STUDIES ON CONCHOSTRACA (CRUSTACEA) OF KERALA

Thesis submitted to the University of Calicut
For the award of the Degree of
Doctor of Philosophy
In Zoology

USHA BALARAMAN

Under the supervision of:

Dr. C. K. Gopinathan Nayar
Former Head of the Department
Department of Zoolgy
Christ college, Irinjalakuda

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CERTIFICATE

Certified that the thesis entitled ***Studies on Conchostraca (Crustacea) of Kerala*** is a bonafide research work carried out by Smt. Usha Balaraman (Lecturer in Zoology, All Saints' College, Thiruvananthapuram) under my guidance and supervision in the Department of Zoology, Christ College, Irinjalakuda, submitted for the award of the degree of Doctor of Philosophy, under the Faculty of Science, University of Calicut.

I also certify that no part of this thesis has been submitted to any other organization for the award of any degree/ diploma or any other similar title.

Dr. C. K. Gopinathan Nayar
Research Supervisor

Irinjalakuda
10.03.2005

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DECLARATION

I, Usha Balaraman, do hereby declare that the present study entitled ***Studies on Conchostrca (Crustacea) of Kerala*** has been conducted by myself at the Department of Zoology, Christ College, Irinjalakuda, under the supervision of Prof. (Dr.) C. K. Gopinathan Nayar. I further declare that this work has neither been published nor has it been submitted for the award of any degree, diploma, fellowship, or any other similar title or recognition.

Usha Balaraman.

Usha Balaraman

Irinjalakuda,

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Usha Balaraman

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Chapter 1

INTRODUCTION

Classification

Habitat Preferences

Morphological features of Conchostraca

Feeding and Reproduction

Conchostraca of Kerala

The members of the order Conchostraca are small, primitive, widely distributed branchiopod crustaceans that have a long geological history dating back to the Devonian period (Hutchinson, 1967). They occur on all continents except Antartica (Belk, 1982). They look like miniature bivalves in general appearance; hence they are popularly referred to as clam 'shrimps'. The order consists of five extant families: Cyclestheriidae Sars, 1899 (monotypic), Cyzicidae Stebbing, 1910 (four genera), Leptestheriidae Daday, 1923 (five genera), Limnadiidae Baird, 1899 (six genera), and Lynceidae Stebbing, 1902 (three genera).

Although conchostracans are common worldwide, they have not been the subject of intense study. The majority of the studies have centered round feeding (Cannon, 1933; Karande & Inamder, 1961; Royan, 1976; Belk, 1982; Martin, 1989; Zeni & Zaffagnini, 1992), reproduction and development (Zaffagnini, 1969; Zeni & Zaffagnini, 1989; Scanabissi Sabelli & Tommasini, 1990; Zucker et al., 1997) and only limited work - focused on other aspects of their biology. Much attention has been devoted to the systematics of these animals (Straškraba, 1965; Royan, 1773; Durga Prasad, 1976; Martin & Belk, 1988; Roessler, 1995a, b, c), and about 200 species are currently recognized. A series of research work on the genetics and evolution of androdioecy (a rare mixed mating system) of *Eulimndia texana* (Sassaman & Weeks, 1993; Sassaman, 1995; Weeks & Bernhardt, 2004), a common clam shrimp of western North America, has generated wide interest.

Classification

The traditional classification (Calman's 1909) of the branchiopod Crustacea into four orders, viz., Anostraca, Notostraca, Conchostraca, and Cladocera was a refinement of the scheme first put forward by Sars in 1867. Calman abandoned Phyllopoda as a taxonomic entity (in Sars' scheme, an order) that included Anostraca, Notostraca and Conchostraca, and elevated them to the ordinal ranks. 'Phyllopoda' is not used now in systematic nomenclature to designate any taxonomic category. On the other hand, 'Phyllopod' is used as a common descriptive term to include Anostraca, Notostraca and Conchostraca. In the scheme of classification put forward by Tasch (1969), Conchostraca is included along with Cladocera in the subclass Diplostraca. In the latest scheme Fryer (1987) recognizes two assemblages of bivalved crustaceans "separated by numerous often trenchant differences and share so few significant features that their close relationships cannot be assumed". Hence he elevates these two assemblages (first recognized as tribes by Linder, 1945) to ordinal ranks - Laevicaudata (consisting of the only family Lynceidae) and Spinicaudata (all other families formerly included as conchostracans). However, Fryer recommends that Conchostraca may still be retained for descriptive purposes, despite its taxonomic invalidity. Fryer also considers Cladocera as consisting of 4 orders so that, according to his classification, Branchiopoda includes eight extant orders and two extinct orders.

Negrea et al. (1999) propose a new scheme of classification for Branchiopoda giving due credit to the "best currently available classification" of Fryer (1987). In this current classification, class Branchiopoda is divided into five super orders and eleven orders. Conchostraca has been reinstated as super order Conchostraca. This super order

Conchstraca includes three orders – Laevicaudata, Spinicaudata, and Cyclestherida. Cyclestheriidae has been separated from Spinicaudata because of the many trenchant differences between the two on the one hand, and the striking similarities it shares with Cladocera on the other hand. Hence Cyclestheriidae has been elevated to ordinal rank.

Fryer's classification is the best currently available, and this classification is followed in the present study.

Superclass Crustacea Lamarck, 1801
Class Branchiopoda Latreille, 1817
Order Laevicaudata Linder, 1945
    Family Lynceidae, Stebbing, 1902*
Order Spinicaudata Linder, 1945
    Family Cyzicidae Stebbing 1910
    Family Leptestheriidae Stebbing, 1902
    Family Limnadiidae Burmeister, 1843*
    Family Cyclestheriidae Sars, 1887*

* Species identified in the present study come under the family

Habitat Preferences

Conchostracans, like other phyllopods Anostraca and Notostraca, are entirely confined to ephemeral fresh water habitats like rain pools, rock pools, rice fields, and flood plain pools. Except for *Cyclestheria*, which occurs in ephemeral ponds as well as in permanent bodies of water, all other conchostracans are restricted to ephemeral habitats. These ephemeral habitats have world wide distribution and occur wherever seasonal precipitation and impervious substrates combine to create surface pools during the wet season. These temporary bodies of water are referred to as temporary pools, rain pools, seasonal ponds, vernal or autumnal pools (in temperate regions), pans, and playas (in arid or semiarid regions). They may have a single wet annual phase or may fill and dry several times a year. Each temporary water body is an accumulation of surface water with no inlet or outlet at any time of the year. They vary in size from small ditches and medium sized pools to vast stretches of water. These habitats are not uniform but vary in depth, duration of existence, substratal conditions in mud, rock, sand bottom etc.

Typically temporary water bodies are of different types based on duration of wet and dry phases. Some are short-term temporary pools with wet phase lasting for about 7 to 20 days only. In Kerala, they form during both the monsoon seasons and the non-seasonal heavy showers. Long-term temporary pools, that have water for 2 to 3 months, form during the monsoon seasons (or during one season) depending on the density of precipitation. Besides, in years of heavy rainfall, during both the monsoon seasons, the surface water will be retained more than 6 to 7 months in the long-term temporary pools (Balaraman & Deepa, 2002).

These habitats, distinguished by fluctuations of physico-chemical conditions, are inhabited by a few but abundant, consistent and predictable assemblages of species (Wiggins et al., 1980). The primary producers are mainly algae and diatoms. A few rooted vascular plants like *Lemna, Wolfia, Pistia, and Eichornia* are found in the long-term temporary pools. Since the favourable milieu prevails only for a short time, there is little chance for numerous trophic levels to evolve; hence, a condensed food chain results.

The apex of the short food pyramid is taken up normally by invertebrate predators, especially insect larvae. Although fishes are rare and no other organism dominates in size, the crustaceans are abundant in the temporary pools. Among the crustaceans, the branchiopods are generally confined to such a habitat and show specialized adaptations like rapid population build up, early maturity, high fecundity (iteroparity), unisexuality, and androdioecy (Wiggins et al., 1980; King et al., 1996).

Rapid population buildup followed by rapid decline is an essential feature of communities living in these transient waters. The speed with which a water body is repopulated when the favourable milieu returns, is in part an expression of the high reproductive potential of its inhabitants and the dispersal mechanisms they rely on. The ecology of these unique animals thus reflects the interplay of a temporally unstable habitat and the different life history strategies of its inhabitants. Evolutionarily ephemeral pools may be important as refuges for certain taxa. At least one group, the large branchiopod crustaceans (including conchostracans), becomes restricted to temporary pools concurrent with the rise of predatory suction-feeding bony fishes in Mesozoic seas and lakes (Hutchinson, 1967; Wiggins et al., 1980).

Morphological features of Conchostraca

The body of a typical conchostracan is laterally compressed and enclosed in a bivalve shell or carapace. The valves are marked by an umbo and concentric growth lines. However, the total number of lines varies with the species and in Lynceidae the carapace lacks an umbo and growth lines. The valves are hinged in Lynceidae and simply folded in all other families. The carapace is connected to the dorsum of the head by a ligament and is closed by powerful adductor muscles in the head region. The body can move freely in the carapace chamber, even when the shell valves are closed. The head region is enormous in Lynceidae while it is relatively smaller in all other families. The anterior portion of the head is known as the rostrum. Usually two closely placed sessile compound eyes are present at the anterior extremity of the head. In Lynceidae, eyes are located far from the anterior extremity of the head. The ocellus, ventral to the eyes, is embedded in tissue beneath the surface of the rostrum. Posterior to the eyes on the dorsal surface is the dorsal organ or neck organ. The first pair of antennae or antennules that flanks the rostrum are short and uniramous and they bear the setae. The setae are sensory in nature and are often referred to as sensory or olfactory setae. The second pair of antennae is biramous and large that varies in morphology among the families. Their function is primarily locomotion but they also function in swimming, burrowing, floating, adhering to surfaces, and grasping the female carapace. The two flagellae are provided with short spines on the dorsal side and long setae on the ventral side. The flagellae are segmented; the number of segments varies with the different species. The free end of the labrum is often marked by prominent spine-like process. The mandibles are large, thick appendages, consisting of a narrow conical dorsal part and a stout ventromedial molar process, thus showing a basic crustacean 'rolling' mandible used for primitive grinding movements. The distal margin differs among the families with two types of triturating surfaces. The first type consists of a slight terminal groove flanked on the outer surface by comb-like scales. These scales fuse and become a smooth surface of connected pits on the slight groove on the triturating surface. This type of mandible is seen in Cyclestheriidae, Cyzicidae, Leptestheriidae, and Limnadiidae. The second type of triturating surface is seen in the Lynceidae. Here the distal mandibular border bears a double row of large teeth. The area between these two rows bears small spinules rather than a pitted surface as seen in the other families. Maxillules and Maxillae are reduced.

The trunk is generally composed of 10-32 segments, each bearing a pair of phyllopodous limbs or thoracopods that decrease in size posteriorly. The thoracopods are never flat but strongly curved at the most convex surface of the appendage with lobes of the exopod bent posteriorly to the outside and endites 1-5 bent posteriorly to the inside. The thoracopods are divided into exopod, epipod, and endopod. Each endopod has five flat setose endites. Sars (1887) treats the endopod as having 6 endites. So also do Martin and Belk (1988) while discussing Laeviacudata. But, according to Olesen et al. (1996), the distal most 6th endite is a reduced 'true' endopod of the limb as there is a clear suture line separating this endite from the protopod. The proximal endite, endite 1 which is more distinct than the others is usually referred to as coxal lobe. The exopod has a dorsal and ventral lobe. The dorsal lobe of exopod is small in all families except in Lynceidae. Thoracopods 9-11, in the females, often have modified dorsal lobes or dorsal filaments of exopod. These structures aid in keeping the eggs in between body and carapace. There is an additional, expanded, thin non-setose epipodite arising near the base of the exopodite. In the males, the first one or two thoracopods bear hook-like endites. These prehensile limbs, called claspers, are used for grasping the female during copulation. Endites 4, 5 and 6 of the clasper are modified into a movable finger (6th endite), the opposing 'palm' or hand (endites 4 and 5) and two palps (one small and one large) from the posterior side of 'palm'.

The trunk of Conchostraca ends in a broad truncate telson which, in turn, terminates in a pair of elongated spines known as the furcal claws. Except in Lynceidae, the telson on its dorsal surface bears a double row of stout spines and terminally a pair of blade-like anal spines. Telsonal filaments (post abdominal setae) usually emerge from the space between the spines. In Lynceidae, the trunk terminates in an anal somite that bears telsonal filaments but lacks furcae. A pair of branched gonads extends the full length of the trunk and opens on the eleventh somite, except for the vasa deferentia of the Lynceidae which opens on the telson. The eggs are carried above the trunk, and are dropped to the bottom of the pond when the animal moults.

Feeding and Reproduction

Conchostracans are mainly benthic, while most species burrow into the substratum and lie with their ventral surfaces pointed upwards, feeding on detritus in suspension. Conchostracans are mainly omnivorous (Martin, 1989). Although only a few studies describe gut contents, those, which do, mention them as non-selective algal and detritus feeders (Karande & Inamdar, 1961; Royan, 1976; Belk, 1982). Royan (1976) has showed that the gut contents of *Leptestheriella madurainesis* included phytoplankton and zooplankton, that varied with the abundance of those foods in the water column. This reflects the non selective bottom grazing feeding habit of this species. All conchostracans possess well-developed coxal lobes that function as gnathobases to move food particles along the ventral food groove towards the mouth (Cannon, 1933; Martin, 1989).

Propagation is generally bisexual, but some species are parthenogenetic or hermaphroditic. More than one mode may occur in some species so that bisexual life cycle, unisexual life cycle, and cyclic parthenogenesis occur within Conchostraca. In bisexual life cycles, both gonochoric (obligatory sexual) and androdioecious (a mixed mating system of sexual and selfing hermaphrodites) conditions exist. In unisexual life cycle, both parthenogenesis and selfing-hermaphroditism occur and in cyclic parthenogenesis, both parthenogenesis and sexual reproduction triggered by environmental cues take place. Inter-conversions between gonochoric and androdioecious and unisexual cycles are common within the Limnadiidae. Lynceidae and the vast majority of Cyzicidae and Leptestheriidae appear to be sexual (Sassaman, 1995). Unisexual species, in which males are totally absent or absent in entire generations of the life cycles and capable of unisexual reproduction, has been reported for *Cyzicus, gynecia* (Sassaman, 1995), and for *C. hislopi* populations (Sars, 1887; Nair, 1968; Paul & Nayar, 1977; Roessler, 1995c).

In most species, eggs are massed and glued together. Package of 'resting eggs' are usually shed when the female moults. In conchostracans, breeding occurs continuously throughout the adult stage. Resting eggs or cysts synchronize life cycles to the variations that occur in the habitat and provide indirect ways for dispersal. Most conchostracans are typified by the production of very resistant, resting-stage eggs or cysts. The cyst is integral to the life cycle of the conchostracans because it allows for the drying (or freezing) of the pools in which they live, and then the 'recolonisation' of the pools when they refill. Conchostraca of temporary ponds have typically only one generation per wet episode and produce dozens to hundreds of cysts per day, to build up a cyst bank. Each batch of eggs is carried for at least a short period by the female. After a few cell divisions, the eggs typically enter diapause. This resting egg (actually a developing embryo) has a dark covering (tertiary envelop or shell) and is prepared to survive drying, heat, freezing and ingestion by birds (Belk & Cole, 1975). Belk (1970) removed the covering of diapausing conchostracan embryos to demonstrate that the shell did not reduce desiccation effects, but may reduce mortality due to abrasion or intense sunlight. Signals that break diapause include temperature, oxygen concentration, pH, and dissolved solutes in water (Belk & Cole, 1975; Wiggins et al., 1980; Brendonck, 1996). Together with early maturation and high fecundity, resting eggs are thought to be highly adaptive to the temporary environment, and hence explain the success of the group. In addition, the patterns of egg-shell sculpturing is species specific (Belk, 1989) and is a useful taxonomic character in the *Eulimnadia* species.

Cysts hatch into typical free swimming nauplius larvae bearing three pairs of appendages and a median eye. Development proceeds through about five nauplius larval stages (Brendonck, 1999). *C. hislopi* is the only conchostracan that has direct development of its offsprings - larval and juvenile stages are passed within the brood chamber.

Conchostraca of Kerala

The State of Kerala, with the Arabian Sea in the west, the Western Ghats in the east, and a network of forty rivers, enjoys a tropical climate. Kerala has two monsoon seasons – the South-west monsoon from June to September, and the North-east monsoon from October to December – interrupted by a dry season from February to May with a few occasional summer showers. A variety of temporary fresh water habitats come into existence with variations between and within regions. However, information on surveys of fauna of the temporary water bodies is lacking in Kerala except for the study of coastal temporary water bodies of Thiruvananthapuram by Balaraman et al. (2000). Consequently, literature on conchostracans of Kerala is scanty.

Of the thirty three species identified in India, only three have been identified from Kerala. These include *C. hislopi* and *L. brachyrus* reported by Nair and Nayar (1968) from Irinjalakuda, Trichur, and *E. michaeli* from Nellai, Trichur by Babu and Nayar (1994). Two other papers by Nair (1968), and Paul and Nayar (1977) deal with the population dynamics of *C. hislopi.*

Unpredictability of the habitats, that makes collections unsuccessful, could be a major factor that has dampened research interest on Conchostraca. Also, rapid loss and pollution of the habitats might have led to the depletion of life and loss of several species, making availability of conchostracans scarce. Nevertheless, obtaining conchostracans can be made more successful if temporary water bodies in which they occur are surveyed prior to collections; also the collections should be planned in accordance with the monsoon seasons and the life cycle of the conchostracan concerned.

Conchostracans are pioneer communities that appear in temporary bodies of water immediately after the rains and complete their short life cycle if the water persists, or die of desiccation. In the transient waters, they feed, attain sexual maturity quickly, and begin to lay eggs or cysts. These eggs remain buried in the mud and after the pools dry, wait for the next rains to rehydrate and hatch them. Reduced stress from biotic factors coupled with rich food resources is a reward for breeding in an unstable and unpredictable environment like the temporary water bodies. For these reasons, conchostracans are indeed fascinating animals for scientific scrutiny. Despite their world wide distribution, ecological interest, morphological, physiological and behavioural adaptations, and evolutionary significance, they have been neglected in taxonomic and other research studies in India, and particularly in Kerala, though they have evoked considerable scientific interest in several other countries in the world.

These unique aquatic animals with their highly specialized survival strategies are rapidly disappearing because of massive loss of habitat not only in Kerala, but also in other parts of the world. Wetlands and swamps are either drained or filled up with substrate. This threatens formation of shallow pools in these areas. Moreover, excessive use of ground water lowers ground water table. Urbanisation leads to rampant pollution. Ninety seven percent of the vernal pools in the highly urbanized coastal areas of San Diego Country have been destroyed (King et al., 1996). Such a loss of habitat portends extinction of many species. For example, Anostraca, once common in the coastal temporary water bodies of Kerala as recorded by Saraswathy Ammal (1977), have disappeared from the localities from where she collected them (Balaraman & Deepa, 2002). In Austria, France, Great Britain, and the USA, conchostracans are considered to be endangered and conservation measures are being adopted (Hodl & Eder, 1996; King et al., 1996).

Like fruit flies that are generally used in genetic research, conchostracans can be easily reared in the laboratory. They are small (size ranges from 1 mm. to 7 mm.), have high fecundity, have only a short generation time, have high tolerance for fluctuating physicochemical factors, and some have rare reproductive systems (androdioecy). Hence they are ideal candidates for research in several fields including genetic and evolution of reproduction.

The uncertain future of the ephemeral habitats wherein the conchostracans live, and the paucity of scientific information on their taxonomy, life history traits and reproductive specialities, present an added need for basic research on these fascinating animals. Hence, "Studies on Conchostraca of Kerala" is chosen as the topic of the present study which is described in the pages that follow.

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Chapter 2

REVIEW OF LITERATURE

Taxonomic Review

Review of Morphological studies and Reproduction

Review of Classification

Taxonomic Review

The pioneer works on conchostracans (made) by Baird (1859, 1860) described five species – viz., *Estheria polita, Caenetheriella boysi, Caenetheriella similis, Cyclestheria hislopi,* and *Eulimnadia compressa.* The conchostracan *Cyclestheria hislopi* was described by Baird (1859) with specimens collected by the Rev. S. Hislop from fresh water pools at Nagpur, India. Baird described it as a new species belonging to the genus *Estheria* on account of the distinctly prominent umbones of the shell. In 1886, Brady recorded the same species from Ceylon but described it as a species of the genus *Limnadia* believing the antennules to have greater resemblance to those in *Limnadia* than to the same organs in *Estheria.* It was, however, Sars (1887) who recognized its distinctive features and erected a separate genus, *Cyclestheria.* Indeed, Sars suggested that it differed distinctly from all described conchostracans and placed it in a new family Cyclestheridae. The other conchostracan *Eulimnadia compressa* described by Baird (1860) was the first Indian species belonging to the genus *Eulimnadia* Packard. Later Sars (1900) described *Eulimnadia similes* from the Sheveroy Hills and *Eulimnadia gibba* from Jamala Hill Fort, both in South India. In this report he has also included the species *Cyclestheria hislopi*, *Leptestheriella nobilis* and *Leptestheriella hendersoni.* In 1906 Gurney described three species found in the collection of the Indian Museum, Calcutta. These included *Estheria indica,* collected by Dr. Annandale from sandy rain pools in Mandapam, Pamben passage, South India, *Estheria davidi* collected by Capt. R.E.Loyd from Gyantse in Tibet, and *Limnetis brachyura* from Shandur lake, Chitral now in Pakistan.

In his monograph on the world Conchostraca, Daday (1913, 1915 & 1923) redescribed several species recorded from India and added two more species: *Caenestheriella annandalei* and *Leptestheriella sarsi* to the then existing list. In 1930 Gurney recorded another new species *Lynceus dendiculatus* from South India. Later Bond (1934) described a new species *Eulimnadia margaratae* from South Arabia. His descriptions were based on specimens handed over to him for examination by Prof. E. Hutchinson, who was the biologist of the Yale North India Expedition (1934). The collections also contained two more new species described by Bond (1934), *Eocyzicus hutchinsoni* from the localities of the tahsil of Kushal, and *Eocyzicus deterrana* from Rawalpindi in Punjab, both in Pakistan now. Further mention of conchostraca in literature was when Tiwari (1959, 1962 & 1966) recorded *Leptestheria jaisalmerensis, Eocyzicus pellucidae, Caenestheria misrai, Caenestheriella roonwali, Leptestheria biswasi* and *Sewellestheria sambharensis* from North India. As the specimens present in the collections made by the Zoological Survey of India. Calcutta in 1958 from Sambhar lake. Rajasthan, could not be assigned to any known genus or species, Tiwari (1966) placed it under a new genus *Sewellestheia* and species *sambharensis*. *Sewellestheria* exhibited two of the five important characters of Leptestheriidae to which it belonged but differed from it in the absence of projections or lobes on the epipodite and structure of telson (Tiwari. 1966). The new genus agreed with *Eoleptestheria* and *Leptestheriella* in the modified 10th and 11th pairs of legs in female. and differed from the former in the structure of occipital angle and from the latter by having margin of flabellum in legs entire. It was thus related to *Eoleptestheria*. differing from it in the structure of telson (Tiwari. 1966). This paper also provided a key to distinguish the four genera included in the family Leptestheriidae. Karande and Inamdar (1961) identified a new species *Leptestheriella gigas* from the Tableland Panchgani in Maharashtra. The authors studied the biology of the species and made an interesting observation on the possibility of *Leptestheriella gigas* laying viable eggs which need no desiccation (Karande & Inamdar 1961). After Tiwari's report (1959 &1966) of 5 new species from North India, Nayar (1965) described 3 new species of conchostracans collected from small ditches in Pilani and Khetri, Rajasthan during the monsoon months of 1962 and 1964. The paper presents descriptions and illustrations of both males and females of *Leptestheria longispinosa* and females of *Eocyzicus acuta* and *Eulimnadia ovata. Leptestheria longispinosa* can be distinguished from other known Indian species by its large rostral spine and 8-9 lines of growth; *Eocyzicus acuta* by its acutely pointed rostrum and *Eulimnadia ovata* by the more elongated shape of the shell and fewer number of spines along the dorsal margin of the telson. Again Nayar and Nair (1968) reported the cosmopolitan species *Lynceus brachyurus* Müller 1776 and *Cyclestheria hislopi* collected from Irinjalakuda. This is the first record of conchostracans from Kerala. They also described *Leptestheriella maduraensis, Caenestheriella indica* and *Eulimnadia michaeli* from samples collected by Dr. George Michael from a quarry pool at Narasingapatti, Madurai, of which *Eulimnadia michaeli* and *Leptestheriella maduraensis* were new. Later, *L. maduraensis* was reported from Pune, Maharastra too (Ghate & Patil, 1995). The next reference to conchostracans from Kerala in literature is the studies on the biology of *Cyclestheria hislopi* by Nair (1968), and Paul and Nayar (1977). Nair (1968) has mentioned that *Cyclestheria hislopi* was the most common conchostracan present almost the year round in the locality of Irinjalakuda, Trichur and was a perennial breeder, with a peak period in

January. No males were noted in his collections. Paul and Nayar (1977) studied the fluctuations of a natural population in relation to physical parameters like temperature, pH and dissolved oxygen. Their paper gives also details on the biology of *Cyclestheria hislopi* like fecundity, duration of development and habitat preference to the aquatic plant *Hydrilla verticellata* (Koyali). In 1994, [illegible] Babu and Nayar described *Eulimnadia michaeli* obtained from a temporary water body at Nellai in Trichur district that contained males of the species. This is not only the first report of this species from Kerala but also the first description of its male

Royan and Alfred (1971) described a new species *Lynceus serratus* from Tamil Nadu. They obtained this species in large numbers from a temporary quarry pool in Narasingampatti, Madurai. Both males and females were examined in detail. In addition to the regular features of *Lynceus,* Royan and Alfred have pointed out the unique features of this species such as the possession of a characteristic row of serrations on the postero ventral margin of the shell, the presence of characteristic spines on the 2nd antenna of both sexes, the serrations on the ventral margin of the female rostrum, the presence of long forked filament and the arrangements of the spines on the telson. Das and Aktar (1971) described *Eocyzicus wulari* from Kashmir. While working on the Branchiopoda of Guntur district, in Andhra Pradesh, Radhakrishnan and Durga Prasad (1976) recorded a new species *Eulimnadia gunturensis* based on both female and male specimens. This is the first time that males were reported for *Eulimnadia.* Again from Guntur district more conchostracans such as *Leptestheriella nobilis, Caenestheriella indica* and *Cyclestheria hislopi* were reported by Durga Prasad

(1981). Battish (1981) described seven species of Conchostraca, collected from the State of Punjab. He described three new species - *Lynceus vasishti*, *Caenestheriella ludhianata* from muddy ponds at Rakra village on Patiala–Nabha road, Punjab, and *Eocyzicus dhilloni* from a roadside ditch at Patiala–Samana road, Punjab. One new sub speies *Eulimnadia ovata inversa* from a small ditch located at the Punjab Agricultural University Campus and two indeterminate species – *Eulimnadia* sp. indet. and *Leptestheriella* sp .indet. were also described by him.

Taxonomic characters used for identification of Indian species are based entirely upon morphological features such as adult carapace shape, appendage structures, and telson. However, scanning microscopy was used for examining the ornamentation in carapace (Samyiah et al. 1985), arrangement of spines in telson (Samyiah et al., 1985; Venkataraman, 1985), and morphology of the egg (Samyiah et al., 1985) on 3 species of conchostracans *Caenestheriella indica, Eulimnadia michaeli,* and *Leptestheriella maduraensis.* These authors are of the opinion that in future, application of SEM to systematic studies of conchostraca can provide a valuable tool to the investigators particularly if micrography of diagnostic characters could be gathered *into* a reference atlas.

Durga Prasad (1991) has compiled works on conchostraca and reported the presence of 33 species in India, belonging the genera: (i) *Cyclestheria* (1 species), (ii) *Lynceus* (4 species) , (iii) *Eulimnadia* (8 species), (iv) *Leptestheriella (5 species) , (v) Leptestheria* (3 species), (vi) *Sewellestheria* (1 species), (vii) *Caenestheria* (1 species), (viii) *Caenestherilla* (6 species) , and (ix) *Eocyzicus* (7 species).

Except for the world revision of conchostraca by Daday (1926) that included descriptions of several species recorded from India, Royan's (1973) short outline on identification of Indian conchostracans is the only one that exists in the literature. His identification is based mainly on seven Indian species (5 reported in South India) belonging to 7 genera.

However, a perusal of the literature reveals a few taxonomic reviews on conchostracan families from other parts of the world. Brendonck (1999) has prepared a chronicle of conchostracans recorded in South Africa, including names of authors, date of original description, and general distribution records. However, he laments that though conchostracans are abundant in Southern Africa, they have been scarcely studied. He has pointed out that species differences were masked by a combination of evolutionary stages and age and environment related morphological variability which made it difficult to distinguish species on the basis of morphology alone. Brendonck's checklist includes also a detailed key with descriptive illustrations to the Southern African families of Conchostraca. The family Lynceidae is represented by only one genus *Lynceus* Müller 1776 and includes 5 species. The cosmopolitan species *Lynceus brachyurus* has not been recorded from Southern Africa. The family Cyclestheriidae contains one species, the cosmopolitan *Cyclestheria hislopi*. The family Limnadiidae includes only one genus *Eulimnadia* Packard 1874 represented by 2 species. The family Cyziaidae contains 2 genera from Southern Africa. The genus *Cyzicus* Audouin 1837 is represented by one species *Cyzicus australis* whereas the genus *Eocyzicus* Daday 1913 by 3 species. Two genera are currently known for the family Leptestheriidae – genus *Leptestheria* Sars 1898 and genus *Leptestherella* Daday 1913. Both include three species each from South Africa. Thus Brendonck (1999) has listed 18 species with their distribution records in Southern Africa.

Roessler (1995a, b & c), who has made a detailed review of conchostracan families of Colombia, gives an overview of the current knowledge of the biology, ecology and distribution pattern of Colombian conchostracans. These studies were carried out from 1977 to 1993 in the warm tropical low land of Colombia. Extensive samplings have revealed that *Cyclestheria hislopi*, a widespread and common species in Colombia, can be found year round in large semi permanent and permanent waters that contain predators like fish, whereas other conchostracans are restricted to temporary waters (Roessler, 1995a). He has also indicated that *C. hislopi* occurs most commonly as parthenogenetically reproducing population that periodically and under certain environmental conditions produces sexually reproducing generations that include males and sexual females that transform into a special type of ephippia (Roessler, 1995a). Roessler's (1995b) important observation is that the branchiopods of Colombia are mainly conchostracans that include 12 species under five families. No members of the families *Cyzicidae Stebbing* 1910, or *Imnadiidae Bontnariuc* and *Orghida* 1941, have been found in Colombia. He has also stressed that morphological features of conchostracans generally used in the classification undergo a sequence of changes in development and are also modulated by environmental conditions (Roessler, 1995c).

Yet another excellent review, restricted to the systematics of the family *Lynceidae Stebbing* 1902 in the America's, has been presented by Martin and Belk (1988). In this detailed review they have listed 31 species identified from all over the world and redescribed all the American species including a new species *Paralimnetis texana.* Morphological characters of the family and various genera are illustrated; affinities, classification and geographical distributions are also discussed. A key to the American species is also provided. This is followed by another review of the species belonging to the genus *Eulimnadia* from Central and South America by Martin (1989), who has listed 8 species, including a new species *Eulimnadia belki*, and its distribution from these regions. Specimens reviewed by him were borrowed from various museums and private collections, and SEM micrographs of the morphology of the egg surface were also done. The new species *Eulimnadia belki* is identified as different from other species based on the morphology of the external egg surface (Martin 1989). Belk (1989) has also used this promising taxonomic character to review the genus *Eulimnadia* of North America. Belk considers the morphology of the external egg surface to be species specific for the genus; though he cautions on its limitation too. Of the 12 species recorded from North America, Belk (1989) regards only 5 as valid; Mattox's 7 nominal species are synonymous and take the name of the first member of the group, *Eulimnadia diversa* Mattox 1937. With the addition of 2 new species, *Eulimnadia cylindrova* and *Eulimnadia astrova*, identified by him, the total number of species in North America reported by Belk is seven. However, recent reviews by Pereira and Garcia (2001) of the family Limnadiidae from Venezuela have shown that though egg morphology is a diagnostic character, species such as *Eulimnadia texana* Packard 1871 and *Eulimnadia geayi* Daday 1926 share a similar egg morphology and in these cases body morphology is to be considered an additional criterion. Illustrations of morphological characters and SEM of egg morphology of 8 species from Venezuela, including a new species *Eulimnadia follisimilis,* are described by them.

One of the earliest reviews on conchostracans of Czechoslovak was by Straškraba (1965) for the family Limnadiidae, Lynceidae, Cyzicidae and Leptestheriidae. Straškraba (1965) has made a comparison of the main characteristics of the genera of the family Limnadiidae and divided the family into 4 subfamilies. He has also commented on the highly variable nature of many of the morpho-taxonomic characters in the above family.

Several papers dealing with conchostracan reviews are mainly included under the overviews of large branchiopod assemblages restricted to specific geographical areas. Hodl and Eder's (1996) studies on the presence and conservation of clam shrimps in Austria show that five of the six known Austrian conchostracan species, that occurred in the Pannonian region and considered extinct, still occur at a restricted number of sites in the Pannonian region of lower Austria. *Leptestheria dahalacensis* Rüppelf 1837, *Eoleptestheria ticinensis* Balsamo-Civelli 1859, and *Cyzicus tetracerus* Krynicki 1830were rediscovered in a shallow lake formed at flood plains of the Morava river. *Leptestheria dahalacensis* , *Limnadia lenticularsis* Linné1761 and *Imnadia yeyetta* Hertzog 1935 were found in two small neighbouring astatic pools in the flood plains of the Morava. But they did not find the cosmopolitan *Lynceus branchyuus*, and is considered extinct. All Austrian clam shrimp species are considered endangered and the main threats, according to the authors, are agricultural activities and artificial changes of the hydrologic conditions. Conservation measures to achieve habitat protection are also discussed (Hodl & Eder 1996).

Brendonck et al. (1990) have documented the systematic morphology of branchiopod collections from different botanical regions of Galapagos Island. In spite of the historical, geographical and climatological isolation and, although fresh water habitats are scarce, the authors have documented 3 branchiopod taxa including a *Eulimnadia* species showing affinity to *E. cylindrova.* Females of the species (male not recorded) have been described using both Light microscope and SEM and systematics of *Eulimnadia* discussed. Along with the Galapagos Eulimnadia specimens they have investigated the morphology of the egg (SEM), carapace, rostrum and telson of 3 mainland population of *Eulimnadia cylindrova* and compared with *E. cylindrova* and concludes that the former differed only slightly from mainland forms. They have also assessed the pros and cons of using egg morphology as a reliable taxonomic criterion within the genus *Eulimnadia.* Finally the distribution of the Galapagos branchiopod fauna and possible connections with mainland populations by means of dispersal are also discussed. Weeks et al.'s (1997) survey of branchiopod crustaceans of temporary water bodies in Ohio (North America) revealed that the rarest branchiopod found there was the conchostracan *Caestherilla gynecia* in addition to conchostracans described earlier indicating that these species might be threatened or extirpated in Ohio.

Maeda-Martinez et al. (1997a) while studying the frequency of large branchiopod associations in North Central Mexico and in Arizona, USA observed that co-occurrence of two or more congeneric species of Anostraca and Notostrca were not uncommon, but with regard to Conchostraca congeners did not co-exist for Spinicaudata and Laevicaudata. A 3-year survey undertaken by Maeda-Martinez (1997b) on the large branchiopods for Mexico of the Baja California peninsula included first records for 2 anostracans, 1 notostracan and spinicaudatans *Eulimnadia cylindrova* Belk, 1989 and *Eulimnadia texana* Packard, 1871 from this area. The conchostracan *Cyclestheria hislopi* Baird, 1859 recorded from the State of Quintana Roo was also another first record from Mexico (Maeda-Martinez 1997b).

