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## REGULAR EGG-POSITIONING BY AN AESHNID SPECIES (ODONATA, AESHNIDAE) WITH COMMENTS ON ITS PHYLOGENETIC VALUE

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**Regular Egg-Positioning by an Aeshnid Species (Odonata, Aeshnidae) with Comments on its Phylogenetic Value.** Matushkina N. A. — Prolarvae and first-instar larvae of an aeshnid anisopteran, probably *Aeshna* sp. or *Anaciaeschna isoceles*, were reared from an endophytic egg-clutch with eggs positioned in line and zigzag orders in stems and flowerstems of *Myriophyllum spicatum* in central Ukraine. Descriptions of the prolarva and the first-instar larva, the distance between neighbouring eggs in the clutch, as well as discussion on evolution of the endophytic egg-laying behaviour in Odonata, are provided.

Key words: Odonata, dragonfly, endophytic oviposition, clutch, Aeshnidae.

**Упорядоченные яйцекладки стрекоз семейства Aeshnidae (Odonata) с комментариями об их филогенетическом значении.** Матушкина Н. А. — В стеблях и цветоносах *Myriophyllum spicatum* были обнаружены кладки стрекоз, содержащие расположенные линейно и зигзагом яйца. Из них в условиях лаборатории получены предличинки и личинки первого возраста стрекоз из семейства Aeshnidae (Anisoptera), возможно относящиеся к виду *Anaciaeschna isoceles* или роду *Aeshna* sp. В работе приведены описания морфологии яйца, личиночных стадий и кладок. Кратко обсуждается эволюция эндофитной откладки яиц у стрекоз.

Ключевые слова: Odonata, стрекозы, откладка яиц, яйцекладка, Aeshnidae.

### Introduction

Two general approaches of the oviposition exist in odonates. Species with well developed ovipositor generally lay their eggs endophytically, i. e., inside the plant tissues. Exophytic oviposition is inherent to the anisopterans with modified ovipositor, which is designed to drop the eggs in water or to deposit them onto the surface of some periaquatic objects, e. g., stones, soil, tufts of grasses, pads of roots, etc. (Corbet, 1999: 21).

Endophytic oviposition is one of the most common approaches of the egg deposition in odonates, recorded for all Zygoptera, Anisozygoptera and some Anisoptera, namely Aeshnidae. The female penetrates the substrate periodically by well-developed cutting ovipositor, and inserts eggs into the slits prepared. Usually, representatives of Zygoptera lay their eggs in a row, which constitutes a peculiar pattern of the clutch. This pattern can strongly depend on substrate characteristics in some damselflies, and therefore demonstrates high variability (e. g., in calopterygid *Phaon iridipennis* (Burmeister): see Miller, Miller, 1988). The egg deposition of other zygopterans is more constant, so that the regular pattern can serve as a family-specific character (Hellmund, Hellmund, 1991) or as a genus-specific character in a few instances, e. g., in some Lestidae (Matushkina, Gorb, 2000). Besides that, the stereotyped consecution of oviposition movements were recorded in *Lestes sponsa* (Hansemann) and *L. parvidens* Artobolevsky, which strictly determine a queue of placing and an inter-orientation of many eggs in one incision, and are controlled by certain mechanoreceptors on the ovipositor (Matushkina, Gorb, 2000, 2001).

Aeshnidae are the only anisopterans, which oviposit endophytically. It was previously supposed, that they lay their eggs predominantly irregularly, not arranged into a certain pattern (Schiemenz, 1953: 79–80, 83–84). There are only few drawings without explanatory text by Gardner (1950) on *Aeshna mixta* Latreille and by P.-A. Robert (1959) on *Ae. juncea* (Linnaeus) (Tafel 14, next to p. 81), *Anax imperator* Leach, and *An. parthenope* Selys (Taf. 16), which show regular patterns of clutches. The present work contains the first detailed description of the regular endophytic clutches of an aeshnid, and provides some arguments for its possible phylogenetic value.

### Material and methods

The emergent stems and flowerstems of *Myriophyllum spicatum* Linnaeus (Halorrhagidaceae) with an odonate egg clutch were collected on June 24, 2002 in a shallow creek of the Kaniv reservoir nearby the Zmyini Islands (vicinity of the Kaniv Nature Reserve, Cherkassy Region, Ukraine, 50°00' N, 31°30' E). All the collected parts of the plant were placed in plastic containers (15 x 10 x 5 cm) with the river water, kept at room temperature and exposed to the sun rays all over the day, for further rearing. The water was changed once per day. The substrata with undeveloped eggs and with the egg shells of the hatched larvae were discolored in the mix of 70% ethanol and concentrated acetic acid (1 : 1) following H. Grunert (1995), and preserved in 70% ethanol for further descriptions and measuring. Some hatched larvae were fixed in 70% ethanol. The materials are currently housed in the author's collection.

