

CHEMOTAXONOMY OF FIVE SPECIES OF THE FAMILY CHIRONOMIDAE (DIPTERA)

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INTRODUCTION

Biochemical techniques such as paper chromatography, starch gel and cellulose acetate have been increasingly used in taxonomic studies (Wood *et al*, 1958 and Wright and Ross, 1965). Electrophoretic techniques have contributed to the solution of many taxonomic problems relating to origin (Sibley, 1960), the discovery of sibling species (Manwell and Baker, 1963 a and Manwell *et. al*, 1967), the status of species and populations (Baker, 1965), the establishment of affinities (Tashian, 1965) and the recognition of hybridization (Desmer *et al*, 1962). Davis and Lindsay (1964, 1967) initiated the use of disc electrophoresis to show that species of distinct genera have distinct electrophoretic patterns and gave a complete analysis of the use, advantage and standardized procedures applicable to molluscan systematics. It is well known that benthic communities in freshwater ecosystems are characterized by the occurrence of large populations of immature stages of many insect groups. Members of the dipteran family Chironomidae represented by their larvae and pupae form one such important group. While these organisms are of undoubted value as indicators of pollution or as fish food, our knowledge of their taxonomy, particularly in the Indian context is still limited. The general objectives of the present investigations were therefore to collect and study these organisms upon which keys could be formulated for the identification of larvae, pupae and adults.

MATERIALS AND METHODS

The five species of larvae used were *Chironomus costatus* (Johannsen) *Nilodorum stupidus* (Johannsen), *Stictochironomus affinis* (Johannsen) *Tanytus photophilus* Kieffer and *Clinotanytus fumipennis* (Kieffer): Three fractions in each species - homogenate, the haemolymph and the body wall without the haemolymph were separately used for electrophoretic studies. All fractions belonged to larvae of the same population of a given species. Five to eight different populations of 4th instar larvae of each species collected at different periods throughout the year were used for all runs.

While developing polyacrylamide gels for protein separations, Davis and Ornstein (1959) introduced the technique of disc electrophoresis which exploited to maximum advantage the mechanisms responsible for the separation of proteins. Since the original introduction of disc electrophoresis by Davis and Ornstein (1961), many variations of the method have appeared. The method followed here is one of their own improvised and modified versions (Davis, 1964; Ornstein, 1964).

RESULTS

The results obtained on the chemotaxonomic studies of the five species of larvae are presented for the three different fractions. Data on the number of discs, the Rf values, number of peaks and the percentage and densities are given for all the species.

Chironomus costatus (Johannsen)

Homogenate : This fraction had 10 discs, with the four largest peaks at Rf values of 0.184, 0.289, 0.383 and 0.528. The first three peaks had high densities of 14.88%, 17.47% and 17.61%, while the fourth peak was represented by a very faint disc with a density of only 9.39%. The disc at Rf 0.866, though not large was similar to the first peak in having a high density of 14.95%.

Haemolymph : 11 discs were seen and as in the homogenate fraction, the first four discs formed the high peaks, at Rf of 0.290, 0.374, 0.449 and 0.517. However, only the 2nd, 4th and 5th peaks with densities of 17.10%, 17.03% and 16.90% respectively, were maximum. Discs 1, 11 and 3 were next in order of decreasing density with values of 10.65%, 10.21% and 10.00%.

Body wall without haemolymph : A total of 13 discs, of which the first, second, fourth and fifth were placed at 0.267, 0.344, 0.426 and 0.574 with densities of 13.85%, 14.27%, 14.41% and 14.38% were found.

Nilodorum stupidus (Johannsen)

Homogenate : This species revealed 13 discs with distinct peaks at one, two, three, four, five, six, ten and thirteen placed at Rf values of 0.171, 0.215, 0.260, 0.333, 0.390, 0.500, 0.710 and 0.950. However, maximum densities were recorded only in the fifth, sixth, tenth and thirteenth discs with 17.59%, 10.59%, 12.71% and 13.21% respectively.