Intensive survey of the Banat Province in Yugoslavia by Petrov and Cvetkovic (1997) during 1991-1995 revealed the richest branchiopod assemblage of 10 species that included 3 conchostracan s (all Spinicaudata) – *Eoleptestheria spinosa* Marincek, 1978, *Leptestheria saetosa* Marincek and Petrov, 1992, and *Imnadia banatica* Marincek and Petrov, 1982. They also recorded 7 co-existing large branchiopod species in a pond that included 3 co-existing conchostracans than previously reported. Again Petrov and Petrov (1997) summarized and published information on large branchiopod – status of the species and their habitat in Yugoslavia, by their study that spreads over a span of 19 years (1977-1996). Their study revealed that among conchostracan species *Imnadia cristata* Marinček *1972, Imnadia bantica* Marinček and Petrov 1982 and *Imnadia panonica* Marinček and Petrov 1984 are endemic to Yugoslavia. While *Eoleptestheria spinosa* is most endangered since it appeared irregularly in small populations, the least endangered with restricted distribution is *Imnadia banatica* as it appeared regularly in large numbers; *Limnadia lenticularis, Imnadia cristata* and *Imnadia panonica* are considered extinct as they have not been found for more than 20 years; *Cyzicus tetracerus* exhibits wide distribution, as they were recorded from several localities; *Leptestheria setosa*, a summer species, occurs in large populations and *Leptestheria dahalacensis*, absent in the survey. Finally the authors have discussed the major disturbing factors since no kind of protection for branchiopods or their habitats exists in Yugoslavia.

Vekhott (1997) has reported Branchiopods including conchostracan from Barents region of Russia, where some of the most extreme polar desert and artic tundra environments for aquatic organisms exist. He has presented both biological and distributional information on these fauna based on both literature and collections. Vekhott (1997) has noted that whereas *Caenestheria sahlbergi* Simon 1886 is an Asia tundra species *Caenestheria propinqua* Sars 1901 is a Eurasian tundra species and penetration of these species into the islands and archipelagos of the Barents region is probably due to the closeness of these lands to the Continent. Each conchostracan species is found in only one locality. *Caenestheria propinqua* is found in several shallow pools on Kolgnev Island and *Caenstheria sahlbergi* in a single pool on Matveev Island (Vekhott 1997). A more recent article on the larger branchiopods of Denmark (5 species including one conchostracan species *Lynceus brachyurus*) by Damagaard and Oleson (1998) indicates that distribution of all species is very restricted probably because of habitat destruction. Though *Lynceus brachyurus* was fairly distributed in Denmark in earlier days, since 1975 it has only been recorded from a single locality.

Review of Morphological studies and Reproduction

The majority of morphological studies on Conchostraca have centered round feeding, reproduction, and development. Cannon (1933) described in detail the feeding mechanism of the Branchiopoda and the phylogenetic inferences in Branchiopoda. Karande and Inamdar (1961) mentioned a predominance of algae and plant detritus in the diet of *Leptestheriella gigas*. Bishop (1969) successfully reared larval and post larval *Limnadia stanleyana* on the alga *Gymnodinium*; this alga was noted also in the guts of field collected fourth and fifth nauplius stages of the clam shrimp. Other rearing studies have employed a supply of dirt from the collecting site for rearing *Eulimnadia antlei* (Belk, 1972), and yeast suspension and fish food for rearing *Eulimnadia texana* (Weeks et al., 1997). The most informative study on conchostracan gut contents is that of Royan (1976) on *Leptestheriella maduraiensis*. Royan showed that the gut contents which included phytoplankton and zooplankton varied with the abundance of those foods in the water column. This reflects the non-selective feeding habits of the species. Different aspects on the feeding biology and feeding structures in Conchostraca have been meticulously reviewed by Martin (1989).

Detailed information is available on early female gametogenesis and egg shell formation in Leptestheriidae (Zeni & Zaffagnini, 1989; Sanabissi Sabelli & Tomnasini, 1990) and in Limnadiidae (Zaffagnini, 1969). Histological examination of the reproductive system of female *Limnadia lenticularis* by Zaffagnini (1969) has revealed scattered testicular regions throughout the length of ovotestis, confirming rudimentary hermaphroditism. Based on these studies he has also reported that automitic parthenogenesis takes place, when restoration of the diploid set of chromosomes takes place through the fusion of the polar nucleus with the secondary oocyte. Zucker et al. (1997) have also provided anatomical evidence for hermaphroditism in the females of *Eulimnadia texana*. They have also observed well developed mature sperms in the lumen of the hermaphroditic gonads of *Eulimnadia texana* showing that self fertilization occurs in hermaphrodites. Scanabissi and Mondini (2000) studied sperm transfer in *Leptestheria dahalacensis*. Their studies reveal that sperm transfer is effected by a primitive spermatophore. It has no true wall so that the sperm protrude beyond the surface. It has neither internal nor external protective structures but is composed of a simple fibrous structure that traps the spermatozoa. Mating time is partly dependent on the adherence of the spermatophore to the female's body. An earlier study by Strenth (1977) has reported the presence of spermatophore in *Eulimnadia texana*; but only in relation to the assumed presence of males in a maleless population. The presence of spermatophore has been cited by other authors (e.g., Sassaman, 1995), though this has never been described. However, Knoll (1995) reported that he did not observe the presence of spermatphores in androdioecious populations of *Eulimnadia texana.*

Most of the works on genetics evolution and aging are mainly restricted to fruit flies and nematodes. However, in the 1990's a series of research involving the species Eulimnadia has focused primarily on the genetics and theoretical implications of its rare mating system, viz., androdioecy. For androdioecy, in *Eulimnadia texana*, Sassaman and Weeks (1993) have proposed that females are instead hermaphrodites which could either self or outcross with males. Sex is proposed to be determined by a simple Mendelian locus with 2 alleles, where a dominant allele codes for hermaphrodites and recessive allele for males. Thus a homozygous dominant hermaphrodite (a monogenic) would produce only hermaphroditic offsprings, whether selfing or out crossing, while a heterozygous hermaphrodite (an amphigenic) would produce 25% males from selfing and 50% males from outcrossing. Due to the nature of this type of sex determination, populations typically consist of many more hermaphrodites than males. Corroborative anatomical evidence for androdioecy in *E. texana* was provided by Zucker et al. (1997) when anatomical study of the gonads of hermaphrodites (once thought to be females) showed the presence of ovotestes. Other than *E. texana* there is only one well documented case of androdioecy in animals – the nematode Caenorhabditis elegance. However, androdioecy has been reported in three other species in the family Limnadiidae: *Limadia lenticularis* (Eder et al., 2000), *Eulimnadia anteli* (Sassman, 1988), and *Eulimnadia agassizi* (Sassaman, 1995), although none of these has been strictly verified as androdioecous. Otto et al. (1993) developed a population genetics model for the androdioecous reproductive system of *E. texana*. Their model explained the conditions under which a mixed mating system comprising all three mating systems – monogenics, amphigenics, and males can be stable. This model consisted of four parameters: the ability of males to fertilize hermaphrodites; the proportion of a hermaphrodite's eggs not fertilized by a male which are then self fertilized; inbreeding depression; and the relative survival of males to hermaphrodite In this model, hermaphrodites can choose either to self fertilize or to outcross with a male. Out crossing is dependent on the likelihood of the two sexes encountering each other. The model assumes that encounters between males and hermaphrodites are directly proportional to the frequency of males in the population. Once an encounter is made, a successful mating will occur on a certain proportion of the time. If no male encounters a hermaphrodite, then the hermaphrodite will self fertilize a proportion of her own eggs. In continuation of the explanation of the dynamics of this mixed mating system, Weeks et al. determined that (a) inbreeding depression ranges between 0.5 and 0.7 (Weeks et al., 1999, 2000a, 2001b); (b) males survive at 70-90% the rate of hermaphrodites (Zucker et al., 2001); (c) hermaphrodites do not experience sperm limitation in the absence of males (Weeks et al., 2001b). The model of Otto et al. (1993) predicts androdioecy to be stable with male frequencies to be 12-36%, monogenics to be 7-55% and amphigenics to be 33-57% of the shrimp population, but Weeks et al. (2001a) found frequencies of 17-24%, 5-13% and 63-75% respectively. Weeks et al. (2000b) used genetically marked mating between males and hermaphrodites to determine if hermaphrodites can store male sperm. If hermaphrodites can, then males can have a higher fertilization success rate, and higher male mortality is less important than is assumed in the model. Their studies reveal that hermaphrodites of *E. texana* are unable to store appreciable no of sperms. A series of studies along this line by Zucker et al. (2001) to assess the selective forces maintaining males in *E. texana* showed that when excessive mating opportunities are provided males live significantly longer, a result contrary to what they had expected. In order to obtain more realistic estimates from natural settings than laboratory estimates of the parameters in the model of Otto et al. (1993), Weeks and Bernhardt (2004) compared inbreeding depression and relative longevity in one population of *E. texana*, reared in artificial pools in Arizona desert. They have found that both estimates of relative male viability and inbreeding depression corresponded well with earlier laboratory estimates of these parameters. Medland et al. (2000) examined encounter behaviour between males and hermaphrodites in *E. texana* by comparing their swimming speeds and locations in the presence or absence of the other sex. They have found that the behaviour of hermaphrodites differs significantly from that of males. Contrary to expectation, it appears that it is hermaphrodites rather than males, which are altering their behaviour in the presence of the other sex.

Males have a lower survival in *E. texana* when reared from a selfing event. Weeks and Bernhardt (2004) have explained it as follows: The S and s genes as originally proposed by Sassaman and Weeks (1993) are more likely S and s linked genes or possibly S and s chromosomes. In these linked genes are likely embedded numerous deleterious recessive genes, such that the resulting homozygous individual would experience reduced fitness. Selfing of amphigenic hermaphrodites (Ss) produces monogenic (SS) and amphigenic (Ss) offsprings that show differences in inbreeding depression. They attribute it to a masking of the deleterious recessives in the Ss individuals and an exposure to these recessives in the SS individuals. The same should be the case for the recessive males (ss) and hence their low survival.

Detailed studies of the life history traits of E. *texana* by Weeks (1997) and behavioural studies like detailed description of mating behaviour by Knoll (1995) have proved useful to supplement the ongoing evolutionary and genetic studies on *E. texana*. Weeks and Marcus (1997) have studied the effect of pond duration shaping life history characters of its inhabitants. Their assumption was that selection pressures in short-lived ponds would lead to faster growth, decreased age at maturity, lower life time fecundity and early senescence, as evolutionary models would predict. However, their results were mixed in relation to their expectations. Except for growth rate, their experiments did not produce any results that correlated evolutionary models of senescence.

An exhaustive review by Sassman (1995) discusses the evolutionary history of unisexuality in the Conchostraca, by explaining the relationship between modes of reproduction and genetic mechanisms of sex determination in this ancient group. His deductions are based on available sex ratio estimates of 60 species belonging to all the families, observed in the natural and laboratory populations of clam shrimp, supplemented by the limited review of literature on these. His data analysis shows that males and females are equally common in most species, but deviations from this pattern occur in four lineages: at least one unisexual species in *Cyzicus* and *Leptestheria*; many species in Limnadiinae; and for many populations of *C. hislopi* in which males are completely absent or appear during later generations of the life cycle. Phylogenetic analysis of patterns of reproduction suggests that sexual reproduction is a primitive condition. Sassman (1995) has explained these modifications of conchostracan life cycles within the context of conservation of sex determination. His model to explain the lack of males in some populations of Cyclestheria, is that from unisexuality a modified sexuality would be restored when males are expressed in a condition dependent manner. Moreover, this evolutionary potential would not exist if the sex-determining mechanism of conchostracans involved male rather than female, heterogamety. In *Eulimnadia* life cycle, both outcrossing and selfing modes of reproduction are employed. If reproduction was exclusively by selfing for a few successive generations, then the male-determining allele would decrease in frequency through time with the dynamical properties of a recessive gene. The equilibrium condition would be fixation on the dominant female-determining allele but the rate of approach to equilibrium would diminish as males become rarer. Periodic circumstances favouring outcrossing would restore the male-determining allele to higher frequencies. His conclusion is that unisexuality in conchostracans has evolved multiple times and by several different routes from different obligatory sexual ancestors.

The direct development of *C. hislopi* with the larval and juvenile stages passed within the brood chamber has been described in detail by Sars (1887). His classical drawings of the larval stages have been reproduced by several authors (e.g., Olesen et al. 1996; Olesen , 1999) in their papers. Olesen's (1999) paper on larval development in *C.*

hislopi examines in detail, with SEM, the developmental stage of *C. hislopi* to provide a detailed basis for a future comparison of its development with that of other branchiopods and, in particular, with other conchostracans and the cladocerans

Review of Classification

The classification of clam shrimps is controversial at all levels ranging from the diagnosis of species to that of order. The traditional classification of branchiopod Crustacea commonly adopted is Calman's (1909) classification, where the branchiopods are grouped into four orders, viz., Anostraca, Notostraca, Conchostraca, and Cladocera. Earlier to Calman, Sars (1890) included Anostraca, Notostraca, Conchostraca within a single order Phyllopoda. A significant difference between the schemes of classification of Sars and Calman is the abandonment of 'Phyllopoda' as a taxonomic entity by the latter. Phyllopoda is now used as a common descriptive term only. Gerstaecker (1866-1879) was the first to unite the Conchostraca and the Cladocera under the name Diplostraca. Earlier Claus (1876) had proposed that Cladocera had evolved by neotony from conchostracans and hence Gerstaecker's procedure was supported. Sars (1887) had also proposed that the aberrant Conchostraca species *C. hislopi* was a 'link species' between the Conchostraca and Cladocera. The next most significant modification of Calman's scheme was made by Linder (1945) who recognized two distinctive groups (tribes) within the Conchostraca and proposed the names Laevicaudata and Spinicaudata.

In the "Treaties of Invertebrate Paleontology", Tash (1969) has outlined the classification of the branchiopods and has treated Diplostraca (Conchostraca and Cladocera) as a subclass. This classification has been adopted by Belk (1982) in an encyclopedic type of work to classify Branchiopoda. In the latest classification of Branchiopoda by Fryer (1987), it is recognized as consisting of eight extant rather than four extant orders. These eight extant groups are the result of recognizing that the 'Conchostraca' consists of two rather different assemblages of bivalved crustaceans, the orders Laevicaudata (family Lynceidae) and Spinicaudata (all other families formerly included as Conchostraca) and that the Cladocera consists of four morphologically disparate taxa. According to Fryer, any attempt to define the Conchostraca is rendered difficult by the many differences between Laevicaudata and Spinicaudata and that these differences were recognized by Linder (1945) who designated the names Laevicaudata and Spinicaudata. Laevicaudata and Spinicaudat*a* are separated by numerous often trenchant differences and share so few significant features that their close relationships cannot be assumed. Their differences may reflect a long period of separation and divergence, but convergence cannot be excluded (Fryer, 1987). According to Fryer, some of the obvious shared characters such as bivalved carapace may have been convergently acquired. Particular attention is drawn by Fryer to a trenchant difference between the Laevicaudata and the Spinicaudata (based on detailed studies of Botnariuc (1947) on the prehensile structure, the clasper of male used for mating. In both orders, these modified 1st pair of trunk limbs looks very similar and suggests a close relationship. However, at least in two families of the Spinicaudata (and by inference in the order as a whole) the distal end of the palm or the apical club is derived from endite 4, whereas in the Laevicaudata it is derived from endite 3. Furthermore, in the Spinicaudata the two palps of the prehensile appendages are true palps of endites 4 and 5 themselves, whereas in Laevicaudata they are the transformed endites 4 and 5 themselves. However, the movable finger (endite 6) is homologous in the two orders. The male prehensile appendage thus provides an excellent example of homoplasy (Fryer, 1987).

The name Conchostraca ceases to have taxonomic validity though it is still used as a general descriptive term (Fryer, 1987). Though most carcinologists have accepted Fryer's classification, he proposed no explicit relationship among the eight extant orders. Sassaman (1995) views Lynceidae (order, Laevicaudata) as the sister group to all other conchostracans rather than as a distinct order with unspecified affinities with conchostracans, cladocerans and notostracans. Martin (1992), while agreeing with Fryer's classification argues that based on naupliar characters of lynceids that were not available to Fryer, Conchostraca might still be retained as a valid taxon, despite the many peculiarities of adult lynceids. Olesen (1996) and Olesen et al. (1997) have discussed the phylogeny of the Conchostraca and Cladocera giving great weight to the dorsal organ of Conchostraca, the head-pores of the Cladocera and the structure of the male clasper in both. A computer-assisted-cladistic analysis on the morphological characters of the Diplostraca (= Conchostraca and Cladocera) was published for the first time by Olesen (1998). If his recommendations based on the results of this analysis can be accepted, then, in addition to several other taxonomic groups, Diplostraca becomes a valid taxonomic unit. A recent classification by Negrea et al. (1999) recognizes class Branchiopoda as consisting of five super orders and eleven orders. Conchostraca has been reinstated as super order Conchostraca. Cyclestheriidae has been elevated to ordinal rank Cyclestherida which along with Laevicaudata and Spinicaudata form the three orders of superorder Conchostraca.

STUDIES ON CONCHOSTRACA (CRUSTACEA) OF KERALA

Thesis submitted to the University of Calicut
For the award of the Degree of
Doctor of Philosophy
In Zoology

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Chapter 3

ORDER: *SPINICAUDATA*

External Morphology

A. Cyclestheria hislopi

B. Eulimnadia colombiensis

ORDER – SPINICAUDATA

External Morphology

The Spinicaudata encompasses those taxa formerly grouped with laevicaudatans in the Conchostraca. There are four extant families, viz.: Cyclestheriidae (monotypic, Cyclestheria hislopi), Limnadiidae (six genera), Leptestheriidae (five genera), and Cyzicidae (four genera). All genera inhabit ephemeral fresh water pools. *Cyclestheria* also frequents permanent bodies of water.

All spinicaudatans have a bivalved carapace permanently enclosing trunk and head. Head is small and not protrusible. The valves are joined dorsally by a simple fold; without a true hinge. All species have external growth lines on the carapace. The eyes are paired but in *Cyclestheria* they are close together. Ocellus is located some distance away from compound eye (except in *Cyclestheria*). The mandibles, belonging to the first type, have a broad mastigatory surface with scales fused to form a smooth surface of connected pits on the triturating surface. The antennules are elongated and subdivided into several lobes, each of which bears sensillae, except in *Cyclestheria* which has straight tubular antennule with sensory setae at the tip. In the family Limnadiidae, the dorsal organ is borne on a peduncle and in Cyclestheriidae it is borne on an elevation with a central pore. The antennae are large and natatory. All the extrinsic antennary muscles orginate at the same side of the body as the appendages they serve. No dorsal lamellae are seen towards posterior end of trunk. More than 12 pairs of trunk limbs (upto 32) are seen. Proximal lobe of exopod is small. Trunk limbs 9, 10, and sometimes 11 of females have dorsal lobe of exopod modified into dorsal filaments. The first and the second trunk limbs of males are modified as claspers (only the first, in the Cyclestheriidae). The 'palm' of clasper is derived from endite 4, and the palps of clasper are true palps of endites 4 and 5. The external openings of the male and the female genital ducts are at the base of the 11th pair of trunk limbs. The telson is massive, armed dorsally with spines and with a pair of furcal claws; but no opercular lamella is seen on the telson.

The two species (*Cyclestheria hislopi* and *Eulimnadia colombiensis*) that belong to Spinicaudata, identified in the present study are described in this chapter.

A. Cyclestheria hislopi

Introduction

Materials and Method

- *(a) Description of sites*
- *(b) Collections and Method*
- *(c) Laboratory Culture*

Diagnosis of Species

- *(a) Description of Male*
- *(b) Description of Female*
- *(c) Discussion*

Morphological forms, Population estimation and Ecology

- *(a) Morphological forms*
- *(b) Population Estimation*
- *(c) Ecology*
- *(d) Discussion*

Figures

A. Cyclestheria hislopi

Class Branchiopoda Laterille, 1817
(Order Conchostraca Sars, 1867)
Order Spinicaudata Linder, 1945
Family Cyclestheriidae Sars, 1887
Genus *Cyclestheria* Sars, 1899
Cyclestheria hislopi Baird, 1859

Introduction

The Spinicaudatan conchostracan *Cyclestheria hislopi* was first described by Baird (1859), who based his descriptions on specimens collected in India and named the species after its collector, Rev. S. Hislop. Baird (1859) originally placed it in the genus *Estheria.* However, it was Sars (1887) who recognized its distinctive features and erected a separate genus. Sars (1887) also suggested that it differed distinctly from all described conchostracans, and established the family Cyclestheriidae which remains to this day monotypic. Although another genus and species belonging to the family have been described from China (*Paracycletheria sinensis* by Shen and Dai (1859)), Olesen (1996) cautions that its validity is in need of verification as the descriptions are lacking in detail, and therefore represent young stages of *C. hislopi.* In the latest classification of Branchiopoda, Negrea et al. (1999) have elevated Cyclestheriidae to ordinal rank, wherein *C. hislopi* is the sole representative of the new conchostracan order Cyclestherida. The three orders Laevicaudata, Spinicaudata, and Cyclestherrida are grouped under super order Conchostraca.

Most of the works on natural history including species descriptions of *C. hislopi* come from Baird (1859) who has given a brief description and Sars (1887) who has given a very detailed description, and later from Roessler (1995c) who has studied its ecological aspects and life history and Olesen et al. (1996) who have described the external morphology of the male and compared it with other conchostracans. *C. hislopi* has a world wide distribution in the tropical and sub-tropical regions of most continents – Africa (Egborge & Ozoro, 1989), Australia (Sars, 1887; Timms, 1986), South America (Roessler, 1995c), North America (Sissom, 1980), and Asia (Sri Lanka: Fernando, 1974; India: Nayar & Nair, 1968; Paul & Nayar; 1977). *C. hislopi is* a common and widespread species that occurs in all types of water bodies except temporary pools of short duration where other conchostracans are common (Roessler, 1995c; Olesen et al., 1996).

Cyclestheria was first reported in Kerala by Nayar and Nair (1977) from a pond at Irinjalakuda, Trichur. In India they were recorded from Nagpur (Baird, 1859), Calcutta (Gurney, 1906) and Ludhiana (Battish, 1981). It is the only conchostracan that undergoes direct development of its offsprings. The eggs are retained in the brood chamber of the female where it undergoes larval development.

Materials and Method

(a) Description of the Sites

The present study is based on eight populations of *Cyclestheria hislopi* that inhabited large permanent ponds, small long-term temporary ponds, and long-term rock pools. A description of the sites is given below:

1. Kawdiar Pond - KP-1

It is a large, permanent, perennial, rectangular pond inside the Kawdiar palace compound, east of the palace building, Thiruvananthapuram. Area is roughly 0.15 km^2. This undisturbed pond has dense vegetation all around. Birds like white breasted water hen, pied king fisher and the little cormorant that mainly feed on fish are commonly seen fishing in the pond. A snake bird (darter) has also been spotted once. Fishes, crabs and tortoises are also seen in the pond. An abandoned flight of steps on the eastern side leading to the pond is the only place from where collections could be done. *C. hislopi* was obtained in all the collection visits, but not any other conchostracans. Laboratory cultures have been established of the *C. hislopi* collected from this pond.

2. All Saints' College pond - ASCP-2

This is a small man-made long-lived temporary pond (seasonal pond) located inside the All Saints' College Campus, Thiruvananthapuram (area: 7 m^2). During the dry season when water-level drops, water would be added to replace water lost by evaporation. Rooted and submerged macrophytes, *Nymphea* and *Hydrilla,* and algae grow in the pond. No other conchostracans except *C. hislopi* were seen in the collections. Quantitative estimation of the *C. hislopi* population is done for the period from July, 2004 to February, 2005. Dissolved oxygen and pH have been recorded during the period of study.

3. Mar Ivanios Campus Pond - MICP-3

This permanent perennial pond inside the Mar Ivanios campus, Thiruvananthapuram has an area of about 0.15 km^2. Banks of the pond are bare with aquatic macrophytes growing sparsely on one side (eastern). *C. hislopi* was present in the collection, but not any other conchostracans.

4. Thoppu Pond - TP- 4

It is a large permanent perennial pond with an area of about 0.15 km^2located at Sreekaryam, Thiruvananthapuram. Two sides of the pond have flights of steps leading into it, the other two sides are inaccessible, and vegetation sparse. *C. hislopi* was obtained in the collections; but, no other conchostracans were found.

5. Akkulam-Kuzhivila Temporary Pond - AKTP–5

Located on the western side of the Bypass of NH-47, this shallow, long-lived, temporary rock pool has an area of 15 m^2, with a maximum depth of 0.5 m. No aquatic macrophytes were found, but algal growth was abundant in the pond. While *C. hislopi* was present in large number, no other conchostracans were found.

6. Thamarakulangara Pond - TKP-6

This is a large perennial lotus (*Nelumbium*) pond on the eastern side of NH-47, Kayamkulam, in Kollam district. The entire pond was covered with lotus plants, where people were seen wading through the pond for plucking lotus flowers. Area is about 0.20 km^2. A number of such small ponds are seen on either side of NH-47 in the above locality. *C. hislopi* was present, but no other conchostracans.

7. Nangyarkulangara Pond - NP-7

This is a small pond (area: 8 m^2; average depth: 0.5 m) on the western side of NH-47, slightly below the road level, close to a large temporary pond from where *L. allepeyensis* were collected (described in Chapter 4). A number of such ponds connected to one another were seen on either side of the road where macrophytes were rare. *C. hislopi* was obtained from this locality, but no other conchostracans were found.

8. Pattikadu Temporary Pond - PPTP-8

This is located at Pattikadu, Mannuthy, in Trichur district. It is a small, shallow, long-lived temporary rock pool (Area: 6 m^2; maximum depth: 0.5 m.). Here, though aquatic macrophytes were absent, algal growth was abundant . *C. hislopi* was collected in large number; no other conchostracans were available.

9. Sasthamkotta Lake - SL-1

The largest fresh water lake (oligotrophic) of Kerala, located in the Kunnathur taluk of Kollam district, has an area of 3.42 km^2. The banks of the lake are bare with small rudimentary clumps of aquatic macrophytes (mainly grass) growing in two or three isolated groups. These areas were chosen for obtaining the sample. A country boat was used for this purpose. No conchostracans were present in the collection.

Other sites

Collections from two permanent ponds, one on the eastern and the other on the western side of NH-17, and three temporary ponds, one on the eastern, and two on the western side, on the road leading from Parappanagadi Railway station to Kozhikode city contained no conchostraca. No conchostracans were present in the collections at that that time. A few ponds (one at Pariyaram, and three temple ponds along the road leading from Pariyaram to Parassinikadavu) in Kannur district were also sampled. But conchostracans were present in none of those, at that time. The Karali wetland in Kollam adjacent to Sasthamkotta lake, and a part of Kole wetland (close to the Karuvannur bridge on the way from Trichur to Irinjalakuda) were also sampled; but, conchostracans were absent in the collections.

(b) Collections and Method

Collections were done by disturbing the roots of macrophytes growing on the banks of the pond, sweeping with a scoop net, and emptying into a bucket of pond water. In the laboratory, specimens were picked up and fixed in 4% formalin/ 70% alcohol, or transferred for culture. For quantitative estimation of the *Cyclestheria* population, collections were standardised. For this, 10 full sweeps of the net were made and emptied into a bucket of pond water. This was repeated at the four corners of the pond. In the laboratory, the *Cyclestheria* were sorted out using a dissection microscope, and sexed. After counting and sexing, they were released back into the pond. Dissolved oxygen was measured using Winkler's method and pH was noted using a pH meter.

For studying life history details, live specimens of *C. hislopi* (females after release of juveniles) were cultured, in small, glass ice-cream bowls filled with pond water and a pinch of cow dung powder, in the laboratory, at room temperature. Cow dung powder was added for the growth of algae, and aged tap water (tap water collected and kept for 24 hrs.) was added intermittently to replace water lost by evaporation. The time taken for hatching of eggs, for development and release of juveniles, and the life span were recorded. Illustrations were made with a compound microscope equipped with camera lucida. Specimens were gold coated with a 10 nm beam sputter coating unit Jeol JFC 1200 and were observed in a Jeol JSM-5600 LV SEM.

(c) *Laboratory Culture of Cyclestheria hislopi*

Two laboratory cultures LC-1 and LC-2 were established from *Cyclestheria* females and algae collected from Site KP-1. LC-1 was maintained in a large aquarium (45 cm x 30 cm x 30 cm) and LC-2 was maintained in a smaller aquarium (30 cm x 15 cm x 15 cm). Small pieces of cow dung that served as manure for the algae were added once a month. The aquaria were maintained on a well lit balcony. Aged tap water was added intermittently to replace water lost by evaporation. Monthly recordings of the number of males and females of each aquarium were done for a period from December 2000 to September 2002 in the case of LC-1, and from September 2001 to September 2002 in the case of LC-2

Diagnosis of Species

(a) Description of Male:

Carapace:Carapace thin, transparent, flexible, almost rounded but not globular; laterally compressed, and nearly as high as long (Fig.3.1A; Fig. 3. 6j). Males smaller than females. Carapace measures 1.38 mm. to 2.56 mm. in length. Lines of growth range from 3 to 5, are arranged in a concentric manner with some distance from one another. Umbo prominent and close to the anterior extremity.

Head region: Head, triangular in side view, tip blunt and minutely serrated with row of teeth increasing in size, distal 2 or 3 teeth stronger and more remote from one another, than the rest which are small and crowded (Fig. 3. 1C; Fig. 3. 8B). Head bears dorsally a keel-like structure, the dorsal carina (Fig. 3. 8A). Dorsally on the head is an incision - the occipital notch dividing head into a pre-oral part bearing antennulae, antennae, labium, eyes, ocellus, and the post oral bearing, the mandibles (Fig. 3. 1C). From the occipital notch along rostral carina, anteriorly, is a swelling bearing the rectangular dorsal organ. It bears a flattened rectangular rim encircling two pores, a large one and a small one close to each other (Fig. 3. 8C). Ocellus large as the eyes (united into a single globular organ), lies inferior and somewhat anterior to the eyes (Fig. 3. 4C).

Antennae: Antennules unsegmented and club-shaped with sensory hairs at their dilated tip (Fig. 3. 1B, C). Antenna has poorly marked scape and two, 7-segmented flagella, with short stiff dorsal setae and long plumose natatory setae (Fig. 3. 8B) on each segment. Mandibles consist of a narrow oblong navicular dorsal part and a stout, incurved ventromedial molar process; mastigatory part almost a right angle, with the body incurved, so as to meet the corresponding part on the other mandible (Fig. 3. 9 I). Triturating surface of mandible (first type) consisting of a smooth surface of connected pits (Fig. 3. 9I).

Thoracopods: Each of eight posterior segments of the trunk (in both males and females) provided with cuticular dorsal process, curving more or less distinctly forward and edged with slender posteriorly pointed setae (Fig. 3. 2G; Fig. 3. 9G). Trunk is composed of 15 well defined segments, each bears a pair of foliaceous legs.

Except the first pair, which is modified into clasper, the thoracopods show serial similarity. Each entire limb strongly concave, with the concavity facing backwards; decreasing successively in length posteriorly, with the posterior most pair being very small. Each thoracopod possesess an elongate exopod, an oval or slightly triangular unarmed balloon-like epipod, and an endopod; endopod with 6 endites. Details of thoracopods are given in the next section on 'Females'.

Thoracopod 1 (Clasper): In the first thoracopod, the distal endites modified into claspers, whereas other parts same as in all the other legs. Coxal segment bears a row of stiff curved setae and two small non-curved setae. Endites 2 and 3 are elongated and bordered by setae (Fig. 3. 1E). Endites 4, 5, and 6 modified into the clasping structure; 6th endite forming the movable finger, and 4th and 5th endites forming the opposing 'palm' with two palps, one larger and the other smaller (Fig. 3. 1E; Fig. 3. 10L, M). At the junction where the inner distal face of 'palm' (endites 4 and 5) opposes the tip of the movable finger the 'palm' bears a field of spine-like setae (Fig. 3. 10L, M). 'Palm' also bears palps; large palp arises near base of movable finger and small palp directly behind (Fig. 3. 10L, M). Large palp bears at its tip a cluster of setae; posterior face of 'palm' bears a row of setae (Fig. 3. 1E; Fig. 3. 10L). The palps and the clasper, as seen in the SEM photographs (Fig. 3. 10L, M), show shrinkage, indicating that they are made of thin cuticle. Movable finger long and curved smoothly with its tip bearing a field of flat teeth like setae (Fig. 3. 1D, E). Dorsal side of the movable finger bears 3-5 long setae (unique to the species) (Fig. 3. 1D, E; Fig. 3. 10L). Exopod elongated; distal end bears a short segment; both segments bear terminal setae (Fig. 3. 1D). Epipod oblong and sac-like. Prehensile character of the 1st pair of legs quite distinct in the young in brood chamber.

Telson :Telson has the form of a broad compressed irregularly triangular structure. (Fig. 3. 2G; Fig. 3. 8D) Ventral edge quite smooth and straight ending in two small denticles (Fig. 3. 2G, i; Fig. 3. 8D). Dorsal edge of telson armed with a double row of spines, successively and rapidly increasing in size; last pair very large and strongly curved whereas first pair very small. On the dorsal side of all spines are rows of small scales. Between the 5th (counting proximal to distal) and the 6th pairs of spines are two rows each of 5 to 6 small spines of small spines, one on either side of telson; these spines directed laterally in a different plane from the paired curved spines of telson (Fig. 3. 8D, F). Angle of orientation of this row of spines approximately 45° away from the dorso-ventral axis, and serves in cleaning when the abdomen is swung between the thoracopods (Olesen, 1996). Each small spine bears 5-6 tiny spines (Fig. 3. 9H). At the point where the posterior trunk setae end and the double row of spines originate (dorsal side of base of telson), is a knob-like projection from which arises a pair of telsonal filaments that are minutely plumose on its distal half (Fig. 3. 2G; Fig. 3. 8D). Furcal claw movably articulated with the telson just below the last pair of telsonal spines. Claws exceedingly elongated and slender, slightly curved, and bear minute denticles along their entire length (Fig. 3. 2G; Fig. 3. 8D; Fig. 3. 9G).

(b) Description of Female:

Carapace: Females distinctly larger than males. Length of carapace valves of adults ranges from 2.97 mm. to 4.11 mm. juveniles, from 1.75 mm. to 2.65 mm. Dorsal line very strongly curved and somewhat angular in the middle (Fig. 3. 1F; Fig. 3. 9K). In older and larger specimens with 10-11 lines of growth, 2 or 3 last lines run parallel to the edge of the shell, and are more adjacent to one another than inner ones. The oval shell gland, composed of three irregular canals, and located in anterior part of valves, partly encircling the insertion of the adductor muscle, clear in the fresh specimens (Fig. 3. 4b).

Head, rostrum, antennules, antennae, and mandibles similar to those of male.

Thoracopods: Trunk bears 16 pairs of foliaceous thoracopods. Between upper side of trunk and dorsal edge of shell, in adult females, is brood chamber for carrying eggs or juveniles. In *C. hislopi,* development is direct with female retaining the direct – developing eggs in brood chamber (Fig. 3. 4d). Thoracopods decrease successively in length posteriorly; first pair being the longest and reaching almost the edge of the shell, posterior pairs very small with the last 3 being smallest. All endites bear sub marginally a row of curved plumose setae; number decreasing on the more posterior thoracopods. Coxal lobe rounded and triangular and bears on its edge delicate curved setae forming a densely crowded comb-like structure (Fig. 3. 2J). Marginally proximal endite also bears 3 strong chitinised setae; uppermost of which longest with two spines on either side. Each of next four succeeding lobes provided with a dense series of plumose setae, arranged in a comb-like manner. They also exhibit another series of setae which are shorter and less numerous. 5th endite narrower than the preceding, and more prominent in 1st pair of thoracopods. Last endite more expanded and obliquely rounded at the end, and provided with a single row of setae, increasing successively in length towards inner side (Fig. 3. 2J). Exopod consists of a straight elongate plate, with evenly spaced plumose setae, the distal expanded (somewhat rectangular) extremity of which reaches beyond the tip of the endopodite. Proximal end of elongate plate is produced above on the outer side of epipodite into a more or less rounded but quadratic and inflated lamella, bordered by 3-12 setae (Fig. 3. 2J). The quadratic dilated part after the 10th thoracopod becomes short, simple and non-dilated. Epipodite is oblong, and sac-like (Fig. 3. 2J). First pair of legs can be distinguished from rest by the presence of a rounded expansion from anterior surface of endopodite, immediately below epipodite (Fig. 3. 2.J). This rounded expansion is bordered by a number of very strong, curved setae. First pair of exopodites very slender and elongate unlike the rest which are expanded. Details of the telson are similar to those of the males (Fig. 3. 8E).

(c) Discussion

All the animals examined easily fit the characteristics of *C. hislopi* as described by Baird (1859), Sars (1887), Roessler (1995c), and Oleson et al. (1996). Thus the shell is thin and rounded with a prominent umbo at the anterior extremity and with growth lines; ocellus is as large as the singular eye; dorsal organ is rectangular with a large central pore (unique to the species) and a small one close to it; antennules are unsegmented and simple with setae at the tip; 2nd antenna is large and biramus, with jointed flagellum, each armed with setae and spines; possess distinctive features of clasper morphology; show sexual dimorphism; and spination of telson.