All measured values are given in mm.

### Results

About two weeks after the collection of egg-clutch (6 and 7 July 2002), the pro-larvae hatched and molted soon afterwards to the first-instar larvae. In total 23 larvae (about 50% of entire number of the collected eggs) hatched out. It was impossible to trace further development of the larvae, because they have died in a few days after the molting.

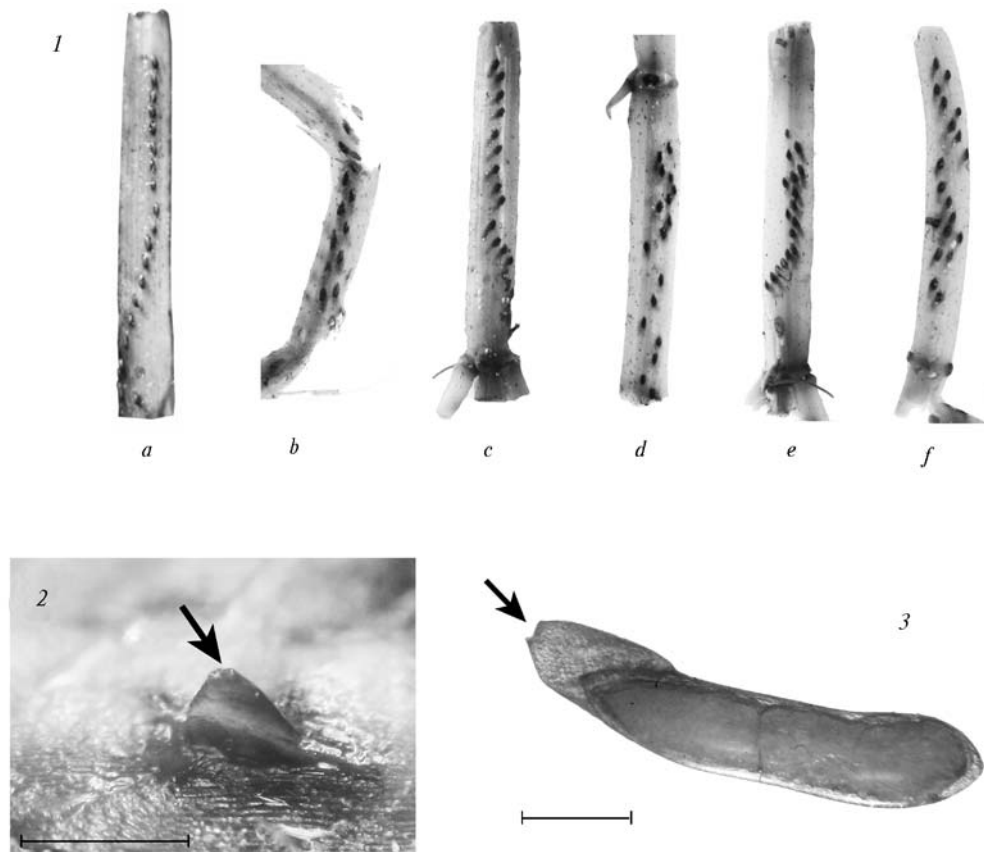


Fig. 1. Parts of studied clutch with different patterns of egg-positioning (1) and details of egg morphology: 2 – free part of elastic exochorion, protruding from egg-hole in alcohol-preserved substrate; 3 – shape of fixed egg. Arrows indicate opening in exochorion. Scale bar 0.5 mm.

Рис. 1. Фрагменты изученных кладок с различным расположением яиц (1) и детали морфологии яйца: 2 – свободная часть эластичного экзохориона, выступающая из отверстия в заспиртованном субстрате; 3 – форма фиксированного яйца. Стрелки указывают на отверстие в экзохорионе. Масштабная линейка 0,5 мм.

## Clutch structure and egg dimensions

The average distance between next eggs in the clutch was 1.33 (s. d. = 0.37, n = 78). The clutch contained the egg-sets with different egg patterns: (1) the row of eggs (fig. 1, *I a*), (2) the simple zigzag with a single egg in a corner of zigzag (fig. 1, *I b*), and (3) the broad zigzag with series of eggs (from 1 to 6) in each row (fig. 1, *I c, d*). Occasionally, the clutch was disordered in some parts, or patterns alternated in some parts of the clutch (fig. 1, *I e, f*).

