

[ORIGINAL PAPER]

A New Type of Egg Burster in *Sialis* and Its Possible Phylogenetic Significance: A Comment on the Article by Ando *et al.* (1985) (Insecta: Megaloptera, Sialidae)

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Abstract

In their article on the embryology of the alderfly *Sialis japonica*, Ando *et al.* (1985) reported the formation of comb-like structures along the midline of the ventral side of the embryonic head of this species, but they did not refer to the function of these structures. By the examination of documentations on *Sialis*, we reconfirm the structures as an egg burster (EB) that is used to tear the eggshell at the time of hatching. We also demonstrate that the EB corresponds to a new type of EB that can be discriminated from other types of EBs. This type of EB is suggested to be widely distributed in another megalopteran family, Corydalidae, and also in the closely related order Neuroptera. The presence of this type of EBs in Megaloptera and Neuroptera affords us further evidence of the sister group relationship between these two orders.

Introduction

On the basis of their scanning electron microscope (SEM) observations, Ando *et al.* (1985) reported the development of a pair of comb-like structures along the midline of the ventral side of the head in an embryonic alderfly, *Sialis japonica* Weele [*S. mitsuhashii* Okamoto is a synonym for this species (Hayashi and Suda, 1995)] (Fig. 1, eb). The structures, which assume the shape of a narrow inverted V, occur in series composed of approximately 10 spines forming two ridges. Previous studies have clearly demonstrated that these structures correspond to the egg burster (EB) -variously referred to as the egg tooth, oviraptor, Eizähne and Eisprenger- that is used to tear the eggshell at the time of hatching (Emden, 1925; Evans, 1972; Canterbury and Neff, 1980). Interestingly, however, Ando *et al.* did not refer to this function of the EB, probably because they were unaware of the previous studies in which the function of this structure was described. We reconfirm that these comb-like structures are indeed the EB, and demonstrate that the EB corresponds to a new type of EB that can be discriminated from other types of EBs. In addition, we propose that this type of EBs is widely distributed in another megalopteran family, Corydalidae, and also in the closely related order Neuroptera.

Egg bursters of *Sialis*

The *Sialis* EB was first reported by Emden (1925).

Subsequently, the EBs were described in 14 North American *Sialis* species by Evans (1972) and Canterbury and Neff (1980). These authors already recognized the following important points regarding the EB: 1) the *Sialis* EB is a sclerotized tooth-like structure that is formed in the embryonic cuticle, 2) the EB is used to tear the eggshell at eclosion, and is shed together with the embryonic cuticle from the body, remaining attached to the teared eggshell after eclosion. Both Emden (1925) and Canterbury and Neff (1980) ended their observations only referring to EBs that remained attached to the eggshells after eclosion, and no direct observations of embryonic EBs were made. Evans (1972) observed EBs on the embryonic heads of four *Sialis* spp. However, none of these studies referred to the origin of the EB, *i.e.*, what parts of the head are involved in the formation of the EB.

Egg burster formation in *Sialis* embryos

According to Ando *et al.* (1985), the EB (referred to by them as comb-like structures) originates in the proximal part of the labial appendages (Fig. 2A–D). In 4-day-old embryos, the labial appendages arise as a pair of robust projections just posterior to the maxillary appendages (Fig. 2A, lb, mx). These labial appendages then elongate medially and their distal halves differentiate into future labial palpi (Fig. 2B, lbp). In 8-day-old embryos, the labial appendages shift anteromedially

and become positioned close to each other in such a way that they are concealed below the developing maxillary appendages (Fig. 2C). In 9-day-old embryos, the EB appears as a narrow inverted V-shaped structure along the midline of the gnathal regions, just posterior to the labrum and between the antennae (Fig. 2D, eb). At this stage, the posterior end or the base of the EB is continuous with the anterior part of the fused labial appendages, which extend posteriorly as labial palpi (Fig. 2D, lbp). Based on these observations, Ando *et al.*

