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## Larval morphology of the water beetle *Ochthebius balfourbrownei* (Coleoptera: Hydraenidae) from marine rockpools of Cape Verde Islands

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### Abstract

The morphology of larval instars I, II and III of the water beetle *Ochthebius* (*Cobalius*) *balfourbrownei* Jäch, 1989 from Cape Verde Islands is described and illustrated for the first time, by scanning electron microscopy, with a special emphasis on their chaetotaxy and porotaxy. Specimens used in this study were collected with adults of the same species in marine rockpools. A detailed description of characters is provided for comparison with the other members of the same genus inhabiting the marine rockpools, and illustrations of the diagnostic features are presented, with the aim of providing information useful for taxonomic and phylogenetic studies of the genus *Ochthebius*.

**Keywords:** Marine rockpools, *Ochthebius*, larval taxonomy, SEM, Cape Verde Islands

### Introduction

*Ochthebius* s.l. is a large genus within the water beetle family Hydraenidae that includes nearly 540 species worldwide (Jäch 1989; Hansen 1991, 1998; Perkins 2007; Villastrigo et al. 2019), generally found at the margins of freshwater habitats, although some species prefer saline or brackish waters, having different degrees of halophilous tendencies (D’Orchymont 1932; Abellán et al. 2009; Perkins 2011; Sabatelli et al. 2016). Among the halophilous species, some *Ochthebius* are known to live for their entire life cycle in hypersaline ephemeral marine rockpool environments, found on rocky shores worldwide.

Phylogenetic relationships of the *Ochthebius* s.l., based on adult morphology, have long been the object of debate. Jäch and Skale (2015) divided the genus into four subgenera: *Asiobates* C. G. Thomson 1859, *Calobius* Wollaston 1854, *Enicocerus* Stephens 1829 and *Ochthebius* (s.str.) Leach 1815. However, a recent phylogenetic study on this group based on

molecular data (Sabatelli et al. 2016) depicted a more complex systematics, later confirmed by Villastrigo et al. (2019), that recognised the presence of 10 subgenera within the genus *Ochthebius*.

A relevant difference between the classifications is that *Cobalius* Rey (1886) represents an effectively separate subgenus (Sabatelli et al. 2016; Villastrigo et al. 2019). *Cobalius* currently includes 15 described species (Jäch 1989; Jäch & Delgado 2017; Ribera & Foster 2018; Sabatelli et al. 2018; Ribera & Cieslak 2019; Villastrigo et al. 2020), all living in marine rockpools with the sole exception of *Ochthebius* (*Cobalius*) *serratus* Rosenhauer 1856, associated with saline streams and pans (in southern Spain and northern Morocco). Members of *Cobalius* are widely distributed in the eastern Atlantic Ocean (British Islands, Atlantic coasts of continental Europe, Cape Verde, Canary Islands, Morocco, Madeira and Azores) and throughout the whole Mediterranean basin.

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Although larval chaetotaxy has been recognised as an informative source of characters in Coleoptera and has provided useful information for taxonomic and phylogenetic studies, as in many other groups of Hydraenidae, the immature stages of *Ochthebius* s.l. are still poorly known. To date, larvae of only a few species belonging to the genus *Ochthebius* have been described: *Ochthebius (Cobalius) lejolisii* Mulsant and Rey, 1861 (D'Orchymont 1913) from Atlantic coasts of Europe, *Ochthebius (Cobalius) subinteger* Mulsant and Rey, 1861 (Delgado & Soler 1996) from North Mediterranean coasts, *Ochthebius (Calobius) quadricollis* Mulsant, 1844 (Delgado & Soler 1997b), *Ochthebius* (s.str.) *auropallens* Fairmaire, 1879 (Delgado & Soler 1997b) from south-western Europe, *Ochthebius* (s.str.) *danjo* Nakane, 1990 (Delgado & Matsui 2000) from Japan, *Ochthebius* (s.str.) *gonggashanensis* Jäch, 2003 from China (Delgado 2003), *Ochthebius* (s.str.) *capicola* Péringuey, 1892 from Cape Peninsula (South Africa) (Sabatelli et al. 2013), *Ochthebius* (s.str.) *attritus* LeConte, 1878 from Cuba (Deler-Hernandez & Delgado 2017) and *Ochthebius* (s.str.) *sasakii* (Yoshitomi, Haruki Karube & Masakazu Hayashi, 2019) from Japan.

The aim of this study is to describe and illustrate all larval instars of *Ochthebius (Cobalius) balfourbrownei* from Cape Verde Islands using light microscopy and scanning electron microscopy (SEM), with a special emphasis on primary chaetotaxy and porotaxy. We also compare this larva with the other known *Ochthebius* larvae dwelling in

marine rockpools, to discuss the adaptive value and convergent evolution of characters possibly related to this peculiar environment.

