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Structure and chemical composition of the cuticle of *Cirolana fluviatilis*, *Sphaeroma walkeri* and *Sphaeroma terebrans*

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Abstract. A comparative study has been made of the cuticular organisation of isopod wood borer *Sphaeroma terebrans*, a fouler *Sphaeroma walkeri* and a free living isopod *Cirolana fluviatilis*. The cuticle of *S. terebrans* shows both structural and chemical peculiarities. In *S. walkeri*, the epicuticle contains fuchsinophilic protein and gives evidence of primary tanning. In *C. fluviatilis* the epicuticle is similar to that of other isopods.

Keywords. Cuticle ; structure and chemical composition ; *Sphaeroma terebrans* ; histochemistry.

1. Introduction

Although the cuticle of arthropods conforms to a basic pattern comprising of an inner procuticle formed of chitin-protein complex and an outer lipo-protein epicuticle, it shows a wide range of modifications in structure and chemical composition in different groups. Dennell (1947) observed that the abbreviation of tanning, occurrence of a two layered epicuticle and calcification of the cuticle of crusta ceans may be related to their aquatic habitat and to the ready availability of calcium in their natural environment.

Earlier work on cuticle of isopods is more limited than on decapod cuticle. The structure and chemical composition of the cuticle of *Porcellio scaber*, *Ligia exotica*, *Armadillidium vulgare* and *Oniscus asellus* have been studied by George and Sheard (1954), Mary (1968), Lagarrigue (1970) and Mary and Krishnan (1974). It is known that there is a general conformity in structure and chemical composition to that of the cuticle of decapod crustaceans. A point of interest is that isopods unlike decapod crustaceans, have a number of adaptive devices for terrestrial life. It is of interest to investigate the nature of modifications in the cuticle structure and chemical composition relevant to their adaptation to semiterrestrial and terrestrial mode of life.

The Sphaeromatidae, which include wood borers and epifoulers, are presumably adapted for their mode of life as borers or as foulers. The nature of the adaptation of the cuticle structure and chemical composition is investigated by a comparative study of a typical borer like *Sphaeroma terebrans* with a closely

allied species *Sphaeroma walkeri* which is not a borer but shows a substratum affinity to sfbmerged wood. The results were compared with the cuticular structure of a free living type, *Cirolana fluviatilis*.

2. Material and methods

Specimens of *S. walkeri* and *S. terebrans* were collected from Madras harbour by immersing timber panels in the sea. Specimens of *C. fluviatilis* were also collected from Madras harbour. The animals were maintained under laboratory conditions by changing the sea water every day.

For histological preparations of the cuticle, the material was fixed in 5% formaldehyde, decalcified in 3% glacial acetic acid or 3% EDTA and embedded in paraffin or celloidin. The stains used were Mallory's triple stain, Masson's trichrome stain and Heidenhain's haematoxylin (Mallory 1938; Pantin 1948; Lillie 1954). Histochemical tests were performed on frozen sections of the cuticle which were prepared by impregnating the specimens with 12½% and 25% gelatin solution and the blocks were hardened in 5% formaldehyde (Carleton and Leach 1938).

For detection of chitin, the tests used were Chitosan test (Campbell 1929) and Schulze test (Clark and Smith 1936). For sulphydryl and disulphide groups, tetrazolium test (Barnett and Saligman 1952), nitroprusside test and ferric ferricyanide test (Lillie 1954; Pearse 1968) were performed. To detect protein constituents the tests included xanthoproteic test, Millon's test (Pearse, 1968), Hg/nitrite test (Lison 1936) and biuret test (Fearon 1946). The presence of lipids was tested by treatment with dyes such as Sudan black *B* (Baker 1946; Lillie 1954). For detecting calcium, alkaline pyrogallon test (Lison 1936), alizarine red-*S* and Vonkossa's test (Lillie 1954) were employed.

