

# Description of the larva and pupa of *Ichnestoma stobbiai* Holm 1992 (Scarabaeidae: Cetoniinae), a range-restricted species of conservation concern

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The Cetoniinae (Coleoptera: Scarabaeidae) genus *Ichnestoma* Gory & Percheron, 1833 currently comprises 13 described species and is endemic to South Africa. The species *I. stobbiai* Holm, 1992 is thought to occur in a very restricted area in and around Gauteng Province. Unlike most cetoniine larvae, the larvae of this species usually occur in dolomitic to cherty, well-drained soils. Here we briefly explain the conservation issues surrounding this species and describe its larvae and pupae.

**Key words:** Cetoniinae, conservation, *Ichnestoma stobbiai*, larvae, pupae, Scarabaeidae, South Africa.

## INTRODUCTION

The larvae of most groups of Scarabaeidae live and feed in the soil where they are thought to have evolved (Scholtz & Chown 1995), and because of the generally stable nature of the habitat, have maintained a somewhat conservative and homogeneous general body form that has possibly remained unchanged for millions of years (Edmonds & Halfpeter 1978). Adults, on the other hand, have radiated expansively in many morphological, ecological and behavioural respects. Their larvae, commonly called 'white grubs', are generally C-shaped and white with yellowish-reddish brown heads, stout bodies and three well developed pairs of legs (Ritcher 1966). Most of the larvae of the subfamilies Cetoniinae (one notable exception is *Desicasta laeivcostata* (van de Poll 1886) that lives in living plant material (Krell *et al.* 2002)), Melolonthinae, Rutelinae and Dynastinae are all free-living in the soil. Since all known cetoniine larvae crawl on their backs (Donaldson 1987, for all South African species examined; Ritcher 1966, for all North American species examined), living larvae are easily separated from all other Scarabaeidae larvae. However, preserved larvae require detailed microscopic study.

Currently there are 13 described species in the genus *Ichnestoma* Gory & Percheron 1833. With the exception of *I. perstriata* Holm and *I. patera* (Gory & Percheron), whose distribution extends into southern Namibia, the genus is endemic to South Africa where the species mostly occur in dry areas and at higher altitudes. The genus is considered a

geographic relict (Holm & Marais 1992, Perissinotto *et al.* 1999) while the constituent species are regarded as extremely range-restricted (Holm & Marais 1992, Perissinotto *et al.* 1999). This is related to their habitat, old and arid mountain ranges, together with the flightlessness of the females of most species and restricted dispersal capabilities of the short-lived males (Holm & Perissinotto (2004) note an entire range of reduction in flight capability amongst females of most species, from species with winged females to species with apterous females).

Most cetoniine larvae live in decaying vegetable matter or moister environments, whereas *I. stobbiai* larvae are usually free-living in dolomitic to cherty, rocky, well drained soils where they might be found up to 50 cm deep. The larvae of *Ichnestoma* species are likely to be important in the breakdown and turnover of detritus in these resource-poor environments (Perissinotto *et al.* 1999).

## CONSERVATION CONCERN

*Ichnestoma stobbiai* Holm, 1992 is a habitat specialist restricted to very small, fragmented areas, mostly in the Gauteng province of South Africa. Within its geographical range it occurs in small populations and only in a few tiny (1.8–31.0 ha), isolated habitat patches. This species has a long generation time (compared to many other Cetoniinae) of one year (Perissinotto *et al.* 1999) and spends most of its life history in the larval stage. As is the general rule for the entire genus, the dispersal capabilities of

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*I. stobbiai* adults are extremely low: they are only active aboveground for a few days in spring after substantial rains (>15 mm) although there might be more than one emergence after subsequent rains, if the first shower was not extensive enough (Perissinotto *et al.* 1999). All the mouthparts (except the mandibles and palpus) of the adults are fused into a plate, thus the adults do not possess functional mouthparts and do not feed. The larvae feed on detritus. The adult females are not capable of flight and therefore have extremely limited dispersal ability; they emerge from the soil, and release pheromones at the emergence site that allow the winged males to locate them. Once one or several males have found a female, she will dig into the soil very close to where she emerged. The male follows her and mating takes place in the soil. The female dies after laying eggs. Despite their ability to fly, the dispersal powers of the males are very restricted.

