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HETERODOXUS SPINIGER (BOOPHIDAE, MALLOPHAGA) FROM *CANIS FAMILIARIS* FROM INDIA IN THE LIGHT AND SCANNING ELECTRON MICROSCOPES*

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HETERODOXUS SPINIGER (BOOPHIDAE, MALLOPHAGA) U *CANIS FAMILIARIS* Z INDII W MIKROSKOPIE ŚWIETLNYM I SKANINGOWYM

Abstract. Knowledge of morphometric characteristics of *Heterodoxus spiniger* was widened on the basis of material collected from dog (*Canis familiaris*) from India. Study, mostly by scanning electron microscope, concerned the males, females and nymphs of *H. spiniger*. The functional role of some elements, particularly observed surface ultrastructures, has been pointed out. The differences between postembrional stages were determined as well as literature data on *H. spiniger* were critically analysed.

INTRODUCTION

The genus *Heterodoxus* LE SOUEF et BULLEN, 1902 was described monotypically with the species *Heterodoxus macropus* LE SOUEF et BULLEN, 1902 as a parasite of „Kangaroos, wallabies etc". KÉLER (1971) reports that the marsupials: *Macropus giganteus* SHAW, *Wallabia agilis* (GOULD) and *Thylogale stigmatica* GOULD — all from the family of Macropodidae — are actually the hosts of that species.

Nowadays, the genus *Heterodoxus* contains 13 species; 12 of them parasitizing in Macropodidae and only one — *H. spiniger* (ENDERLEIN, 1909), morphologically related to the other ones, unexpectly is noted from carnivorous mammals (Carnivora: Canidae and Viverridae) (KÉLER 1971, EMERSON and PRICE 1975, 1981). Dog (*Canis familiaris*) is typical host (terra typica: Bechuane, Kalahari desert in Africa). EMERSON and PRICE (1981) list for *H. spiniger* the following hosts: *Canis familiaris* L. (includes the Dingo),

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C. latrans SAY, *C. aureus* L., *C. adustus* SUND., *Urocyon cinereoargenteus* (SCHREB.), *Genetta victoriae* THOMAS and *Viverra civetta* (SCHREB.). All faunistic--zoogeographical data indicate that *H. spiniger* is distributed in the area of hot climate. According to EICHLER's (1963) map it occurs in various parts of Africa, Malay Peninsula, Malay Islands, Japan and in the south-east part of Australia as well as in the Middle America, South America and southern part of the North America. KÉLER (1971) writes that *H. spiniger* was noted on *C. familiaris* from all continents, with the exception of Europe and Antarctic Continent.

Up to now, the descriptions of *H. spiniger* (including the data on its synonyms) concern only the imagines investigated in the light microscope. Detailed description of that species is given by KÉLER (1971) and the complete list of its hosts — by EMERSON and PRICE (1975). Also early SYMMON's (1952) paper with the numerous morphological and anatomical details of the head of *H. spiniger* should be noted. CLAY (1979) presents the SEM pictures of the representatives of Boopiidae, also of *H. longitarsus*** from the unknown host.

The purpose of that paper was the investigation by the scanning microscope of that interesting species with the regard to its characteristics and distribution. Having in hand all developmental stages (nymphs and imagines) of *Heterodoxus spiniger* from the dog from India we think that presentation of characters changing during the ontogenesis hastens the findings of some developmental regularities in the lice. Similar subjects have been already undertaken in Poland with regard to the surface ultrastructures of lice of Amblycera and Ischnocera from birds (MODRZEJEWSKA and ZŁOTORZYCKA 1987, ZŁOTORZYCKA and MODRZEJEWSKA 1992) as well as of the Ischnocera of mammals (ZŁOTORZYCKA 1990).

Material and methods

Some dozens of specimens of *H. spiniger* collected from dogs in India were investigated. Part of that material fixed in 70% ethyl alcohol was closed by conventional method in Canadian balsam and investigated in the light microscope (LM). The remaining specimens were prepared for the study in the scanning electron microscope (SEM) by the CPD method (Critical Point Drying). Photograms were made in SEM Tesla BS 300. We would like to express our sincere thanks to Mr MAREK CHMIELEWSKI for the making of the microphotographies in the Electron Microscope Laboratory of Microbiological Institute at the University of Wrocław.

** *H. longitarsus* PIAGET, 1880 was described from *Macropus giganteus*, but it was mistaken for very similar species *H. spiniger*, according to KÉLER's (1971) paper.