The length of the alcohol-preserved eggs ranged 1.6–1.9 ( $\bar{x}$  = 1.71, s. d. = 0.08, n = 21). They were spindle-shaped with dark-colored apical ends. Each egg was covered by membranous pellicle, which was distinctly larger than the egg body (fig. 1, 2, 3) and represented an elastic exochorion (Sahlén, 1994). The membrane was projected partly from the plant tissues, and such an apical free part had an opening. The surface of the membrane had clearly hexagonal sculpture.

## Descriptions of instars

Prolarva ca 3.20 long (fig. 2, *1*). Elongate conical head 0.66 long and 0.49 of maximum across diameter. Tip of head slightly bent ventrally. Straightened labium 0.55

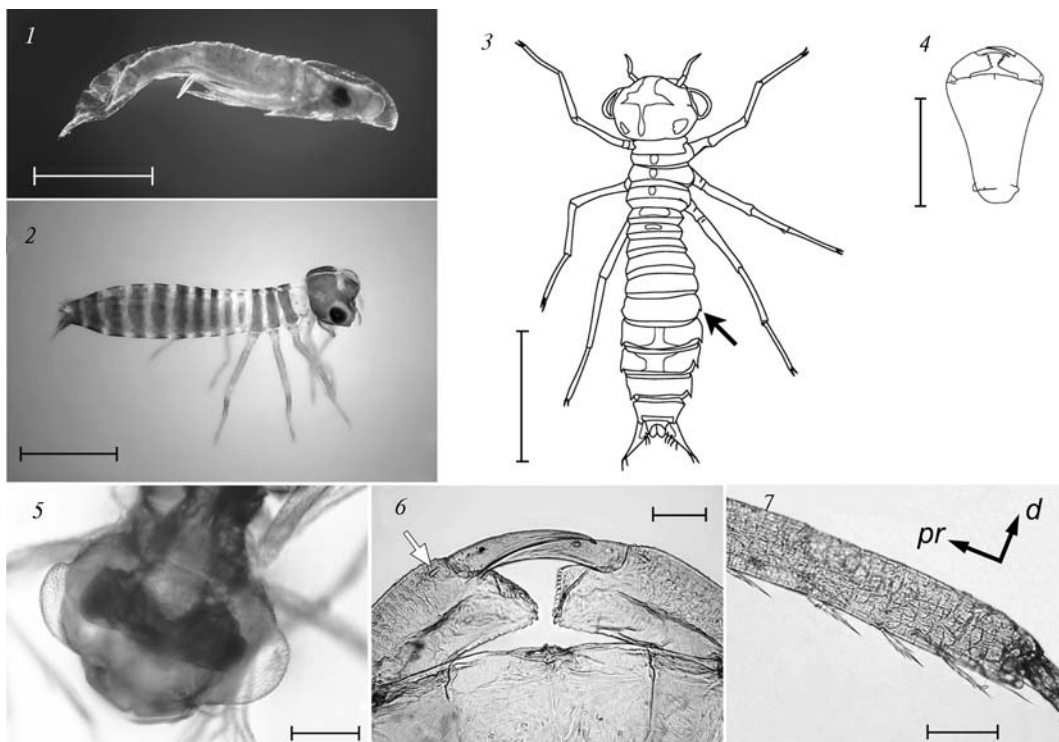


Fig. 2. Aeshnid instars reared from regular clutch: 1 – prolarva; 2–7 – first larva: 2, 3 – habitus, 4 – form of mask, 5 – head, 6 – labial palpi, 7 – first tarsus (*d* – dorsal direction, *pr* – proximal direction). Black arrow indicates lateral thorn on S6, which is diagnostic character of some *Aeshna* species and *Anaciaeschna isocetes*. White arrow indicates short labial hair. Scale bars: 1, 2, 4 – 1.0 mm; 3, 6, 7 – 0.5 mm; 5 – 0.2 mm.

Рис. 2. Личиночные стадии Aeshnidae, выращенные из упорядоченных кладок: 1 – предличинка, 2–7 – личинка первого возраста: 2, 3 – общий вид, 4 – форма маски, 5 – голова, 6 – боковые лопасти маски, 7 – лапка передней ноги (*d* – дорсальное направление, *pr* – проксимальное направление). Черная стрелка указывает на латеральный шип на шестом сегменте, который является диагностическим признаком некоторых видов рода *Aeshna* и *Anaciaeschna isocetes*. Белая стрелка указывает на короткий лабиальный волосок. Масштабные линейки: 1, 2, 4 – 1,0 мм; 3, 6, 7 – 0,5 мм; 5 – 0,2 мм.

long; legs 0.94–1.11 long; anal pyramid 0.28 long. Prolarvae molt shortly after hatching or even yet in egg hole, emerging from transparent skin, partly projecting from egg hole.

