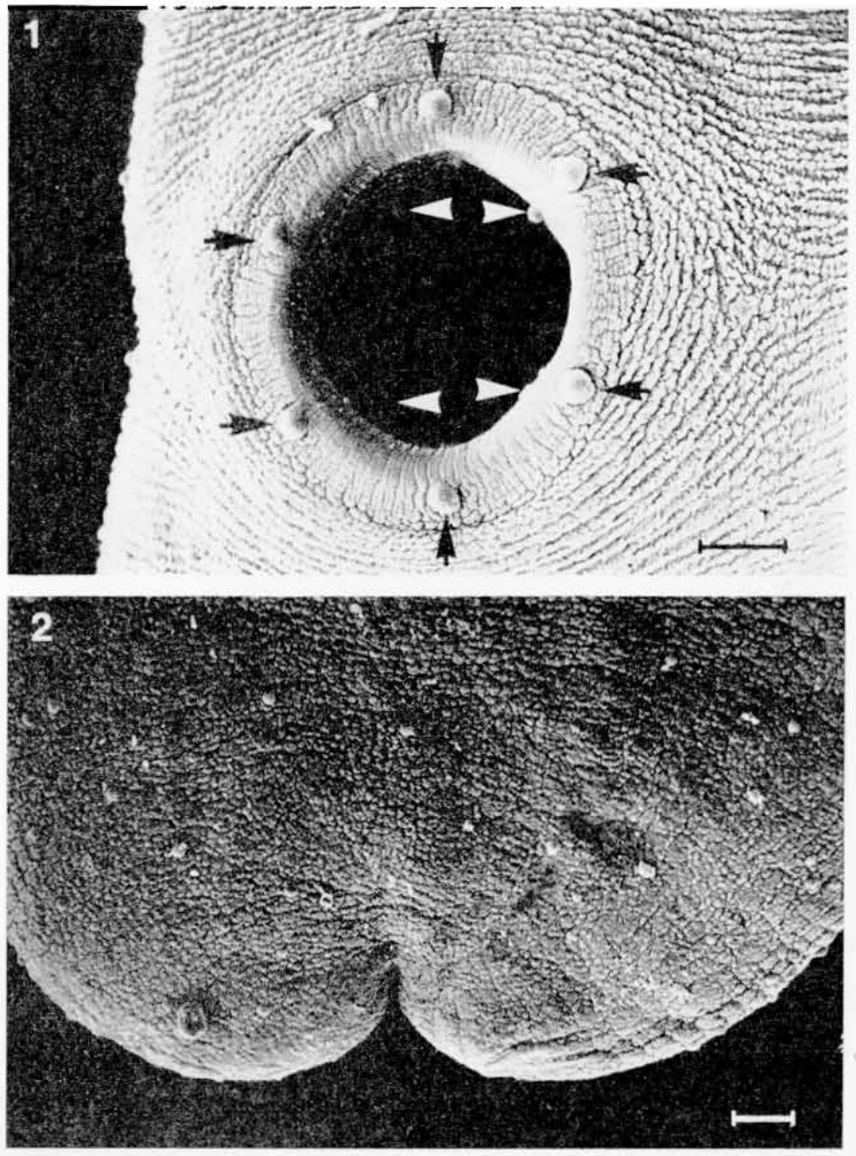


Fig. 1. The papillary arrangement on the ventral sucker of *P. folium*, comprising the 6 consistent papillae on the rim (black arrows) and the 4 consistent papillae within the sucker (white arrows).
Fig. 2. The posterior notch as seen from the ventral side (Scale bars 20 μm).

