Title: *Colophon* larvae: descriptions and phylogenetic implications.

Short title: *Colophon* larvae (Coleoptera: Lucanidae): descriptions and phylogenetic implications.

Authors: Angelika K. Switala[1], Catherine L. Sole[1] & Clarke H. Scholtz[1]

Institute: [1] Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield, 0028, Pretoria, South Africa

Abstract:

The aim of the *Colophon* larval study was to enable researchers to identify the species found in the field; to use larvae as an alternative for adults in molecular studies; to comment on possible phylogenetic information that may contribute to the sub-familial placement of the genus; and to obtain habitat preference data. To achieve this, larvae of four *Colophon* species were examined and their main diagnostic morphological characters identified. Larvae live in a fairly homogeneous micro-habitat of moist, humus-rich soil in protected places such as under rocky overhangs and amongst the roots and tussocks of Restionaceae.. *Colophon* larvae show small inter-specific differences and larval characters contributed little equivocal information from which phylogenetic support for family placement could be deduced. Apparently, as with many scarabaeoid groups (Trogidae, Scarabaeidae), larval morphology may not have diversified much from the basal ground-plan and it remains for other (adult) phylogenetically significant morphological characters or DNA to provide more clarity on *Colophon*'s subfamilial placement.

Keywords: Cape Floristic Region; *Colophon*; Endangered; Larval description; Lucanidae

Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za

Introduction

As a global biodiversity hotspot, the Cape floristic region (CFR) is known for its floral species richness and high levels of endemism (Cowling *et al.* 1989; Myers *et al.* 2000; Midgley *et al.* 2003). This is in large part due to both the unique reigning climate and climatic history of the area (Midgley *et al.* 2001). Associated with global warming, there has been a steady increase in temperature and decrease of rainfall within the CFR (Tyson *et al.* 2002). It is therefore becoming desirable to study the responses of species to these climatic changes. High mountain fauna and flora worldwide are especially threatened by global warming (Parmesan & Yohe 2003), more so when the mountain peaks are high enough to differ substantially in weather patterns when compared to the lower slopes. The Cape Fold Mountains (CFM) qualify as such mountains. During the summer months, strong south-easterly winds, blowing off the ocean in late afternoons, generate updrafts of cool moist air against the mountain slopes which condenses and regularly covers the peaks in a dense cold mist. During winter, cyclonic fronts and north-westerly winds bring rain and snow storms, often leaving the highest peaks covered in a blanket of snow. The CFM also provides habitat to a diversity of insect species (Cowling 1992; Linder 2003; Giliomee 2003; Galley & Linder 2006; Cowling et al. 2009). One such insect species group is the enigmatic Cape high-mountain stag beetle genus *Colophon* Gray.

Endemic to the CFR and geographically isolated to the high mountains of the Western Cape, this ancient, apterous genus is represented by 17 species. Endrödy-Younga (1988) proposed that *Colophon* is a relict of a past temperate climate regime that prevailed at lower elevations and which with increasing global temperatures since the Plio-Pleistocene have been forced up the mountains. This led to speciation and the current localised distribution of individual species on more hospitable mountain tops. It has been shown that over the past century,

Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za

montane species worldwide are withdrawing an average of 60m up the slopes of their mountain habitats due to the effects of global warming (Parmesan & Yohe 2003). All *Colophon* species are restricted to the highest peaks on the mountain ranges, often in very small habitat fragments, making their survival tenuous considering the conditions predicted to prevail in future due to global warming (e.g. Lutjeharms *et al.* 2001; Meadows 2006). *Colophon* survival is not only threatened by predicted future climatic scenarios, but also by on-going collection pressures from beetle collectors, who have traded specimens for large sums of money (Gess & Gess 1993; Melisch & Schütz 2000).

Colophon species were declared “protected” by South African Cape Provincial law in 1992 and listed by CITES (Convention on International Trade in Endangered Species) in Appendix III in 2000. Fourteen of the 17 *Colophon* species are also included on the IUCN Red list (http://www.iucnredlist.org) and the genus as a whole was placed on the South African ToPS (Threatened or Protected Species) list in 2007. However, these assessments of *Colophon* are largely outdated and in need of revision (New 2012). Initially listed by CITES, (Anonymous 1994) to protect them from commercial exploitation, this listing has only succeeded in drawing more attention to the beetles and possibly increasing their black market value (Gess & Gess 1993; Melisch & Schütz 2000; Geertsema & Owen 2007).