First-instar larva ca 3.22 long, head width 0.78. Integument with brown-and-white pattern (fig. 2, 2, 3). Head with clear cross-shaped pale spot on dorsal surface and with pair of obscure small spots behind compound eyes. Sclerites mainly dark brown, but also bearing pale medial spots of different configuration on all thoracic tergites as well as on abdominal tergites 1–2, 4 and 7–8. Intersegmental membranes always pale. All legs mostly pale, but femora, tibiae and tarsi with transverse darkenings. Paraprocts dark with pale basal spots. Epiproct pale.

Head round, with swollen vertex (fig. 2, 2, 5). Eye distinctly protruded forward, projecting over sides of head and forming slightly more than one half of head lateral surface (fig. 2, 5). Eye longer than broad. Antenna two-segmented; distal segment elongated and curved laterally. Prementum gradually widened distally (fig. 2, 4). Labium not reaching middle coxae in folded position. Toothed edge of labial palpus with 12 equal denticles (fig. 2, 6). Prementum with a few thin setae on the and labial palpus with one short hair (fig. 2, 6). Thoracic segments equal in size and shape. Prothorax lacking any discernible supracoxal armatures. Tarsi unisegmented with two ventral rows of normal and branched setae (fig. 2, 7). Abdominal segments 6–9 with lateral spines (fig. 2, 3). Paraprocti sharp with foveae, surrounded by long hairs, located on bases of their internal surfaces. Epiproct about 1/3 as long as paraproct. Apex of epiproct bilobed.

## Discussion

Identification of most Odonata species based on external morphology of their early instars is still problematic. Although the morphology of all stages has been described for some species (see Corbet, 2002), the available keys either refer to the exuvia or, more rarely, to final or penultimate larval instars (e. g. Heidemann, Seidenbusch, 1993; Norling, Sahlén, 1997; Gerken, Sternberg, 1999). Thus, we can only suppose, which dragonfly species laid the studied egg-clutch.

The habitus and the form of the mask of the reared larvae are characteristic to Aeshnidae, and some other features, e. g. the shape of the head and eyes, the presence of the lateral thorns on S6, allow to presume that this is neither an *Anax* nor a *Brachytron* species, nor *Aeshna juncea* (Heidemann, Seidenbusch, 1993; Matushkina, Khrokalo, 2002).

Six aeshnid species have been recorded in the Cherkassy Region earlier: *Aeshna affinis* Vander Linden, *Ae. grandis* (Linnaeus), *Ae. mixta*, *Anaciaeschna isocetes* (Müller), *Anax imperator* and *Brachytron pratensis* (Müller) (Khrokalo, Matushkina, 1999; Gorb et al., 2001). All these species have been also registered in the studied locality. However, other Ukrainian aeshnids, e. g. *Ae. cyanea* (Müller) and *Ae. viridis* Eversmann, are expected in this locality.

The pattern of the studied aeshnid clutch and its morphometrical features are compared with other odonates in table 1. The ordered egg clutch may be characterized by two main outlines of egg positioning, in line and in zigzag (fig. 3). Linear pattern has been considered inherent to some Lestinae, namely species of the genus *Lestes* (Matushkina, Gorb, 2000). Such clutches consist of a straight row of eggs constituting a simple linear pattern in *L. barbarus* (fig. 3, 3), or a row of egg-sets, each of which containing 2–8 eggs (fig. 3, 1, 2). The latter constitutes a complex linear pattern described for *L. sponsa* and *L. parvidens*. The *Lestes* species chiefly use the long upright growing parts of semiaquatic plants, like a flower-stems of *Scirpus*, *Juncus*, *Butomus*, or leaves of *Typha*, *Glyceria* etc., for oviposition (Jödicke, 1997: S. 228–229; Matushkina, Gorb, 2002). The pattern of some parts in the studied clutch of Aeshnidae fits the category of simple linear endophytic clutches.

