FINE EXTERNAL MORPHOLOGY OF THE EGGS OF *EPHORON ALBUM* (SAY) AND *EPHORON SHIGAE* (TAKAHASHI) (EPHEMEROPTERA, POLYMITARCYIDAE)

ELDA GAINO
Istituto di Zoologia dell' Università, Via Elce di Sotto, 06100 Perugia, Italy

and JOHN FLANNAGAN
Freshwater Institute, Central and Arctic Region, Canada Department of Fisheries and Oceans, 501 University Crescent, Winnipeg, Manitoba, Canada R3T 2N6

Abstract

The Canadian Entomologist **127**: 527–533 (1995)

Eggs of *Ephoron album* and *E. shigae* were studied by scanning electron microscopy. They showed similar structure of both polar cap and micropyles, but had different chorionic patterns. Micropyles were of the tagenoform type and, whereas in most mayflies they are equatorial or subequatorial, in these eggs they were located close to the apical pole. The chorion was smooth in *E. album* and had a large mesh reticulation in *E. shigae*. The effect of water in causing the cap to swell and expand was tested by fixing eggs of *E. album* at different time intervals after their mechanical deposition in water. The polar cap was a composite apparatus, consisting of finger-like projections with slight terminal enlargements, which arose from a common hood. The latter was connected via a thread bundle to the chorionic surface. In turn, the thread bundle arose from a chorionic collar located immediately underneath the hood. Each finger-like projection was composed of a large number of thin threads packed together, each ending in a small terminal knob. The function of the polar cap was discussed in relation to egg–water interaction.

Gaino, E., et J. Flannagan. 1995. Morphologie externe des oeufs d'*Ephoron album* (Say) et d'*Ephoron shigae* (Takahashi) (Ephemeroptera, Polymitarcyidae) à l'échelle microscopique. *The Canadian Entomologist* **127**: 527–533.

Résumé

Les oeufs d'*Ephoron album* et d'*Ephoron shigae* ont été étudiés au microscope électronique à balayage. La structure de la calotte polaire et des micropyles de l'oeuf est semblable chez les deux expèces, mais la structure du chorion n'est pas la même. Les micropyles sont de type tagénoforme et sont situés près du pôle apical, contrairement à ce qui prévaut chez les autres éphémères où ils sont généralement en position équatoriale ou subéquatoriale. Le chorion est lisse chez *E. album* alors qu'il comporte une réticulation à larges mailles chez *E. shigae*. L'effet de l'eau sur le renflement et l'expansion de la calotte a été éprouvé par fixation des oeufs d'*E. album* à divers intervalles après leur plongée dans l'eau. La calotte polaire est un appareil complexe composé de prolongements digitiformes légèrement renflés dans leur partie terminale et issus d'une coiffe commune. Celle-ci est reliée par un faisceau de filamentes à la surface du chorion. Le faisceau de filaments est lui-même rattaché à un collet chorionique situé directement sous la coiffe. Chaque prolongement digitiforme est composé d'un grand nombre de filaments minces, serrés les uns sur les autres, chacun portant une petite massue terminale. Le rôle de la calotte polaire est examiné en relation avec l'action de l'eau sur l'oeuf.

[Traduit par la Rédaction]

Introduction

The surface structure of mayfly eggs, as revealed by scanning electron microscopy, has provided a set of characters used for taxonomic purposes (Gaino and Mazzini 1984; Gaino et al. 1989, 1993; Malzacher 1982, 1986), complementing those exhibited by larvae and adults. As a result, articles describing new species of mayfly, or clarifying the phylogenetical relationships among groups, include figures of egg structure (Alba-Tercedor and Sowa 1987; Sartori and Gillies 1990; Peters and Campbell 1991).

Characteristics of mayfly's egg were stressed in early studies in light microscopy (Degrange 1960; Koss 1968; Koss and Edmunds 1974). In addition, the investigation of chorionic microcharacters has enhanced our knowledge on the adhesive function performed by both egg envelopes (Gaino and Bongiovanni 1992*a*) and fibrous attachment structures (Gaino and Mazzini 1987, 1988; Gaino and Bongiovanni 1992*b*).

