SCANNING ELECTRON MICROSCOPIC STUDIES ON THE CORDON STRUCTURES OF SEVEN ACUARIID GENERA (NEMATODA: ACUARIIDAE)

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Abstract. The cordon structures of seven acuariid species belonging to seven different genera, namely Paracuaria tridentata, Dispharynx nasuta, Cosmocephalus obvelatus, Decuratorialia dioanthes, Acuria anthuri, Chevreuxia rosickyi and Skrjabinocara squamata, have been studied under SEM. Of interest are three cord-like structures forming the cordon in D. dioanthes, and absence of transverse rows of minute spines covering the cordon in S. squamata. The present hypothesis believes that evolution of Acuariinae followed primarily two lines with Paracuaria at the base. Acuria with Skrjabinocera form an independent branch. From Dispharynx two evolutionary lines are evident. One is followed by Synthirinae, Decuratorialia, Chevreuxia, Echinuria and Skrjabinocara, while the other by Symphistis, Pactinospira, Cosmocephalus, Sexansocara, Skrjabinocara and Desportesius.

Cordon-pattern is the most important diagnostic and systematic separating feature of the genera belonging to Acuariinae (Yamaguti 1961, Skrjabin et al. 1965). Stress has been laid on specific type-oriented cordon leading to the establishment of a genus. Though a number of acuariid species have been described dealing with cordon structures, yet there are some lacunae in the ultrastructure of this cephalic feature which will entail a relationship and phylogeny of this subfamily (Osehe 1955, 1968; Chabaud and Petter 1959). The purpose of this paper is to describe the results of examination with scanning electron microscope (SEM) of the cordon structure of seven acuariid species belonging to seven genera as also the cervical papillae of Paracuaria tridentata. An attempt has also been made to draw and discuss the mode of evolution in this subfamily. It is the first endeavours of its kind to make a comprehensive study with SEM on the ultrastructure of nematodes of Acuariinae and its application in taxonomy.

MATERIAL AND METHODS

Specimens of Chevreuxia rosickyi Baruš et Majumdar, 1975 — host: Threskiornis melanocephalus from India and Dispharynx nasuta (Rudolphi, 1819) — host: Clamator jacobinus and Alecto atthis from India which had previously been fixed in 70% alcohol, as also Skrjabinocara squamata (Linstow, 1883) — host: Phalacrocorax auritus from Cuba; Cosmocephalus obvelatus (Crepin, 1825) — host: Larus argentatus and Ajaja ajaja from Cuba, Acuria anthuri (Rudolphi, 1918) — host: Corvus frugilegus from Czechoslovakia and Paracuaria tridentata (Linstow, 1877) — host: Larus argentatus from Cuba previously fixed in 4% formalin were used in this study. The first two species were dehydrated in graded alcohols. Specimens belonging to the latter five species were washed thoroughly in distilled water and then processed through upgraded alcohols, and thus dehydrated. Samples of about

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0.5 mm in length were prepared from the anterior part of body of each species with a base under a binocular and fixed on copper specimen stubs already containing minute quantity of glue prepared from adhesive of collophane tape to which acetone or xylene was pipetted. Orientation of the samples was done while fixing the specimens on the stubs. On drying, these stubs containing samples were quenched in liquid nitrogen for about 1—2 minutes. The samples were coated first with a thin layer of carbon and then with a layer of gold in a vacuum evaporator. The preparations were examined in the ASID-1 scanning device used with the JEM 100B electron microscope at an accelerating voltage of 20 kV (for C. rosickyi) and 40 kV (for rest of the species) and micrographs were taken. Magnifications of micrographs are specified in each case.

RESULTS

Observations on the fine structures of the cordons were made along with that of the ultrastructure of the cervical papilla of *P. tridentata*. Following are the results based on SEM studies and are arranged in accordance with their phase of complexities in cordon structures.