All of these factors favour to an increased extinction risk in a declining species (Purvis *et al.* 2000). *Ichnestoma stobbiai* has been assessed by the Gauteng Department of Agriculture, Conservation and Environment (GDACE) and qualifies as 'endangered: B1+2ab' (For explanation of criteria, see Standards and Petitions Working Group 2006). This is in the process of being submitted to the International Union for Conservation of Nature (IUCN).

The habitat requirements of *I. stobbiai* are poorly known and since they spend most of their lifecycle underground as either larvae or pupae, it is difficult to confirm the presence of a population at any particular locality for conservation or environmental purposes (e.g. Environmental Impact Assessments). To compound this, adults sometimes fail to emerge under apparently suitable conditions, as with the poor emergence in the spring of 2007 season (pers. obs.). These factors may result in erroneous conclusions about the species' presence or absence. It is therefore important to have reliable identification characteristics for the larvae and pupae since they are theoretically present for most of the year.

Because it is difficult to separate *I. stobbiai* larvae and pupae from other Cetoniinae immatures without study under a microscope and detailed information on the distinguishing characters, this paper provides the required morphological description of the larval and pupal stages to be able to do so. This project is part of a larger, com-

prehensive study of the ecology and conservation genetics of *I. stobbiai* being undertaken by members of the Scarab Research Group at the University of Pretoria.

## MATERIAL AND METHODS

In October 2006 a total of 60 adults (30 females, 30 males) were collected from a site to the west of Gauteng. This site has a relatively high censused population size as opposed to other known populations. Adult males were followed till they landed close to a female, and both were collected. The beetles were taken to the breeding laboratory of the Scarab Research Group at the University of Pretoria.

Fifteen pairs were placed in buckets of c. 21 × 21 × 28 cm in autoclaved, sifted (5 × 5 mm) loamy soil from the collecting site and the rest in unautoclaved, sifted (5 × 5 mm) loamy soil, also from the collecting site. All buckets were filled to c. 15 cm. All the detritus was still present as food for the larvae. Adults immediately started breeding and were left undisturbed for one month. The containers were kept moist to prevent desiccation of the larvae. After this, the buckets were inspected monthly. The larvae progressed to the third instar within two months, when 10 were killed in near boiling water and preserved in 70 % ethanol.

The final inspection of the contents of the buckets was on 6 September 2007 and a very high larval mortality rate was recorded. Only three live larvae (all from autoclaved sand) and five cocoons (three from autoclaved and two from unautoclaved sand) were recovered. The surviving larvae were kept in fresh soil from the collecting site and inspected on 23 February 2008. All three had pupated. The cocoons were hard and brittle. Five were soaked in water for 30 minutes before they could carefully be opened. A live pupa was found in each. They were placed between tissue paper in a Petri dish inside a small cardboard box and kept moist. After they had sclerotized, the pupae were killed and preserved as described above.

All specimens are stored in the University of Pretoria Scarab Collection (UPSA). Terminology used in the descriptions is based on Ritcher (1966), Smith *et al.* (1998) and Perissinotto *et al.* (1999). For the larval description we follow Perissinotto *et al.* (1999) where the only other *Ichnestoma* larvae are described.

## RESULTS

### Description of third instar larvae (Figs 1–3)

*Material examined.* Larvae collected from breeding colony, seven complete in 70 % ethanol, three dried and dissected (UPSA).

*Thorax and habitus.* Larvae are back crawlers and free-living in the ground. Body scarabaeiform, length 33–40 mm ( $n = 10$ ). Head capsule width, 2.9–3.2 mm ( $n = 15$ ) Pronotal shield evident, triangular with deep median depression. Spiracles distinct. Spiracular plate C-shaped, open anteriorly except pronotal one which opens posteriorly. All spiracles more or less the same size except last which is smaller. All segments with mostly short yellow-brown irregular spaced setae interspersed with some long setae.

*Legs.* Coxa, trochanter, femur and tibiotarsus of all legs with medium length fine setae. Tarsunguli of all legs falcate with five to seven setae at tip of protarsungulus and five at meso- and metatarsunguli, all setae about the same size, except hind dorsal seta of metatarsungulus which is extremely short.

*Cranium.* Ovoid, yellowish brown, except at precoxae and where antennae are attached, dark brown. Clypeofrontal suture distinct and dark brown. Epicranial suture distinct, extending from epicranial stem further along frons up to same level as posterior frontal setae. Frons with one pair each of anterior frontal setae, posterior frontal setae and exterior frontal setae. Frons with some irregular dark brown pores. Anterior angles each with one seta. Epicranium with some short irregular dorsoepicranial setae and two pairs of lateral setae. Ocelli absent.