In this work we describe the eggs of *Ephoron album* and compare them with those of *E. shigae*, a species that is not found in North America, to demonstrate that eggs of species of this genus, even geographically widely separated, have a similar polar device and that chorionic decoration can be used in this genus to separate species.

Eggs of the genus *Ephoron* are unique among Ehemeroptera in having a multi-unit cap, consisting of threads compacted into several distinct units. They do not merge together to form a common terminal knob, as in other genera having eggs with polar caps (Koss and Edmunds 1974).

Material and Methods

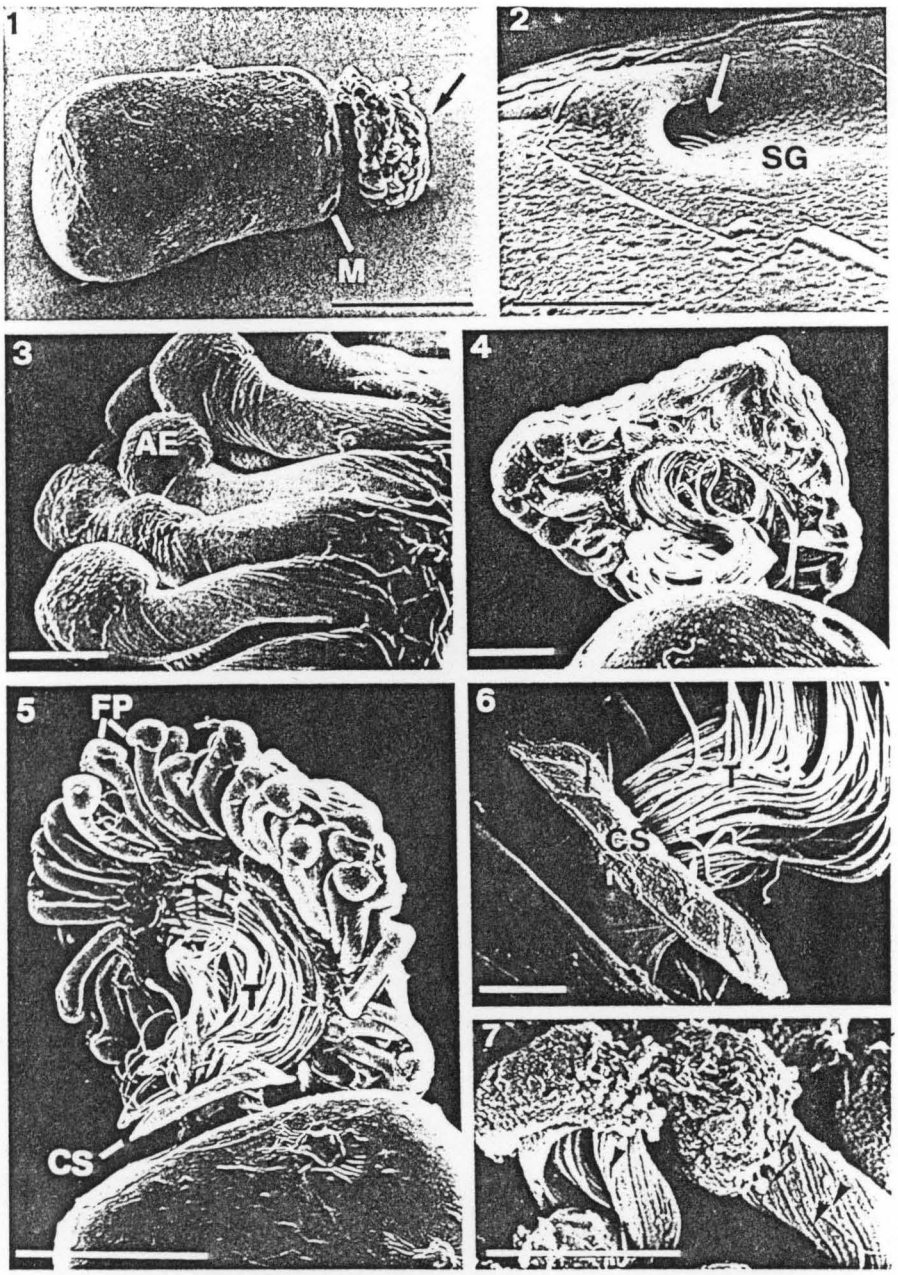
Eggs of *E. album* were obtained from adults collected along the Assiniboine River in summer 1993 (at Lido Plage Road, near St. Francois Xavier, Manitoba), for examination by scanning electron microscopy (SEM). Female abdomens were gently pressed to induce oviposition and eggs were allowed to settle in water-containing Petri dishes. In contact with water, egg polar devices undergo gradual changes giving inception to egg adhesion to the coverslips. Eggs were fixed in Karnovky's medium (1965) in cacodilate buffer (pH 7.2), at different times after deposition in water. Selected material was rinsed in the same buffer and dehydrated in an ethyl alcohol series. Eggs of *E. shigae* were taken from alcohol-preserved adults collected along the Asahi River (N. Watanabe leg., 14.9.1993), located close to Okayama (Japan). Eggs of both species were critical-point-dried using CO_2 in a Bomar apparatus, mounted with double-sided tape on SEM stubs, and sputter-coated with gold-palladium in a Balzers Union evaporator. They were observed with a Philips EM 515 electron microscope at 23 kV.

