Int. J. Insect Morphol. & Embryol., Vol. 17, No. 2, pp. 113–120, 1988
Printed in Great Britain

0020–7322/88 $3.00 + .00
Pergamon Press plc

FINE STRUCTURE OF THE CHORIONIC PROJECTIONS OF THE EGG OF *RHITHROGENA KIMMINSI* THOMAS (EPHEMEROPTERA : HEPTAGENIIDAE) AND THEIR ROLE IN EGG ADHESION*

ELDA GAINO

Istituto di Zoologia, Via Balbi 5, 16126 Genova, Italy

and

MASSIMO MAZZINI

Istituto di Zoologia, Via Muroni 25, 07100 Sassari, Italy

(*Accepted* 30 *September* 1987)

Abstract—Scanning (SEM) and transmission electron microscopical (TEM) observations of the eggs of *Rhithrogena kimminsi* (Ephemeroptera : Heptageniidae), a species of the *alpestris* group, revealed 2 kinds of chorionic projections, both characterized by knob-terminated coiled threads (KCTs). The former are concentrated at one egg pole, and arise directly from the shell surface. The latter are scattered on the egg chorion and are supported by a basal excrescence, giving them a peglike feature. At TEM level, KCTs, arising directly from the chorion, appear to be composed of fibers that are enveloped by filaments. The fibers are tightly twisted together and joined at their apicals, which end in a terminal knob. KCTs, supported by peglike projections, show a similar organization, but each thread derives from a single coiled fiber. The different numbers of fibers form wider threads at the egg polar region and thinner ones on the peglike projections. The involvement of both kinds of KCTs in egg adhesion is documented through the discharge of their threads.

Index descriptors (in addition to those in title): Ootaxonomy, egg chorion, attachment structures, scanning electron microscope.

INTRODUCTION

EPHEMEROPTERAN eggs are characterized by chorionic structures categorized according to their morphology and arrangement (Koss, 1968; Koss and Edmunds, 1974). A character state of attachment structures is represented by knob-terminated coiled threads (KCTs), which are frequently found on egg shells, and are considered to be adhesive devices. A recent SEM analysis of the egg chorion of the mayfly, *Electrogena*

* Research supported by the Italian National Research Council (CNR) under "Gruppo Nazionale di Biologia Naturalistica" Contrib. No. 3359340; by Ministry for Public Education (MPI) under 'Biogeografia delle terre del Mediterraneo Occidentale" and MPI "60%" funds.

zebrata confirmed their involvement in egg adhesion (Gaino and Mazzini, 1987). The presence of chorionic attachment structures is especially important in the Ephemeroptera, as it prevents most of the eggs from being carried away to an environment potentially unfavorable for their development.

Although the KCTs were described as composed of a large number of fibers closely twisted together (see review in Koss and Edmunds, 1974; Gaino and Mazzini, 1987), by both light microscopy and SEM, no data were available about their fine organisation by TEM.

We have attempted to document at SEM and TEM levels the chorionic pattern of the egg of *Rhithrogena kimminsi*, a species of the *R. alpestris* group, in which KCTs represent a common type of attachment structure (Gaino *et al.*, 1987b).

MATERIALS AND METHODS

Fully developed eggs were obtained from preserved mature nymphs of *Rhithrogena kimminsi*, a gift from Dr A. Thomas, University of Grenoble and from Professor R. Sowa, University of Krakow. These specimens were collected in Ruisseau d'Ourtigué at 1180 m (20 August 1986) and Ruisseau de la Mousquère at 950 m (19 July 1985), in France.

For SEM analysis, the eggs, stored in alcohol, were dried, mounted on a specimen holder, placed in a vacuum evaporator, sputtered with gold–palladium in a Balzer's Union evaporator and observed in a Philips 505 SEM.

For TEM observations, some eggs were fixed again for 1 hr in 5% glutaraldehyde–4% formaldehyde in 0.1 M cacodylate buffer at pH 7.2. After rinsing in the buffer, eggs were postfixed for 1 hr in 1% osmium tetroxide in 0.1 M cacodylate buffer at pH 7.2. They were dehydrated in a graded series of ethanols, and embedded in an Epon–Araldite mixture. Silver-to-pale gold sections were cut on a Reichert ultramicrotome and mounted on Formvar-coated copper grids. After staining in aqueous uranyl acetate and lead citrate, sections were examined in Philips 300 and 400 electron microscopes.