Some of the unique ultra structural characters of C. *hislopi* revealed by the present study are details of the clasper – the presence of spine-like setae on the 'palm' that opposes the tip of the movable finger; the two palps of the 'palm'; the presence of setae on the outer margin of the movable finger; setal row on the 'palm'and the presence of double pores at the centre of the dorsal organ. The only other detailed description of the external morphological characters of a male *C. hislopi* is that by Olesen (1996). Comparison at the ultra structural level shows no differences between the two studies. Olesen has also described the presence of double pores at the centre of the dorsal organ but is unable to explain its significance. The clasper morphology of *Cyclestheria* is clearly of the spinicaudatan type. *C. hislopi* thus exhibits characters, some unique and others typical of Spinicaudata.

Olesen (1996) finds that the clasper of C.hislopi is most likely to be derived from a normal thoracopod in the same way as in other spinicaudatans. According to him the 'hand' or 'palm' is derived from endite 4 & 5 and the movable finger from the endopod (as in all conchostracans both spinicaudatans and laevicaudatans) and the palps are true palps on the endites (as in other spinicaudatans). Commenting on the phylogeny of *C. hislopi*, he argues that since *Cyclestheria* does not have true palps on the basic thoracopods., presence of these palps could be used for an alliance of non-*Cyclestheria* families. A striking difference between the clasper of *Cyclestheria* and the three other spinicaudatan families is that *Cyclestheria* has preserved more of the setations of the normal thoracopods, such as the long setae on the movable finger and setal row on the 'palm'. It is likely that the preservation of the setae on the clasper should be interpreted as a primitive condition and their loss as an apomorphy for the other three spinicaudatan families. He also points out that one pair of thoracopods modified as claspers in *Cyclestheria* is more likely pleisomorphic compared to having two pairs of claspers modified (in other spinicaudatans).

Morphological Forms, Population Estimation and Ecology

(a) Morphological Forms

Four morphological forms of *C. hislopi* as described by Roessler (1995c) have been obtained from the present laboratory culture and field collections. All morphs are structurally similar as described above except that during the reproductive cycle they undergo specific changes.

(1) Parthenogenetic forms

The females that hatch from diapause eggs are the parthenogenetic females that reproduce parthenogenetically. These females become sexually mature when they have four to five carapace lines. The parthenogenetic eggs mature within the ovaries and are deposited in the brood chamber (Fig 3. 4d). The larvae hatch and develop in the brood chamber into juveniles with 16 post maxillary appendages (Fig. 3. 4e). The parthenogenetic females reproduce during the entire life span to produce parthenogenetic generations successively. The eggs are spherical with a diameter of 0.26 mm. to 0.29 mm. The number of eggs in the brood chamber ranges from 1 to 22. Sars (1887) referred to such eggs as summer eggs. The longevity of a female in the laboratory is 30-35 days, with 5-6 broods of parthenogenetic eggs produced during its life span. The eggs deposited in the brood chamber take 4-5 days to hatch, develop and release juveniles with adult number of limbs. Parthenogenetic females are found to be the most numerous of the population throughout the year (Fig. 3.11, 3.12 & 3.13), as also noted by other investigators (Sars, 1887; Nair, 1968; Paul & Nayar, 1977; Roessler, 1995). These parthenogenetic females are found to produce males in December with the onset of the dry season (Fig.3.11, 3. 12 & 3.13). They produce males and females in varying ratios within and between successive broods. Parthenogenetic females with only males (1 to 4) or only females (sexual and parthenogenetic) or varying combinations of males and females in a brood was noted. In the subsequent generations also, these parthenogenetic females are seen to produce males. In most of the male-female combinations, females are found to be parthenogenetic.

(2) Sexual females

The sexual females are observed to produce diapause eggs and form ephippia. They have only 5 to 6 growth lines, but with respect to other structural details they are similar to parthenogenetic females (Fig. 3. 7k, l). The sexual females, which are generated by parthenogenetic females in the same way as males, are seen only after the appearance of males in the population (Fig. 3. 11, 3.12 & 3.13), and are identified 20-21 days after the males are first seen in the population. After release from the brood chamber of the parthenogenetic female, the sexual females are seen to undergo marked changes in their morphology. The ends and joints of appendages undergo strong pigmentation (Fig. 3. 2H, I). The carapace undergoes a particular reinforcement (Roessler, 1995) of the external plates of the bivalved carapace that gets converted later into a special type of ephippium (Fig. 3. 5f; Fig. 3. 7l). At this stage, the sexual female can be easily distinguished from the parthenogenetic female as the carapace of the former appears opaque. These changes cause the female to become feeble and remain buried, finally leading to her death and deposition of the ephippium. Sexual females produce one generation of diapause eggs, and die with the eggs.

Mating was not observed, but when males and females were isolated for counting, males and young females appeared to pair but broke away quickly when disturbed. Mating has not been reported in the literature (Roessler, 1995c; Olesen et al., 1996), and Roessler has speculated that sperms may be transmitted by water currents. The parthenogenetic females, when isolated just before males appeared in the population, produced in the next generation, sexual females that develop into ephippia with diapause eggs These ephippia contain only a few eggs (Fig. 3. 3K; Fig. 3. 5h). Whether these diapause eggs hatch or not could not be confirmed. Roessler (1995c) found that if sexual females do not encounter males, they develop into ephippia with diapause eggs that do not hatch. .

Ephippium --The ephippium consists of the modified carapace of the sexual female that becomes comparatively hard and opaque for protecting the fertilized eggs or diapause eggs. The ephippium contains 1 to 7 eggs that are larger than the parthenogenetic eggs (diameter of diapause eggs: 0.29 mm to 0.34 mm.) (Fig 3. 3L; Fig 3. 5f). The carapace is strengthened by the deposition of shell material more on the dorsal and dorso-lateral parts and to a lesser extent on the anterior and peripheral parts. Deposition of shell material begins with sexual maturity of sexual females, and continues after oviposition until their death, when the valves get tightly closed locking the eggs inside. This type of formation of ephippium is in contrast to the cladocerans of the order Anomopoda where only a larger or smaller part of the carapace is shed and where death of female is not necessary when the ephippium is deposited (Olesen et al., 1996).

When ephippia were dried and rehydrated they hatched into parthenogenetic females. Twenty five ephippia with algal mat and detritus from LC-1 were dried for three months and rehydrated with aged tap water (pH = 7.1). After 3 weeks, 8 *C. hislopi* (females) were seen. All the females were found to reproduce parthenogenetically.

(3) Females with hypodermic corpuscles

Females with whitish valves have also been obtained from the present laboratory cultures, but not from the field (Fig 3. 5g). In the aquarium LC-1 towards the end of April when the sexual females and the males disappeared, 8 white in colour, opaque females as described by Roessler (1995c) were obtained in the culture. These females bear large eggs (1 or 2) that resemble the diapause eggs (Fig 3. 5g). Accumulation of ovoid corpuscles within the hypodermic cells of the carapace valves gives them a whitish appearance, caused by the total reflection of light (Roessler, 1995c).

(4) Males

Males are usually green in colour, distinctly smaller and more active than females. They can be easily mistaken for juveniles unless specifically identified by the clasper with the aid of a microscope (Fig 3. 6f, i, j). The male would be already mature when it leaves the brood chamber. Roessler (1995c) found that males in the brood chamber had fully developed spermatozoa. Population estimation of the cultures - both laboratory and field - shows that males appear in the population during December (Fig. 3. 11, 3. 12 & 3. 13) when the parthenogenetic females and males combination was at its highest level. The male-female ratio was found to be 1:6 for LC-2, and 1:4.5 for LC-1. For the field population (ASCP-2), the ratio was 1:5.8. In all the populations, males occur in December, after the NE monsoon with the onset of dry season. Maximum number of males were seen to occur in December, followed by a decline (with slight fluctuations) during the next four months when dry season continued; no males were further recorded until after both the subsequent monsoon seasons were over (Fig. 3. 11).

This is the first report of males from the Indian subcontinent.

(b) Population Estimation

Population estimation of the LC-1 population shows a marked periodicity with respect to the appearance of males (Fig. 3.11) which is also seen for populations from LC-2 and ASCP-2 (Fig. 3. 12 & 3.13). The parthenogenetic forms are seen throughout the year with slight fluctuations in their population (Fig. 3. 11, 3. 12 & 3. 13). The three populations show a general increase in the density of population (parthenogenetic forms) with a peak in December when males appear (Fig. 3. 11, 3. 12 & 3. 13). A similar increase in the population, consisting entirely of females from October to January, was noted by Nair (1968), and Paul and Nayar (1977) also. This increase in population and the appearance of males persist for January, February and March. However, males begin to decline in number and disappear by April/May. In the three parthenogenetic populations, sexual females are seen after the appearance of males (Fig. 3.11, 3.12 & 3.13). Sexual females develop in the population 20 to 21 days after males are present in the population. The appearance of males is closely related to a particular season – the beginning of dry season. The maximum number of males observed in December, January and February – both in field and laboratory – is related to the seasonal cycle of the aquatic habitat which begins to contract with the dry season. Males disappear by April/May which is the end of dry season and the beginning of the rainy season. The levels of dissolved oxygen of the water have been generally low, and have showed a marked variation within a narrow range (2.434 mg/l to 2.028 mg/l), the lowest being noted in December (Fig. 3. 15); pH also has fluctuated within a narrow range (7.02 to 8.20), the highest level being in December (Fig. 3. 14). In the present study, the three ponds in which males occurred are small astatic water bodies showing fluctuations in water levels, without drying completely.

(c) Some aspects of Ecology

Sampling of different types of fresh water bodies of water within an area 1 to 2 km. east and west of NH-47, from Thiruvananthapuram, Kollam, and Alleppey districts has revealed that 4 large permanent ponds, 2 seasonal ponds and 2 rock pools contained *C. hislopi*. *C. hislopi* has already been reported to be the most common conchostracan that occur in abundance in the seasonal pools of Irijalakuda, in Trichur (Nayar & Nair, 1967; Nair, 1968; Paul & Nayar, 1977). *C. hislopi* is also seen in a rock pool (PPTD-8) in Mannuthy, Trichur. Most of the ponds sampled during the 3 year study were confined to Thiruvananthapuram, Kollam, Alleppey, and Trichur districts. Only extensive sampling over a longer period of time will help map the distribution of *Cyclestheria* in Kerala. Roessler's (1995) work, for example, on Colombian *Cyclestheria* is based on 15 years of extensive sampling of the tropical lowlands of Colombia.

Though extensive sampling was not done in other parts of Kerala, C. *hislopi* can be considered to be apparently widespread in Kerala. However, it is found to be absent in the very short-term temporary water bodies we studied in Kerala, where other conchostracans are common. *C. hislopi* were not obtained in our collections from Karali wetland, in Kollam, and Kole wetlands in Trichur. These areas are rich in fresh water fishes – majority of them being carnivorous. Conchostracans are usually not present along with carnivorous fishes (Pennak, 1953). In the long-lived temporary pools (ASCP-2, AKTP-5 & PPTP-8) where fishes are absent, *C. hislopi* are found in abundance. When they occur in permanent ponds where fishes also occur, C. *hislopi* show specific habitat preferences as they are always found among the roots of submerged macrophytes while absent in open waters. They also do not occur in large lakes, as was stated by Pennak.(1953). *C. hislopi* was absent in the oligotrophic Sasthamkotta lake. Conchostracans were also not present in the collections of Joseph and Prakasam (2002) who studied the flora and fauna of the Sasthamkotta lake. In the laboratory cultures, they are invariably found buried in the algal mat at the bottom of the aquarium. Females are frequently seen swimming just above the algal mat. Males when present can easily be collected as they frequently swim to the surface water of the aquarium.

Paul and Nayar (1977) have noted that *C. hislopi* has a preference for the aquatic plant *Hydrilla verticellata* (L. 1781) to which it attaches itself. Timms (1986) has observed the same preference of C. *hislopi* for *Hydrilla verticellata* in Australia, and Egborge and Ozoro (1989) have observed a less frequent preference of it to *Eichornia* in Africa. Other than from *Hydrilla* and *Eichornia*, *C. hislopi* has also been obtained, in our collections, from *Nelumbium, Nymphaea*, and *Ipomoea*. *C. hislopi* has been collected from rock pools (AKTP-5 & PPTP-8) which had no aquatic macrophytes but a thick algal growth. Sissom (1980) also obtained C. *hislopi* from algal mat.

(d) Discussion

The findings of the present study, which gives the first report of males from the Indian sub continent, unfold several unique aspects of the life cycle and reproductive biology of the species. *C. hislopi* has a biphasic life cycle with two distinct cycles of reproduction, one parthenogenetic and the other in which sexual reproduction takes place (Sars, 1887; Roessler, 1995c). It was Sars who first observed that *C. hislopi* reproduced parthenogenetically with a change to sexual reproduction, and that the appearance of males coincides with the beginning of unfavourable conditions. He also observed some empty shells, with upper part opaque, that reminded him of ephippia of Cladocera. However, it was Roessler (1995c) who confirmed the presence of cyclic parthenogenesis in Colombian populations, and described the sexual female, the male, and the ephippium. As described earlier, the sexual females undergo complete transformation into a special type of ephippia with fertilized eggs, culminating in their death. This same method of producing ephippium has been described in the case of the Colombian population (Roessler, 1995c).

Drying and refilling of the habitats trigger hatching and development. Artificial hatching of the ephippia produced females in the present population as well as in the Colombian population. Direct development of both diapause eggs and parthenogenetic eggs occurs both in the present and in the Colombian populations. Thus unlike other conchostraca, the life cycle of *C. hislopi* includes two distinct modes of egg production. One mode leads to direct developing eggs (subitaneous) that are brooded by the female until juveniles hatch, and the other sexual reproduction that leads to diapause eggs or ephippium. The diapause eggs have to undergo drying before hatching. Both types of eggs are direct developing, that hatch into either all female clutches or mixed sex cultures, apparently in response to environmental stimuli. However, Roessler (1995c) has observed ephippial eggs developing without total dessication.

Roessler (1995c) has given an explanation for the presence of whitish females with hypodermic corpuscles and diapause eggs, towards the end of the sexual cycle when males disappear at the end of April. They could be parthenogenetic forms transforming into ephippia. He speculates that in the whitish females there could be switching over of development of parthenogenetic eggs into diapause eggs as seen in modern cladocerans. Roessler failed to hatch these ephippia; in the present study also they failed to hatch.

Males of *Cyclestheria* have long been considered rare and so far have been reported from only four sites so far: one young specimen in a culture from Australian mud samples by Sars (1887); one specimen among the hundreds collected from Australia by Daday (1926); a few specimens from a population in Texas by Sissom (1980); and in large number from Colombia by Roessler (1995c). No males have been reported from India. Extensive sampling of seasonal ponds near Christ College, Irinjalakuda, Kerala by Nair (1968), and Paul and Nayar (1977) failed to find any males in their collections. On account of this, Sassaman (1995) and Olesen et al. (1996) are of the view, that not only the *C. hislopi* population of India but also that of Asia are unisexual. However, in the present study, regular field sampling (Site: ASCP-2) and laboratory cultures of *C. hislopi* have recorded an abundance of males (Fig. 3. 11, 3. 12 & 3.13). Males appear in large numbers during the months of December, January and February when the dry season begins, and disappear by the end of the dry season (April/May). As seen in Fig. 3. 15, there is a drop in the level of dissolved oxygen by December and January; and pH tends to become highly alkaline during the same period (Fig. 3.14). These changes could be signalling the onset of the unfavourable dry season. These environmental cues could have initiated a modified form of egg production leading to the appearance of males and sexual females. Sampling of permanent ponds has yielded only females in the collections. However, sampling was not done throughout the year.

Female *Cyclestheria* collected from Site TP-4 (Date of collection: December 30, 2000) when cultured in the laboratory yielded males in the next generation (Date of culture: January 31, 2001). This suggests that males could occur in larger numbers elsewhere at appropriate times. Males appeared in LC-1 and LC-2 that were populations started with females collected from KP-1. Whether males would have appeared in the natural population of the above pond could not be confirmed. Difficulties in collecting the samples (sampling could be done only on one side that had a flight of steps) and presence of predators, like fish, could be the reasons for the absence of males in the collections. But when females from this pond were cultured in the laboratory, males made their appearance at the appropriate time (onset of dry season). These cultures have revealed that natural populations have the inherent ability to produce males, and that sex determination is related to environmental factors. Sassaman (1995) has presented a model to explain sex determination in the cyclestherian life cycle as a modification of the same genetically based life cycle operating in other conchostracans. Females are heterozygous for the sex determining locus (S/s). For iterative reproduction to occur without expression of males, they must reproduce parthenogenetically. Environmental stimuli induce a modified form of egg production in which segregation of male determining factors occur, leading to appearance of males and sexual females (Sassaman, 1995). A factor other than environmental stimuli, that could induce a modified egg production, may be the presence of fish or predator presence. *C. hislopi* responds to predator presence using several strategies - restriction to particular environments and attempts to increase the number of offsprings, etc. The laboratory cultures in which males appear were started with females taken from KP-1 that has plenty of fish in it. Furthermore, lotic habitats (Egborge & Ozoro, 1989) have only female Cyclestheria populations. Hence predator presence could also be an important factor influencing the life cycle of *C. hislopi.*

Cyclestheria are found in both lotic habitats (Egborge & Ozoro, 1989) and lentic habitats (Sars, 1887; Paul & Nayar, 1977; Sissom, 1980; Timms, 1986; Roessler, 1995c). Only females are found in lotic habitats and they produce only female generations – unisexual. In the lentic habitat, there is an initial generation of females but as the dry season or summer season sets in, males are included in the subsequent generations. Roessler (1995c) has termed this type of reproductive cycle as cyclic parthenogenesis wherein a series of unisexual female generation is interrupted by the appearance of sexual females and males in the generations, whose mating produce the diapause or resting eggs. In the lotic habitats, the cycle is uninterrupted by environmental decline and only female generations are produced.

Figures

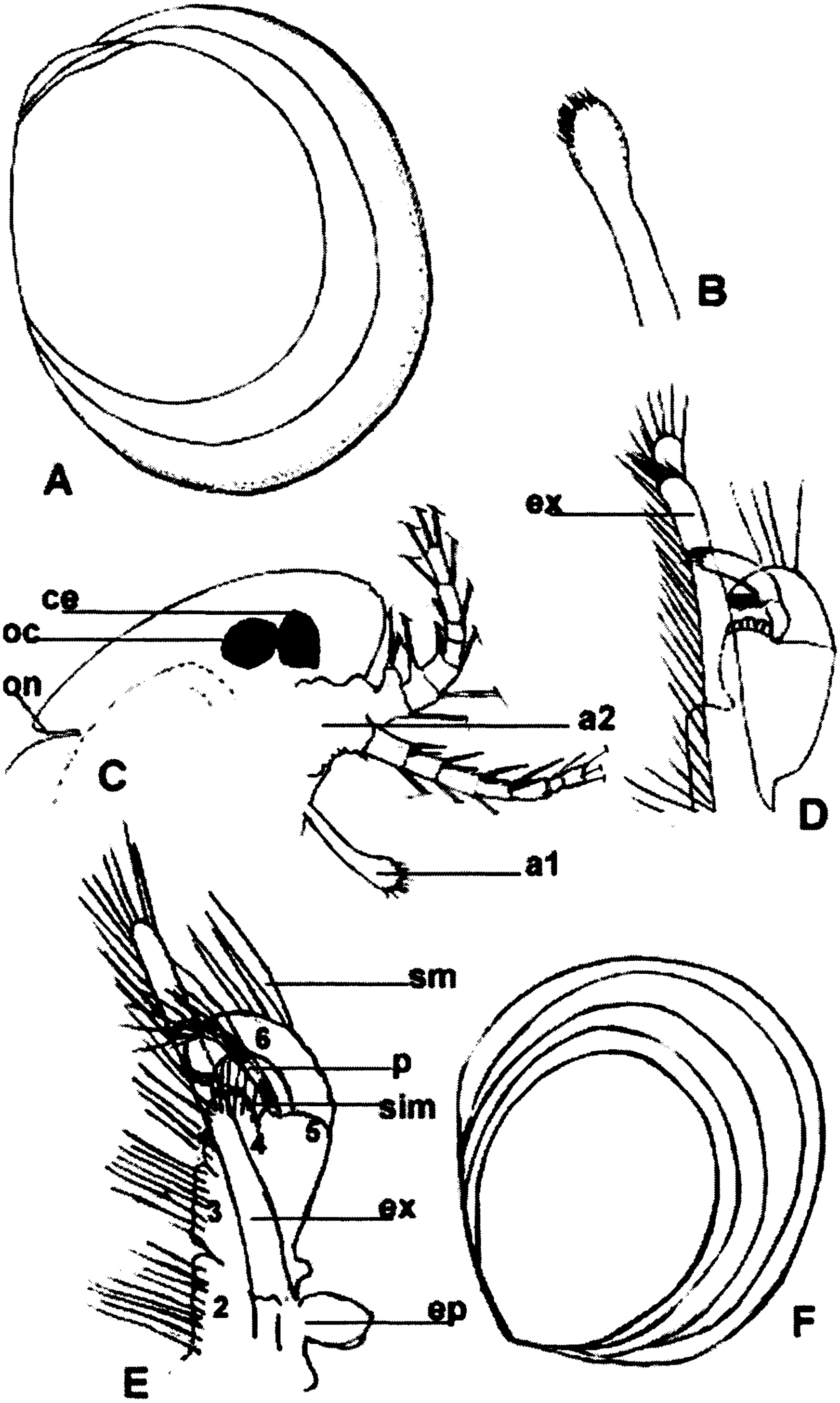

Fig. 3. 1. *C. hislopi*. A, shell ♂; B, 1st antenna ♂; C, lateral view head ♂; D & E, clasper of ♂; F, shell ♀. Abbreviations, a1, antennule, a2, antenna; ep, epipodite; ex, Exopodite; p, palp; sim, setae of immovable finger; sm, setae of movable finger ce, compound eye; oc, ocellus; on, occipital notch.

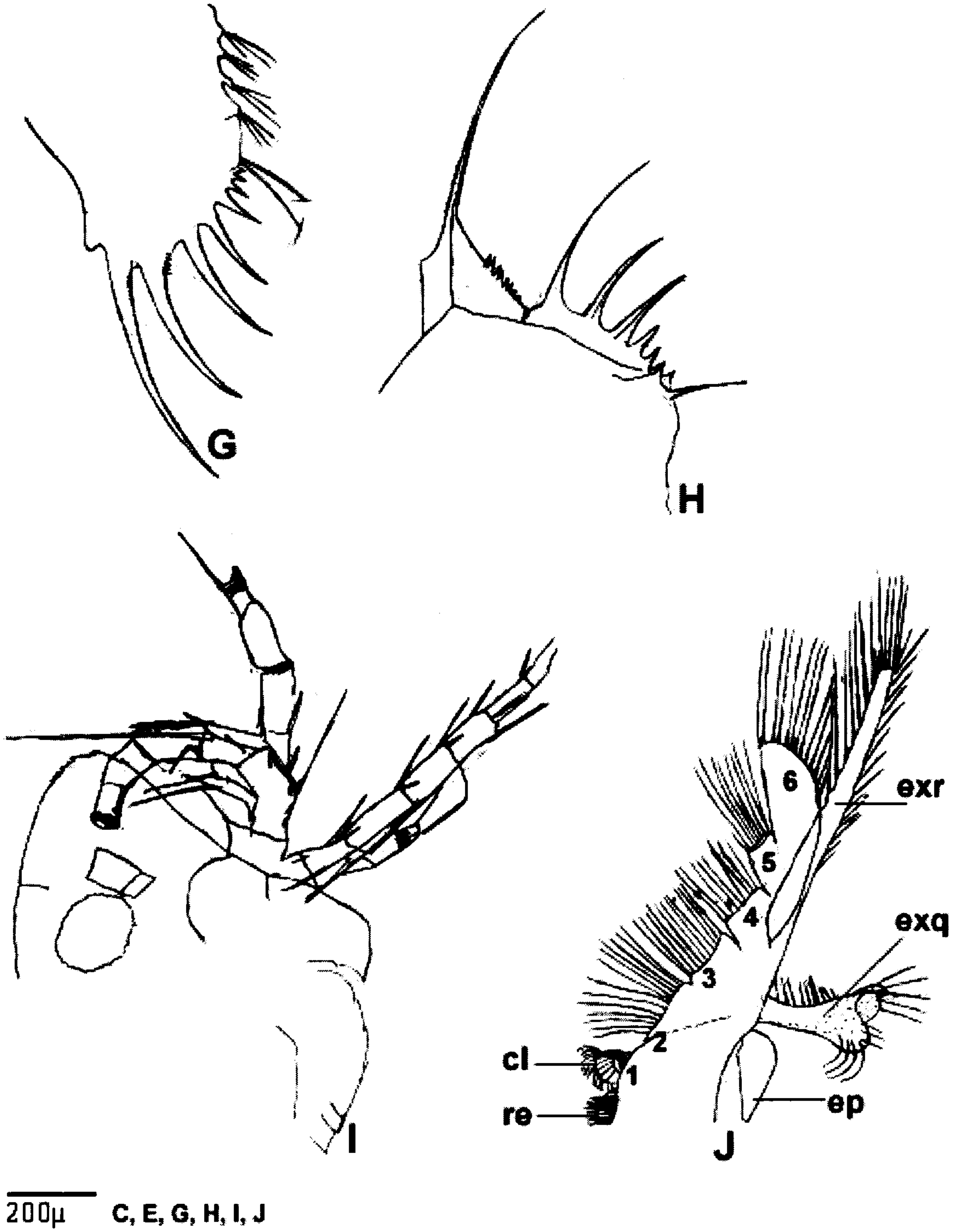

Fig 3. 2 . *Cyclestheria hislopi* . G, telson ♂; H, telson sexual ♀; I, lateral view of head sexual ♀; J, first thoracopod ♀. Abbreviations: cl, coxal lobe; ep, epipodite; exq, rounded quadrate lobe of exopodite; exr, rectangular lobe of exopodite. Arabic numbers refer to endites.

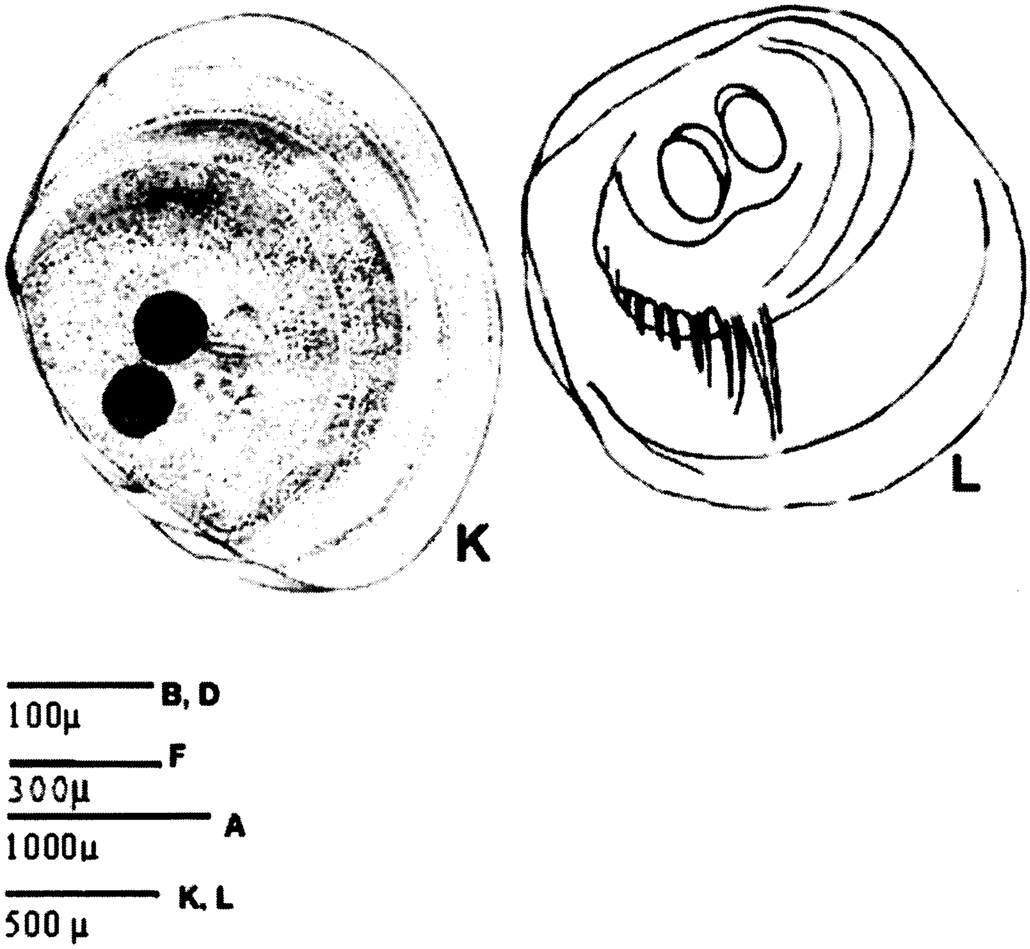

Fig. 3. 3. *Cyclestheria hislopi*. K, ephippium of sexual ♀with no ♂ encounter; L, Ephippiumwith diapause eggs.

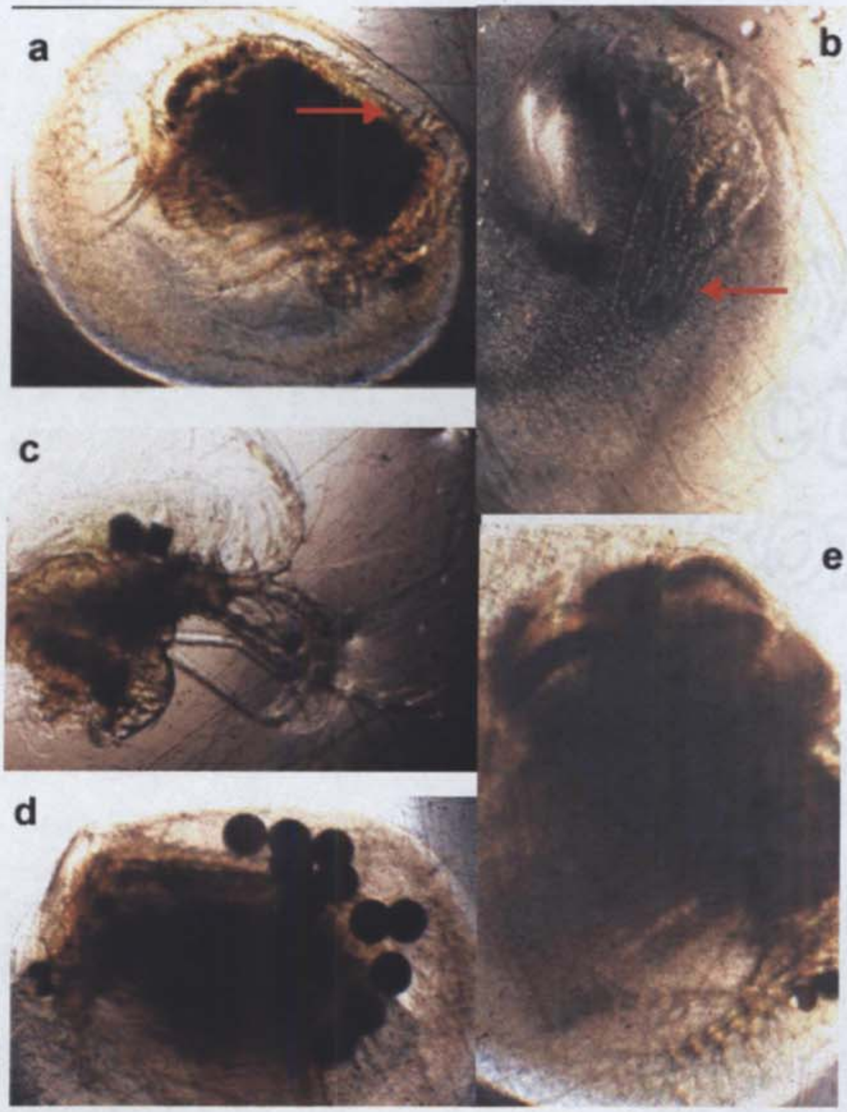

Fig.3. 4. *Cyclestheria hislopi.* a, mature ♀, arrow indicates cervical ligament; b, shell With shell gland, arrow indicates shell gland; c, latter view of head ♀; d, ♀ with eggs in brood chamber; e, ♀ with embryos in brood chamber (Scale in Fig.3. 7).

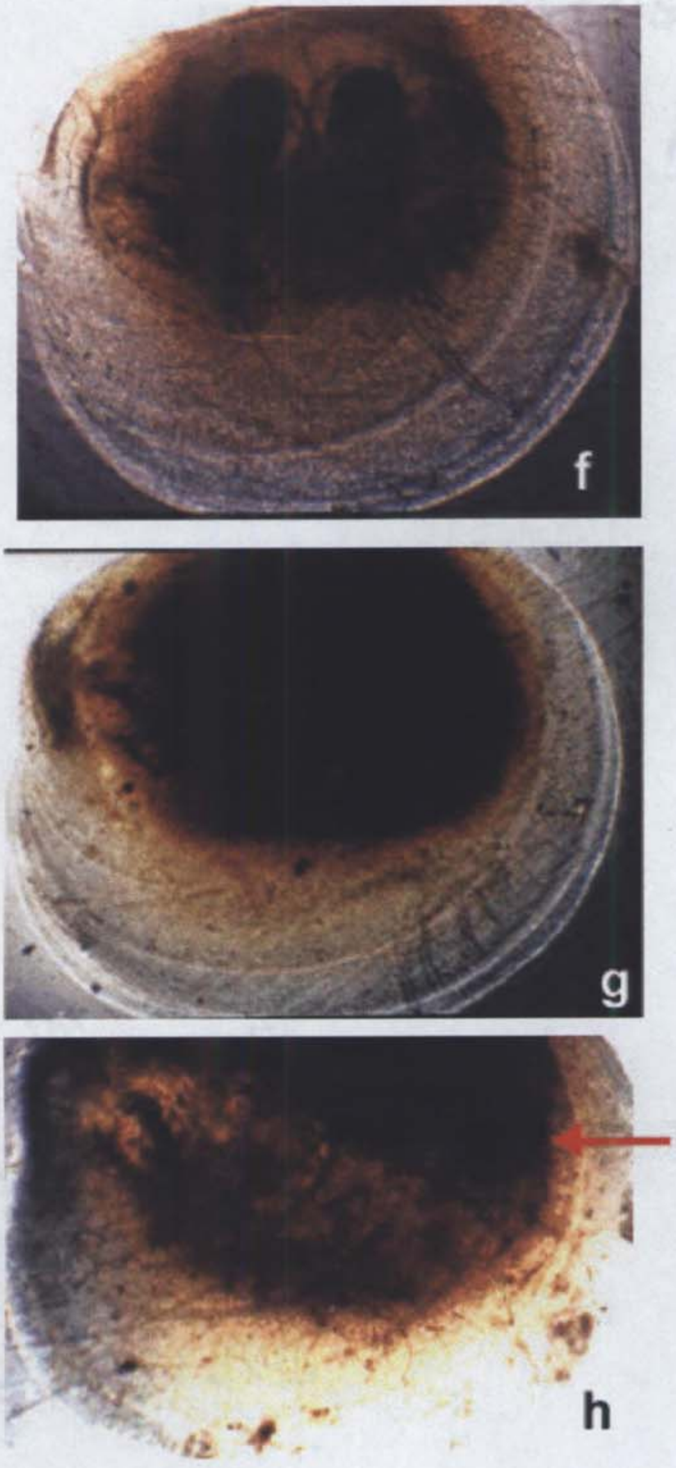

Fig. 3. 5. *Cyclestheria hislopi*. f, ephippium of sexual ♀; g, white ♀; h, ephippium of sexual ♀ with no ♂encounter.; arrow indicates diapause eggs (Scale in Fig.3. 7).

