The eggs of *Afronurus* LESTAGE, 1924 (Ephemeroptera: Heptageniidae): A cue for phylogenetic relationships

CARLO BELFIORE
Dipartimento di Zoologia Università di Napoli, Via Mezzocannone 8, 80134 Napoli, Italy

HELEN BARBER-JAMES
Albany Museum, Somerset St., Grahamstown, 6139, South Africa

ELDA GAINO
Dipartimento Biologia Animale ed Ecologia Università di Perugia, Via Elce di Sotto, 06123 Perugia, Italy

Abstract

Scanning electron microscopy (SEM) investigation carried out on the eggs of *Afronurus scotti, A. harrisoni, A. peringueyi,* and *A. ugandanus,* has provided some chorionic details useful for a comparison between these species. In all the species, the egg chorion is decorated with sparse granules and shows two kinds of knob-terminated coiled threads (KCTs). The first kind is represented by small KCTs (5-7 µm in diameter) densely concentrated at each egg pole; the second kind encompasses large KCTs (60-80 µm in length) situated equatorially. The micropyle has an oval to round sperm guide (15-20 µm long; 12-18 µm wide), and a well-defined rim. The polar KCTs expand towards the equatorial KCTs in *A. scotti*, *A. harrisoni,* and *A. peringueyi*, but in *A. ugandanus* there is a large clear area between polar and equatorial KCTs. A chorionic pattern very close to that of these *Afronurus* species is shared only by species belonging to the Asiatic genus C*inygmina.* In addition, examination of the labrum of the nymphs belonging to both genera, indicates a very similar morphology, in the curved sclerification in the basal region of the labrum, a feature that contrasts with the almost straight shape presented by other heptageniid genera (e.g. *Electrogena* species). Egg and labrum characteristics are advocated to hypothesise a close phylogenetic relationship between the South-African *Afronurus* and the Asiatic *Cinygmina* genera.

Keywords: chorionic pattern, knob-terminated coiled threads, *Cinygmina* genus, SEM.

Introduction

A provisional checklist of the South African Ephemeroptera was presented by McCafferty and de Moor (1995). A more recent checklist, including synonyms, can be seen at an active website (Barber-James, 2002). Such lists provide a useful start for evaluating the state of systematics in an area such as southern Africa, where there is a high biodiversity but many species are still undescribed. These checklists provide the basis for understanding the historical biogeography of the Southern Hemisphere mayflies in relation to the evolution of the world lineages. The lists include several species belonging to the genus *Afronurus,* namely *A. barnardi* SCHOONBEE, 1968, *A. harrisoni* BARNARD, 1932, *A. oliffi* SCHOONBEE, 1968, *A. peringueyi* (ESBEN-PETERSEN, 1913), *A. scotti* SCHOONBEE, 1968, *A. ugandanus* KIMMINS, 1956, and another undescribed species.

The genus *Afronurus* was erected by Lestage, 1924 for some South African representatives belonging to the Heptageniidae. Later, some other species from Eastern Asia (i.e., Flowers and Pescador, 1984; Kang and Yang, 1994) were referred to this genus.

The eggs of *Afronurus peringueyi* were formerly described by Koss and Edmunds (1974) using light microscopy. These authors considered the *Afronurus* eggs to be very similar to those of *Ecdyonurus yoshidae* and *Cinygmina*? sp. and question the generic assignment of *E. yoshidae*. Only the egg of *Cinygmina*? sp. was figured *in toto*.

Cinygmina is an Asiatic genus, characterised mainly by the penis arrangement in male imagines (lateral lobes expanded, presence of titillators). We confer with the suggestion of Koss and Edmunds that *E. yoshidae* is incorrectly placed. On the basis of the diagnosis of *Cinygmina*, also *E. yoshidae* should be transferred to this genus (You *et al.*, 1981).

In this paper we present the SEM pictures of the eggs of some South African species of *Afronurus* and discuss the affinities and differences between *Afronurus* and *Cinygmina,* also taking into account the morphology of the nymphal labrum of some of the species belonging to both genera and comparing this to the shape shown by *Electrogena* species, where the structure is seen to be different.