RESULTS

The eggs of *R. kimminsi* are oblong, measuring about 220 × 115 μm, and have a chorionic pattern of short peglike projections arranged to form longitudinal rows (Fig. 1). The micropyles (3 or 4) are generally located in the subequatorial area, and consist of both a chorionic depression, called the sperm guide, and a tunnel-like micropylar canal penetrating into the egg. The junction point between sperm guide and micropylar canal is known as the micropylar opening. Each peglike projection is characterized by a basal cylindrical formation, emerging from the chorion, 7 μm high and 3 μm wide, terminating in a distinct tightly spiraled thread, covered apically by a thin circular knob, 2.4 μm in diameter (Fig. 2). The diameter of the thread is much less (0.6 μm) than that of the supporting excrescence. In fact, a clear division occurs between the 2 different parts (Figs 2; 3). The basal cylinder-shaped structure has the same rugose surface as the chorionic background but lacks any trace of the tubercles that are scattered on the chorion (Figs 2–6). All the peglike structures are arranged with their basal excrescences bent towards the same polar region (Fig. 4) owing to the adhesion of threads to the chorion (Fig. 4). Despite this adhesion, each thread still clearly stands out from the

FIG 3. Peglike projections showing a clear separation (arrows) between basal excrescence (B) and knob-terminated coiled thread (KCT). × 4500.

FIG 4. Peglike projections bent over and arranged with their knob-terminated coiled threads directed towards same polar region. × 4100.

FIG 5. A peglike projection sends out its knob-terminated coiled thread. × 4300.

FIG 6. Peglike projection in which knob-terminated coiled thread shows its extended configuration (arrows). × 2200.

FIG. 1. SEM of an egg of *R. kimminsi* showing chorionic pattern of peglike projections and 2 micropyles (arrows). × 380.

FIG. 2. Three peglike projections, each characterized by a basal excrescence (B) supporting a knob-terminated coiled thread (KCT). × 6700.