## Material and methods

### Sampling

*Ochthebius balfourbrownei* (Figure 1(b)) from Cape Verde Islands was found on the island of Sal (Ilha do Sal), in hypersaline pools in shallow depressions on rock surfaces above the high tide line (Figure 1(a)), but within the splash zone. The pools contained variable numbers of the beetles; they were usually found on the submerged rock surfaces of the pools, in fissures or among submerged algal vegetation.

The larvae described below were collected by the first author together with F. Mosconi in February 2014 from two localities of the island of Sal (Murdeira and Buracona), with adults of the same species, and identified *ex societate imaginis*. They were collected from the pools by hand with soft forceps or, after vigorous agitation of the water, from the water surface.

### Examined material

A total of 20 larvae of *Ochthebius balfourbrownei* were collected: three first instars, seven second instars and 10 third instars. All larvae were initially preserved in 70% ethanol and deposited in the Sapienza University (Rome, Italy) Zoological Collection (MZUR).



Figure 1. (a) Hypersaline pool in shallow depressions on rock surfaces, Murdeira, Island of Sal, Cabo Verde; (b) habitus of *Ochthebius balfourbrownei*.

Three specimens (1 first instar and 2 third instars) of *O. balfourbrownnei* were rehydrated, cleared in 10% KOH, transferred to hot lactic acid, dehydrated through a series of EtOH baths of increasing concentration (10, 20, 50, 70, 90, 95 and 100%), left overnight in a clove oil bath and mounted on slides with Canada balsam. They were studied and measured using an Olympus BX51 light microscope, equipped with a drawing tube.

For the SEM analysis, seven specimens (2 first instars, 3 second instars and 2 third instars) of *O. balfourbrownnei* were dehydrated through a series of EtOH baths of increasing concentration (70, 80, 90, 95 and 100%), critical point dried (Bal-Tec CPD 030), mounted on a stub (using self-adhesive carbon disks), sputtered with gold (Emitech k550 sputter coater) and observed with the SEM column of a Dualbeam FIB/SEM Helios Nanolab 600 (FEI) (LIME Laboratory, University of Roma Tre, Rome).

In this paper, the general terminology of larval structures follows Lawrence (1991), while the chaetotaxy standards follow Delgado and Soler (1996, 1997a, 1997b, 1997c), Delgado and Archangelsky (2005) and Deler-Hernandez and Delgado (2017).

## Results

Larval description of *Ochthebius balfourbrownnei* Jäch, 1989

### Diagnosis

The larva of *Ochthebius balfourbrownnei* shows the following unique combination of characters: egg-bursters (EB) extremely reduced; head temporal regions without setae T2; head lateral regions without campaniform sensillum (LC1); head ventral regions with one seta (V1), V2 absent; antennomere II with one campaniform sensillum (IIC1) subapically, mesodorsal; mandibles with one conspicuous triangular premolar tooth; second labial palpomer with very small seta; pronotum and mesonotum of same length; metanotum slightly shorter than others; pronotum with one additional small seta posterior to A1; sternite I with one discal seta (D1), pygopod without anal hooks.

### First instar

#### Habitus and colouration

Elongate, slender. Head and body sclerites moderately sclerotised. Colour of sclerites uniformly

pale brown. Total body length about 1.4 mm (Figure 2(a)).

### Head

Head capsule width  $0.23 \text{ mm} \pm 0.01$  (mean  $\pm$  Stand. Dev.;  $n = 3$ ); head transverse, slightly narrowed posteriorly and distinctly curved laterally. Ecdysial suture Y-shaped; five stemmata are present on each ocular area. Chaetotaxy of head capsule as in Figure 3. Frontal region (Figure 3(a)) on either side with five setae, two frontodorsal (Fd1, Fd2), two frontolateral (Fl1, Fl2), one frontomarginal (Fm1) much shorter and thinner than the others (Figure 3(a)). Campaniform sensillum (FC1) present and clearly visible in all specimens. Egg-bursters (EB) extremely reduced, an anterior pair not clearly detected in all specimens and a posterior pair subtriangular, slightly produced, medial to Fl2, just anterior to FC1. Frontoclypeal suture clearly distinct. Clypeus (Cl) (Figure 3(a, c)) with three setae on either side (Cl1–Cl3) transversally lined anteriorly, decreasing in length from medial (Cl1) to lateral (Cl3). Epicranial regions (Figure 3(a)) each with two campaniform sensilla (EC1, EC2), one cephalic gland (CG) (one pair) and 10 setae: a row of four minute posterior setae (P1–P4), two epicranial dorsal setae (Ed1, Ed2), two epicranial lateral setae (El1, El2) and two epicranial marginal setae (Em1, Em2). Temporal regions (Figure 3(a, c)) each with three setae (T1, T3 and T4), T4 very short; T2 absent. Lateral regions (as in Figure 3(b, c)), campaniform sensillum (LC1) absent and two setae (L1, L2). Ventral regions (as in Figure 3(b)) each with one seta (V1), V2 absent; gula wider than long. Labrum cordiform (as in Figures 3(b, d), 4(c, d)) with the basic pattern of chaetotaxy of other hydraenid species, with two campaniform sensilla (C1, C2) on either side and seven setae arranged in two rows: two discal setae (Ld1, Ld2) and five marginal setae (Lm1–Lm5); setae Lm1, Lm3 and Lm5 situated dorsolaterally, Lm2 and Lm4 ventrolaterally; setae Lm2 (Figures 3(b, d), 4(c, d)) pectinate. Antennae (Figures 3(a–d), 4(b)) 0.37 times as long as head capsule width, antennomere II about 3 times as long as antennomere I, with four setae (IIA1–IIA4) and two solenidia (IIS1 and IIS2) and one campaniform sensillum (C1) subapically, mesodorsal; solenidium IIS1 long, slender, situated on distal end; solenidium IIS2 shorter, positioned ventrally on centre of antennomere; antennomere III 1.20 times as long as II, with four setae (IIIA1–IIIA4), IIIA4 very small, subapical, and three apical solenidia (IIIS1–IIIS3); IIIS1 longer than IIIS2 and IIIS3. Mandibles (as in Figures 3(c), 4(d, e)) similar in size and shape, with two campaniform sensilla (C1 and C2) and two setae of

