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Note that the title below was later changed to the one above.

Two non-destructive techniques for sex determination of live adult *Hylobius warreni*

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Abstract

Two non-destructive sexing techniques, suitable for use in the field, are described for the Warren rootcollar weevil *Hylobius warreni* Wood (Coleoptera: Curculionidae). On the 2nd visible abdominal sternite of males, there is a longitudinal depression which is absent on females. In addition, hairs on the last visible abdominal sternite of females are arranged longitudinally, while hairs on males are arranged radially. Accuracy for the first character was 90%, and sex could be determined without the aid of magnification. The latter character was slightly more reliable with a 95% accuracy, but required some magnification.

Keywords *Hylobius warreni*, sex determination, sexual dimorphism, Warren rootcollar weevil

Résumé

Deux techniques non destructives, permattant le sexage lors d'une étude de terrain, sont décrites pour le charançon de Warren *Hylobius warreni* Wood (Coleoptera : Curculionidae).Sur le 2ème sternite abdominal visible des mâles, il y a une dépression longitudinal qui est absente sur les females. De plus, les soies du dernier sternite abdominal visible, sont placées longitudinalement sur les femelles, tandis que les soies des mâles sont placées radialement. La fiabilité du premier caractère est de 90% et le sexe peut être déterminé sans l'aide d'un grossissement. Le dernier caractère mentionné est légèrement plus fiable avec une exactitude de 95% mais nécessite un grossissement.

Mots clés *Hylobius warreni,* détermination du sexe, dimorphisme sexuel charançon de Warren

The Warren rootcollar weevil, *Hylobius warreni* Wood (Coleoptera: Curculionidae), is distributed widely throughout the boreal forest in Canada, where it attacks several conifer species (Wood 1957). Live, healthy trees are susceptible to attack from *H. warreni* from a young age (Cerezke 1970). Larvae feed on phloem in the root and root collar region of them host trees. This may predispose trees to infection by root and stem diseases, and in young trees it may cause complete girdling, killing the tree (Warren 1956, Cerezke 1970).

Widespread regeneration of the main host in British Columbias, lodgepole pine (*Pinus contorta* var. *latifolia*) has led to increasing incidence (Schroff et al. 2006), and hence a renewed interest in this insect. Behavioural and ecological research on *H. warreni* could benefit from an in-field, non-invasive sexing technique, but no. No reliable technique is non-invasive techniques are available, even though the external anatomy of the weevil has been described in great detail (Wood 1957,Warren 1960). Thus, the Invasive techniques that do not involve sacrificing insects include identifying apodeme genital tissues in females, which may be observed by examining the anal opening with fine tweezers. Similarly, gender may also be determined by observing differences in the relative orientation of internal markings on the 8th sternite (Hopkins et al. 2008). The aim of this study was to use non-invasive techniques to identify external sex-specific characters that are reliable and convenient to use in the field.

Materials and methods

Adult weevils used for the study were caught in a heavily infested plot of planted, 8-year old lodgepole pine in the city of Prince George, British Columbia. Adult

weevils for this study were captured in a novel stem trap (Björklund and Lindgren, in prep) when they ascended trees for feeding. The traps were emptied on 22 June and 11 July 2006 and weevils were stored in a freezer for later examination.

Sexual dimorphism of *H. warreni* was assessed by examining a number of sex-determining external characters previously used for sexing other species. The following characters were assessed: The length of the snout (examined for *Anthonomus grandis*, Sappington and Spurgeon 2000) and the pronotum; presence/absence of any thorns on tarsal claws (Anthonomine, Kovarik 1983); the density, length and arrangement of scales on metasternum (*Euscepes postfasciatus*, Baba and Yoneda 2000); a convex abdomen for females and a saucerlike depression in last sternite for males (*H. abietis*, Anonymous 1952, and *H. radicis*, Wilson 1966); the arrangement of hairs on the last abdominal sternite (the scolytine genus *Hylastes*, Grocholski et al. 1976); and longitudinally depressed abdominal sternites (present to some degree in males of all North American *Hylobius*, Wilson 1966, and in the Cacao weevil borer *Pantorhytes szentivanyi*, Hassan 1973).

Weevils were examined under a stereomicroscope at 16× magnification. As a first step 20 weevils were examined for each of the above characters. As a second step, promising characters were examined on an additional 80 weevils. After examination of the characters mentioned above, the weevils were dissected under a stereomicroscope at 6.4× magnification to determine their sex based on male- or female genitals (Nordenhem 1989), and the accuracy of sex classification using

these characters were established. Each weevil was marked with a unique label allowing us to cross reference the different techniques.

Results and Discussion

The overall sex ratio as determined by dissection was 40/60 male/female. Due to the broad overlap between males and females it was not possible to determine the sex based on the length of the snout (females, N = 14, average = 2.94 mm, range = 2.50 - 3.20: males, N = 6, average = 2.84 mm, range = 2.60 - 3.05) or the pronotum (females, N = 14, average = 3.70 mm, range = 3.20 - 4.35: males, N = 6, average = 3.64 mm, range = 3.20 - 3.90). Furthermore, males and females did not differ with respect to the presence or absence of tarsal claw thorns or the density, length or arrangement of scales on the metasternum. The presence of a convex abdomen was not a reliable female character of *H. warreni*, since an overlapping gradient between the sexes made it impossible to classify the sexes as either convex or concave.

Two characters were found that would be useful for separating the sexes of *H. warreni* (Table 1): (1) on the 1st visible abdominal sternite there is a longitudinal depression which in males continues without interruption to the 2nd sternite, forming a sulcus along the midline (fig. 1); and (2) on the last abdominal sternite the hairs of females are arranged longitudinally, whereas hairs on males are arranged radially (fig. 2).

Ninety out of 100 weevils were sexed correctly based on the presence or absence of the longitudinal depression on the 2nd sternite. Some of the females had a small round depression on the 2nd sternite not to be mistaken for the longitudinal

depression. This character was best seen when tilting the weevil underneath a light spot without any use of magnification. Ninety-five out of 100 weevils were sexed correctly based on the longitudinally or radially arranged hairs on the last sternite. Many of the females also had a few radially arranged hairs on the last sternite, thus, to classify a weevil as a male the hairs have to be radially arranged in more than one row of hairs. Ninety out of ninety-three weevils (97 %) were sexed correctly based on combining the two methods and exclude weevils where the male/female identification did not agree between the two.

Since it is possible to see the longitudinal depression on the 2nd visible abdominal sternite of *H. warreni* without magnification this method can be used in the field for convenient sex determination with a reasonable accuracy. If a higher accuracy is necessary it would be better to view the weevils with at least a 12× magnification to view the orientation of the hairs on the last visible sternite.

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180 Table 1. Keys for sexing adults of *Hylobius warreni*.
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Character	Female	Male
2nd sternite	No depression on the 2nd sternite or a small saucerlike depression separated from the 1st sternite.	Longitudinal depression in 2nd sternite continuing from 1st sternite forming a sulcus
Arrangement of hairs on last sternite	Longitudinal	Radial

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Figure-caption list

Fig. 1. Imago of *Hylobius warreni* showing the abdominal longitudinal depression absent on the 2nd sternite of females (left) and present on on males (right).

Fig. 2. Part of abdomen of *Hylobius warreni* showing the longitudinal arrangement of hairs on the last sternite of females (left) and the radial arrangement on males (right).

191 Fig.1

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Fig. 2