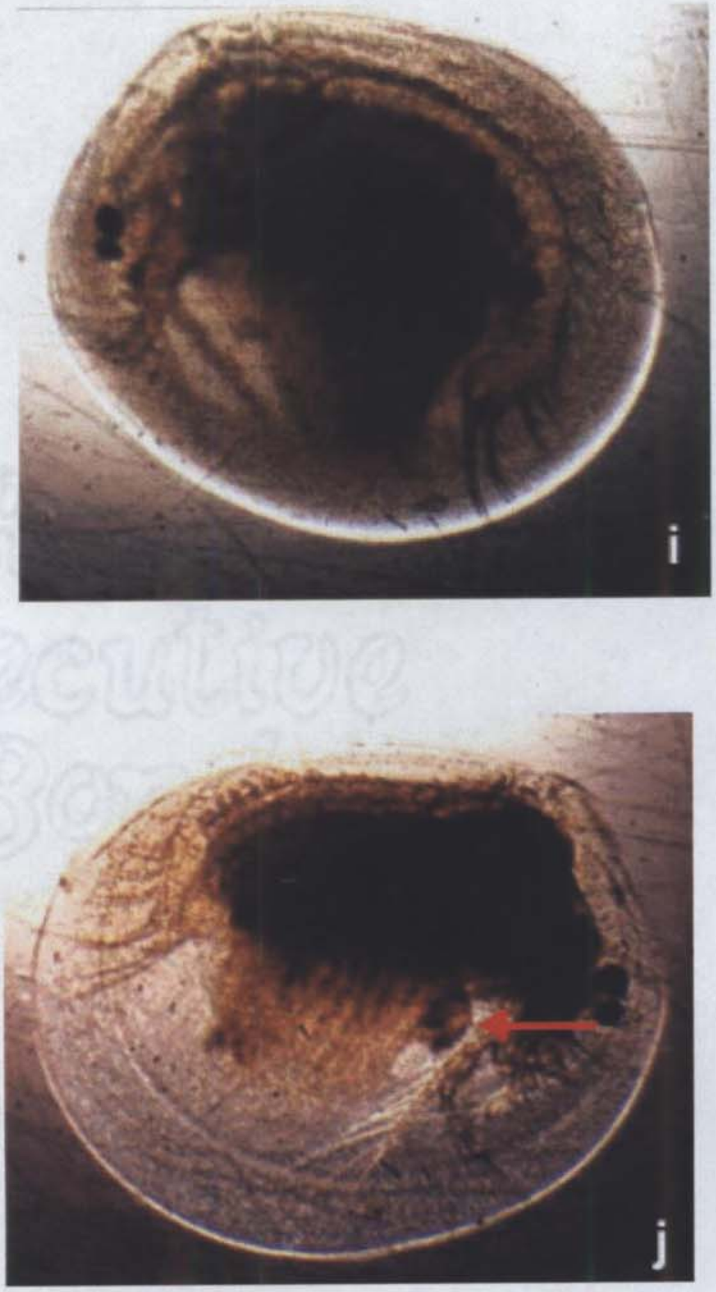

Fig. 3 6. *Cyclestheria hislopi*. i, entire view young ♀; j, entire view ♂; arrow indicates Clasper (Scale in Fig.3. 7).

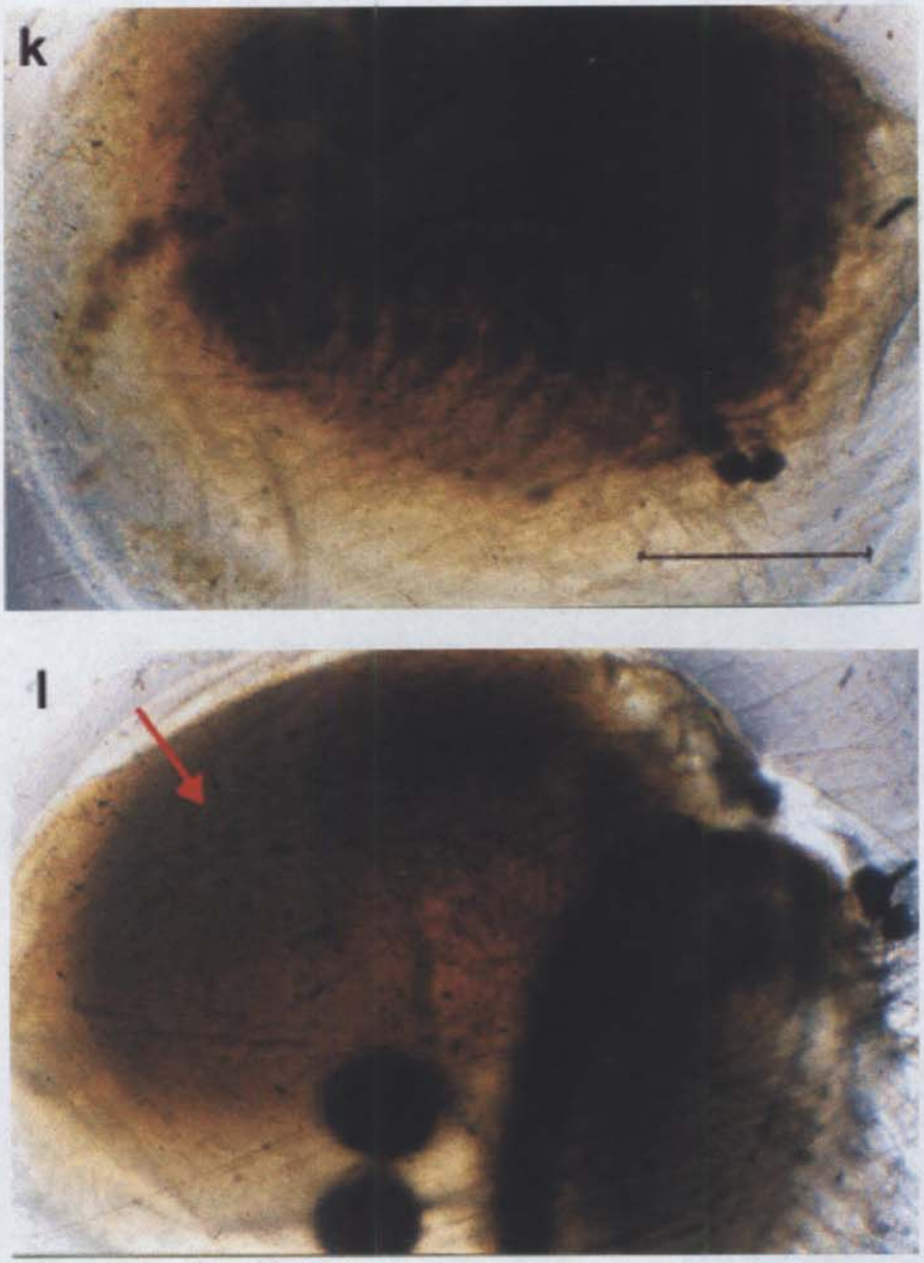

Fig. 3.7. Cyclestheria hislopi. k & l, sexual ♀. Arrow indicates opaqueness of carapace. Strongly pigmented nature of ♀and diapause eggs visible. Scale bar = 0.5 mm. This scale applies to Fig 3.4, 3.5, 3.6, & l.

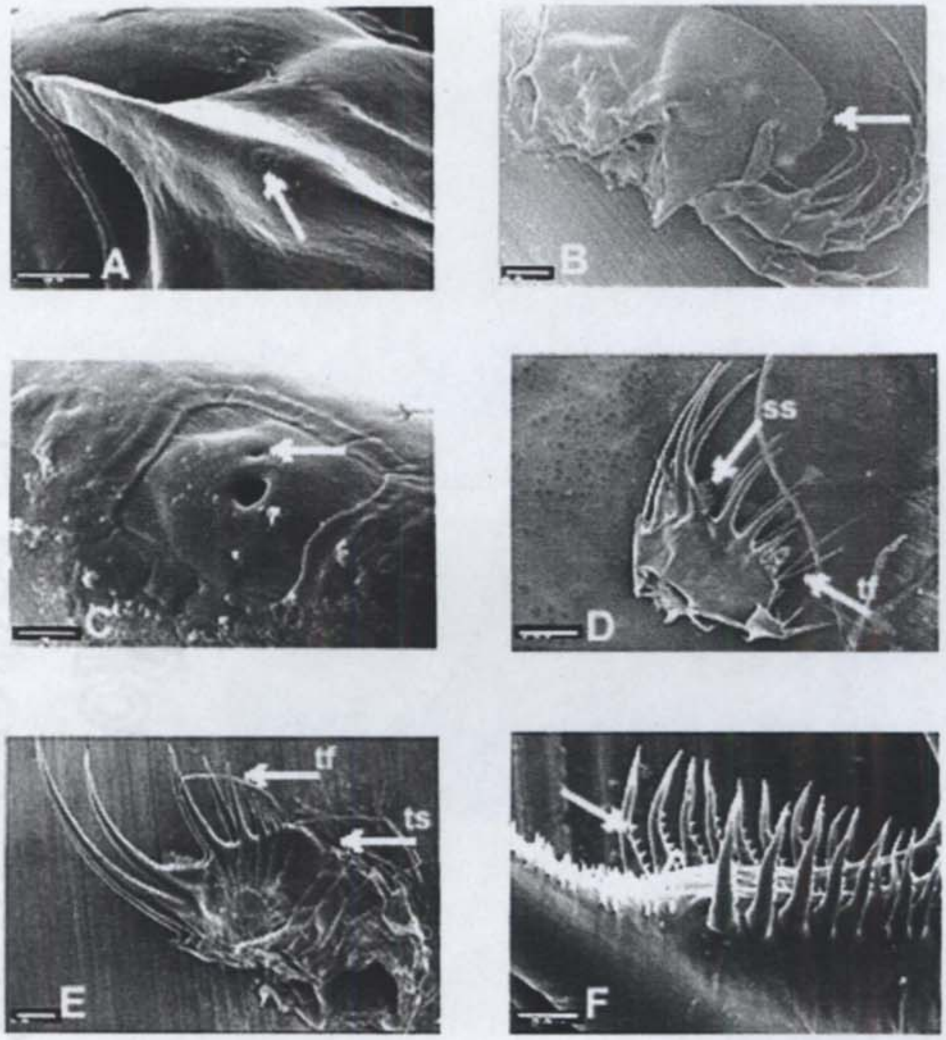

Fig. 3. 8. *Cyclestheria hislopi*. SEM of head and caudal region. A, dorsal view of head ♂ arrow indicates dorsal organ (scale bar = 50 µm); B, lateral view of head ♂ arrorw indicates serration (scale bar = 100µm); C, dorsal organ ♂(scale bar = 5 µm) arrow indicates small pore; D, lateral view of telson and furca ♂(scale bar = 200 µm); E, lateral view of telson and furca ♀ (scale bar = 100 µm); F, small spines on either side of telson (arrow indicates laterally directed tiny spines) (scale bar = 20 µm). Abbreviations: ss, small spines; tf, telsonal filament; ts, trunk setae.

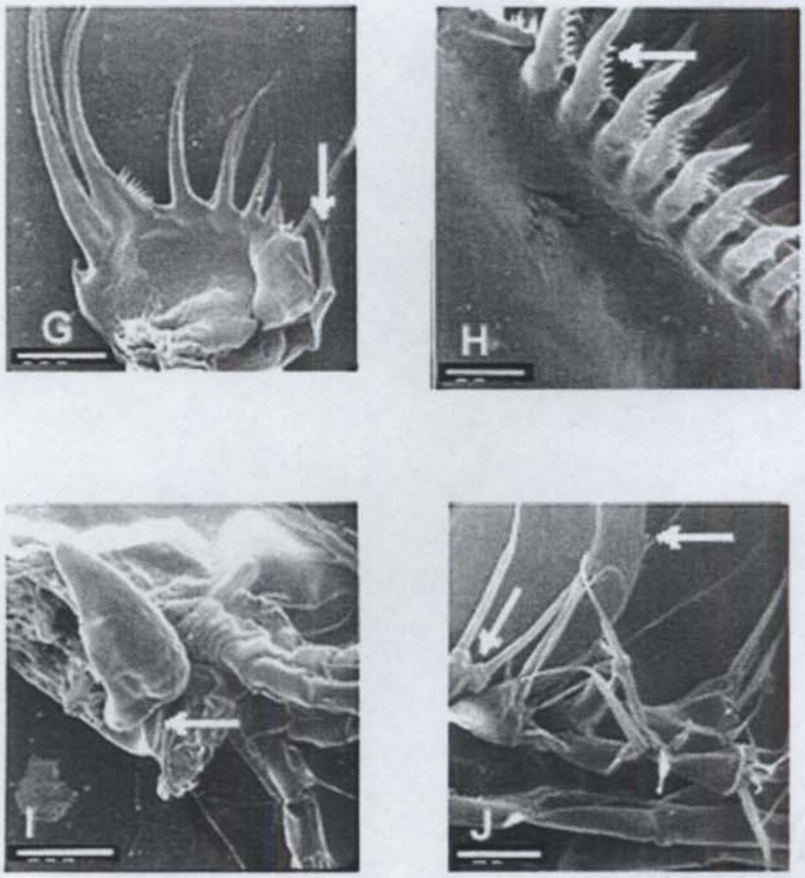

Fig. 3. 9. *Cyclestheria hislopi*. SEM. G, Lateral view of telson and furca (scale bar = 200µm) arrow indicates dorsal somite setae; H, small spines of telson (arrow indicates tiny spines) (scale bar = 20µm); I, mandibles and antennae ♂, arrow indicates triturating surface (scale bar = 200µm); J, part of and head antennae showing plumose setae and dorsal spines, horizontal arrow indicates serration of head, oblique arrow indicates base of stiff setae (scale bar = 50µm).

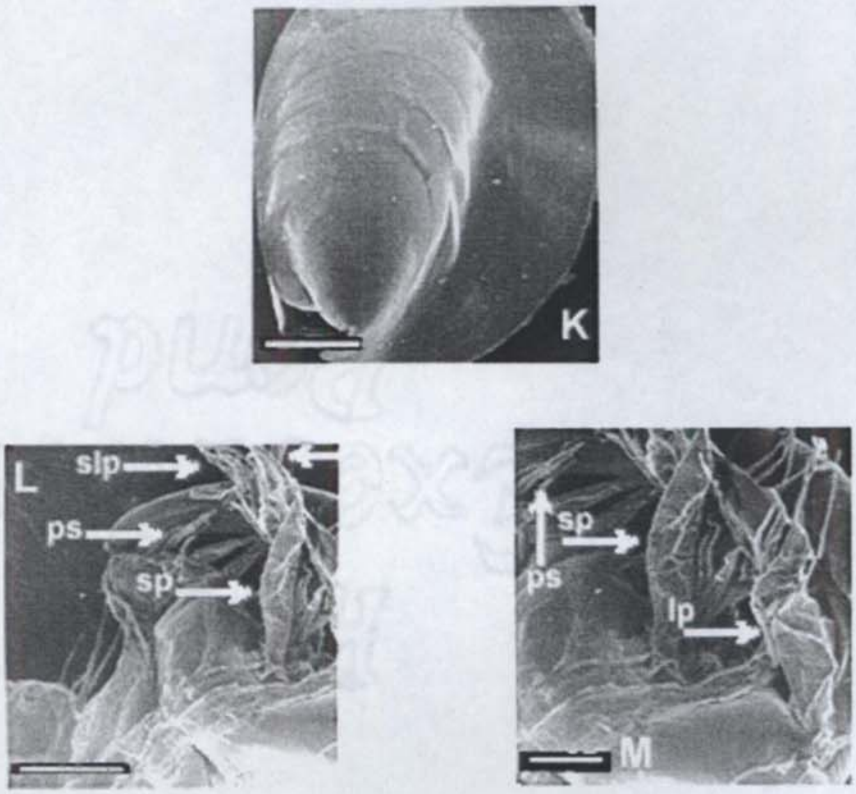

Fig. 3. 10 *Cyclestheria hislopi*. SEM of shell and clasper. K, shell ♀(scale bar = 500µm); L, posterior view of clasper. Unmarked arrow indicates setae on outer edge of movable finger. Note curve of movable finger, (scale bar =100µm); M, higher magnification of L. Note 'palm' at the base of photo, and curve of movable finger, (scale bar = 50µm). Abbreviations: lp,large palp; ps, setae of 'palm';slp, setae of large palp; sp, small palp.

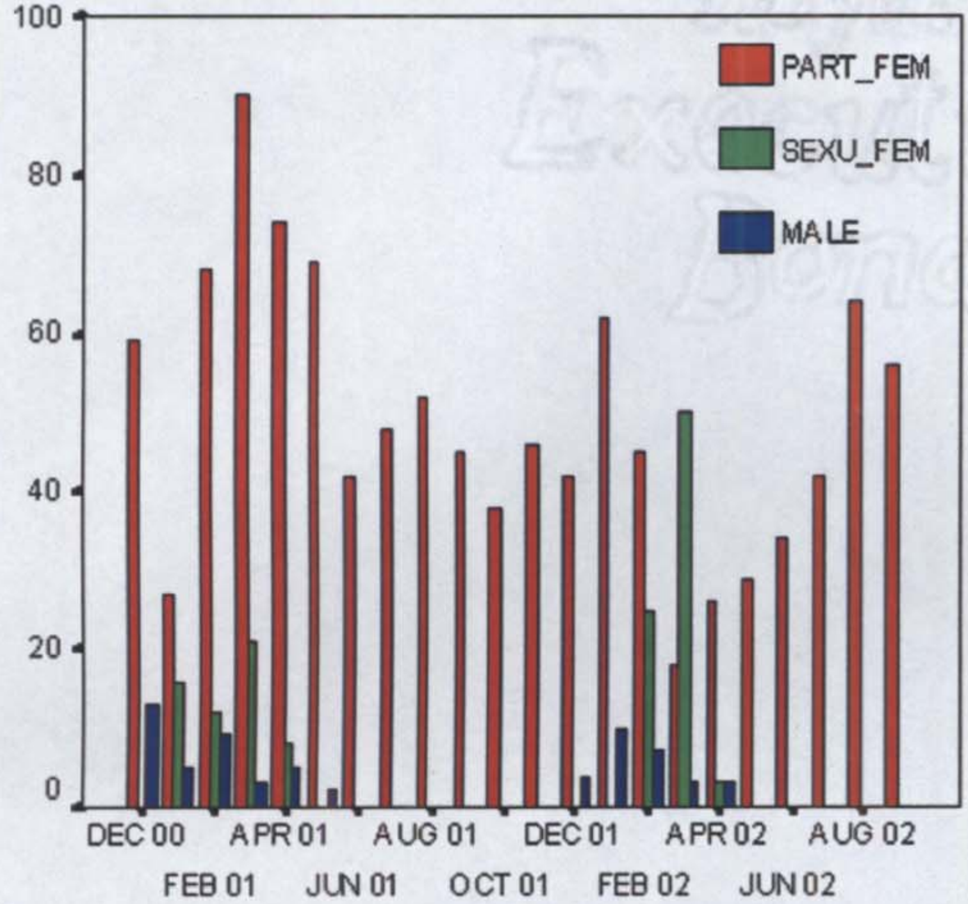

Fig. 3.11. *Cyclestheria hislopi*. Monthly variations in the number of the 3 types individuals for LC-1 population (December, 2000 to August, 2002). (Sexual females include ephippia too)

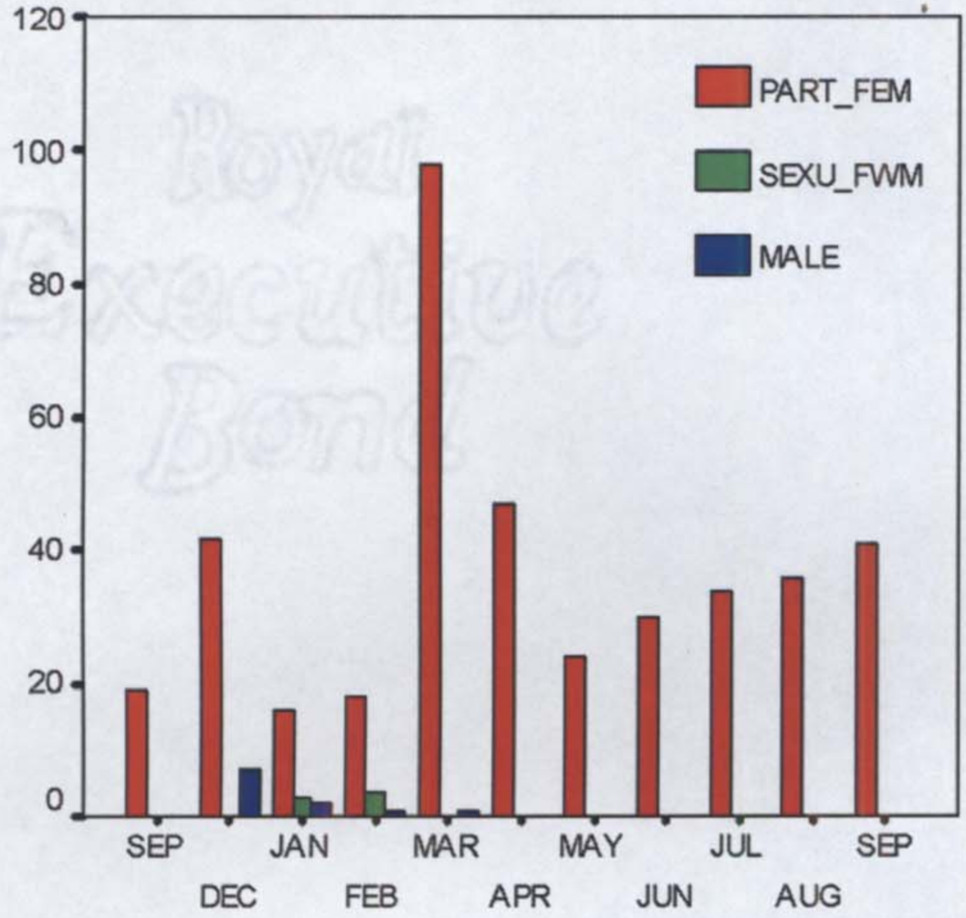

Fig. 3.12. *Cyclestheria hislopi*. Monthly variations in the number of the 3 types individuals for LC-2 population September, 2000 to September, 2001(Sexual females include ephippia too).

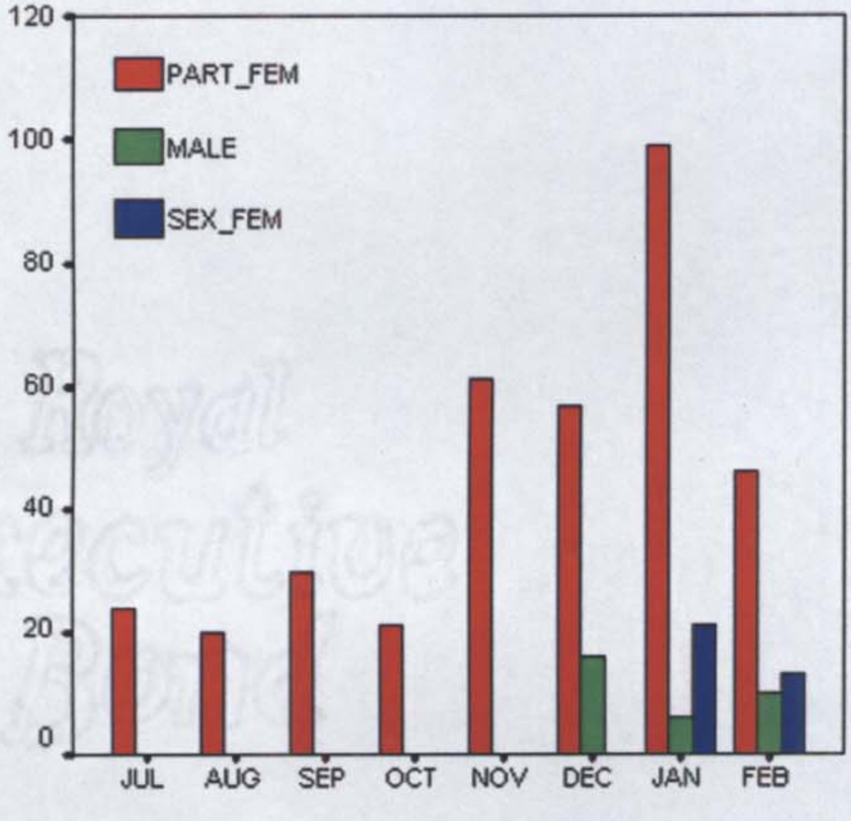

Fig. 3.13. *Cyclestheria hislopi*. Monthly variations in the number of the three types of individuals for ASCP-2 population (from July, 2004 to February,2005). Sexual females include ephippia too.

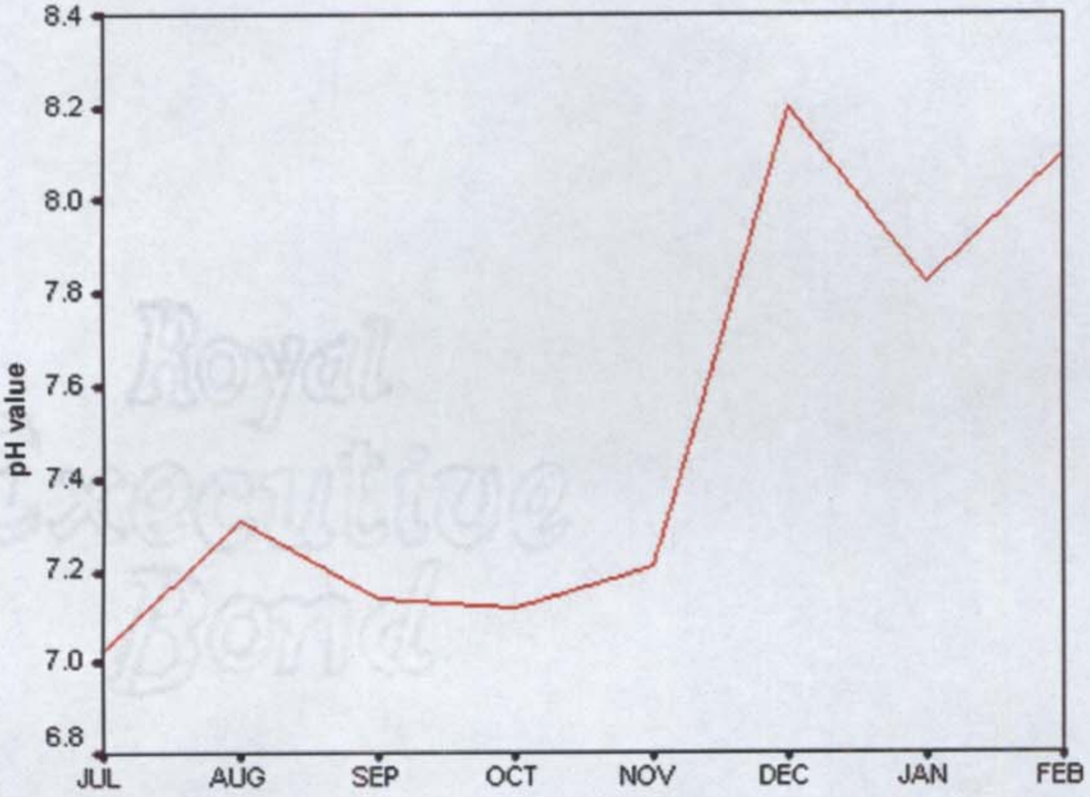

Fig. 3. 14. Monthly variations in pH (from July, 2004 to February, 2005 for ASCP-2).

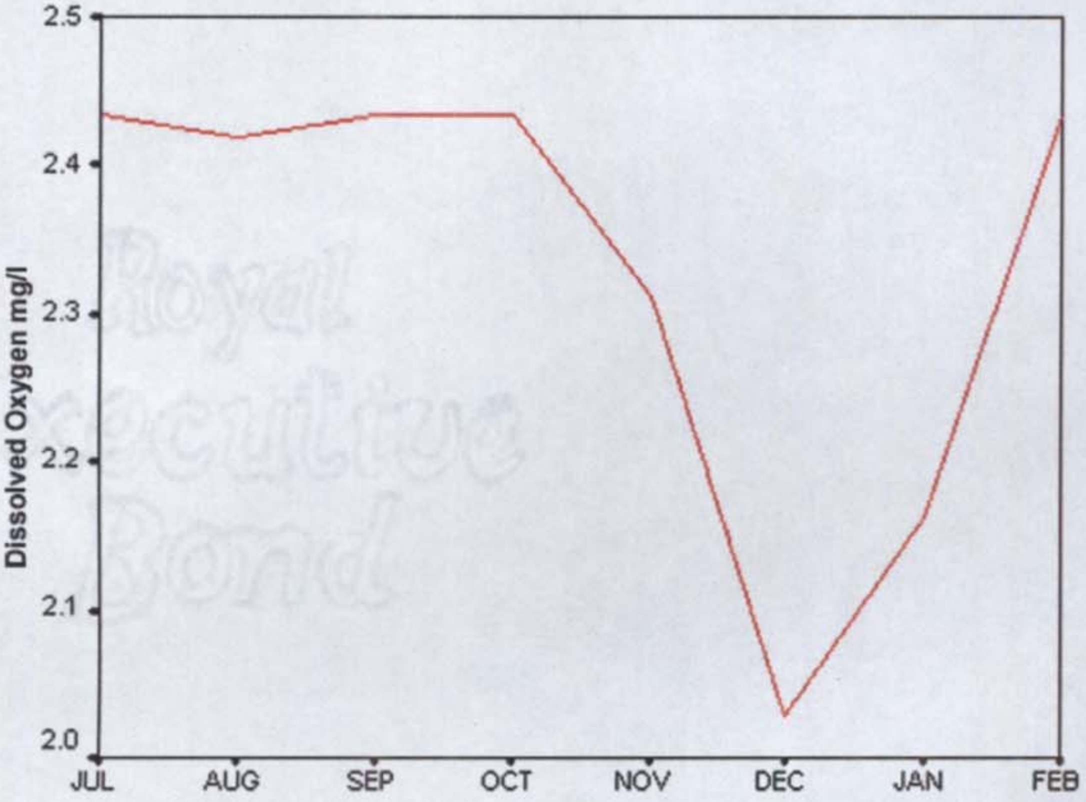

Fig. 3. 15. Monthly variations in dissolved oxygen (from July, 2004 to February, 2005).

B. Eulimnadia colombiensis

Introduction

Materials and Method

- *(a) Description of sites*
- *(b) Methods of collection*
- *(c) General rearing methods*
- *(d) Rearing methods for life history measures*
- *(e) Rearing methods for androdioecy analysis*
- *(f) Gut content analysis*

Diagnosis

- *(a) Systematic account of Species*
- *(b) Population estimation and Habitat specification*

Life history traits and androdioecy analysis

- **(a)** *Life history traits*
  - *Discussion*

- **(b)** *Androdioecy analysis – Progeny testing*
  - *Discussion*

Ecology and Observationof habits

Figures

B. Eulimnadia colombiensis

Class Branchiopoda Laterille, 1817

(Order Conchostraca Sars, 1867)

Order Spinicaudata Linder, 1945

Family Limnadiidae Burmeister, 1843

Genus Eulimnadia Packard, 1874

Species Eulimnadia colombiensis, Roessler, 1989

Introduction

The members of the Family Limnadiidae are unique in having a large pedunculate frontal organ located on the mid-dorsal surface of the head, the absence of fornix on either side of the rostrum, and the presence of a few to many concentric growth lines on the laterally compressed valves. The family is cosmopolitan in distribution; but it has not been reported from Antartica (Belk, 1982). The family contains 6 genera. The genus *Eulimnadia* comprising about 45 described species in the most diverse genus, not only of Limnadiidae but possibly of the entire Conchostraca (Straškraba, 1965; Belk, 1982). As the taxonomy of genus *Eulimnadia* is confusing, it is in need of revision (Belk, 1989; Martin, 1989). In *Eulimnadia*, egg morphology is considered species-specific and a more conservative taxonomic character than the traditionally employed characters like the carapace line number, and the telson spine number (Belk, 1982). The present status of the genera *Eulimnadia* Packard and *Limnadia* Brongniart is unclear. Belk (1989) and Pereira & Garcia (2001) consider that both genera should be kept separated.

The first record of the genus *Eulimnadia* from India was when Baird (1860) described *E. compressa* from Central, western and southern India. Seven more species have been recorded from India, with E. *indrocylindrova* being the latest addition from Andhra Pradesh (Durga Prasad and Simhachalam 2004). This is the only genus recorded from India so far.

A rare mating system in animals, viz., androdioecy has recently been described in E. texana (Sassaman & Weeks, 1993; Zucker et al., 1997). In this system, males coexist with hermaphrodites of two phenotypically similar but genetically different types – amphigenic and monogenic hermaphrodites. This androdioecious mating system is considered common within *Eulimnadia* (Sassaman, 1991; Weeks et al., 2001b).

Materials and Method

(a) Description of the Sites

The present study is based on three populations of *E. colombiensis* that inhabited temporary water bodies at three sites referred to as: (1) KKM-1 (Karikkakom), (2) ASCC - 2 (All Saints' College Campus), and (3) KRA-3 (Karuvatta).

KKM-1 and ASCC-2 are located about 3 kms. away from each other, and 9 km. from Thiruvananthapuram city. KKM- 1 (Fig. 3. 26E, F) is located on the western side of NH-47 (National Highway) bypass road, 3 km. north of Chackai Bypass Bridge. From the locality, conchostracan populations were collected from 2 temporary isolated water bodies, on the southern side of the access road from Veli to the NH-47 bypass. This area lies about 3 m. below the road level and is bordered by a stone wall of a house. These ephemeral pools are shallow with an average depth of 0.2 m. and having an area of about

7.5 m² with a sandy bottom. Vegetation consists mainly of grass species and wild herbs – like *Eupatorium, Cassia tora, Cassia oxidentalis* etc. that are also seen growing among the coconut palms. The wet phase of the temporary water bodies lasted only 7 to 9 days during the study period (September 2000 to September 2001). During the dry phase the area is used for grazing cattle.

ASCC-2 (Fig. 3. 24; Fig3.25c) is located within the All Saints' College campus, situated on the access road to Airport 2 km. from the NH-47 bypass. ASSC-2 consists of several small and large temporary water bodies that formed in the All Saints' College campus during the rainy season. Most of these ephemeral pools are in the form of ditches or distinct depressions (area: 3 to 6 m²), having a depth of not more than 0.3 m. at the deepest point. Temporary water bodies having conchostracan populations are also found in the coconut palm ditches that are dug around the base of the palm for manuring with dung and composts. The wet period of the temporary pools was not more than 6 days during the study period (October 2000 to October 2001). The soil of ASCC-2 is more sandy than that of KKM-1 site and vegetation is sparse with a few species of grass and with plants like *Drosera, Mimosa, Tephrosia, Evolvulus* etc. (typical of a semi arid region).. This site is relatively high and does not flood as often as KKM-1. In addition to conchostracans, the pools in these two sites were also having populations of cladocerans, insect larvae of Hydrophilidae and *Chironomus*, Naucoridae, Corixidae, Vellidae and amphibian tadpole larva (Balaraman 2001).

The 3rd population of *E. colombiensis* (KRA-3; Fig. 3. 25d; Fig. 3.27G, H) was collected from a site located on the western side of NH-47 at Nangyarkulangara, Alleppey district. It is an abandoned paddy field of about 8000 m² that gets dry up during summer, and gets inundated by seasonal rains to form a large temporary water body. It is a grassy and weedy pool rich in algae, insect larvae, aquatic insects, cladocerans, copepods, etc. The sample collection was made on 8th June at about 12 noon when the water temperature was 29°, pH 6.7 and salinity was nil. However, there were no conchostracans when the collections were made in the subsequent year (2002) from the same site. On enquiry it was noted that flocks of ducks were allowed to graze a few days prior to our visit.

(b) Methods of collection

Collections were done with a dip-net fitted with bolting silk provided with a handle. For shallow pools, buckets of pool water were hauled up and poured through the net. In the laboratory, conchostracans were sorted out and fixed in 4% formalin or 70% alcohol. For quantitative estimation of population, 10 full sweeps of the same net were made and emptied into a bucket of pool water. In the laboratory, organisms were sorted out, counted and sexed. Dissolved oxygen (mg/litre) was estimated using the Winkler's method; pH was noted with pH meter (digital model), and salinity (%) was calculated using the Argentometric method (APHA-98). Illustrations were made with a compound microscope equipped with camera lucida. Specimens were gold coated with a 10 nm beam sputter coating unit Jeol JFC 1200 and were observed in a Jeol JSM-5600 LV SEM.

(c) *General Rearing Methods*

Several aspects of the biology of the species *E. colombiensis* like survivorship curves, growth rate, egg production, molts, and a method of reproduction (androdioecy) have been studied. The data for these traits were measured on clam shrimps reared in the laboratory under optimal conditions. All the shrimps used in these studies were reared from field- collected soil from site KKM-1. This site was selected because the clam shrimps had been recorded from this site for a long period (June 2000 to June 2001), confirming presence of their eggs in the soil. The soil samples were collected from the top soil up to a depth of 1 to 5 cm, a week after the wet phase. Soil samples were kept in plastic bags in dark and used for hydration experiments, two weeks after collection.