3. Results

The cuticle of *Cirolana fluviatilis* varies in thickness in different regions from 10 to 30 μ. Sections passing through the tergite reveal two well defined regions in the cuticle corresponding to epicuticle and procuticle. An outer, thin homogeneous layer, 7 to 10 μ thick is different in appearance and colour from a thicker lamellated region which may be subdivided into three distinct layers in the intermoult stage.

The epicuticular nature of the outer thin part is confirmed by treatment with chlorated nitric acid which separates the epicuticle from the procuticle by the differential solubility of the two layers in this reagent. At this stage the epicuticle is not light yellow coloured; the procuticle is not distinguishable into sub-divisions. When stained with Mallory, the epicuticle may be divisible into two regions, an outer thin blue staining membrane and below it, a fuchsinophil region (figure 1). The two parts correspond to outer epicuticle and inner epicuticle of other arthropods, The procuticle stains uniformly blue in Mallory and green in Masson's stain. Tests for protein show that the inner epicuticle contains a protein containing phenyl groups (table 1). The protein in the procuticle on the other hand is negative to these tests but reacts positively to biuret test. In this respect the protein constituents of the cuticle conform to those reported in the cuticle of decapod crustaceans and insects (Dennell 1947; Wigglesworth 1948). A feature of the

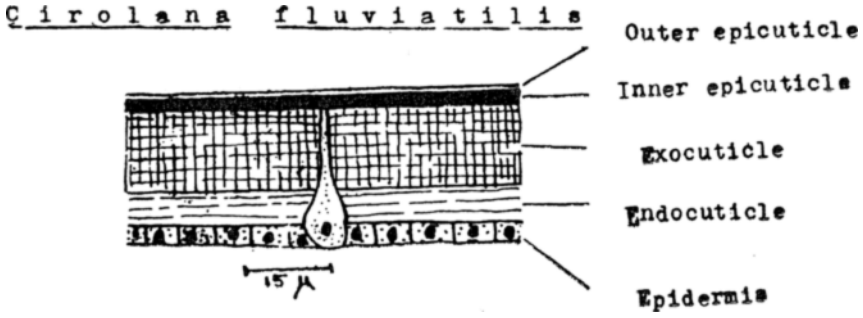

Figure 1. Transverse section through the intermoult cuticle, stained in Mallory's triple stain.

Table 1. Results of staining reactions and histochemical tests obtained with the late freshmoult cuticle of *Cirolana fluviatilis*.

No.	Stains and tests	References	Epicuticle		Procuticle
			Outer layer	Inner layer	
1.	Mallory's triple stain	Mallory 1938	Blue	Red	Blue
2.	Masson's trichrome stain	Trim 1941	Green	Red	Green
3.	Heidenhain's haematoxylin	Lillie 1954	Blue black	Grey	–
4.	Chitosan test	Campbell 1929	–	–	+
5.	Schultz modified test	Clark and Smith 1936	–	–	+
6.	Sudan Black B	Baker 1946	+	–	–
7.	Liebermann-Burchardt test	Lison 1953	+	–	–
8.	Biuret test	Fearon 1946	–	–	+
9.	Xanthoproteic test	Lillie 1954	–	+	–
10.	Millon's test	Pearse 1968	–	+	–
11.	Hg/nitrite test	Baker 1946	–	+	–
12.	Argentaffin test	Lison 1936	–	+	–
13.	Ferric chloride test	Lison 1936	–	+	–
14.	Blue tetrazolium test	Barnett and Seligman 1952	–	–	–
15.	Ferric ferrycyanide test	Pearse 1968	–	–	–
16.	Alkaline pyrogallol test	Lison 1936	–	–	–
17.	Alizarin red-*S*	Lillie 1954	–	–	–
18.	Vonkossa's test	Lillie 1954	–	–	–

+ positive reaction ; – negative reaction.

protein of the cuticle of the isopod studied above, is the negative reaction to biuret test in the epicuticle which is positive to the Million and xanthoproteic tests.