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RESULTS

Morphometric characters of the imagines and nymphs

The general shape of body of male and female of *H. spiniger* in LM is presented at the Figs 1 and 2. Measurements of adults and nymphs are given in the Table. The most important morphological characters of nymphs are as

TABLE
Measurements (in mm) of *Heterodoxus spiniger* from India

Measurements	males $n=10$	females $n=15$	Nymphs I inst. $n=3$	II inst. $n=1$	III inst. $n=7$
Length of head	0.38–0.45	0.39–0.45	0.24–0.27	0.30	0.37–0.40
Width of head	0.58–0.62	0.59–0.64	0.29–0.35	0.37	0.46–0.54
Width of prothorax	0.50–0.54	0.52–0.58	0.24–0.28	0.34	0.43–0.48
Width of abdomen	0.83–0.98	0.84–1.00	0.32–0.39	0.57	0.60–0.71
Total body length	2.20–2.70	2.29–2.63	0.77–0.99	1.27	1.58–1.89

follows: sufficiently wide and anteriorly rounded head of the nymphs I instar and short, sharply ended postpalpal processes. There are numerous setae of various length on the head, except for gular region. There is poor chaetotaxy on the thorax and abdomen. In the nymphs of II and III instars two pairs of setae appear on gular region. Also the number of setae increases on the edge of orbital grooves. In the adults postpalpal processes become elongated, very long and sharply ended. Chaetotaxy of the thorax and abdomen of those nymphs becomes gradually abundant in comparison with the nymphs of I instar.

Surface ultrastructures

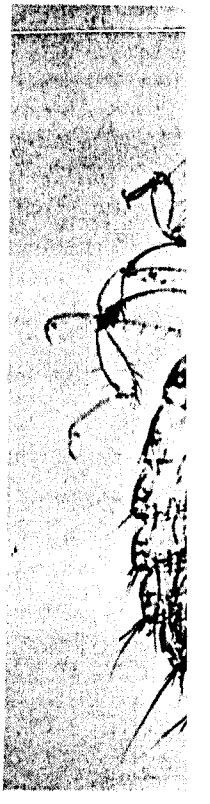
Ventral side of head is characterized, among others, by the presence of two postpalpal processes growing immediately behind the maxillary palpi. Those processes are considerably longer in relation to the head dimensions of adults (Fig. 3) than in the nymphs. Four-segmented maxillary palpi, like the whole mouth apparatus, are located in the anterior part of the head. The biggest is basal part of labium, but lying nearly its edge one segmented labial palpi are small and distinguishable from basal part only with over 2000 × magnification. There are numerous sensillae both on labial palpi as well as on anterior part of labium. The mandibles are not visible on the microphotograms of all developmental stages including the adults. They are covered by distally rugged sclerite of hypopharynx. This sclerite sometimes covers narrow, arched labrum. The labrum is turned inside out a little and close to anterior edge of clypeus. Behind the mouth apparatus the lyre-shaped big gular plate extends. It is more distinctly sculptured than the rest of the head. The sculpture in form of wide

covered scales is similar in the nymph and adult specimens. The antennary fossae with antennae are presented in the SEM in the peculiar and interesting way. It can be seen that the antennary fossa consists of two parts: anterior basin with antenna, and posterior large and empty basin behind the deep transverse groove. The antennae in all preparations have only two first segments. For example it is seen in one of the nymphs of the first instar (Figs 4 and 5). Both of two first segments are characterized by various surface sculptures consisting of tiny spines and protuberances as well as not numerous microchaetes. The second segment of antenna is nearly round, in distal part with quite numerous protuberances. Similar sculptures are seen on the adjacent surface at the bottom of antennary fossa.

Legs, whose picture in the LM does not show nothing peculiar, have variety adaptations to successful fixing of lice on host hairs. Indubitably, oblong area on the ventral side of tarsus with finger- and spine-like processes can serve this purpose (Fig. 6) as well as sculpture on ventral sides of both claws. The claws are not smooth but have thick, transverse and undulated furrows forming specific „grater". All those elements are arranged along ventral side of claw and are convex on sides and concave along middle line of claw. Furthermore, endings of claws are curved; the opposite sclerite ended with two spines can be seen at their base (Fig. 7). This sclerite seems to correspond to empodium or sucker known within other Amblycera. However, it is not membraneous form and its sculpture suggests quite well formed sclerotization. Another detail of unknown role is very small ring (sensillum?) visible between the mentioned spines.

The whole abdomen of I instar nymph is covered by tiny spines (Fig. 8). In the older developmental stages the sets of such spines occupy smaller and smaller parts of abdomen, and in the end, in the imagines, they are limited to narrow fields between sternites and pleurites. There they have complicated shapes (Fig. 9). Groups of spines, similar in appearance, have been noted also on the prosternal plate of the thorax (Fig. 10.). Adjacent surfaces between neighbouring pleurites in all developmental stages are provided with dense triangular spines going over laterally and gradually into tile-like plates. The abdomen of female has almost all sternites rectangle with the exception of trapezium-like one lying before short (reduced) last segment (IXth). All intersegmental sutures are straight. On the other hand, in the nymph of first instar the last abdominal segments are curved (Fig. 11). In the next developmental stages they become straight. Moreover, sculpture of ventral side of abdomen in N_I consists of central area between rows of double microchaetes with the tile-like sculpture. The remaining lateral surfaces are covered with minute spines. In the older nymphs and adult specimens the sculpture differentiates and the central area with weakly marked tile-like structures expands.

