Delimitation difficulties in species splits: a morphometric case study on the *Euxoa tritici* complex (Lepidoptera, Noctuidae)

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**Abstract.** Genital characteristics tend to vary greatly between Lepidoptera species, providing helpful features for species delimitation. The differences between species are usually remarkable and suspicions about species identity never arise. However, fairly often, and possibly increasingly, taxa are elevated to species rank on the basis of very slight morphological differences, often without quantitative support. *Euxoa tritici* (Linnaeus) is a typical example of a variable species split into several morphologically similar species. The present study tested whether the diagnostic genital characters of the current classification, based on nonquantitative methodology, provide safe identification of species. Both traditional distance morphometrics as well as modern geometric morphometrics, which also enables quantitative shape exploration, were used. Moreover, whether the study specimens can be unambiguously categorized into several species with visual comparisons was tested independently using four specialist entomologists. Genital types of several named species as well as considerable variation in genitalia were found, but no support was found for the presence of several morphologically distinguishable species with quantitative morphometric analyses. Neither were study specimens categorized unambiguously by specialists. The results suggest that pure visual comparisons may lead to unsound taxonomic conclusions and that a quantitative approach in critical cases should be used more frequently.

**Introduction**

Insect species-level taxonomy is often based on differences in genitalia, which usually evolve comparatively rapidly and divergently (Eberhard, 1985; Shapiro & Porter, 1989; Arnqvist, 1997), thus providing good diagnostic characters. Insects differing in genitalia are considered as distinct species regardless of whether or not their ranges overlap. Thus, genital characteristics are treated differently to many nongenital morphometric characters, which are often recognized as varying substantially both locally and geographically. A good example concerns moth wing patterns that may vary greatly within species, e.g. among allopatric populations. The assumption that genital morphology implies genetic isolation is based on the lock-and-key hypothesis, although often unconsciously as taxonomists seldom discuss theoretical underpinnings in revisionary studies. According to this hypothesis, mechanical or sensory mismatch in genitalia between the sexes of different species works as a reproductive isolation system (Eberhard, 1985).

The European moth fauna is well known, probably better than in any other area in the world. Within the last two decades, new species discoveries have become scarce, especially in so-called ‘macrolepidoptera’, such as noctuid and geometrid moths. At the same time, more and more new species descriptions have not been ‘true’ discoveries, but due to species group revisions and consequent splitting of one species into two or more. As a consequence, the new species are often superficially similar and difficult to identify. Diagnostic characters between such sibling species are typically relative rather than absolute or discrete in nature, and are usually located in the genitalia. The presence/absence characters or otherwise unambiguously discontinuous, well-defined characters are not always presented. There are evidently reproductively isolated sibling species with or without minor genital differences (Itämies et al., 2003) but critical splitting may easily increase the risk of type I error.

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(nonexistent species become described as good species) in taxonomic decisions.

European noctuid taxonomy provides examples of increasing species splitting: since the 1980s, species have been split and several uncertain sibling taxa elevated to species level. Such actions have provoked debate among those interested in noctuids, for example that of Diarsia rubi (Vieweg, 1790), which is accepted as consisting of two species in Europe; the nominal species and its sibling D. florida (F. Schmidt, 1859) (Skou, 1991; Fibiger, 1993). Slight genital differences have been described, but variation has been insufficiently assessed. Similarly, the stability and reliability of differences have not been validated with morphometric statistics, and are thus subjective.

The aim of this study was to explore genital variation and reliability of nonquantitative methodology in taxonomically problematic cases, using both subjective and quantitative morphometric approaches. The Euxoa tritici species complex, which has gradually been split into six species during many decades (Fibiger, 1990, 1997), was used. As this study was not a taxonomic revision, the study was restricted to a certain area of E. tritici’s whole range, namely south-western Finland. In the study area, three species have been reported living in sympatry (Hulden et al., 2000; Kullberg et al., 2001). First, it was investigated whether the study material was comprised of several genital types (‘species’) as described in current taxonomic literature, and whether the samples could be categorized unambiguously into several morphologically distinguishable groups by subjective comparisons. Subjective classifications were compared with each other and each classification was compared with the results of morphometric analyses. Second, whether the study material could be categorized into several groups with traditional distance measurements, morphometrics and multivariate statistics was tested. Third, the shapes of two taxonomically important genital structures were investigated using modern geometric morphometric methods. Although morphometric methodology has advanced significantly (Bookstein, 1991; Rohlf & Marcus, 1993), until recently these methods have been quite rare in taxonomy (Fulford & Rutherford, 2000; Pretorius & Clarke, 2001; Pretorius & Scholtz, 2001; Querino et al., 2002; Gumiel et al., 2003). Traditional distance measurements apply well to size explorations, but contain limited information about the shapes of structures. Conversely, geometric morphometric methods also retain most shape variation and enable quantitative shape explorations. This is important, because typically taxonomic characters concern variation in shape rather than size.

