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Updates on the little-known *Firminus maltensis*, with revisited classification based on the anatomy of everted endophallus (Coleoptera: Scarabaeidae, Melolonthinae)

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Abstract
New information on the almost unknown rhizotrogine *Firminus maltensis* Rössner & Zorn, 2013, endemic to the Maltese Archipelago, is provided, based on new specimens recently collected. The species is transferred to the genus *Rhizotrogus*, introducing *Rhizotrogus maltensis* (Rössner & Zorn, 2013) as **comb. nov.,** based on new evidence provided by the morphology of the endophallus, whose everted structure is addressed for the first time in the genera *Firminus* and *Rhizotrogus*. A comparative diagnostic characterization for the endophalli of the two genera is proposed. Some morphological traits are also discussed, and the first behavioural and ecological observations are provided.

Keywords. Maltese Archipelago, *Rhizotrogus*, *Rhizotrogus maltensis,* taxonomy, morphology, ecology, endophallus.

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Introduction

Firminus maltensis Rössner & Zorn, 2013 is a little-known rhizotrogine species apparently endemic to the Maltese Archipelago, with records so far restricted to Gozo Island. To date, only two males collected in 1979 with no precise locality available, and a female specimen from Xlendi, originally identified as *Amphimallon* cfr. *scutellare* (P.H. Lucas, 1847) and whose identity is however uncertain, are known (Rössner & Zorn 2013).

In 2019, encouraged by recent observations of Rhizotrogini remains in the area (R. Rattu, pers. comm. to authors), one of us (VG) looked for the species around Dwejra. The search was successful, allowing to gather first observations on its habitat and on adult behaviour, and to expand knowledge on its morphological traits, also reporting some anomalies frequently affecting its antennomeres.

In the framework of a broad exploration of the endophallic anatomy of the Palaearctic Rhizotrogini, the endophallus of *Firminus maltensis* was examined and compared to that of related, or supposedly related, species: its generic assignment is discussed on this original basis, and evidence is provided for its correct placement in the genus *Rhizotrogus* Latreille, 1825.

Materials and methods

Acronyms of collections
MUCC: private collection Marco Uliana, Codevigo, Italy
VGCS: private collection Valerio Gallerati, San Pietro in Casale, Italy
MLHU: Martin-Luther-Universität, Zoologische Sammlung, Halle, Germany

Comparative material assessed. The inflated endophallus of *Firminus maltensis* has been compared to that of the supposedly most similar species [*F. bellieri* (Reiche, 1862)], as well as to that of the type species of both taxa involved in the problematic genus-level attribution of the discussed species, namely *Firminus* [type species *Firminus punicus* (Burmeister, 1855) and *Rhizotrogus* (type species *Rhizotrogus aestivus* (Olivier, 1789)]. The following specimens were examined:

Firminus bellieri (Reiche, 1862): • 2 ♂♂, **France**, Corsica, Lago di Capitello, 26.VIII.1982, Leg. Meregalli, MUCC (both endophalli inflated).

Firminus punicus (Burmeister, 1855):

Syntypes: • 1 ♂, 1 ♀, [**Algeria**], Const. [= Constantine], MLHU (endophallus dissected by previous worker, not inflated).
Other specimens • 1 ♂, **Algeria**, G. Kabylie, Forêt Akfadou, m 1250, 2.V.1983, leg. Osella, MUCC (endophallus inflated).

Rhizotrogus aestivus (Olivier, 1789): • 2 ♂♂, **Greece**, Metsovo (Epirus), 4-5 Km Nort-west Metsovo, N 39° 46' E 21°09', 30.V.2010, m 1400, leg. Z. Lucbauer, MUCC (both endophalli inflated) • 1 ♂, **Greece**, Pindo, Metsovon, Katara, 1.VI.2013, leg. C. Sola, MUCC (endophallus inflated) • 1 ♂, **Ukraine** (eastern), Donetzk Region, Severski Donetz river, Zakotnoje vil., 11.v.1997, Leg. O. Pak, MUCC (endophallus inflated).

The endophallic morphology of all other species of *Firminus* was also taken into account in the discussion, based on currently unpublished data (MU, in preparation).

Treatment of endophalli. Endophalli of examined specimens have been rehydrated, softened with NaOH solution, rinsed, and inflated by injection of white UV curable gel.

Terminology. Anatomical terminology according to Cristóvão & Vaz de Mello (2020), and Coca-Abia & Martin-Piera (1998) for the specific terms applied to Rhizotrogini. For anatomical portions of endophalli never figured or addressed before, necessary descriptive terminology was introduced directly in the discussion.