Results

Ephoron album (Figs. 1–7). Each egg is about 290 μm long and 125 μm wide at its equator. It has a smooth chorionic surface and tends to slightly enlarge toward the anterior pole where no chorionic differentiations are present. In contrast, the posterior pole shows distinct units gathered together to form a cap (Fig. 1). Immediately underneath the capped end of the egg, as many as five micropyles of the tagenoform type are visible. They are characterized by a sperm guide that leads to the proximal, expanded, micropylar canal raised above the chorionic surface (Fig. 2).

The polar cap consists of numerous finger-like protrusions, 30–32 μm long and 4–6 μm wide, each slightly enlarged toward its apex (Fig. 3).

When females are experimentally induced to lay eggs and these are fixed at different times after deposition in water (see Material and Methods), the fine organization of the polar cap can be investigated. Indeed, water contact triggers progressive extension of the polar cap, thereby revealing its complex architecture. This polar device consists of a common hood that supports the finger-like protrusions (Fig. 4). The hood is connected with the egg surface by a thread bundle (T, in Fig. 5), whose units are collectively twisted on themselves and compacted inside the space occurring between cap and egg surface (Fig. 5). This thread bundle emerges from a chorionic collar-shaped structure (CS, in Fig. 5), ending in an outwardly folded peripheral rim (Fig. 5). The collar is characterized by a series of thin rows arranged to form a sequence of lines (Fig. 6). Each finger-like protrusion stands out as a single unit (Fig. 3) and swells after eggs enter the water thus revealing it to be composed of an uncountable number of tightly packed, thin threads (Fig. 7). Each thread ends in a small



FIGS 1–7. 1, egg of *Ephoron album* [note the posterior pole with polar cap (arrow) and micropyles (M)] (scale bar = 100 μm); 2, detail of the tagenoform micropyle with the sperm guide (SG) leading to the proximal expanded micropylar canal (arrow) in relief on the chorionic surface (scale bar = 10 μm); 3, a sector of the polar cap showing a few finger-like projections slightly enlarging in their apical ends (AE) (scale bar = 10 μm); 4, polar cap about to lift from the chorionic surface (scale bar = 25 μm); 5, composite organization of the polar cap revealed by egg entering the water [note the row of finger-like projections (FP) raising from the common hood (arrows) connected with the chorionic surface via a thread bundle (T) sprouting out from a collar-shaped structure (CS)] (scale bar = 50 μm); 6, detail of the collar-shaped structure (CS), which envelops the thread bundle (T), showing its peripheral rim with a pattern of lines (arrows) (scale bar = 10 μm); 7, swelling of two finger-like projections that reveals the organization of a countless number of thin threads (arrowheads) ending in a small knob (arrows) (scale bar = 10 μm).

knob, this feature producing the granular appearance of the finger-like projections of this region.

