Anatomy of the Feather-Winged Beetles *Acrotrichis montandoni* and *Ptilium myrmecophilum* (Coleoptera, Ptiliidae)

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Abstract—The internal morphology of adults and larvae of *Acrotrichis montandoni* and adults of *Ptilium myrmecophilum* is described on the basis of the study of LM and TEM of serial sections and total preparations. Specific structural traits related to miniaturization are revealed, the most essential ones are the absence of midgut muscles, reduction of two Malpighian tubules, absence of heart, general reduction of the circulatory system (which is replaced by the fat body), absence of tracheal system in the larval abdomen, strong oligomerization and concentration of the nervous system in the adult; reduced size and number of nerve cells; and reduction of the right testis and ovary. The possible factors limiting the further miniaturization in Ptiliidae are the egg size and the volume occupied by the nervous system.

The family Ptiliidae comprises the smallest coleopterans and the smallest non-parasitic insects. Representatives of this family are only 300 μm long, exceeding only some parasitic hymenopterans. The body size, especially approaching the lower limit, is an important parameter that largely determines the morphology, physiology, and biology of a species. The anatomical traits related to miniaturization have been considered for many vertebrates, but are still practically unknown for insects (Schmidt-Nielsen, 1987; Hanken and Wake, 1993). The problem of factors limiting the size of insects is important from the viewpoint of general entomology as well. There are many hypotheses explaining the upper size limits (Chernyshev, 1996), whereas the lower limits are suggested to be set by the number and size of cells in the insect body. Thus, the anatomy of Ptiliidae may be of considerable theoretical importance.

The internal morphology of adult feather-winged beetles is practically unknown. There are only two publications on the structure of the genitalia (De Coninck and Coessens, 1982; De Marzo, 1992), some specific data on the sperm morphology (Dybas and Dybas, 1981, 1987), and several descriptions of the metendosternite in some representatives of the family (Crowson, 1938, 1944; Sorensen, 1997; Hall, 1999). The only report dealing with the larval anatomy covers the internal topography (Grebbennikov and Beutel, 2002). Considering the recent views on the taxonomic position of Ptiliidae (Lawrence and Newton, 1995; Hansen, 1997), the data obtained for feather-winged beetles will be compared with those for other members of Staphylinioidea.

**MATERIALS AND METHODS**

I have studied adults and larvae of *Acrotrichis montandoni* (Allibert 1844) and adults of *Ptilium myrmecophilum* (Allibert 1844), collected in Moscow Province in July 2002. The insects were fixed and embedded in Epon 812, following standard procedures (Valovaya and Kavtaradze, 1993; Mironov et al., 1994).

The anatomical studies proceeded in two stages. First, series of longitudinal and transversal semithin (3 μm) sections were obtained using an LKB Pyramitome. These sections were stained with methylene blue and eosin, embedded in Epon, and examined using light microscopy. Details of internal morphology were further studied in ultrathin sections using TEM. In addition, total preparations of some organ systems were studied.

Designations in figures: *ag*, abdominal ganglion; *ar*, anterior tentorial rami; *ct*, corporotentorium; *cx*, coxa; *dlm*, dorsal longitudinal muscle; *dr*, dorsal tentorial rami; *dvcm*, dorsoventral muscle; *dwvm*, dorsoventral wing muscle; *e*, eye; *ec*, egg cavity; *es*, egg sac; *fb*, fat body; *il*, ileum; *lg*, labial gland; *mg*, midgut; *mgp*, midgut pouch; *mpm*, musculature of mouthparts; *mt*, Malpighian tubule; *mtg*, mesothoracic ganglion; *mv*, microvilli; *np*, neuropile; *oes*, oesophagus; *ov*, ovari-ole; *pc*, pouch cavity; *ph*, pharynx; *phm*, pharyngeal muscle; *pr*, posterior tentorial rami; *ptg*, prothoracic ganglion; *ptm*, peritrophic membrane; *rc*, rectum; *spg*,
subpharyngeal ganglion; sppg, suprapharyngeal ganglion; t, tentorium; vlm, ventral longitudinal muscle.