**Table 1. Comparison of regular endophytic clutches in some Odonata**  
**Таблица 1. Сравнение упорядоченных кладок некоторых стрекоз**

| Species and source of information                                      | Distance between next eggs/egg sets in a clutch<br>[ ± s. d., in mm] | Pattern of clutch                              |
|--|--|--|
| <i>Calopteryx splendens</i> (Harris)<br>(Lindeboom, 1996)              | 1.16 ± 0.12<br>n = 10  | Simple zigzag, i. e. zigzag with a single eggs |
| <i>Lestes barbarus</i> (Fabricius)<br>(Matushkina, Gorb, 2000)         | 2.39 ± 0.08<br>n = 71  | Simple linear, i. e. row of single eggs        |
| <i>Lestes sponsa</i> (Hansemann)<br>(Matushkina, Gorb, 2000)           | 1.44 ± 0.53<br>n = 111   | Complex linear, i. e. row of egg-sets          |
| <i>Enallagma cyathigerum</i> (Charpentier)<br>(Matushkina, Gorb, 2000) | 0.82 ± 0.15<br>n = 78  | Broad zigzag, i. e. zigzag with series of eggs |
| <i>Erythromma najas</i> (Hansemann)<br>(Matushkina, Gorb, 2000)        | 0.56 ± 0.01<br>n = 196   | Broad zigzag, i. e. zigzag with series of eggs |
| Aeshnidae gen. sp. (this study)  | 1.34 ± 0.37<br>n = 78  | Simple linear, simple zigzag, broad zigzag     |

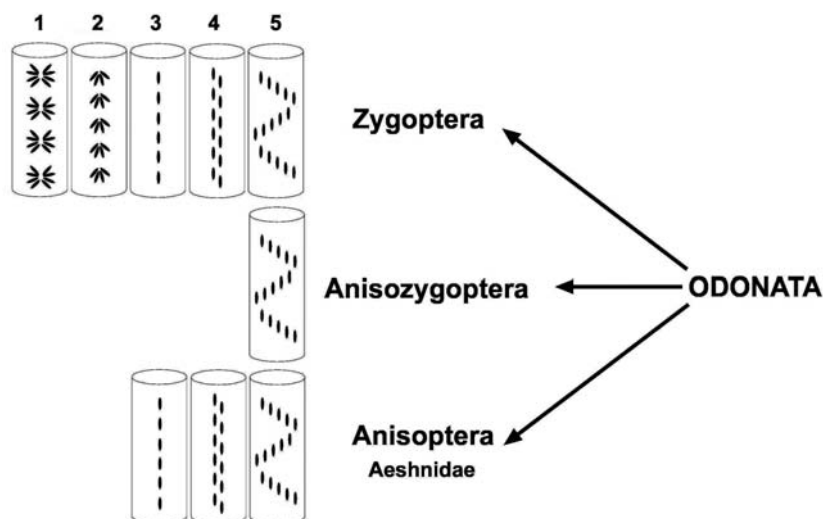


Fig. 3. Distribution of main patterns of regular endophytic clutches in recent odonate suborders: 1, 2 – complex linear, 3 – simple linear, 4 – simple zigzag, 5 – broad zigzag. According to Matushkina, Gorb (2000) for Zygoptera, Shimura (2005) for Anisozygoptera, and this study for Anisoptera.

Рис. 3. Распределение основных типов упорядоченных эндофитных кладок среди современных подотрядов стрекоз: 1, 2 – сложная линейная; 3 – простая линейная; 4 – простая зигзаговидная; 5 – широкая зигзаговидная. По: Матушкина, Горб (2000) для Zygoptera, Shimura (2005) для Anisozygoptera, и данное исследование – для Anisoptera.

Zigzag-like clutches are known from many zygopterans of Calopterygidae, Coenagrionidae, Platycnemididae (Matushkina, Gorb, 2000), as well as from the lestid genus *Sympsectra* (e. g. Martens, 1996; Reinhardt, Gerighausen, 2001), and from the anisozygopteran *Epiophlebia superstes* (Selys) (Shimura, 2005). Many zygopterans place their eggs in broad zigzag (fig. 3, 5), rows of which consist of different number of eggs. Some of such clutches are of semicircular shape, if the female lays her eggs inside the broad floating leaves of Nymphaeaceae (Schiemenz, 1953: fig. 12). The pattern of some other clutches is formed by a single egg in the corner of the zigzag, so, one may have an impression that the clutch contains two parallel lines of eggs (fig. 3, 4). Such mode of egg positioning is referred as «a simple zigzag» and was previously described for *Calopteryx splendens* (Lindeboom, 1996) and *Platycnemis latipes* (Rambur) (Heymer, 1966).

Thus, the regular pattern, i. e., well-organized positioning of eggs in the endophytic clutches, was reported for the representatives of all recent suborders of Odonata (fig. 3). This may suggest that the regular endophytic oviposition was rather descent from a common ancestor, than originated independently within established lineages of Odonata (i. e. at least three times). This assumption requires either confirmation or rejection depending on results of further phylogeny reconstructions.

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