Threads greatly swell in water and give rise to a polar network anchoring each egg to the substrate. The adhesive properties of the threads remain even after drastic treatments. Indeed, the adhesiveness of previously frozen eggs is so efficient that they cannot be dislodged from their substrate by the fasted flowing tap water.

Ephoron shigae (Figs. 8–14). Eggs of this species are about 280 μm long and 160 μm wide. Their morphology is very similar to that of *E. album* in regards to micropyle location, micropyle structure, and polar cap organization (Fig. 8), but eggs of *E. shigae* do not enlarge toward the anterior pole, and are thus more ovoidal. The finger-like projections (FP, in Fig. 9) are similar to those of *E. album*, although in *E. shigae* they tend to be longer (42–45 μm) and wider (6–9 μm). The pattern of tightly packed thin threads is evident even in alcohol-preserved material (Fig. 10) and has been confirmed by cross-sectioning them (Fig. 11). In contrast, the chorionic surface is not smooth but shows a large mesh reticulation (Fig. 8). Adhesive layers sometimes cover the egg surface or part of it, masking its chorionic pattern (Fig. 12). The reticulations delimit cavities (Fig. 13) that are included within the thickness of the chorion and contain adhesive material (Fig. 14).

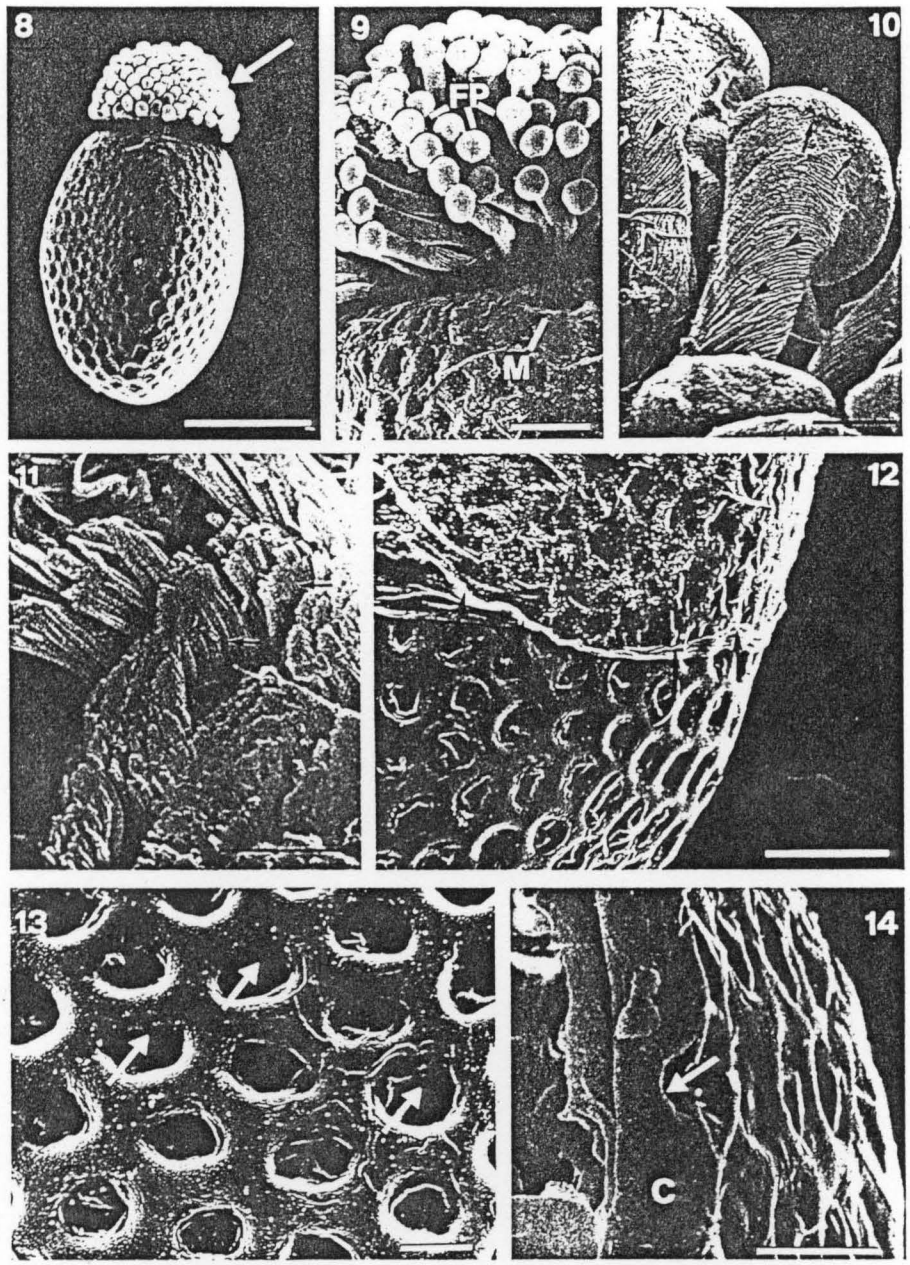
Discussion

The uniqueness of the polar cap of eggs of *Ephoron* was previously noticed by Degrange (1960) who described it as a complex of tubular-shaped projections arising from a ring connecting them to the chorionic surface. He emphasized the thread composition of each tubular-shaped projection, which was confirmed by later studies (Koss 1968; Koss and Edmunds 1974). The possible effect of water in causing the cap to swell and expand, however, was merely a guess. The present study showed that egg–water interaction gives rise to dynamic changes in the constitutive parts of the polar cap. This