To
Mrs. J. G. Peters
with kind regards
K. G. Sivaramakrishnan

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Biosystematic studies of south Indian Leptophlebiidae and Heptageniidae in relation to egg ultrastructure and phylogenetic interpretations

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Abstract. Previous work on the importance of egg ultrastructure to the taxonomic and phylogenetic studies of Ephemeroptera with special emphasis on the two schistonotan families, Leptophlebiidae and Heptageniidae is reviewed. The chorionic sculpturings, the types of micropyles and attachment structures of the eggs of south Indian mayfly species belonging to 8 genera of Leptophlebiidae and 3 genera of Heptageniidae have been studied through scanning electron microscope.

The present study confirms the earlier observations that the Leptophlebiidae are most certainly of enough distant relationship with the Heptageniidae to warrent inclusion in separate superfamilies viz Leptophlebioidea and Baetoidea. Whereas the structural modifications on the eggs are significant in contributing to the taxonomy of both the families at the generic level, submicroscopic examinations of chorionic structures provide data for the separation of different species and species complexes in Leptophlebiidae.

Keywords. Biosystematics; Leptophlebiidae; Heptageniidae; egg ultrastructure; phylogeny.

1. Introduction

Biosystematic studies of the Ephemeroptera have gained much significance in recent years because of their importance as fish food, of pollution indication in freshwater ecosystems and in being ideal objects for biogeographic analysis due to their conservative dispersal habits. This group of insects is best suited for integrated approach to phylogeny, classification and biogeography due to its compact size enabling systematic studies on a global perspective. Furthermore, systematic conclusions can be arrived at through the study of a wide range of characters from different sources which include exoskeletal, soft anatomical, physiological and behavioural data from imaginal, nymphal and egg stages (Mc Cafferty and Edmunds 1979). The Ephemeroptera have various life history stages that have semi-independent genetic control, separate selection pressures and hence different rates of divergence. These differential rates of evolution in various stages are a powerful tool in the reconstruction of the sequence of branching in phylogeny. Differential rates of semi-independent or independent character systems within a life history stage can provide equally powerful data (Edmunds 1975). However, interpretations become rather difficult due to niche specialization, niche overlap and parallelisms. In such intriguing situations, electrophoretic (Kownacki and Starmach 1984; Matha and Sula 1984; Zurwerra *et al* 1984), karyological (Kiauta and Mol 1977) and egg ultrastructural (Koss 1968; Koss and Edmunds 1974) studies assume crucial importance as valuable tools in resolving enigmatic generic and species complexes.

Through a study of the ultrastructure of approximately 100 of the known mayfly genera, Koss (1973) made it evident that this group of insects can provide valuable data for taxonomic and phylogenetic studies of the order. One very important and outstanding feature of the mayfly reproductive cycle makes the egg stage ideally

suited for systematic studies. Unlike most insect groups, mayfly eggs mature during the nymphal or immature part of the life cycle, and therefore, eggs can be dissected from mature, black wing-padded nymphs for study. Also, those eggs may be compared to eggs dissected from adult specimens, and this procedure can be very helpful when trying to associate nymphs with adults when rearing is not possible and one of these stages is undescribed. The eggs possess morphological characters which are far more dependable than the body colouration of the adult.

Almost unique among mayflies is the fact that most adult females lay their eggs freely on the surface of the water rather than personally attaching them to any fixed objects. Because of the egg laying behaviour of the adult mayfly and the environment to which the eggs are released, these eggs are quite strongly subjected to the pressure of natural selection. These selection pressures have caused the evolution of a great variety of external features on ephemeroptera eggs to contribute valuable information for phylogenetic interpretations of the order. These features include, the chorionic sculpturings, the types of micropyles and attachment structures. The egg ultrastructural data provide for further testing of the numerous hypotheses involved in proposed phylogenies, and suggests revision of some of the present concepts of ephemeropteran phylogeny.