RESULTS

Adult Morphology

The two species studied, *Acrotrichis montandoni* and *Ptilium myrmecophilum*, belong to different subfamilies (Acrotrichinae and Ptiliinae), represent different ecological groups (eurybionts and myrmecophilous stenobionts, respectively), and clearly differ in body size (0.8 and 0.5 mm, respectively). However, their internal morphology, except for dimensions, was largely the same.

As can be seen from the general scheme (Fig. 1), the median area of the body cavity is occupied by the intestine; muscles occupy most of the thorax, and the space between all the organs is filled with the fat body. The only large cavity in the body is the egg sac in females; the nervous system is strongly oligomerized and concentrated in the thorax; the genital system occupies a considerable portion of the posterior abdominal segments; the heart and septa are absent.

Individual organ systems are considered in detail below.

The integument of feather-winged beetles consists of the cuticle, hypoderm, and basal lamina. The cuticle is 3–6 μm thick and consists of the epicuticle (about 70 nm) and indistinctly separated exo- and endocuticle. The last two layers have a lamellar structure except for intersegmental areas where they are homoge-
Fig. 3. Internal morphology of adult of *A. montandoni* (transversal sections): (1) anterior part of head; (2) mesothorax; (3) base of abdomen; (4) apical portion of abdomen. Scale bar 0.1 mm.
The hypoderm is 2–3 μm thick and built of flattened cells.

The main *endoskeletal* elements of adult coleopterans are the tentorium and metendosternite. The tentorium of feather-winged beetles, as well as in other members of Staphylinoidea, consists of the base, corporotentorium, and anterior, dorsal, and posterior rami (Fig. 2, 1); however, this group differs from other Staphylinoidea in the absence of the laminotentorium (Naomi, 1987). The metendosternite, which is regarded as a conservative structure in the higher taxonomy of beetles (Crowsen, 1938, 1944), is represented by several variants in Ptiliidae. The metendosternite of most of Ptiliidae has two broadly separated branches (Fig. 2, 2), and in Nanosellinae it is strongly reduced (Hall, 1999). All the variants are characterized by the absence of a common stalk, which distinguishes Ptiliidae from the majority of Staphylinoidea (Naomi, 1989). This feature may be explained by the small body size and broadly separated coxae of feather-winged beetles.

The *digestive system* of feather-winged beetles generally follows the structural plan common to all insects. The alimentary canal is subdivided into the foregut, midgut, and hindgut. The fore- and hindgut are of ectodermal origin and have thin cuticuline lining; the midgut is endodermal and has no such a lining. The alimentary canal is somewhat longer than the body and forms a loop in the metathorax. Of the digestive glands, only labial salivary glands were found in feather-winged beetles (Fig. 3, 1).

The foregut is subdivided into the pharynx and oesophagus, while the crop and muscular stomach are absent. The pharynx is rather wide and has powerful muscles (Fig. 3, 1), which may indicate ingestion of semi-liquid food (Pavlovsky, 1956). The straight oesophagus is 25–35 μm in diameter in *A. montandoni* and 20–25 μm in *P. myrmecophilum*. It has one layer of circular muscles and thus differs from the oesophagus of other beetles, which also possesses a layer of longitudinal muscles (Crowsen, 1981).

The midgut is shaped as a tube with numerous short pouches, which are somewhat longer and directed forwards in the cardial area. The diameter of midgut is 110–150 μm in *A. montandoni* and 70–90 μm in *P. myrmecophilum*. The midgut wall consists of a single homogenous layer of epithelial cells. A transversal section reveals 10–15 cells (Figs. 1; 3, 2; 4, 1, 2), which is less than in other insects. A thin monolayered peritrophic membrane is present along the entire midgut. No midgut muscles have been found, and propulsion of food may be performed by the somatic muscles. This feature distinguishes Ptiliidae from other beetles, which possess two layers of midgut muscles (Crowsen, 1981).

The hindgut is subdivided into the ileum and rectum (Fig. 3, 3, 4). The ileum is 30–40 μm in diameter in *A. montandoni* and 20–30 μm in *P. myrmecophilum*, while the cross-section of the rectum is oval-shaped (70 × 30 μm and 35 × 22 μm, respectively).