feature can be regarded as a strategy to enhance egg survival in water. Indeed, when it is immersed in water the polar device, apparently consisting of a solid structure, uncoils into two tiers of threads, one of which is joined to the egg chorion. It is possible that when the egg enters the water, the swelling of its polar device gives rise to the uncoiled configuration of the thread bundles supporting the hood with the finger-like projections. In such a configuration, the polar device may modulate egg descent in water allowing the eggs to disperse before settling on the substrate. Subsequently, thread swelling may create a network facilitating egg anchoring to the substrate. Indeed, in experimental conditions, Watanabe and Takao (1991) stated that the eggs of *E. shigae* became attached to the bottom of Petri dishes a while after their deposition in water.

The polar devices, referred to in the literature as "epithema" (Bengtsson 1913) or "polar caps" (Koss 1968; Koss and Edmunds 1974), have various configurations in mayflies, and have apparently evolved independently several times. They are considered to be apomorphic attachment structures evolved to anchor eggs so that they elicit the least resistance to water flow. Indeed, an adhesive function of the chorionic threads has been documented in several species (Degrange 1960; Gaino and Mazzini 1987; Provonsha 1990). These devices have been considered markers of adhesiveness. In other mayfly species, eggs lack differentiated structures for adhesion, and substrate anchorage is ensured by adhesive membranes (Gaino and Bongiovanni 1992*a*). These membranes keep the egg uplifted (Kosova 1967). Egg adhesion to the substrate seems to be crucial for successful egg hatching in *Ephoron* (N. Watanabe, personal communication).

Attachment disks have been described in some Plecopteran eggs whose elastic protein components take part in adhesion to the substrate (Rosciszewska 1991).