different length, M1 shorter than M2 on outer edge of mandible; apex multidentate, with two major apical teeth and dorsal row of three minor teeth; one conspicuous, triangular premolar tooth present (Pt), slightly curved towards base (as in Figure 4(e)); both mandibles with a prosthema (Pr) long, slender and curved without any basal denticle, ending in four small teeth; penicillum extremely reduced (Pe); molar area with two rows, one dorsal and one ventral, of small denticulate microsculpture basally directed (as in Figure 4(e)); fringe of minute hairs present at base of mola; outer edge of mandible with two setae; ventral part of mandibular base with a rounded condyle and without any accessory process. Maxillae (as in Figures 3(b–d), 4(c, d)): cardines longer than wide and widely separated from each other by submentum, each with one seta (Cdo1); stipes longer than wide, with four setae (Stp1–Stp4) and one campaniform sensillum; palpifer with a small clearly defined sclerite, and with one seta (Pf1); both galea and lacinia fixed; galea (Ga) hyaline, falciform and slender; lacinia slightly wider than galea, with six stout setae along outer edge and two elongate, dorsal, sub-basal setae; maxillary palp three-segmented: segment 1 with two campaniform sensilla, segment 2 with two setae (Pm1, Pm2), segment 3 with a long, digitiform, sensorial appendage on external surface (SD) (as in Figures 3(b–d), 4(c)), and a small, pointed sensorial structure on its apex. Labium (as in Figures 3(b, d), 4(c) consisting of three sclerites: submentum (Smnt) with two setae, mentum (Mnt) with four setae and two campaniform sensilla, and praementum (Pmnt) with four setae as well as two inconspicuous campaniform sensilla; ligula (Lg) well developed, composed by two globose lobes, bearing four small dorsal sub-apical sensilla; labial palps each with a cupuliform sensillum bearing several small papillae and pores and one rounded sensorial appendage (as in Figure 4(d, f)). Second labial palpomer with very short seta, medially directed.

#### Thorax

Tergites transverse, similar in width, pronotum and mesonotum similar in length; metanotum slightly shorter than others; ecdysial suture present in all tergites. Pronotum (Figures 2(a), 5(a)) with 14 setae and five campaniform sensilla on either side. Setae: four in anterior row A(A1–A4), A1 and A4 shorter, and one additional small seta posterior to A1 (A); three in lateral row L (L1–L3), L2 shortest; four in posterior row P (P1–P4), P1 and P4 shorter; one in each of the three discal groups (Da1, Db1, Dc1); all discal setae well developed and similar in length, transversally lined. Pronotal campaniform sensilla C1 to C5 present. One tergal gland (Tg) on either side of pronotum. Mesonotum (Figure 2(a)) with 14 setae and three campaniform sensilla (C3–5)

on either side. Setae: four minute setae in anterior row A (A1–A4); three longer setae in lateral row L (L1–L3), L2 shortest; four in posterior row P (P1–P4); one in each of the three discal groups (Da1, Db1, Dc1); discal setae on mesonotum not reduced. Mesonotal campaniform sensilla C1 and C2 absent. Metanotum similar to mesonotum. Legs: Prothoracic leg (as in Figure 5(c,d)). Coxa with 14 setae: 1D, 2 Ad, 4 Al, 1 Av, 1Pd, 4 PI and 1 Pv. Trochanter with seven campaniform sensilla and eight setae: 1 Ad, 2Al, 2 Av, 1 PI, 1 Pv and 1 V. Femur with two campaniform sensilla and eight setae: 1 D, 1 Ad, 1 Al, 2 Av, 1 Pd, 1 PI and 1 V. Tibia with one campaniform sensillum and eight setae: 2D, 1Al, 1Av, 2Pd, 1PI and 1Pv. Tibiotarsal setae Ad1 absent. Tarsungulus with two minute setae (Figure 5(d)).