At the boundary of mid- and hindgut, there are two *Malphigian tubules*. In this feature Ptiliidae differ from the rest of Staphylinoidea, which have four tubules. The tubules are straight and extend in parallel to the hindgut, nearly reaching the abdomen apex (Figs. 1; 3, 3, 4). Their diameter is 20–25 μm in *A. montandoni* and 15–20 μm in *P. myrmecophilum*.

The *internal tissues* of insects are the hemolymph and the fat body. Because of very small size, the circulatory system of Ptiliidae is strongly reduced, the heart is absent, and the hemolymph occupies a small fraction of the body cavity. This reduction may be accounted for by the fact that transport by diffusion is...
sufficient for a small-sized organism. The fat body in feather-winged beetles occupies almost all cavities, replacing the hemolymph (Figs. 1, 3). A similar situation is observed in many mites, especially small ones, in which the hemolymph is completely replaced by parenchymatous tissue that functions as both the fat body and the hemolymph (Silvere and Stein-Margolina, 1976). The fat body consists of variously shaped cells 8 to 20 μm in size with electron-lucent inclusions (Fig. 5, 1). As in other insects, including Staphylinidae (Carstens and Storch, 1980), the volume of the fat body strongly depends on the physiological state of an organism. The body cavity was found to contain oenocytes; these cells are present in many insects (Shvannvich, 1949) but their functions are still obscure.

The tracheal system of Ptiliidae is strongly reduced. There are only few weakly branching tracheae originating from the spiracles. The longitudinal and transversal branches as well as the air sacs are reduced. With such a small size, simple diffusion is sufficient for oxygen transport. The tracheae have a typical structure. They consist of the hypoderm and intima, and the latter includes spiral thickenings, or tenidia.

The nervous system consists of the central, peripheral, and sympathetic parts. The CNS of feather-winged beetles shows strong oligomerization and concentration. The suprapharyngeal ganglion occupies almost the entire posterior half of the head, while the subpharyngeal ganglion has shifted into the prothorax and merged with the prothoracic ganglion. The mesothoracic ganglion is separate, and the abdominal ones are merged with the metathoracic ganglion. The morphology of the ganglia in Ptiliidae follows the standard pattern, with a central neuropile and peripherally arranged neuron bodies (Fig. 5, 2, 3). The neuropile is homogenous, without distinct nuclei. The neuron bodies in feather-winged beetles are 2–4 μm in diameter, whereas in other insects their size is 6–50 μm (Plotnikova, 1979; Svidersky, 1980). Neurons of similar size (2 μm) were found in Tetrapodili mites (Silvere and Stein-Margolina, 1976). The nucleus occupies 80–90% of the neuron body. The approximate number of neurons in a ganglion can be determined from the mean size of the neuron body and the number of nuclei revealed in a transversal section. According to these calculations, A. montandoni has about 3000 neurons in the suprapharyngeal ganglion, about 2500 neurons in the subpharyngeal–prothoracic synganglion, about 500 neurons in the mesothoracic ganglion, and about 1200 neurons in the metathoracic–abdominal synganglion. Thus, the CNS of Ptiliidae contains approximately 10⁴ neurons; this number is considerably smaller than the average one for insects (10⁵–10⁶) and much smaller than that for higher vertebrates (10¹⁰) (Svidersky, 1980; Mandelstam, 1983). Despite the small size and number of neurons, the CNS occupies a relatively large volume. In particular, the brain occupies 1/70 of the body volume in A. montandoni and 1/50 of the

![Fig. 5. Ultrastructure of adult of A. montandoni (TEM): (1) cells of fat body; (2) peripheral and (3) intermediate zones of suprapharyngeal ganglion. Scale bar 5 μm.](image)
body volume in *P. myrmecophilum*. This fraction is much greater than in other insects; for example, the brain-to-body volume ratio is 1/4200 in *Dytiscus*, 1/280 in *Formica*, and 1/174 in *Apis* (Wigglesworth, 1953).

Because of the very small size of the insects, their peripheral nervous system could not be studied. Elements of the sympathetic system were not revealed, either; it is possible that they merge with the CNS.

