

Recent trends in the biosystematics of Entognathous Apterygota with special reference to Collembola

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Abstract. In recent years increasing number of studies have been undertaken involving modern techniques like DNA hybridization, analysis of enzymic and chromosomal polymorphism in conspecific populations, ecophysiology and behaviour in individual species etc with far reaching implications in the systematics of Entognathous Apterygota, particularly the Collembola. Both scanning and transmission electron microscopes have been used extensively for the study of surface structure, sense organs, spermatozoa and gut providing considerable insight into phylogenetic relationship of higher taxa as well as inter-relationships at the generic and lower taxonomic levels.

Keywords. Insect biosystematics; Collembola; Protura; Diplura.

1. Introduction

Collembola, Protura and Diplura are regular inhabitants of the soil of natural forests and grasslands as well as others having good plant cover. Collembola are numerically most abundant and faunistically most diverse of the 3 groups. They have been found to colonize not only almost any type of soil with sufficient moisture but also the bark of trees, moss and to some extent fresh water bodies and marine systems (Prabhoo 1970, 1971a, b, c). An ecological study of soil invertebrates could hardly be complete without due consideration being given to the 3 groups mentioned above. Naturally meaningful ecological investigations can be undertaken especially on community structure, species diversity and niche, only with a proper understanding of the biosystematics of the groups.

Ever since the group Collembola has been established in 1862 by Lubbock, only morphological characters were utilised in the systematics of this group. Morphological variations were observed in geographical isolates (Prabhoo 1967) and local populations (Prabhoo 1976). With many new species being added to the existing list annually and with the accumulation of interesting information on biology and ecology of Collembola, systematists started looking up on the species from different angles for their delimitation and for understanding their genesis and phylogenetic position. An early attempt in this direction was the ecological categorisation of Collembola based on the concept of life forms made by Gisin (1943), subsequently modified by Cassagnau (1961) and Christiansen (1964). These categorisations, mostly based on external features were found to be inadequate especially in the case of cave dwelling forms in which specific features of biology and ecophysiology were recommended for study (Thibaud and Vannier 1986). Further, considerable information has become available on the chromosomal and protein polymorphism, ultra-structure and behaviour of Collembola in the recent past. An assessment of the studies carried out in the past 25 years to improve the species concept, phylogenetic relationship at various levels of taxa as well as speciation mechanisms is attempted

here so that an integrated approach in the systematics of these groups would be possible in future.

2. Cross hybridization of human and collembolan DNA

A variety of techniques are now available for hybridization of any species of DNA or RNA using labelled probes (Southern 1975; Britten and Davidson 1985). Stable and complete hybridization of two homologous DNA strands is possible under very stringent conditions, while partially homologous strands will hybridize under low stringency conditions (Howley *et al* 1979). The latter technique would be especially useful to study the degree of sequence homology between distantly related DNA's.

Fodde *et al* (1986) employed the human haptoglobin gene (DNA segment) to isolate from the Collembola *Thaumanura ruffoi* the DNA sequence sharing high degree of homology with the former. The authors incubated filter immobilised single stranded DNA from different species of animals, including *T. ruffoi*, with labelled Haptoglobin complementary DNA (Hp cDNA) at various stringencies. Autoradiographs showed that at the lowest stringency (washing at 42°C) a distinct band (6 kb) appeared with *T. ruffoi* genomic DNA as well as with those of some other species, with considerable amount of background formed by nonspecific hybridization. In two further sets of experiments the stringency was increased by increasing the temperature of washing to 55° and 62°C. This resulted in progressive disappearance of all nonspecific background in the autoradiographs. The band exhibited by *T. ruffoi* genome was found to be stable even at the highest stringency.

