

Scanning electron microscopy study of the eggs of some rare mayfly (Ephemeroptera) species: *Ametropus fragilis*, *Isonychia ignota* and *Neoephemera maxima*

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Abstract

Eggs of three rare lowland mayfly (Ephemeroptera) species: *Ametropus fragilis*, *Isonychia ignota* and *Neoephemera maxima* were studied by scanning electron microscopy (SEM). Eggs of *A. fragilis* are characterized by a polar cap structure (threads coiled around one pole of the egg) and large mesh chorionic reticulation. Eggs of *I. ignota* are spherical with differently sited knob-terminated coiled threads, densely located in one hemisphere and scattered in the other. Eggs of *N. maxima* have a fibrous envelope with finger-like projections consisting of numerous thin threads ending in small apical mushroom-like structures.

Keywords: Ephemeroptera, eggs, morphology, scanning electron microscopy (SEM).

Introduction

Eggs of Ephemeroptera are characterized by many structures, differing in morphology and arrangement. Egg adhesive layers and attachment devices have a mainly adhesive function (the attachment of eggs to the substratum). Chorionic pattern, attachment devices and micropyle organisation can also be used for taxonomical purposes, as well as, for understanding phylogenetic relationships between mayfly species (Gaino and Flannagan, 1995; Gaino and Mazzini, 1987, 1988; Gaino *et al.*, 1987, 1989; Mazzini and Gaino, 1990). Egg morphology of numerous mayfly species was described using light microscopy (Degrange, 1960; Koss, 1968; Koss and Edmunds, 1974). The use of scanning electron microscopy (SEM) enabled researchers to carry out a detailed study on mayfly egg fine structure.

In this work, we describe the eggs of three rare species inhabiting large and middle sized rivers: *Ametropus fragilis* ALBARDA, 1878, *Isonychia ignota* (WALKER, 1853) and *Neoephemera maxima* (JOLY, 1870). Original habitats are usually critically endangered or have become extinct owing to the pollution and degradation of the environment. These species are usually recorded as small isolated populations. SEM morphology of the eggs of these species allowed us to describe the structural features of their chorion. Eggs of *I. ignota* and *N. maxima* are described for the first time, and a description of *A. fragilis* eggs is completed here.

Material and Methods

Eggs were removed from: *Ametropus fragilis* - 2 female subimagines (Poland, Pilica river, near Sulejów, 18.03.1971, coll. T. Jażdżewska) and 4 female mature nymphs (Poland, Warta river, Rychłocie, 18.04.1968, coll. T. Jażdżewska); *Isonychia ignota* - 4 female mature nymphs (Poland, Warta river, near Strońsk, 9.07.1964, coll. T. Jażdżewska); *Neoephemera maxima* - 2 female imagines (Poland, Pilica river, near Koniecpol, 28.05.1972, coll. T. Jażdżewska). All were preserved in 75% alcohol. For the scanning electron microscopy examination, about 100 eggs from each species were dehydrated in the ethyl alcohol series, and critical-point dried using CO₂ in a Bomar apparatus, mounted with double-sided tape on SEM stubs and coated with carbon-gold. They were observed with a JEOL JSM 5410 electron microscope at 25 kV.

Terminology of egg structure follows Koss and Edmunds (1974).

Results

Ametropus fragilis (Figs. 1-5)

The egg is oval. Its dimensions are: length 210.5-242.1 μm , width 147.4-158.0 μm (Fig. 1). It is characterized by a chorionic surface with a pattern of large mesh reticulation (Figs. 1, 2). At high magnification, both fibrous structures of chorionic surface and mesh costae are visible (Fig. 3). The polar cap at one egg pole (Fig. 4a) is composed of many long, compactly coiled threads. The apical part of the polar cap shows many groups of threads with fibrous endings (Figs. 4a, b). A thin rim around the polar cap is evident (Fig. 4d). One micropyle of tageniform type is situated near the pole without a polar cap. The sperm guide is oval, chorionic-suprachorionic, 8.6-10.5 μm long and 4.8-5.7 μm wide, with a star-like chorionic pattern (Fig. 5).

Isonychia ignota (Figs. 6-10)

The egg is spherical, without obvious poles. Its diameter is 153.4-168.2 μm (Figs. 6a, b). The chorion is smooth and decorated with KCT attachment structures of two types: large (3.8-4.6 μm in diameter) and small (1.9-2.7 μm in diameter). Large KCTs densely cover one hemisphere (0.8-1.5 μm distance between them) (Figs. 7, 8a, b); small KCTs are scattered on the other hemisphere (3.0-6.2 μm distance between them) (Figs. 9a, b). Between both hemispheres, a smooth band measuring 37-53 μm wide is visible. Two or three micropyles of the tageniform type are located on the hemisphere with small KCTs. The sperm guide is fairly round, chorionic, 9.2-12.0 μm long and 8.5-12.0 μm wide. The micropylar rim is narrow (Fig. 10).