The outer epicuticle reacts to tests for lipids and sterols. The inner epicuticle is only feebly reactive to these tests. It shows a positive reaction to argentaffin test which may be indicative of the presence of reducing substances which in the present context, considering this reaction together with the positive reaction obtained in the region with ferric chloride, may suggest that the reacting materials may be diphenols or polyphenols.

The structural features and staining reactions as well as the chemical composition of the cuticle differ in intermoult stage (figure 1). The epicuticular region in a section shows, an amber colouration and is unreactive to stains. The outer lipid epicuticle is less prominently seen in the sections. The procuticle is now distinguishable into an outer region which is amber colour and an inner region in which the lamellations are still clearly seen and still below is another region in which the lamellations are closely set. The results of histochemical tests are given in table 2. It is seen that the chemical composition of the epicuticle conforms to that in a number of decapod crustaceans in undergoing tanning resulting in acquisition of rigidity and resistance to chemical reagents.

In the procuticle prominent changes are brought about by the formation of an outer amber region giving rise to exocuticle and the part of the procuticle under it appears to be calcified and this region reacts to tests for calcium, like Vonkossa's test, alkaline pyrogallol and alizarin red-*S*. A region immediately below the calcified procuticle is free from calcium and is designated as the non-calcified layer. Results of tests applied for protein in the procuticle show that at this stage in addition to biuret positive protein and a protein involved in tanning, there is evidence of another protein which reacts positively to the blue tetrazolium and ferric ferricyanide tests. The presence of such a protein containing organic sulphur associated with calcified region has been earlier reported in decapod crustaceans like *Orconectes virilis* (Travis 1965). This author suggested that in the absence of tanning in this region the protein containing the SH group may play a role in facilitating calcification.

To examine how far the cuticular organisation of a closely allied fouler associated with wood differs from a free living form (described above) a detailed study of the cuticle of *S. walkeri* was made. Examinations of the stained and unstained sections of the cuticle of *S. walkeri* in the freshmoult condition showed epicuticle as in *Cirolana fluviatilis* distinguished by its homogeneity and in being formed of an outer thin membrane of the outer epicuticle (figure 2). The procuticle conforms in all respects to the condition reported in the corresponding stages of moult cycle of *Cirolana fluviatilis* (table 3). But in the intermoult stage there are seen marked differences in chemical features of the cuticle compared to those of intermoult cuticle of *Cirolana fluviatilis* (table 4). Unlike in *C. fluviatilis* the inner epicuticle does not undergo tanning. It however stains red in Mallory's and reacts positively to tests for protein like xanthoproteic and Millon's. Similarly in the procuticle, the outer part is not differentiated into an exocuticle but the middle region of the procuticle undergoes calcification and reacts to tests for calcium like Vonkossa's alizarin red-*S* and alkaline pyrogallol tests (table 4). From a comparative study of the intensity of the reaction to tests for calcium it may appear that calcium content is more than what was noted in the allied type. The region of the pro-

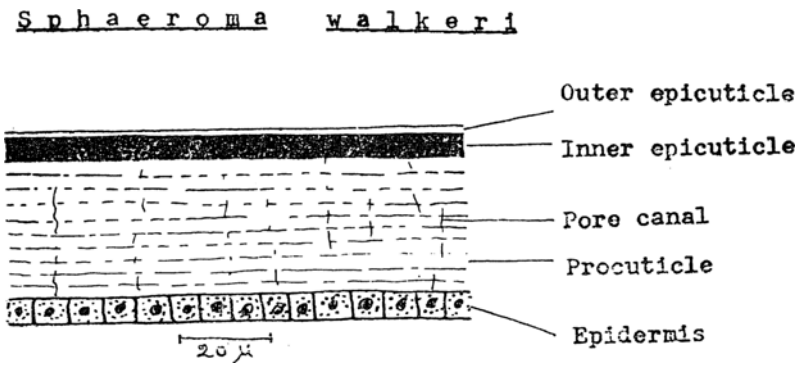

Figure 2. Transverse section through the freshmoult cuticle, stained in Mallory's triple stain.