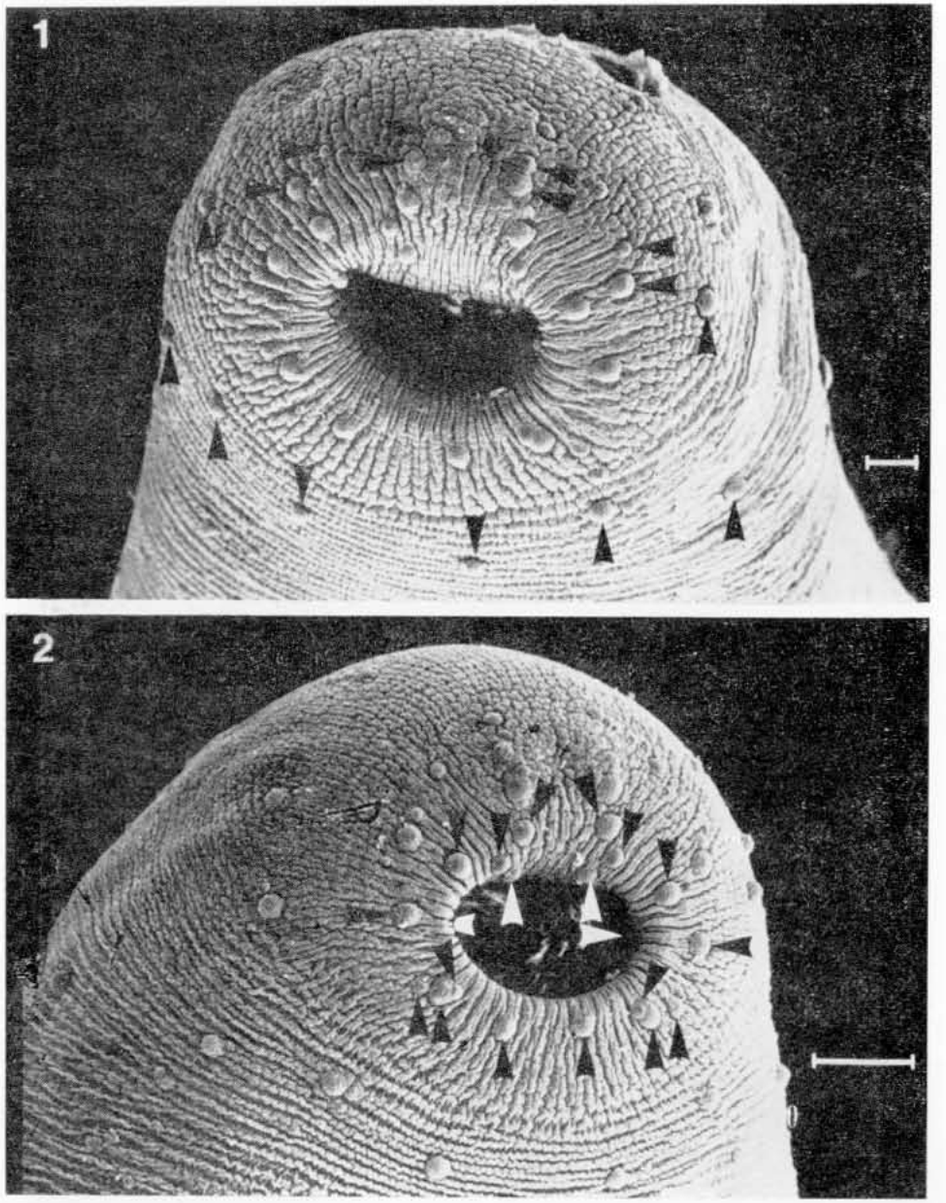
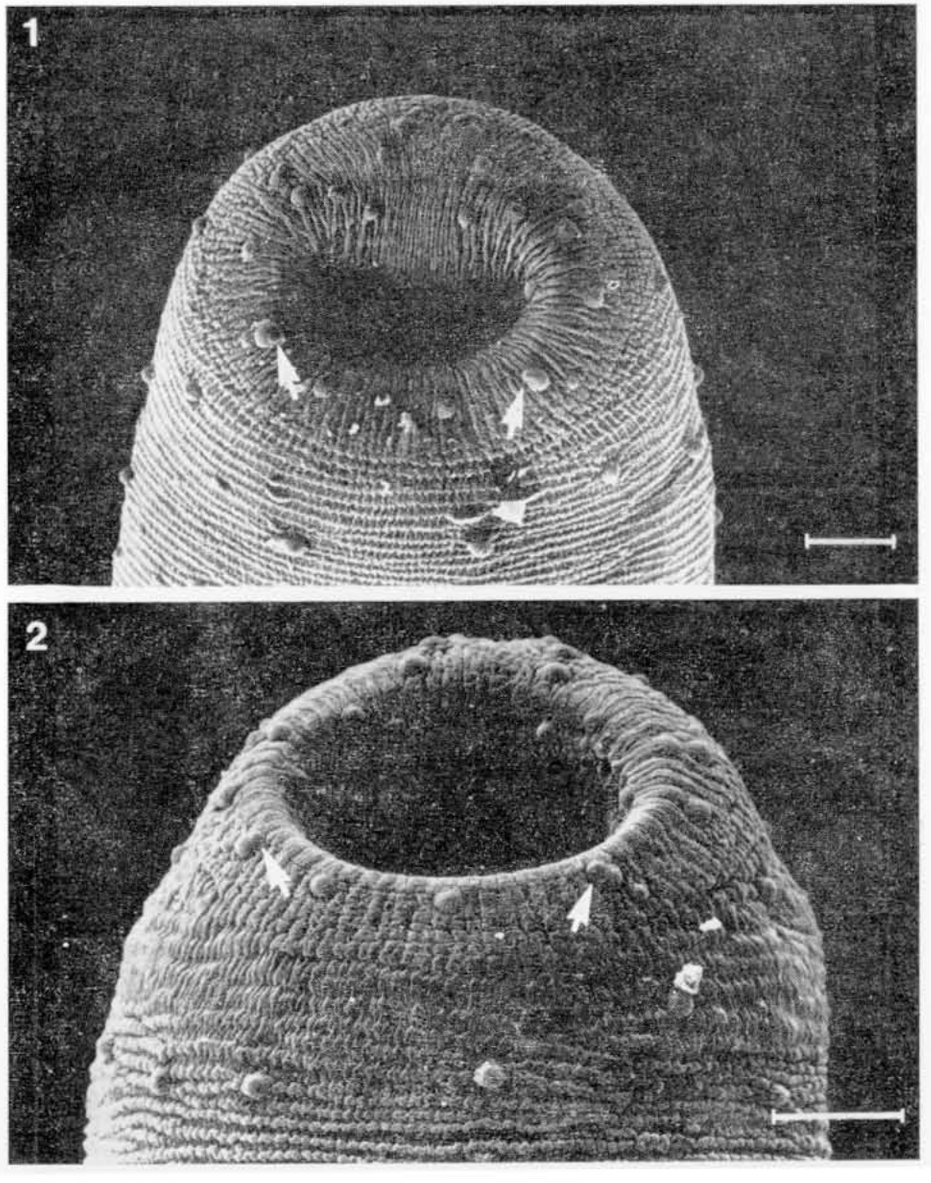