*Clypeus.* Sub-rectangular. Postclypeus more sclerotized and longer than preclypeus. One pair of anterior clypeal setae, rooted in deep indentations situated paramedially and close to proximal end of post clypeus. Two pairs of exterior clypeal setae on lateral margin of postclypeus, one medially and one at fusion point with preclypeus. Irregular postclypeal pores, more numerous distally, no preclypeal pores present.

*Labrum.* Trilobed, not well defined, symmetrical with rounded lateral margins. Some irregular pores present, proximally, medially and at distal lateral margins. Three pairs of proximal setae, two proximally and one paramedially. One pair of setae at lateral margin. Apical and lateral lobes with numerous setae.

*Antennae.* Distinctly four-segmented, glabrous.

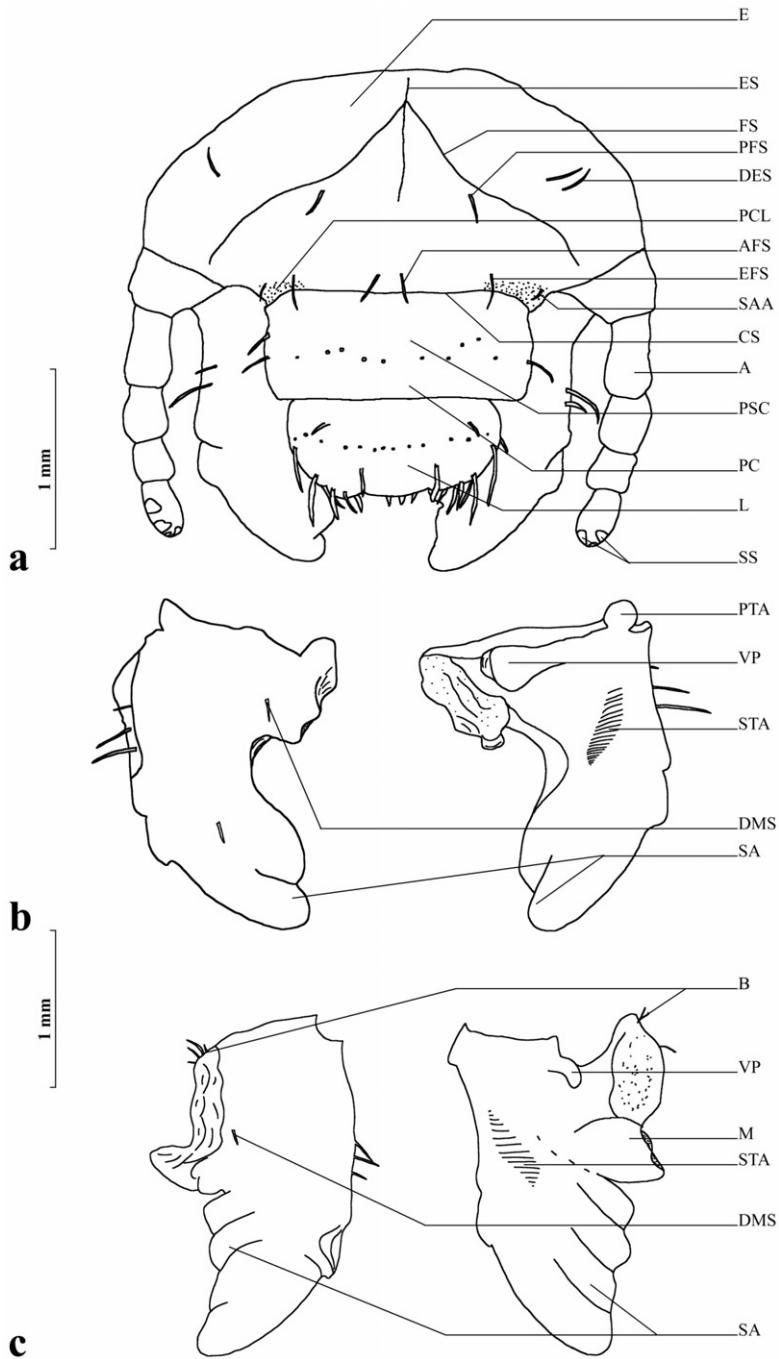
First segment longest, second two-thirds the length of first and third two-thirds as long as second. First three segments each yellow brown proximally, becoming pale-white distally. Third segment with one sensory spot protruding distally on inside lateral edge. Fourth segment yellow brown, three oval to round sensory spots at distal end and two on outside lateral edge.