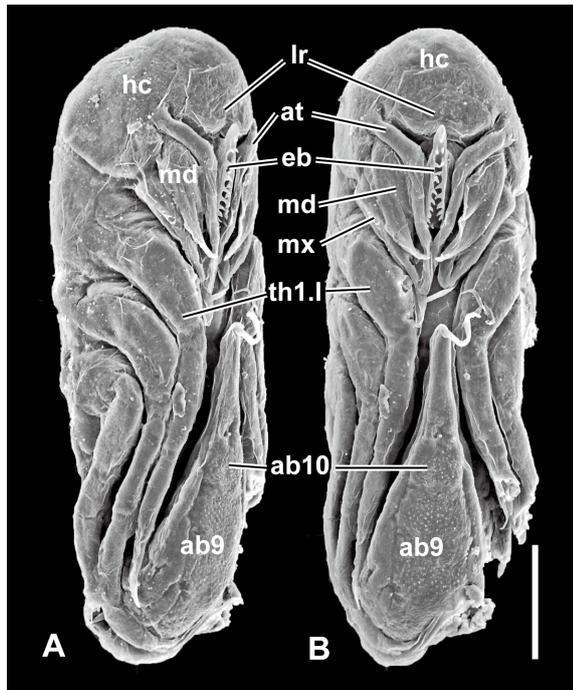


Fig. 1 Latero-ventral (A) and ventral (B) views of a 10-day-old *Sialis japonica* embryo, showing the egg burster (eb) (Ando *et al.*'s comb-like structures). Modified from Ando *et al.* (1985) with permission from The Arthropodan Embryological Society of Japan. ab9 and 10: ninth and tenth abdominal segments, at: antenna, hc: head capsule, lr: labrum, md: mandible, mx: maxilla, th1.l: prothoracic leg. Scale = 100 μ m.

(1985) concluded, "This structure (EB) is originated from the anterior part of the labial rudiments." (p. 198). They also regarded the structure as a 'dentate ligula' (p. 200).

The labial origin of the *Sialis* EB asserted by Ando *et al.* (1985) appears to be natural as the general morphogenetic movement of the cephalognathal region containing the proximal part of the fused labial appendages is strongly shifted anteriorly, resulting in this region sometimes coming into contact with the labrum (*e.g.*, trichopteran embryogenesis, Kobayashi and Ando, 1990). It is likely that Ando *et al.* (1985) did not directly observe the primordial EB before the formation of the V-shaped structure, as this primordial EB would have been concealed by the developing antennae (Fig. 2C, circle with white stippled line) in 8-day-old embryos. We presume that the most proximal endites of labial appendages (Fig. 2B, C, arrows), or glossae, are fused to each other and develop into the EB. However, in a discussion concerning the origin of the EB with Dr. Rolf G. Beutel, he addressed that this part (Fig. 2C, circle with white stippled line) is the future hypopharynx and not the labium, which means that the *Sialis* EB is hypopharyngeal in origin (Rolf G. Beutel, *pers. comm.*). In thysanopteran embryogenesis, the hypopharynx develops from the epidermis of the mandibular and maxillary segments, and possibly also the intercalary segment (Heming, 1980). In the embryonic whirligig beetle *Dineutus mellyi*, the future hypopharynx, which is probably derived from the sterna of the mandibular and maxillary segments, is located just anterior to the fused labial appendages (see Fig. 5 in Komatsu and Kobayashi, 2012). Thus, while we are still inclined to regard the EB as being labial in origin, the possibility that some hypopharyngeal elements participate in the formation of the EB should not be excluded completely. For the determination of the exact origin of the *Sialis* EB, histological observations on the serial sections of the parts concerned need to be conducted. However, in the interim, we propose that the EB of *Sialis* should be tentatively called the *Sialis*-type EB, without referring to its embryonic origin.

