



The Variegated Mud-Loving Beetles of Europe (second part) (Coleoptera: Heteroceridae)

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Abstract. Data on the biology and ethology of adults and larvae of the variegated mud-loving beetles, their parasites (Ascomycetes, Protozoa, Nematoda, Acari, Hymenoptera Chalcidoidea), collecting methods, preparation and conservation are provided. For the first time the presence of *Botryandromyces heteroceri* (Maire) was discovered on *Augyles flavidus* (Rossi, 1794), from Italy, and on *Heterocerus obsoletus* Curtis, 1828, from Germany.

Riassunto. *Eteroceridi d'Europa (seconda parte) (Coleoptera: Heteroceridae).* Vengono forniti dati sulla biologia ed etologia di adulti e larve, sui loro parassiti (Ascomycetes, Protozoa, Nematoda, Acari, Hymenoptera Chalcidoidea), sui metodi di raccolta, preparazione e conservazione. Viene segnalata, per la prima volta, la presenza di Botryandromyces heteroceri (Maire) su Augyles flavidus (Rossi, 1794) in Italia e su Heterocerus obsoletus Curtis, 1828 in Germania.

Key words. Coleoptera, Byrrhoidea, Heteroceridae, biology, ethology, parasites, *Botryandromyces heteroceri*, collecting methods, preparation, conservation.

Introduction

After the first part on the variegated mud-loving beetles (MASCAGNI, 2014), this second part considers their biology and ethology, their numerous parasites and collecting, preparation and conservation methods. The discovery, for the first time, of the parasite *Botryandromyces heteroceri* (Maire) (Ascomycetes, Laboulbeniales) on *Augyles flavidus* (Rossi, 1794) from Italy and on *Heterocerus obsoletus* Curtis, 1828 from Germany, is interesting.

Biology and ethology

Heterocerid beetles are riparian insects which live near fresh, salt and brackish water. They live and reproduce in narrow winding burrows dug in damp mud at the water's edge (Fig. 1) (SCHIÖDTE, 1866). When the water level rises or falls, these insects extend their burrows until they reach terrain with the correct amount of humidity, that is with a water content between 30% and 60%. Flooding or drought can cause entire populations of these insects to take flight and migrate several kilometres far away. Apart from cases of emergency, heterocerids usually do not inhabit sandy areas without mud. Following flooding, heterocerids abandon their normal shoreline habitat and initially migrate to humid sandy terrain even if this is not perfectly suitable, and only later they establish burrows in terrain that is predominantly muddy. A predominantly muddy habitat with no underlying layer of sand is preferred by males and non-reproductive females, while a sandier habitat is required by egg laying females (KAUFMANN & STANSLY, 1979). The insects dig with their mandibles and robust anterior legs provided with lateral spines which increase their burrowing capacity (Fig. 2); these insects are capable of burying themselves completely in the mud in 2-3 seconds.

Four different types of burrows have been identified: those for adults and larvae (Figs 1, 3), those for pupal cells (Fig. 4), those for egg incubation (Fig. 5), and those for hibernation (Fig. 6).

Egg laying females and young larvae prefer a boggy soil, while the pupae, larvae, males, and old or nonegg laying females prefer a drier soil (KAUFMANN & STANSLY, 1979). The burrows are horizontal and close to the surface and appear as ridges easily visible above ground (Fig. 1); these ridges also make it possible to follow the insect during the construction process. The exits and entrances used during the construction of the burrow remain open but are not normally reused (CLAYCOMB, 1919). Although the insect enters, and exits, the tunnel anywhere simply by burrowing through a wall, these burrows not only hide the insect from predators, but also provide it innumerable escape directions while enhancing its chance of meeting a member of the opposite sex.

The larval tunnels differ from those constructed by the adults only in being smaller (narrower) (Fig. 3). The pupal cells are built by the larvae in the final stage inside their latest tunnel (Fig. 7); TERZANI *et al.* (2010) have recently studied the morphological characteristics of a series of 138 *Heterocerus fenestratus* (Thunberg, 1784) pupal cells from Tuscany and Marche. The incubation chambers resemble the pupal cells in form and size and are dug in loose terrain by the females ready to lay their eggs (Fig. 5). With the approach of winter the specimens of the latest generation construct hibernating cells a few centimeters under the surface of the sand or mud where they overwinter (Fig. 6); it does not appear that the larvae pass the winter. The insects abandon these hibernating cells during the first months of the year.