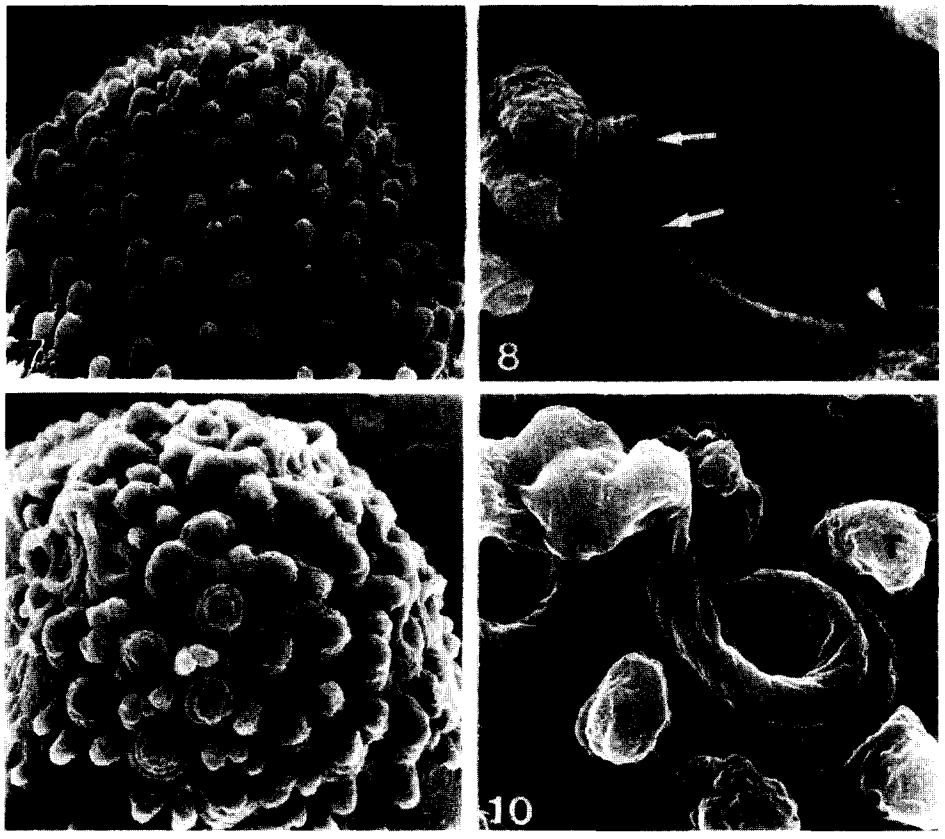

FIG. 7. Extended peglike projections covering anterior pole of egg. × 1500.
FIG. 8. Mucous coat (arrows) around knob-terminated coiled threads of peglike projections connecting them to micropylar rim. Arrowhead shows the micropylar opening. × 3700.
FIG. 9. Polar cap of knob-terminated coiled threads at posterior pole of egg. × 1100.
FIG. 10. Twisted shape of an extended thread among chorionic tubercles (T) at polar region. × 4100.

chorionic background. The threads are seen to gradually extend (Fig. 5) until they are completely uncoiled (Fig. 6). When the thread is completely extended, it is about 3 times as long as the basal excrescence (Fig. 6). The peglike structures completely cover the anterior pole of the egg (Fig. 7) and may also occur near the micropylar sperm guide (Fig. 8). The folding down of the peglike projections is sometimes associated with a sort of mucous coat around the coiled thread, which connects it to the chorion (Fig. 8).

Knob-terminated coiled threads (KCTs) are concentrated at the posterior polar region of the egg where the peglike structures disappear (Fig. 9). The threads of the KCTs' chorionic attachment structures are much larger than those of the peglike projections. Their extended configuration reveals the twisted shape of each thread surrounded by chorionic tubercles (Fig. 10).

Analysis at TEM level of both the posterior polar region, characterized by KCTs attached to the egg chorion, and the remaining part of shell surface where each KCT is supported by a chorionic excrescence, revealed the fine organization of KCTs. In the polar region, KCTs are arranged side-by-side among electron-opaque polygonal structures (Fig. 11), corresponding to the chorionic tubercles visible in the SEM. Each

KCT's complex organization consists of a coiled thread composed of clustered and coiled fibers supporting the terminal knob (Fig. 12). Fibers are closely associated with a central area of the supported terminal knob where they appear fused together, sustaining this structure (Fig. 13). About 8 or 9 fibers are seen to join in this region (Fig. 11). Terminal knob can be divided morphologically into 3 distinct regions (Fig. 13): the outer one is smooth and consists of amorphous material that is strongly electron-opaque; the middle one forms a transparent band; the inner one shows a sequence of membrane-like structures, which extend from the surface to form slender projections (Fig. 13). Every single fiber of about 0.3 μm in diameter, shows an electron-dense core of amorphous material, completely enveloped by filaments of 50 nm in diameter, tightly coiled around it (Fig. 13). At high magnification, each filament presents a dotted aspect, thus revealing a characteristic feature of periodic units (Fig. 14).

Analysing the peglike projections, their supported KCTs show an organization very similar to that of the polar ones, but the former derive from a single coiled fiber and are very thin (Fig. 15).

DISCUSSION

Attachment structures are commonly found in the Ephemeroptera. The different morphology and distribution over the egg surface of these chorionic projections can be utilized as a taxonomic character (Degrange, 1960; Flowers, 1980, 1986; Kopelke, 1980; Koss, 1968, 1970; Malzacher, 1982; Gaino and Mazzini, 1984; Gaino *et al.*, 1987 a, b; Sowa and Soldàn, 1984, 1986). A comparative evaluation of the data could also demonstrate some phylogenetic relationships inside the Ephemeroptera (Koss, 1968; Koss and Edmunds, 1974), albeit with some allowances made for possible convergence mechanisms.

Our observations on eggs of *R. kimminsi* by SEM and TEM, gave evidence of the fine organization of knob-terminated coiled threads (KCTs). They present different sizes and distribution on egg surface, according to their origin directly from the chorion, or supported by excrescences. In addition to this, KCTs differ in number of fibers. In fact, polar KCTs are composed of fewer fibers twisted together to compose a thread, while a single fiber constitutes the KCTs of peglike projections. Number of fibers and their degree of coiling may represent the result of different evolutionary trends.

