

Novel method for determining sex of live adult *Laricobius nigrinus* (Coleoptera: Derodontidae)

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Abstract—A method for determining the sex of live adult *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) is described. Beetles were briefly chilled and positioned ventral-side-up under a dissecting microscope. Two forceps with blunted ends were used to gently brace the beetle and press on the centre of the abdomen to extrude its terminal segments. Male beetles were distinguished by a sclerotised, reticulate ninth abdominal sternite. In females, the distinct ovipositor (tergite, valvifers (ninth sternite), and laterotergites of the ninth abdominal segment) was visible. The procedure was rapid and harmed only a small number of individuals (fewer than 5%).

Résumé—Nous décrivons une méthode pour déterminer le sexe chez des adultes vivants de *Laricobius nigrinus* Fender (Coleoptera: Derodontidae). Les coléoptères sont brièvement refroidis et placés sur le dos sous une loupe microscopique. Deux brucelles à bouts émoussés permettent de délicatement immobiliser l'insecte et appuyer sur le centre de l'abdomen pour faire sortir les segments terminaux. Les coléoptères mâles se reconnaissent par leur neuvième sternite abdominal sclérifié et réticulé. Chez les femelles, l'ovipositeur est bien visible; il se compose du tergite, des valvifères (neuvième sternite) et des latérotergites du neuvième segment abdominal. La procédure est rapide et ne blesse qu'un petit nombre d'individus (moins de 5%).

Laricobius nigrinus Fender (Coleoptera: Derodontidae) is a predator native to western North America that feeds on the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). This predator is being used in classical biological control of *A. tsugae* in the eastern United States of America where the adelgid is exotic and is causing extensive mortality to eastern hemlock, *Tsuga canadensis* (Linnaeus) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelman (Pinaceae) (Mausel *et al.* 2010). Studies of *L. nigrinus* behaviour and chemical ecology often require the use of live beetles, separated by sex (*e.g.*, Zilahi-Balogh *et al.* 2003). Currently there are no rapid, nondestructive methods for distinguishing between male and female adult beetles.

Methods described in the literature for determining the sex of live *L. nigrinus* are either

time-consuming or require access to the pupal stage. One sexing technique involves looking for the presence of predator eggs in *A. tsugae* egg sacs to identify isolated female beetles (Vieira *et al.* 2012). However, this method requires days of observation, and a lack of oviposition does not distinguish between males and females that did not lay eggs. Copulatory behaviour cannot be reliably used to sex beetles, as males will attempt to mate with other males, and females with other females (personal observations). The sexes of *L. nigrinus* can also be distinguished by examining the developing genitalia of the pupae (Zilahi-Balogh *et al.* 2006). However, this method risks damaging the pupae and cannot be used for the wild-captured adults used in many experiments.

We have developed an alternative method for sexing live *L. nigrinus* adults. First, beetles were

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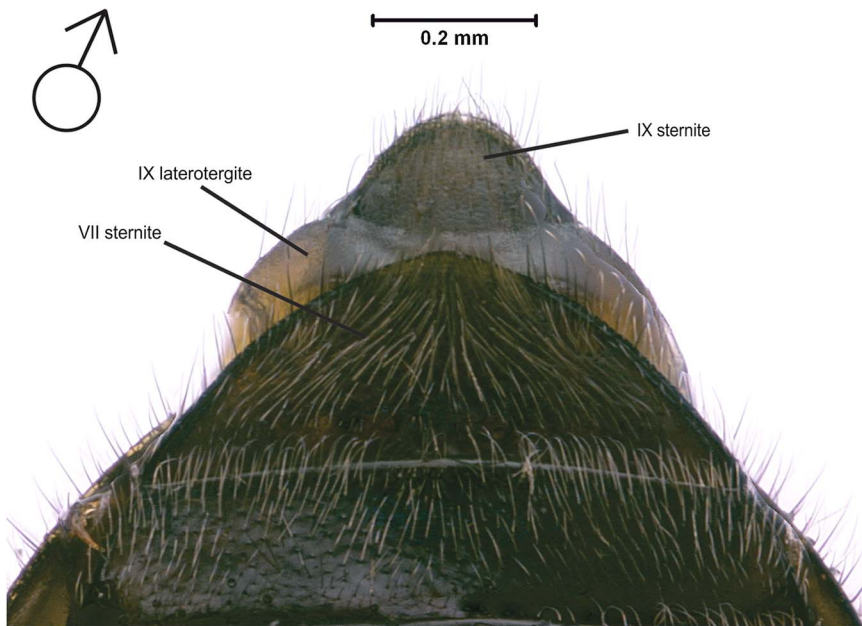
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Fig. 1. Ventral view of the apex of the abdomen of male *Laricobius nigrinus* after slight pressure was applied to the venter with blunt forceps. Males were distinguished by the sclerotised, reticulate ninth abdominal sternite. Photographed by E. Vallery, United States Department of Agriculture Forest Service.