During every stage of their life cycle, but particularly during the imago stage, heterocerids are profusely covered with water repellent hairs. These hairs retain a thin cushion of air in contact with the stigma (MESSNER, 1973), which allows the beetles to breathe in conditions where it would otherwise be impossible. MESSNER (1973) observed that this air cushion allows adult *Heterocerus parallelus* Gebler, 1830 to withstand prolonged flooding of their habitat, and *Heterocerus flexuosus* Stephens, 1828 to overwinter buried in mud. Besides acting like a permanent physical gill, these same hairs keep the insect afloat if it falls into the water, and keep its body surface free of mud. The flight of heterocerids is fast and takes place immediately after their exit from the ground, if forced to leave. The movements in flight do not normally exceed three or four meters, and if the achieved area is not adequate, the insect takes flight and continues in this way until it finds a suitable place to stay. When the insect touches the ground, because of the spots present on the elytra, becomes similar to the substrate and thus protected from predators. If instead these beetles fall in the water, they are able to stand up quickly in flight aided by the presence of the coating of water-repellent hairs that guarantees a perfect flotation; flights are intensified if the temperature is higher.

The Heteroceridae are limnivorous beetles which feed mainly on plankton. Many species of Heteroceridae produce several generations during the year and the adult and larval populations reach monthly peaks lasting a few days. Copulation almost always occurs within the burrows and thus is quite difficult to be observed. After copulation the females move closer to the water and build incubation cells in very wet mud where they lay a single mass of eggs. Apparently the females watch over the eggs and keep them clean until hatching; during this period the females consume less food in order to care for their eggs. This behaviour is an uncommon example of parental care in this order of insects. When the eggs hatch, the females abandon the incubation chambers and move to firmer ground.

The larvae, which can move swiftly either above or below ground, are campodeiform with a cylindrical body; on the surface, they have a typical jerky movement, continually changing their direction and spacing out the movement with sudden stops. The larval period lasts 7-10 days, the pupal period 3 to 7 days according to the species (PORTEVIN, 1896; LARSEN, 1936; PIERRE, 1946). The imago emerges from a short cylindrical duct in the anterior part of the cell (Fig. 7). Larvae and pupae are very susceptible to changes in water level; flooding can wipe out an entire population of eggs, larvae and pupae. If flooding occurs gradually and not beyond a certain level the larvae are capable of moving rapidly over the surface to safe ground. The adults are excellent flyers and very sensitive to light (particularly UV). The species *Augyles niloticus* (Grouvelle, 1896) intensifies or diminishes its nighttime flights according to temperature, humidity and wind (HANNA, 1969a) and, it seems, also to lunar phases (HANNA, 1969b).

Because the habitat of heterocerids is a zone in contact between different ecosystems (ecotones) it is important for them to be able to remain in, and return to, their elected zone along the quickest route, that is perpendicular to the borders of the zone. This capacity for zonal recovery has been documented experimentally in the species *Augyles flavidus* and *Heterocerus fenestratus* (SCAPINI *et al.*, 1993): riverside populations of *H. fenestratus*, which show no positive phototaxis, orient by landscape cues, colours and the inhomogeneity of the visual field in order to return home; instead, swamp populations of the same species

show positive phototaxis. This capacity to orient is apparently learned as the larvae show no such capacity. Furthermore, the same species shows habitat-linked differences while different species (*H. fenestratus* and *A. flavidus*) sharing the same habitat do not show the same differences.

Parasites

Ascomycetes

Heteroceridae host the parasite fungus *Botryandromyces heteroceri* (Maire) (Fig. 8) belonging to Ascomycetes Laboulbeniales; this highly specialised fungus apparently does not cause any harm to the insect. The parasite, which protrudes by the exoskeleton of the beetle with all the mycelium, easily frees the spores within the galleries contaminating a large number of specimens. In Europe and Mediterranean Africa this parasite has been found on the following species (TAVARES & MAJEWSKI, 1976; MASCAGNI, 1988; MASCAGNI & GIARDINI, 2005):

Augyliini:

Augyles flavidus (Rossi, 1794): Italy (<u>first record</u>). *Augyles intermedius* (Kiesenwetter, 1843): Poland. *Augyles maritimus* (Guérin-Méneville, 1844): Algeria. *Augyles pruinosus* (Kiesenwetter, 1851): Greece.