Images. All-in-focus images were produced after stacks of photos taken a Pentax K-1D camera moved by semiautomatic system MJKZZ QOOL Rail 250 rail, stacked with CombineZP software and enhanced with Photoshop CS6 software. Photos of the habitus and of external anatomic details were taken with a Rodenstock Rodagon 2,8/50mm lens, photos of antennomeres and details of raspulae were taken with a 10x Leitz Wetzlar objective for compound microscope; in both cases a bellow extendable up to 290 mm was used.

Results and discussion

Rhizotrogus maltensis (Rössner & Zorn, 2013), **comb. nov.** (Figs 1-3, 10-13)
Firminus maltensis Rössner & Zorn, 2013: 243.

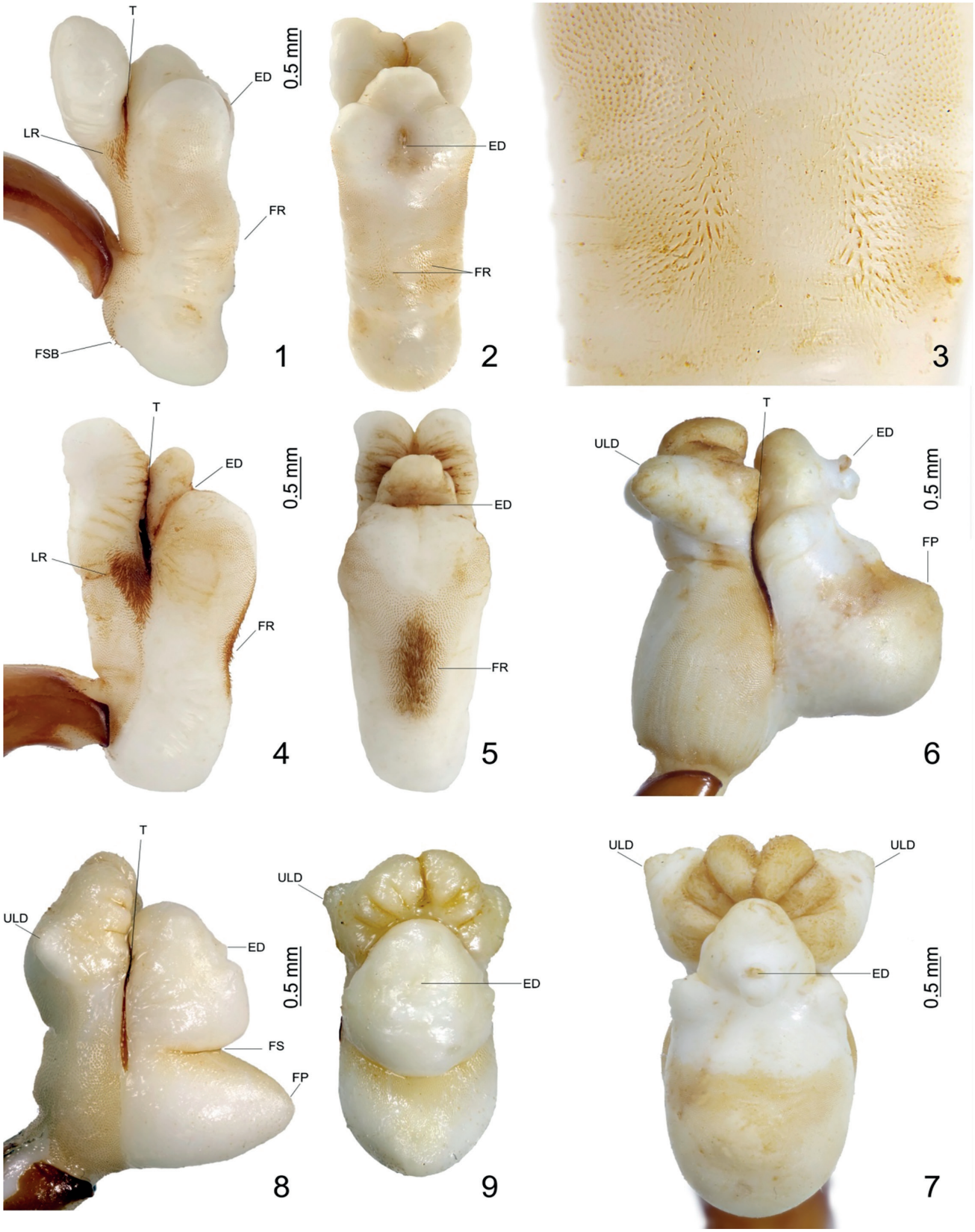
Material examined
• 6 ♂♂, **Malta** (Maltese Archipelago), Gozo, Dwejra Bay, 36.050, 14.191, 2 November 2019, leg. V. Gallerati, MUCC and VGSC.