Table 2. Results of staining reactions and histochemical tests obtained with the intermoult cuticle of *Cirolana fluviatillis*.

No.	Stains and tests	Reference	Epicuticle			Procuticle	
			Outer layer	Inner layer	Exocuticle	Calcified layer	Uncalcified layer
1.	Mallory's triple stain	Mallory 1938	Blue	Amber	Amber	Blue	Light blue
2.	Masson's trichrome stain	Trim 1941	Green	Amber	Amber	Green	Light blue
3.	Heidenhain's haematoxylin	Litlie 1954	Blue backk	Grey	—	—	—
4.	Chitosan test	Campbell 1929	—	—	+	+	+
5.	Schultz modified test	Clark and Smith 1936	—	—	+	+	+
6.	Sudan Black B	Baker 1946	++	+	—	—	—
7.	Liebermann Burchardt	Lison 1953	++	+	—	—	—
8.	Biuret test	Fearon 1946	—	—	—	+	+
9.	Xanthoproteic test	Lillie 1954	—	+	+	—	—
10.	Millon's test	Pearse 1968	—	+	+	—	—
11.	Hg/nitrite test	Baker 1946	—	+	+	—	—
12.	Argentaffin test	Lison 1936	—	+	+	—	—
13.	Ferric chloride test	Lison 1936	—	—	—	—	—
14.	Blue tetrazolium test	Barnett and Seligman 1952	—	—	+	+	—
15.	Ferric ferrycyanide test	Pearse 1968	—	—	+	+	—
16.	Alkaline pyrogallol test	Lison 1936	—	—	+	+	—
17.	Alizarin red-*S*	Lillie 1954	—	—	+	+	—
18.	Vonkossa's test	Lillie 1954	—	—	+	+	—

+ positive reaction ; ++ intensely positive ; — negative reaction.

cuticle reacting to calcium test is also abbreviated. In other respects it recalls the condition noted in the cuticle of *C. fluviatilis*. In the cuticle of *S. terebrans* which is a borer, the epicuticle shows a further deviation from the condition reported in *C. fluviatilis* (figure 3). These differences refer to the protein compound of the epicuticle which unlike in *S. walkeri* is not the fuchsinophil tyrosine containing protein. There is evidence of only the basal protein which is biuret positive, stains blue with Mallory's and green in Masson's stain. Complete absence of tanning is a feature of the epicuticle of this animal in all the stages of moult cycle (tables 5, 6).

Table 3. Results of staining reactions and histochemical tests obtained with the late freshmoult cuticle of *Sphaeroma walkeri*.

No.	Stains and tests	References	Epicuticle		Procuticle
			Outer layer	Inner layer	
1.	Mallory's triple stain	Mallory 1938	Blue	Red	Blue
2.	Masson's trichrome stain	Trim 1941	Green	Red	Green
3.	Heidenhain's haematoxylin	Lillie 1954	Blue black	Grey	—
4.	Chitosan test	Campbell 1929	—	—	+
5.	Schultz modified test	Clark and Smith 1936	—	—	+
6.	Sudan black B	Baker 1946	+	—	—
7.	Liebermann Burchardt	Lison 1953	+	—	—
8.	Biuret test	Fearon 1946	—	—	+
9.	Xanthoproteic test	Lillie 1954	—	+	—
10.	Millon's test	Pearse 1968	—	+	—
11.	Hg/nitrite test	Baker 1946	—	+	—
12.	Argentaffin test	Lison 1936	—	—	—
13.	Ferric chloride test	Lison 1936	—	—	—
14.	Blue tetrazolium test	Barnett and Seligman 1952	—	—	—
15.	Ferric ferrycyanide test	Pearse 1968	—	—	—
16.	Alkaline pyrogallol test	Lison 1936	—	—	—
17.	Alizarin red-S	Lillie 1954	—	—	—
18.	Vonkossa's test	Lillie 1954	—	—	—

+ positive reaction ; — negative reaction.