*Mandibles.* Asymmetrical, left mandible slightly longer and less robust. Scissorial area very sclerotized. Outside lateral margin of both mandibles in some specimens with a single prominent hook medially. Scissorial area of left mandible having four small scissorial teeth and right with two robust scissorial teeth and a single seta at base. Angle between dorsal and outside-lateral surface is a uniformly round right-angle, with a row of three setae on lateral surface. Angle between dorsal and outside-lateral surface right-angled, angle uniformly round, with a row of three setae on lateral surface. Molar area of both mandibles heavily sclerotized. Molar area part of right mandible with three parallel ridges, proximal ridge longest, bending almost 90 degrees proximally, middle ridge more robust and distal ridge small and short. Single seta at dorsal base of molar area. Molar area of left mandible large, concave with a big, sharp protrusion distally and a very small, round protrusion laterally. Brustia present only ventrally at base of molar area of both mandibles. Stridulatory area big and clearly visible (for alignment and shape of ridges see Fig. 1b,c). Ventral process of left mandible smaller than right mandible. Postartis same size on both mandibles.

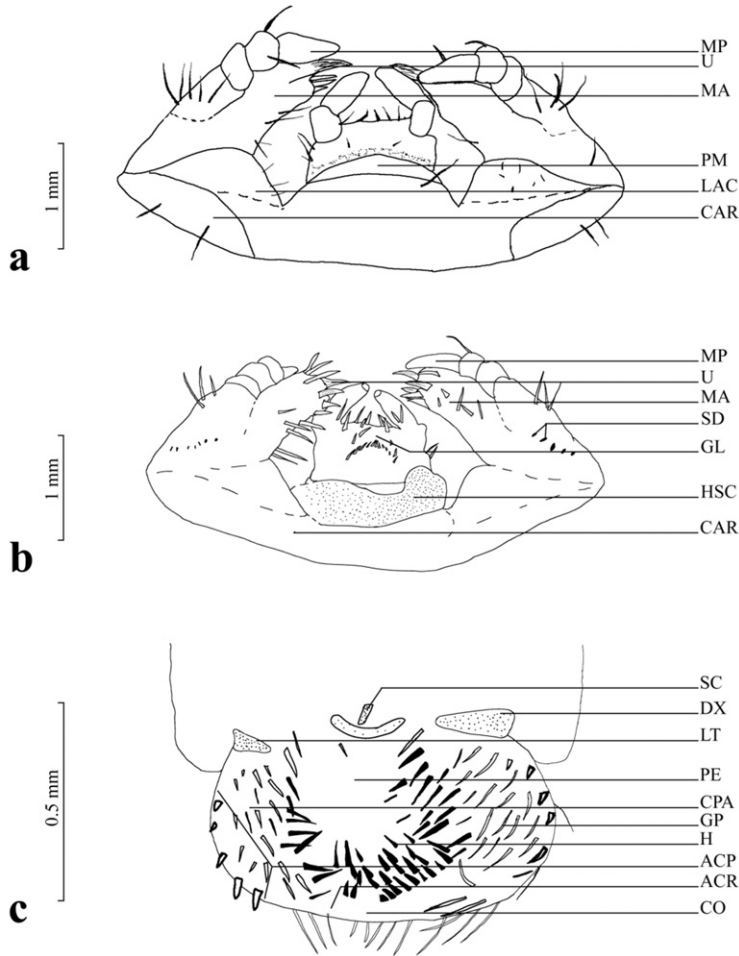
*Maxillae.* Galea and lacina fused into mala, suture obvious. Cardo with few irregular short setae. Lateral surface with two stipes. Distal-lateral area with few long setae. Dorsolateral edge with few short, stout stridulatory teeth. Mala with three unci, two larger unci at tip and one smaller below. Sensilla absent. Maxillary palp with three segments, sensory area at distal tip of terminal segment. Eight stridulatory teeth on left and right stipes. Proximal five sharp pointed and last three blunt.

*Labium.* Five pairs of long setae proximally on prementum. Postmentum with one pair of short setae and some irregular pores. Glossa with medium length setae medially glabrous, some sensilla proximally. Hypopharangeal sclerome asymmetrical. Truncate process obvious on right side. No second process.

