



Distinguishing between *Leptidea sinapis* and *L. reali* (Lepidoptera: Pieridae) using a morphometric approach: impact of measurement error on the discriminative characters

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Abstract

A morphometric approach was used to test the possibility of discriminating between *L. sinapis* and *L. reali* by taking into account some new genitalic characters in addition to those used in previous surveys. Principal component analysis, performed on the size-and-shape data sets and on the size-adjusted data sets, has allowed two completely separate morphotypes to be detected, both in males and in females. Discriminant analysis has confirmed the separation of previously detected morphotypes and has correctly classified 100% of the specimens in both sexes with six discriminative characters being identified in males and two in females. However, some of these discriminative characters were not considered reliable enough because of the high associated measurement error and the scarce discriminative power. Reliable discriminative characters were: *vinculum* (\approx *valve*) width, length of *phallus* (\approx *aedoeagus* \approx *aedeagus*), *saccus* and *uncus* in males and *ductus bursae* length in females. The main topics discussed are: a comparison of the discriminative characters with previous studies, the sources of measurement error and the devices used to reduce it, as well as the between and within-species variability of the characters.

Key words: sibling species, repeatability, size-adjustments, principal component analysis, discriminant analysis

Introduction

Since Lorkovic (1993) reported that males of *Leptidea sinapis* (Linnaeus, 1758) and those of *Leptidea reali* (Reissinger, 1990) could be identified by means of genitalic measurements, because they distinguish between two morphotypes, many surveys have dealt with these two species (Vila *et al.* 2003 and Beneš *et al.* 2003 provide a full list of the published papers). Many of these surveys, which are based exclusively on a morphological approach, confirm the existence of two separate morphotypes (interpreted as probable distinct sibling species), both in males and females (Laštůvka *et al.* 1995; Ábrahám 1996; Embacher 1996; Karsholt 1999; Mazel & Leestmans 1999; Nelson *et al.* 2001; Gianti & Gallo 2002; Freese & Fiedler 2004; Cupedo & Hoen 2006). However, some uncertainties in assigning some individuals, mainly males, to *L. sinapis* or *L. reali* have arisen in some studies (Neumayr & Segerer 1995; Göhl & Buchsbaum 1996, 1997; Hauser 1997). These uncertainties have also contributed to maintaining doubts regarding the effective reproductive isolation of the two species (Hauser 1997; Kudrna 2001). Freese and Fiedler (2002) stated that pre-mating isolation processes may exist between *L. sinapis* and *L. reali* and Martin *et al.* (2003) demonstrated the lacking of gene flow between the two species.

It seems that much of the still existing uncertainty in classifying some individuals of the *Leptidea sinapis* species complex on the basis of morphological characters is the result of the methods used in previous studies. In many instances the sample size was too small (Lorkovic 1993; Skalski 1995; Embacher 1996; Ábrahám 1996; Hauser 1997; Karsholt 1999; Mazel & Leestmans 1999; Nelson *et al.* 2001) and hence did not ade-