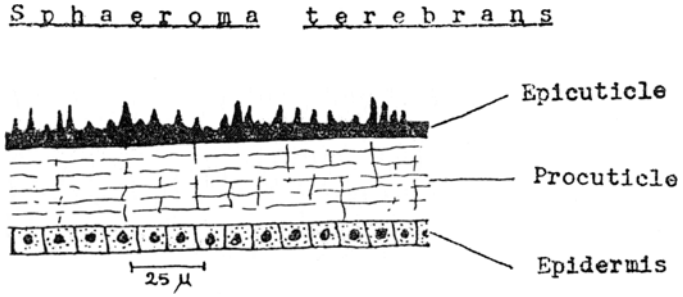

Figure 3. Transverse section through the freshmoult cuticle, stained in Mallory's triple stain.

Table 4. Results of staining reactions and histochemical tests obtained with the intermoult cuticle of *Sphaerome walkeri*.

No.	Stains and tests	References	Epicuticle		Procuticle	
			Outer layer	Inner layer	Calcified layer	Uncalcified layer
1.	Mallory's triple stain	Mallory 1938	Blue	Red	Blue	Light blue
2.	Masson's trichrome stain	Trim 1941	Green	Red	Green	Light bluee
3.	Heidenhain's haematoxylin	Lillie 1954	Blue black	Grey	—	—
4.	Chitosan test	Campbell 1929	—	—	+	+
5.	Schultz modified test	Clark and Smith 1936	—	—	+	+
6.	Sudan black B	Baker 1946	+	—	—	—
7.	Liebermann-Burchardt test	Lison 1953	+	—	—	—
8.	Biuret test	Fearon 1946	—	—	—	+
9.	Xanthopr teic test	Lillie 1954	—	+	—	—
10.	Millon's test	Pearse 1968	—	+	—	—
11.	Hg/nitrite test	Baker 1946	—	+	—	—
12.	Argentaffin test	Lison 1936	—	—	—	—
13.	Ferric chloride test	Lison 1936	—	—	—	—
14.	Blue tetrazolium test	Barnett and Seligman 1952	—	—	+	—
15.	Ferric ferricyanide test	Pearse 1968	—	—	+	—
16.	Alkaline pyrogallol	Lison 1936	—	—	+	—
17.	Alizarin red-*S*	Lillie 1954	—	—	+	—
18.	Vonkossa's test	Lillie 1954	—	—	+	—

+ positive reaction ; — negative reaction.

Table 5. Results of staining reactions and histochemical tests obtained with the freshmoult cuitcle of *Sphaeroma terebrans*.

No.	Stain and tests	References	Epicuticle	Procuticle
1.	Mallory's triple stain	Mallory 1938	Blue	Light blue
2.	Masson's trichrome stain	Trim 1941	Green	Light green
3.	Heidenhain's hasmotaoxylin	Lillie 1954	Light blue	—
4.	Chitosan test	Campbell 1929	—	+
5.	Schultz modified test	Clark and Smith 1936	—	+
6.	Sudan Black B	Baker 1946	—	—
7.	Liebermann-Burchadrt test	Lison 1953	—	—
8.	Biuret test	Fearon 1946	+	+
9.	Xanthoproteic test	Lillie 1954	—	—
10.	Millon's test	Pearse 1968	—	—
11.	Hg/nitrite test	Baker 1946	—	—
12.	Argentaffin test	Lison 1936	—	—
13.	Ferric chloride test	Lison 1936	—	—
14.	Blue tetrazolium test	Barrnett an2 Seligman 1952	—	—
15.	Ferric ferrycyanide test	Pearse 1968	—	—
16.	Alkaline pyrogallel	Lison 1936	—	—
17.	Alizarin red-*S*	Lillie 1954	—	—
18.	Vonkossa's test	Lillie 1954	—	—

+ positive reaction ; — negative reaction.