Research Update on Ephemeroptera & Plecoptera
2003, E. Gaino (Ed.), University of Perugia, Perugia, Italy.

Material and Methods

The eggs examined using SEM were taken from specimens collected in South Africa and preserved in alcohol, as follows:

Afronurus peringueyi (ESBEN PETERSEN, 1913): Cunene River, mature female nymph, 17°01'12"S 12°56'42"E, 20/XI/1997; *A. harrisoni* BARNARD, 1932: Elandspadkloof River female subimago, 33°45'S 19°07'E, 7/III/1961; *A. ugandanus* KIMMINS, 1956: Cunene River, female subimago, 17°00'18"S 13°25'52"E, 17/XI/1997; *A. scotti* SCHOONBEE, 1968: Elands River, mature female nymph, 25°34' 03"S 30°39'30"E, 06/IX/1991.

For scanning electron microscopy (SEM) observations, selected material was dehydrated by using ethanol gradients, followed by critical-point drying in a CO_2 Pabish CPD apparatus. Specimens were mounted on stubs with silver conducting paint, sputter-coated with gold-palladium in a Balzer Union Evaporator, and observed with a Philips EM XL 30 at the Electron Microscopy Centre of the University of Perugia.

For comparative analysis, the structure of the labrum of several species of Ecdyonurini, was examined. The following species were considered: *Afronurus harrisoni*, *A. peringueyi*, *A. oliffi*, *A. scotti*; *Cinygmina cf. rubromaculata* YOU, WU, GUI & HSU, 1981 (Hong Kong); *Electrogena galileae* (DEMOULIN, 1973) (Israel), *E. affinis* (EATON, 1883) (Poland); *E.* sp. (Turkey). Eggs were also dissected from a female subimago of *Cinygmina cf. rubromaculata* (China, Hong Kong, Tan Shan River, 13.2.1998, Tong Xiaoli leg.) for comparing egg morphology of this genus with that of *Afronurus*.

Results

Egg size (the reported values must be considered merely indicative owing to the limited number of eggs that were measured) - *A. scotti*: 154x109 µm; *A. harrisoni*: 157x114 µm; *A. peringueyi*: 152x103 µm; *A. ugandanus*: 140x125 µm.