Neoephemera maxima (Figs. 11 - 19)

The egg is oval and its dimensions are: length 185.1-203.7 μm ; width 103.7-130.0 μm (Fig. 11). Dense thin threads cover the egg, forming a fibrous adhesive layer. These threads terminate in a small knob (diameter 0.2-0.4 μm) (Fig. 12). Finger-like projections (19.2-27.0 μm long) are situated at both egg poles and in 4 transversal rows around the egg: two rows below egg poles and the other two rows in a subequatorial area (Figs. 11, 13). The distance between these structures are 15.4-38.1 μm . These finger-like projections consist of many thin threads (like those covering the chorion) (Fig. 14). At the basal part of these projections, groups of thin threads are visible (Figs. 14, 15); in the middle and apical part

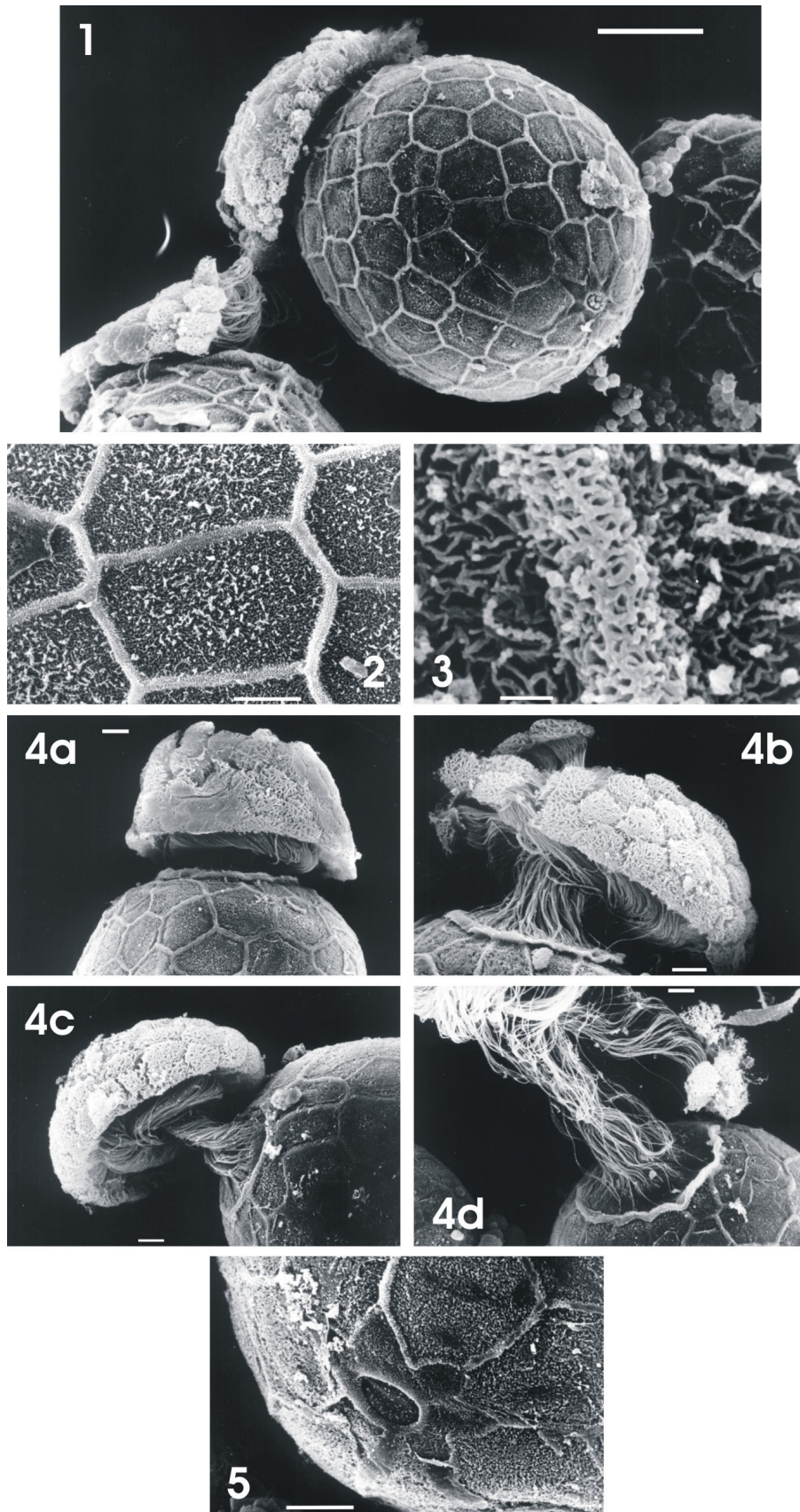
of the projections, the threads end in mushroom-like structures (diameter 0.75-1.0 μm) (Figs. 16, 17). Three or four micropyles of poorly developed tageniform type are located in the equatorial area (Fig. 18). The sperm guide is oval, suprachorionic, 22.0-28.6 μm long and 15.2-17.1 μm wide (Fig. 19).