The experiments confirmed the presence of human haptoglobin like sequence in the Collembolan *T. ruffoi*. It is possible to use additional DNA probes to identify other base sequences in this insect. By isolating and cloning such fragments of established base sequence it is hoped to employ them in DNA hybridization studies which would lead to a better understanding of the systematic position and inter-relationship of over 1500 species of Collembola. Fodde *et al* (1986) suggest that nucleotide sequence comparison and restriction fragment length polymorphism analysis used by other workers (Helm-Bychowski and Wilson 1986; Sibley and Ahlquist 1986) to reconstruct bird phylogeny could be employed once *T. ruffoi* probes become available. This would be a new approach for the study of systematics and genetics of Collembola.

3. Use of electrophoresis in Collembolan population analysis

Advances in electrophoretic techniques now permit one to analyse multiple fractions of proteins in a sample by staining methods and show that to a great extent such variations (allozymic) are inherited in a Mendelian fashion. Large number of proteins in the same species can thus be studied for understanding genic variability and population structure (Goettlibe 1971). The rationale behind electrophoresis of proteins (enzymes) is not difficult to understand (Avisé 1974). Proteins migrate through an electric field according to the net charge and also depending upon the molecular weight. Those migrating to different distances usually differ at least by one amino acid residue. The relationship between amino acid sequence in proteins and DNA base sequence is well established. Hence electrophoretic mobility of proteins provides indirect information about DNA.

An attempt was made by Dallai *et al* (1983) to measure the genetic distance among Italian populations of two Neanurid species *Bilobella aurantiaca* and *T. ruffoi*. Only enzymes that could be separated electrophoretically were selected for the study, 4 in the first and 15 in the second species. One of the populations was considered as the control for measuring genetic distance among local populations. They found that *T. ruffoi* was monomorphic at 6 of the 15 loci studied while the remaining 9 loci were polymorphic for two or more alleles. Low genetic distance [calculated employing Nei's (1972) method], was noted between Siena and Barbione populations which were closely situated geographically. High level of genetic distance was evident between Siena/Barbione and Naples population which was comparable to that between well differentiated species which were reproductively isolated (Ayala 1975; Avise 1974). In yet another study of *T. ruffoi* populations from 3 more localities and in *Lathriopyga longiseta* (Fanciulli *et al* 1986a, b) it was found that the coefficient of genetic identity and genetic distance indicated that the populations had reached a high degree of genetic differentiation. According to the above workers if one considers that the value of genetic divergence permitting to attain reproductive isolation without morphological differentiation is the same as that in other organisms (Ayala 1983), then it is only proper to consider that the different populations at least of *T. ruffoi* were evolving independently and should probably be considered as belonging to different taxa. In *L. longiseta* a cline was suspected. It is evident from the above studies that species of Neanurinae with limited powers of dispersal could form isolated small populations wherein several factors including drift could play an important role in producing genetic divergence among populations.

3.1 *Polytene chromosome in Collembola*

Polytene chromosomes so characteristic of salivary gland cells and few other tissues of larval Diptera, played an important role in the understanding of the systematics and evolution of many dipteran families. In Collembola such chromosomes were discovered (Prabhoo 1961) in a neanurid species in the adult salivary gland cells. Basic configuration of the salivary gland complex and polytene chromosomes of *B. aurantiaca* has been described by Cassagnau (1968a). Considerable diversity has been noted in the gross morphology and cytology of the salivary glands, features that appear to be characteristic at the generic level, but in some cases at the species level (Lee 1985a). Species with polytene chromosomes always have stylet like mouth parts (Cassagnau 1968b) though the converse is not true. Thus pseudachorutinae and Caputanurinae with stylet like mouth parts do not have polytene chromosomes.

3.2 *Population variations in polytene chromosome structure*

Features characteristic of the polytene chromosomes are the puffs (Balbiani rings) and the heterochromatin of two types— α - and β -heterochromatin of which the latter is less condensed and takes up less intense stain but generally occupies a major part of the chromosome. Somatic pairing and a diffuse chromocenter are characteristically absent in the polytene chromosomes of Collembola.