#### Abdomen

Subcylindrical, with subparallel sides, tapering posteriorly. Abdominal terga and sterna I–VIII each with a well sclerotised plate; abdominal pleura with two small sclerites, a dorsopleural and a ventropleural sclerite, on either side. Tergites I–VIII as in Figures 2(a), 5(b). Number of setae and campaniform sensilla reduced: setae A2, Db1, Dc1, P2 and sensilla C1, C2, C4 absent. Remaining setae arranged in a transverse row. Dorsopleural sclerites each with a spiracle (SP) and two setae (DP1, DP2) (Figure 5(e)). Ventropleural sclerites of segment I each with one seta: VP1. Ventropleural sclerites of segments II to VIII each with two setae (VP1, VP2) (as in Figures 2(c), 5(c)). Sternite I (Figure 5(c)) with three setae on either side: one small pre-sternal seta (PS1), one discal seta (D1), and one posterior seta (P2); P1 and P3 absent; subprimary seta lateral on sternum absent; additional seta (D2) absent. Segment IX bearing a pair of urogomphi, without dorsopleural or ventropleural sclerites. Setal homologies on segment IX and X (Figure 5(f)) and with reduced chaetotaxy. Urogomphi (Figure 5(f)) two-segmented: segment I (URI) mobile, not fused to tergite IX, with six setae (U1–U6) and four campaniform sensilla (C1–C4); segment II (URII) 0.32 times as long as segment I, with an apical seta (AS). Abdominal segment X (Figure 5(f)), forming a completely sclerotised ring, with three long ventrolateral setae of unknown homology on either side. Pygopod (Figure 5(f)) moderately developed, without anal hooks (AH), with three pairs of very short setae.

#### Second instar

Body length 1.52 mm. Head capsule width: 0.31 mm  $\pm$  0.02 (mean  $\pm$  Stand. Dev.; n = 7)