Hydration Experiment. About 20 liters of aged tap water (tap water collected and kept for 24 hours, pH 7.3 to pH 7.6), or rain water was added to approximately 200 ml of soil sample in an aquarium and stirred well. Though distilled water facilitates the hatching process (Belk 1972) aged tap water was found to be ideal for hatching *Eulimnadia* cysts in laboratory studies (Sassaman & Weeks 1993; Knoll & Zucker 1995; Sassaman 1995; Weeks et al. 1997; Zucker et al. 2001; Weeks & Bernhardt 2004). The aquarium was placed in a well lit airy room. The bottom of the rearing aquarium was lined with 1 cm. of clean steam sterilised soil. Aged tap water was added intermittently to replace water loss by evaporation. Twenty four hours after wetting, 1 table spoon of dried and finely powdered cow dung was added to the water and stirred well. This was repeated the next day too. Bacteria and algae began to colonize the aquarium that formed the food for the clam shrimps, which were filter feeders (Royan 1976). Bakers' yeast solution and fish food usually used as feed, for rearing of *E. texana* (Sassaman & Weeks 1993; Knoll & Zucker 1995; Sassaman 1995; Weeks et al. 1997; Zucker et al. 2001; Weeks & Bernhardt 2004) was found unsuccessful in stabilising the present culture. In the laboratory, none of the larvae survived after the 2nd day when Bakers' yeast solution and fish food were used as feed. In the natural field, algae and bacteria become active soon after the pools fill with rain water and are abundant by the time the conchostracan eggs hatch. Nauplii subsist on yolk until the 3rd nauplius stage (Bishop 1969) and die thereafter of starvation, if suitable food is not provided.

Under these laboratory conditions eggs hatched within 24-36 hours of wetting. Hatching rather than hydration was counted as Day 1 and hydration as Day 0. The shrimps were found to mature on the 3rd day when they could be readily sexed.

Sexing of shrimps – Sexing was done by observing under the simple dissection microscope. Pronounced dimorphism allows easy identification of males and females. In the males, the first two pairs of thoracic appendages have been modified into clspers for grasping the females. Females have straight anterior thoracic limbs and the paired reproductive organs can be seen on either side of the digestive tract. When fertilization occurs, the eggs move into a brood chamber where they are contained in a mucous mass between the female's dorsum and the carapace. The eggs are visible to the naked eye and aid in sexing. Sassaman and Weeks (1993) have shown that E. *texana* females are actually functional hermaphrodites as is the case in *Limnadia lenticularis* (Zaffagnni 1969; Scanabissi & Mondini 2002)) and have categorized the two morphotypes as males (males rare in *Limnadia lenticularis)* and hermaphrodites. Histological evidence also suggests that *Eulimnadia* populations consist of mixture of males and self-compatible hermaphrodites\females (Sassaman & Weeks 1993; Zucker et al. 1997). The ovo-testis of the hermaphrodites\females in *E. texana* consists primarily of ovarian tissue and a small posterior portion of testicular tissue (Zucker et al. 1997)

(d) Rearing Methods for Life History Measures

For determination of individual life history measures - growth, molting, fecundity, and longevity - a pair of male and female was isolated separately on the 3rd day in a 1000 ml. glass bowl filled with water taken from the rearing tank. The water was filtered through a double plastic-meshed common sieve. Half a tea spoon dried and finely powdered cow dung was added only for the first day. Continuous addition of cow-dung powder caused excess growth of bacteria and algae. Moults were found intact at the bottom of the bowl and were removed daily and recorded.

Isolations were conducted for a total of 20 isolate pairs (male and female). Growth was measured by removing the individual shrimps on to a clean glass slide along with a drop of culture water, and size measured under the dissection microscope. The shrimps were quickly put back to the culture. For counting of eggs, females with eggs were transferred into a large glass cavity block along with its culture water. As soon as the eggs and the exuvium were released, the shrimps were returned to their culture bowl. If release of eggs and exuvium was delayed by more than 6 hrs., a pinch of feed was added to the cavity block. Using a clean fine glass dropper, eggs were transferred on to a clean glass slide and counted under the simple dissection microscope. Later, eggs were transferred to small vials and stored in aged tap water.

Daily growth increment was calculated for each shrimp by subtracting its size at Day X from its size at Day X+1. Overall growth rates were calculated for individuals by regressing size on the log of age. The slope of the regression was used as a measure of overall growth rate

For studying life span, groups of males and females were hatched from KKM-1 soil samples (45 females and 19 males) in glass aquaria and daily checked for number of shrimps surviving. Behavioural observations were also done daily for 1 hour between days 3 and 7. For the survivorship curves or longevity curves, age in days was plotted against percentage survival. Percentage survival was daily survival as a percent of their initial number.

(e) Rearing Methods for Androdioecy Analysis

Determining androdioecy is a straight forward process of raising and isolating female shrimps, collecting their eggs, rearing them to maturity and recording their sex ratio – this is progeny testing (Sassaman & Weeks 1993). What is done here is merely checking to see whether female *E. colombiensis* can produce viable offsprings in isolation, i.e., they are self-compatible females, some percentage of whom produce ~25% males among their offsprings, indicating that they are 'amphigenic' females.

Experimental shrimps reared from soil samples collected from Site KKM-1 and Site KRA-3 respectively, were hydrated in two separate rearing aquaria marked KKM and KRA. On the second day of hydration 1tb/sp of finely powdered cow dung was added and stirred well.

Isolation culture - Shrimps for isolation culture were taken from the above two rearing tanks. Female juveniles for androdioecy analysis and isolation pairs for studying life history measures were taken from the rearing aquaria are isolated on the 2nd day (before reaching sexual maturity) and isolated in 500 ml. glass bowls filled with water from rearing aquaria. For outcrossing, a male was included along with the female isolate. Aged tap water was also added intermittently to replace water lost by evaporation. Half a tea spoon of dried and finely powdered cow dung was added once. The shrimps, reared to maturity, were allowed to lay 5 to 6 batches of eggs. For collecting eggs, the brooded females were removed to large cavity block containing water from the bowls. After release of eggs the females were transferred back to the bowl.

Rearing of Laboratory clutches – These eggs from the above female isolates were then counted and allowed to dry (at room temperature in the cavity block) for two days. After drying, they were rehydrated in large plastic trays or basins with aged tap water/ rain water, and cow-dung was added as feed. The eggs hatched within 24-36 hrs. and were reared to maturity; when sexing of offsprings was done, and segregation data tabulated.

(f) Gut Content Analysis

The alimentary canals of 5 shrimps each (fixed in formalin) from 5 collections (KKM-1) were removed ,squash prepared and the items identified, enumerated and expressed as percentage composition. The phytoplankton present were grouped into Chlorophyceae, Bacillariophyceae, Euglenaceae, and Cyanophyceae. Broken legs and body parts of zooplankton were grouped as miscellaneous. Detritus was also counted.

Diagnosis

(a) Systematic account of species

Description of Male:

Carapace: Shell oval, bilaterally compressed, thin translucent and with granulations seen only close to the growth lines (Fig. 3. 16D; Fig. 3. 22a); size of the shell ranges from 2 to 6.5 mm. and bears 7 to 8 growth lines, with lines on the ventral margin more close to one another. Maximum width of the shell somewhat in the middle, greater than half its length. Umbo absent and the hinge-line of the shell is not serrate. Dorsal profile of the shell is slightly convex, of which the posterior half is less convex and straight than anterior half. Ventral profile more rounded than dorsal profile, dorso-posterior end produced into a minute acute angle, more pronounced in males (Fig. 3.16D). Shell in older specimen covered with abundant growth of epizoic algae (Fig. 3. 19B, C).

Head: Head small and bears on mid-dorsal side the typical frontal organ of genus *Eulimnadia*. Frontal organ pyriform in shape, placed on a slightly elevated convex ridge, and posterior to compound eye (Fig.3.16B; Fig. 3. 22a, b). Rostrum short, triangular, terminating in an acute point (Fig. 3. 16B). Antennulae, having 11 to 12 papillae bearing minute setae, indistinctly segmented extending to fifth or sixth segment of posterior antennal flagellum (Fig. 3. 16C). Peduncle of antennae indistinctly segmented with 8-9 segments on both flagella, each segment bearing 2 – 6 short dorsal spines with lesser number of spines towards distal end and long natatory plumose ventral setae (Fig. 3. 16A). Number of dorsal spines and setae vary between the two rami (Fig. 3. 16A).

Mandibles - Crescent shaped with narrow conical dorsal part and stout ventro-medial molar process (characteristic of Spinicaudata (Martin, 1989b) (Fig. 3. 19E, G, F). Outer lateral wall of body of mandible not smooth but with numerous shallow depressions (Fig. 3. 19F). Triturating surface has comb-like scales that fuse and become a smooth surface of connected pits (Fig. 3.19I). Unlike in other spinicaudata, pitted surface not slightly grooved but elevated (Fig. 3.19I).

Thoracopods: Truunk bears 17 pairs of thoracopods or swimming legs. All thoracopods similar except first and second modified as claspers for holding female during mating. Thoracopods diminish in size posteriorly, being somewhat the same size up to 10th, after which descendingly smaller – last three smaller, and last thoracopod the smallest. Each thoracopod consists of 6 endites, an elongated exopod and an elongated oval epipodite (Fig. 3. 17I). First lobe of the endites or coxal lobe a complicated structure oriented at a different angle from more distal endites (Fig. 3. 17J). Its setae are directed towards mid-ventral food groove. This endite responsible for mechanical movement of food particles anteriorly (Martin & Christiansan, 1985). Coxal lobe roughly triangular with sides of the triangle curved towards body and terminates in a blunt claw and a long curved spine. From distal anterior end arises a ridge bearing a row of closely packed, curved, comb-like setae. On hinder margin of gnathobase or coxal lobe are a row of stout straight setae widely separated and increase in length towards middle (Fig. 3. 17J). Arrangement of various setae on gnathobase helps in straining and food collection. The 2nd to 6th endites similarly armed with long filtatory setae that bear comb setules. These setae set close together and run parallel to each other; in addition, endites also bear stout and short setae. Proximal lobe of exopd narrow and small (Fig. 3.17I).

In first thoracopod or clasper of male, endites 2 and 3 almost equal in size and bear rows of plumose setae. Clasper consists of the palm, the opposing "movable finger" and two palps – one larger and other smaller. Endites 4, 5 & 6 modified into the clasper structures (Fig. 3. 16E; Fig. 3. 20M). Palm smooth without any setae and extends towards distal part. Palm shows indentation proximal to area bearing stout setae (Fig. 3. 16E; Fig. 3. 20J, L, M). Inner distal face of palm extend to an apical club (Fryer 1987) distally covered with stumpy flat tipped setae and long plumose setae at distal end (Fig. 3. 16E; Fig. 3.20K). Movable finger (endite 6) long and curved smoothly, with dorso-distal cup-like or sucker-like projection (Fig. 3. 20J, M, R). Movable finger bears a field of smaller round flat cup/sucker-like structures opposed to field of short setae on apical club (Fig. 3. 16E; Fig 3. 20R). These sucker-like structures appear like miniatures of sucker found on the dorso-distal end. Sucker-like dorso-distal projection characteristic of all Limnadiidae (Olesen et al. 1996). Both palps arise from posterior part of distal face of palm. Larger palp (endite 5) is 2-segmented, with each segment bearing terminal setae, and total length of palp exceeds length of movable finger (Fig 3. 16E, Fig. 3. 20L). Smaller 1-segmented palp arises from apical club and bears setae at tip (Fig. 3. 16E; Fig. 3.17F). Second thorocopod similar in general shape and spination but with 2-segmented palp much longer than first thoracopod (Fig. 3. 17F; Fig. 3.20-O). Combined length of segments nearly twice the length of movable finger (Fig. 3. 17F; Fig. 3.20-O). Third pair also shows a small modification with tactile process or endopodistal palp two segmented, with broader basal segment and longer and slender distal segment that bears setae at its tip (Fig.3. 18N).

Telson: Telson bears one unpaired spine and 14 to 15 paired unequal spines. Caudal furca provided with 10 to 12 plumose setae and small spines at distal end, with first spine larger and a little apart from rest in majority of species (Fig. 3. 18L; Fig. 3. 20N, Q). Telson filament delicate and arises at level of 3rd, 4th or 5th posterior spine. Ventral margin of telson ends in a blunt spine (Fig. 3. 18L; Fig. 3. 20N, Q).

Description of Female*:*

Carapace: Carapace larger than that in males (females with carapace size 7 mm. recorded), and with more growth lines 7- 12 (Fig. 3.17G). Head region similar to that of male, but rostrum is more rounded (Fig. 3. 17H; Fig. 3. 22a, b). Roundness of rostrum variable (Fig. 3. 28A, C, D, E, F & G). Rostrum bears internally ocellus varying in shape (Fig. 3. 28 A, C, E, F, G). Hepatopancreas extends from bases of antennulae to dorsal end of head (Fig. 3. 28G). Mandibles, antennules and antennae similar to those of males. The trunk bears 17 pairs of appendages. Appendage similar to that in male except 9th and 10th limbs that bear long dorsal filaments of exopod (Fig. 3.18 K). Attached to base of 10th or 11th thoracic appendage is a pair of small slightly curved club-like brown spermatophore. All females do not have these structures. Only females from bisexual population – KKM-1 and ASCC-2 – with brooded eggs or eggs in reproductive organs bear spermatophore (Fig 3.23). Telson of female similar to that of male (Fig. 3. 20P)

Eggs -- Mature females bear eggs in brood chamber between dorsum and carapace.

Measurement of eggs: Length (cylindrical part) : 174.04 ± 8.44 μm

Diameter : 173.00 ± 21.44 μm

Under the light microscope, eggs appear orange-brown, drum shaped, and cylindrical with egg diameter more toward ends of the cylinder than in middle. Each clutch of eggs consists (on each side) of 2 to 9 rows (depending on the size of the clutch – a clutch of 204 eggs had 7 rows with upper row having 13 eggs on one side) with the largest number of eggs in the upper row and the lesser number in the lower row. Pattern of arrangement for large batches of eggs as well as smaller ones same.

SEM analysis of eggs reveals the following features. Rims of the eggs are well defined, with smooth ridges and narrow valleys that extend parallel along the length of the cylinder (Fig. 3. 21W, Y, Z). Ends of the eggs are flat with parallel grooves and ridges similar to those on the body of the eggs (Fig. 3. 21 T, V). In some cases one end may be slightly doomed (Fig. 3. 21X). Arrangement of the ridges on the flat end more irregular, with straight and branched ribs, some parallel to one another or radiating from the centre. Egg surface appears to be textured with numerous minute pores (Fig. 3. 21a).

Discussion: The genus *Eulimnadia* comprising about 40 described species is the most diverse genus not only of the Limnadiidae but possibly of the entire order Conchostraca (Straškraba 1965; Belk 1987). Morphological characters like carapace features, number of body segments, morphology of antenna, armature of telson etc., used to distinguish between species of the genus *Eulimnadia,* are highly variable. This ambiguity, according to Sissom (1971), is mainly the result of taxonomic interpretations that have been based on a few specimens usually from a single population. Sissom, after measuring the extent of morphological variations statistically (by testing F-values) in *E. texana*, concludes that majority of the morphological characters are of little use in defining species. However, if a highly variable character is as useful in the description of a species as those that are less variable, and if it can also be shown that in two species there is no significant overlap in the mode of and range of that character, then that character may still be of value. For example, in *E. texana* the number of carapace lines range from 3 to 13. This range includes the range of four other species. However, it does not include *E. alineata* (which has no carapace lines) or *E. antillarum* (which has a maximum of 2 carapace lines); nor does it include *E. diversa* (which has a maximum of 3 in males and 4 in females). Belk (1989) and Martin (1989a) have attempted to clarify this ambiguity by proposing scanning electron microscopy of egg morphology as a diagnostic character. However, species such as *E. texana, E. geayi* and *E. ovisimilis* (Martin & Belk, 1989) share similar egg morphology and in these cases body morphology gives an additional different criterion. This need for caution was emphasised by Belk (1989) in anticipation of this sort of problem. Nevertheless, SEM of egg morphology is essentially needed for defining species but cannot be used exclusively to the exclusion of other morphological characters (Martin & Belk 1989). Based on both traditionally employed morphological characters and SEM of egg morphology the present species bears the nearest resemblance to *E. colombiensis*. This is the first report of this species from Kerala as well as India.

A comparative account of the morphological characters of the 8 species recorded earlier from India, in relation to the present species, is given in Table 3.1. The *E. colombiensis* species described here is different from the rest in the following characters:

(1) acute posterodorso angle of shell,

(2) sexual dimorphism of rostal morphology,

(3) 17 pairs swimming legs,

(4) telson filament arises at level of the 3 rd, 4 th or 5 th posterior spine,

(5) SEM of egg morphology,

(6) details of of clasper morphology, and

(7) spermatphore package.

When taking morphological characters into account the present species shows closer proximity to *E. gunturensis* (Radhakrishnan & Durga Prasad, 1976) with presence of acute angle in carapace structure and, rostral morphology. However, with regard to clasper morphology, the 2-segmented palp of the 5th endite is of equal length in the 1st clasper and in the 2nd clasper. The 2nd segment is twice the length of the first in both species. With regard to egg morphology, they are distinctly different, with *E. gunturensis* having spherical eggs and *E. colombiensis* having cylindrical eggs. Egg morphology shows two patterns for the Indian species: cylindrical eggs in *E. colombiensis* and *E. indrcylindrova*, and spherical eggs in *E. michaeli, E. gunturensis, E. ovata, E. similes, E. gibba, and E. compressa.* Though *E. indrocylindrova* and the present species *E. colombiensis* have cylindrical eggs, they differ with details of SEM of egg morphology. While in *E. colombeinsis* the rims of eggs are inflated, the ends flat and the shell surface porous, in *E. indrocylindrova*

Table 3. 1. Comparative account of the morphological characters of Indian species

Character	*E. compressa*	*E. gibba*	*E. similis*	*E. ovata*	*E. ovata inversa*	*E michaeli*	*E. gunturensis*	*E. indrocylindrova*	*E. colombiensis*
Shell size Length	5 -9 mm	6 mm	6-6.5 mm	7.5 mm	6.5 mm	6 mm	4.5/3 mm	♀ 7.09-8.14 mm ♂ 5-6 mm	**2-7 mm**
Growth lines	5-12	8/6-9	3-4	4-6	4-5	5	4-9	♀ 7-14 ♂ 6-8	**♀7-12** **♂ 7-8**
Shape ofshell	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	**Oval**
Postero-Dorsal angle of shell	Present	No angle	--	No angle	--	Present	Present ♂ angle with protuberance	Present	**Acute angle** **♂ more acute**
Rostrum	Pointed	Not pointed	--	--	--	Rounded anteriorly		♀ Blunt ♂ Long pointed	**♀Rounded** **♂ Pointed**
First antenna sensory lobes	5-8	7	4-5	8	8	6	♀ 5-8 ♂ 8-10	♀ 8-9 ♂ 10-12	**11-12**
Second antenna segments	6-10	5-9	7-8	8-9	8-9	8-9	7-9	9-10	**9-9**
Thoracopods (pairs)	18-20	18-22	18-20	18	18	18	16-17	♀ 19-20 ♂ 19	**17**
Spines of telson	9-14	8-9	8-11	14-15	14 or 15	17-18	13-15	♀ 19-20 ♂16-18	**14-15**
Position of forked filament (between)	1 & 2 or 2 & 3	2 & 3	2 & 3	2 &3	3 & 4	6 & 7	♀ 4 & 5 or 5 & 6 ♂ 4 & 5	♀ 4 &5 or 5& 6 ♂ 5 &6	**3 & 4 or 4 & 5**
Setae on cercopod	8-10	7	9-11	16	16	15	♀ 8-13 ♂ 10-14	♀18-20 ♂19-20	**10-12**
♂ 1st clasper	--	--	--	--	--	Large palp with unequal segments. Total length exceeds movable finger.	Large palps 2 equal segments. Total length of palp exceeds movable finger.	Large palp with unequal segments. Total length exceeds movable finger.	**Large palp with equal segments. Total length exceeds movable finger.**
2nd clasper	--	--	--	--	--	Total length of large palp exceeds twice the length of moval finger.	Total length of palp segments exceeds length of movable finger, but not twice its length	Total length of palp exceeds twice length of movable finger.	**Total length of palp exceeds twice the length of movable finger.**

Egg morphology	Round	Round	Round	Round	Not Known	Round	Round, narrow ridges, polygonal and hexagonal surface	Cylindrical	**Cylindrical**
SEM	--	--	--	--	--	Surface with deep fissure like pockets with prominent raised ridges (Samyiah et al. 1985)		Rims not flared, one end doom shaped, other end flat, shell surface not porous.	**Ends flared and flat, surface porous**

the rims are not flared nor are the ends flat and egg surface porous. The two species also differ with regard to clasper morphology. In *E. indrocylindrova* the 2-segmented palp of the 5th endite in both claspers are of unequal length, while in *E. colombiensis* only the palp of the 2nd clasper is of unequal length while in the 1st clasper they are of equal length. Rostral morphology also shows two patterns for the Indian species: round and pointed. The females of *E. gibba, E. ovata, E. michaeli, E. gunturensis, E. indrocylindrova* and *E. colombiensis* have rounded rostrums, while the males of *E. gunturensis, E. indrocylindrova* and *E. colombiensis* have pointed rostrums. However, in *E. colombeinsis* the rostrum of the males is less pointed when compared with the other two species. A morphological trait common to all the Indian species is the conspicuous first spinula (longer than rest of the spinules) of the caudal furca.

E. colombiensis described here differs only in one aspect from *E. colombiensis* first described by Roessler (1989b) from Colombia, and later described from Venezuela by Pereira and Garcia (2001). In *E. colombiensis* collected from the above two regions, the first spinule is minute whereas in the Indian *E. colombiensis*, the first spinule is large.

But a few with small spinule have also been obtained in our collection. In addition, the present species is larger (with larger females reaching a size of 7mm. and smaller, 2 mm.), whereas the Venezuelan species has a size of 3.1 to 3.7 mm. in females and also with less growth lines. Since the above two characters show high intraspecific variability (Sissom, 1971; Roessler, 1995b) they do not have the taxonomic importance formerly attributed to them. These two characters are more dependent on age and environmental conditions (Pennak, 1953; Belk, 1989; Roessler, 1995b; Pereira & Garcia, 2001). Rostral morphology, clasper morphology and egg morphology (SEM) are identical in all the three *E. colombiensis* described – Colombian, Venezuelan, and Indian. Clasper details like length and serrations of palps are similar in all the three. These details like shape and spinatian of claspers are distinguishing characters, more specific and ideal for species recognition in *Eulimnadia,* according to Pereira and Garcia (2001). With respect to SEM of egg morphology in all the three, the eggs are cylindrical with developed, inflated and flared rims and rounded ridges defining valleys, extending parallel along length of cylinder. *E. belki* (Martin, 1989a) from Mexico also has an egg shell morphology similar to that of *E. colombiensis* described here. The clasper morphology is also similar in the two. In *E. belki* (Martin, 1989a) the area dorsal to the compound eye of head has a large 'empty' space, and male rostrum drawn out into a long narrow projection. These characters are not seen in *E. colombiensis.*

The other non Indian species that bear cylindrical eggs are *E. cylindrova, E. texana, E. ovisimilis, and E. geayi. E. cylindrova* is the only species which has oblique ridges on its cylinder (Belk 1989; Brendonck et al. 1990; Pereira & Garcia 2001). *E. texana* and *E. ovisimilis* have stout cylindrical eggs with acute ridges and with rims that are not marked

(Martin & Belk, 1989a). In *E. geayi* one end of the cylindrical egg is slightly wider than the other. The wider end has raised ridges and the opposite end flat ridges (Pereira & Garcia, 2001). The male rostrum of *E. texana* is similar to that of the present species, clasper morphology also agrees well, but with egg morphology they can be distinguished as two species. Though there is a slight uncertainty due to the variability shown in a few morphological characters, there is little doubt that the species described here is *E. colombiensis*.

(b) Population estimation and Habitat specification

Population estimation of the various macro fauna of the temporary water body formed at KKM-1 was started on the third day of pool formation and continued for the 9 day wet phase of the pool. This study shows that *E. colombiensis* was the earliest to colonize the pool and the major community of the temporary pool (Fig. 3. 29). *E. colombiensis* appears on the third day along with amphibian tadpoles and insect larvae as seen from Fig. 3. 29. During the period of study, salinity of water was found to be nil, pH ranged from 7.4 to 7.6, temperature varied from 26° to 30°, and dissolved oxygen from 5.60 to 5.82 mg/l. The pool was formed on 30th September and existed till 8th October 2000. During this period, Thiruvananthapuram district recorded a mean rain fall of 138 mm., and relative humidity varied between 74 and 96. This heavy rain fall led to the inundation of the water body under study. Gravid females of *E. colombiensis* were present in the collections made on the 3rd day and were present in all the subsequent collections till the last day. Among the macroinvertebrates collected, *E. colombiensis* belonged to Wiggins' Group I (Wiggins et al. 1980) or over-wintering residents or resident specialists of temporary water bodies (Balaraman & Deepa, 2002). Group I includes inhabitants that aestivate by producing resting eggs or cysts and are the first to hatch when pool formation takes place. Wiggins et al. (1980) divided temporary pool communities into four groups based on the survival strategies adopted by these communities to escape dry period and seasonal pattern of recruitment. Insect larvae of the genus *Hydrophilus* and *Nasiaeshna* collected from the pool belonged to Wiggins Group II or over-wintering recruits that are capable of dispersal as adult insects but oviposit as the pools begin to fill. Amphibian tadpoles (Family Ranidae) collected from the pool belong to Wiggins Group IV – the non-overwintering migrants, with well developed powers of dispersal. They metamorphose as the pool begins to dry and pass the dry phase in permanent water, returning to oviposit in the temporary water body during the following rainy season.

A similar community pattern for macroinvertebrates is also seen in the temporary pools of ASCC-2 with *E. colombiensis* prevailing throughout the wet period of the temporary pools

Discussion: The first animals to appear as the pools get flooded are mostly detritivors, feeding on the rich food resource provided by plant detritus. Wiggins et al. (1980) have shown that protein levels in decaying plant material were higher in temporary water bodies (vernal pools) than in permanent pools. Dense vegetation invading the dry basin increases the amount and diversity of plant detritus whose decay by fungi is enhanced by aerobic conditions during the wet phase. When flooding takes place rich food resources are therefore available for detritivores. This regular cycle maintains the ecosystem in an early successional stage in which efficient colonizers will have an advantage (Wiggins et al., 1980). The optimal strategies for colonization are rapid development and early maturation; that is, short generation time and high fecundity (Wiggins et al., 1980; Seaman et al., 1995; King et al., 1996; Weeks et al., 1997). *E. colombiensis,* an efficient colonizer and a typical (dominant) inhabitant of temporary water bodies, exhibits these traits as revealed in the present study (in subsequent Sections).

Life history traits and androdioecy analysis

(a) Life History Traits - Results

When the shrimps are juveniles, daily handling of them increases mortality; hence, growth was not measured until the third day of hatching. Accordingly, early growth is not recorded but can be assumed to be quite high on account of the size attained on the third day (Fig. 3. 30). Average daily growth increment was high in early life. The most remarkable decrease in daily growth occurred during the initiation of reproduction (Days 5-7) after which growth was slow and irregular, with no further growth recorded after Day 16 (Fig. 3. 30).

Egg production started on either Day 3 or 4. Egg production began low and then steadily increased until Days 7, and remained more or less the same until Day 11; thereafter a rapid decline was noted. Maximum egg production was between Day 6 and Day 11 (Fig. 3. 30). The decline in fecundity after Day 11 could have been due to the more fecund hermaphrodites dying at a higher rate, leaving behind only the less fecund individuals. However, females showed the same pattern of decline in fecundity at later ages as is seen in (Fig. 3. 30) Hence it can be deduced that females underwent reproductive senescence after one week of life. During the last two or three days of its life the female did not release eggs. Majority of the females died in this state. The range of egg production per day was from 0 to 538 (the latter produced by the largest ♀ size 7 mm.) with an average of 110 ± 35 per day. The range of total egg production was 380 to 2859 per female with an average of 1412 ± 923. Daily growth increment and fecundity records in *E.texana* (Weeks et al. 1997) also follow a similar pattern. In *E. texana* high fecundity was maintained for 10 days whereas in *E. colombiensis* it was recorded for a shorter period.

Egg production was found to be linearly related to size ($r = 0.639$; $p < .01$) in the size range examined (2.8 mm. to 7 mm. carapace length; Fig.3. 32).

Survivorship, high during the early period, began to decrease steadily after Day 9 in females and after Day 16 in males (Fig.1). Mortality during the nauplius stage was quite high as in the case of other crustaceans, and handling of shrimps during this stage could have caused even higher mortality. Hence counting of shrimps in the survivorship experiment was done only from Day 3, when the shrimps begin to mature. As is evident from the data, survivorship was high near the age of maturity, and declined a few days after maturity. Average life span for females was 20.38±8.45 days and for males 23.79±7.79 days, with earliest death being noted from Day 9 for females and Day 10 for males; none of the shrimps surviving after Day 34. The longevity of males was found to be the same as that of the females (the last of the males and females dying on the same day – Day 34). However, in the case of *E. texana* male longevity was reported to be two days less than that of females (Weeks et al., 1997; Zucker et al., 2001; Weeks & Bernhardt, 2004). Meanwhile, in the same shrimp, male longevity was relatively higher when females were excess in number to males (Zucker et al. 2001). Mating behaviour has been reported to reduce female growth and survival rate in a related conchostracan *Limnadia orinoquiensis* (Roessler, 1995b). A somewhat equal life span for males and females, or a higher one for males, is advantageous because males that outlive other males would have less competition and more chance to mate older females that are larger and have more eggs. Hence natural selection should tend to increase the average male life span (Zucker et al., 2001).

On an average the shrimps moulted only once in 24 hrs. with no molts during the last 2 -3 days of its life. Though moulting is necessary for growth, in these shrimps molting seems to bring about egg shedding and to occur even after the shrimps have ceased to increase in size. It means that moulting is more related to egg production than to increase in size. This relationship is evident from Table 3. 2, and is also reported to be so in the case of E. *texana* (Knoll 1995; Weeks et al. 1997) also.

Discussion

The life history measures reported herein are those for shrimps grown in laboratory under available optimal conditions. Light and temperature were not controlled and the experimental shrimps could have been subjected to day and night fluctuations that simulate the habitat conditions. Feeding also was chosen to mimic the natural field conditions, where cow dung was natural manure for these habitats. In the natural conditions, when summer sets in, grass and weeds grow and are grazed by cattle, the droppings of which form the manure for the temporary water bodies during the rainy season. Hence dried cow dung was chosen as the ideal manure and feed for culturing the shrimps in the laboratory.

Growth increment pattern indicates that it is high before maturity (Day 4) but when daily egg production is maximal, growth increment declines rapidly, then becomes irregular and reaches zero (Fig. 3.30). No correlation is obtained between growth rate and either daily egg production or total egg production (Table 3. 2). This Table indicates that egg production is independent of growth rate; but it was found to be positively related to size (r = 0.639; p< 0.01). In *E. texana*, Weeks et al. (1997) reported a negative correlation between growth rate and egg production. However, in *E. colombiensis,* growth rate indicates a negative trend (though not significant) in its relationship with age at maturity - those with lower growth rate tend to reach maturity at a higher age. In *E. texana,* growth rate was found uncorrelated with age at maturity. Nevertheless, both daily and life-time egg productions are positively correlated with longevity in both these species, indicating no trade-off between reproduction and life span. The number of moults per day is not significantly correlated with growth rate; yet, it shows a positive trend in its relation with average daily-egg-production, indicating that molting may be related more to egg production than to increase in size (Table 3. 2). This is in agreement with the finding of Weeks et al. (1997) who reported a high correlation between egg

Table 3. 2 Correlation coefficients for the 6 life history measures in *E. colombiensis*

	1	2	3	4	5	6
1. Overall growth rate		-.049	-.024	.020	-.335	.058
2. Average daily egg Production			.911**	.231	.146	.402
3. Total egg production				-.051	-.056	.733*
4. Average daily moults					-.048	-.451
5. Age at maturity						-.298
6. Longevity						

**= P< 0.01; *=P<0.05

production and moulting. Also, moulting shows a positive trend in its relationship with longevity, implying that increased longevity is more associated with relatively less daily molts than with longevity. This suggests that moulting is more related to egg production (which is also low or nil during the last few days of long living shrimps). Age at maturity is uncorrelated with any of the other life history traits except longevity (Table 3. 2). An apparent trade-off at Day 5-6 between growth and reproduction observed in the case of *E. colombiensis* was also discernible in *E. texana* (Weeks et al., 1997). Again, as recorded in the case of *E. texana,* no apparent trade-off exists between any other life history traits and longevity in *E. colombiensis.* The only significant correlation is with total egg production, indicating that longer lived shrimps had higher egg production. Though not significant the pattern of relationship among the different life history measures in *E. colombiensis* reveals a close correspondence with that in *E. texana.*

The above findings, based on correlation coefficients, are only suggestive as the number of cases used for the analysis is only 8. (Though an attempt was made to rear 20 female isolates initially, difficulties in handling shrimps for counting of eggs, measuring size etc. enhanced their mortality resulting in complete data of all the 6 measures available for 8 shrimps only). Weeks et al. (1997) have observed the same trends, though significantly, in their study, with a relatively large number of shrimps - the reason being the more sophisticated techniques available to them, like image analysis system for measuring growth, counting of eggs etc. without actually handling the shrimps.

Fecundity patterns were typical of 'early colonists' species, having a short time to maturity and, producing a large number of eggs per individual (Wiggins et al. 1980; Seaman et al., 1995; King et al., 1996; Weeks et al., 1997). Egg production begins on

Day 3 and reaches maximum on Day 7, 8 or 9, after which it begins to decline. *E. colombiensis* is iteroparous; so, once egg production has begun, it continued till death of the organism, usually as a result of desiccation. This means also that high fecundity or reproductive efficiency extends for 4 to 7 days after which senescence sets in leading to a drop in the reproductive efficiency. These data are consistent with the idea that strong natural selection for rapid growth and early reproduction leads to early senescence and shorter life span. Early maturity and iteroparity are survival strategies of animals specialized to live in temporary water bodies that have a short wet period of maximum three weeks. Based on the available inundation data, during the period of this study (2000 to 2003), the wet phase of the temporary water bodies was an average 8 days for KKM-1 site and 20 days for the KRA-3 site.

The life history strategies for successful survival in an ephemeral habitat are well exemplified in *E. colombiensis*. The shrimps hatch within 24-36 hrs. of hydration, begin reproduction by the 3rd to the 4th day producing dozens to hundreds of eggs per day, thereafter till death, natural death being rare. Death occurs when the inundation period of the temporary water bodies are over. The successful life history strategies that help the shrimps to survive in the unpredictable habitat are the short time taken for maturity and iteroparity which firstly allow the organism to reproduce while the aquatic habitat is available and secondly to keep putting eggs into the 'egg bank' as long as the habitat allows. However, these life history strategies have their drawbacks too, primarily in terms of early senescence and reduced longevity (Wiggins et al., 1980; King et al., 1996).

(b)Androdioecy analysis – Progeny testing results

Androdioecy *E. colombiensis* like many of its congeners is characterized by a female biased sex ratio; i.e., clutch sex ratios are not 1 female: 1 male. The female sex ratio in the natural population of KRA-3 was 13:0 (N=13), that of the KKM-1 was 7:1 (N=186), and of ASCC-2 was 6:1 (N = 82) (Table 3. 3). In *E. texana* this bias is a consequence of the combination of two features in the reproductive biology of these clam shrimps, an autosomal recessive genetic factor that determines male phenotype and a dominant factor that determines for hermaphrodites/females with facultative hermaphroditism (Sassaman & Weeks, 1993) Facultative hermaphroditism means that the females are capable of out-crossing in the presence of, and selfing in the absence of males. While, analyzing the sex ratio of the progeny of female isolates, Sassaman & Weeks (1993) have concluded that *E. texana* populations are actually mixtures of males and females/hermaphrodites – a condition called androdioecy. The sex ratio of the offsprings of the female isolates or selfed-females helps to distinguish between the two types of phenotypically indistinguishable but genotypically distinct females. If no males are produced and all the offsprings are females, then the female isolate is a monogenic. On the other hand, if there are ~ 25% male offspring the female isolate is an amphigenic (Sassaman & Weeks, 1993). The presence of amphigenic gives the species an androdioecious status. The outcrossing offsprings of monogenics are all amphigenics, and the outcrossed offsprings of amphigenics are females and males in a 1:1 ratio (Sassaman & Weeks, 1993).

Table 3. 3 Sex ratio data of *E. colombiensis* from field collection

Site: KKM-1		
Date: August 2001		
Sample	Male	Female
15	6	9
9	2	7
Date: October 2001		
39	2	37
40	4	36
29	6	23
40	5	35
9	1	8
5	1	4
Site: ASCC-2		
Date: July 2001		
28	5	23
23	3	20
31	4	27
Site: KRA-3		
Date: June 2001		
13	0	13

Progeny testing of offsprings from female isolates belonging to two populations from the two sites (KRA-3 & KKM-1) was done in this study for *E. colombiensis* (Table 3. 4). A total of 44 isolation hatching experiments were done for female isolates from the KKM rearing tanks. Five isolation hatching experiments and 5 cross isolation hatching experiments were done for females from the KRA rearing tanks. The segregation data of number and sex of offsprings of the 44 female isolates and 5 isolations and 5 crosses for KRA are given in Table 3. 4.