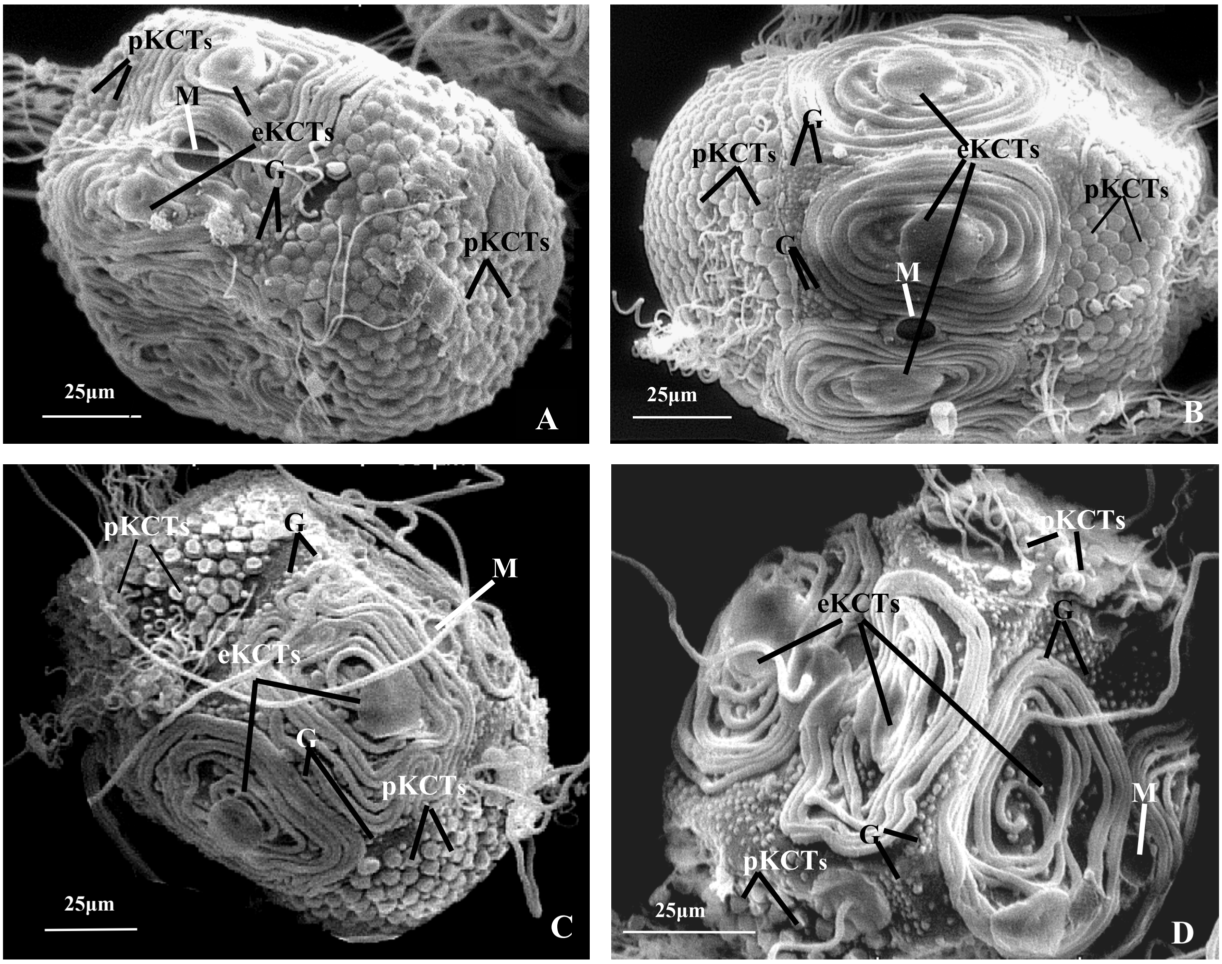

Fig. 1 - SEM images of *Afronurus* eggs: A) *Afronurus scotti*; B) *A. harrisoni*; C) *A. peringueyi*; D) *A. ugandanus*. Note the polar KCTs (pKCTs) and the large sized equatorial KCTs (eKCTs). G, granules; M, micropyle.

The chorionic pattern of the eggs of all the examined species is decorated with granules and with two kinds of knob-terminated coiled threads (KCTs):

1) small KCTs (coils 5-7 μm in diameter) concentrated at each pole;

2) much larger oval KCTs (coils 60-80 μm in length) located equatorially (Fig. 1A-D).

Whereas the polar KCTs coil about their own base and the terminal knob completely covers them, the equatorially KCTs assume a plano-spiral configuration in such a way that, proceeding from the centre of each KCT towards its periphery, each coil is completely enveloped by the next one. As a consequence, being limited to the central part of the coil, the terminal knob leaves most of the threads uncovered. In addition, SEM images show that equatorial threads are longer and wider than the polar ones.

The micropyle has an ovoid to round sperm guide (15-20 μm long; 12-18 μm wide), with a well-defined rim (Fig. 1A, B). The micropyle is interposed between adjacent equatorial KCTs.

Both kinds of KCTs become unravelled easily, thus making the eggs difficult to manipulate for SEM observations. Consequently, eggs often appear distorted, partially wrapped in their extended threads forming a complex network entrapping numerous eggs.