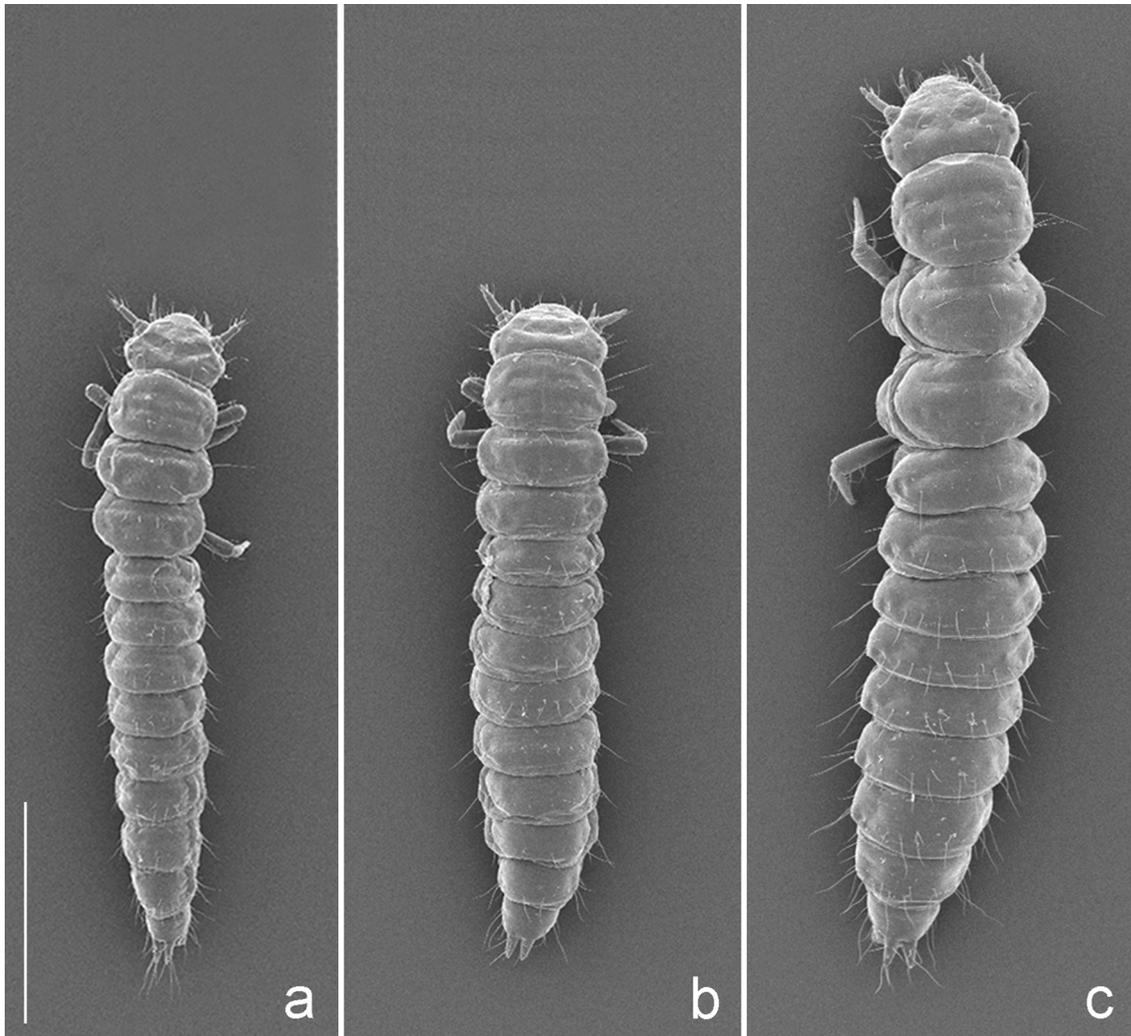


Figure 2. *Ochthebius balfourbrowniei* larvae. Scanning electron microscopy micrographs of habitus, dorsal view: (a) first instar; (b) second instar; (c) third instar. Scale bar: 1 mm.

(Figure 2(b)). Clearly distinguishable from the first instar due to the lack of cephalic egg-bursters (EB). It differs from the third instar by the presence of dorsopleurites and ventropleurites not yet fused to tergi and abdominal sternal. Chaetotaxy identical to first instar.

#### *Third instar*

Body length 1.93 mm. Head capsule width:  $0.40 \text{ mm} \pm 0.02$  (mean  $\pm$  Stand. Dev.;  $n = 10$ ) (Figure 2(c)). Chaetotaxy identical to those of first and second instars except for an additional pair of setae on meso- and metanotum internal to Da1. Abdominal dorsopleurites and ventropleurites fused to dorsal and ventral sclerites.

#### Discussion

Larvae of *Ochthebius (Cobalius) balfourbrowniei* show different characters present in other larvae of the genus *Ochthebius* inhabiting marine rockpools (Delgado & Soler 1996, 1997a, 1997b; Delgado & Matsui 2000; Sabatelli et al. 2013). Herein, we excluded the larva of *O. lejolisii* from the comparison, because it was not described with modern and comparable criteria (D'Orchymont 1913). In comparison to the only larva of the *Cobalius* group to date studied according to modern criteria, *O. subinteger* (Delgado & Soler 1996), the larvae of *O. balfourbrowniei* show numerous shared characters: presence of campaniform sensillum (FC1); presence of egg-bursters (EB), here extremely reduced;