3.3 Population variation in polytene chromosome structure

Several workers (Cassagnau 1976; Dalens 1976, 1977, 1979; Dallai 1979; Deharveng 1982) have studied chromosomal polymorphism in *B. aurantiaca*. Proper understanding of the phenomenon proved difficult because of the difficulty in tracing homologous sites in the chromosomes. In a more recent study of the above species Deharveng and Lee (1984) devised a system of nomenclature of the heterochromatin bands based on their location in 32 sites of the 7 chromosomes ($n=7$) in the Sainte Baume population (France). Comparison of this population with Gredos population (Spain) indicated a marked stabilisation of the karyotype with increasing heterochromatin load, a feature noted by Dallai (1979) in the Italian population of the species. Further heterochromatic load was more important in the northern (north Italy, southern France) and Atlantic (northern Portugal) races than in races living in more xeric and warmer environments (Spain, southern Portugal, southern Italy, Morocco) of the distribution of the species. It has already been shown earlier that heterochromatic load increased in *Deutonura monticola* with the environmental situation becoming unfavourable (Cassagnau 1974; Deharveng 1982).

3.4 Species differences in polytene chromosomes

Haploid number of chromosomes in several species of *Bilobella* was found to be 7. Hardly any karyotypic difference could be made out in the dot chromosomes characteristic of Collembola. On the other hand based on the band sequence in the polytene chromosomes and other features like the relative length of the chromosomal arms and taking *B. aurantiaca* as the reference species, Cassagnau *et al* (1985) categorized the 7 chromosomes in 7 species of *Bilobella* into 11 categories. Each species possessed a more or less unique combination of 11 categories of chromosomes such that the 7 species of *Bilobella*, with relatively uniform external features, fell into 3 groups, members of a group being more closely related to each other than to members of other groups. The 3 groups were *B. proxima* and *B. matsakisi*; *B. massoudi* and *B. zekoi* and lastly *B. albanica*, *B. aurantiaca* and *B. digitata*. Polytene karyotypic studies could thus help understanding interrelationship of congeneric species.

4. Electron microscopic studies

4.1 Cuticular structure in Collembola

Two types of granules, primary or single elementary granule triangular in form and secondary granule formed by the association of certain primary granules were recognized by Massoud (1969) in the epicuticle of Collembola. This study stimulated further work on many species (Lawrence and Massoud 1973; Lawrence 1975; Dallai 1977). In Entomobryidae one finds the primitive pattern of triangular primary granules arranged hexagonally. This pattern is noted in other families in restricted regions of the body like head, antennae, legs, furca etc. Isotomidae basically have closely connected quadrangular granules. The cuticular pattern is more or less constant in

many genera of Hypogastruridae, Neanuridae and Onychiuridae (Massoud 1969). It is possible that the cuticular pattern is intimately related to ecological factors (Dallai 1977). If so it will not help much in the understanding of the phylogeny. On the other hand the cuticular structure does not change in the course of development and hence it can be of considerable value in classification and discrimination of taxa. Cuticle morphology, however, has the same pattern in members of Entomobryidae, Cyphoderidae, Tomoceridae and Oncopoduridae. On the other hand in the tribe Sminthurini almost each genus has a peculiar pattern (Dallai 1977). Cuticular pattern of *Sminthurinus echinatus*, *S. marginatus* and *S. bremondi* is very different from that of other species of the genus so that the creation of the genus *Caprainea* for accommodating the above 3 species on the basis of other features like the presence of trochanteral organs and postantennal sensory hair seems justified (Dallai 1977).

4.2 Postantennal organ of Collembola

The postantennal organ situated just behind the antennae in the lower families of Arthropleona viz Hypogastruridae, Onychiuridae and Isotomidae. Light microscope studies revealed a limited diversity of structure of these organs and the characters were widely used in the systematics of the groups. The organ is supposed to be sensory in function. Scanning electron microscopic (SEM) studies (Dallai 1971) showed that the cuticle is continuous and undulated in Hypogastruridae but is perforated in Onychiuridae and Isotomidae. Further TEM studies of the organ in *Anurida maritima* indicated that it is olfactory in function (Dallai 1971).