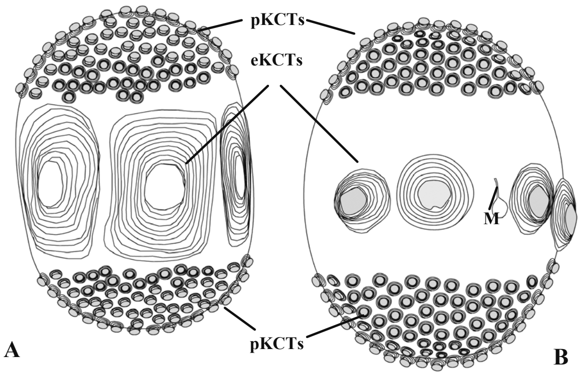

Fig. 2 – Schematic drawing of eggs of *Afronurus peringueyi* (A) and *Cinygmina cf. rubromaculata* (B). Note the different size of the equatorial KCTs; pKCTs, polar KCTs; eKCTs, equatorial KCTs; M, micropyle.

On the basis of the polar and equatorial KCT arrangement, the eggs are subdivided, as follows: (i) the eggs of *A. scotti* (Fig. 1A), *A. harrisoni* (Fig. 1B) and *A. peringueyi* (Fig. 1C) share a similar arrangement, being characterised by polar KCTs tightly adherent to one another and uniformly covering the polar region. Polar KCTs extend towards the equatorial surface and reach the proximity of the large KCTs; (ii) the eggs of *A. ugandanus* (Fig. 1D) differ from the other species owing to the presence of a clear cut region between the polar and equatorial KCTs. In particular, this region of the chorionic surface that lacks polar KCTs shows a sparse covering of granules.

By comparing the eggs of *Afronurus* with those of *Cinygmina* (Fig. 2), it is evident that in *Afronurus* the egg chorionic surface is almost completely covered with KCTs, a feature mainly due to the large sized equatorial KCTs (Fig. 2A), whereas in *Cinygmina* equatorial KCT's are smaller, thereby leaving a wide area of the chorion uncovered (Fig. 2B).

The difference at generic level, based on the chorionic pattern, is not confirmed by the morphological analysis carried out on the nymphal stages. Indeed, nymphs of the whole complex of *Afronurus*-*Cinygmina* species are very similar. We report here on the morphology of the labrum, where the curved sclerotised bases characteristically distinguish this complex from the very similar nymphs of *Electrogena* where this region is almost straight (Fig. 3).

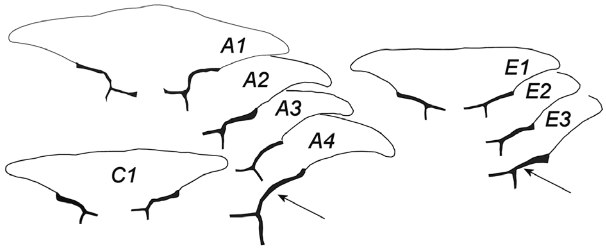

Fig. 3 – Labrum of several species of Ecdyonurini showing either the curved or straight shape of the sclerotized base: A1) *Afronurus harrisoni*; A2) *A. peringueyi*; A3) *A. oliffi*; A4) *A. scotti*; C1) *Cinygmina cf. rubromaculata* (Hong Kong); E1) *Electrogena galileae* (Israel); E2) *E. affinis* (Poland); E3) *E.* sp. (Turkey).

Discussion

Within the Heptageniidae, the large equatorial KCTs have so far only been recorded in *Afronurus* and *Cinygmina* species. Indeed, in the eggs of other genera of heptageniids having a chorion decorated with KCTs (i.e., *Ecdyonurus*, *Rhithrogena*, *Electrogena*), these structures are reduced in size (like those present at the egg poles of *Afronurus*) and the terminal knob, independently from the KCT's position on the chorionic surface, completely covers the coiled threads underneath (Gaino and Mazzini, 1987; Gaino *et al.*, 1987, 1989). Nevertheless, the size of these peculiar large equatorial KCTs differ when comparing the eggs of African species with those of the Asian ones (either *Afronurus* or *Cinygmina*), being larger in the former than in the latter. This feature can also be deduced by observing the SEM image of the egg of *A. philippinensis* (Flowers and Pescador, 1984).