Materials and methods

Study species

Species of the genus Euxoa are typical members of the subfamily Noctuinae. The European members of the genus were recently revised by Fibiger (1990, 1997), who elevated several taxa of the earlier E. tritici to species rank. According to Fibiger, the E. tritici complex consists of five sibling species in Europe: E. tritici (Linnaeus, 1761), E. nigrofusca (Esper, 1788), E. eruta (Hübner, 1817), E. diaphora Boursin, 1928 and E. segnilis (Duponchel, 1836). Furthermore, although Fibiger (1997) treated E. montivaga as a sister species of E. obelisca (Denis & Schiffermüller, 1775), both external and genital features clearly show that it belongs to the E. tritici complex. Differences among the species are subtle, the most important diagnostic characteristics being genitalic. Three of these species, E. tritici, E. nigrofusca and E. eruta, occur in the study area (Kullberg et al., 2001). The genital structures of both sexes are presented in Figs 1 and 2, and the main genitalic differences among the three species are described in Table 1. According to Fibiger (1990, 1997), the differences should be constant, but no morphometric or statistical support was presented.

Samples

The study samples were collected from the south and west coasts of Finland. The specimens were collected by bait and light traps and selected randomly for the study. In total, ninety-two males from five localities (nineteen specimens...
from Oulu area, twenty-two from Lohtaja, twenty from Dragsfjärd, thirteen from Helsinki and eighteen from Hamina) and fifty females (ten per locality) were analysed in the study.

Genital preparations

All parts of the genitalia of each specimen were dissected. The male vesica is difficult to prepare and is often everted incompletely, but only perfectly dissected specimens were analysed. Thus, many more specimens were dissected than indicated by the final (ninety-two) sample size. Male external genitalia were stained and mounted on microscopic slides in Euparal in a manner used routinely in insect taxonomy. Internal genitalia (mainly aedeagus + vesica tube) were prepared following Dang’s (1993) procedure and preserved in liquid Euparal, which was observed to best retain their shape. The cylindrical female ovipositor lobes were cut and rolled open to facilitate measurements. Other parts of the female genitalia except corpus bursae were mounted on microscope slides. Corpora bursae were inflated and their shape fixed with isopropanol. The three-dimensional shapes of female corpus bursae were preserved in liquid Euparal. All genitalia were stained with Chlorazol Black (female genitalia and vesica) or Eosin Red (male external genitalia).

Statistical and morphometrical analyses

Measurement error. The calculation to evaluate the degree of measurement error was made by using double-blind repeat measurements for each male genital trait for fifty males of *E. tritici*. The repeatability of those measurements was very high (range 98.84–99.93%). Such low levels of measurement error are insignificant (cf. Yezerinac et al., 1992), and thus other measurements were not repeated.

Subjective classifications of individuals. The subjective classification of individuals was carried out independently by four lepidopterists. Each had experience in moth identification by genital characters either with *Euxoa* moths or other moth taxa or both. Their task was to categorize the study material into species as defined in current literature. Because the current classification (Fibiger, 1990, 1997) is based predominantly on genital characteristics (there should be some subtle average differences in their wing patterns and size as well), the identification was carried out on the basis of digital photographs of both external and internal genitalia of each study specimen.

The congruence between independent classifications was tested, to examine if several ‘species’ were represented in the

Table 1. The most significant genital differences of species of the *Euxoa tritici* complex (after Fibiger, 1990, 1997 and Baungaard & Fibiger, 2002). For terms, see Figs 1 and 2.

<table>
<thead>
<tr>
<th>Character</th>
<th><em>E. nigrofusca</em></th>
<th><em>E. tritici</em></th>
<th><em>E. eruta</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of subbasal diverticulum</td>
<td>Smaller than in other species</td>
<td>Larger than in other species</td>
<td>Between <em>E. nigrofusca</em> and <em>tritici</em></td>
</tr>
<tr>
<td>Size of additional subbasal diverticulum</td>
<td>Smaller than in <em>E. eruta</em></td>
<td>Smaller than in <em>E. eruta</em></td>
<td>Larger than in other species</td>
</tr>
<tr>
<td>Vesica curve</td>
<td>Relatively weak</td>
<td>Strong</td>
<td>Relatively weak?</td>
</tr>
<tr>
<td>Length of sacculus extension</td>
<td>Shorter than in <em>E. tritici</em></td>
<td>Longer than in <em>E. nigrofusca</em></td>
<td>Like in <em>E. tritici</em></td>
</tr>
<tr>
<td>Valva width</td>
<td>Broader than in <em>E. tritici</em></td>
<td>Comparatively narrow</td>
<td>Broader than in <em>E. tritici</em></td>
</tr>
<tr>
<td>Bursa shape</td>
<td>Almost straight on the side of ductus seminalis</td>
<td>Clearly concave on the side of ductus seminalis</td>
<td>Like in <em>E. nigrofusca</em></td>
</tr>
<tr>
<td>Ovipositor setae</td>
<td>Nine to ten, shorter and narrower than in <em>E. eruta</em></td>
<td>Nine to ten, shorter and narrower than in <em>E. eruta</em></td>
<td>About thirteen, longer and broader than in other species</td>
</tr>
</tbody>
</table>