4.3 Pseudoculi in Protura

Protura lack antennae, but in their place are found a pair of curious structures called pseudoculi, certain features of which are utilised in the systematics of the group. SEM and TEM studies (Bedini and Tongiorgi 1971; Yin *et al* 1986) confirmed the sensory nature of these organs and besides indicated that there are mainly two types of pseudoculi. The first type has striations and furrows on the surface and is the most common type found in *Eosentomon*, *Neocondellum*, *Hesperentomon*, *Acerentomon*, *Acerentulus*, *Gracilentulus* and *Baculentulus*. There are pore canals at the bottom of the furrows connected directly to the dendrites of 3 large sensory cells. In the second type the surface shows an orderly array of pores, each with a tubule and there are 6–8 sensory cells. This type is found in *Fujientomon* and *Sinentomon*, throwing light on the phylogenetic position of the former.

4.4 Ultrastructure of spermatozoa

Ultrastructure of spermatozoa has received special attention in the study of phylogeny of Arthropoda (Baccetti 1979). In insects the primitive 9+2 filaments in the axoneme (composed of tubulin and dynein) is found only in Collembola and some Diplura. In Collembola the sperm is encysted (Dallai 1969) but motile, and in Japygidae (Diplura) the condition is more or less similar but the sperm has an extra set of microtubules in the mitochondrial compartment. This latter model has

apparently paved the way for the evolution of the classical insectan sperm with axonemal complex of $9+9+2$ filaments, found in Campodeidae (Diplura), Thysanura and Pterygota. The proturan sperm is not in the direct line of evolution of the insectan sperm being specialised and evolved with loss of dynein and hence has become immotile with the axoneme pattern $12+0$ in *Acerentulus* and $14+0$ in *Acerentomon* and finally becoming aflagellate and disc shaped in Eosentomidae by the loss of acrosome and axonemal complex and in *Sinentomon* it has become aflagellate and spherical. These studies throw light on the phylogenetic position of Protura and relationship within the group. Based on sperm ultrastructure it would be difficult to agree with the classical view that Eosentomidae are more primitive than Acerentomidae.

4.5 Gut ultrastructure and phylogenetic interrelationship of Entognathous groups

Recent work on the ultrastructure of the gut of Entognathous Apterygota has thrown some light on the interrelationship within the group (Dallai 1976; Dallai and Callani 1979; Dallai and Burroni 1982). Comparative study of the ultrastructure of the midgut and hind gut indicates that the Protura and Collembola are aberrant groups, the former due to the possession of a peculiar pyloric chamber with specialised cells bearing long microvilli behind the sphincter and the latter because of the possession of a peculiar pyloric ring and connecting cells in the pyloric region just behind the midgut. Diplura possess some features of one or the other of the above two groups. Thus *Campodea* has similarities with Protura in possessing the Malpighian papillae but there is no pyloric chamber while *Japyx* has similarities with Collembola in having the connecting cells but there is no pyloric sphincter. Nor is there any trace of Malpighian papillae. Tuxen (1972) and Kristensen (1975) consider Protura and Collembola as sister groups of Diplura. Probably the ultrastructure of the gut lends support to this view.

5. Cladistic analysis of taxonomic characters

Cladistics has its origin in the Henning School (Henning 1966) which recognizes relationships among taxa at all levels on the basis of dichotomous branching (Klados = branch). In this system the characters are considered to be mainly of two types, those inherited from the ancestor are plesiomorphous and those that are special ones are apomorphous. Shared apomorphous characters are synapomorphous. Relationship among taxa is sought to be understood only on this basis.

