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Repeatability and precision in proboscis length measurements for long proboscid flies

SHELAH I. MORITA
Department of Entomology, North Carolina State University, Raleigh, NC, 27695-7613
**Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC, 20560-0169.*
E-mail: moritas@si.edu
**Address to which correspondence should be sent.*

Abstract

Methods for measuring proboscides in long-tongued fly pollinators are examined with respect to fly morphology and behavior. Most ecological studies aim to measure the functional proboscis length as a response or predictor variable. Here I suggest a proxy for this, the Maximum Functional Length (MFL), obtained by combining the lengths of the prementum and labrum. I also quantify errors in proboscis measurements due to a lack of consideration of the extensible ventral rostral membrane (VRM). Results show that naïve measurements allow for substantial unrepeatable variation (mean: 7mm; maximum 14mm).

Key words: Proboscis, long-tongued fly, Tabanidae, Nemestrinidae, measurement, morphology, co-divergence, pollination ecology

Introduction

Entomologists and botanists alike are interested in proboscis lengths (PL). Numerous studies have used measures of this morphological trait to explore floral morphology and evolution, speciation and coevolution, not only in insects, but also in birds (Campbell *et al.*, 1994; 1996; Goldblatt & Manning, 1999), and mammals (Muchhala, 2006; 2007). Studies of PL in weevils (Toju, 2008; Toju & Sota, 2006), moths (Alexandersson & Johnson, 2002; Johnson *et al.*, 2002; Johnson & Steiner, 1995; Krenn, 1998), true bugs (Carroll & Boyd, 1992; Carroll *et al.*, 2001) and long-tongued flies (LTF) (Anderson *et al.*, 2005; Goldblatt & Manning, 2000; Johnson, 2000; Johnson & Morita, 2006; Johnson & Steiner, 1997; Morita, 2008; Pauw *et al.*, 2009; Potgieter *et al.*, 1999) have employed this metric as a response or predictor variable in their studies. Most ecological studies that include proboscis length as a response variable are interested in the proboscis length that the organism uses to interact with its environment, essentially, the functional proboscis length (FPL) (Anderson & Johnson, 2006; Anderson *et al.*, 2005; Archibald *et al.*, 2004; Bloch & Erhardt, 2008; Borrell & Krenn, 2006; Johnson, 1995; Johnson & Steiner, 1997; Manning & Goldblatt, 1996; Molleman *et al.*, 2005; Potgieter *et al.*, 1999; Szucsich & Krenn, 2002). The distance between pollinator contact with floral reproductive structures and the proffered reward is the metric predicted to be under selection. Thus, for flowers, selection would act on corolla length, and for pollinators, on FPL.

Accurate measurements of the functional proboscis length in long-tongued flies should incorporate morphology as well as fly behavior related to proboscis extension while feeding. In general, morphological studies are explicit about the structures and reference points used to obtain measurements. There may be, however, substantial differences recorded in proboscis length if preserved, or even live specimens with varying degrees of extension are examined (Grimaldi, 1988; Kearns & Inouye, 1993; Stuck, 1997; Johnson & Midgley, 1997). In most studies, insect PL is a simple linear measurement from the base of the clypeus or its analog (the labio-clypeal suture) to the distal end of the mouthparts (e.g. Combs & Pauw, 2009; Potgieter *et al.*, 2009), though the metric is not often explicitly stated (e.g. Anderson & Johnson, 2008; Manning & Goldblatt, 2005; Stang *et al.*, 2006). Structurally, this measurement, clypeus to tip distance (C2T), captures the length of the labium. Stretching of the ventral rostral

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