Fig. 1, 2. The papillary arrangement on the anterior ventral end on and around the oral sucker of *P. folium*, demonstrating the 16 consistent papillae around (Fig. 1, black arrows) and the 20 consistent papillae on the rim and within the oral sucker (Fig. 2, respectively black and white arrows) (Scale bars 20 μm).



Two specimens of *P. folium* demonstrating the little variability in the oral sucker papilla arrangement. White arrows, see text (Scale bars 20 μm).

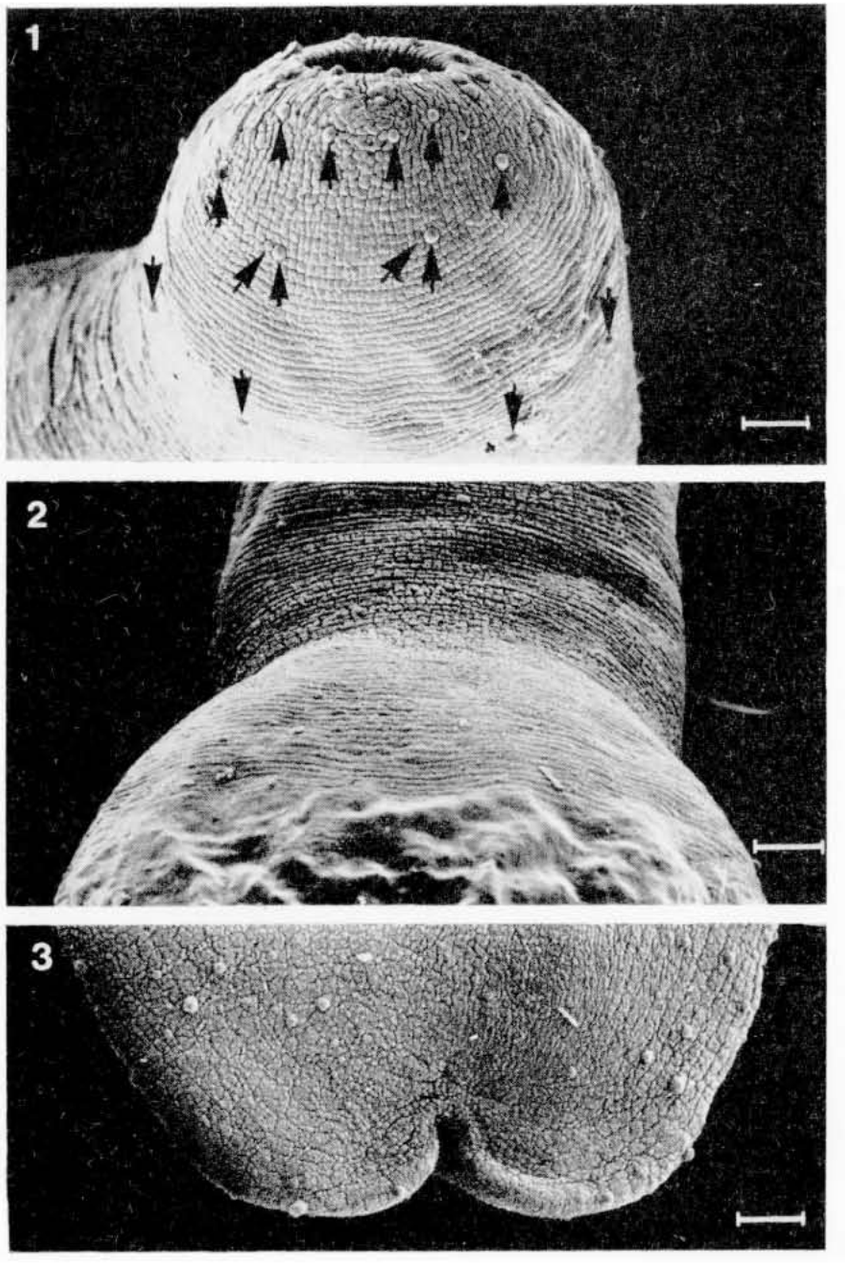


Fig. 1. The papillary arrangement dorsally on the anterior end of *P. folium*, demonstrating the 14 consistent papillae (black arrows). **Fig. 2.** The dorsal surface microtopography of the neck and posterior part, at level of shoulders. **Fig. 3.** The posterior notch as seen from the dorsal side (Scale bars 20 μm).