Fig. 2. Schematic presentation of *Euxoa (E. tritici s.l.)* female genitalia (dorsal view). Traits measured for traditional morphometric analysis are shown by arrows; traits 1 and 2 are shown in magnification on ovipositor lobes. Measurement 1 is the length of the longest seta of the left ovipositor lobe. cb = corpus bursae; o = ovipositor.
samples. As it soon became apparent that there were considerable differences in species identifications between experts, any parallelism (correlation) between the four classifications was determined. The species were numbered from one to three (a total of three ‘species’ was found by classifiers) and the ‘average identification value’ for each specimen was calculated. Then the absolute deviations of each classification from the average identification value were added together, specimen by specimen. The sum of these deviation values was considered an index for the unity of classifications. The lower the value the less disagreement between identifiers. The random distribution of deviation values was then produced by 1000 iterations where classifications were randomized. The null hypothesis of random classification is rejected if the observed deviation value falls within the fifty ($\alpha = 0.05$) smallest values of the 1000.

Traditional morphometrics. In total, eight distance measurements from male and six from female genitalia were taken for traditional morphometric analysis. The characters that should differ between species (cf. Fibiger, 1990, 1997) were emphasized, but some other features were also measured (see Table 1; measurements shown in Figs 1 and 2). Some presumed diagnostic characters were not analysed due to practical difficulties in quantification. For example, the number of ovipositor setae could not be evaluated unambiguously, as they formed a continuum from long and stout setae to minute ones. For measurements, all the preparations were photographed by Olympus Camedia C-3030 ZOOM digital camera. The measurements were performed by the IMAGEJ JAVA image-processing software.

First, it was determined whether the measured traits showed bi- or polymodal, rather than normal, distributions, as is supposed if the complex consists of several morphologically distinguishable species. Normality of the distribution of each trait was tested independently. Assuming that the material comprised several species, the distributions of the traits should reveal several peaks, especially because most of the measured traits are argued to differ among species (Fibiger, 1990, 1997; Baungaard & Fibiger, 2002). Assuming that these traits alone can be used as safe diagnostic characters, distributions should show several discrete peaks with no overlap. Normality was also tested with Shapiro–Wilkinson tests of normality.

Second, it was determined if principal component analysis (PCA) can differentiate the assumed species. Plotting specimens on PCA axes illustrates if morphometric data consist of several morphologically distinguishable clusters. The analysis reduces the number of original variables into fewer orthogonal variables. The first usually reflects the size and the second the shape of the measured object in morphometric data (Ricklefs & Travis, 1980), as in the present data in which all original variables were highly positively correlated with the first component, and the original variables of the second component showed several strong contrasts. Specimens were thus plotted on these two axes to illustrate patterns of genital morphometry and to reveal potential clusters.

Geometric morphometrics. Modern geometric morphometric methods are based on geometric configurations of landmarks and their relative positions in coordination, but also enable analyses of outlines where homologous landmarks cannot be defined (Bookstein, 1997). The landmarks are first located into the picture and digitized, after which the raw coordinate data of the landmarks is superimposed. This eliminates nonshape variation from the landmark data (done when only shape variation is addressed). The non-shape variation contains variation due to the location, rotation and size of an object. After superimposition only the shape variation of landmarks is retained and the data can be dealt with statistically. Normal multivariate statistical analyses can be applied. In exploratory analyses, the relative warp analysis is certainly applicable. The relative warp analysis is actually a PCA of the partial warps of the landmarks. Partial warps are sets of principal warps forming a set of basis vectors for shape tangent space and characterize individual specimens. Principal warps are eigenvectors of the bending energy matrix that are determined by the reference configuration of landmarks. The results of the relative warp analysis can be visualized by many ways. Thin-plate spline deformation grids, which enable visual comparisons of shape differences, e.g. between species or populations, were used in this study. The theoretical background of the geometric morphometrics is treated in detail by Bookstein (1991).