Generic placement. In the frame of a still unclear characterization of the genus *Firminus* Coca-Abia, 2003 and other close genera, including *Rhizotrogus*, *F. maltensis* was originally treated as a member of the genus *Firminus*, although admittedly atypical (Rössner & Zorn 2013). The original generic placement was decided based on the overall similarity with the Sardo-Corse endemite *Firminus bellieri* but acknowledging the unusual presence of tooth-like projections (albeit scarce and rather small) at the insertion of the setae along the dorsal side of metatibiae, a condition regularly observed in *Rhizotrogus* but never in *Firminus* (Coca-Abia 2003).

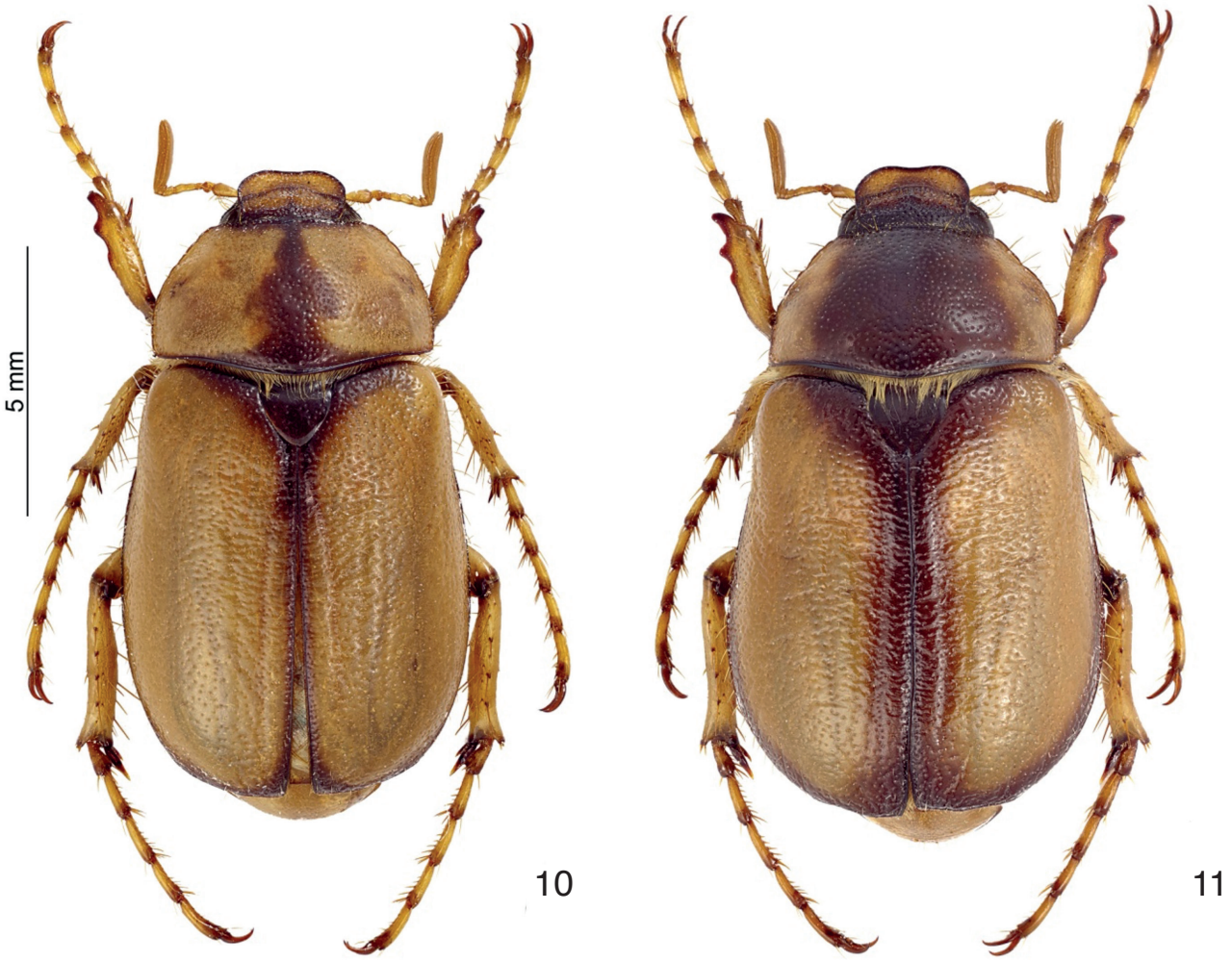
The everted endophallus of *Firminus maltensis* (Figs 1-3) resulted to be not similar to that of *F. bellieri* (Figs 8-9), nor with that of any other species of the genus *Firminus*, including the type species of the genus, *F. punicus* (Figs 6-7). Conversely, it resulted well-fitting with one of the endophallic morphotypes encountered among *Rhizotrogus*, and in particular with the one which is also shared by type species of the genus, *R. aestivus* (Figs 4-5, "morphotype II" according to Coca-Abia & Martin Piera 1998).