Discussion

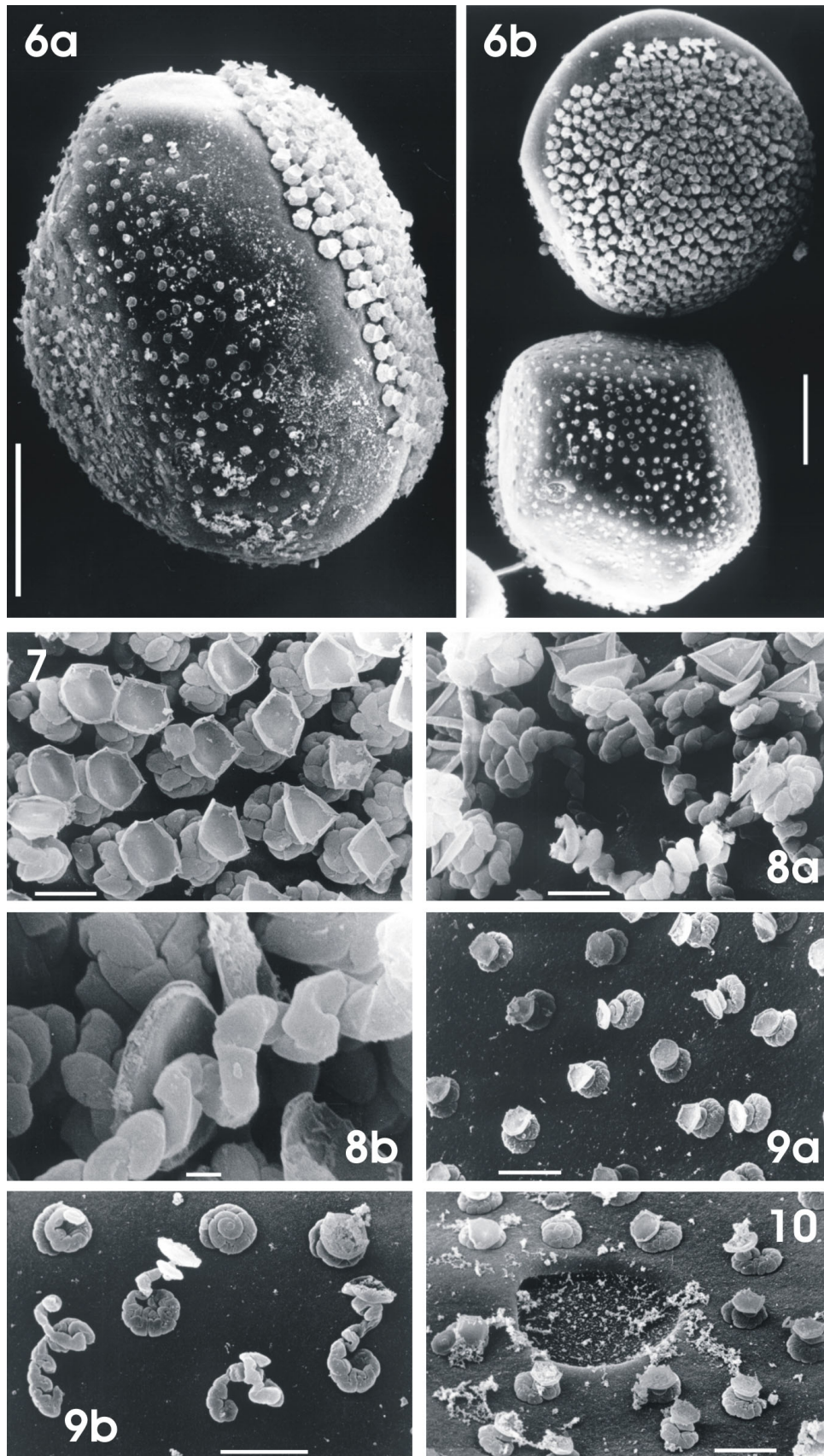
The eggs of *Ametropus fragilis* were partly described by Jażdżewska (1973), in light microscopy. Koss and Edmunds (1974) schematically described the egg structures in the genera *Ametropus*, *Isonychia* and *Neoephemera*, also on the basis of light microscopy studies.