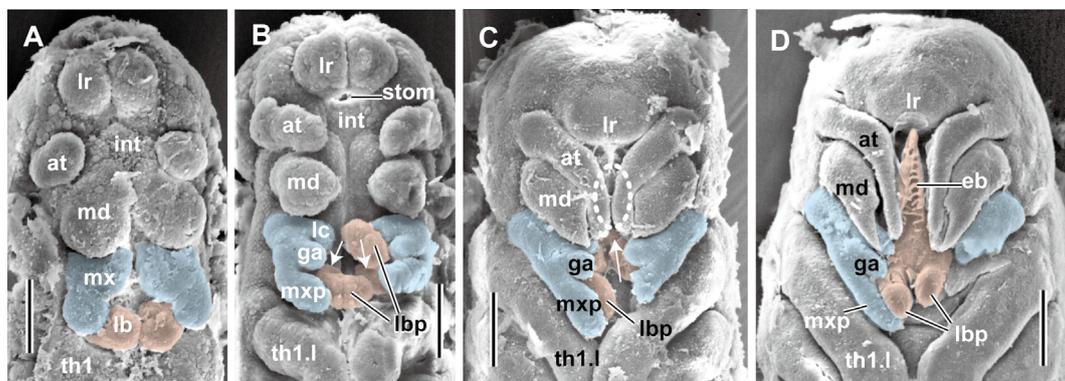


Fig. 2 Successive stages of developing *Sialis japonica* embryos (ventral views), showing the morphogenetic movement of the cephalognathal region. Modified from Ando *et al.* (1985) with permission from The Arthropodan Embryological Society of Japan. A. 4-day-old embryo. B. 6-day-old embryo. C. 8-day-old embryo. The left maxillary palp and left labial palp were removed. D. 9-day-old embryo. The regions corresponding to the maxillary appendages and labial appendages are highlighted in blue and red, respectively. at: antenna, eb: egg burster, ga: galea, int: intercalary segment, lb: labial rudiment, lbp: labial palp, lc: lacinia, lr: labrum, md: mandible, mx: maxillary rudiment, mxp: maxillary palp, stom: stomodaeum, th1: prothoracic segment, th1.l: prothoracic leg, white arrow: glossa, white stippled line: see text. Scales = 50 μ m.

Egg bursters of Corydalidae

In another megalopteran family, Corydalidae, or dobsonflies, the presence of an EB has been reported in four genera (*Corydalus*, *Chauliodes*, *Neohermes*, and *Orohermes*) by Smith (1922) and Evans (1972). Each of the corydalid EBs, unlike those of *Sialis*, assumes as a single blade with minute irregularly shaped teeth. The EB is located on the ventral side of the labrum, but they are not connected.

Egg bursters of Neuroptera

Among the 17 families in Neuroptera, EBs have been identified in eight; the Sisyridae, Osmylidae, Coniopterygidae, Mantispidae, Hemerobiidae, Chrysopidae, Nemopteridae, and Nymphidae (Hagen, 1852; Smith, 1922; Withycombe, 1923, 1925; Emden, 1925; Kuroko, 1961; New, 1983; Popov, 2002; Candan *et al.*, 2005; Konopová and Zrzavý, 2005). Based on the

absence of an EB in embryos of *Ascalaphus ramburi*, it is considered that EB formation probably does not occur in the Ascalaphidae (Kamiya and Ando, 1985). Similarly, the Myrmeleontidae is said to lack an EB (Hinton, 1981). Thus, although the state of the EB has not been examined in seven other neuropteran families to date, EBs are presumed to be widely distributed in Neuroptera. The neuropteran EB, as in the Corydalidae, is located on the ventral side of the embryonic head, posterior to the labrum and between the antennae where it assumes as a single blade or ridged structure with mostly minute teeth; however, the shape of the blade is highly variable among genera (Figs. 3, 4A-H); e.g., the blade is long and thin in *Osmylus* (Osmylidae) and *Sisyra* (Sisyridae), and thick and robust in *Semidalis* (Coniopterygidae) and *Hemerobius* (Hemerobiidae). The EB of *Nemoptera bipennis* is conspicuously hill-shaped without a blade (Fig. 4H). As in the Megaloptera, neuropteran EBs are also shed with the eggshell at eclosion.