Different character conditions of this endophallic morphotype are here identified as diagnostic against *Firminus*:
- the general shape, which is evidently more elongated and slenderer than in *Firminus*.
- the lower part is simple, regularly shaped, lacking a frontal projection that is present in all members of *Firminus*, with shape ranging from a broad bulge to a digitiform diverticule (cfr. Figs 6 and 8, FP).
- the lack of a frontal split, in form of fold or deep constriction, that separates the portion next to the eiaculatory ductus from the lower part of the endophallus (Fig. 8, FS) and is present in all members of *Firminus*, although this character state in *F. punicus* is ambiguous (Fig. 6).
- the lack of the upper lateral diverticula on the posterior portion of the endophallus, which are present in all members of *Firminus* (Figs 6-9, ULD).
- the lower posterior part of the endophallus (between the tigilla and the apex of parameres, opposite to the FP) is scarcely developed, while it is very developed in most *Firminus*, including the type species.
- peculiar structures on the endophallus surface, as detailed below.

In both genera, large portions of the endophallus are covered by a texture of dense and minute granulations whose local hyperdevelopment may produce derived structures such as the "filiform setal band" and the "raspulae", that is setae-like structures arranged, respectively, in a row or in patches, the latter often appearing as dense tufts of stiff bristles. Such derived structures are a trait common to most *Rhizotrogus* (see Coca-Abia & Martin-Piera 1998), including *R. aestivus* (Figs 4-5) while they are absent in all members of *Firminus* (Coca-Abia 2003; Uliana unpublished), in which granular texture is undifferentiated. They are also present on *Firminus maltensis*, although not much developed: the lateral raspulae are still well recognisable



Figs 1-9 – Everted endophalli in lateral view (1, 4, 6, 8) and frontal view (2, 3, 5, 7, 9), with detail of frontal raspulae (3, not to scale); 1-3, *Rhizotrogus maltensis* (Gozo, Dwejra bay); 4-5, *R. aestivus* (Greece, Metosvon, Katara); 6-7, *Firminus punicus* (Algerie, Forêt Akfadou); 8-9, *F. bellieri* (Corsica, Lago di Capitello). **ED**: ejaculatory ductus, **FP**: frontal projection, **FR**: frontal raspulae, **FS**: frontal split, **FSB**: filiform setal band, **LR**: lateral raspulae, **T**: tigillum, **ULD**: upper lateral diverticula.



Figs 10-11 – *Rhizotrogus maltensis*, males (Gozo, Dwejra Bay). Habitus and variability of the dorsal pattern.

(Fig. 1, LR), while the ventral raspulae (Fig. 2, FR and Fig. 3) range from poorly developed (that is, poorly differentiated from the granulated texture) to practically absent, which is fitting with the variability documented in other species of the genus, such as *R. pallidipennis* (Blanchard, 1851) (Martin-Piera & Coca-Abia 1992; Coca-Abia & Martin-Piera 1998).

It is unclear whether the term "endophallites" should apply to the diffuse granulation described above. Such granulation presumably corresponds to the "fine rugosities or raspules" believed likely to have different ontogenetic origin than endophallites by Génier (2019), and matches the "fine ultrastructure" explicitely mentioned to be other than endophallites by Cristóvão & Vaz de Mello (2020: 28). However, the latter authors also included the raspulae among types of endophallites (2020: 19), while in the present work we evidence (see Fig. 3) that there is no break of continuity between such granulation and raspulae as intended in the genus *Rhizotrogus*, as already described by Coca-Abia & Martin-Piera (1998: 29).

In conclusion, the structure of endophallus of *Firminus maltensis* does not support a relation with members of the genus *Firminus*, while a strong similarity with *Rhizotrogus* was evidenced both by shape and by superficial structures. Thus, we introduce *Rhizotrogus maltensis* (Rössner & Zorn, 2013) as **new combination**.