Lee (1985b) tried to establish phylogenetic relationship at the subfamily level within Neanuridae by cladistic analysis using character weighted (Schaeffer *et al* 1972) and character unweighted (Kluge and Farris 1969) methods. Twenty characters were used for the purpose and the family Hypogastruridae was taken as the ancestral out group for character comparison and for computing branching sequence. Distance of various subfamilies of Neanuridae from the ancestor is then determined (Kluge and Farris 1969; Wiley 1981) and the cladograms obtained following the two methods although looked different, the position and arrangement of the taxa with respect to the ancestor and in relation to each other was very similar except for the fact that the position of the Neanurinae and Caputanurinae was reversed. It appears

that the above methods could be employed fruitfully for understanding the phylogeny of especially higher categories of taxa.

6. Behavioural studies and systematics of Collembola

Behavioural studies have contributed much to the clarification of problems in systematics. As behaviour constitutes most diverse of phenotypic characters and this feature is perhaps more strongly selected than morphology or physiology it can provide important criteria for characterising taxa at different levels.

Complicated behaviour patterns related to reproduction are shown by different groups of animals including insects. One may feel that the degree of complication is related to the evolutionary status of the groups determined on the basis of other characteristics chiefly morphological. Reproductive behaviour in Collembola is particularly associated with sperm transfer (Schaller 1971). These insects do not have external genitalia and hence the sperm transfer is shown to be indirect through a sperm packet or spermatophore. Mechanism of spermatophore production appears to be complicated as is shown in the case of *Orchesella cincta* by Doring (1986). Two ways of spermatophore transfer is recognized. The first one is effected without mating, considered as primitive and is noted in almost all Arthropleona and a few Symphypleona studied so far. Reviewing the mechanism of sperm transfer in Bourletiellinae Betsch (1974) suggested that the subfamily could be divided into two. The Bourletiellinae should comprise of the genera *Bourletiella*, *Deuterosminthurus*, *Heterosminthurus* and others in which the sperm transfer occurs with mating (Bretfeld 1971). In the other group of genera *Bourletiellitas*, *Anjavidiella* and *Vatomadiella* as well as *Parabourletiella* and *Paulianitas* (Betsch 1980), all confined to Madagascar, the males deposit the spermatophores at random and females pick them up without mating. This group according to Betsch (1974, 1980) should be recognised as a separate subfamily Parabourletiellinae. Bretfeld's (1971, 1973, 1976a, b) studies showed how reproductive behaviour related to sperm transfer in the 4 species of *Heterosminthurus*, viz *novemlineatus*, *insignis*, *lineatus* and *clviger* is specific and can serve as diagnostic features.

A second type of behaviour is concerned with protection against desiccation and is revealed as behaviour in nest construction or anhydrobiosis. Information on the nest building behaviour in a few species of Collembola is given by Poinot (1971). Consideration of these behaviour patterns has helped distinguishing species of some genera like *Folsomides* in which overlapping of characters due to variability of certain morphological features like ocelli create a certain degree of uncertainty in determination at the species level. Poinot-Balaguer and Barra (1982) used morphological features in combination with behaviour to distinguish *Folsomides americanus*, *F. angularis*, *F. deserticola*, *F. sexophthalma*, *F. novacerradensis* and *F. corsicus* and to support the contention that *F. deserticola* is a synonym of *F. sexophthalma*.

7. Conclusion

It appears from the above that there is an urgent need to undertake study of population phenomena in Entognathous Apterygota. This will help understanding the

mechanism of speciation on the one hand and elucidation of ecological importance of the species on the other leading to discovery of species that indicate specific ecological conditions. Sibling species are also likely to be brought to light. Evidently these objectives can be realised only by using modern methods of research. Cytological studies of polytene chromosomes can be made only in some genera of Neauridae which possess polytene chromosomes. On the other hand study of enzyme polymorphism employing sensitive electrophoretic techniques can be undertaken in all groups wherever necessary. Similarly the study of behaviour and ecophysiology of all important species should be undertaken along with electron microscopic studies. An integrated approach to the study of taxa appears extremely desirable.

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