Previous light microscopical observations have demonstrated the presence in some Leptophlebiidae of the so-called peglike chorionic structures, considered as adhesive devices (for review see Koss, 1968; Koss and Edmunds, 1974). Peglike projections were also found in 2 American species of *Rhithrogena* and were likewise presumed to be for attachment (Koss and Edmunds, 1974), although there were no data about their fine morphology or their mechanism of adhesion to the substrate. According to Koss and Edmunds (1974), peglike projections are included in the non-fibrous attachment structures, the most plesiomorphic type being found in the order. But their fine organization, at least in *R. kimminsi,* shows that each peglike structure has 2 different parts: a coiled thread deriving from an excrescence attached to the chorion. This feature is surprising and suggests that it would be more correct to include these chorionic projections in the group of fibrous attachment structures, namely coiled types with terminal knob.

The mucous coat connecting the apical thread of each peglike projection to the chorion, may play an important role in preserving the coiled configuration of the threads

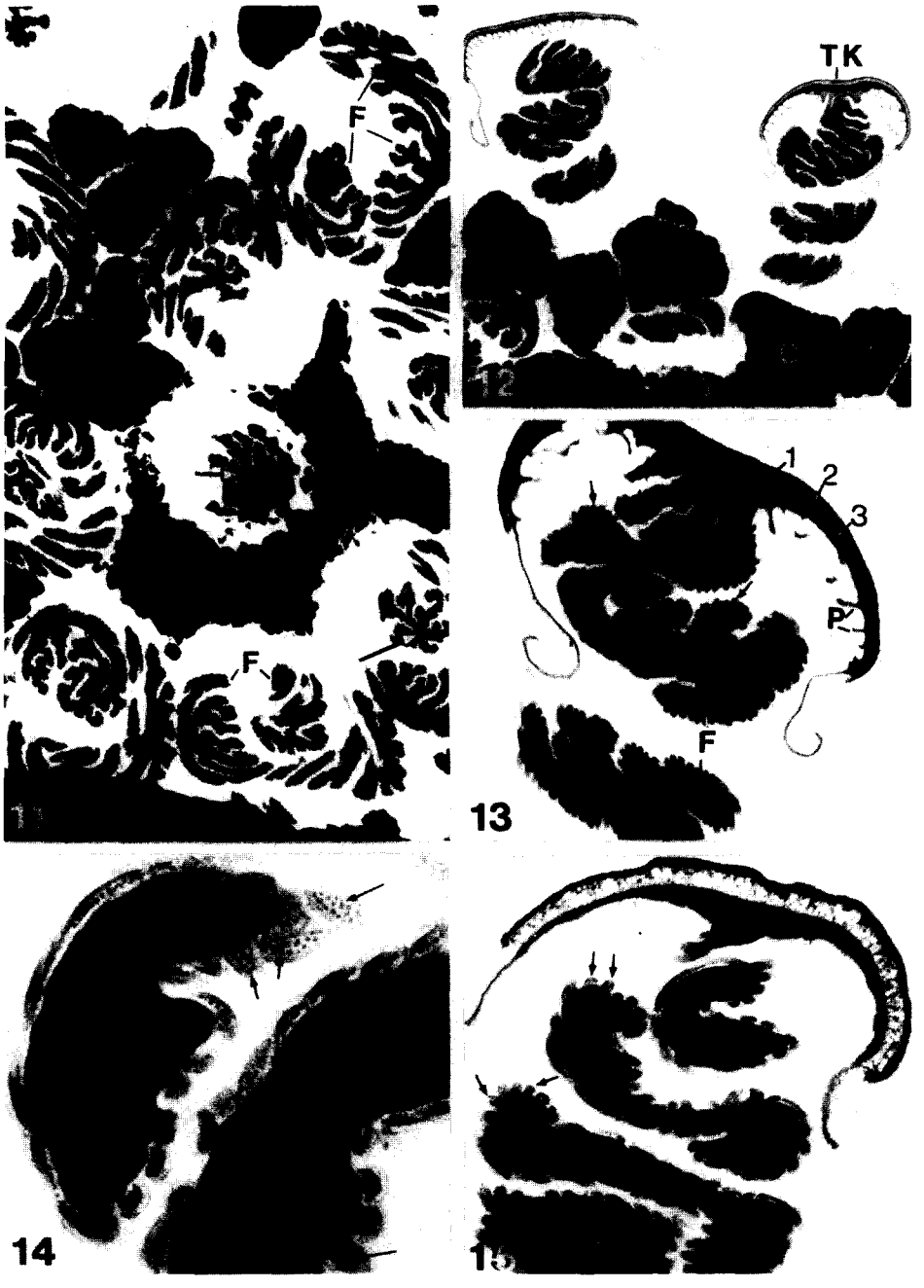

FIG. 11. A transversal section of knob-terminated coiled threads arranged at posterior egg pole. Each KCT, surrounded by chorionic tubercles (T) shows its organisation of tight spiraling fibers (F) which join together (arrows) in supporting terminal knob. × 2800.