On the dorsal side of body, among others, the spiracles were observed: one pair of thoracic spiracles (Fig. 12) and six pairs of abdominal ones (Fig. 13). The



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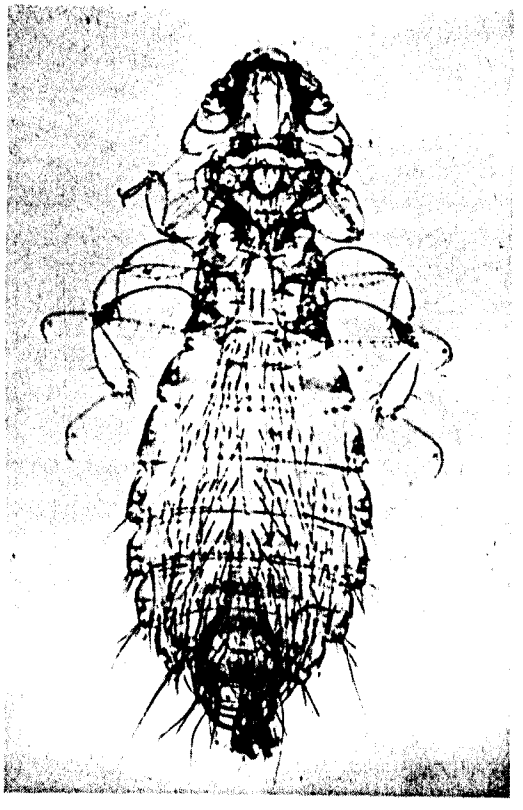

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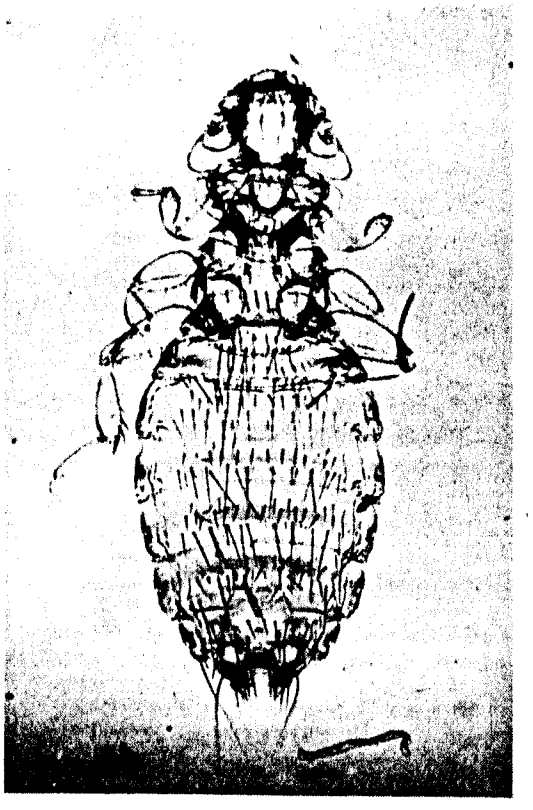

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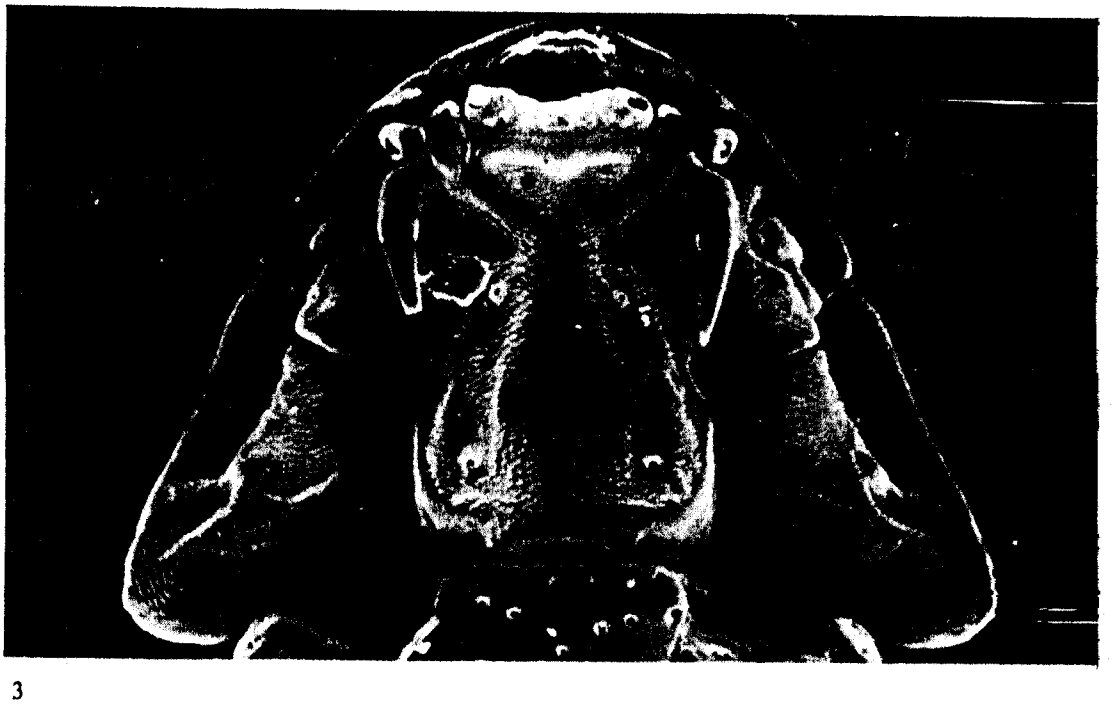