1. *P. tridentata*: The two lips and two pseudolips are connected by the translucent membrane-like cordons extending beyond the cephalic papillae. The cordons are situated deeper in cuticle (Plate I, Fig. 1) and are not identical with other acuarids. The membranous cordon exhibits irregular and discontinuous longitudinal as also sparsely arranged transverse prominences (Plate I, Fig. 2). Tricuspid cervical papilla bears three caudally directed prongs. Two lateral prongs are incurved and have concave inner margins. The middle prong is highly characteristic. The proximal part of this prong projects posteriorly a little from the common base appearing as a piller ultimately leading to a sharply demarcated conical projection distally (Plate I, Fig. 3). It appears that at the junction of this conical projection with that of the proximal piller-like base of the mid-prong are two horn-like protuberances on the dorsal and ventral sides.

2. *D. nasuta*: Serpentine, transversely striated, recurrent and non-anastomosing cordons (Plate I, Figs. 4, 5, 6) are characteristic of the genus *Dispharynx*. In *D. nasuta* figures are available where a continuous longitudinal marking extends from the tip to the terminus of each cordon in addition to cross striations. Under SEM the longitudinal marking representing any groove, swelling or similar structure is found to be wanting. Fine structure of the cordon reveals an irregular outline of the apparently even margins of a cordon. This is due to discontinuous, transversely arranged cuticular prominences each alternating a depression in the cordon (Plate I, Fig. 7). The cordon, though tubular appears to retain membranous nature.

3. *C. obelatus*: Each cordon is long, recurrent, laterally anastomosing and applied to the margin of a membranous plate (Plate I, Figs. 8, 9). Though not sharply demarcated from the plate, each cordon appears to retain membranous nature. The surface of cordon presents no cuticular ornamentation. But the membranous plate exhibits both transverse and longitudinal markings resulting in an appearance of numerous outwardly directed crystalline cuticular ornamentations (Plate I, Fig. 10). While the surface of the cordon proper is smooth, that of membranous plate is obviously uneven.

4. *D. diacantha*: Transverse ridge-like bands are the characteristics of the elongated cordons. Each cordon bears two closely set longitudinal grooves. This leads to the formation of three longitudinally arranged cord-like components of which the central one is narrower and both the peripheral ones are wider (Plate II, Fig. 11). The cordon is relatively broader, too (Plate II, Figs. 12, 13).

5. *A. anthuris*: Presence of four caudally directed long cordons in the form of ridges or grooves is the definition of *Acvaria*. In *A. anthuris* each cordon is located a little within the body rather than on the margins. Each cordon is set in a shallow gutter-like groove (Plate II, Fig. 14). The components of a cordon are two rows of discontinuous
longitudinal ridges running parallely which are symmetrically arranged and uniformly spaced corresponding to the annulations of the body cuticle (Plate II, Fig. 15). A ridge may be rectangular or provided with a convexity towards body margin. There is a definite space between two ridges forming a pair in the parallel order, as also between succeeding pairs of ridges. The bordiers of each ridge are not highly even.

6. *C. rosickyi*: The cordon's run caudally and each pair unites on dorsal and ventral sides and these junctions lie on the collarette. The cordon's are also flanked on the boarder by a strip of highly striated cuticle (Plate III, Fig. 16). The primary components of each cordon are ridges and membrane (Plate III, Figs. 17, 18). The membranes are lateral to the ridges which are central in position. The ridges are rectangular in shape and lie transversely along the whole length of the cordon. Though each ridge is separated from its preceding and succeeding ones by a gap, a number of finer cuticular fibril-like structures interconnect this with the neighbouring ones (Plate III, Fig. 18). Moreover, the surface of the ridges is not smooth. The collarette at its posterior boarder towards dorsal and ventral sides comes out a little from the body margin of the nematode forming a backwardly directed conical process on each of the respective sides. The cuticle of the collarette bears distinct longitudinal annulations (Plate III, Fig. 16). The surface of the cuticular collarette is also not smooth.

7. *S. squamata*: The generic definition includes the possession of transverse rows of minute spines covering the cordon. The spines are found to be wanting under SEM. Instead, each cordon is observed to be composed of a number of vertically placed transverse plates (Plate IV, Fig. 20). These plates, though distinct and separate from each other, are connected with adjacent ones by a number of projections coming out from the posterior surface of each of such plate (Plate III, Fig. 19; Plate IV, Fig. 21). Thus each cordon-plate connects its succeeding one by such cuticular projections forming a bridge-like structure throughout the length of the cordon (Plate III, Fig. 19; Plate IV, Figs. 21, 22).