Haemolymph : 17 discs were present and the prominent peaks were one, three, four, nine and seventeen placed at 0.279, 0.395, 0.468, 0.756 and 0.970. These revealed densities of 13.71%, 10.31%, 18.03%, 11.49% and 9.32% respectively. However, disc seven at Rf value of 0.618, though lacking a high peak had a density of 11.71%.

Body wall without haemolymph : There were 18 discs, with the highest peak placed at Rf 0.438, the sixth which also had the maximum density of 15.09%. Discs one, two, three, four, five, six and eighteen in that order of peak heights were placed at

Rf values of 0.222, 0.242, 0.269, 0.317, 0.371, 0.438 and 0.973 respectively. The discs 9 and 18 showed a density above 11%. The densities of the other discs were insignificant.

Stictochironomus affinis (Johannsen)

Homogenate : Of a total of fifteen discs, one, two, three, four, eight and fifteen had prominent peaks at Rf values of 0.222, 0.313, 0.404, 0.529, 0.697 and 0.965. However maximum density was seen only at disc eight which was 15.63%, with disc fifteen following closely with 14.29%. Discs two with 11.44% and three with 10.48% were next in order of densities.

Haemolymph : This fraction had 13 discs having prominent peaks at two, three, four, five, six, seven and thirteen with Rf values of 0.286, 0.392, 0.464, 0.537, 0.649, 0.708 and 0.969. Discs two, three, four, seven and thirteen recorded high densities of 15.11%, 17.76%, 15.29%, 11.72% and 9.83% respectively.

Body wall without haemolymph : 17 discs were recorded in this fraction with discs one to seven and seventeen at Rf of 0.141, 0.213, 0.300, 0.403, 0.492, 0.567, 0.665 and 0.960 having high peaks. Discs two, three, four, seven and seventeen had densities of 11.21%, 13.39%, 13.16%, 11.18% and 12.78%. The highest peak observed for disc one which had a density of only 9.38%.

Tanypus photophilus (Kieffer)

Homogenate : There were 19 discs with high peaks at one, two, three, five, fifteen, seventeen and nineteen at Rf values of 0.122, 0.210, 0.300, 0.388, 0.782, 0.877 and 0.953. High densities were recorded only in the three discs of one, two and nineteen with 13.42%, 12.90% and 13.72% respectively.

Haemolymph : 15 discs with prominent peaks at one, two, three, four, and fifteen placed at 0.164, 0.242, 0.324, 0.436 and 0.956 and with densities of 9.52%, 10.59%, 8.63%, 14.21% and 21.63% were recorded. Disc seven, though failing to show a prominent peak had a density of 10.80%.

Body wall without haemolymph : There were 17 discs with the prominent peak at disc seventeen with an Rf of 0.983 and density of 5.94%. Discs three, seven, thirteen and fourteen at 0.350, 0.570, 0.670, and 0.808 had high densities of 12.60%, 12.75%, 10.67% and 21.96% respectively though they showed no prominent peaks.

Clinotanypus fumipennis (Kieffer)

Homogenate : 10 discs were present in this fraction with high peaks at one, two, three, eight, nine and ten placed at Rf values of 0.233, 0.324, 0.400, 0.800, 0.902 and 0.921 respectively. High densities were recorded in discs one, three and eight with 18%, 12.2% and 14.8%. Discs six at Rf 0.678 though showed a comparatively small peak had a density of 13.2%.

Haemolymph : This fraction showed 12 discs with large peaks at discs one, two, three, four and twelve at Rf 0.145, 0.216, 0.300, 0.403 and 0.950. Only the fourth and the twelfth discs showed high densities of 13.27% and 16.07% respectively.

Body wall without haemolymph : Of the 12 discs the large peaks were one, two, three, four, six and twelve at Rf of 0.171, 0.253, 0.328, 0.405, 0.558 and 0.910. Discs two, four, six and eight recorded densities of 10.86%, 14.78%, 11.60% and 11.60% being the maximum.