Heterocerini:

Heterocerus fenestratus (Thunberg, 1784): Italy, Greece, Germany, Poland. Heterocerus flexuosus Stephens, 1828: Greece. Heterocerus fusculus Kiesenwetter, 1843: France, Germany, Poland. Heterocerus holosericeus Rosenhauer, 1856: Italy. Heterocerus obsoletus Curtis, 1828: Germany (first record).

I have found the perithecium of this fungus on several parts of the insect: head, thorax, abdomen, elytra and legs.

Protozoa

Adult *Augyles sericans* (Kiesenwetter, 1843) host the unicellular endoparasite *Gregarina heteroceri* Rauchalles, 1969 belonging to the Sporozoasida Gregarinidae (GEUS, 1969).

Nematoda

Both larvae and adults are parasitized by the following species of Nematoda Spiruria: *Goffartia heteroceri* Hirschmann, 1952, *Goffartia variabilis* (Micoletzky, 1922), *Pristionchus lheritieri* (Maupas, 1919), and *Protylenchus heteroceri* Wachek, 1955, all of which have been found on *Heterocerus fenestratus*, *H. fusculus* and *H. marginatus* (Fabricius, 1787) (HIRSCHMANN, 1952).

Arthropoda

The adults are parasitized by mites, often in large quantities, while the pupae are parasitized by Hymenoptera Chalcidoidea. I observed frequently *Heterocerus fenestratus* specimens infested by a high number of mites, arranged, mainly, on the thoracic and ventral region. In addition, from pupal cells still closed, collected by me and stored in suitable containers, I noticed, after a few days, the presence of adults of Hymenoptera Chalcidoidea emerged from the pupal cells.

Collecting methods

Adults and larvae are found anywhere from a few centimetres to a few metres away from the edge of water according to the degree of terrain humidity. In order to catch these beetles, which are normally submerged in slime, they must be made go out. One method is to simulate flooding – easily done by pouring water on the ground – which forces the beetles to come to the surface and, after a few seconds, to take flight. In those few seconds the insects can be collected with light tweezers, an aspirator, or a strainer if they have

fallen in the water. A second method is to stamp on the ground and examine the footprint for specimens: terrain compression compels both adults and larvae to exit. A third method is to use an UV light (the light source which they are most sensitive to), which can attract several thousand specimens in the space of a few hours but which, obviously, does not allow to establish their exact origin. A fourth method is to use an aeroplankton net mounted on a car roof.

Preparation and conservation

Due to their morphological homogeneity, positive identification of many Heteroceridae cannot depend on external morphology alone. The only exception to this among the three genera present in Europe (Micilus Mulsant & Rey, Augyles Schiödte and Heterocerus Fabricius) is Micilus murinus (Kiesenwetter, 1843) (the only species belonging to this genus), which is easily recognized because it is so much smaller than all the other European species of Heteroceridae. For many of the other European species, positive identification depends on extracting and observing the aedeagus. To prepare the aedeagus for microscope observation, soak the extracted organ in a cold solution of 10% KOH for 4-8 hours, then place it in 70° alcohol for 8-9 minutes and then in 100° alcohol. I advise to include the aedeagus together with the ninth sternite, separated from each other, in a single drop of Canadian Balsam or better yet in water soluble dimethyl hydantoin formaldehyde (DMHF). The adults can be conserved in 70° alcohol but are better mounted on cards, being careful to extend the head, antennae and legs. It is also advisable to detach the abdomen of one of the specimens in a series and mount it upside down, as the ventral surface of the abdomen contains several important taxonomic characters (MASCAGNI, 2014: p. 80 and Fig. 7). It is also a good habit to remove any eventual slime or sand from the insect, particularly on the elytra, using hot water and a lot of patience and never removing the dry crust with a needle or stiff brush, which could yank out hairs such as those on the elytra. The larvae should be conserved exclusively in 70° alcohol.

Addenda and corrigenda to first part (MASCAGNI, 2014)

ADDENDA:

Augyles (Augyles) pruinosus (Kiesenwetter, 1851): 286 Distribution: Portugal (Faro, Calico, 2009, coll. Mantič - CZ) (first record)

Augyles (Augyles) sericans (Kiesenwetter, 1843): 212 Distribution: Russia (LITOVKIN *et al.*, 2013)

Heterocerus jelineki Skalický, 1998: 7 synonym of Heterocerus magnus Mamitza, 1933: 83 (SKALICKÝ, 2004)

Follow additional contributions to the first part (MASCAGNI, 2014):

RATCLIFFE & FAGERSTROM (1980), FRIDAY (1988), DE MARZO (2008), FOSTER (2010), HENDRICH *et al.* (2011), MASCAGNI (2011), HACKSTON (2013), QUENEY *et al.* (2013), BAMEUL & QUENEY (2014), SKALICKÝ & EZER (2014), TAŞAR (2014), TAŞAR & MASCAGNI (2014).