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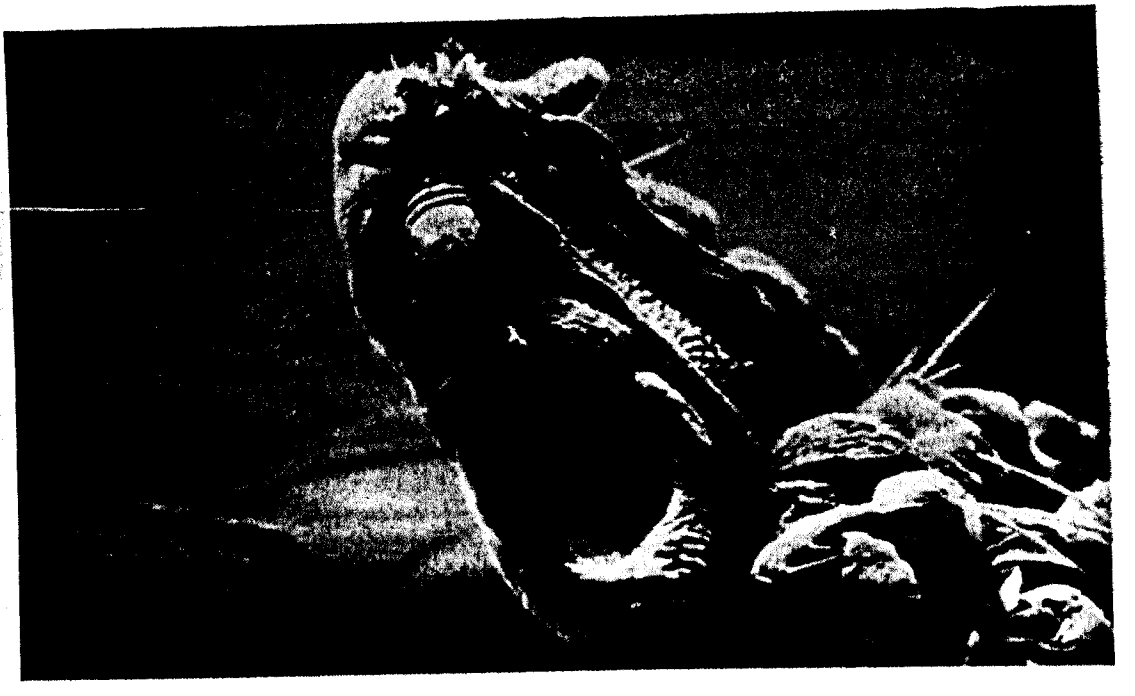