DISCUSSION

The values of the three fractions, homogenate, the haemolymph and the body wall without the haemolymph were compared with a view to choose that tissue fraction which would reveal the most stable results. In four of the species, the body wall without haemolymph fraction showed maximum number of discs than the other two fractions (Table I). It is not quite clear why the body wall fraction alone yielded more discs. The muscle content present in an unmixed condition should possibly be the cause as was also shown earlier for foot muscles in molluscs (Davis and Lindsay, 1967), and for fish muscle myogens (Devadasan and Nair, 1971). In the present study, peaks occurred at different levels in the three fractions even within the same species. Their densities expressed as percentages also vary likewise. It is known that heights of peaks could alter depending upon the amount of protein component, since faint bands with low densities could form a high peak by spreading (Davis and Lindsay, 1967). Thus these two parameters of peak heights and density percentages were likely to show variations. Irrespective of these differences, peaks were always located by their Rf values.

TABLE - I

Total number of discs in the three fractions of all species

	<i>Homogenate</i>	<i>Haemolymph</i>	<i>Body wall</i>
<i>Chironomus costatus</i> (Johannsen)	10	11	13
<i>Nilodorum stupidus</i> (Johannsen)	13	17	18
<i>Stictochironomus affinis</i> (Johannsen)	15	13	17
<i>Tanytus photophilus</i> (Kieffer)	19	15	17
<i>Clinotanypus fumipennis</i> (Kieffer)	10	12	12

An interesting fact emerging from the study of the peaks is that in all the five species usually the first four discs and the last disc showed prominent peaks. This being one of the pioneering investigations in the chemotaxonomic approach to chironomid taxonomy, one is prompted to suggest that the fore part of the gel and the last peak

close to the front of separation were probably the most important regions for taxonomic considerations in this group. Similar demarcations of gel regions for molluscan chemotaxonomy exist (Davis and Lindsay, 1967).

Irrespective of the species, the Rf values for a given disc were more or less the same. All the discs recorded were serially arranged and compared for the five species and for each fraction with a view to locate commonly occurring discs among the species and to relate them for possible taxonomic relationships. Even though the homogenate and haemolymph fractions were given more consideration as they showed the largest number of discs. Among the species belonging to the Tribe Chironomini (sub-family Chironominae) discs 19, 20, 23, 25 and 29 were common for all the three species. Likewise discs 3, 8, 18, 19 and 29 were common for the species of the sub-family *Tanypodinae*. These two sub-families Chironominae and Tanypodinae appeared chemotaxonomically different groups. When the relationship was estimated among the three species of Chironominae by the occurrence of common discs between any two species, it was seen that *Chironomus costatus* (Johannsen) had ten discs in common with *Nilodorum stupidus* (Johannsen) while it had only six discs in common with *Stictochironomus affinis* (Johannsen), whereas *Nilodurim stupidus* had nine discs common with the latter. Thus *Chironomus costatus* (Johannsen) and *Nilodorum stupidus* (Johannsen) showed more affinity with one another than with *Stictochironomus affinis* (Johannsen). This chemotaxonomic relationship shown between the genus *Chironomus* and *Nilodorum* are in confirmity with the morphotaxonomic findings. Since availability of knowhow of chemotaxonomic techniques prompted the application of the same to the present group of organisms. The results are more exploratory rather than conclusive in nature, as there are very few published works available on the chemotaxonomic studies on Chironomidae.

SUMMARY

Members of the Dipteran family Chironomidae, represented by their larvae and pupae for an important group in benthic communities of freshwater ecosystems. While these organisms are of value as fish food or pollution indicators, the knowledge of their taxonomy is limited particularly with reference to tropical latitudes. Chemotaxonomic studies were done using polyacrylamide gel electrophoretic technique. The fractions such as homogenate, the haemolymph and the body wall without the haemolymph were used separately for five species from the fourth instar larvae. The two sub-families Chironominae and Tanypodinae appeared as distinct chemotaxonomically different groups. Among the three species of Chironominae (Tribe: Chironomini) it was seen that *Chironomus costatus* (Johannsen) and *Nilodorum stupidus* (Johannsen) show more affinity with one another than with *Stictochironomus affinis* (Johannsen).

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