In the past, Polymitarcyidae was considered a lineage derived from ancestral Euthyplociidae (Koss and Edmunds 1974). Representatives of this latter family can be traced back to



FIGS. 8–14. 8, egg shape of *Ephoron shigae* [note the polar cap at the posterior pole (arrow)] (scale bar = 100 μm); 9, capped pole of the egg showing finger-like projections (FP) and one micropyle (M) underneath them (scale bar = 25 μm); 10, detail of two finger-like projections showing their pattern of thin threads (arrowheads) collectively merging in the enlarged terminal part (arrows) (scale bar = 5 μm); 11, cross section of the finger-like protrusions showing their thin constitutive threads (arrows) (scale bar = 5 μm); 12, adhesive layer (arrows) covering the chorionic surface (scale bar = 25 μm); 13, detail of the chorionic surface showing its pattern of large mesh reticulation delimiting chorionic cavities (arrows) (scale bar = 10 μm); 14, cross section of the eggshell pointing out the thickness of the chorion (C) with a cavity (arrow) partially covered by adhesive material (scale bar = 10 μm).

at least the Lower Cretaceous (McCafferty 1990). Non-burrowing Euthyplociidae was supposed to give rise to true burrowing Polymitarcyidae (Edmunds 1973). In a recent phylogenetic revision of Ephemeroidea, McCafferty (1991) considers traditional Polymitarcyidae as holophyletic, which also encompasses the former Euthyplociidae. In this study six subfamilies are included in Polymitarcyidae, which comprises Polymitarcyinae with the genus *Ephoron*.

As far as we know, *Ephoron* is the only genus whose members are characterized by polar openings for egg–sperm interaction, because in mayflies the location of micropyles is typically equatorial or subequatorial. This feature stresses the occurrence of derived traits in Polymitarcyinae that may be useful for tracing phyletic diagrams.

The different morphology of the chorionic pattern in *E. album* and *E. shigae* stresses the contribution of eggs to insect taxonomy.

The sequence of events following entry of the egg of *E. album* into water has allowed us to demonstrate (1) that egg-to-water interaction is crucial for revealing the complexity of the polar device and (2) that the threads are the basic constitutive components. A possible function of some of these components is adhesion to substrate.

Acknowledgments

We thank Franco Di Lauro for technical assistance, Naoshi Watanabe for providing eggs of *E. shigae*, and J. Alba-Tercedor, C. Pesce, D. Cobb, H. Kling, and P.M. Flannagan for reviewing the manuscript. This research was financially supported by M.U.R.S.T.

References

Alba-Tercedor, J., and R. Sowa. 1987. New representatives of the *Rhithrogena diaphana*-group from Continental Europe, with a redescription of *R. diaphana* Navas, 1917 (Ephemeroptera: Heptageniidae). *Aquatic Insects* **9**: 65–83.

Bengtsson, S. 1913. Undersökningar öfver äggen hos Ephemeriderna. *Entomologisk Tidskrift* **34**: 271–320.

Degrange, C. 1960. Recherches sur la reproduction des Ephéméroptères. *Travaux du Laboratoire de Hydrobiologie et Pisciculture de l'Université de Grenoble* **50/51**: 7–193.

Edmunds, G.F., Jr. 1973. Some critical problems of family relationships in the Ephemeroptera. pp. 145–154 *in* Peters, W.L., and J.G. Peters (Eds.), Proceeding 1st International Conference on Ephemeroptera. E.J. Brill, Leiden. 312 pp.

Gaino, E., and E. Bongiovanni. 1992*a*. Scanning electron microscopy of the eggs of *Palingenia longicauda* (Olivier) (Ephemeroptera: Palingeniidae). *International Journal of Insect Morphology and Embryology* **22**: 41–48.

——— 1992*b*. Comparative morphology of Epithemata (polar chorionic structures) in the eggs of *Ephemerella ignita* (Ephemeroptera: Ephemerellidae). *Transactions of the American Microscopical Society* **111**: 255–265.

Gaino, E., and M. Mazzini. 1984. Scanning electron microscope study of the eggs of some *Habrophlebia* and *Habroleptoides* species (Ephemeroptera, Leptophlebiidae). pp. 193–202 *in* Landa, V., T. Soldán, and M. Tonner (Eds.), Proceeding 4th International Conference on Ephemeroptera. Bechynè. Czechoslovak Academy of Sciences. 345 pp.