*Epipharynx.* Lateral margins rounded. Apical



**Fig. 1.** Line diagrams showing head capsule and mandibles of *Ichnestoma stobbiai* larvae. **a**, Head capsule: E, epicranium; ES, epicranial suture; FS, frontal suture; PFS, posterior frontal seta; DES, dorsoepicranial setae; PCL, precolia; AFS, anterior frontal seta; EFS, exterior frontal seta; SAA, seta at anterofrontal angle; CS, clypeofrontal suture; A, antenna; PSC, postclypeus; PC, preclypeus; L, labrum; SS, sensory spots. **b**, Right mandible, left, dorsal view; right, ventral view; PTA, postartis; VP, ventral process; STA, stridulatory area; DMS, dorsomolar seta; SA, scissorial area. **c**, Left mandible, left, dorsal view; right, ventral view: B, brustia; VP, ventral process; M, molar; STA, stridulatory area; DMS, dorsomolar seta; SA, scissorial area.



**Fig. 2.** Line diagrams showing maxilla and epipharynx *Ichnestoma stobbiai* larvae. **a.** Dorsal surface of maxillae; MP, maxillary palp; U, uncus; MA, mala; PM, prementum; LAC, labacoria; CAR, cardo. **b.** Ventral surface of maxillae; MP, maxillary palp; U, uncus; MA, mala; SD, stridulatory teeth; GL, glossa; HSC, hypopharangeal sclerome; CAR, cardo. **c.** Epipharynx; SC, sense cone; DX, dexitorma; LT, laetorma; PE, pedium; CPA, chaetoparia; GP, gymnoparia; H, heli; ACP, acanthoparia; ACR, acroparia; CO, corypha.

margin in region of corypha bulging ventrally and with acroparia carrying long setae. No epizygum, zygum, clithra or plegmatium present. Acanthopariae with six to seven short, spiny setae. Gymnoparia with irregular medium slender setae and chaetoparia with longer thicker setae. Pedium strongly concave. Haptomerum with three irregular rows of heli. Laetorma sclerotized, triangular; dexitorma sclerotized, elongated. Haptolachus with faintly sclerotized crepis. Sensory cone present.

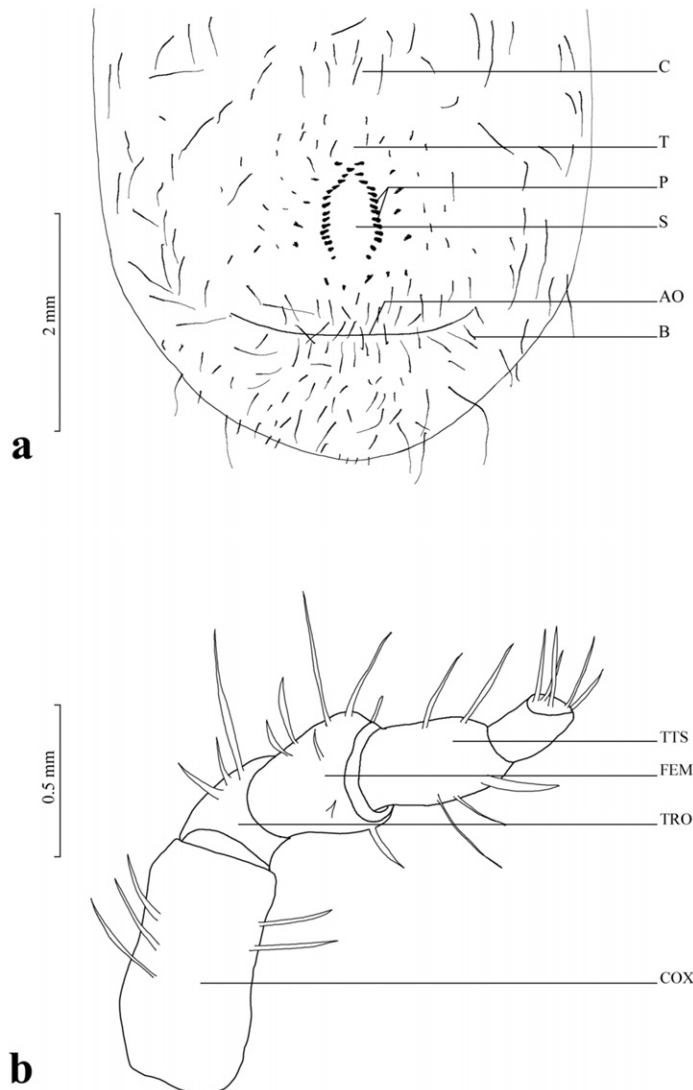
*Raster.* Septula present, extending laterally over medial third of last abdominal segment, two times longer than width. Palidium V-shaped pointing anteriorly with more than 16 irregularly paired