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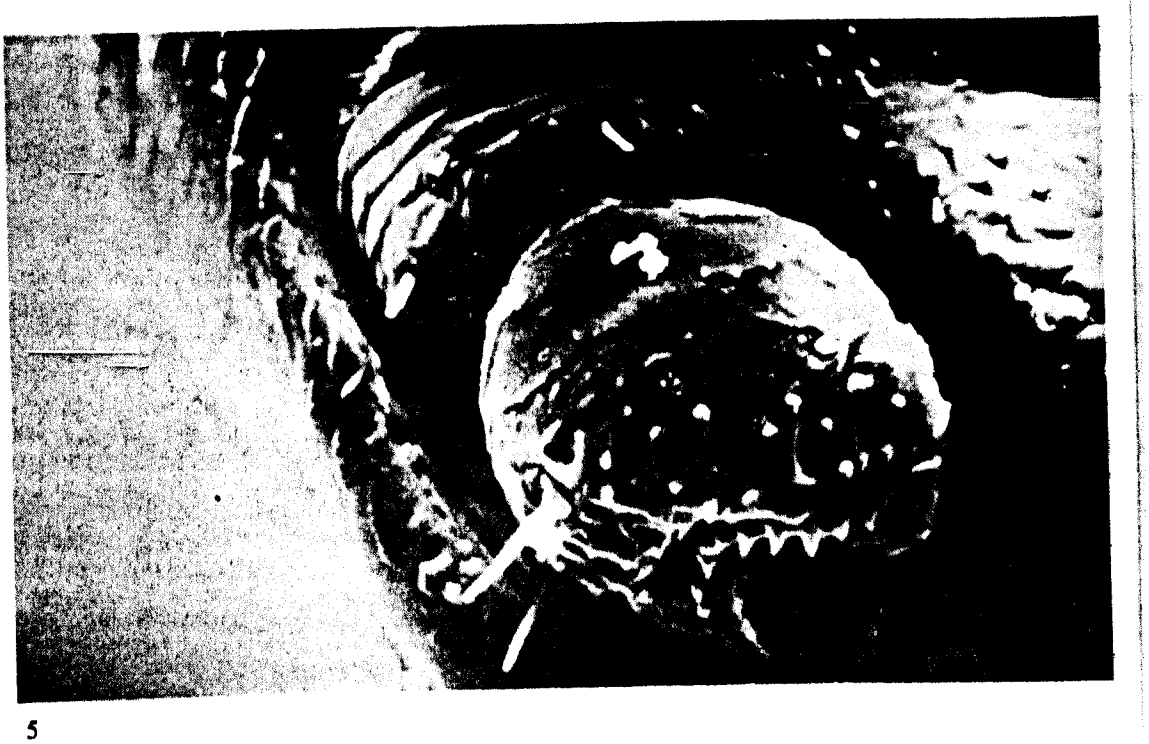

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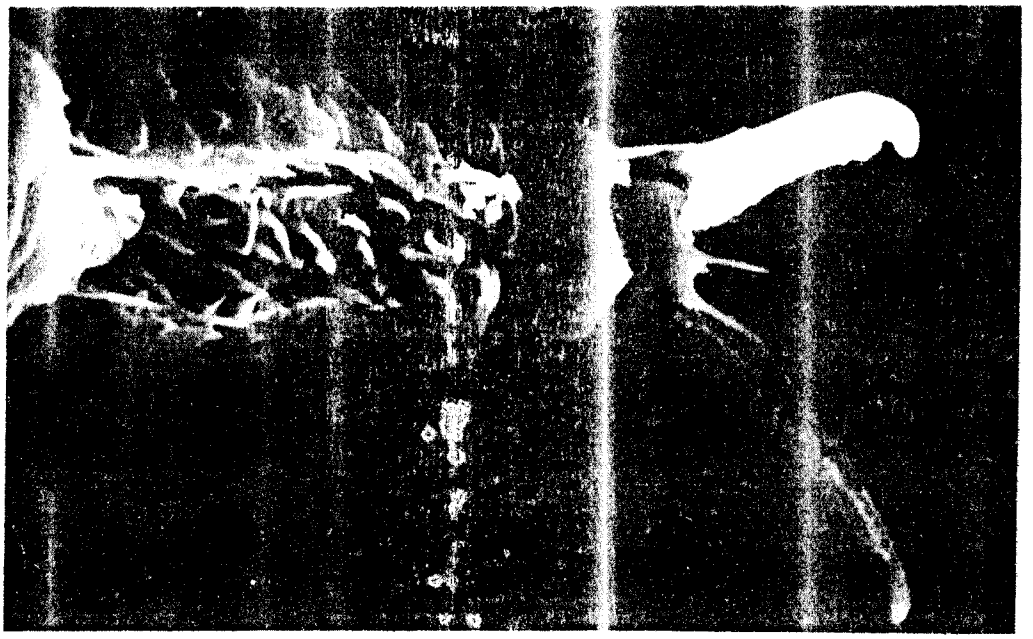