SEM investigation on the eggs of *A. fragilis* showed chorionic fibrous reticulation form an adhesive layer and attached the eggs to the substratum. A similar pattern of large mesh reticulation can be seen on the surface of the chorion adhesive layers of *Caenis lactea* (BURMEISTER) and *Caenis robusta* EATON (Malzacher, 1982), as well as, some North American mayfly genera: *Tricorythodes* (Tricorythidae), *Eatonica* and *Pseudeatonica* (Ephemeridae) (Koss and Edmunds, 1974). The polar cap organisation is similar to that observed in some species of the Polymitarcyidae family. In *Ephoron album* (SAY), *Ephoron virgo* (OLIVIER) and *Ephoron shigae* (TAKAHASHI), the polar cap is situated at one egg pole and composed of numerous compactly coiled threads, creating finger-like protrusions (Degrange, 1960; Koss and Edmunds, 1974; Gaino and Flannagan, 1995).

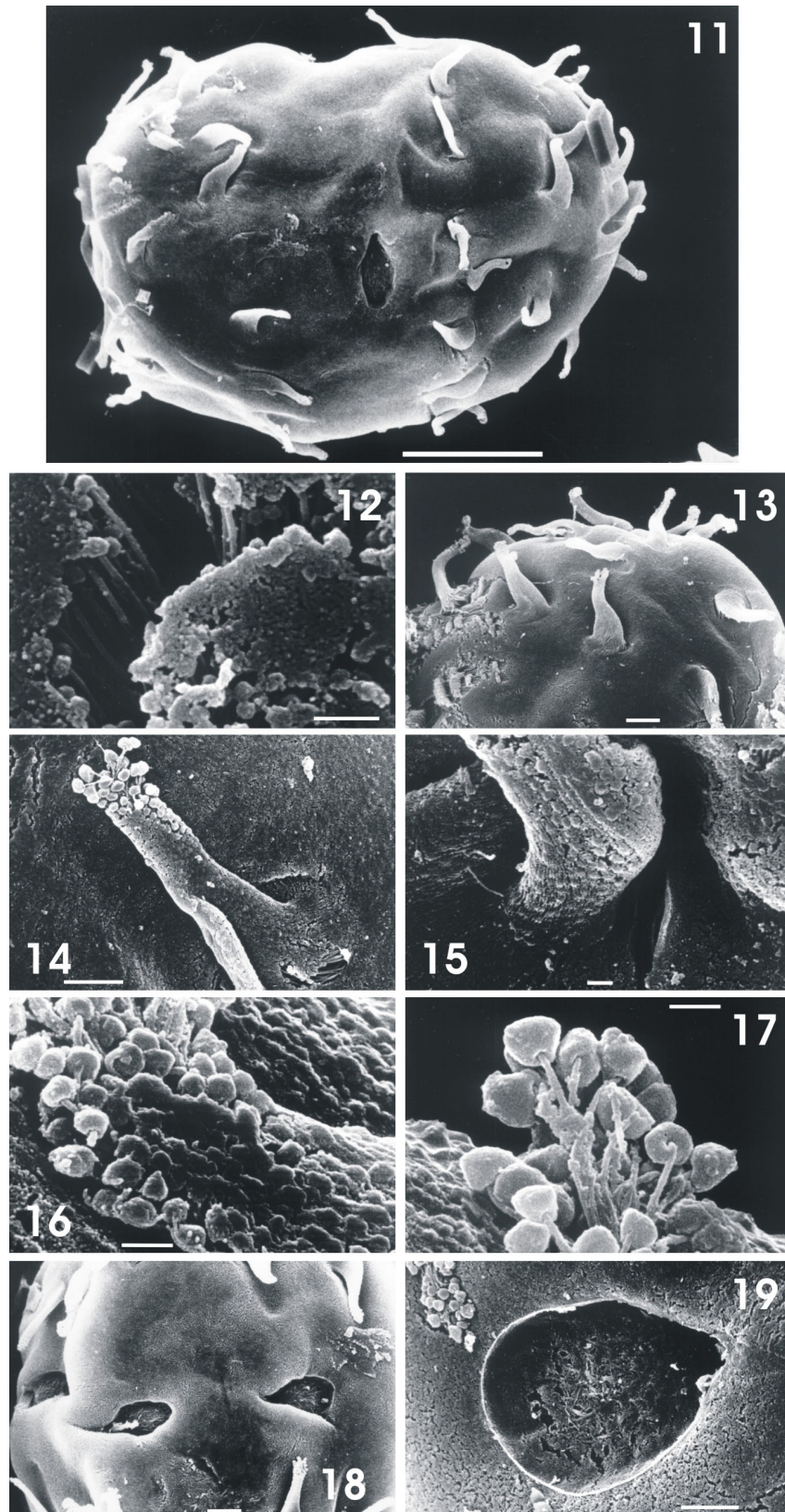
The eggs of *I. ignota* have KCT attachment devices and micropyle of the tageniform type with chorionic sperm guide, much like other species of *Isonychia* genus (Koss and Edmunds, 1974). Kondratieff and Voshell (1984) studied eggs morphology of many North and Central American *Isonychia* species using SEM. Their investigation shows that *Isonychia* eggs are always spherical with no obvious poles. KCT attachment structures are distributed in several ways: 1) a uniform layer of KCTs covering the entire egg or one hemisphere; 2) KCTs are concentrated in one region of the egg; 3) KCTs are scattered over the surface of the egg; 4) KCTs are densely packed on one hemisphere and scattered on the other (Kondratieff and Voshell, 1984).