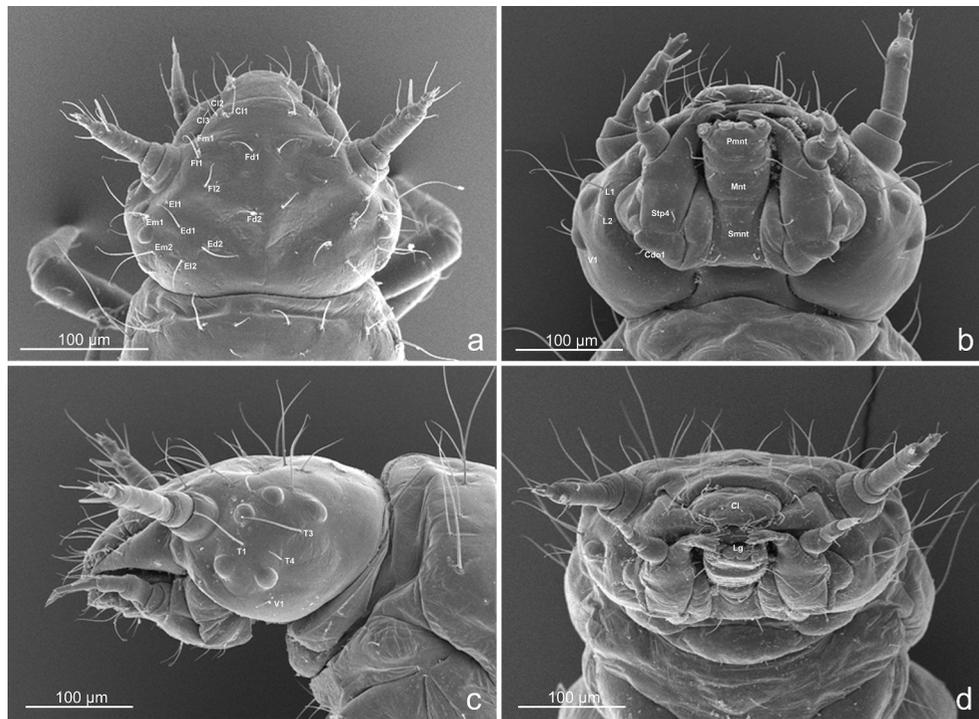


Figure 3. *Ochthebius balfourbrownnei* larva. Scanning electron microscopy micrographs of head capsule: (a) first instar, dorsal view; (b) second instar, ventral view; (c) second instar, lateral view; (d) second instar, frontal view. Abbreviations: Cdo, cardines setae; Cl, clypeus; C11–3, clypeal setae; Ed1–2, epicranial discal setae; E11–2, epicranial lateral setae; Em1–2, epicranial marginal setae; Fd1–2, frontal discal setae; F11–2, frontolateral setae; Fm1, frontomarginal setae; L1–2, cephalic lateral setae; Lg, ligula; Mnt, mentum; Pmnt, prementum; Smnt, submentum; Stp, setae of the stipes T1, 3–4, temporal setae; V1, ventral setae.

absence of temporal seta T2, a character that so far, as suggested by Delgado and Soler (1996) and Delgado and Archangelsky (2005), could constitute an autapomorphy for *Cobalius*; absence of campaniform sensillum (LC1) on lateral regions; absence of V2 on ventral regions; absence of tibiotarsal setae Ad1; presence of setae DP1 and DP2 on dorso-pleural sclerites; sternite I without setae P1 and P3; absence of additional seta (D2) and pygopod without anal hooks (AH).

However, the larvae of *O. balfourbrownnei* may be distinguished from larvae of *O. subinteger* by the subapical, mesodorsal presence on antennomere II of one campaniform sensillum (IIC1), which seems to be an autapomorphic character of this species; Ligula (Lg) well developed, composed by two globose lobes; presence on mandibles of one conspicuous, triangular premolar tooth, slightly curved towards base; penicillus extremely reduced; molar area with two rows, one dorsal and one ventral, of small, denticulate microsculpture, basally directed; second labial palpomer with very small seta, medially directed; pronotum with one additional small seta posterior to A1 (A); mesonotal campaniform sensilla C1 and C2 absent and sternite I with one discal seta (D1).

On the other hand, some characters that are considered phylogenetically informative are shared with species belonging to *Calobius* and *Ochthebius capicola*, which share the marine rockpool habitat with *O. balfourbrownnei*. The following characters are synapomorphic: (1) presence of mandibular penicillum; (2) absence of tibiotarsal setae Ad1; (3) absence of additional seta (D2); and (4) absence of anal hooks. The larvae of *O. balfourbrownnei* may be distinguished from larvae of *O. danjo* by the different state of all the above-mentioned characters, as shown below, despite both living in the marine rockpools. In conclusion, the results of our study provide additional support to the phylogenetic relationship between *O. balfourbrownnei* and the species belonging to *Calobius* and *Ochthebius*. Likewise, this study highlighted the close relationships of *O. balfourbrownnei* and *O. subinteger*, as confirmed using the molecular analyses of Sabatelli et al. (2016) and Villastrigo et al. (2019, 2020), with which it shares numerous characters, particularly the loss of temporal setae T2, considered at present an autapomorphic character for the genus *Cobalius* by Delgado and Soler (1996) and Delgado and Archangelsky (2005).