An attempt has been made to review the previous work on the importance of egg ultrastructure to the taxonomic and phylogenetic studies of the order with special emphasis on the two schistonotan families, Leptophlebiidae and Heptageniidae and to examine the importance of this study in providing valuable data for the separation of generic and species complexes in the light of the scanning electron microscopic study of the eggs of south Indian mayfly species belonging to 8 genera of Leptophlebiidae and 3 genera of Heptageniidae.

2. Perspectives and overview

Pioneering studies of importance of the eggs to insect taxonomy were made by Onsager and Mulkern (1963) for Orthoptera; Ross and Horsfall (1965) for Culicidae; Knight *et al* (1965a, b) for Plecoptera; Degrange (1960), Koss (1968) and Koss and Edmunds (1974) for Ephemeroptera; and Southwood (1956) for Heteroptera. Recently, numerous scanning electron microscope (SEM) studies have been performed on insect eggs (Hinton 1981). Most of these works revealed diagnostic characteristics useful in the assessment of phylogenetic relationships.

Previous SEM studies of the eggs of the Ephemeroptera have included Baetidae (Kopelke and Müller-Liebenau 1981a, b, 1982), Caenidae (Kopelke 1980; Malzacher 1982), Heptageniidae (Flowers 1980a, b; Kopelke 1980; Landa and Soldan 1982), Leptophlebiidae (Kopelke 1980; Pescador and Peters 1982; Gaino and Mazzini 1984; Mazzini and Gaino 1985; Towns 1983; Towns and Peters 1978, 1979a, b) and Tricorythidae (Kopelke 1980). These studies have revealed features of the chorionic sculpturing, micropyle and attachment structures that can be useful in understanding the phylogeny of Ephemeroptera.

Koss and Edmunds (1974) studied the eggs of representative genera of all families of Ephemeroptera except the Palingeniidae and found out that the archetypical Ephemeroptera egg is considered to have been round, with a smooth chorion, nonfibrous adhesive layer, funnelform micropyle, and a suprachorionic sperm guide.

Subsequent evolution resulted in several different micropyles and many different chorionic sculpturings and attachment structures. The ultrastructural features of the eggs of the superfamilies of Ephemeroptera are given in table 1. This is apparently in conformity with the phylogeny (figure 1) of the extant superfamilies and suborders of Ephemeroptera as suggested by Mc Cafferty and Edmunds (1979). The evidence from the study of Koss and Edmunds (1974) has suggested that within the

Table 1. Ultrastructural features of the eggs of superfamilies of Ephemeroptera.

Superfamily	Attachment structures	Micropyle (Chorionic sculpturing—variable)
Baetoidea	Lack of polar caps other than types IV and V	Tagenoform
Leptophlebioidea	Polar caps absent*	Funnelform
Ephemeroidea	Polar caps other than type II absent	Funnelform
Ephemerelloidea	Type I polar cap	Tagenoform
Caenoidea	Type III polar cap or absent	Linear or poorly developed tagenoform
Prosopistomatoidea	Type III polar cap, undersized, unorganized	Tagenoform

*Type I, Non-coiled, single unit cap; type II, non-coiled, multi-unit cap; type III, multi-threaded coiled cap; type IV, single-threaded coiled cap; type V, KCT cap (knob-terminated coiled threads).

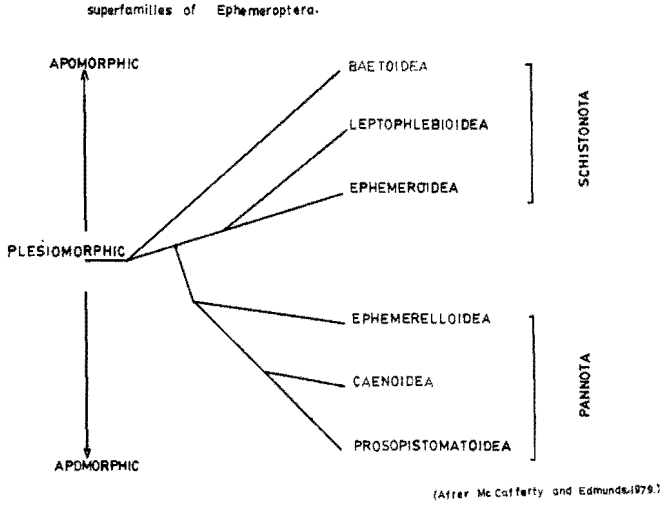


Figure 1. Phyletic diagram representing the probable phylogeny of the superfamilies of Ephemeroptera (after Mc Cafferty and Edmunds 1979).

