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Sexing method of adults (living and dead) abbreviated wireworm, *Hypnoidus abbreviatus*

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Abstract

Click beetles (Coleoptera Elateridae) are the ninth most diverse beetle family, with nearly 10,000 species described worldwide. They are mostly known for the economic importance of their long-lived larval stage, commonly called wireworms, on crops. In Canada, 20 species are considered to be a major pest. Not all provinces in Canada have the same level of documentation about the biodiversity of wireworms in field and which species can be considerate a pest or not. In 2017, in Quebec, 72% of the wireworms found that were identified as *Hypnoidus abbreviatus* (Say), which seems to be specific to the region. In the scientific literature, little information is found on this species. This study aims to describe the structures of the reproductive systems of *H. abbreviatus* and present an effective method for sexing adults, while keeping them alive and undamaged.

Key words: Elateridae, abbreviated wireworm, click beetle, sexing technique.

Wireworms are soil-inhabiting organisms known to be important pests in many crops (Rawlins, 1940; Parker and Seeney, 1997; Toth *et al.*, 2003; van Herk and Vernon, 2013; Vernon and van Herk, 2013; 2017; Traugott *et al.*, 2015; Furlan *et al.*, 2017). In the province of Quebec in Canada, the main species found is the abbreviated wireworm, *Hypnoidus abbreviatus* (Say), representing 72% of the Elateridae assemblage in agricultural areas (Saguez *et al.*, 2017). Considered predominant in Quebec since the 1960s (Lafrance and Cartier, 1964), there is, as yet, no information available about its basic biology or damage potential. The morphology of the genitals of some click beetle species have been described in the scientific literature (Zacharuk, 1958; Leseigneur, 1972; Costa, 1982; Casari, 2004), but most of these studies are only based on morphological observations of dead specimens and none allows effective sex determination of *H. abbreviatus*. Some click beetle species have sexual dimorphism. This can be manifested by differences in body colour, shape, and / or size. The main structures affected are antennas, elytra, tibia, pronotum and prothorax (Jeuniaux, 1955; Leseigneur, 1972; Costa, 1982; Furlan, 1996; Merivee *et al.*, 1998; 1999; Ren *et al.*, 2014; Sufyan *et al.*, 2014). Nonetheless, *H. abbreviatus* does not present a sexual dimorphism. In such situations, the only way for effective sexing of living individuals is to carefully examine abdomen and genital organs (Sufyan *et al.*, 2014). Depending of the species, it can be quite difficult to do so without damaging specimens (Leseigneur, 1972). Since sexing adults reliably and quickly is useful both for research (eg. life cycle and reproduction studies) and practical (eg. sampling, pheromone traps, etc.; integrated pest management [IPM]) purposes (Furlan *et al.*, 2017; Vernon and van Herk, 2017), this study aim to provide an effective sexing method suitable for *H. abbreviatus.* The method described here has been developed during experiments carried out to characterize the life cycle of the insect. It constitutes an effective sexing method, although delicate, for living specimens.

The easiest and safest way to determine the sex of an adult *H. abbreviatus* is by exposing the genital organs. By removing the elytra, it is possible to observe the genital armour consisting of one or more mobile tergites. This mobility makes it possible for adults to take out the external parts of their copulatory member during mating. The morphology of the genital armour varies between the sexes. The male genital armour consists of a first shorter section (tergite 8) leaving behind a second part of the armour longer, thinner and less opaque (tergites 9,10) (figure 1a). This second structure is not present in females, their genital armour consists only of the tergite 8 (figure 2a). By lifting the genital armour, the reproductive organs become clearly visible. In the male, the aedeagus is divided into three parts; a median lobe with a pointed end and two rounded flat side lobes (aka. parameres) surrounding the median lobe (figure 1c). In females, the ovipositor, a membranous and translucent tube, is finished by two coxites surmounted by lateral styles trimmed with bristles (figure 2c). The coxites move apart to give access to the genital orifice.

With living individuals, since the adults of the species do not fly and rarely separate elytra, it is difficult to observe the external parts of the copulatory organs without causing permanent injury. The presence of a space between the elytra and the abdominal tergites often indicates that the individual is carrying eggs which, as they grow, slightly push the wings upwards. Nevertheless, this indication only works for mature females and therefore does not distinguish a male from a freshly emerged female. For this reason, the observation of the reproductive organs remains the only solution. Although difficult to perform and requiring much practice, the method shown in figure 3 allows to highlight the end of the aedeagus (figure 1b) or the ovipositor (figure 2b) thus clearly indicating the sex of the individual without damaging it. This requires, by observing with a binocular microscope, to 1) pass a brush composed of a single hair