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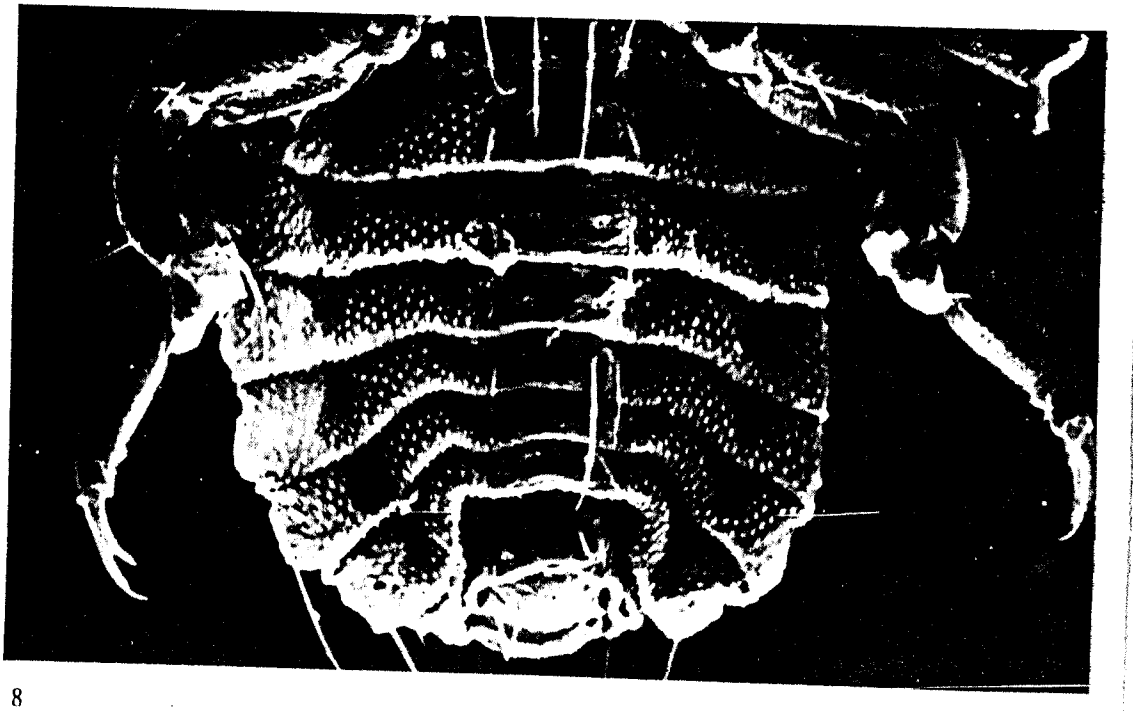

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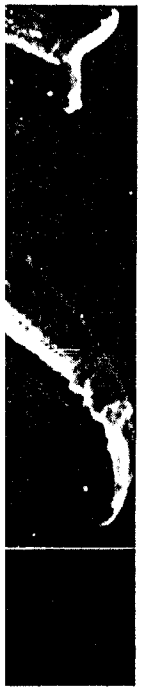

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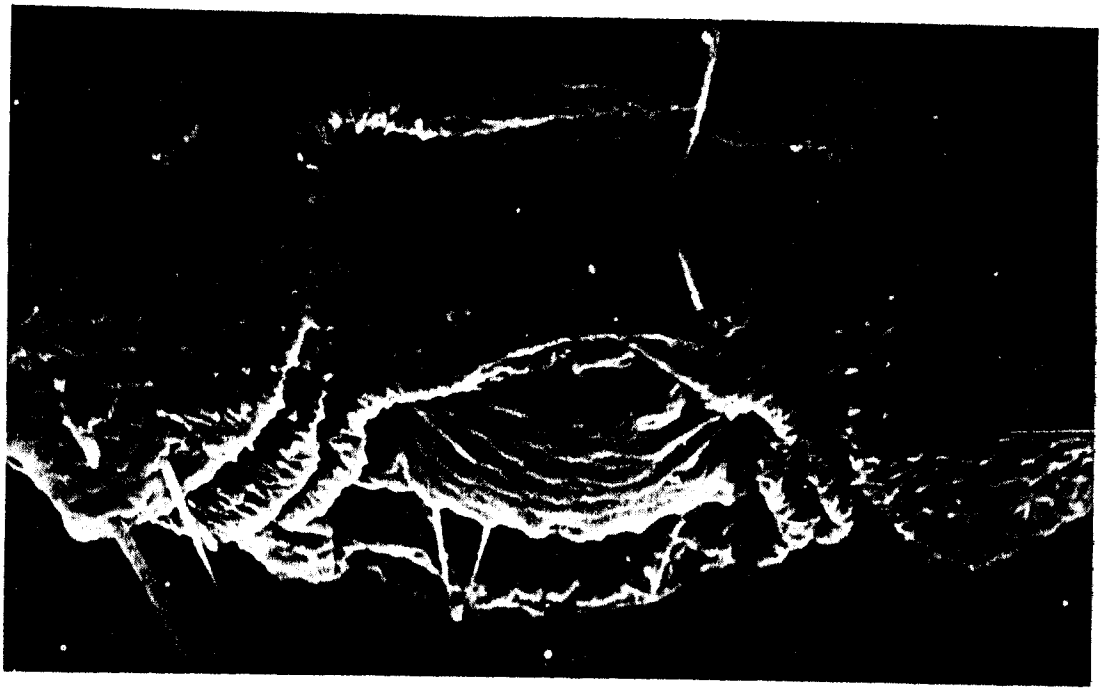

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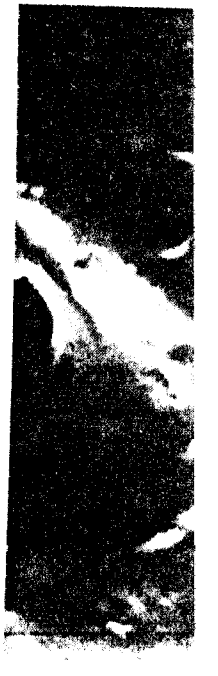

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Heterodoxus spiniger (ENDER.)