Ephemeroptera, chorionic sculpturing evolved independently in nearly every family and superfamily; therefore, it has little use as a tool for reconstructing a phylogeny indicating familial relationships. The micropyles and attachment structures supply the bulk of the data useful for phylogenetic studies of the order.

However, the sculptural modifications of chorion of the eggs have allowed significant contributions to the taxonomy of Heptageniidae at the generic level and of Leptophlebiidae at the generic and species level. Working on the Ecdyonurid complex of the Nearctic *Heptagenia*, Flowers (1980b) has established two new genera viz *Leucrocuta* (*maculipennis* group) and *Nixe* (*lucidipennis—simplicioides* group). Among several other characters, the chorionic sculpturing of eggs have played a vital role in the separation of these two genera and in the establishment of two subgenera

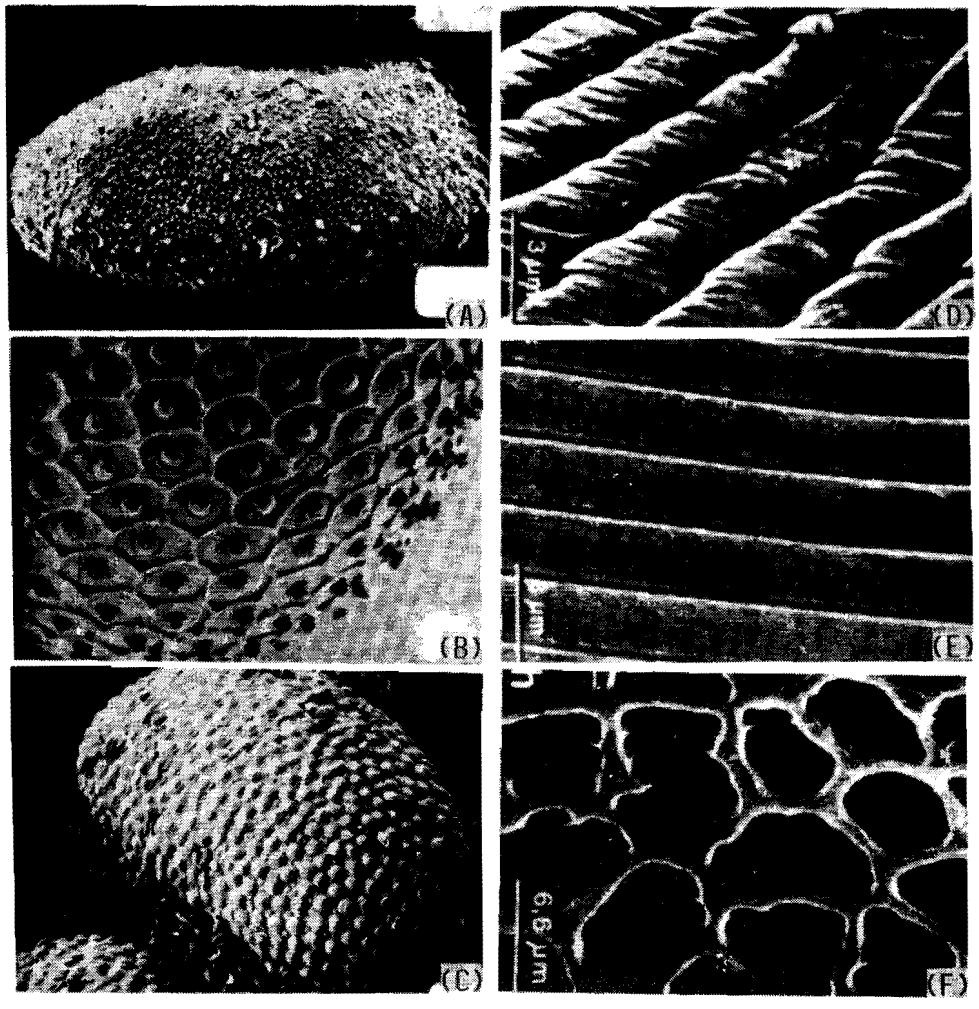
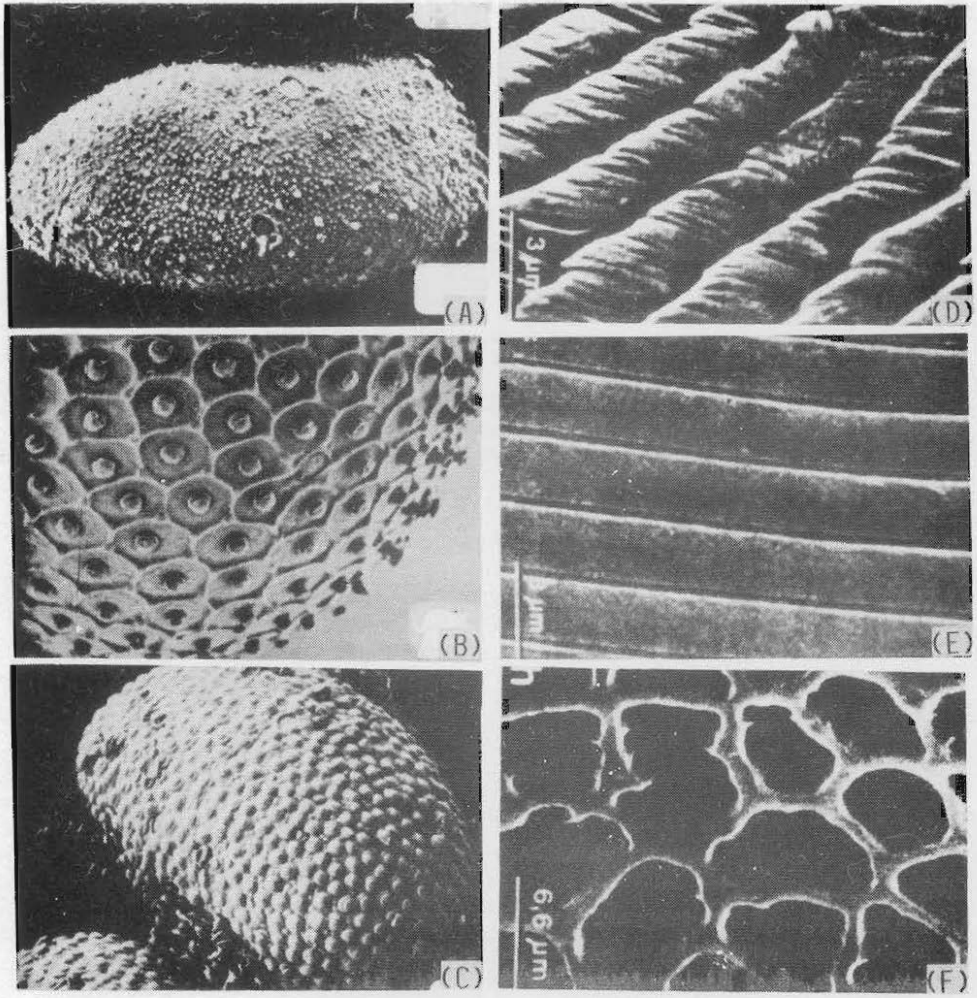


Figure 2. SEM of eggs (**A–C**, after Flowers 1980b; **D–F**, after Gaino and Mazzini 1984). **A.** *Leucrocuta juno*. **B.** *Nixe* (*Nixe*) sp. **C.** *Nixe* (*Akkarion*) *criddlei*. **D.** *Habroleptoides modesta* (chorionic details). **E.** *Habroleptoides umbratilis* (chorionic details). **F.** *Habroleptoides auberti* (chorionic details).