Adults of all species are strongly sexually dimorphic, with males boasting large mandibles, as is typical for many members of Lucanidae. The optimal weather conditions for surface activity have been defined as cool misty conditions during late afternoons and early mornings (Barnard 1929; Endrödy-Younga 1988). However, specimens of several species have been collected in the heat of day following a misty evening/morning (personal observation). A study by Roets *et al.* (2012) on the abiotic variables influencing adult *Colophon westwoodi* activity showed that their activity was significantly influenced by illuminance (suggesting

Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za

they might be nocturnal) and the presence of mist (moisture) during the day. Adults appear not to feed and circumstantial evidence suggests that larvae are soil and humus feeding (Endrödy-Younga 1988). *Colophon* larvae live in humus-rich soil beneath stones and amongst the roots of tussocks of the Restionaceae which dominate on suitable patches of soil on the mountain peaks. They are long-lived, with a 3rd-instar larva of *Colophon neli* kept in the laboratory showing no signs of growth over a six-month period (Scholtz & Endrödy-Younga 1994). Another large, possibly 3rd-instar larva, originally thought to be that of a fruit-chafer (Scarabaeidae: Cetoniinae) and kept in humus-rich soil indoors, yielded a female specimen of *Colophon cameroni* approximately one year later (Tony Brinkman, pers. comm.).

Since adult *Colophon* beetles mainly occur on largely inaccessible mountain peaks and are mostly active during ideal conditions, finding specimens is usually difficult. Larvae, however, although sparsely distributed and usually buried in the soil, are often a more dependable source of research material. They are the topic of this paper.

Colophon larvae found in the field can be ascribed to species since in most cases only one species occurs on each mountain peak. On the basis of this, specimens of *C. neli* were collected and described by Scholtz and Endrödy-Younga (1994). This was the first, and until the current study, the only larva of any species of the genus to be described. The purpose of the present larval study is fourfold: to enable researchers to identify the species found in the field; to use larvae as an alternative for adults in molecular studies; to comment on possible phylogenetic information that may contribute to the sub-familial placement of the genus; and to obtain habitat preference data. The latter is of special importance for future studies if predicted climate change starts to impact on larval habitat availability and suitability.

Methodology

Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za

Specimens studied

Several scarabaeiform larvae were collected from various Western Cape mountains (Table 1, Fig. 1) and identified as Lucanidae based on the shape of the anal opening (Ritcher 1966). *Colophon* is the only member of the family on these mountains. Larvae were mostly found under rocky overhangs and amongst the roots and tussocks of Restionaceae, in moist, humus-rich soils. Those collected where ascribed to species based on their locality and associated adults. Larvae were described and subsequently preserved in absolute alcohol for DNA sequence isolation and phylogenetic studies. *Colophon neli* larvae from the Scholtz & Endrödy-Younga (1994) study, housed in the Ditsong Museum (formerly Transvaal Museum), were re-examined and included in the present study (Table 1).

Several characters essential to distinguish the larvae of major groups of Lucanidae (Lawrence 1981; Scholtz & Endrödy-Younga 1994) where examined under a dissecting microscope. For examination of the mouthparts, heads were dissected, main structures removed, dried, gold-coated and studied in a scanning electron microscope (SEM). Ritcher's (1966) terminology was used to describe the larvae. Voucher specimens are currently deposited in the University of Pretoria Insect collection (UPSA).

General Larval Description

The four *Colophon* species' larvae differed only slightly in character from each other, thus a general description for the genus *Colophon* is provided with species differences highlighted in Table 2.

Head capsule. Maximum width: 6.3mm. Antenna 3-segmented; reduced distal segment with dorsal sensory spot (Table 2). Surface of head capsule smooth, brown, with preclypeus a slightly lighter colour than the brown labrum and postclypeus. Labrum slightly asymmetrical.

Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za

Primary frontal setae on each side consist of one or two exterior frontal setae (EFS), one anterior frontal angle seta (AA) and one anterior frontal seta (AFS). Posterior frontal setae (PFS) are absent. Isolated setae present on epicranium.

Mandibles. Left mandible with 3 distinct teeth on incisor edge, with a scissorial notch. No teeth on inner surface of mandible, with a blade-like slope between molar and scissorial area. One triangular molar tooth present (Table 2, Fig. 2a-d). Right mandible without prominent teeth. Blade-like scissorial area present. Inner surface of mandible with a small notch and no prominent molar teeth.

Epipharynx. Elevated pedium. Chaetoparia well developed with about 20 pointed setae. Haptolachus with three nesia. Distinct, united, symmetrical pternitorma (Table 2, Fig. 2e).

Hypopharynx. Distinct asymmetrical sclerome, right side with prominent truncate process (Fig. 2f).

Maxilla. Palpus 4-segmented with slight dark colouration around anterior end of second segment and without setae on last segment. Distinctly separated galea and lacinia. Lacinia with single, terminal uncus and a fringe of about 10 stiff setae; galea with two subequal and one small basal unci and a fringe of stiff setae. No maxillary stridulation area on stipes (Fig. 2f).

Thorax. Transverse row of sparse long and short setae on dorsal side of thorax, with no anterior process on prothorax.

Abdomen. Row of sparse long setae on tergites of segments 1-3, third segment with a broader band of short, stiff setae. Large field of short stiff, and long isolated, setae on segments 4-7. Segments 8-9 with isolated long setae. Abdominal segment 10 greatly constricted. Vertical anal slit with bulbous lateral lobes and a well-developed raster.

Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za

Spiracles. Thoracic and abdominal spiracles with reniform plate, concavity facing cephalad. Spiracles on abdominal segment 1-3 larger than spiracles on abdominal segments 4-8.

Tarsangulus. Well-developed claw with 2 setae.

Stridulatory organs. Mesocoxal stridulatory organ (pars stridens) consisting of a main row of large, dark tubercles, with less defined area of small, pale granules outside this (Fig. 2g). Metatrochanteral stridulatory organ (plectrum) consisting of a row of about 50 granular carinae, close together on the anterior surface (Fig. 2h).

Discussion and conclusion

The larvae of each of the species collected were found in similar micro-habitats on the mountains. These consisted of moist, humus-rich soil in protected places such as under rocky overhangs and amongst the dense roots and bases of plants, mainly Restionaceae. From this study, and the little information provided by Scholtz and Endrödy-Younga (1994) and Tony Brinkman's unpublished record, there is fairly compelling evidence to suggest that the larvae are humus-feeders that are dependent on an accumulation of protected, moist decomposing vegetation. Moisture is obviously critical for larval survival; hence their occurrence in sheltered places where desiccation is less severe than in exposed places. Increased temperatures and drier conditions predicted by global warming scenarios are likely to have far-reaching effects for larvae and it is perhaps in this area that future studies of habitat suitability and change should concentrate.

Colophon larvae show only small inter-specific differences, something in common with the larvae of several scarabaeoid groups (Browne & Scholtz 1999; Grebennikov & Scholtz 2004). We ascribe this to the fact that the larvae of most of the groups live in fairly homogeneous micro-habitats such as humus, rotting wood and dung, somewhat removed

Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za

from the selective pressures that necessitate major change from the basal larval type in the group. That *Colophon* larvae of the different species are geographically isolated also precludes inter-species competition that might necessitate habitat shift and corresponding morphological adaptive change.

The taxonomic placement of *Colophon* has been riddled with ambiguity. Originally placed in the subfamily Lucaninae (Lacordaire, 1856), the genus was later transferred by Perry (1864) to the subfamily Chiasognathinae (which included Lampriminae at this stage). Didier and Séguy (1953) considered the subfamilies Chiasognathinae and Lampriminae as separate, placing *Colophon* in the latter. A later examination of the structure of the male genitalia by Holloway (1960) resulted in *Colophon* being transferred back to Lucaninae, with which Endrödy-Younga (1988) tentatively agreed. Scholtz and Endrödy-Younga (1994) discussed in some detail *Colophon* [and the east coast forest species, *Prosopocoilus natalensis*] larval morphology from a Lucanidae phylogenetic perspective, so only major points pertinent to this study are discussed here.