The **musculature** of feather-winged beetles, despite their small size, follows the pattern common to all insects. All the main groups of muscles are present: muscles of the mouthparts; thoracic longitudinal (dorsal and ventral), dorsoventral, and pleural muscles as well as leg muscles; abdominal longitudinal (dorsal and ventral) and dorsoventral muscles; muscles of the genitalia. The main difference lies in the number of myofibrils. The wing musculature is well developed and occupies a considerable part of the thorax. The sarcomere length is about 4 μm, which is close to the average value for insects (Mandelstam, 1983).

The **male reproductive system** consists of an unpaired (left) testis, sperm duct, and copulatory apparatus. The spermatozoid of *A. montandoni* is 8–9 μm long, though in some species of *Ptiliidae* spermatozoa can be longer than body (Dybas and Dybas, 1981, 1987; De Marzo, 1992).

The **female reproductive system** consists of the unpaired (left) ovary, oviduct, receptaculum seminis (spermatheca), and accessory glands (Fig. 1, 3, 3). The ovary comprises 2–4 teletrophic ovarioles. The oviduct dilates into the egg sac where egg development takes place. Only one egg undergoes development at any given moment; a mature egg prior to oviposition occupies up to 1/3 of the body volume.

**Larval Morphology**

The internal morphology of the larva of *Acrotrichis montandoni* differs only insignificantly from that of *Pitella tenella*, described in the literature (Grebennikov and Beutel, 2002), and therefore it will be only briefly considered here. A large part of the body cavity is occupied by the gut (Fig. 6). Muscles occupy a considerable part of the thorax, the space between all the organs is filled with the fat body, and the heart and septa are absent.

The **integument** of feather-winged beetle larvae consists of the cuticle, hypoderm, and basal lamina. The cuticle is very thin (1–3 μm) and weakly sclerotized.

The main **endoskeletal** element of coleopteran larvae is the tentorium. In feather-winged beetles, as in the rest of Staphylinoida, it consists of the base, corporotentorium, and dorsal and posterior rami; the difference from other Staphylinoida is the absence of anterior rami (Beutel and Mølenda, 1997). Similar structure of the tentorium was described for the larvae of other small beetles, for example, *Sphaeriusidae* (Beutel et al., 1999).

The **digestive system** of feather-winged beetle larvae generally follows the structural plan common to all insects, similar to what is observed in the adult.

The foregut is subdivided into the pharynx and oesophagus; the crop and muscular stomach are lacking. The oesophagus is straight, 10–18 μm in diameter. Unlike other beetles, it has one layer of circular muscles (Crowson, 1981).

The midgut is tubular and has two lateral pouches in the cardiac area, directed anterior (Fig. 6, 1, 2). The midgut is 65–80 μm wide in its anterior and middle portions, and slightly narrows backwards. Its wall consists of a monolayered epithelium; a transversal section reveals 8–14 similar cells, which is much less than in other insects. A thin monolayered peritrophic membrane is present along the entire midgut. Midgut muscles were not found.

The hindgut is subdivided into the ileum and rectum, which are 25–30 and 35–45 μm in diameter, respectively.

Two **Malpighian tubules** originate at the boundary between mid- and hindgut. They run parallel to the ileum and bend together with it (Fig. 6, 1, 3). The diameter of the tubules is 20–25 μm. *Pitella tenella* was reported to have four Malpighian tubules (Grebennikov and Beutel, 2002), which may indicate either variability of this character within the family, or an error in reconstruction.

As in adult feather-winged beetles, the **circulatory system** of the larvae is reduced, the heart is absent, and hemolymph occupies only a very small fraction of the body cavity. The **fat body** occupies almost all cavities, replacing the hemolymph.

The **tracheal system** is strongly reduced. Larvae of most species of *Ptiliidae* have only one pair of spiracles, located between the pro- and mesothorax, and a few tracheae in the head and prothorax. Other thoracic segments and the abdomen have no tracheae.
Since the larval integument is thin and weakly sclerotized, one may assume that the larvae have a mixed tracheo-cutaneous type of respiration.