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viz *Nixe* (*Nixe*), which belongs to the former *lucidipennis* group and *Nixe* (*Akkarion*) which belongs to the former *simplicioides* group. The chorion of *Leucrocuta juno* shows fine granular structures (figure 2A), that of *Nixe* (*Nixe*) sp. has large mesh reticulation (figure 2B) and that of *Nixe* (*Akkarion*) *criddlei* has small knobs (figure 2C) and there is no reticulated mesh. Gaino and Mazzini (1984) have shown the importance of chorionic sculpturing of the eggs in the separation of 3 species of *Habroleptoides*. *H. modesta* has slightly raised costae, about 1·8 μm wide, slightly twisted along its longitudinal axis appearing as a thin banding with very small interspaces between adjacent costae (figure 2D). *H. umbratilis* egg shows slightly raised costae arranged very close to each other on the chorion. Each costa is 1·6 μm wide, and unlike the previous species, shows no banding (figure 2E). On the other hand, the chorionic surface of the egg of *H. auberti* shows a reticulation made up of irregular polygons the sides of which vary in length but are about 1μm wide and thick (figure 2F).

3. Materials and methods

Eggs from the final instar nymphs of investigated species (from an undescribed female imago in the case of *Nathanella indica*, collected from Palni hills) were dis-

Table 2. Ultrastructural features of the eggs of some south Indian Leptophlebiidae and Heptageniidae.

	Chorion	Attachment structures	Micropyle
Family Leptophlebiidae			
Petersula courtallensis	Punctate with floristic pattern of ridges	Absent	Funnelform
Choroterpes (*Euthraulus*) sp	Ornamented with a pattern of star-shaped bosses, each with 8–9 irregular raised ribs radiating from the centre	Absent	Funnelform
Thraulus gopalani	Thick without ornamentation	Each pole of the oval egg with 8 very long, stout curved spinous processes	"
Indialis badia	Ornamented with longitudinal undulating ridges	Circular, sucker-like at both poles	"
Genus nov. sp. nov.	Tuberculate	Peglike structures	"
Nathanella indica	Reticulate	Absent	Not observed
Notophlebia jobi	Tuberculate	Uniformly clothed with cylindrical, slender rods	Funnelform
Isca (*Isca*) sp.	Circular, floristic patterns	Absent	Not observed
Family Heptageniidae			
Epeorus sp.	Smooth	Absent	Tagenoform
Cinygmina sp.	Tuberculate	Large KCT equatorial	"
Thalerosphyrus sp.	Tuberculate	KCT concentrated at poles	"

sected out into 95% ethyl alcohol and transferred to 50/50 ethyl alcohol—amyl acetate and then on to 100% amyl acetate. Eggs were dried with CO_2. SEM studies were carried out in the School of Biological Sciences, Madurai Kamaraj University, Madurai.

4. Results and discussion

The ultrastructural features of the eggs of investigated species of south Indian Leptophlebiidae and Heptageniidae are given in table 2.

The only characters typical of the 3 heptageniid genera studied are a tagenoform, usually entirely chorionic micropyle and a lack of polar caps other than type V. The type of attachment structure along with the chorionic ultrastructure give some clue to distinguish the 3 investigated genera (figure 3A–C) at least in the regional peninsular Indian context.

The eggs of the investigated species of south Indian Leptophlebiidae have the greatest diversity in chorionic sculpturing (figure 4A–H). The chorion may be smooth (figure 4C), tuberculate (figure 4E), punctate with floristic pattern of ridges (figure 4A), reticulate (figure 4F) or even sculptured with a floristic pattern of ridges