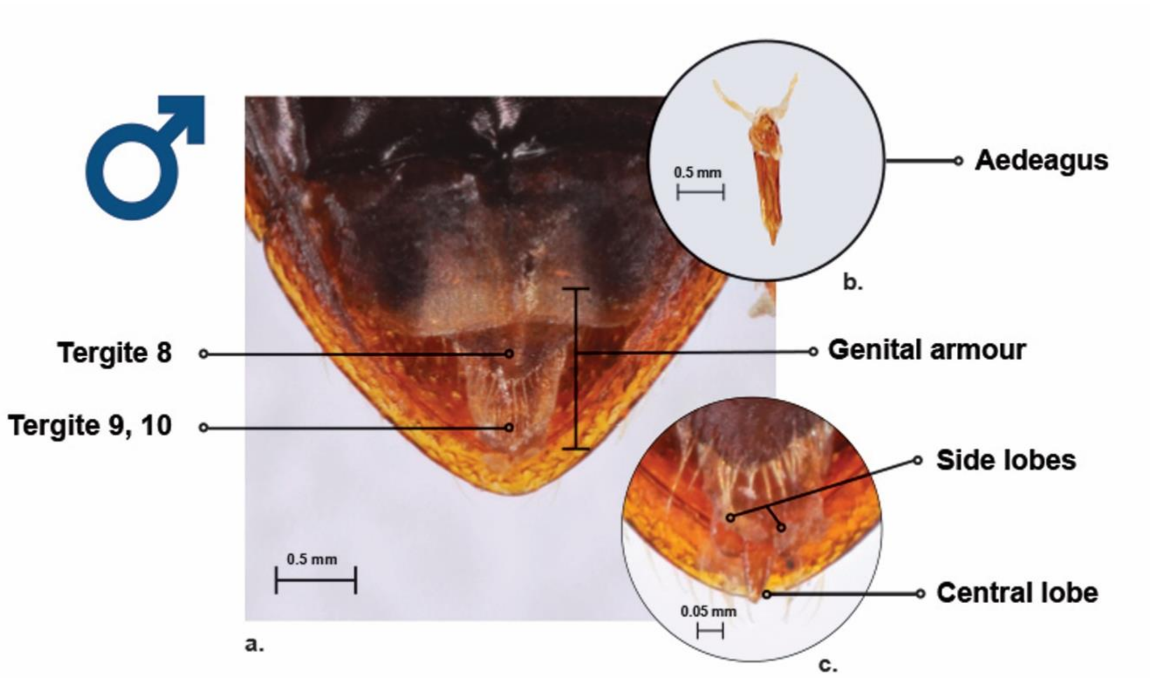

Figure 1. *H. abbreviatus* male adult: **(a)** abdomen dorsal surface without elytra; **(b)** aedeagus; **(c)** end of the aedeagus.