——— 1987. Scanning electron microscopy of the egg attachment structures of *Electrogena zebrata* (Ephemeroptera: Heptageniidae). *Transactions of the American Microscopical Society* **106**: 114–119.

——— 1988. Fine structure of the chorionic projections of the egg of *Rhithrogena kimminsi* Thomas (Ephemeroptera: Heptageniidae) and their role in egg adhesion. *International Journal of Insect Morphology and Embryology* **17**: 113–120.

Gaino, E., M. Mazzani, C. Degrange, and R. Sowa. 1989. Etude en microscopie à balayage des oeufs de quelques espèces de *Rhithrogena* Eaton groupe *alpestris* (Ephemeroptera, Heptageniidae). *Vie et Milieu* **39**: 219–229.

Gaino, E., M. Mazzini, and M. Sartori. 1993. Comparative analysis of the chorionic pattern in *Habroleptoides* species (Ephemeroptera, Leptophlebiidae). *Bollettino di Zoologia* **60**: 155–162.

Karnovsky, M.J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology* **27**: 137A–138A.

Kosova, A.A. 1967. A contribution on the ecology of the mayfly *Palingenia sublongicauda* Tshern. in the Volga delta. *Zoologicheskii Zhurnal* **46**: 1856–1859.

Koss, R.W. 1968. Morphology and taxonomic use of Ephemeroptera eggs. *Annals of the Entomological Society of America* **61**: 696–721.

Koss, R.W., and G.F. Edmunds, Jr. 1974. Ephemeroptera eggs and their contribution to phylogenetic studies of the order. *Zoological Journal of the Linnean Society* **55**: 267–349.

Malzacher, P. 1982. Eistrukturen europäischer Caenidae (Insecta, Ephemeroptera). *Stuttgarter Beitraege zur Naturkunde Serie A* **356**: 1–15.

——— 1986. Caenidae aus dem Amazonasgebiet (Insecta, Ephemeroptera). *Spixiana* **9**: 83–103.

McCafferty, W.P. 1990. Chapter 2. Ephemeroptera. pp. 20–50 *in* Grimaldi, D.A. (Ed.). Insects from the Santana Formation, Lower Cretaceous, of Brazil. *Bulletin of the American Museum of Natural History* **195**: 191 pp.

——— 1991. Toward a phylogenetic classification of the Ephemeroptera (Insecta): A commentary on systematics. *Annals of the Entomological Society of America* **84**(4): 343–360.

Peters, W.L., and I.C. Campbell. 1991. Ephemeroptera (Mayflies). pp. 279–293 *in* Naumann, I.D., et al. (Eds.), The Insects of Australia. Vol. 1, CSIRO, 2nd Edition of the Division of Entomology. Melbourne University Press, Melbourne. 542 pp.

Provonsha, A.V. 1990. A revision of the genus *Caenis* in North America (Ephemeroptera: Caenidae). *Transactions of the American Entomological Society* **116**(4): 801–884.

Rosciszewska, E. 1991. Ultrastructural and histochemical studies of egg capsules of *Perla marginata* (Panzer, 1799) and *Dinocras cephalotes* (Curtis, 1827) (Plecoptera: Perlidae). *International Journal of Insect Morphology and Embryology* **20**: 189–203.

Sartori, M., and M.T. Gillies. 1990. Further records of mayflies (Insecta: Ephemeroptera) from the Arabian Peninsula. Leptophlebiidae and Baetidae. *Fauna of South Arabia* **11**: 10–17.

Watanabe, N.C., and S. Takao. 1991. Effect of a low temperature period on the egg hatching of the japanese burrowing mayfly, *Ephoron shigae*. pp. 439–445 *in* Alba-Tercedor, J., and A. Sanchez-Ortega (Eds.), Overview and Strategies of Ephemeroptera and Plecoptera. Sandhill Crane Press, Gainsville, FL. 588 pp.

(Date received: 19 April 1994; date accepted: 2 March 1995)