pali, infrequently only 10–11. Pali spatulate, medium length. Tegilla with sparse covering of long and short setae from barbulae to palidia. Barbulae with some long setae and campus with some short setae. Anal opening transverse, long and straight, surrounded with mostly short setae.

#### *Description of pupae* (Fig. 4)

*Material examined.* 5 pupae, 3♂ and 2♀, UPSA.

Cocoon average size ( $n = 5$ )  $16 \times 11$  mm, made from larval faeces. Pupae exarate, creamy white to dark brown depending on state of sclerotization; size variable, males (3) length: 12.9–16.5 mm and width: 10.0–12.2 mm and females (2) length:



**Fig. 3.** Line diagrams showing raster and middle leg of *Ichneostoma stobbiai* larvae. **a.** Raster; C, campus; T, tegilla; P, palidia; S, septula; AO, anal opening; B, barbula. **b.** Middle leg; TTS, tibiotarsus; FEM, femur; TRO trochanter; COX, coxa.

13.2–14.5 mm and width: 9.9–10.6 mm.

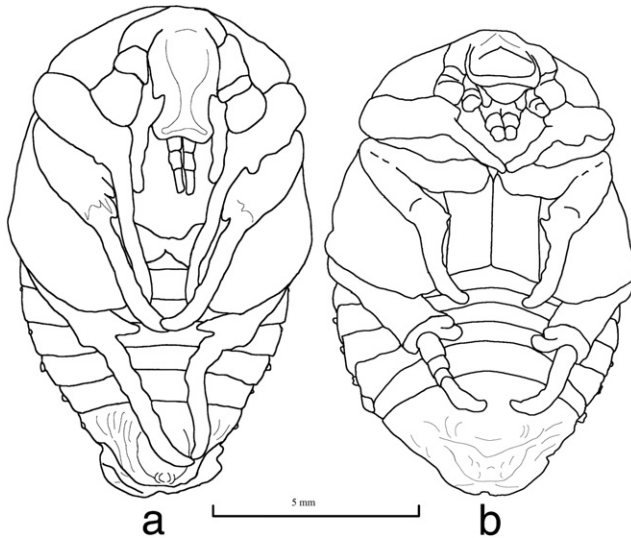
**Head.** Deflexed downward. Antenna and mouthparts clearly recognizable. Sexual dimorphism in clypeus evident, male with armed, bifurcated clypeal horns and female, concave, bilobed and lateral anterior margins slightly upturned. Eyes well differentiated.

**Thorax.** Pronotal disk slightly convex, punctate with punctures just smaller than distance between them, lateral corners anteriorly rounded and posteriorly angulate. Strongly convex projections

at posterior lateral corners in both sexes. Very fine and short setae, visible under high magnification, covering disk. Scutellum big, triangular. Meso- and metanota well developed with mesometasternal process small ending bluntly. Coxae well differentiated. Pteroteca free, folded around body, covering to half the nota, under middle legs. Hind wing teca in males slightly longer than elytron teca and in females the hind wing teca not even reaching the mesocoxa.

**Abdomen.** No dioneiform organs present. Tergites





**Fig. 4.** *Ichnestoma stobbiai* pupal habitus, ventral view. **a**, Male; **b**, female. No dorsal sexual differentiation was observed.

almost convex with median fold line. Tergites I to VI with conical projections adjacent to tergo-lateral margin. No transverse ridges on tergites present. Tergo-lateral tubercles present on tergites II–VIII. Sternites more or less convex. Genital ampulla large, C-shaped.

*Legs.* Well differentiated with all denticles, tarsomeres and spurs defined.

## DISCUSSION

The falcate mesotarsungulus and medium-sized head capsule places this species in the Goliathini (Donaldson 1987). The genus can not be confused with any other known Goliathini larvae of the same size, while *I. stobbiai* is the only *Ichnestoma* sp. to occur in the area. Pupae can be identified by the shape of the clypeal armour of the males and similar size to the adults.

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