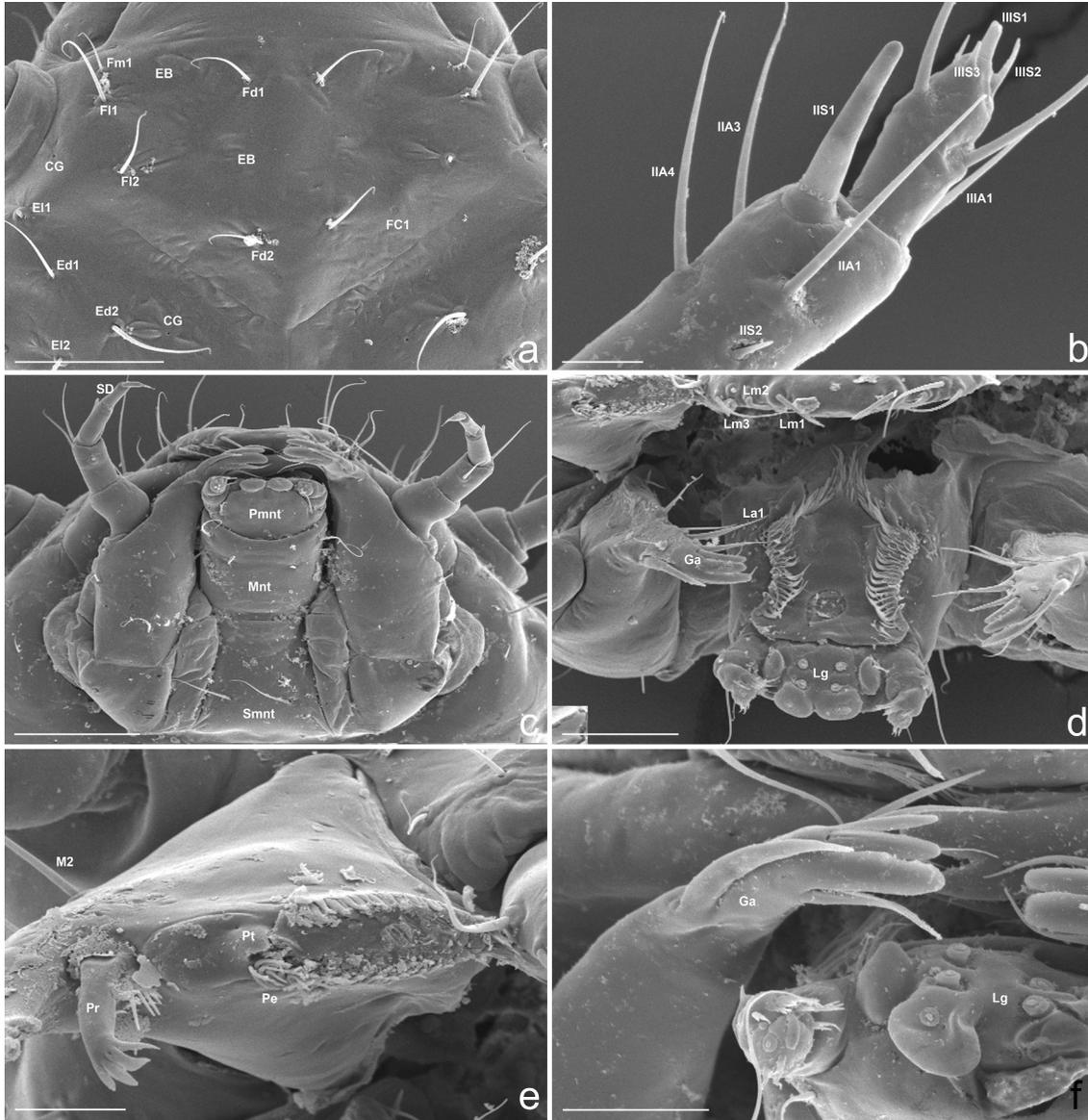


Figure 4. *Ochthebius balfourbrowniei* second instar larva. Scanning electron microscopy micrographs of head appendages: (a) apex of left antenna, ventral view; (b) labrum, frontal view; (c) left labial palp with elongate seta medially directed, frontal view; (d) left maxilla, setiferous medial lobe; (e) apex of left mandible; (f) triangular premolar tooth and penicillus. Abbreviations: CG, cephalic glands; Ed1–2, epicranial discal setae; El1–2, epicranial lateral setae; Em1–2, epicranial marginal setae; Fd1–2, frontal discal setae; F11–2, frontolateral setae; Fm1, frontomarginal setae; Ga, galea; La1, lacinial seta 1; Lg, ligula; Lm1–3, labral marginal setae; M2, mandibular setae; Mnt, mentum; Pe, mandibular penicillum; Pmnt, prementum; Pr, prostheca; Pt, triangular premolar tooth; SD, sensory digitiform sensilla; Smnt, submentum; IIA, antennal setae of segment II; IIIA, antennal setae of segment III; IIS1–2, antennal solenidia of segment II; IIIS1–3, antennal solenidia of segment III. Scale bars: a–b = 20 µm; c–e = 10 µm; f = 5 µm.

**Key to *Ochthebius* larvae from marine rockpools**

1. Mandibular penicillum absent; tibiotarsal setae Ad1 present; abdominal sternal setae D2 present; anal hooks well developed (Delgado & Matsui 2000) ....  
 ..... *O. danjo*

- Mandibular penicillum present; tibiotarsal setae Ad1 absent, abdominal sternal setae D2 absent; anal hooks absent ..... 2  
 2. Labral marginal setae Lm1 pectinate; pygopodial setae absent; urogomphal campaniform sensilla absent; long urogomphal setae U6 (Delgado & Soler 1997a. .... *O. quadricollis*