The central nervous system of feather-winged beetle larvae shows some oligomerization and concentration of ganglia, though to a much lesser degree than in the adults. The suprapharyngeal ganglion occupies most of the posterior half of the head and a considerable portion of the anterior half of the prothorax (Fig. 6, 1), as it is observed in the larvae of other small beetles (Beutel and Haas, 1998; Beutel et al., 1999).

The subpharyngeal ganglion is located in the prothorax and almost merged with the prothoracic ganglion, while the meso- and metathoracic ganglia are separated. The ganglia of five anterior abdominal segments are separate, and those of segments VI–VIII are merged and located in segment VI. The ganglia of larval Ptiliidae, as well as those of the adult forms, have the usual morphology. The neuron bodies lie in the periphery, with neuropile in the central area. The neuropile is homogenous, without distinct nuclei. The average size of neuron bodies is 1–3 μm.
Because of very small size, the peripheral nervous system of the larvae could not be studied. Elements of the sympathetic system were not revealed; they may merge with the CNS.

The musculature of feather-winged beetles, despite their small size, follows the general pattern of all insects. All the main groups of muscles are present: muscles of the mouthparts; thoracic longitudinal (dorsal and ventral), dorsoventral, pleural, and leg muscles; abdominal longitudinal (dorsal and ventral) and dorsoventral muscles. The main differences are observed in the number of myofibrils.

**DISCUSSION**

The results obtained indicate that miniaturization leads to the most extensive transformation of the systems related to metabolism (digestive, circulatory, and tracheal ones), whereas the nervous and reproductive systems undergo the least changes.

With small body size, effective circulation of the hemolymph is hampered by capillary forces, because of which the heart in feather-winged beetles is reduced and the hemolymph is replaced by the fat body. The absence of a specific transport system is compensated for by high efficiency of diffusion. A similar situation is observed in many mites, especially small ones (Silvere and Stein-Margolina, 1976).

Miniaturization leads to reduction of the relative surface area of the gut epithelium. In feather-winged beetles, this reduction is compensated for by the development of numerous short midgut pouches. In addition to absorption, the pouches may facilitate transport of nutrients, reducing the distance from the gut to other organs.

Very small body size, on the one hand, increases the efficiency of passive tracheal respiration, and on the other hand, does not allow active aeration of the tracheal system. Consequently, this system is simplified in the adult beetles, which usually have 9 pairs of spiracles; the larvae, possessing only one pair of spiracles, partly utilize cutaneous respiration, facilitated by 100% ambient humidity and thin non-sclerotized integument.

Thus, the main changes in the metabolism-related systems can be explained by increased efficiency of diffusion accompanying miniaturization. The absence of qualitative changes in the nervous system indicates that principal modifications of this system are impossible.

The factors limiting miniaturization should be considered not only for Ptiiidae but also by comparing them with smaller insect groups (Mymaridae and Trichogrammatidae) and the smallest mites (Tetrapodili).

The first hypothesis concerning these factors deals with the size of egg, which decreases at a lower rate than the body size. The egg of Ptiiidae occupies up to 1/3 of the body volume, and it may be the egg size that limits further miniaturization in this group. This limit is less evident in small parasitic hymenopterans, whose larva develops in the host body and whose eggs can therefore store only a small reserve of nutrients. The same problem is solved in a different way in Tetrapodili: the intestine of these mites is resorbed during egg development, so that the egg occupies most of the body volume (Silvere and Stein-Margolina, 1976).

The second hypothesis considers the size of the nervous system. The neurons in Ptiiidae are much smaller than those in other insects, and approach the minimum value corresponding to the size of the nucleus. The number of neurons in feather-winged beetles also decreases but the relative volume of the CNS is still greater than in other insects. It may be concluded that further decrease in the volume occupied by the nervous system is impossible. The body size of Tetrapodili can be reduced below this limit due to their simple behavior and different structure of their CNS. The structure of the nervous system in small parasitic hymenopterans is poorly known; therefore its miniaturization cannot be discussed.

Thus, the hypothesis stating that miniaturization of insects is limited by the number and size of cells, can be confirmed by the example of Ptiiidae only for the nervous system.

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