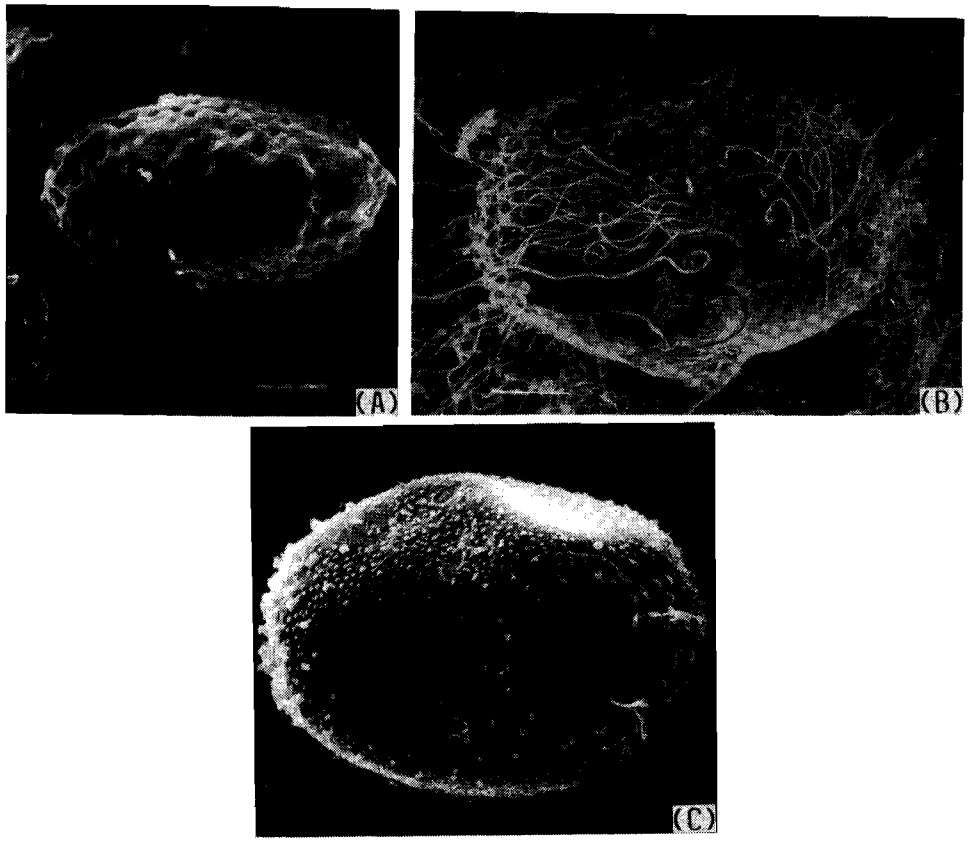
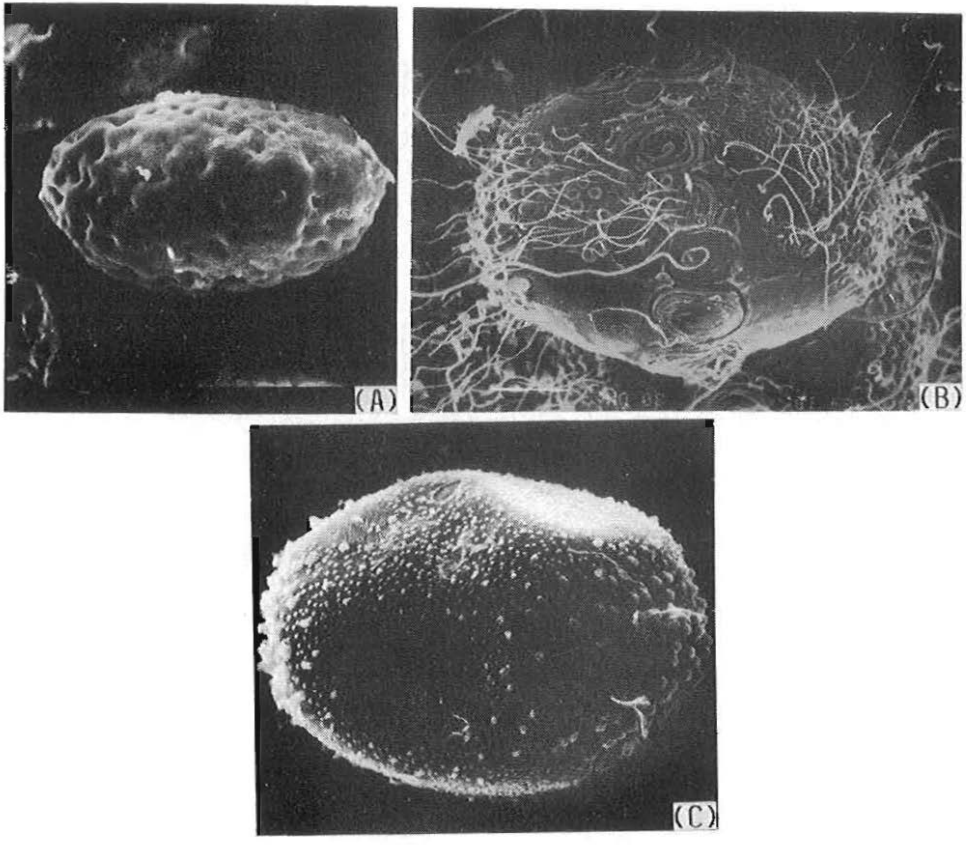