Colophon larvae are characterised by 3-segmented antennae (this is the basal condition in Lucanidae [and Scarabaeoidea]) and precludes them from inclusion in the Lucaninae based on it – Lucaninae have 4-segmented antennae, with the last segment greatly reduced in size. The epipharynx is generally similar in all studied species but distinct from other known lucanid species. The left mandible has teeth on the incisor edge between the apical teeth and mola, a character that places *Colophon* amongst other members of the subfamily Lucaninae. All Lucaninae have teeth in this position, a character unique to the group (Ritcher 1966). Scholtz and Endrödy-Younga (1994) appear to have misinterpreted the extent of the scissorial area, leading to the conclusion that there are no teeth in this area, which, they concluded also placed *Colophon* outside of the Lucaninae. The meso- and meta-trochanteral stridulatory

Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za

organs are similar to those of various other lucanine groups so are of little phylogenetic use. The soil-living and humus-feeding habit of *Colophon* larvae is comparable to those recorded for *Sphaenognathus* (Bartolozzi & Onore 1993) and *Altitatiayus* (Nagel 1934; Grossi & Almeida 2010), two high montane Lucanidae from South America. An ongoing study by David Hawks (University of California, Riverside) and Matt Paulsen (University of Nebraska, Nebraska), on the phylogeny of the world Lucanidae will give more insight into the family placement of *Colophon*.

From our study it would appear that *Colophon* species are morphologically rather similar which would suggests a phylogenetically conservative suit of characters (for similar conclusions see references to Trogidae and Scarabainae). Furthermore, in view of the dearth of comparative studies on the phylogenetic placement of Lucanidae larvae, we are unable to equivocally comment on *Colophon* phylogenetic placement and it remains for other [adult] phylogenetically significant morphological characters or DNA to provide more clarity on *Colophon*'s subfamilial placement.

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Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za

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e-mail: akswitala@zoology.up.ac.za

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e-mail: akswitala@zoology.up.ac.za

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Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za

Table and Figures

Table 1. *Colophon* species and number of larval specimens examined and used in the analyses.

Species	**Location**	**Second instar**	**Third instar**
Colophon haughtoni	Matroosberg Mountain	-	2
Colophon cameroni	Waaihoek Mountain	4	7
Colophon eastmani	Ben Heatlie Mountain	-	1
Colophon neli	Swartberg Mountain	-	2

Table 2. Structural differences between larvae of four *Colophon* species.

	Colophon haughtoni	*Colophon cameroni*	*Colophon eastmani*	*Colophon neli*
Left mandible	3 distinct teeth on incisor edge	3 distinct teeth on incisor edge	S2 and S3 semi fused.	3 distinct teeth on incisor edge
	Prominent scissorial notch	Prominent scissorial notch	Small scissorial notch	Prominent scissorial notch
	Sloping (>90˚) angle between molar and scissorial area	Sloping (>90˚) angle between molar and scissorial area	Strong bladelike slope between molar and scissorial area	Region between molar and scissorial area slightly more angulated
	One prominent triangular molar tooth	One prominent triangular molar tooth	Small rounded molar tooth	One prominent triangular molar tooth
Antenna	2 setae on distal segment	No setae on distal segment	2 setae on distal segment	2 setae on distal segment
Epipharynx	Proto-, dexio- and laephoba present	Proto-, dexio- and laephoba present	Proto-, dexio- and laephoba present	No phoba
	No epitorma	No epitorma	No epitorma	Faint epitorma
	Haptomerum with 2 sensilla	Haptomerum with 2 sensilla	Haptomerum with 2 sensilla	No sensilla on haptomerum

Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za

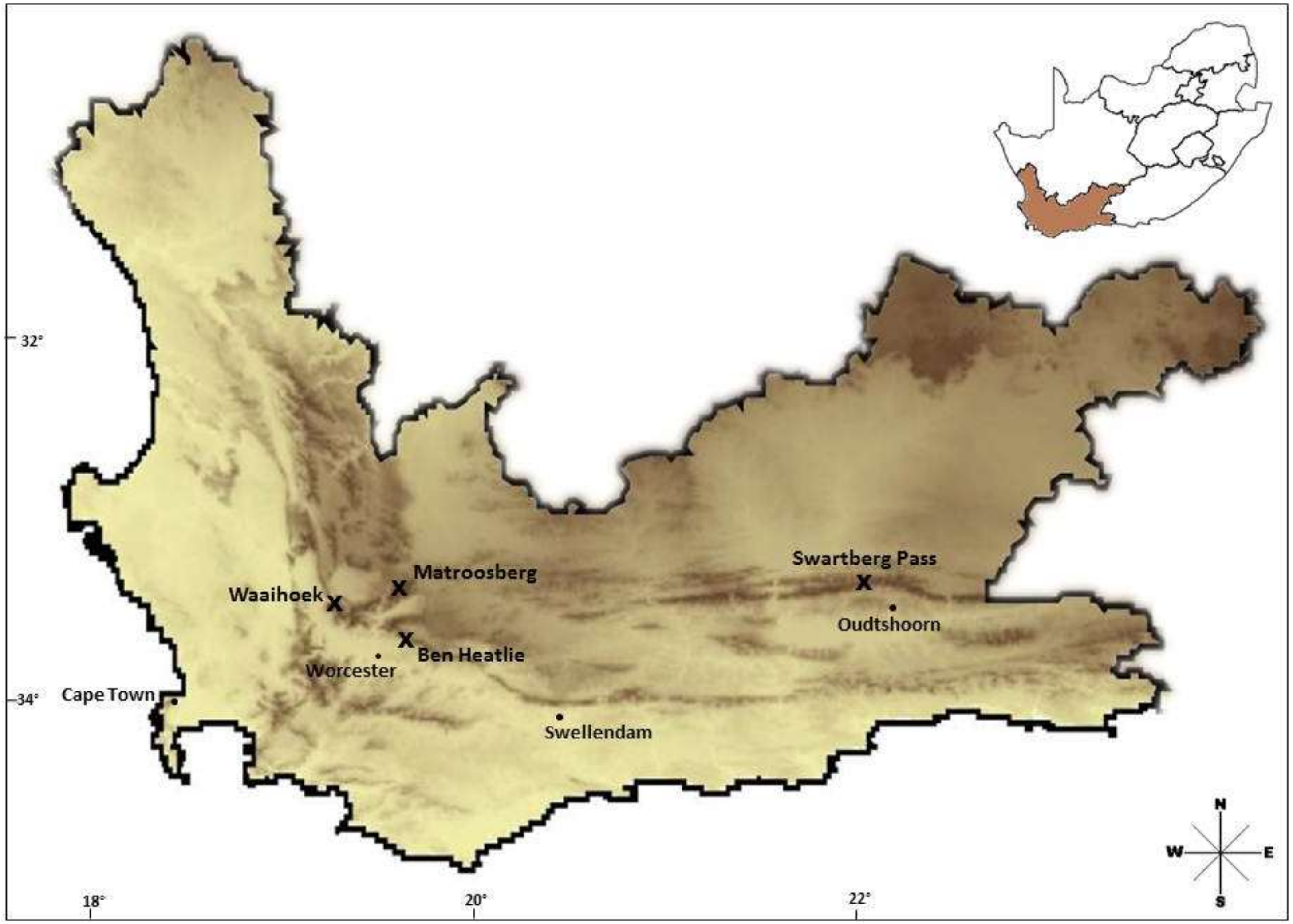

Figure 1. Map indicating the Mountain Peaks where *Colophon* larvae have been collected in the Western Cape of South Africa.

Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za

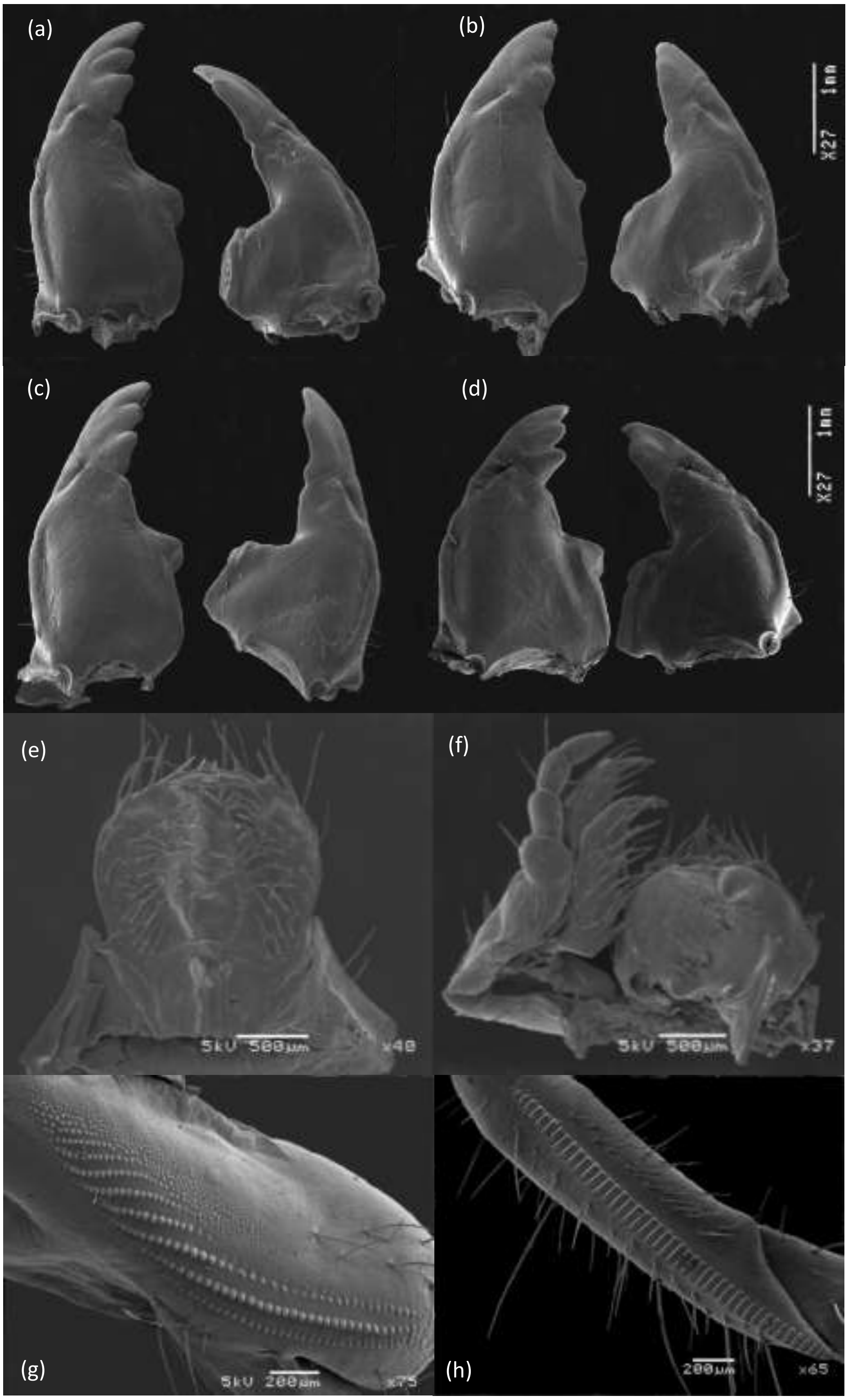

Figure 2. Structures of *Colophon* larvae. (**a-d**) Left and right larval mandibles of (a) *C. cameroni*; (b) *C. eastmani*; (c) *C. haughtoni* and (d) *C. neli*; (**e**) epipharynx of *C. cameroni*; (**f**) hypopharynx and maxilla of *C. cameroni*; (**g**) posterior view of the Mesocoxal stridulatory organ (pars stidens) of *C. eastmani*; (**h**) anterior view of the Metatrochanteral stridulatory organ (plectrum) of *C. eastmani*.

Corresponding author: A.K Switala
e-mail: akswitala@zoology.up.ac.za