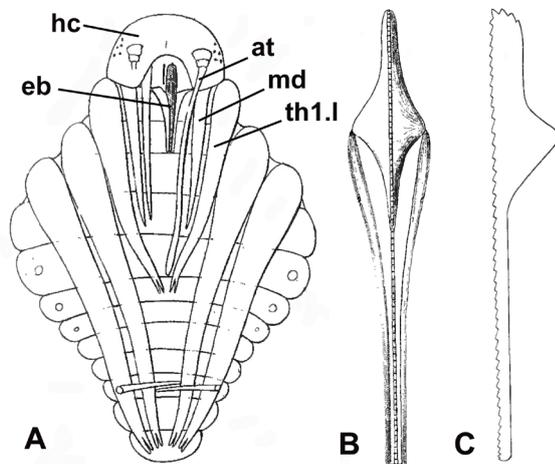


Fig. 3 Ten-day-old embryo (A) of *Osmylus chrysops* Linné [= *Osmylus fulvicephalus* (Scopoli)] and its egg burster (eb) (B, ventral view; C, left side view) (After Hagen, 1852).
at: antenna, hc: head capsule, md: mandible, th1.l: prothoracic leg.

Various types of egg bursters in Dicondylia

Extending our view to all hexapods, the presence of an EB has been reported in 22 of the 29 dicondylial insect orders. Emden (1946) classified hexapod EBs into three types according to their location and relation to the embryonic cuticle. The first type is an acral or frontal EB, located on the frontal region of the head. This type of EB is confined to the embryonic stage and shed with the embryonic cuticle immediately after the rupture of the chorion at eclosion. The second type is a persistent frontal EB, that is also located on the embryonic head; however, this type is retained during the first larval stage. The EBs of the third type are scattered on the dorsa of the thoracic and abdominal segments, and are retained during the first larval stage. The third type has only been recorded in several polyphagan coleopteran families (Chrysomelidae, Coccinellidae, *etc.*).

Since Emden's classification, EBs have been discovered

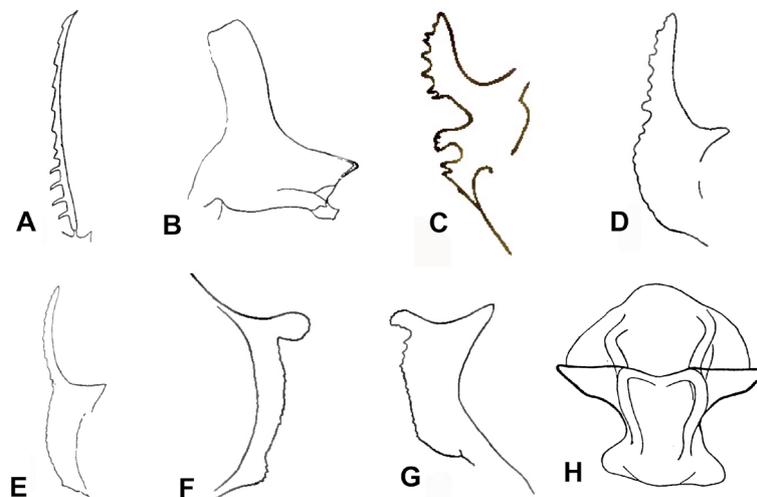


Fig. 4 Egg bursters of neuropteran species (A, C, D, E and G, right-side views; B and F, left-side views; H, ventral view) (A - G, after Withycombe, 1923; H, after Withycombe, 1925). A. *Sisyra fuseata* (Sisyridae). B. *Semidalis aleurodiiformis* (Coniopterygidae). C. *Symphorobius pygmaeus* (Hemerobiidae). D. *Hemerobius lutescens* (Hemerobiidae). E. *Hemerobius paganus* (Hemerobiidae). F. *Nothochrysa capitata* (Chrysopidae). G. *Chrysopa flavifrons* (Chrysopidae). H. *Nemoptera bipennis* (Nemopteridae).

in many orders, and EBs of the first type have been recorded in 19 orders including Megaloptera and Neuroptera. Emden (1925, 1946) already regarded the megalopteran and neuropteran EBs as being associated with the head. Subsequent interpretations of EB location in orders excluding the Megaloptera and Neuroptera have revealed that 17 other orders have EBs located on the vertex of the head capsule, frons or clypeus (*e.g.*, Kobayashi and Ando, 1990; Mashimo *et al.*, 2014). On the other hand, as mentioned previously, since the megalopteran and neuropteran EBs have been observed on the ventral side of a head capsule, they should be discriminated from the first type.