KCTs have been considered for a long time as attachment structures presumably preventing eggs from being dragged into an unfavourable environment (Koss and Edmunds, 1974). The adhesive function of KCTs has been tested in heptageniids along with their fine organisation (Gaino and Mazzini, 1987, 1988). It seems reasonable to assume that these structures should function to assure egg anchorage to the substrate, adhering directly to it with their terminal knobs or wrapping eggs into a network of threads promoting their marked cohesion in masses. Egg cohesion in masses after deposition in water is fairly common in Ephemeroptera and represents a successful mechanism protecting eggs from shear stress (Gaino and Rebora, 2001).

The shared large KCTs can be considered a synapomorphy of *Afronurus* and *Cinygmina* and highlight the close relationship between the two genera. This fact, together with nymphal similarities, could substantiate a synonymy between *Afronurus* and *Cinygmina*.

Male genitalia (Belfiore, personal observations) are of very disparate kinds: they lack titillators in all African species and in some Asian ones. Shapes are varied and not very useful for grouping species.

Acknowledgements

We wish to thank Tong Xiaoli (Hong Kong), Mustafa Tanatmis (Eskisehir), Alain Thomas (Toulouse) and Michel Sartori (Lausanne) for the loan of *Cinygmina* and *Electrogena* specimens.

Afronurus material was borrowed from the Albany Museum, Grahamstown, South Africa and the Directorate of Museum and Heritage Resources, Eastern Cape, is thanked for supporting mayfly research.

References

Barber-James H.M., 2002. A checklist of South African Mayflies. http://www.ru.ac.za/academic/departments/zooento/Martin/EphemeropteraAfrica.html.

Flowers R. W., Pescador M. L., 1984. A new *Afronurus* (Ephemeroptera: Heptageniidae) from the Philippines. Int. J. Entomol. 26(4): 362-365.

Gaino E., Belfiore C., Mazzini M., 1987. Ootaxonomic investigation of the Italian species of the genus *Electrogena* (Ephemeroptera, Heptageniidae). Boll. Zool. 54: 169-175.

Gaino E., Mazzini M., 1987. Scanning electron microscopy of the egg attachment structures of *Electrogena zebrata* (Ephemerotera: Heptageniidae). Trans. Am. Microsc. Soc. 106: 114- 119.

Gaino E., Mazzini M., 1988. Fine structure of the of the chorionic projections of the egg of *Rhithrogena kimminsi* Thomas (Ephemeroptera: Heptageniidae) and their role in egg adhesion. Int. J. Insect Morphol. & Embryol. 17: 1-7.

Gaino E., Mazzini M., Degrange C., Sowa R., 1989 – Etude en microscopie balayage des oeufs de quelque especies de *Rhithrogena* Eaton groupe *alpestris*.Vie Milieu 39: 219-229.

Gaino E., Rebora M., 2001. Synthesis and function of the fibrous layers covering the eggs of Siphlonurus lacustris (Ephemeropetra, Siphlonuridae). Acta Zool. 82: 41-48.

Kang S. C., Yang C. T., 1994. Heptageniidae of Taiwan (Ephemeroptera). J. Taiwan Mus. 47(1): 5-36.

Koss R. W., Edmunds G. F., 1974. Ephemeroptera eggs and their contribution to phylogenetic studies on the order. Zool. J. Linn. Soc. 55: 267-349.

Lestage J. A., 1924. Les ephemeres de l'Afrique du Sud. Catalogue critique et systematique des espèces connues et description de trois genres nouveaux et de sept espèces nouvelles. Rev. Zool. Afr. 12: 316-352.

McCafferty W.P., de Moor F.C., 1995. South African Ephemeroptera: Problems and priority. In: Corkum L.D. and Ciborowski J.J.H., (eds). Current directions in research on Ephemeroptera. Canadian Scholars' Press Inc., Toronto. pp. 463-476.

You D., Wu T., Gui H., Hsu Y. 1981. Two new species and diagnosis characters of genus *Cinygmina* (Ephemeroptera: Ecdyonuridae). J. Nanjing Normal College (Nature Sciences) 3: 26-32.