DNA identification of *Busseola* (Lepidoptera: Noctuidae) larvae in Ethiopian sugarcane

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Noctuidae is one of the largest lepidopteran families, encompassing about 20 000 species (Holloway 1998). Some 157 described species of Noctuidae are known to be cereal stem borers in the Afrotropical region (Moyal 2006) and the most economically important of these belong to the genera *Busseola* and *Sesamia*. Accurate identification of pest species is the first and most fundamental step to developing sound pest management strategies (Szalanski et al. 2003). However, many of these closely related stem borers are difficult to distinguish from each other morphologically, and no key is available to cover all noctuid stem borers (Holloway 1998).

Misidentifications of stem borers have occurred frequently, resulting in the publication of misleading data that are perpetuated, often for decades (Polaszek 1992). This problem is pronounced when identification of larvae is considered. Morphological structures such as setae are easily broken and frequently missing from alcohol-preserved and deep-frozen material. This makes identification even more difficult and results can be unreliable (Meijerman & Ulenberg 1998). Moreover, even when all of the setae are present, differences between species at the larval stage are minor and often it is not possible to identify larvae to species level. For instance, in the case of noctuid stem borers, species belonging to different genera, e.g. *Busseola fusca* Fuller and *Sesamia calamistis* Hampson, can have exactly the same long and microscopic setae, and the only difference in larval morphology is a slight change in the position of two setae on one segment (Moyal & Tran 1989). Distinction of species within these genera is generally more difficult and is usually not possible.

Hebert *et al.* (2003) proposed that the analysis of sequence diversity in *cytochrome-c oxidase* I (COI) gene of the mitochondrial DNA could serve as the core of a global identification system in the animal kingdom (‘DNA barcoding’). The potential of DNA barcoding has been demonstrated in many recent studies, reviewed by Vogler & Monaghan (2007) and Waugh (2007). Although this approach is not without controversy (Cognato 2004; Will *et al.* 2005; Brower 2006), the lack of adequate morphological taxonomic services makes the molecular approach an attractive alternative. The use of DNA-based technologies has been suggested as a good option to solve the current problems in identification of field-collected material (Hogg & Hebert 2004; Janzen *et al.* 2005; Hajibabaei *et al.* 2006). However, these papers did not adequately address the important question of whether the COI gene has the discriminatory power to correctly identify closely related species (Will *et al.* 2005). In this paper, we demonstrate the utility of DNA barcodes for identification of Ethiopian *Busseola* species.

*Busseola* larvae were collected from sugarcane and wild host plants bordering sugarcane fields of small-scale farms and commercial estates in Ethiopia (Table 1) on two occasions, November 2003 and February 2004. Specimens were placed into 30 ml plastic vials each containing a piece of sugarcane stalk or artificial diet in which to complete their development. However, none of the collected specimens developed to adult stage. The dead larvae were taken out of the stalks and/or artificial diet and kept in 95 % ethyl alcohol in sealed screw-top 30 ml glass vials.

Identification of adult *Busseola* specimens was carried out by comparison of male and female genitalia with those of the type specimens deposited in the Natural History Museum (BMNH, London). *Busseola fusca* and *Busseola phaia* Bowden...