Fig. 1. Male in the light microscope
Fig. 2. Female in the light microscope
Fig. 3. Female's head, ventral view, in the SEM; magn. 380 ×
Fig. 4. Nymph's head, diagonal view, in the SEM; magn. 590 ×
Fig. 5. N_I antenna (fragment), in the SEM; magn. 3400 ×
Fig. 6. Tarsus with claws in N_I, ventral view, in the SEM; magn. 3200 ×
Fig. 7. Male's tarsus with claws, lateral view, in the SEM; magn. 1100 ×
Fig. 8. N_I abdomen, ventrally, in the SEM; magn. 460 ×
Fig. 9. Area with protuberances between pleurite and tergite of segment VII, in the SEM; magn. 6000 ×
Fig. 10. Protuberances on N_I prosternal plate, in the SEM; magn. 2000 ×
Fig. 11. The posterior part of N_I abdomen, ventral view, in the SEM; magn. 1220 ×
Fig. 12. Spiracle on the male thorax, in the SEM; magn. 7200 ×
Fig. 13. Spiracle on the segment II of male abdomen, in the SEM; magn. 2300 ×
Fig. 14. Sensillum with the thin hair on the pleurite of the segment II of male thorax, in the SEM; magn. 10 000 ×
Fig. 15. Two spines embracing the macrochaeta from behind; the central abdominal pleurite, in the SEM; magn. 5800 ×

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opening of thoracic spiracle is smooth, whereas all abdominal around the opening have the crown of protruding finger-like micro processes (they are visible only using above 2000× magnification). Moreover, at the picture of SEM the surrounding of the spiracles, particularly the abdominal ones, was always lighter than the rest of the body. In caudal part of the first three abdominal pleurites there is the round opening of similar magnitude as the nearby spiracle. A very thin and relatively long hair runs out of this opening (Fig. 14). Certainly all these structures together form a sense organ. Nearby, on the edges of every somatic pleurite, there are normal setae, visible also in the light microscope. The pleurites of segment IV do not comprise the mentioned sensillum with hair, on the other hand, where it was supposed two spines close each other arise. Both embracing from behind the macrochaeta located in row on caudal edge of the pleurites (Fig. 15). This pair of spines has the sensoric function, of course. At the ventral side of abdomen, where the pleurites adjoin either sternites and also at dorsal side tergites, there are oblong alveola. Their bottoms have similar sculptures as the neighbouring areas.

Discussion

The general shape and diagnostic characters of the males and females of *Heterodoxus spiniger* from the dog (*Canis familiaris*) from India correspond with the description and illustrations given by KÉLER (1971, Figs 17–20 and Fig. 118) and EMERSON and PRICE (1975, Figs 1–4). KÉLER (1971) reports also genital apparatus (Fig. 118) as well as posterior part of abdomen of male *H. spiniger* from *Wallabia agilis* (Fig. 119). However, the characters demonstrated on both figures seem to be not quite congruent with analogical details on the Fig. 4 of EMERSON and PRICE (1975) which has been cited for *H. spiniger* from *C. familiaris* in Venezuela in WERNECK's (1946) paper.

Metrical characters of *H. spiniger* from *C. familiaris* according to literature (KÉLER 1971) are a little different in the populations coming from various areas: total length of male and female from Paraguay is 2.64–2.83 and 2.94–3.07, respectively; from Ceylon (Sri-Lanka) – 2.62 mm of one examined male specimen; from Israel – 3.07 and 3.19 mm for one male and one female specimens, respectively. Our metrical data of *H. spiniger* males from India (Tab.) are only partly contained in those given by KÉLER (op. cit.), the female specimens being remarkably smaller. Similar zoogeographical relationships as well as metrical differences between one-species populations of lice from the Polish and Indian hens were shown by LONC et al. (1992).