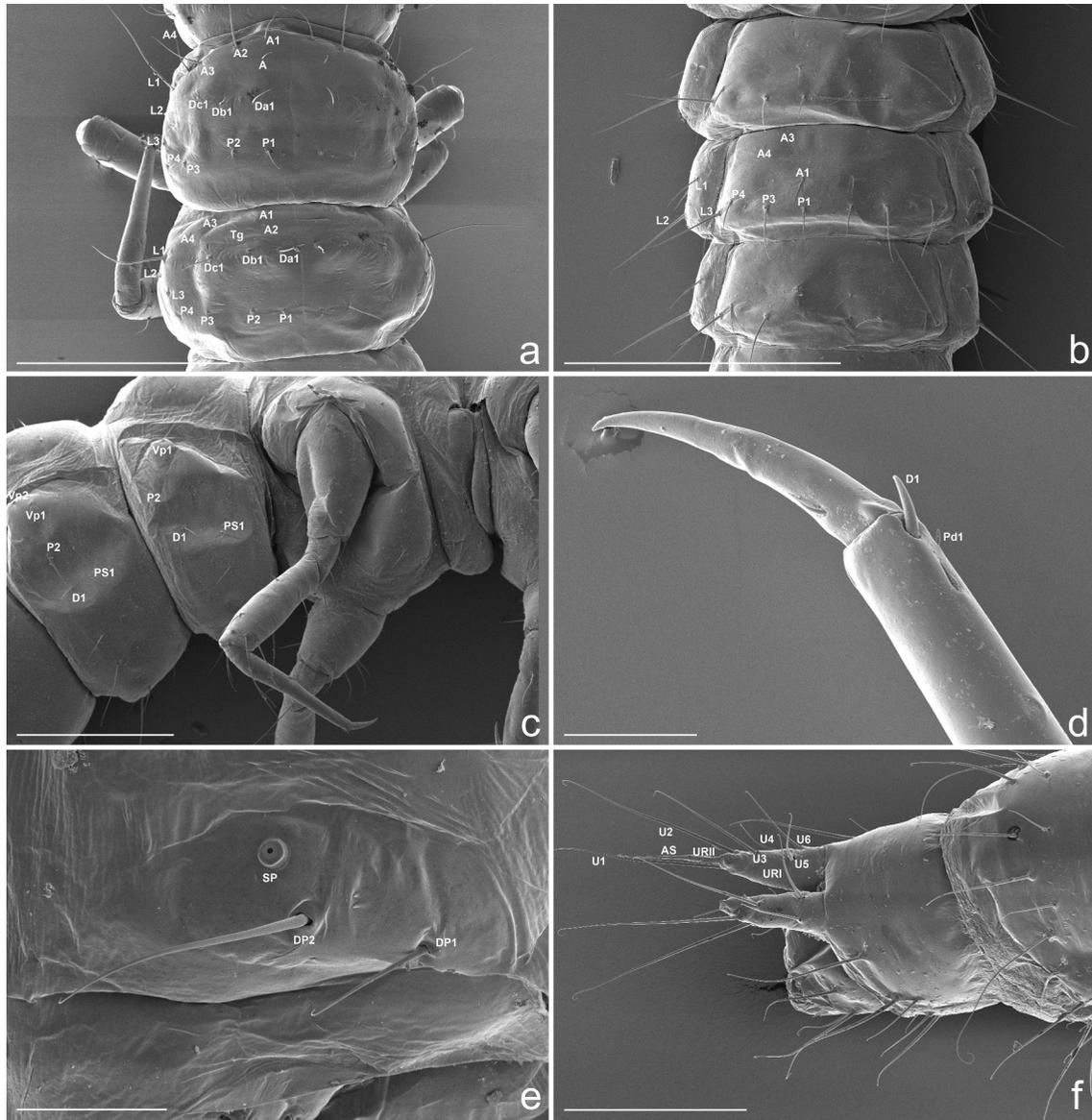


Figure 5. *Ochthebius balfourbrownei* larvae. Scanning electron microscopy micrographs of: (a) pronotum, first instar; (b) tergites I–VIII, second instar; (c) metathoracic leg in ventrolateral view, third instar; (d) metathoracic tarsungulus (close-up of the former), second instar; (e) abdominal segment III of first instar larva, left lateral view; (f) abdominal apex of first instar larva, dorsolateral view and urogomphus. Abbreviations: A1–4, anterior setae; A11, anterolateral seta; AS, apical seta; Av1, anteroventral seta; D1, sternal discal seta; Da1, Db1, Dc1, primary dorsal discal setae; DP1–2, dorsopleural setae; L1–3, lateral setae; P1–4, posterior setae; Pd1, posterodorsal setae; SP, spiracle; Tg, tergal gland; U1–6, urogomphal setae; URI–II, urogomphal segments; Vp1–2, ventro-pleural setae. Scale bars: a–c = 200 µm; b = 3000 µm; d = 20 µm; e = 25 µm; f = 100 µm.

- Labral marginal setae Lm1 of normal shape; pygopodial setae present; urogomphal campaniform sensilla present; short urogomphal setae U6 ..... 3  
 3. Cephalic temporal setae T2 present; cephalic ventral setae V2 present; pronotum with one pair of additional setae posterior to A1; meso- and metanotum with one pair of additional setae close to ecdysial line; sternum with one new lateral subprimary seta L' (Sabatelli et al. 2013) ..... *O. capicola*

- Cephalic temporal setae T2 absent; Cephalic ventral setae V2 absent ..... 4  
 4. Shape of ligula digitate; abdominal sternal setae D1 absent (Delgado & Soler 1996) .....  
 ..... *O. subinteger*  
 - Shape of ligula globose; abdominal sternal setae D1 present; presence on antennomere II of one campaniform sensillum (IIC1) subapically, meso-dorsal ..... *O. balfourbrownei*

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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