In the absence of a tyrosine containing protein which is the precursor of tanning of the cuticle, *S. terebrans* may be said to lack the essential mechanism for tanning. In the procuticle also there are seen marked deviations from the condition noted in *C. fluviatilis*. This refers to the non-differentiation of outer part of the procuticle into an exocuticle or a mesocuticle. Although there is evidence of calcification, compared to the other two types studied, it is much abbreviated. The results of tests are recorded in table 6.

4. Discussion

It deserves to be noted here that the cuticle of the wood borer *S. terebrans* is devoid of the outer epicuticle while those of *S. walkeri* which is a fouler, and of the free living *C. fluviatilis*, have this layer. The outer epicuticle is formed of lipid and is believed to check evaporation of water from the surface of the body (Beament 1961, 1964). Recent work has shown that the cuticle lining the gut in decapod crustacean like *Ocypoda platytarsis* lacks an outer lipid epicuticle, which accounts for the increased permeability to water through the layer (Mary and Krishnan 1974). The significance of the absence of an outer epicuticle in the wodo

Table 6. Results of staining reactions and histochemical tests obtained with the intermoult cuticle of *Sphaeroma terebrans*.

No.	Stains and tests	References	Epicuticle	Procuticle
1.	Mallory's triple stain	Mallory 1938	Blue	Light blue
2.	Masson's trichrome stain	Trim 1941	Green	Light green
3.	Heidenhain's haematoxylin	Lillie 1954	Light blue	—
4.	Chitosan test	Campbell 1929	—	+
5.	Schultz modified test	Clark and Smith 1936	—	+
6.	Sudan Black B	Baker 1946	+	—
7.	Liebermann Burchardt test	Lison 1953	—	—
8.	Biuret test	Fearon 1946	+	+
9.	Xanthoproteic test	Lillie 1954	—	—
10.	Millon's test	Pearse 1968	—	—
11.	Hg/nitrite test	Baker 1946	—	—
12.	Argentaffin test	Lison 1936	—	—
13.	Ferric chloride test	Lison 1936	—	—
14.	Blue tetrazolium test	Barnett and Seligman 1952	—	+
15.	Ferric ferrycyanide test	Pearse 1968	—	+
16.	Alkaline pyrogallol	Lison 1936	—	+
17.	Alizarin red-S	Lillie 1954	—	+
18.	Vonkossa's test	Lillie 1954	—	+

+ positive reaction ; — negative reaction.

borer *Sphaeroma terebrans* may be that the cuticle in it is more permeable than that of the closely allied species *Sphaeroma walkeri*. This species in its natural habitat within the wood may not be exposed to fluctuations in ambient temperatures, to need protective devices against water loss. Similarly the absence of an outer epicuticle which would restrict the permeability to water and possibly ions, may not be an important and a necessary factor as the borer unlike the free living forms living within a restricted environment.

The wood boring species *S. terebrans* is characterized by the occurrence of spines on the cuticular surface. Spines are absent in *S. walkeri*. It thus appears that the presence of cuticular spines is somehow related with boring habit. The precise functional role and the spines in boring is not known.

The inner epicuticle which may undergo tanning is important in bringing about a restraint on water loss. In the wood borer *S. terebrans* the protein composition of the cuticle is very different from the allied species *S. walkeri* and *C. fluviatilis* in the absence of the fuchsinophil protein and the presence of only a biuret positive protein in the epicuticle. This is an unusual feature in an intermoult cuticle. Immediately after moulting or in preecdysial cuticle it has been reported that the epicuticle may contain only a biuret positive protein which is seen over-

laid by a fuchsinophil protein which is a precursor of tanning (Dennell and Malek 1955). Tanned protein and precursors of tanning are known to prevent water loss (Sundararajulu and Krishnan 1968; Mary 1968).

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