## DISCUSSION

The translucid membranous cordon's of *P. tridentata* are highly atypical of an acuarid. But the existence of tricuspid cervical papillae adds circumstantial evidence in favour of placement of the species under Acuarini. After Chabaud and Czaplinsky (1961) it is highly probable that this translucid membrane connecting the lips and pseudo-lips may be the beginning of a cuticular cordon structure in subsequent acuarid genera. This opinion also includes the typical topography of the caudal papillae in male *P. tridentata*. As such, *Paracuaria* forms the basic stock for evolution of Acuarini.

Cordon structure of *D. nasuta* has been reported to exhibit an even margin and bear a longitudinal marking passing through the central position of a cordon. Under SEM the margins of a cordon are found to be uneven and any structure representing a marking through the mid-region along the long axis of a cordon is found to be absent. However, discontinuous transversely arranged cuticular prominences (but not striations) are present in a cordon.

The apparently transversely striated cordon of *C. obvelatus*, in fact, represents cordon proper and a membranous plate. The cordon proper is smooth and bears no transverse striae, while the plate exhibits discontinuous transverse and longitudinal markings.

Interesting observations on the cordon structure of *D. diacantha* centre around the existence of two closely placed longitudinal grooves passing along the long axis of each cordon near its central position. This results in the formation of three longitudinally
arranged cord-components of a cordon of which the peripheral ones are much wider than the central narrower one.

In *A. anthuris* each cordon is set in a shallow gutter-like groove of the cephalic cuticle. The parallel and symmetrically arranged rectangular longitudinal ridges are set in the aforesaid groove. The ridges are not in any way connected with each other.

The cordon of *C. rosickyi* is in consonance with that of the observations made under light microscope excepting that the neighbouring bands of the cordons are interconnected with each other by a number of fibril-like cuticular structures. The surface of the ridges as also of the collarette are not even.

Since the cordon of *S. squamata* is composed of vertical plates arranged along the long axis of the body, under light microscope their boarders exhibit the appearance of spinous structures along the margin of the cordon, which do not exist, in fact. Therefore, the definition of the genus has to be emended. Another interesting finding on the species lies in the development of definite and highly prominent cuticular

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**Fig. 1.** Schematic hypothetical evolutionary relations of the genera belonging to the subfamily Acuarinae. (Genera in continuous line of the rectangles were studied under SEM.)
projections coming out from the posterior surface of each of such vertical plates and connected with the succeeding plate.

Evolutionary sequence of Acariiniace has been attempted to be traced by a few authors (Osehe 1955, 1960; Chabaud and Petter 1959; Chabaud and Czapliński 1961). Osehe (1955) considered Syncuaria and Chevruzia to be two most closely related genera derived from the same stock. Moreover, Acuaria being the basic stock, gave rise to two distinct lines of evolution: one through Dispharynx, Synthimantis and Stammerinema, and the other through Desportesius, Cosmocephalus and Sexanocara (cf. Osehe 1960). Though the hypothesis of Chabaud and Petter (1959) agrees in general with that of Osehe (1960) so far as Dispharynx and its succeeding genera are concerned, the difference lies with the position of Stammerinema which the former authors derive from Syncuaria (Decortaria)-group.

Like the above referred authors, we consider the shape of the cordon to be of fundamental importance and a significant criterion for establishing phylogenetic relationships between different genera of Acariiniace. However, we lay more emphasis on the ultrastructure of the cordon than on their shapes while expressing mutual affinities amongst the genera concerned. It is considered that the simple structure of the cordon (as in Paracuaria and Dispharynx) exhibits primitive signs contrary to the more complicated pattern (as in Skrjabinoecara, Chevruzia, Cosmocephalus, Decortaria). In consonance with the analysis of Osehe (1955, 1960), and Chabaud and Petter (1959) the not-anastomosing cordon are regarded as typical of the primitive forms, and the joined cordon are, consequently, considered a feature of evolutionarily higher forms. In addition to these signs, attention has been paid to the shape and size of the cervical papillae (vid Osehe 1955). It is equally necessary to take into account the typical prevailing location of the species in the definitive hosts (microhabitats). In some cases it is possible to draw affinities based on comparative location of the vulva, anus and other features. Based on the present studies on the ultrastructure of cordon of members of seven acuarid genera, as also by using other literary data of the authors cited above, a preliminary attempt has been made at the present occasion to express the evolutionary sequence of a total of fifteen genera of Acariiniace (Fig. 1). Contrary to Skrjabini et al. (1965), genera Echinuria Soloviev, 1912 and Skrjabinocara Sobolev, 1943 are not considered to be members of the independent subfamily Echinuriinae Sobolev, 1943. But in accordance with the opinion of Chabaud and Petter (1959) these two genera are retained in the subfamily Acariiniace. Chabaud and Petter's (1959) listing of the genus Aviculariella Wehr, 1931 within the subfamily Seuratiinae Chitwood and Wehr, 1932 is also agreed upon.