Morphological notes. Consistently with other species of *Rhizotrogus* having dark areas on the dorsal side, *R. maltensis* expresses a marked variability in the degree of brown patterns. Specifically, the medial pronotal band may be much broader than what so far ascertained in male (cfr. Figs 10-11), and matching the pattern of the presumed female illustrated by Pivotti et al. 2011 (sub *Amphimallon scutellare*).

Rössner & Zorn (2013) mentioned that antennomeres 4 and 5 tend to merge, a condition that arguably led to the misattribution to the genus *Amphimallon* of the specimen studied by Pivotti et al. (2011). A misattribution with the same origin was recently verified by Rattu et al. (2020), concerning specimens of *Firminus* with antennomeres 4-5

non-disjointed, that led to the erroneous identification as *Amphimallon*. We do confirm the high frequency of this anomaly in *R. maltensis*: out of the six specimens studied by us, only two had a normally developed 10-jointed antenna (Fig. 12), while four expressed the mentioned anomaly, producing a 9-jointed antenna (Fig. 13). Antennal conditions observed by us appeared overall symmetric, with scarcely noticeable differences between the two sides. The non-disjunction of antennomeres 4 and 5 produces a longer antennomere, where the setation of the two expected articles can be still recognised and may serve for the correct interpretation of the morphology. Such an anomalous condition is documented in various other species of *Rhizotrogus* (Baraud 1977), with at least another known case where the teratology is widespread within a population: out of 35 males *Rhizotrogus iglesiasi* (Baguena Corella, 1955) collected in two close localities of northern Portugal, only 3 had normal antennae, the rest having one or both with two or three non-disjointed antennomeres (Baraud 1977, sub *R. villiersi* Baraud 1970, identification then corrected by Branco 1990).

Phenological and ecological observations. *Rhizotrogus maltensis* is active after the sunset, and is fototropic. On November 2, with local sunset at 17.06 and astronomical dusk at 18.33 (GMT+1), the flight area has been visited by one of us (VG) since the late afternoon, and no specimens have been observed during the daytime. Two light sources (10W UV led stripes placed against a white vertical screen) were lit at the sunset, allowing to attract two males before complete darkness, the first one at 17.30. The area surrounding light traps was explored by using a head torch, leading to spot four other males that were standing on rocks, with stretched antennae (Figs 16-17), presumably searching for chemical trails released by females. The last male was observed just before 24.00, evidencing a daily activity of at least six hours starting from the dusk. However, since the arrivals in flight to the light only happened at the beginning of the twilight, we suppose that the dispersal flight of males may only, or mostly, occur at the onset of the evening and last only for a short time, consistently with the behaviour of other Mediterranean Rhizotrogini.

The collecting area (Figs 14-15) was 100-150 m distant from the coastline and characterized by widespread calcareous outcrops of the Globigerina Limestone Formation (Prampolini et al. 2018), with largely discontinuous soil coverage and sparse garrigue vegetation. Garrigue habitat was also mentioned by Pivotti et al. (2011) as the collecting environment for their specimen.

Digging just below the surface in grassy spots of the same area (Fig. 18), allowed to collect four third-instar larvae of Melolonthinae that were standing among roots. Although our attempt of breeding failed, we confidently identify them as *R. maltensis*, based on the association with adults, and on the fact that this species is the only member of Melolonthinae currently recorded for Gozo island (Pivotti et al. 2011).

Figs 12-13 – *Rhizotrogus maltensis*, right antenna of male; 12, normal antenna; 13, anomalous antenna, with antennomeres 4-5 non disjointed.

Conclusions

Rhizotrogus maltensis is a fototropic species active after the sunset, with flight possibly limited to the twilight. Its antennae are frequently subject to non-disjunction of two antennomeres, a condition uncommon but not new to other members of this genus.

The new placement of this species in the genus *Rhizotrogus* is evidenced by the structure of the everted endophallus.

The study of the latter has been so far only marginally addressed in Rhizotrogini, the endophallus being commonly studied squeezed and observations limited to the sclerified structures. The present study, however, points out that its tridimensional morphology may provide useful characters for the assessment of taxonomic and phylogenetic relationships.

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Figs 14-18 – Collecting site of *Rhizotrogus maltensis* (14) and general view of the area (15), 23.XI.2017 (photos L.F. Cassar); 16-17, active males of *R. maltensis* crawling on rocks in the dark; 18, detail of the collecting site of larvae.

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