Table3. 4 Progeny testing for Androdioecy Analysis

Site	Mud hydrated	Males	Females	Total	% Male
KRA-3		0	28	28	0.0
KKM-1		4	23	27	14.8

A. Progeny testing: KRA-3

				Observed
Isolations	Males	Females	Total	% Male
1	0	45	45	0
2	0	23	23	0
3	0	14	14	0
4	0	17	17	0
5	0	26	26	0

				Observed
Crosses	Males	Females	Total	% Male
1	0 hatching			
2	0 hatching			
3	0 hatching			
4	0	3	3	0
5	0	1	1	0

B. Progeny testing: KKM-1

		Observed				*Expected*			
Isola	Eggs hydrated	Males	Females	Total	% Male	Males	Females	Chi-suare	P-value
1	190	0	4	4	0	1	3	1.3	
2	250	0	42	42	0	11	32	14	
3	103	4	15	19	21.1	5	14	0.2	
4		3	33	36	8.3	9	27	5.3	*
5		3	15	18	16.7	5	14	0.7	
6		2	8	10	20	3	8	0.1	
7	150	1	6	7	14.3	2	5	0.4	
8	40	6	15	21	28.6	5	16	0.1	
9	24	0	2	2	0	1	2	0.7	
10	40	1	2	3	33.3	1	2	0.1	
11	42	1	10	11	9.1	3	8	1.5	
12	50	0	5	5	0	1	4	1.7	
Total		21	157	178	11.79	47	135		
Total Amphi		21	104	125					
				% Amphigenic	**66.67**				
13	250	0	2	2	0	1	2	0.7	
14	67	0	5	5	0	1	4	1.7	
15	75	0	2	2	0	1	2	0.7	

16	38	0	12	12	0	3	9	4	
17	98	0	11	11	0	3	8	3.7	
18	30	1	1	2	50	1	2	0.7	
19	20	1	1	2	50	1	2	0.7	
20	120	3	4	7	42.9	2	5	1.2	
21	185	8	21	29	27.6	7	22	0.1	
22	100	1	7	8	12.5	2	6	0.7	
23	130	4	19	23	17.4	6	17	0.7	
24	450	0	25	25	0	6	19	8.3	
25	247	3	15	18	16.7	5	14	0.7	
26	255	1	53	54	1.9	14	41	15.4	***
27	310	36	120	156	23.1	39	117	0.3	
28	244	0	10	10	0	3	8	3.3	
29	350	0	40	40	0	10	30	13.3	
30	320	9	19	28	32.1	7	21	0.8	
31	570	8	44	52	15.4	13	39	2.6	
32	360	15	54	69	21.7	17	52	0.4	
33	402	0	37	37	0	9	28	12.3	
34	350	11	29	40	27.5	10	30	0.1	
35	360	0	32	32	0	8	24	10.7	
36	280	0	41	41	0	10	31	13.7	
37	180	4	11	15	26.7	4	11	0	
38	160	6	21	27	22.2	7	20	0.1	
39	450	0	126	126	0	32	95	42	
40	138	8	23	31	25.8	8	23	0	
41	75	1	5	6	16.7	2	5	0.2	
42	148	4	23	27	14.8	7	20	1.5	
43	115	2	3	5	40	1	4	0.6	
44	150	3	11	14	21.4	4	11	0.1	
	Total	150	984	1134	13.2				
Total Amph		150	588	738	20.3	185	554	8.6	**
% Amphigenic 63.64									

A. Results of Progeny testing of Females from Site KRA-3

The pedigree chart for the KRA-3 population begins with - ten females that hatched from the KRA aquarium, of which 5 were isolated and progeny tested by rearing their offsprings to maturity (Table 3. 4A). A total of 129 females from the female isolates were obtained. An all female progeny in this analysis is indicative of monogenic nature of females from Site KRA-3. The rest 5 females were outcrossed with males. Of the five, 3 produced eggs, but failed to hatch and the rest 2 produced a total of 4 females. Even without the outcrosses the KRA females can be confirmed as monogenic from the first step isolation hatches (Sassaman & Weeks, 1993).

B. Results of Progeny testing of females from Site KKM-1

Two sets of the progeny testing of female isolates from Site KKM-1 was carried out – the first to check for androdioecy and the other to confirm it (Table 3. 4B).

In the first set of 12 KKM females isolated and progeny tested, there were 2 types of females based on their progeny sex ratio - 8 amphigenics and 4 monogenics; i.e., ~ 67% amphigenic which is right in the ball park of the estimates of amphigenics in *E. texana* (Sassaman & Weeks, 1993). The 4 monogenic females produced an all female clutch of 53 females, confirming its monogenic status. The amphigenic females produced a mixed clutch of 125 offsprings, of which 104 were females and 21 males. Out of the 8 amphigenic females, only 1 deviated from the 3:1 offspring sex ratio at a probabilty criterion of $p< 0.05$. The sex ratio of offsprings from the 8 amphigenic parents is ~ 30.1% males, which is slightly different from the expected 25%, but may not be biologically different. The same sort of discrepancy was noted in the *E. texana* model proposed by Sassaman & Weeks (1993). Looking at the statistics per hydration, 1 of the 8 (No. 4, in Table 3. 4B.) amphigenics has significantly fewer males than expected from the *E. texana* model; three others (No. 5, 7 & 11) have fewer males than expected, and the rest 4 (No. 3, 6, 8 & 10) have almost as many males as expected from the model of Sassaman and Weeks (1993).

The second set of 32 KKM females were isolated and progeny tested (Table 3. 4B). Of the 32 females, 20 were amphigenic and 12 monogenic (i.e., 63% amphigenic). The 12 monogenic females produced an aggregate of 343 females, and the 20 amphigenic females produced a mixed clutch of 484 females and 129 males. Looking at the statistics per hydration, one (No. 26 in Table 3. 4B) of the amphigenics deviated at a probability criterion of $p<0.001$, 3 of them (No. 20, 31 & 42) had slightly different number of males and females from the *E. texana* model, and the remaining 16 had more or less the same ratio predicted by the model.

Discussion

Female isolates from KKM produced both female and male-female among their offsprings. The KKM females thus consisted of two categories: those that produced some proportion of males among their offsprings, and those that produced only female offsprings.

A total of 1134 offsprings were reared from 44 KM female (2 sets of 12 and 32) isolates belonging to the polymorphic KKM-1 population. Twenty eight of the KKM isolates produced a total of 150 male and 641 female offsprings (Table 3. 4B), and 16 of the isolates produced 416 females and no males. Thus, considering the offspring sex ratio pattern of females from the KKM-1 population, 28 were amphigenic, and 16 monogenic. This puts the total at ~64% amphigenic (which is similar to the estimates of amphigenics in *E. trxana*). The total sex ratio of offsprings from amphigenic parents is 20.3% males which is slightly different from the expected frequency of 25% (Sassaman & Weeks 1993) with a probability of $p<0.01$ (Table 3. 4B). A lower than expected male proportion is due to the lower survival rate of males under laboratory rearing conditions(Sassaman & Weeks 1993 ; Zucker et al. 2001).

The data presented in (Table 3. 4) clearly predicts a strong case of androdioecy in *E. colombiensis*. This is the first report of androdioecy in this species.

Having a mating system that provides both selfing and outcrossing is of advantage to a colonizing species like clam shrimp *E. colombiensis*. The ability to self fertilize may be extremely important for the successful colonization of new habitats with a single cyst. Also the ability of amphigenics to produce males gives added advantage to a colonizing species by permitting dispersed zygote to be progenitor of a sexual population which may increase the persistence of genetic variability in newly established populations (Sassaman & Weeks, 1993). Androdioecy has also been reported in 10 other species of *Eulimnadia* (Weeks et al., 2004), indicating the prevalence of this rare mating system in the family Limnadiidae, thus making it the largest group of androdioecious animals known. Hence it can be considered a common, and perhaps a characteristic feature of this genus. With complete selfing there would be rapid elimination of the **s** allele for maleness (in Sassaman and Weeks' (1993) model of sex determination in *Eulimnadia*, males are genotypically homozygous recessive for the alleles – **ss**, amphigenic females are heterozygous – **Ss,** and monogenics are homozygous – **SS,** respectively) which would eliminate both males and amphigenics from the population. Hence in most natural populations both types of females are maintained (as indicated by the female biased sex ratios in *E. colombiensis* – Table 1) suggesting the prevalence of a mixed mating system of both outcrossing and selfing. This mixed mating system is an effective reproductive strategy for surviving in an unpredictable habitat that these shrimps colonize.

The data reported here formed also part of a collaborative work of the present author with Weeks et al. (2004) for progeny testing of 10 species in the genus *Eulimnadia* to see whether they have an androdioecious mating system as found in *E. texana.* Androdioecy thus appears to be the predominating mating system among *Eulimnadia* and this genus also has a global distribution, being reported from every continent except Antarctica (Weeks et al., 2004). This wide spread distribution is not due to exceptional dispersal capabilities of these crustaceans, but is rather due to their ancient origin - estimated at ~280 million years ago for the family Limnadiidae, thus placing them on the super continent Pangaea before its breakup (Weeks et al., 2004). An attempt has been made to investigate the evolutionary history of the androdioecious genus by examining the current species distribution and map this distribution on to continental movement (manuscript under preparation). This is to explain that the clam shrimps were continuous at the time they were fossilized. The current species of *Eulimnadia* were collected from India, Venezuela, Galapogos, Mexico, Japan, Taiwan, Australia and the U.S.A. They belong to four continents and 8 countries. The last time period when these localities were contiguous was when the ancestor to the modern *Eulumnadia* was androdioecious and was distributed across Pangaea. This form of reproduction has been stable in these shrimps for millions of generations. If androdioecy is the ancestral reproductive mode in *Eulimnadia*, this places the minimum of age of androdioecy in these shrimps at the origin of the genus. Fossil evidence for genus *Eulimnadia* is at least 24-26 million years old if not older. This long term stability is not predicted in any of the current models of the evolution of this mating system. Earlier (Sassaman, 1995) had argued that androdioecy was likely to be an intermediate stage between obligatory sexual reproduction and unisexuality in Eulimndiidae. But, then, the notion that androdioecy is only a short lived transitory phase between hermaphrodite and dioecy (or vice versa) is not accepted by Weeks et al. (2004) who argue that only a truly comprehensive model of the evolution and maintenance of androdioecy can explain androdioecy in the ancient *Eulimnadia* crustacean.

Ecology and observation of habits

Epizootic algae and ecto commensol protozoa are found growing on the shell of many fresh water crustaceans (Pennak, 1953; Thiery & Cazubon, 1992). However, epibionts on conchostracan are less known except for the report by Thiery and Cazaubon (1992) on the fresh water branchiopods in temporary ponds in Morocco. They have distinguished 3 types of ciliates belonging to the subclass Peritrichia and 2 types of green algae belonging to the genus *Stigeoclonium* Kutzing, 1843 and Oedogonium Link, 1820 growing on the spinicaudatan *Leptestheria mayeti* Simon 1885 and *Cyzicus bucheti* Daday 1914.

Observations of the clam shrimp *E. colombiensis* collected from a number of temporary water bodies from KKM-1 site and ASSC-2 site showed a frequent colonization of the carapace by green algae and protozoa. Filamentous green algae Oedogoniales , genus *Oedogonium* were seen attached to the valve mainly to the hinge by their straight filaments and small sized blue-green algal cells belonging to the genus *Clastidium* Kirchner (cells terminating in hair-like extension of the sheath (Fig.3.19B, C) and Bacillariophyceae (diatoms or unicellular algae) belonging to the *Synedra* Ehr (valves without costae, neither bent nor arcuate) (Fig.3.19B, C)) were found crowded on the carapace of *E. colombiensis*. These algae have been identified from those temporary water bodies from where *E. colombiensis* was collected (Balaraman et al., 2001). Two types of ciliated protozoans belonging to the subclass Peritrichia, the solitary stalked Vorticella and the colonial *Zoothamnium* were identified as attached to the carapace and sometimes inside, on the anterior thoracic appendages of *E. colombiensis.* The epizootic algae and protozoans were benefited by this association as they have an easy access to the nutritive and oxygenated water current created by the beating of the appendages of the shrimps. Moreover, by colonizing the carapace, mainly the hinge, epizootic algae were exposed to light, as these shrimps were found in the shallow parts of the pond and frequently swam to the surface. Alga and protozoan colonization was linked to the mode of life of the host and exposition to light as revealed by the study of epizootic algae and protozoa on fresh water branchiopods, by Thiery and Cazaubon (1992).

In addition to epizootic algae and protozoa that live as commensols on *E. colombiensis*, the rotifer belonging to the genus Philodina was also found in close association with the clam shrimps. When the eggs of *E. colombiensis* were counted, invariably these rotifers would be seen moving among the legs. Though rotifers belonging to six genera have been identified from these temporary water bodies (unpublished STEC report, 2004) this particular species was most frequently seen moving over the eggs of *E. colombiensis.*

Pond size and Longevity. Longevity of females (cultured in laboratory) that belong to the KRA-3 population was a maximum of 30 days and that of the KKM-1 population was a maximum of 34 days. As given in the site description, the temporary pond in KRA-3 site is larger and of longer duration whereas the temporary pools in KKA-1 site are smaller and of shorter duration. Weeks and Marcus (1997) studied the relationship of pond size and duration to several life history traits including longevity in *E. texana* to test whether shrimps inhabiting temporary ponds that dry quickly had a shorter expected life span than ones inhabiting slower drying ponds. Longevity of females in *E. colombiensis* from the two different sites was somewhat same despite the size difference of the pools from which the samples were collected. Though no statistical analysis could be attempted, the available data seem to be in agreement with those of Weeks and Marcus (1997) who found no correlation between pond size and longevity of females in *E. texana*.

(a) Feeding habits.

Study of the gut contents of the present species reveals that they are detritus and algal feeders with Detritus forming major part of the gut 38.2% followed by Chlorophyceae 27.7%, Bacillariophyceae 24.6%, Euglenaceae 4.6%, Cyanophyceae 2.4% and others 2.5%. (Fig 3. 33). The Bacillariophyceae identified were *Pinnularia, Navicula* and *Anabaena*; Euglenaceae were *Phacus* and *Lepocynclis*; Cyanophyceae were *Volvox, Chlamydomonas, Pediastrum, Closterium* and *Cosmarium.* Miscellaneous included broken pieces of spines, carapace and rotifers.

The fact that detritus is the major food consumed by the clam shrimps is evidence for the observation on the feeding behaviour that major part of the time is spent feeding at the bottom of the culture bowls, with their appendages in rapid and constant movement. Swimming is also a food gathering activity that brings about filtering of food particles from the water column (Pennak, 1953). They have also been observed to swim frequently towards the surface, attach with their antenna to the surface film of water and hang down or attach to the sides of the culture bowl. In these positions their appendages are seen to constantly beat setting up water current that brings in detritus algae etc. found in the overlying water. In the laboratory, these animals were also seen attaching and dragging floating pieces of detritus to the bottom. When not swimming they remain buried in the mud at the bottom. *E. colombiensis* were also seen swimming frequently with the dorsal side down and their appendages up and beating constantly. Zooplankters like cladoceran and copepods and ostracods although absent in the gut were present in the above temporary water bodies. This could be because these zooplankters are larger and mobile, and hence cannot be easily wafted into the gut by the feeding currents of this organism. Similar feeding pattern has also been recorded for *Leptestheriella maduraiensis* by Royan (1976). One of the main advantages of feeding in temporary water bodies is the rich food resource in the form of detritus available in the environment (Wggins et al., 1990; King et al. 1996). As mentioned earlier, the pulse-stability of the ecosystem i.e, the annual dry phase followed by rapid aerobic decomposition during the wet phase releases a pulse of plant nutrients on flooding and a bloom of new vegetation. *E. colombiensis*, the detritivorous and non-selective algal feeders, are the earliest species to hatch on flooding of the temporary water bodies that efficiently utilize the rich resource of this habitat and thus show a typical trait of an efficient temporary pool colonizer.

(b) Behaviour of shrimps

Behavioural observation of *E. colombiensis* shows that it performs mainly four types of activities: (1) Swimming and grazing or feeding behaviours – setting up a water current by the constant beating of the appendages, remaining attached by the antenna to the underside of the water film or sides of aquarium, or lying at the bottom or just below the water surface, beating its appendages. Females spend more time for grazing and resting than do males; males spent more time for swimming. (2) Resting, lying on their side at the bottom of the aquarium. (3) Burying behaviour – when not swimming, grazing or mating males and females bury themselves in the mud at the bottom of the container. The females retain their eggs in the brood chamber for up to 24 hrs. before burying them in the soil. Gravid females bury themselves in the mud and release their eggs. (4) Mating behaviour (as described below):

Mating or outcrossing Behaviour - Males of *E. colombiensis* spend most of their time swimming, and they swim at a faster rate than females. *E. texana* males use this tactic to increase their potential encounter rate with slower moving females (Medland et al. 2000). In *E. colombiensis*, mating begins with males clasping females. They clasp not only females but gravid females (unreceptive females), males and juveniles as well that they encounter. However, the unreceptive ones are released by the male as they struggle to disengage him.This signifies that the males are unable to discriminate between the presence and absence of females and only haphazardly encounter females.Thus males encounter females mainly by random encounter, facilitated by increased swimming speed. This also means that males do not require any special sensory mechanism to locate females because the latter occur in higher frequencies. In *E. texana* (Medland et al., 2000), it appears that it is the female rather than males which alter their behavior in the presence of males as well as other females. The females increase encounter rates with more rapid random movements, in the presence of other shrimps. These tactics suggest that the females have evolved encounter behaviour that increases the likelihood of outcrossing (Medland et al., 2000). The above behavioural tactics employed by females were observed in *E. colombiensis* as well.

Mating behaviour is observed on the 3rd day after hatching. The male, after trial and error, attaches to the two edges of the carapace of non-gravid females, with the first two pairs of thoracic appendages, that are modified to form claspers for holding the female (Fig3.16E; Fig.3.17 F) This is pre-copulatory mating behaviour, seen in many conchostracans (Knoll, 1995). If the female struggles when clasped, the male releases his hold. But at other times the female does not struggle when clasped by the male, but instead passively allows the male to push it around or remain at the bottom. The female's legs would continue to beat suggesting active feeding, but the antenna would remain motionless, suggesting that the male is actively pushing and causing movement. After about 2-5 minutes of pushing about the female, the male begins jerky thrusting movements. The male coils its posterior end and thrusts it into the carapace of the female near the ovaries. Sperm transfer is assumed to be taking place during the thrusting movements. After performing thrusting movements twice or thrice the male releases its hold on the female and outcrossing is over. A previous study by Strenth (1977) in *E. texana* has reported that males transfer a spermatophore like package to the 11th thoracic segment of the female during outcrossing. Belk (1987) has also observed spermatophores as defined by Strenth (1977) in 6 of the 24 mature females of *E. astrva* examined by him. In a related shrimp *Leptestheria dahalacensis* (Scanabissi & Mondini, 2000) a sticky mass containing sperms, the spermatophore is seen attached to the female's epipodite during mating. In *E. colombiensis,* described here, several of the females were seen to have spermatophore-like package attached to the 10th or 11th thoracic appendage of the female (Fig. 3. 22b; Fig. 3. 23c). The spermatophore is attached by a stalk to the female's appendage and is covered by a very thin wall across which the spherical spermatozoa can be seen (Fig. 3. 23d). The males release sperms directly into the bivalved carapace rather than injecting sperm directly into the gonophore (Zucker et al. 2001). In *Eulimnadia*, egg fertilization is external rather than internal, and fertilization occurs immediately after egg release or in the brood chamber (Strenth, 1977; Knoll, 1995; Zucker et al., 2001; Weeks et al., 2002b).

Outcrossing was never observed between a male and struggling female, which was also observed in the case of *E. texana* (Knoll, 1995). Observations of Knoll (1995) suggest that females can control the mode of fertilization – either selfing or outcrossing. Her observations, based on laboratory population, showed that when males were present selfing was relatively rare compared to outcrossing. Prior to selfing, the *E. texana* females would lie motionless on the substrate for several hours (Knoll 1995). In *E. colombiensis*, outcrossing is more frequent, and females lying on the substrate are mostly gravid females. Very few non-gravid females are seen lying motionless on the substrate for long periods.

Aggressive interactions are also observed between a clasping male and another male that attempts to clasp its female. The clasping male is seen coiling its telson and pushing off the other male, forcefully with it. Sometimes another male clasps the carapace of the clasping male and tags along. All these observations are based on both laboratory populations of *E. colombiensis.* Aggressive interactions and tagging behaviour have also been observed in laboratory populations of *E. texana* (Knoll, 1995). However, the pre-outcrossing clasp time during mating was longer for *E. texana* when compared to *E. colombiensis.*

Figures

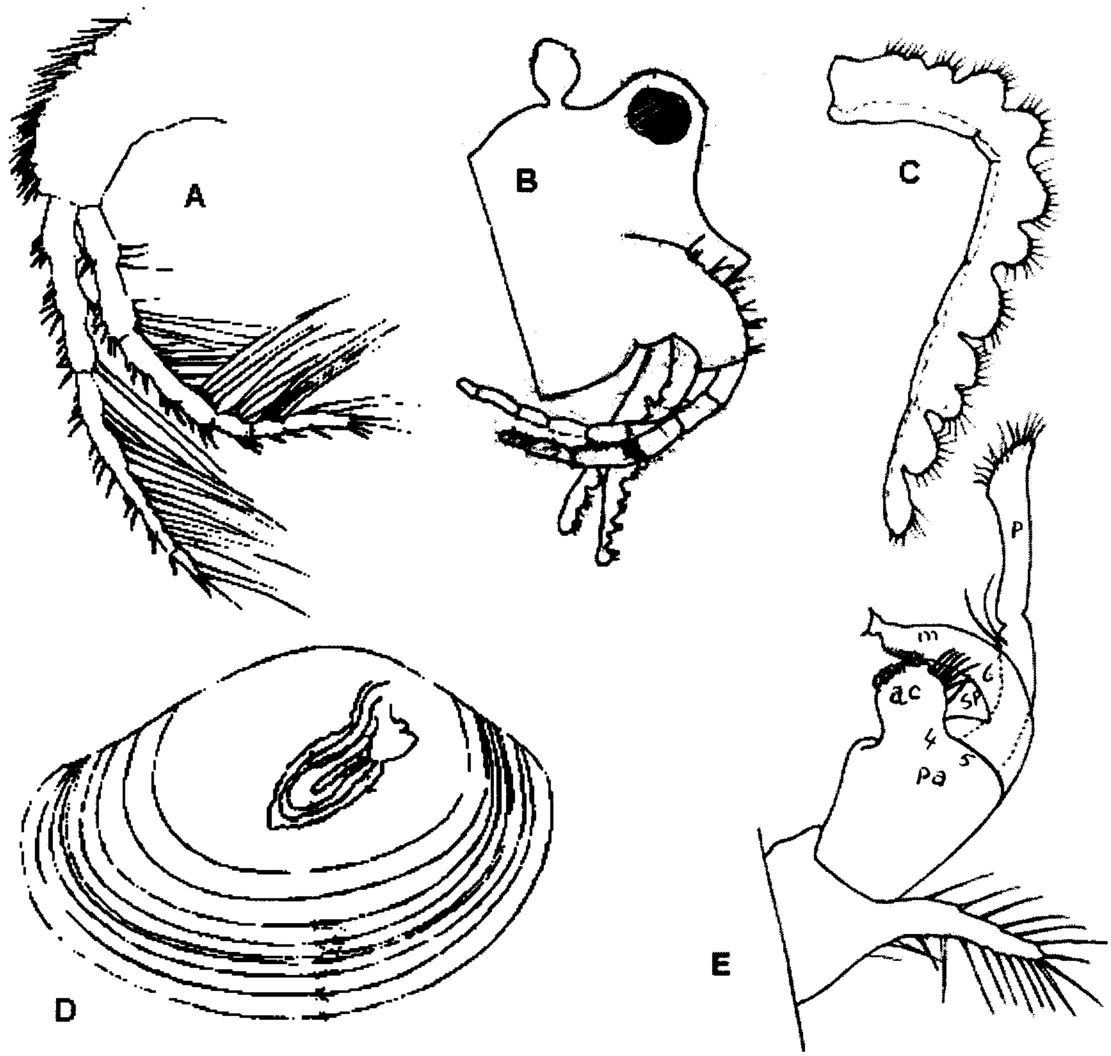

Fig.3.16. *Eulimnadia colombiensis.* A, antenna ♂; B, lateral view of head region ♂; C, antennule♂; D, lateral view of carapace ♂; E clasper of 1st thoracopod ♂. Abbreviations: ac, apical club; m, movable finger; p, palp; pa, palm; sp. small palp. Arabic numerals specify endites.

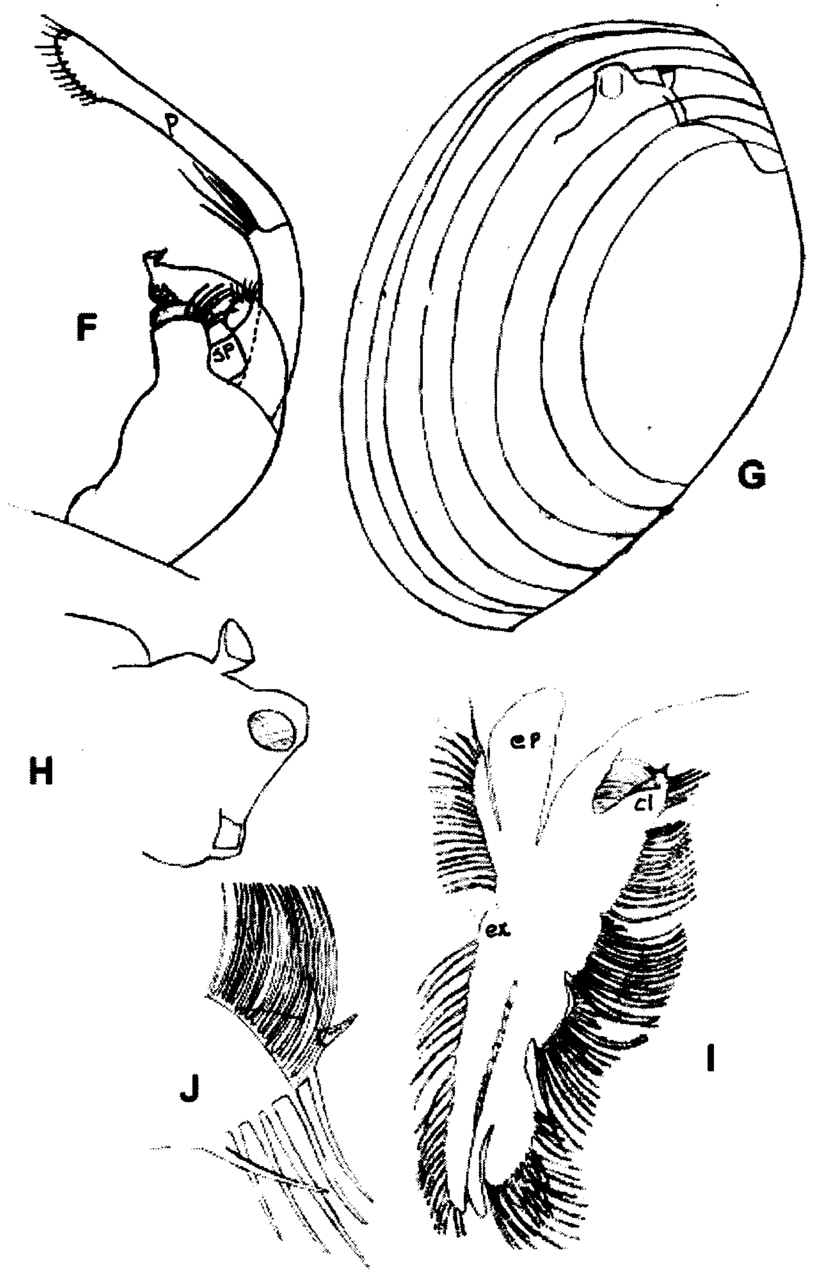

Fig. 3.17. *Eulimnadia colombiensis*. F, clasper of 2nd thoracopod ♂; G, lateral view of carapace ♀;H, lateral view of head ♀; I, 1st thoracopod ♀; J, coxal lobe of 1st thoracopod ♀. Abbreviations: cl, coxal lobe; ep, epipod; ex, exopod; p. palp; sp, small palp.

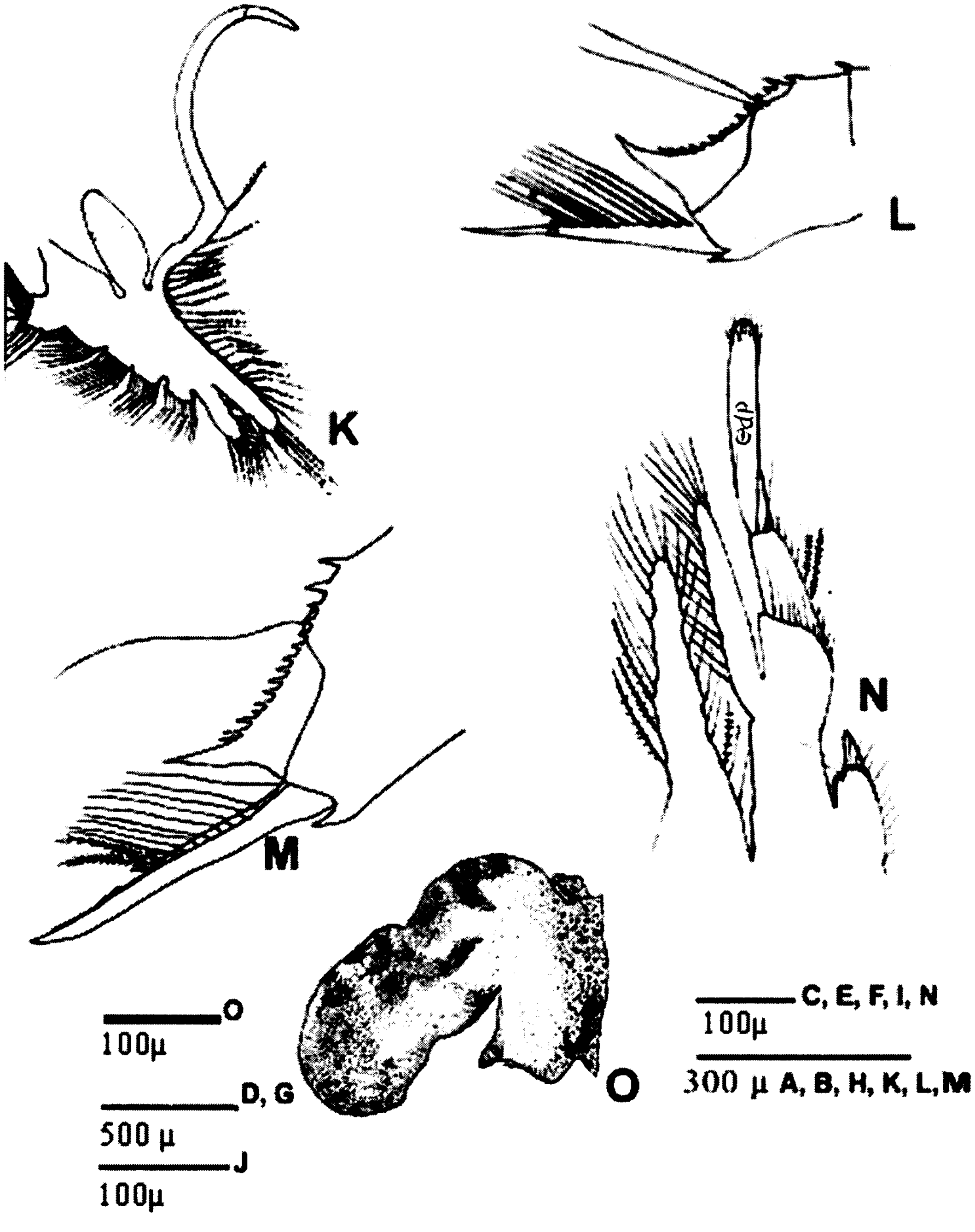

Fig. 3.18. *Eulimnadia colombiensis.* K, 9th thoracopod ♀; L, telson ♂; M, telson ♀; N, distal region of 3rd thoracopod ♂; O, spermatophore; edp, endopodistal palp

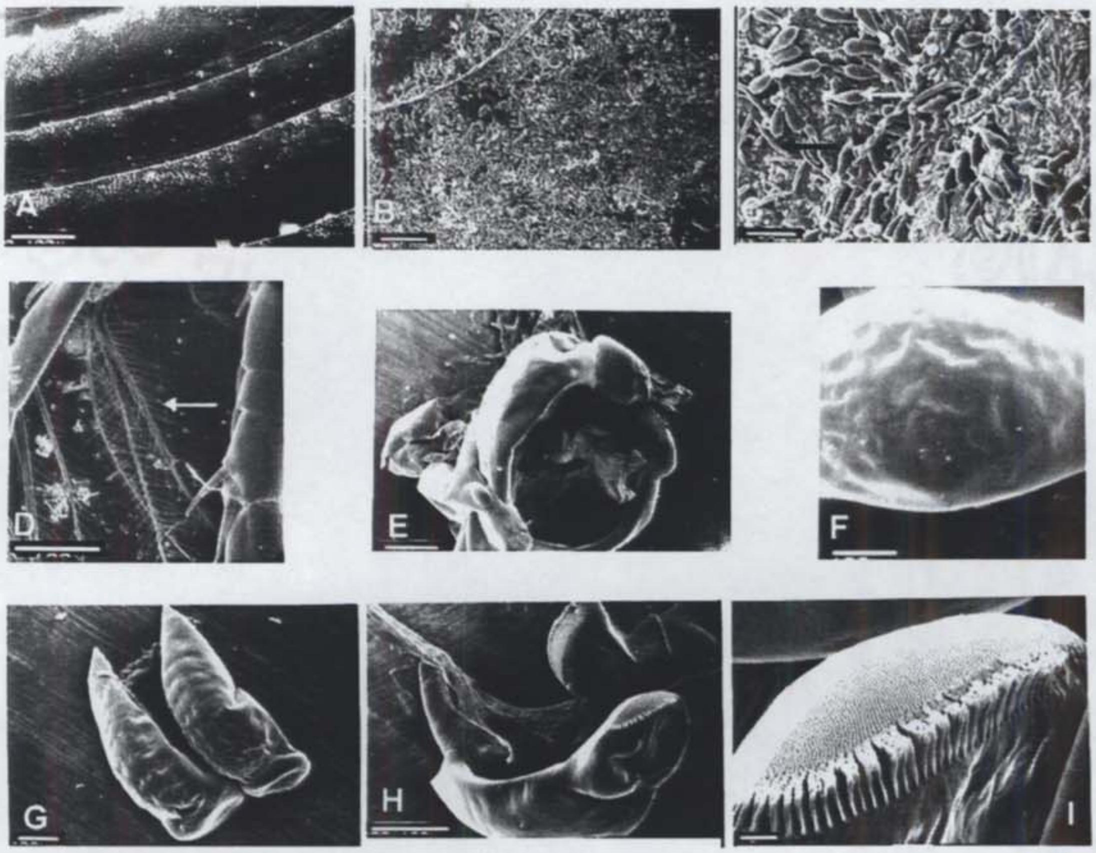

Fig. 3. 19. *Eulimnadia colombiensis*. A, shell with shell lines; B, algae on carapace; C, alga *Clastidium* and diatom *Synedra* on carapace. White Arrow indicates *Clastidium*; D, part of antenna with spines and plumose setae. Arrow indicates plumose setae; E, mandible pair; F, lateral view of body of mandible; G, mandible pair; H, distal end of mandible; I, details of distal end of mandible. Scale bar = 100μ A, D, E, F, G, H; 50μ B; 20μ C; 10μ I.