Following the authors who solved this problem we also consider the species listed to the genus Paracuaria to be members of the primitive forms of Acariiniace. Further evolution is assumed to have taken place in two lines. One evolutionary direction is represented by the genus Acuaria which is very rich in species, the other direction by the genus Dispharynx. Contrary to the foregoing authors the genus Acuaria (and also closely related Skrjabinoecera) is now considered to form an independent evolutionary branch. The structure of cordon of the acuaroid type of these two genera is specific and markedly differs from the rest of the genera of this subfamily. The genus Acuaria embraces a large number of species having wide variations. The species listed in it appear to be in different stages of evolution (e.g. equal, subequal or unequal spicules; cordon of different lengths and apparently also of different microstructures; presence of pseudocordons). The species of this genus are adapted to parasitizing under the cuticle of the muscular stomach of birds. They have usually small and simple cervical papillae (Osehe 1955). The genus Skrjabinoecera exhibits some of these signs which bring it close to the genus Acuaria (presumed cordon structure) and also to Paracuaria.
(in shape of cervical papillae). The tail of female possesses cuticular processes (in **Skrjabinocerca**) which is to a certain extent analogous with the tail forms of some larvae of invasive stage in some species of the genus *Acuaria*.

According to our opinion, the second evolutionary line is followed by the genus *Dispharynx*. In this genus the cordons are already well developed and remain not-anastomosing where cuticular formations elevate from the body surface. Cervical papillae range from small to middle sizes (which are simple, bicuspid or tricuspid). The usual site of occurrence is the glandular stomach of birds (where they remain by boring their anterior part of the body into its walls). From this genus we derive evolutionary lines which are characterised by the anastomosing cordons (mostly wider) with more complicated structures.

One line leads probably to the genus *Syncuaria* (and further to *Decorataria* and *Chevreuxia*). The base of the other line is probably the genus *Synhimantus* from which *Cosmocephalus, Pectinospirura, Sexansocara, Skrjabinocara* and *Desportesius* are thought to have evolved.

The *Syncuaria — Decorataria — Chevreuxia* line is generally identical with the presumption of *Chabaud and Petter* (1959) who derived it, however, from the genus *Acuaria*. According to our comparative studies on the structure of cordons, it is possible to presume affinities rather in relation to the genera *Dispharynx — Syncuaria — Decorataria — Chevreuxia*. The genera *Skrjabinocara* and *Pectinospirura* are not in the same line of evolution as that of the preceding one, which is contrary to *Chabaud and Petter* (1959). The structure of cordons in *Skrjabinocara* is rather in relation with more specialised genera of the second evolutionary line. Similar relation we suppose to be present in the genus *Pectinospirura*. The genera *Syncuaria, Decorataria, Chevreuxia* are characterised further by cervical papillae of small size and simple shape (only rarely bicuspid). The cordons of *Chevreuxia* and *Decorataria* have already complicated structure. Most of the species of these genera parasitize underneath the cuticle of muscular stomach.