Figs. 1-5 - *Ametropus fragilis*, egg; 1: general view, scale bar=50 μm ; 2: chorionic surface with large mesh reticulation, scale bar=10 μm ; 3: details of mesh reticulation-fibrous structures of chorionic surface are visible, scale bar=1 μm ; 4a, 4b, 4c, 4d: egg pole with polar cap, scale bars=10 μm ; 5: detail of the micropyle with star-like chorionic pattern, scale bar=10 μm .



Figs. 6-10 - *Isonychia ignota*, egg; 6a, 6b: general view, scale bar=50 μ m; 7: large KCT attachment structures, scale bar=5 μ m; 8a: extended large KCT attachment structures, scale bar=5 μ m), 8b: details of the extended coiled threads, scale bar=1 μ m; 9a, 9b: chorionic surface with small KCT attachment structures, scale bar=5 μ m; 10: detail of the micropyle with the sperm guide, scale bar=10 μ m.



Figs. 11-19 - *Neophemera maxima*, egg: 11: general view, scale bar=50 μ m; 12: chorionic surface composed of thin threads ending with a small knob, scale bar=1 μ m; 13: egg pole with finger-like projections, scale bar=10 μ m; 14: finger-like projection of the chorionic surface, scale bar=5 μ m; 15: basal part of a finger-like projection, scale bar=1 μ m; 16: middle part of a finger-like projection, scale bar=1 μ m; 17: apical part of a finger-like projection. Note the thin threads ending in mushroom-like structures, scale bar=1 μ m; 18: micropyles in the equatorial area, scale bar=10 μ m; 19: detail of the micropyle with the sperm guide, scale bar=5 μ m.

It is worth stressing that KCT attachment devices in *I. ignota* differ in size and cover both hemispheres (large KCTs densely cover one hemisphere, and small KCTs are scattered on the other hemisphere).

A similar KCT distribution was observed in *Isonychia edmundsi* KONDRATIEFF and VOSHELL, *Isonychia campestris* McDUNNOUGH, and *Isonychia intermedia* (EATON). The small tubercles scarcely distributed on the chorion surface also occur in these species (Kondratieff and Voshell, 1984). The chorion of *I. ignota* is smooth, without any tubercles. Although the eggs of *Isonychia* are spherical with a plesiomorphic character, both the structure of attachment devices and the organisation of the micropyle have apomorphic characters (Koss and Edmunds, 1974).

Bae and McCafferty (1998) gave a general description of the eggs of the Neoephemeridae family and reported the occurrence of a single micropyle. These authors provided figures of the eggs of two *Neoephemera* species: *Neoephemera purpurea* (TRAVER) and *Neoephemera youngi* BERNER, but did not furnish details of the chorionic decorations. It is evident that these eggs' surface is decorated with finger-like projections. They are very short on the eggs of *N. purpurea*. In *N. youngi*, they are scattered on the chorionic surface (Bae and McCafferty, 1998). In contrast, the finger-like projections of *N. maxima* eggs are longer and concentrated on both egg poles and transversal rows around the eggs. The fibrillar component of the finger-like projections in *N. maxima* suggests that these devices perform an adhesive functions along with the fibrous envelope. A dense fibrous adhesive layer and a suprachorionic sperm guide, as they occur in *N. maxima*, are considered to have the most plesiomorphic character in the Caenoidea superfamily (Koss and Edmunds, 1974).

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