FIG. 12. A longitudinal section of 2 KCTs arising from chorion (C) showing their coiled thread and terminal knob (TK). × 3700.

during the accumulation of eggs in the oviduct where each thread could stand out. Previous SEM investigations of *Electrogena zebrata* showed that the KCTs are coiled in their respective chorionic recesses and the breakdown of terminal knobs is involved in egg adhesion and triggers the spring-like uncoiling of the threads (Gaino and Mazzini, 1987). Also in *R. kimminsi* the transition to the uncoiled shape of both KCT kinds may be triggered by swelling in water after oviposition.

According to Koss and Edmunds (1974), the KCTs are the most apomorphic fibrous attachment structures. The peglike projections described here may be considered as an intermediate between more complex KCTs and simple tubercles.

The different distribution over the egg surface of KCTs and the peglike projections may be an advantage for survival of the eggs after deposition in water. *R. kimminsi*, a species included in the *alpestris* group, oviposits in running water and the speed of the current might carry the eggs downstream before they settle on the bottom. The attachment structures may hold the eggs firmly to the substratum. The most probable sequence involved in adhesion to the substrate is a rapid extension of the peglike knob-terminated coiled threads to anchor the eggs and to orient them in the position of least resistance to the water flow. Subsequently, permanent attachment is probably assured by the more specialized polar KCTs, as is evident from their thicker, stronger threads and from their wide terminal knobs.

Two kinds of chorionic projections can also be distinguished in other species of *Rhithrogena* of the *alpestris* group and the different morphology of the projections attached to a basal excrescence of the chorion, may furnish characters used in taxonomy at specific level (Gaino *et al.*, 1987b).

External sculpture-forming, club-like structures appears on the smooth chorion of Lepidoptera after egg deposition but, in this particular case, these structures result from exudation of the oocyte (Chauvin and Chauvin, 1980).

Further SEM and TEM analysis might be necessary to ascertain whether the so-called peglike projections observed in light microscopy in other species of Ephemeroptera are similar in shape to those of *R. kimminsi*; this should clarify whether the peglike organization observed in this species constitutes the typical feature of such chorionic sculpturing.

REFERENCES

CHAUVIN, J. T. and G. CHAUVIN. 1980. Formation des reliefs externes de l'oeuf de *Micropteryx calthella* L. (Lepidoptera : Micropterigidae). *Can. J. Zool.* **58:** 761–66.

DEGRANGE, CH. 1960. Recherches sur la reproduction des Ephéméroptères. *Trav. Lab. Hydrobiol. Pisc. Univ. Grenoble* **51:** 7–193.

FLOWERS, R. W. 1980. A review of the Neartic *Heptagenia* (Heptageniidae, Ephemeroptera), pp. 93–102. *In* J. F. FLANNAGAN and K. E. MARSHALL (eds). *Advances in Ephemeroptera Biology*. Plenum Press, New York, London.

FIG. 13. Three-layered terminal knob (TK) with outer (1), middle (2) and inner (3) region. In this latter arrangement of slender projections (P) is interrupted by fiber (F) junction. Fibers are enveloped by coiled filaments (arrows). × 13,000.

FIG. 14. High magnification of a fiber enveloped by coiled filaments (arrows) showing their dotted core. × 68,000.

FIG. 15. A knob-terminated coiled thread of a peglike chorionic projection, which shows its thread formed by a single coiled fiber (F) enveloped by filaments (arrows). × 28,500.

FLOWERS, R. W. 1986. Holarctic distributions of three taxa of Heptageniidae (Ephemeroptera). *Entomol. News* **97:** 193–97.

GAINO, E. and M. MAZZINI. 1984. Scanning electron microscope study of the eggs of some *Habrophlebia* and *Haproleptoides* species (Ephemeroptera, Leptophlebiidae), pp. 193–202. *In* V. LANDA *et al.*, (eds). *Proc. 4th Int. Conf. Ephemeroptera, Bechynè.* Czechoslovak Academy of Sciences.