Also in the genera of the second line *Synhimantus — Cosmocephalus — Skrjabinocara — Desportesius* (and derived from them *Pectinospirura* and *Sexansocara*) the cordons are of complicated structure and are relatively wide. Cervical papillae of these genera are comparatively large and mostly tricuspid in nature (in *Cosmocephalus* there are combinations of simple, bicuspid or tricuspid). These species are usually located in the glandular stomach of birds, while the genus *Synhimantus* under the cuticle of muscular stomach. We consider *Desportesius, Skrjabinocara, and Sexansocara* to be the most specialised genera of this line. The most primitive forms include *Cosmocephalus* (relatively simple structure of cordons) and *Pectinospirura*.

We agree with the opinion of *Chabaud and Petter* (1959) in that the genera *Echinura and Skrjabinoclava* form an independent line initiated from the genus *Syncuaria*. Both the genera are well characterised in their shape of cordons and in having longitudinal rows of additional spines. We did not study their detail structure of cordons and, therefore, we cannot correlate them with other genera of *Acuariinae*.

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ИЗУЧЕНИЕ СТРУКТУРЫ КАНАТИКОВ СЕМИ РОДОВ АКУАРИИД (NEMATODA: ACUARIIDAE) МЕТОДОМ СКАНИРУЮЩЕЙ ЭЛЕКТРОННОЙ МИКРОСКОПИИ

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Резюме. При помощи сканирующего электронного микроскопа изучали структуры канатиков семи видов аквариумов, принадлежащих к семи разным родам: Paracuaria tridentata, Dispharynx nasuta, Cosmocephalus ocellatus, Decoratariia diacantha, Acuaria anthuris, Chevreuxia rosickyi и Skrjabinocara squamata. Интересными являлись три верхние видные образования, состоящие из канатиков D. diacantha и отсутствие непрерывных рядов мелких шипиков, покрывающих канатики S. squamata. По современной гипотезе развитие аквариумов протекало в двух линиях, исходя из Paracuaria, Acuaria и Skrjabinocara образуют самостоятельную ветвь. Из Dispharynx они имеют две линии развития; к одной из них принадлежат Syncuaria, Decoratariia, Chevreuxia, Echinuria и Skrjabinocara и к другой Synthimansus, Pectinospirura, Cosmocephalus, Sexansocara, Skrjabinocara и Desportesius.

REFERENCES


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Plate I

*Paracuaria tridentata* (Figs. 1-3), *Diapharynx nasuta* (Figs. 4-7) and *Cosmocephalus obvelatus* (Figs. 8-10). Fig. 1. Anterior end showing deep seated cordon in cephalic cuticle (×3000); Fig. 2. Prominences in membranous cordon (×10000); Fig. 3. Tricuspid cervical papilla (×10000); Figs. 4, 5 and 6. Recurrent serpentine cordon (×1000); Fig. 7. Cordon magnified showing transverse prominences and furrows (×6000); Fig. 8. Lateral anastomosing cords (×1000); Fig. 9. Cordon applied to marginal plate (×3000); Fig. 10. Crystalline ornamentations in membranous plate (×3000).
Plate II

*Decorataria diaelectra* (Figs. 11–13), *Acuaria anthoris* (Figs. 14–15). Fig. 11. Cordon on body margin showing grooves (× 1 000); Figs. 12 and 13. Cordon magnified showing central narrower and lateral broader cord-components (× 3 000); Fig. 14. Cordonal ridges in a shallow gutter on the cuticle (× 1 000); Fig. 15. Two ridges of the same order magnified (their long axis correspond to that of the body) — (× 3 000).
Plate III

*Chevreucia rosickyi* (Figs. 16—18), and *Skrijabinocara squamata* (Fig. 19). Fig. 16. Collarette showing highly flanked cuticle bearing longitudinal annulations ($\times 10,000$); Fig. 17. Ridges of a cordon and adjacent membrane ($\times 3,000$); Fig. 18. Ridges magnified showing space between each of them and interconnections with fibrils ($\times 10,000$); Fig. 19. Projections conming out of the vertical plates and joining succeeding one ($\times 3,000$).
Plate IV

*Skrjabinocara squamata* (Figs. 20–22). **Fig. 20.** Vertical plates constitute the cordon (the absence of spines in cordon may be noted) — (×1 000); **Fig. 21.** Projections from posterior surface of cordon plates joining the succeeding ones (×3 000); **Fig. 22.** Bridge-like structure formed of projections and vertical plates of a cordon (×3 000).