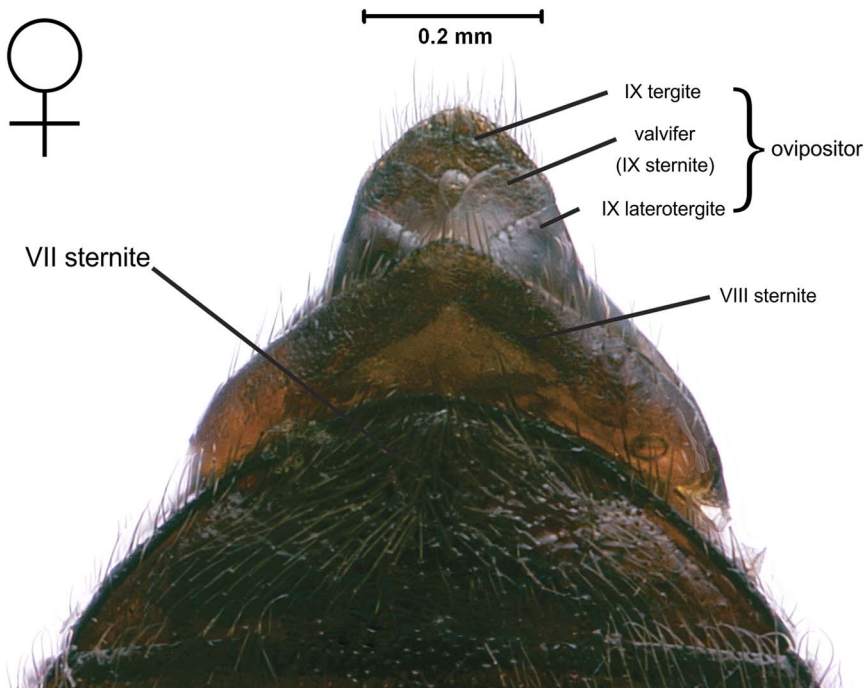
chilled in a 120 mL plastic specimen cup that had been placed on a paper towel inside an ice-filled Styrofoam container. After 30 minutes of cooling (which reduced insect movement), each beetle was positioned ventral-side-up under a dissecting microscope. Two #5 fine straight forceps with the ends slightly blunted were used to gently press the centre of the mesosternum and abdomen to cause the terminal abdominal segments to be extruded. The forceps tip on the mesosternum was used primarily to anchor the beetle while pressure was gradually increased on the abdomen with the second forceps tip until the terminal sclerites became visible. To ensure proper identification of sex, it was necessary for the terminal segments to protrude 0.1–0.2 mm beyond the seventh sternite of the abdomen. The genitalia belong to the eighth and ninth sternites and become visible only when they are protracted (Franz 1958). Pressure necessary to make all the sexually dimorphic structures visible damaged the beetles, and thus pressure was increased only until sufficient to view those needed to determine the sex. We used descriptions and photographs of *Laricobius osakensis* Montgomery and Shiyake (Montgomery *et al.* 2011) and

Laricobius erichsonii Rosenhauer (Franz 1958) to identify homologous morphological features of *L. nigrinus*.

Before pressure was applied with the forceps, only the seventh sternite was visible at the apex of the abdomen (Figs. 1, 2). The ninth abdominal segment, ~0.2 mm in width in both sexes, was most useful for distinguishing the sexes. In male beetles, typically only the apex of the ninth sternite was visible, but its distinctive sclerotisation and reticulation was sufficient for recognising its sex (Fig. 1). In female beetles, the ovipositor (tergite, valvifers (ninth sternite), and laterotergites of the ninth abdominal segment) was visible (Fig. 2). Although usually not visible in the male, the laterotergites of the ninth segment were twice as wide in the male as the female.

With 2–3 hours of practice, the time needed to sufficiently examine the terminal sclerites was decreased to as little as 1–2 seconds, and such rapidity in the examination appeared to reduce beetle damage and stress. Beetles that became too active for examination following the period of chilling could be relaxed temporarily by gently tapping the mesosternum with a forceps tip.

Fig. 2. Identical view of female as in Fig. 1. Females were distinguished by the exposed ovipositor, consisting of the tergite, valvifers (ninth sternite), and laterotergites of the ninth abdominal segment. Although visible in this photograph, the eighth abdominal sternite is usually not exposed when the abdomen is gently pressed. Photographed by E. Vallery, United States Department of Agriculture Forest Service.



We have found no evidence that our methods significantly harmed the insects since both male and female beetles exhibited no abnormal behaviours following sex determination: they were active, walked normally, and continued to feed and mate. However, further research is required to determine if this procedure has negative effects on *L. nigrinus* reproductive success (*i.e.*, mating frequency, fecundity, and % egg hatch). Several, albeit infrequent, problems have occurred with our procedure. Occasionally the terminal sclerites did not extend sufficiently to allow examination, and subsequent, more forceful attempts to cause extrusion harmed the beetle. Additionally, there could be a delay of up to several minutes before the terminal sclerites were withdrawn back inside the abdomen. During this time the exposed sclerites were at risk of becoming damaged (although no such damage was observed). With practice, however, fewer than 5% (7 out of 162) of *L. nigrinus* adults examined had any indication of not being fully functional. All sex determinations

were successful, as verified by dissection of genitalia following beetle death.

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