A new type of egg burster in Megaloptera and Neuroptera: A potential synapomorphy between these two orders

Relatively little is known about the embryonic origin of the EB in the Corydalidae and Neuroptera. In their article examining the formation of the embryonic cuticle in several insect orders, Konopová and Zrzavý (2005) investigated the formation of the EB in the neuropteran *Chrysopa perla*. In the caption of their “Figs. 7 and 8”, they used the phrase “Note the labral egg-tooth” (p. 350) and the term ‘labral egg-tooth’ was also used in the text of the article (p. 349). It therefore seems clear that Konopová and Zrzavý (2005) consider the EB as belonging to the labrum. However, judging from its location between the antennae it is strongly suggested that the *Chrysopa* EB could also be classified as a *Sialis*-type EB with no connection to the labrum. It is therefore presumed that the EBs of Megaloptera and Neuroptera are derived from the same embryonic part, but the exact part is not yet been identified; the EBs of these two orders could therefore be regarded as the *Sialis*-type EB.

It is also possible that the *Sialis*-type EB could be a new type of EBs that is only known in Megaloptera and Neuroptera, and that its presence could be regarded as a potential synapomorphy between these two orders. Although the Megaloptera, Neuroptera, and Raphidioptera are known to constitute the superorder Neuropterida (Mickoleit, 1973), the phylogenetic relationships among these orders have been highly debated, with some authors questioning whether the Megaloptera are monophyletic (see Kristensen, 1995; Aspöck *et al.*, 2012). Traditionally, the Megaloptera and Raphidioptera have been considered sister taxa (Hennig, 1953), primarily due to similarities in skeletomuscular characteristics of the basal abdominal segments (Achtelig, 1975) and the structure of the female postabdomen (Hünefeld *et al.*, 2012). On the basis of comprehensive morphological and molecular evidence, Beutel *et al.* (2010) also proposed the clade Megaloptera + Raphidioptera, although in their analysis the Megaloptera are paraphyletic [*i.e.*, Sialidae + (Corydalidae + Raphidioptera)]. Although some molecular phylogenetic analyses have provided evidence for the clade Megaloptera + Raphidioptera (Whiting, 2002; Wiegmann *et al.*, 2009), the hypothesis for the sister-group relationship of Megaloptera +

Neuroptera that was first proposed by Boudreaux (1979) has been strengthened by an increasing body of morphological (Aspöck *et al.*, 2001; Aspöck, 2002; Krenn, 2007; Aspöck and Aspöck, 2008; Zhao *et al.*, 2014) and molecular phylogenetic evidence (Haring and Aspöck, 2004; Kjer *et al.*, 2006; Cameron *et al.*, 2009; Peters *et al.*, 2014). Consequently, the clade Megaloptera + Neuroptera has gained increasing acceptance, as has the monophyly of the Megaloptera. Indeed, the presence of the *Sialis*-type EB in both the Megaloptera and Neuroptera would provide further evidence for the sister group relationship between these two orders (*i.e.*, synapomorphy). However, in order to resolve this argument, the state (presence or absence) of the EB in Raphidioptera should be investigated. From an examination of the figures of the fully grown *Inocellia japonica* embryo (Tsutsumi, 2008), who first described embryogenesis of Raphidioptera, an EB is indeed absent in this species (his Fig. 26A'). The absence of an EB in *Inocellia* therefore adds evidence favorable for the clade Megaloptera + Neuroptera to the phylogenetic discussion of Neuropterida.

Acknowledgments: We extend our sincere gratitude to the late Prof. Emeritus Hiroshi Ando, the late Dr. Kozo Miyakawa, and Mr. Satoru Shimizu, for providing us with numerous SEM micrographs of *Sialis*. We are grateful to Prof. Rolf G. Beutel and for his comments on the origin of the EB of *Sialis*. We also thank Prof. Ryuichiro Machida and Mr. Akimasa Kamiya for their help in many aspects and critical comments on the manuscript.

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