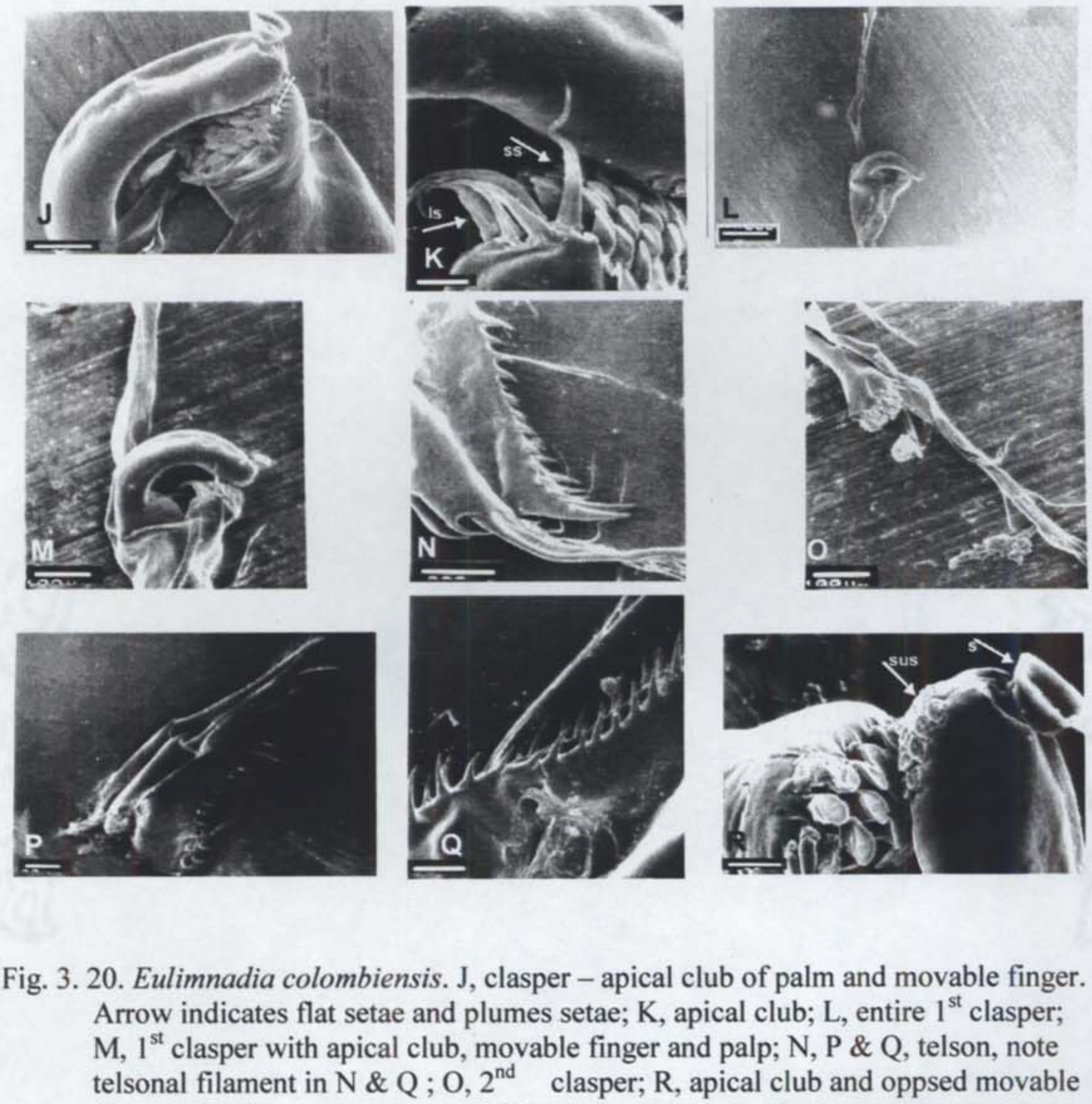

Fig. 3. 20. *Eulimnadia colombiensis*. J, clasper – apical club of palm and movable finger. Arrow indicates flat setae and plumes setae; K, apical club; L, entire 1st clasper; M, 1st clasper with apical club, movable finger and palp; N, P & Q, telson, note telsonal filament in N & Q ; O, 2nd clasper; R, apical club and oppsed movable finger with clasping structures. Abbreviations: ls, long plumose setae; s,dorsodistal sucker-like prominence; ss, stout seate; sus, sucker-like setae. Scale bar = 200µ L, N, P; 100µ M, O, Q; 50µ J, R; 20µ K.

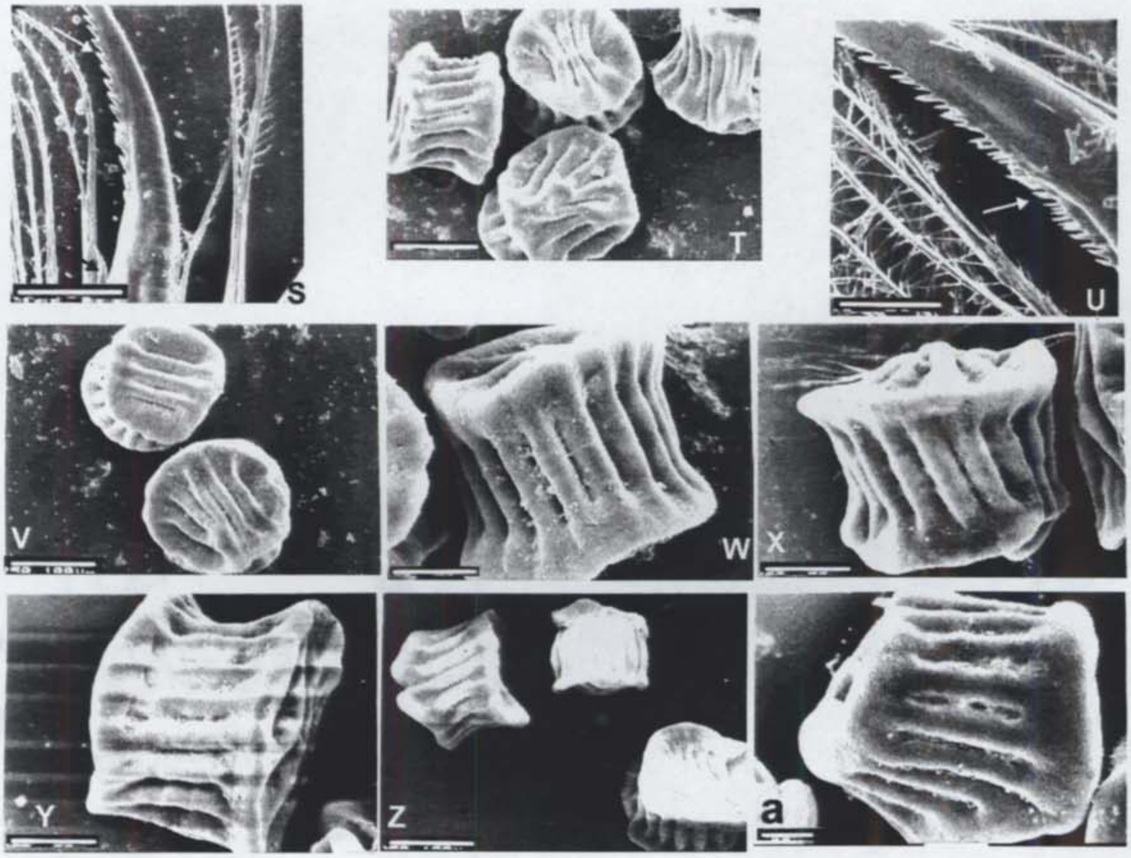

Fig. 3.21. *Eulimnadia colombiensis*. S & U, distal region of caudal furca. Black aarrow indicates 1st large spine, white arrow small spines. Note plumose setae; T & V, egg showing flat ends with parallel and branched grooves; W, Y, Z & a, lateral view of egg with smooth ridges and valleys; X, egg with one end doomed. Scale bar = 100µ S, T, U, V, Z; 50µ W,X,Y; 20µ a.

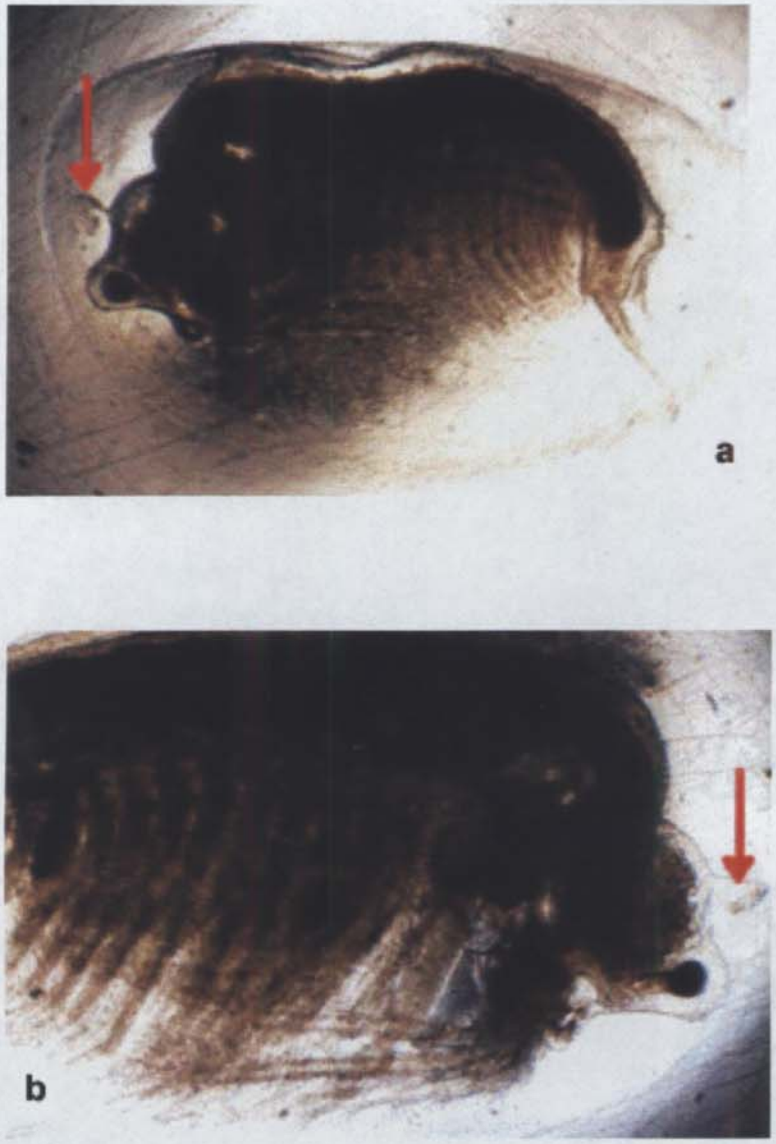

Fig. 3. 22. *Eulimnadia colombiensis*. a, entire ♀ ; b, hepato pancreas seen extending from base of antenna to dorsal end of head ♀. Arrows indicate frontal organ. Spermatophore seen attached to base of 10th thoracopod. Scale bar for a & b in Fig. 3. 7k.

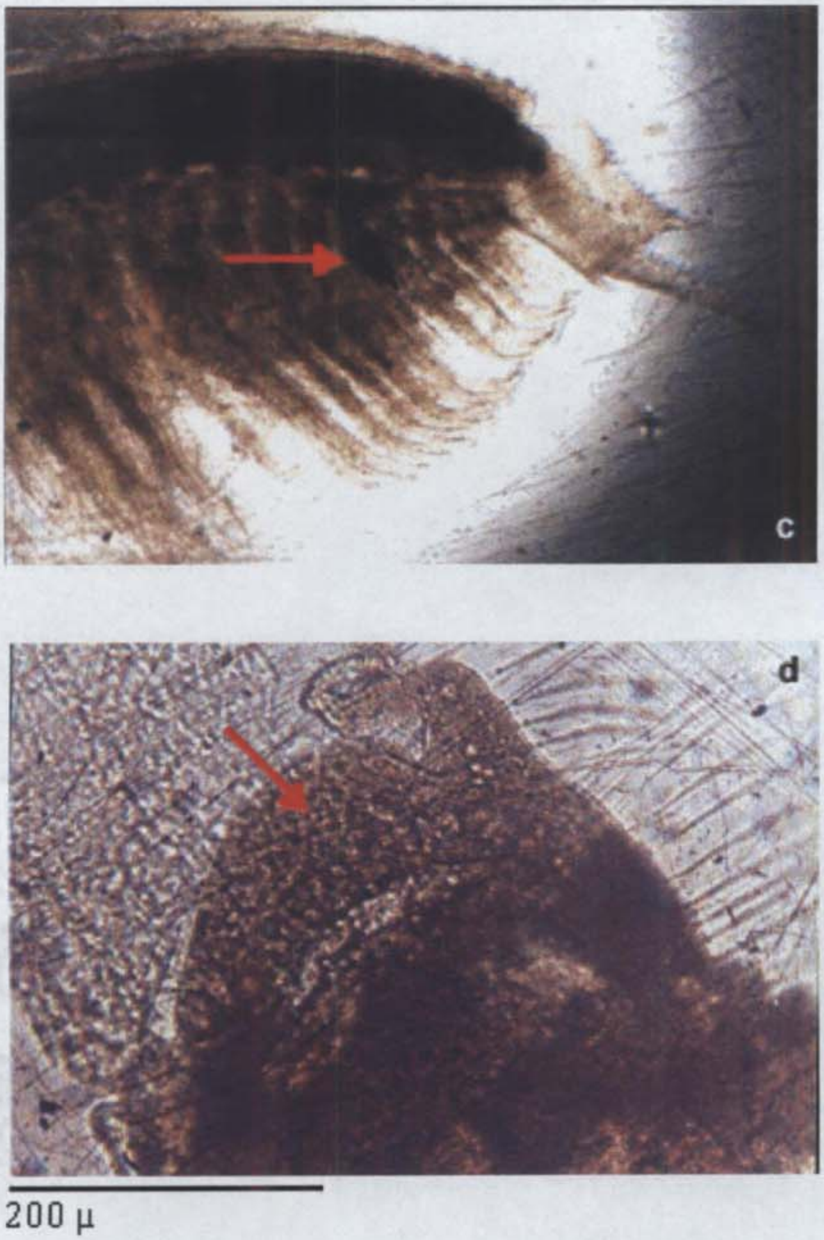

Fig. 3. 23. *Eulimnadia colombiensis*. c, female. Arrow indicates spermatophore. d, spermatophore filled with spermatozoa. Arrow indicates spermatozoa. Scale bar for c in Fig. 3. 7k.

Fig. 3. 24. Small temporary pool at ASCC-2. Circle shows *E. colombiensis* at the bottom of the pool.

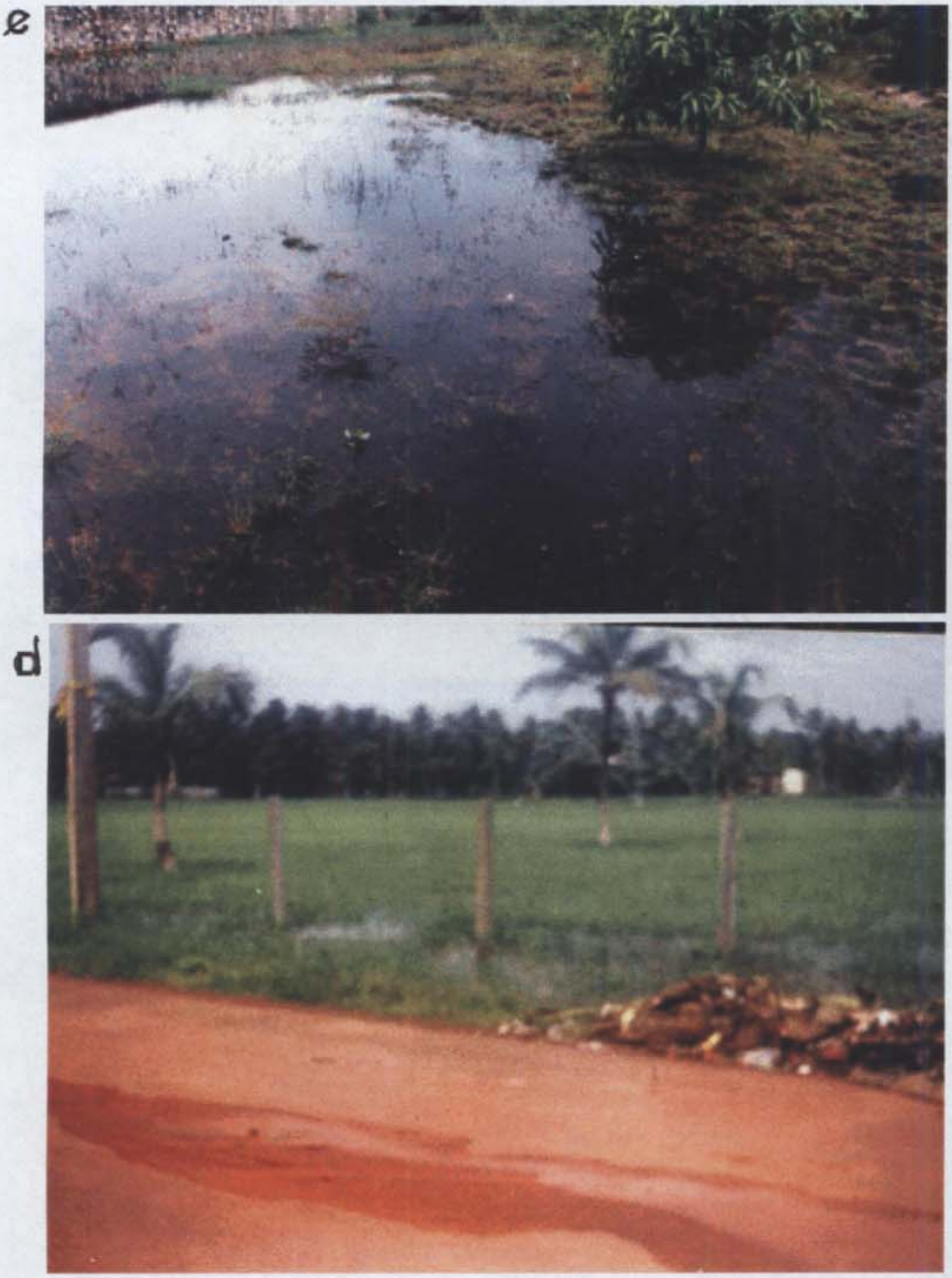

Fig.3. 25. c, large temporary pool at ASCC-2; d, large temporary pool at KRA-3

Fig. 3.26. E & F, temporary pool at KKM-1

Fig. 3. 27. G & H, views of large temporary pool from its southern and northern sides of site KRA-3.

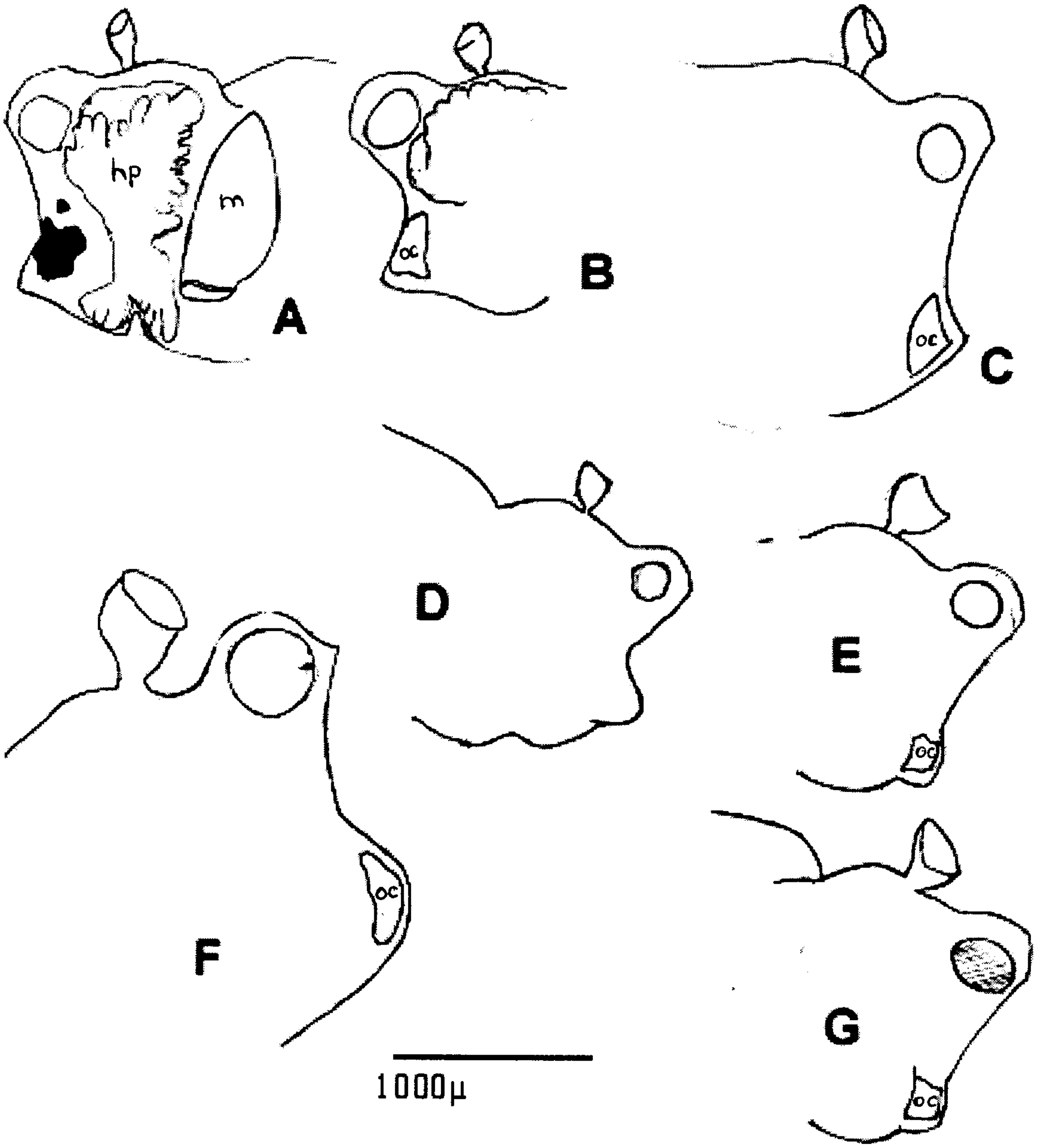

Fig. 3. 28. *Eulimnadia colombiensis*. A, C, D, E, F & G, profiles of ♀; B, profile of ♂. Abbreviations: hp, hepatopancreas; m, mandible, oc, ocellus.

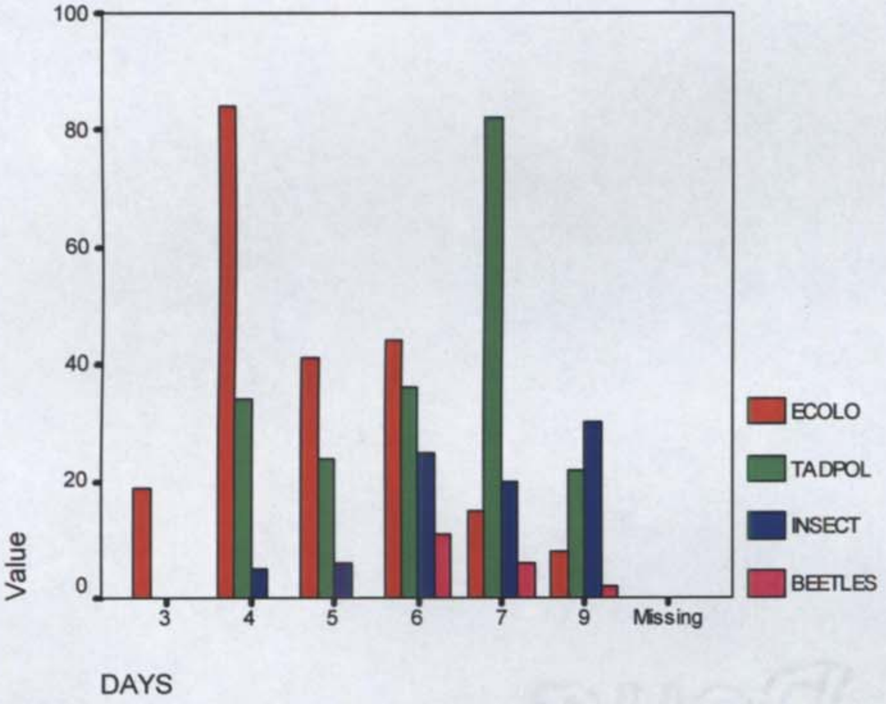

Fig. 3. 29. Population estimation of communities in a temporary water body from KKM-1

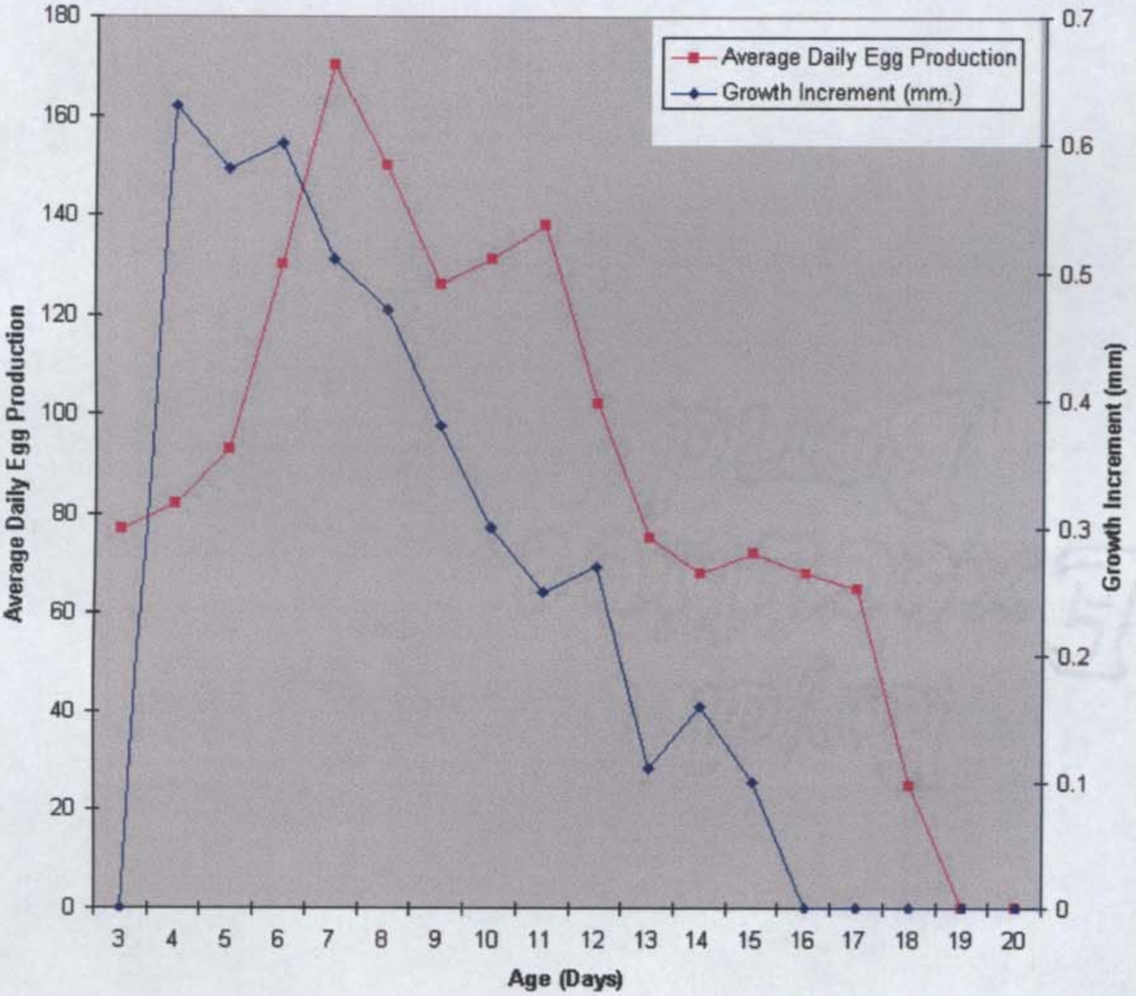

Fig. 3. 30. Average daily growth increment and average daily egg production for *E. colombiensis*

Fig. 3. 31. Survivorship curves of male and female *E. colombiensis*

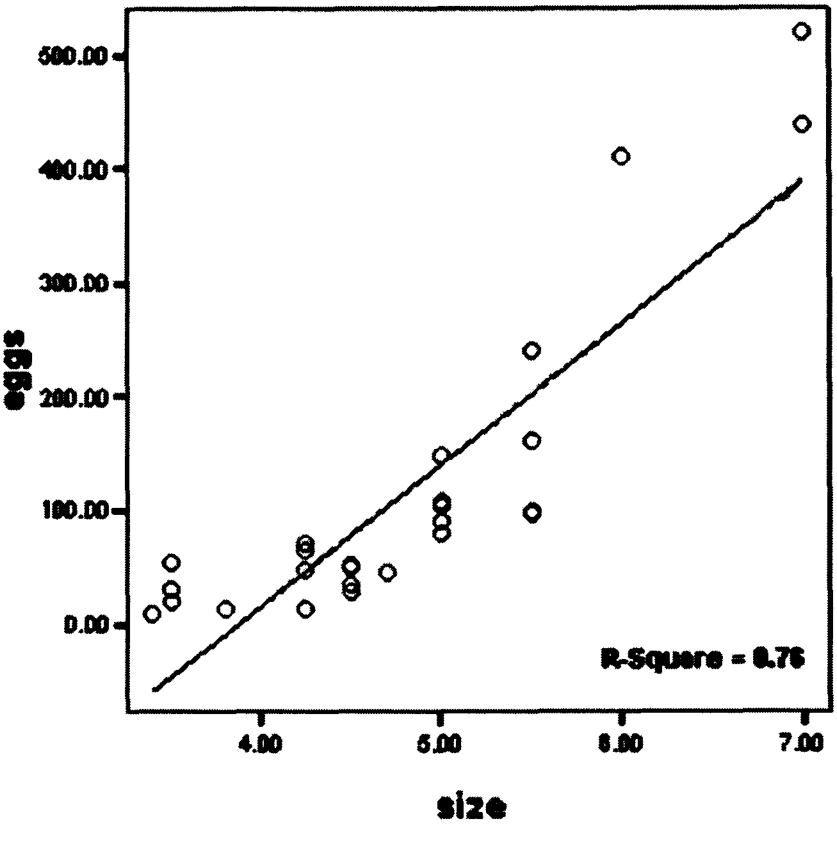

Fig. 3.32. Egg count related to carapace length. Line represents the least square fit of the two variables.

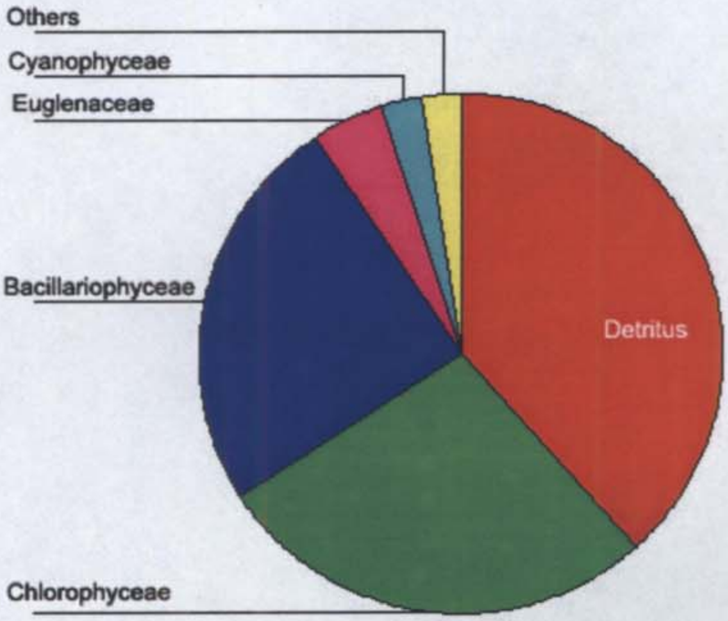

Fig.3.33. Percentage composition of the gut content of *E. colombiensis.*

STUDIES ON CONCHOSTRACA (CRUSTACEA) OF KERALA

Thesis submitted to the University of Calicut
For the award of the Degree of
Doctor of Philosophy
In Zoology

USHA BALARAMAN

Under the supervision of:

Dr. C. K. Gopinathan Nayar
Former Head of the Department
Department of Zoolgy
Christ college, Irinjalakuda

University of Calicut
2005

Chapter 4

LAEVICAUDATA

External morphology

***Lynceus alleppeyensis* sp. nov** (paper published)

Abstract

Introduction and Methods

Taxonomy

Discussion

Figures

Order : Laevicaudata

External Morphology

This order contains one extant family the Lynceidae with approximately 40 species (Martin & Belk, 1988) in three genera: Lynceus, Lynceiopsis, and Paralimnetis. The bivalved carapace is globose, and lacks an umbo or any growth lines or external ornamentation. The two valves are joined dorsally by a true hinge, although the valves are not entirely separate, but are fused for a short distance along dorsal border. The head is large and free to protrude from the enclosing carapace. The head is produced into an elongate rostrum, more so than in other clam shrimps. The eyes are located far from anterior extremity of the head. The ocellus is located near compound eye and bears distinctive paired field of sensory setae. The antennule is shorter than that in Spinicaudata, uniramous, two-segmented , and bears sensory setae on the expanded distal segment. The antennae are large and biramous. Some of the extrinsic muscles of antennae originate on the opposite side of body to appendage served. The labrum is large and fleshy. The mandibles are large and heavy and belong to the second type that has a narrow triturating surface of double row of large teeth. The number of trunk segments is fixed. There are no spines on the dorsal surface of the trunk filaments. Twelve pairs of trunk appendages are seen in females and ten in males. The first trunk appendage is modified in males into a clasper. The second male appendage is sometimes modified as well (*Paralimnetis, Lynceiopsis*) but not as a clasper. The 'palm' of the clasper is derived from endite 3 and the palps derived from endites 4 and 5. The proximal lobe of exopod is large. The trunk terminates in an anal somite (telson feebly developed) that bears telsonal filaments, but lacks caudal rami and is ventrally shielded by opercular lamellae of the penultimate trunk somite. Oviducts of female open on the 11th somite and those of male open either along side the anus or at the base of a posterior trunk limb. In the females, unique lateral flaps of the body wall and dorsal filaments of exopods of trunk limbs 9 and 10, support the egg mass.

*

A NEW SPECIES OF THE CLAM SHRIMP GENUS *LYNCEUS* (BRANCHIOPODA, CONCHOSTRACA, LAEVICAUDATA) FROM KERALA, INDIA

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ABSTRACT

A new species of Conchostraca belonging to the genus *Lynceus* Müller, 1776, obtained from a rain-filled paddy field in Kerala, is described and illustrated. The account includes morphological details of the carapace, rostrum, antenna, thoracopod, opercular lamella, and dorsal lamina. SEM examination of the carapace, mandible, and egg has been done, and comparison with other described species of the genus *Lynceus* is made.

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RÉSUMÉ

Une nouvelle espèce de Conchostraca apartenant au genre *Lynceus* Müller, 1776 obtenue d'une rizière remplie de l'eau de pluie au Kerala est décrite et illustrée. Ce récit inclut des détails morphologiques de la carapace, rostrum, thoracopod, opercular lamella et dorsal lamina. L'examination SEM de la carapace, la mandibule et l'œuf ont été faits et la comparaison avec les autres **espèces** du genus *Lynceus* est faite.

INTRODUCTION AND METHODS

Among the three conchostracan genera belonging to the family Lynceidae Stebbing, 1902, only the genus *Lynceus* Müller, 1776 is widely known (Mattox, 1939, 1959; Belk, 1982; Martin & Belk, 1988; Roessler, 1995a; Damgaard & Olesen, 1998). This genus is represented by several species and occurs on all continents except Antarctica (Belk, 1982). Of the other two, *Paralimnetis* Gurney, 1931 is known from North, Central, and South America (Martin & Belk, 1988; Roessler, 1995a; Maeda-Martinez et al., 1997) and *Lynceiopsis* Daday, 1912 is known only from Africa (Martin & Belk, 1988). In India, this genus was first recorded by Gurney (1906) from Shandur Lake Chitral, British India, now in Pakistan. Later the species *Lynceus denticulatus* was described by Gurney in 1930. In the first report from south India on this genus, Nayar & Nair (1968) described *Lynceus brachyurus* Müller 1776, a cosmopolitan species, obtained from Irinjalakuda, Kerala. Another species, *Lynceus serratus* Royan & Alfred, 1971, collected from Madurai was also described as new (Royan & Alfred, 1971), but, according to Durga Prasad (1991), *Lynceus serratus* and *Lynceus denticulatus (*Gurney, 1930) are synonymous. Battish (1981) described a new species as *Lynceus vasishti* from Punjab, north India.

Members of the family Lynceidae inhabit temporary water bodies. They can be distinguished from other families of the Conchostraca by a pea-like, smooth carapace without growth lines, a true hinge, a large head with distinct fornix, setose sensory fields, 2-segmented 1st antennae, the absence of a furca, the first pair of legs modified as claspers in the male (Pennak, 1953; Mattox, 1959; Belk; 1982; Dodson & Frey; 1991; Martin, 1992), and by their clasper morphology (Fryer, 1987; Olesen et al., 1996).

Illustrations made with a compound microscope equipped with camera lucida. Specimens gold coated with a 10 nm beam sputter coating unit Jeol JFC 1200 and observed in a Jeol JSM-5600 LV SEM. Descriptions follow the scheme of Martin and Belk (1989).