Figure 3. SEM of eggs. **A.** *Epeorus* sp. **B.** *Cinygmina* sp. **C.** *Thalerosphyrus* sp.



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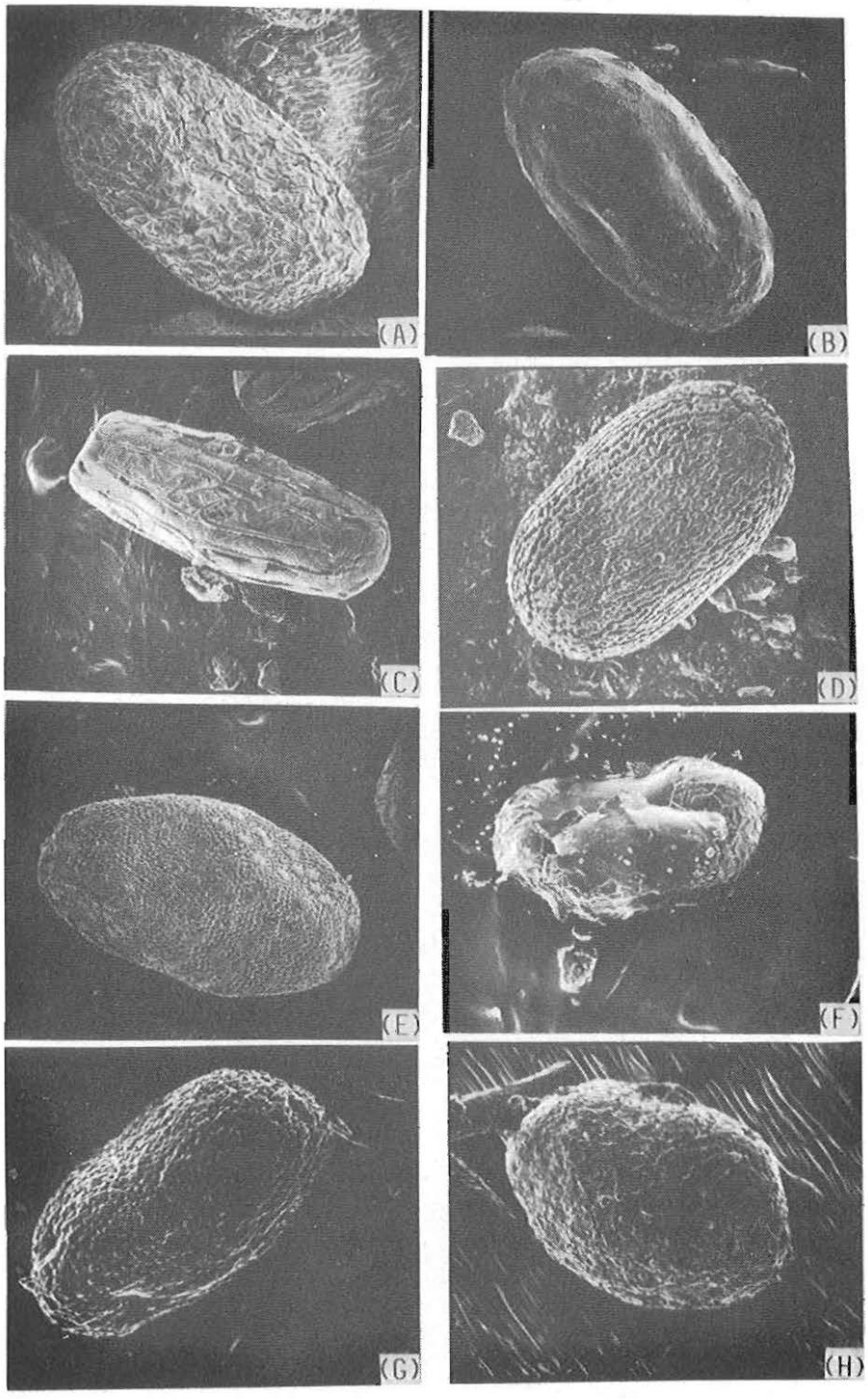