The pictures of *H. spiniger* obtained in SEM allowed to distinguish more precisely the details on the ventral and dorsal body surfaces. Without any doubt it has been noted that big processes on the head are orbital grooves on ventral side of head, contrary to EICHLER's (1963) Abb. 134 of *H. spiniger* with this processes visible on the dorsal side. Moreover, both the pictures of the LM

and the drawings of ventral side of head, (e.g. SYMMONS 1952, Fig. 27) show the mandibles, whereas in all now investigated specimens they are hidden under hypopharyngeal sclerite. The same author demonstrates the mandibles covered in big part by sclerites (probably of hypopharynx). It can be seen at his pictures showing ventral sides of head of *Gliricola porcelli* (Fig. 25), *Trimenopon jenningsi* (=*T. hispidum*) (Fig. 26) belonging also to Amblycera from the mammals. It can be concluded that this is the character appearing rather among the Amblycera because it appears in the representatives of Gliricolidae and Trimenoponidae. Both families together with Boopiidae are joined within one superfamily Gyropoidea by EICHLER (1963). The apparently similar location of mandibles in this superfamily has to have functional grounds. The picture of *H. spiniger* seen now in the SEM and earlier by KÉLER (1971, Fig. 82) shows the biting apparatus surrounded by obtuse hypopharyngeal sclerites and by clypeus from the opposite side. Probably these appendices play a role supporting the perforation of host's epidermis by mandibles. At the KÉLER's figure (op. cit.) the whole four-segmented antenna is seen in the antennary fossa. The fossa is also deep, what we have succeeded to observe in SEM, though only two first segments of antenna were preserved in the investigated specimens. Whole antennae were not preserved also in all conventionally-made preparations for the observations in the LM. It was surely caused by notable narrowing between the second and third segment of antenna. Furthermore, at the Fig. 82 of KÉLER (1971) it can be seen that this is the point where the antenna is curved to the outside. The apical end of last segment of antenna in all biting lice species compries numerous sensillae. It can be expected that they look similar in *H. spiniger* as in *H. longitarsus* (CLAY 1970, Pl. I, Fig. 1) i.e. there are various forms of them (spines, cones etc.). Along fields with spine-like processes on ventral side of tarsi in all legs as well as furrowed inside surfaces of claws visible in SEM are not exclusive characters of *H. spiniger*. Similar structures in *Boopia grandis* were presented by CLAY (1970, Pl. 5, Fig. 25). The sculpture on ventral surface of claws is well visible in the LM in those Amblycera of mammals which have large, single claws, i.e. in Gyropidae. It can be seen on numerous figures in paper of EMERSON and PRICE (1975). Now it is difficult to establish the range of occurrence of „brushes" on ventral side of tarsi in Gyropoidea. Yet, the conclusion on their function can be drawn: they support the power of legs' sticking to host's hair. Analogical, but not homological, „brushes" on the legs (but on femora) are met in some Ischnocera. They were noted in *Docophoroides brevis* (Docophoroididae) by ZŁOTORZYCKA and MODRZEJEWSKA (1992). The grasp of claws in *H. spiniger* is also conditioned by the presence of their curved ends and by opposed sclerite (empodium) ended with two spines.

Sculpture of the thorax and abdomen in *H. spiniger*, especially the variety of the surface ultrastructures, shows the series of similarities to the ultrastructure of *H. longitarsus* and *Paraheterodoxus insignis* illustrated by CLAY (1970,

Pl. 5, Figs 26 and 27). According to our own studies the development and differentiation of the ornamentation into the plates and groups of spines near intersegmental surfaces occur in *H. spiniger* gradually during postembrional development. Interesting is our observation that the monomorphic spine-like structures are the first form of sculpture (presented on the whole abdomen in the nymph I instar) which disappear partly in central part of abdomen and differentiate with regard to shape and length on the sides of the older nymphs. In the adults they occupy only the spaces between pleurites.

Particular attention in *H. spiniger* can be paid to single sensillum consistig of long thin hair arising from vast opening. This organ is not homological with hair on pleurite II of abdomen in *H. longitarsus* (see CLAY 1970, Fig. 29) because of the lack of two small setae present in the CLAY's figure. Such short double setae were stated in our SEM pictures near other pleurite setae on those segments which did not posses the mentioned sensillum with long thin hair.

Spiracles on abdomen are visible both on CLAY's photos as well as on our pictures from SEM in much brighter area than remaining background. The frame of microviiii crown around each spiracle belongs to new details found by us. Perhaps they protect respiratory system against penetration of strange particles.

Male genital apparatus, in lice usually hidden inside the abdomen, in *H. spiniger* was otfen protruding. Thanks to this, it was possible to trace its end part turned dorsally. It indicates indirectly the male disposition to subfeminal position during the copulation. This position is known in many Ischnocera lice from mammals (ZŁOTORZYCKA 1994). In Amblycera it may be different, i.e. subfeminal or suprafeminal male position. Unfortunately even those scanty data do not concern Boopiidae family.

Conclusions

1. Differentiation of successive nymphal instars in *Heterodoxus spiniger* is possible even when using the light microscope. Pictures from the scanning electron microscope add the complementary information.

2. Described surface ultrastructures of *H. spiniger* allow for the interpretation of their functions. Particularly, it concerns the structures supporting the sticking of parasites to host hairs.

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