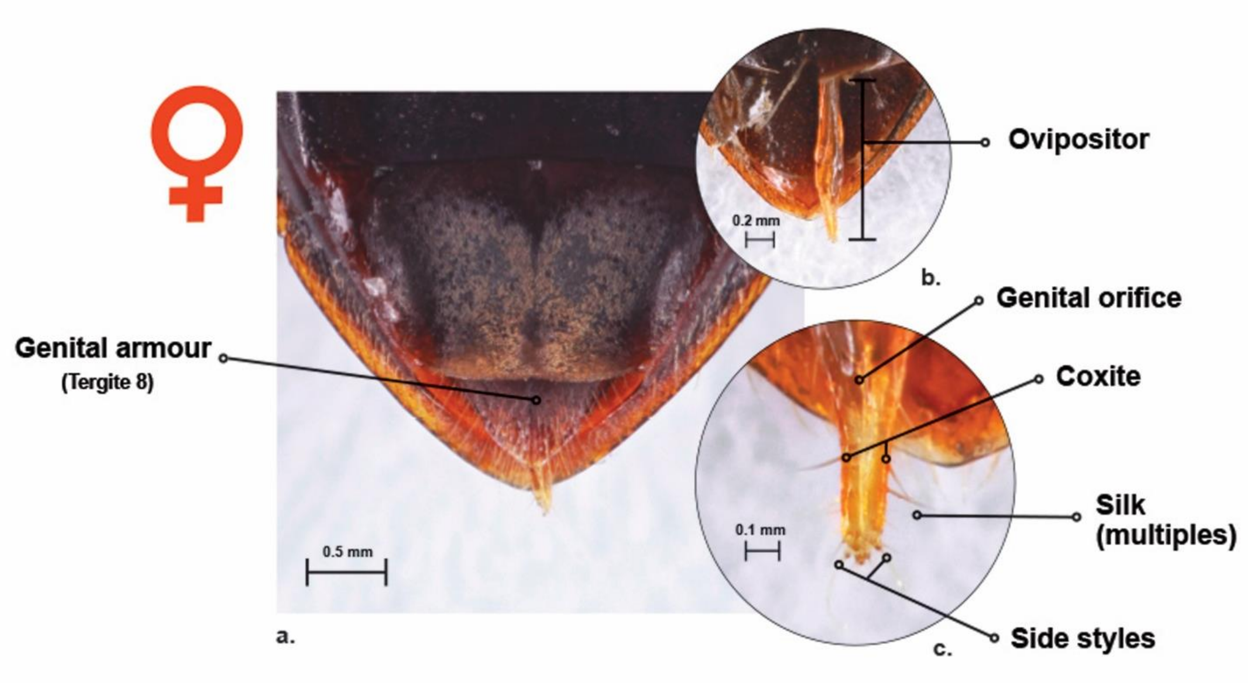

Figure 2. *H. abbreviatus* adult female: **(a)** abdomen dorsal face without elytra; **(b)** ovipositor; **(c)** extremity of the ovipositor.

(or a fine and flexible entomological needle) under the elytra, 2) apply a slight pressure with the brush on the abdominal tergites to move the genital armour outwards, and 3) observe the end of the reproductive organ protruding from the end of the abdomen in order to identify sex, sclerotized tip in males (figure 1c) or tube gelatinous with bristles in females (figure 2c). Be careful not to apply too much pressure on the genital armour (damaging, undesirable). In some individuals, this would force the armour out of the abdomen, damaging the armour itself and the genitals it protects. Damaged armour or genitals reduce the fitness of the affected adult, making them unsuitable for reproduction testing. After practicing the method on about forty individuals (about 3 hours of observation), less than 5% of sexed adults were damaged.

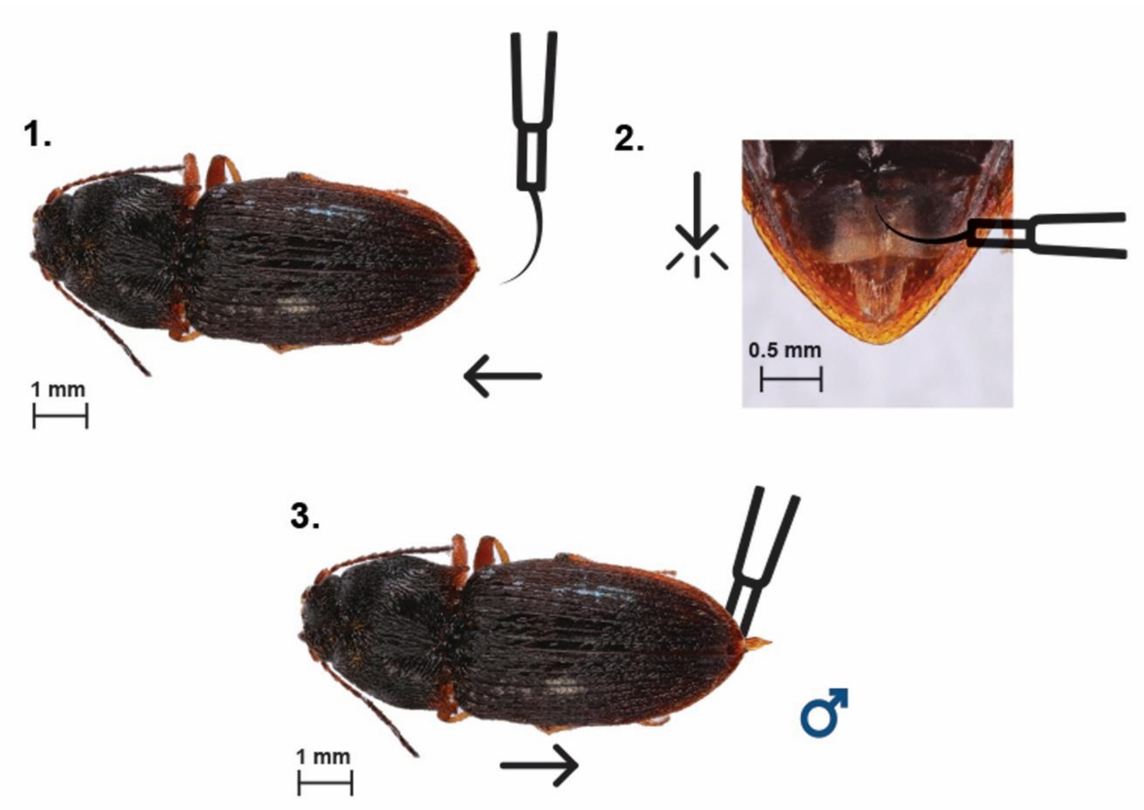

Figure 3. Diagram describing the only technique for sexing *H. abbreviatus* live adults; the example above indicates that it is a male.

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