Figure 4. SEM of eggs. **A.** *Petersula courtallensis*. **B.** *Choroterpes* (*Euthraulus*) sp. **C.** *Thraulus gopalani*. **D.** *Indialis badia*. **E.** Genus nov. sp. nov. **F.** *Nathanella indica*. **G.** *Notophlebia jobi*. **H.** *Isca* (*Isca*) sp.

(figure 4H) or ornamented with longitudinal undulating ridges (figure 4D). Attachment structures are rather rare. Peglike structures (figure 4E), long, stout, curved, spinous processes around each pole (figure 4C), circular, sucker-like structures at both poles (figure 4D) and cylindrical slender rods (figure 4G) have been observed. Polar caps apparently do not occur. A funnelform micropyle (figure 4D) and lack of polar caps are the only features that are constant on all the observed species of Leptophlebiidae eggs. The chorionic sculpturing of the eggs is very useful in differentiating 8 genera of Leptophlebiidae under investigation and the data from the egg ultrastructure are consistent with the phylogenetic scheme proposed by Peters and Edmunds (1970) for eastern hemisphere Leptophlebiidae (figure 5).

Total egg data in the present study appears to be consistent with the classification and with the phylogenetic scheme (figure 1) proposed by Edmunds and his co-workers (Edmunds and Traver 1954; Edmunds 1962, 1972; Edmunds *et al* 1963; Mc Cafferty and Edmunds 1979) for the superfamilies of Ephemeroptera. The phylogeny suggested by the egg data is inconsistent with the classification proposed by Demoulin (1958). The present study confirms the earlier observation of Koss and Edmunds (1974) that the Leptophlebiidae with their funnelform micropyle are most certainly of enough distant relationship with the Heptageniidae which have tagenoform micropyle to warrent inclusion in separate superfamilies viz Leptophlebioidea and Baetoidea.

The subfamily Atalophlebiinae of the family Leptophlebiidae has undergone extensive adaptive radiation in peninsular India. Many genera like *Choroterpes, Thraulus, Indialis, Notophlebia* and *Isca* apparently have several species complexes and judicious use of data from egg ultrastructure along with other modern biosystematic tools is bound to help in solving many taxonomic and phylogenetic puzzles in future.

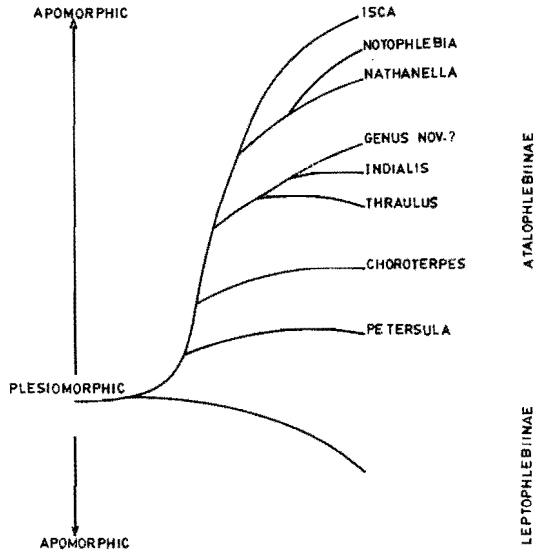

Figure 5. Phyletic diagram representing the probable phylogeny of the genera of south Indian Leptophlebiidae (modified from Peters and Edmunds 1970).

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