GAINO, E. and M. MAZZINI. 1987. Scanning electron microscopy of the egg attachment structures of *Electrogena zebrata* (Ephemeroptera: Heptageniidae). *Trans. Amer. microsc. Soc.* **106:** 114–19.

GAINO, E., C. BELFIORE and M. MAZZINI. 1987a. Ootaxonomic investigations of the Italian species of the genus *Electrogena* (Ephemeroptera, Heptageniidae). *Boll. Zool.* **54:** 169–75.

GAINO, E., M. MAZZINI, CH. DEGRANGE and R. SOWA. 1987b. Les oeufs de quelques espèces de *Rhithrogena* Eaton du groupe *alpestris* (Ephemeroptera). Un étude en microscopie à balayage. *Vie Milieu* (in press).

KOPELKE, J. P. 1980. Morphologische Studien an den Eiern der Eintagsfliegen (Ephemeroptera) aus der Emergenz der zentralafrikanischen Bergbaches Kalengo. *Mitt. Schweitz. Entomol. Ges.* **53:** 297–311.

KOPELKE, J. P. and I. MÜLLER-LIEBENAU. 1981a. Eistrukturen bei Ephemeroptera und deren Bedeutung für die Aufstellung von Artengruppen am Beispiel der europäischen Arten der Gattung *Baetis* Leach, 1815 (Insecta : Baetidae). Teil II: *rhodani-vernus-* und *fuscatus-* Gruppe. *Spixiana* **4:** 39–54.

KOPELKE, J. P. and I. MÜLLER-LIEBENAU. 1981b. Eistrukturen bei Ephemeroptera und deren Bedeutung für die Aufstellung von Artengruppen am Beispiel der europäischen Arten der Gattung *Baetis* Leach, 1815 (Insecta : Baetidae). Teil III: *buceratus-*, *atrebatinus-*, *niger-*, *gracilis-* und *muticus-* Gruppe. *Dtsch. Entomol. Z. (N.F.)* **28:** 1–6.

KOPELKE, J. P. and I. MÜLLER-LIEBENAU. 1982. Eistrukturen bei Ephemeroptera und deren Bedeutung für die Aufstellung von Artengruppen am Beispiel der europäischen Arten der Gattung *Baetis* Leach, 1815 (Insecta : Baetidae). Teil I: *alpinus-*, *lutheri-*, *pavidus-* und *lapponicus-* Gruppe. *Gew. Abw.* **68–69:** 7–25.

KOSS, R. W. 1968. Morphology and taxonomic use of Ephemeroptera eggs. *Ann. Entomol. Soc. Amer.* **61:** 696–721.

KOSS, R. W. 1970. Ephemeroptera eggs: sperm guide morphology and adhesive layer formation. *Trans. Amer. Microsc. Soc.* **89:** 62–9.

KOSS, R. W. and G. F. EDMUNDS. 1974. Ephemeroptera eggs and their contribution to phylogenetic studies of the order. *Zool. J. Linn. Soc.* **55:** 267–349.

MALZACHER, P. 1982. Eistrukturen europäischer Caenidae (Insecta, Ephemeroptera). *Stuttg. Beitr. Naturk.* (Ser. A) **356:** 1–15.

MAZZINI, M. and E. GAINO. 1985. Fine structure of the egg shells of *Habrophlebia fusca* (Curtis) and *H. consiglioi* Biancheri (Ephemeroptera : Leptophlebiidae). *Int. J. Insect Morphol. Embryol.* **14:** 327–334.

SOWA, R. and T. SOLDÀN. 1984. Two species of *Rhithrogena* Eaton (Ephemeroptera, Heptageniidae) from Central Europe, pp. 75–84. *In* V. LANDA *et al.*, (eds). *Proc. 4th Int. Conf. Ephemeroptera, Bechynè.* Czechoslovak Academy of Sciences.

SOWA, R. and T. SOLDÀN. 1986. Three new species of the *Rhithrogena hybrida* group from Poland and Czechoslovakia with a supplementary description of *R. hercynia* Landa, 1969 (*Ephemeroptera, Heptageniidae*). *Bull. Entomol. Pologne* **56:** 557–72.

THOMAS, A. 1970. Sur la taxonomie de deux *Rhithrogena* des Pyrénées (Ephemeroptera, Heptageniidae). *Ann. Limnol.* **6:** 305–15.