CORRIGENDA:

Errata: MASCAGNI, 2005b (pp.: 82, 85, 86, 87, 88, 89, 91, 92, 93, 94, 95). Corrige: MASCAGNI & GIARDINI, 2005 (pp.: 82, 85, 86, 87, 88, 89, 91, 92, 93, 94, 95).

References:

Errata: MASCAGNI A., 2005b. Heteroceridae della Penisola Balcanica (Insecta: Coleoptera). *Onychium*, 3: 21-33 (p. 98).

Corrige: MASCAGNI A. & GIARDINI S., 2005. Heteroceridae della penisola Balcanica (Insecta: Coleoptera). *Onychium*, 3: 21-33 (p. 98).

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Fig. 1. Tunnels of Heterocerus fenestratus (Thunberg) dug into the wet mud (photo A. Mascagni).

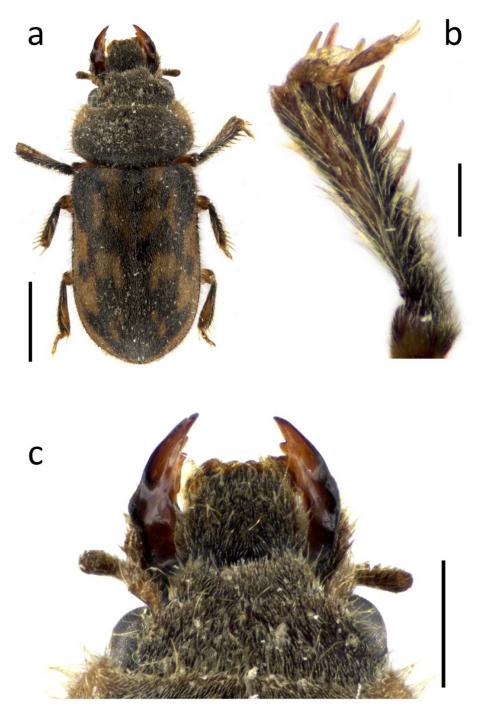


Fig. 2. *Heterocerus fenestratus* (Thunberg). a) habitus (scale bar: 1 mm); b) detail of the right front leg (scale bar: 0.2 mm); c) detail of the mandibles (scale bar: 0.5 mm) (photo F. Cianferoni).

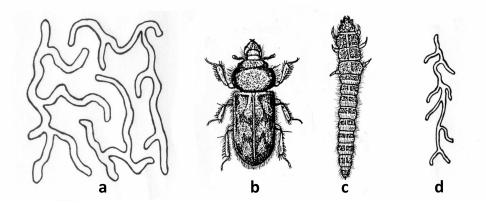


Fig. 3. Adult and larva with their tunnels. a) tunnel of adult; b) habitus of adult; c) habitus of larva; d) tunnel of larva.



Fig. 4. Pupal cell.

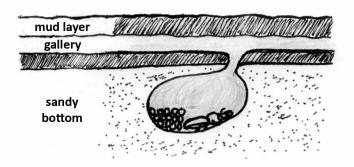


Fig. 5. Incubation cell.

Onychium, 11: 117-126

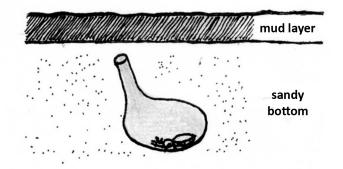


Fig. 6. Hibernation cell.



Fig. 7. Pupal cell of Heterocerus fenestratus (Thunberg) (photo S. Bambi).

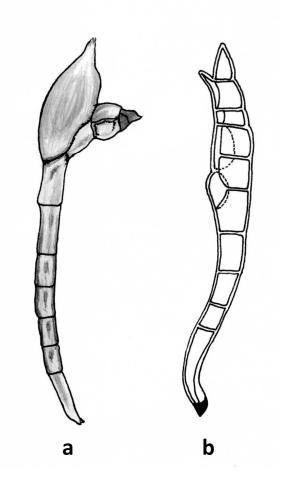


Fig. 8. Botryandromyces heteroceri (Maire) (Ascomycetes, Laboulbeniales). a) fungus and b) section of the thallus (redrawn from TAVARES & MAJEWSKI, 1976).

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