TAXONOMY

Lynceus alleppeyensis sp. nov. (figs 1-4)

Material examined. – One holotype ♀ (disarticulate; reg. no. CC7ZSI/SRS), 1 allotype ♂ (disarticulate; reg. no. CC8ZSI/SRS), and 4 paratype ♀ ♀ ♀ ♂ (reg. no. CC6ZSI/SRS) deposited with Southern Regional Station, Zoological Survey of India, Chennai; collected by Usha Balaraman, on 8th June, 2001. Specimens preserved in 7% formalin / 70% alcohol.

Type locality. - Temporary monsoon rainpool located on western side of N. H. – 47 at Nangyarkulangara, Alleppey district, Kerala, India, 9.5° N 76.5° E.

Diagnosis.- Bivalved carapace globose, hinged, more or less rounded in lateral view, and without an umbo, growth lines, or external ornamentation. The carapace is thin, transparent, and leathery, and measures 3.5 to 4.5 mm in length in the female and 3.5 to 4.0 mm in the male. Rims flattened (fig. 4A), and pressed against each other tightly to enclose the entire animal. Site of attachment of adductor muscle visible with compound microscope and partially surrounded by coils of maxillary glands in 3 - 4 oval rings (fig. 1A, fig. 2N). Head large, articulating with trunk, and able to swing forward from between the valves of the carapace.

Female. – Carapace. Shell in living specimen orange-red in colour and swollen, especially when carrying eggs. Minute serrations present along flattened border of shell valves (fig. 4A) on the posteroventral region, for a short length (fig. 4B).

Head region. Rostrum slightly larger than that of male, distal border of rostrum broad, spatulate, and serrated, bearing fine setae. In frontal view, fornix reaches apex of the spatulate rostrum in both sexes, rostral carina entire with single rostral keel, apex of dorsal carina terminates just short of the anterior border of the head region (fig. 1B, D, 4C). Its posterior end terminates in front of the two closely adjacent compound eyes, separating two distinct fields of olfactory setae (fig. 1D) that lie anterior to the compound eyes. Ocellus situated anteriorly of and close to compound eye. Frontal pore present between the setose fields (fig. 1C); posterior to that pore and just anterior to the occipital notch on the midline of the head region the dorsal organ is situated (fig. 4C).

Antennae. First antenna small and two-segmented with distal segment elongated, bearing short setae with terminal pores (fig. 3T). Second antenna large and biramous (see description in male).

Labrum large and plough-shaped, with 2 oval glands and bearing simple setae (figs. 2P, 4C. 1).

Mandibles large and stout, with proximal part conical and masticatory surface narrow, bearing 11 teeth (fig. 4D). Triturating surface of mandible typical of the Lynceidae (cf. Martin, 1989), posterior tooth the largest, conical, stout and slightly apart from the rest, which decreasing in size to anterior, with thin, chisel-shaped edges curved inwards with pointed corners, and bearing minute denticles (fig. 4E, F, G). Besides, a cluster of small sharp, needle-like teeth present at the distal dorsal border (fig. 4G).

Thoracopods. The trunk bears 12 pairs of thoracic appendages, gradually decreasing in size after the 4th appendage; 12th thoracopod the smallest and greatly reduced, with both lobes of the exopod lost (fig. 2L). All thoracopods thin, folded longitudinally into a V-shape and divided into many lobes and endites (fig. 1E, F). Exopod divided into an elongated, tapering ventral lobe and a broader dorsal lobe, covered with long setae. Endopod divided into six endites. The first endite or coxal lobe short, blade-like, bearing serrated spines dorsally, and short sharp spines with serrations at their tips that do not resemble any setal type described by Martin et al. (1986) (fig. 1G). Second and third endites broad, the 2nd larger than the rest and the 6th finger-like. All endites covered by long setae. Epipod dorsal, smooth, long, and as large as the exopod (fig. 1E, F). The 9th and the 10th thoracopods (only the 10th drawn in detail) differ from the rest: the dorsal exopod is modified into a long, laterally curved hook-like structure that distally ends in minute setae (fig. 2K). While the ventral exopod bears long setae, the endites bear short, both non-serrated and serrated spines and setae (fig. 2K).

Opercular lamella. The opercular lamella or subanal plate is a thin, bilobed flap that extends beneath the telson (fig. 1H).

Lamina abdominalis. The lamina abdominalis (dorsal lamina), an extension of the posterolateral trunk somites that projects laterally to fold the egg mass, has curved, tentacle-like processes studded with minute spines (fig.1I, J).

Caudal region. The telson bears a short spine at the lower posterior end and is covered in spinules on the posterodorsal margin. Long telson filaments are seen, surrounded by long setae at their base (fig. 3U).

Eggs. - Egg mass large; number of eggs varying from 200 to 350; eggs spherical with a diameter of 141μm, showing distinct design. Under the compound microscope the egg shell appears covered by intersecting ridges (fig. 2M). The SEM morphology of the eggs shows ornamentation in the form of thin, ribbon-like, frilled and raised ridges that intersect, producing a polygonal or honey comb pattern (fig. 4H). Egg design nearly resembles that of the anostracan *Chirocephalus salinus* Daday, 1913 (cf. Thiery & Gasc, 1991). According to Martin & Belk (1988), in lynceids the egg shell is either smooth or slightly granulated, but SEM examination of the eggs of the new species reveals a distinct egg design, though not highly sculptured as in the genus *Eulimnadia,* wherein it is species-specific (Belk, 1989).

Male. – Carapace. Shell more pale and transparent than in female. Unlike in female, there are no serrations on the shell border (fig. 2N). Rostrum typically broad and spatulate, serrations absent; setae larger and denser than in female (fig. 2O, P). Apex of dorsal rostral carina terminates at the anterior border of the head region (fig. 2O).

Antennae etc. First and second antennae, labrum, and mandibles similar to those of female. The anterior and posterior flagella of the 2nd antenna have 18 and 21 segments, respectively, bearing long plumose setae directed posteriorly; the anterior flagellum, in addition, bears serrate spines along the dorsal edge, except on the four distal segments (fig. 2Q). The first three distal spines are unpaired, whereas the rest are paired. The distal most segment of the anterior flagella bears one short and one long, non-serrated spine-like setae and a plumose seta at its end. The basal segment bears spines near the base of the anterior flagellum. Spines absent in posterior flagellum (fig. 2Q).

Thoracopods. The trunk bears 10 pairs of thoracic appendages. First thoracopods modified as prehensile claspers (fig. 3R), second pair unmodified (fig. 3S). Right and left claspers similar in size and morphology; movable finger (endite 6) of clasper sickle-shaped, gently curved, approximately of equal length to that of immovable finger (endite 3), and it does not extend beyond the setose margin (fig. 3R). Distal palp (endite 5) of clasper less than twice the length of proximal palp (endite 4), immovable finger bears serrate stout spines on the side facing the movable finger, and some short setae proximally (fig. 3R). Numerous rows of minute granules seen on the margin of the enlarged 3rd endite, facing the exopod (fig. 3V). Telson similar to that of female (fig. 3U).

Etymology. - This species has been named after the Alleppey district from where it has been collected, whence the name *alleppeyensis* is an adjective agreeing in gender with the (masculine) generic name.

Habitat and life style. - An abandoned paddy field, about 5 ha, dry during the summer season, and after heavy seasonal rains inundated with rain water to form a large temporary water body: a grassy and weedy pool rich in algae, insect larvae, aquatic insects, cladocerans, copepods, etc. The water temperature was 29°C, pH 6.7, and salinity nil. Presence of another undescribed conchostracan, *Eulimnadia* sp. nov. belonging to the order Spinicaudata was also noted in the collection. This is an incidence of non-congeneric species co-existing, supporting the observation made by Maeda-Martinez et al. (1997) for conchostracan assemblages. In the laboratory, these animals were frequently seen swimming towards the surface and dragging small floating blades of grass to the bottom to feed on them. When not feeding, they remained half buried in the mud. The animals remained alive in the laboratory for 10 weeks.

DISCUSSION

Taxonomic studies on conchostracans are based mainly on morphological characters of rostrum, clasper, telson, and ornamentation of the egg shell (Pennak, 1953; Mattox, 1959;

Martin & Belk, 1988; Roessler, 1995b; Brendonck, 1999) using both light microscopy and scanning electron microscopy.

The rostral morphology of *Lynceus alleppeyensis* sp. nov. resembles that of *L. brachyurus* (Martin & Belk, 1988), and *L. brevifrons* Packard, 1877 (Martin & Belk, 1988) in some details like setated rostrum and presence of single rostral keel in both sexes, but differs in several other details. *L. brachyurus* differs in having a medium indentation in the rostrum of the male and a tridentate rostrum in the female, and *L. brevifrons* differs in the shape of the distal rostral border (which is concave), and in the absence of rostral setae in the female. The rostral morphology of *L. brevifrons* is similar to that of another Indian species *L. denticulatus* (Gurney, 1930) as mentioned by Martin & Belk (1988*)*. *L. bicarinatus* Barnard, 1924 (Brendonck, 1999) is similar to the new species in the shape of the rostrum but differs in having 2 dorsal rostral keels in both sexes. With regard to clasper morphology, *L. alleppeyensis* resembles the above species in general appearance; but differs in the length of the movable finger - whereas in *L. alleppeyensis* the movable finger does not extend beyond the setose margin of the immovable finger, in *L. brachyurus* it extends beyond and in *L. brevifrons,* it is much shorter.

L. alleppeyensis resembles two Indian species *L. serratus* (Royan & Alfred, 1971) and *L. vasishti* (Battish, 1981) with respect to the shape of carapace, rostrum, male clasper, and (in L. vasishti only) setae at the base of the telson filaments. *L. alleppeyensis* differs from the above two species in having: (1) serrations on carapace and rostrum only in the female (in *L. serratus,* serrations on carapace seen in both sexes, and serrations on rostrum only in the female; in *L. vasishti*, serrations on carapace and rostrum seen in both sexes); (2) rostrum fringed with setae in both the male and the female (which are absent in both *L. serratus* and

L. vasishti); (3) 2nd antenna bears 9-9 segments more when compared to *L. serratus*, and 5-6 more when compared to *L. vasishti* ; (4) movable finger of clasper not showing a bump at base as seen in the clasper of *L. serratus*; (5) telson covered with spinnules on the posterodorsal margin (in *L. serratus*, spinules occur on the ventral side, and in *L. vasishti*, they are found all over the telson); (6) Egg mass large and eggs ornamented with ridges (in *L. serratus* the egg mass small, consisting of 50 eggs, and eggs finely dotted).

Based on the above comparative account of the features used for identifying the species of the family Lynceidae, the present species can be distinguished as a new species by : (1) sexual dimorphism in carapace and rostral morphology, (2) morphological details of the male claspers, (3) dorsal exopod of 9th and 10th thoracopod in the female modified into a long hook, (4) dorsal lamina with tentacle-like process studded with spines, (5) bilobed opercular lamella, (6) telson covered with spinnules on the posterodorsal margin, and (7) egg shell surface ornamented with ridges

FIGURES

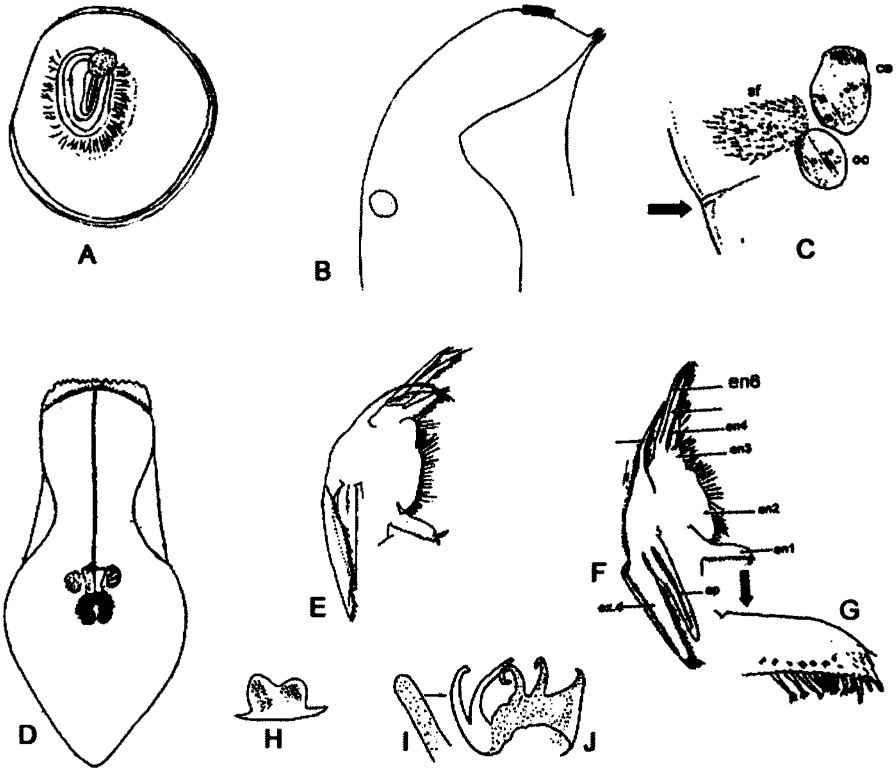

Fig. 1 *Lynceus alleppeyensis* sp. nov. A, shell ♀; B, lateral view of head ♀; C, lateral view of head, enlarged, near fontal pore; D, frontal view of head ♀; E, first thoracopod ♀; F, second thoracopod ♀; G, coxal lobe of second thoracopod; H, opercular lamella ♀; I, enlarged tentacle of dorsal lamina ♀; J, dorsal lamina ♀. Abbreviations: ce, compound eye; en, endites; ep, epipod; ex.d, dorsal lobe of exopod; ex.v, ventral lobe of exopod; oc, ocellus; rc, rostral carina; sf, setose field. For scales, see fig. 3

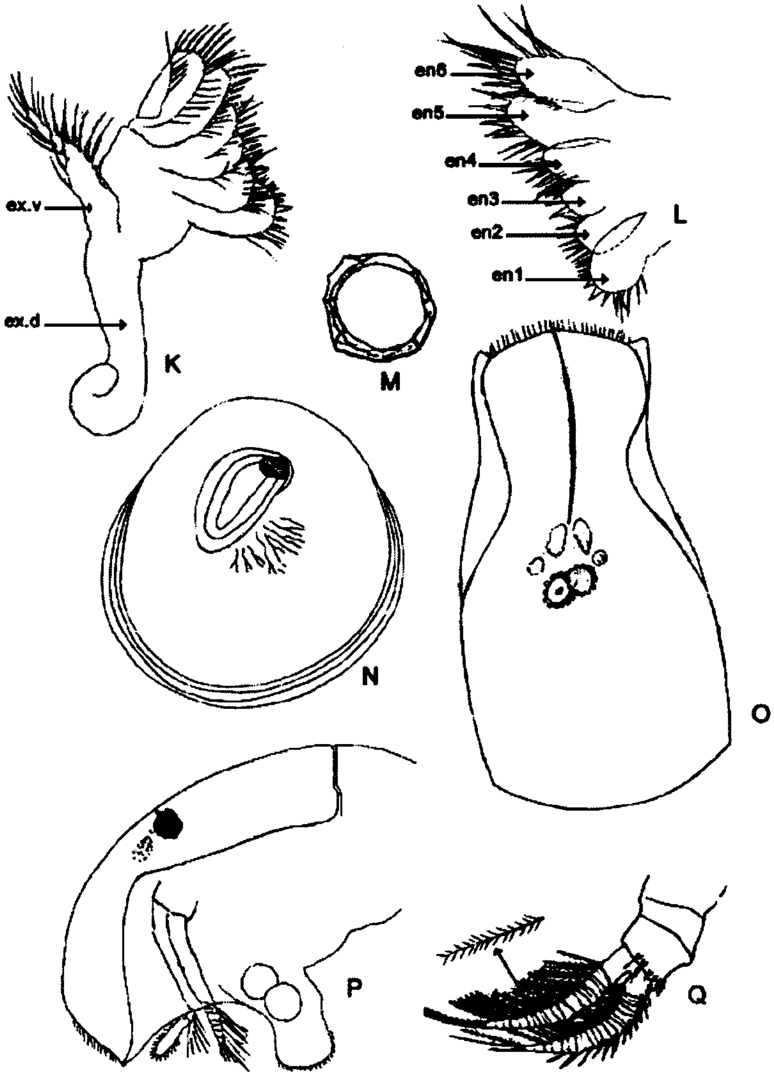

Fig. 2 *Lynceus alleppeyensis* sp.nov. K, tenth thorocopod ♀; L, twelfth thorocopod ♀; M, egg; N, shell ♂ ; O, frontal view of head ♂; P, lateral view of head♂ ; Q, second antenna ♂. Abbreviations as in fig. 1. For scales, see fig. 3

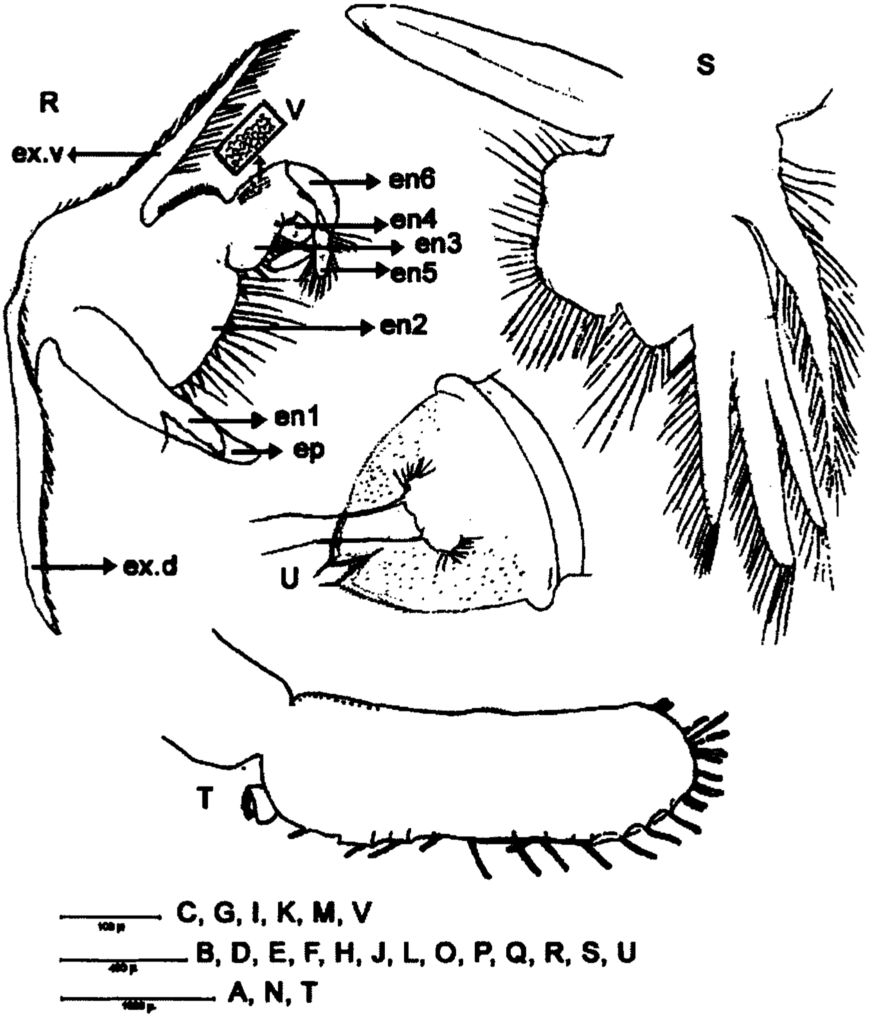

Fig. 3 *Lynceus alleppeyensis* sp.nov. R, first thorocopod ♂; S, second thorocopod ♂; T, first antenna ♂; U, telson ♂. Abbreviations as in fig. 1.

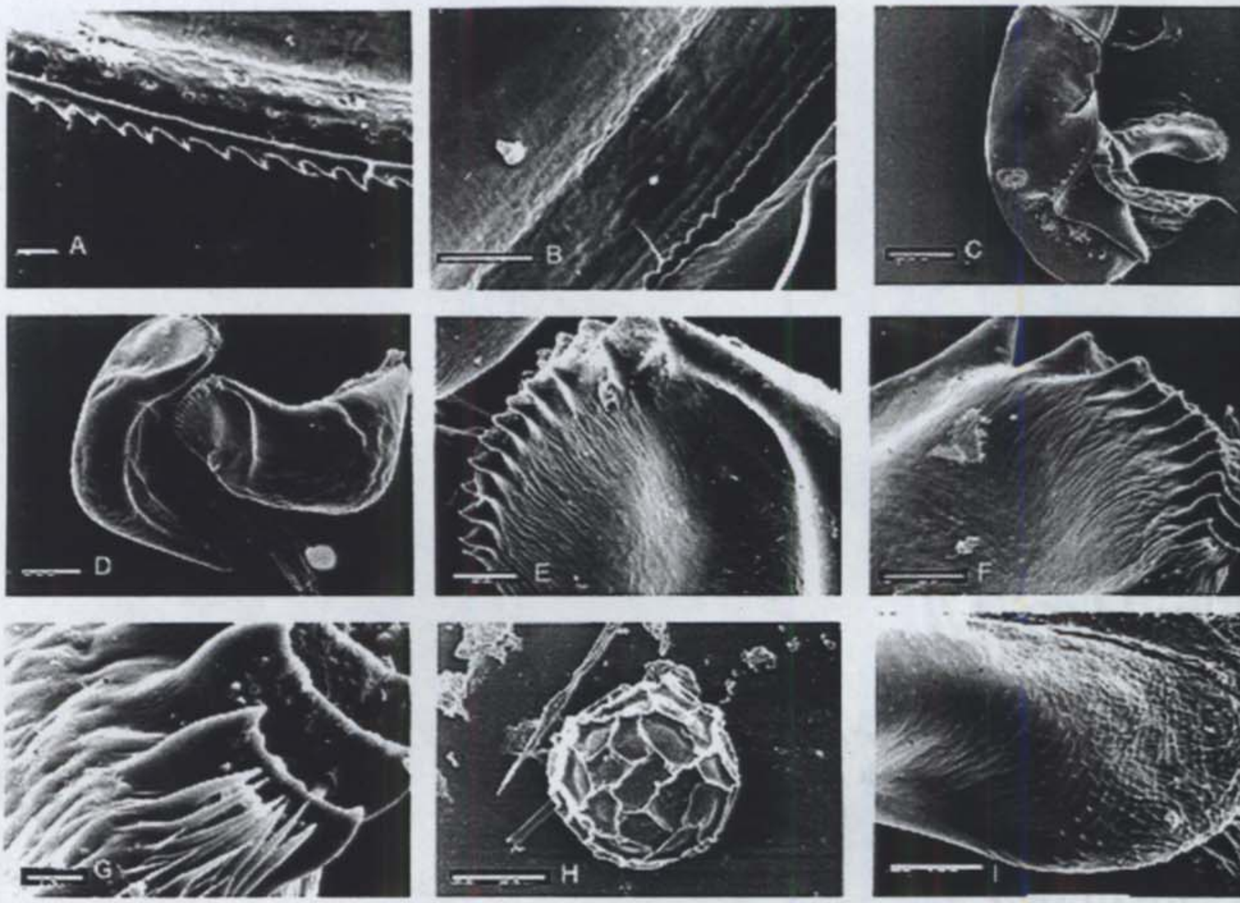

Fig. 4 *Lynceus alleppeyensis* sp. nov. Scanning electron micrographs of selected characters. A, posteroventral region of shell showing serration ♀. Scale bar = 20 µm; B, posteroventral serrations becoming entire. Scale bar = 50 µm; C, lateral view of head ♀. Scale bar = 500 µm; D, pair of mandibles ♀. Scale bar = 200 µm; E & F, left and right mandibles, respectively, viewed from opposite ends. Scale bar = 50 µm; G, anterodistal end of triturating surface of mandible. Scale bar = 10 µm; H, egg. Scale bar = 50 µm; I, labrum. Scale bar = 100 µm. Abbreviations: an, antenna 2; do, dorsal organ; dr, damaged region; la, labrum; on, occipital notch; s, setae; sf, sensory field.

STUDIES ON CONCHOSTRACA (CRUSTACEA) OF KERALA

Thesis submitted to the University of Calicut
For the award of the Degree of
Doctor of Philosophy
In Zoology

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2005

Chapter 5

SUMMARY AND CONCLUSIONS

Habitat Prediction

Findings and Significance

Taxonomic key to the species of Conchostraca, Kerala

only some means of bridging the dry phase but also to detect a wet phase of sufficient duration.

Table 5.1 Physical, chemical and biological features of the major water bodies sampled in the study.

Hydro period & nature of habitat	Site	Area*	Depth*	Dominant vegetation	pH	DO mg/l	Temp. (° C)	Salinity	Species present
2 to 3 weeks T. pool	KRA-3	8000 m^2	0.80 m	Grass	6.7	5.21	29	Nil	*L. allepeyensis, E. colombiensis*
7 to 9 days T. pools	KKM-1	40 m^2	0.20 m	Grass and herbs	7.4 to 7.8	5.60 to 9.22	28 to30	Nil	*E. colombiensis*
6 to 7 days T. pool	ASCC-2 small	3 m^2	0.25 m	Grass	7.4 to 7.8	3.27 to 9.69	28 to 34	Nil	*E. colombiensis*
	ASCC-2 large	60 m^2	0.30 m	Grass	7.2 to 7.7	5.61 to 9.52	29 to 30	Nil	*E. colombiensis*
7 months water level high. Seasonal pool	ASCP-2 Seasonal pond	7 m^2	0.40 m	Submerged macrophytes, Nymphea, Hydrilla	7.02 to 8.2	2.03 to 2.23	25 to 27	Nil	*C. hislopi*
Perennial Permanent Pond	KP-1	0.15 k m^2	4 m	Grass, Hydrilla,Ipomoea and Other rooted macrphytes	7.1	3.01	27 to 29	Nil	*C. hislopi*

*Approximate; Temp: Temperature; T: Temporary
Salinity below 0.02 was taken as Nil

Eggs/cysts laid from an early stage sink to the bottom of the pond to form the cyst/egg bank. These cysts do not hatch immediately, but pass through the dry phase and hatch later during the next flooding of the pools.

Habitat Prediction.

Table 5. 1 lists the various physical, chemical and biological factors of the major water bodies sampled in this study. From Table 5. 1 and available literature the following assumptions are made for habitat preferences for Conchostraca of Kerala:

1. The more temporary the water body, the more likely it is to include conchostracans. Conchostracans in Kerala, with the exception of C. hislopi, occur in warm, clear, freshwater temporary water bodies that have a neutral to slightly basic pH, with DO above 5 mg/l.

2. The shorter the inundation or wet phase (6 to 9 days), the more likely that species with shorter life-cycles will prevail over those with longer life cycles. Pools that hold water for less than a month are dominated by conchostracans . *E. colombiensis* and *L. alleppeyensis* are temporary-pool inhabitants, of which *L. alleppeyensis* develop in temporary water bodies that have a longer wet phase while *E. colombiensis* with its longevity of 34 days and sexual maturity on the 3rd or 4th day can survive in short-lived temporary pools as well as in relatively long-lived pools. If the wet phase persists, *E. colombiensis* completes its life cycle, or else in the shorter temporary pools death follows desiccation of the pools. *L. alleppeyensis*, with its longevity up to 10 weeks survives in long-lived temporary pools (longevity data are under laboratory conditions for both the species). Under natural conditions, their longevity can be completed only if the habitat persists (under extreme conditions).

3. Seasonal pools that hold water for more than a month are successfully inhabited by conchostracans that form a part of the fauna of permanent water. *C. hislopi* is found both in seasonal pools and in permanent waters.

Findings and Significance

The conchostracan fauna of Kerala includes four species representing the two orders of Conchostraca the Laevicaudata and the Spinicaudata. The three species identified in the present study *Lynceus alleppeyensis* Balaraman and Nayar 2004, *Eulimnadia colombiensis,* Roessler, 1989 and *Cyclestheria hislopi,* Baird, 1859 belong to the three families Lynceidae, Limnadiidae, and Cyclestheriidae respectively. *L. alleppeyensis* is a new species desribed from Kerala during the present study. *E. colombiensis* is decribed for the first time, the life cycle and the presence of males of *C. hislopi* are reported and described, for the first time.

E. colombiensis and *L. allepeyensis* were collected from coastal areas 2 to 3 kilo metres from the coastal line located in Thiruvananthapuram, Kollam, and Allepppey districts of Kerala. However, samples collected from the Poovar - Vizhinjam coastal belt of Thiruvananthapuram, did not yield any conchostracans. High density of population along the coastal belt of these areas have led to large scale habitat destruction.

Due to the unpredictable nature of the habitats, there are many difficulties in collecting conchostracans from the ephemeral pools. These ephemeral habitats periodically disappear and reappear and are colonized primarily by the 'egg banks' rather than by dispersal from other habitats. Construction activities, engineering interventions on terrain i.e., digging, soil dumping etc. cause destruction of these egg banks

.Collections have to be done as soon as the pools make their appearance. If the hydroperiod of the pool is prolonged, the inhabitants have to confront predation from predacious insects and fishes, and most of them are exterminated before the temporary habitat dries out. Moreover, if the normal quantity of the seasonal rains is not received, normal filling of the pools will not take place.

Developmental activities, changing agricultural practices, reclamation of backwaters and wetlands, euptrophication, dumping of domestic and industrial wastes, pollution etc. that have threatened aquatic habitats have also taken their toll of the temporary water bodies. While many branchiopods, including conchostracan species, are being listed as threatened, conservation measures are being planned and implemented in several countries of the world. In India, particularly in Kerala, documentation of conchostracans based on surveys of temporary water bodies and the communities therein are absent in the scientific literature, except for a few sporadic reports on Conchostraca. Moreover, these groups of crustaceans have been overlooked in majority of the limnological text books. The present study, though limited to the conchostracans, is hoped will act as a sound foundation upon which other studies not only of Conchostraca but also of other unique communities present in these temporary habitats of Kerala and India will be undertaken

The findings and significance of this study are:

1. Identification of a new species *L. allepeyensis* sp. nov. obtained from a rain filled abandoned paddy field in Nangyarkulangara, in Alleppey district. Information are given on the morphology and eggs supported by scanning micrographs. Temporary

pools show endemism for many crustacean species (King et al., 1996). *L. brachyurus* reported from Irinjalakuda, Kerala, is a cosmopolitan species. It was also found in the vernal pools of Northern California (King et al., 1996). *L. serratus* has been reported from temporary water bodies in Madurai, Tamil Nadu (Royan, 1971), and *L. vasishti* (Battish, 1981) from Punjab, North India.

2. *E. colombiensis* is described for the first time from Kerala. Diagnosis of the species includes scanning electron micrographs of the key characters of taxonomic importance like egg morphology, male clasper, telson, and mandibles. Spermatophore is also described. The ecology, feeding, and mating behaviour have also been studied and discussed.
3. Life history traits of *E. colombiensis* are typical of 'early colonist' species. Hatching occurs within 24-36 hours of hydration. Egg production begins on the 3rd or 4th day, and continues till death. Females undergo reproductive senescence after one week of egg production. The range of total egg production is 380 to 2859 per female, with an average of 110 ± 35 per day. The average life span of females is 20.38 ± 8.45 days. Gut analysis of *E. colombiensis*, shows that these early colonizers of temporary pools are mainly detritivores
4. The technique of culturing *E. colombiensis* in the laboratory has been standardized. Eggs have to pass through a dry phase of minimum of 3 days before hatching by rehydration. *E. colombiensis* can be easily reared in the laborartory. They are small, have high fecundity, short life span and hence ideal for research in genetics, evolution, and other allied studies.

5. Pedigree analysis of *E. colombiensis* was done, to test for androdioecy. Androdioecy is a rare type of mating system in which males and self compatible hermaphrodites coexist, and is an effective reproductive strategy for surviving in an unpredictable habitat. Progeny testing of offsprings from female isolates belonging to populations from the two respective sites showed that the population from KRA-3 site was monogenic and that from KKM-1 site was amphigenic. This is evidence for androdioecy in *E. colombiensis*, and this forms the first report of androdioecy in this species. The data generated in this study of androdioecy have also been used as a collaborative work with S. C. Weeks , University of Akron, USA, for progeny testing of 10 species in the genus *Eulimnadia* to see whether they have an androdioecious mating system as found in *E. texana.*
6. Males of *C. hislopi* have been described for the first time. Descriptions of the male include scanning electron micrographs of male clasper and dorsal organ unique characters of *Cyclestheria* that are of phylogenetic importance and photographs of its morphological forms – parthenogenetic female, sexual female, white female, and male. In literature, *C. hislopi* is quoted as a unisexual species in India and Asia. In our study, males occur in the population but are of a periodic nature. The periodicity of appearance of male is linked to environmental changes such as onset of dry season, that the species is able to detect and bring corresponding changes in its life cycle by altering its egg production, leading to the appearance of sexual forms, the male and the female, resulting in sexual reproduction and production of diapause eggs or resting eggs. *C. hislopi* exhibits a different strategy for life in a seasonal pond. A

seasonally controlled production of diapause eggs or ephippia is a feature of its life cycle.

7. In Conchostraca, the reproductive mode of individual species can be inferred by the absence or presence or proportionate abundance of males in the population samples. When males are abundant, the species is considered to be sexual or gonochoric (as in *L. alleppeyensis*); when males are absent, the species is considered to be unisexual, with either parthenogenetic (as in *C. hislopi*) and selfing hermaphroditic (as in *E. colombiensis*, population from KRA site. When males are rare, or if males are present in some generations only, the species is considered to show cyclic parthenogenesis with parthenogenesis being prominent and sexual reproduction occurring with the onset of dry season as in *C. hislopi*. When males are less than females/hermaphrodites (female biased sex ratio) as in *E. colombiensis,* a mixed mating system occurs in which females/hermaphrodites, can self fertilize or mate with males but cannot outcross with other female/hermaphrodites.
8. Representative species for the order Spinicaudata and Laevicaudata have been described.
9. Conchostracans of Kerala occur in ephemeral pools, but *Cyclestheria* occurs in permanent waters too.

TAXONOMIC KEY TO THE SPECIES OF CONCHOSTRACA, KERALA

1. Size 1 to 7 mm; body soft and flexible, covered by a chitinous hard bivalved clam like shell; 10 to 32 pairs of legs; second antennae large and biramous........................ Order CONCHOSTRACA Sars, 1867......**2**

2. a. Dorsal union of the carapace valves a true hinge with a groove; shell without growth lines; head free to protrude from enclosing carapace; telson feebly developed and covered ventrally by opercular lamella......................
.... .Order LAEVICAUDATA Linder, 1945 .. **3**

   b. Dorsal union of valves a simple fold; shell with growth line; head not protrusible; no opercular lamella on telson. ....
....Order SPINICAUDATA Linder 1945 .. **5**

3. Shell globular and smooth; head large in relation to body size; caudal furca absent. .... LYNCEIDAE Stebbing, 1902
second male thoracopod unmodified and similar to posterior thoracopod; rostral carina entire. .... LYNCEUS Müller, 1776........**4**

4. a. Rostrum broad, spatulate and serrated in female; serrations absent in male; movable finger of clasper sickle shaped, equal to that of immovable finger; minute granules on the margin of second endite of clasper; spherical eggs with frilled ridges that intersect. ....
.... **Lynceus alleppeyensis** Balaraman & Nayar 2004

b. Rostrum broad and truncate in male; tridentate in female; movable finger of clasper sickle shaped, extends beyond the setose margin of the immovable finger. ………… **Lynceus brachyurus** Müller, 1776

5. a. Shell almost circular; first antennae unsegmented, club-shaped with sensory hairs at tip; first pair of thorocopod modified into clasper in male ………………………………… … CYCLESTHERIIDAE Sars, 1887 ….. **6**

b. Shell ovate, laterally compressed; frontal organ pedunculate; first two pairs of thoracopods modified into claspers in male. …………….. ………………………………... LIMNADIDAE Burmeister, 1843 …..7

6. Ocellus close to compound eyes;serrations on head; larval development inside brood pouch of female. ….. …….. **Cyclestheria hislopi Baird,** 1859

7. a. Rostrum rounded in female and pointed in male; large palp of first clasper with equal segments; eggs cylindrical with ridges and valleys, rim flared and ends flat. ………………. **Eulimnadia colombiensis** Roessler, 1989

b. Rostrum rounded; large palp of clasper with unequal segments; egg round deep fissure like pockets and raised ridges. … …………………………… ………… **Eulimnadia michaeli** Nayar & Nair 1968

STUDIES ON CONCHOSTRACA (CRUSTACEA) OF KERALA

Thesis submitted to the University of Calicut
For the award of the Degree of
Doctor of Philosophy
In Zoology

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