Male subbasal diverticulum and female bursa copulatrix were chosen for detailed shape variation study, because differences in their shapes have been emphasized in current taxonomic classification of the study species (Fibiger, 1990, 1997). Digital photographs of the structures were used for landmark positioning. The landmark digitizing was carried out using TPSDIG software (Rohlf, 2004a). Well-defined homologous landmarks were scarce; three well-defined landmarks could be defined in male and two in female genital structures (Figs 3 and 4). Due to the lack of homologous landmarks, a combination of homologous and so-called sliding semilandmarks (Bookstein, 1997) was used. Sliding semilandmarks are intended to capture outline information in the absence of well-defined homologous structures for true landmarks, and they may slide along the outline to optimally match with the corresponding points of the reference (= average) specimen. The number of semilandmarks must be constant throughout the specimens. The semilandmarks were defined using the TPSULTRA computer program (Rohlf, 2004b). For the male structure, five semilandmarks were used, with nineteen for the female (Figs 3 and 4). The landmark configurations of the specimens were then superimposed by generalized Procrustes analysis (= generalized least squares). When superimposed, the data were analysed using relative warp analysis. This exploratory analysis was used because the data could not be classified into groups prior to the analysis. The changes in geometric configurations of landmarks were visualized by thin-plate spline deformation grids. The superimposition and relative warp analysis were conducted with TPSRELW software (Rohlf, 2003).
Results

Independent subjective classifications of individuals

The four experts could not identify species unambiguously on the basis of taxonomic literature. Of ninety-two males, only seven specimens were categorized into the same species by all classifiers. Of females, four out of fifty specimens were identified unambiguously. Of the remaining eighty-five male specimens, thirty-four were identified unambiguously by three out of four classifiers. For females, the corresponding value was twenty-two out of forty-six specimens. No classifier bias was present as deviating identifications were made by all classifiers (see Supplementary material). The 1000 random iterations indicated that in males the categorizations were essentially random ($P = 0.642$), whereas in females the result deviated from random classification ($P = 0.031$). The results indicate that neither males nor females were categorized by their genitalia unambiguously, but in females the variation was similarly delimited by the classifiers. Variation in male vesica and female corpus bursae phenotypes are shown in Figs 5 and 6. When this variation is compared with the type genitalia of different species (shown by males in Fig. 7), the variation observed in study material apparently well exceeds that of between-species variation presented in the literature.

Traditional morphometric analyses

The distributions of distance measurement traits were unimodal and normally distributed. This was supported by the normality tests; only one exception from normality was found in males (trait 2 (male valva width), $P = 0.020$, otherwise all $P > 0.160$) and none in females (all $P = 0.244$). The exception from normality in male trait 2 was caused by a slight skewness, not multimodality.

In males, the first component of the PCA explained 42.4% (eigenvalue 3.392) and the second 16.3% (eigenvalue 1.305) of the total variance. In females, the values were 44.2% (2.651) and 20.7% (1.243), respectively. Plotting specimens on the two components showed continuous variation in both sexes (Fig. 8).

Geometric morphometric analyses

The superimposed configurations of the male subbasal diverticuli show that the plots of each landmark and semilandmark formed rather evenly rounded scatters (Fig. 9A). The first and second relative warps explained 48.6% and 30.8% (together 79.5%), respectively, and the third 9.8% of the shape variation of this structure. A scatter plot of these two relative warps revealed no discrete phenotype clusters of male subbasal diverticulum shape (Fig. 10). As shown by thin-plate splines (Fig. 10), the first relative warp ($x$-axis) reflects contraction of the lower part (= ‘toe’) of the diverticulum and elongation of the upper part (= ‘heel’). Shape differences in these structures between species (see Table 1) have been claimed to exist. The second relative warp describes the change in the relative width of the diverticulum (Fig. 10). As a whole, the total shape variation...
seems wider than the variation between the named species (cf. Fig. 7), but no clustering was present.

The superimposed configurations of female bursa landmarks indicated most evident variation on the right side (viewed ventrally) of the bursa copulatrix (in Fig. 2 on the left side due to dorsal view) (Fig. 9B). However, the alleged diagnostic features should appear especially in the concavity on the left (see Table 1). The three first relative warps explained 39.8, 21.0 and 10.5% (together 71.3%) of the total variance of the bursa shape, respectively. According to thin-plate spline visualizations (Fig. 11A), variation of the first relative warp is mainly due to concavity/convexity of the right side of the bursa and the angle between upper and lower halves. The second relative warp describes the change in the overall width of the bursa (Fig. 11A). The width of the bursa corresponds to some degree to the concavity of the right side of the bursa so that narrower bursae tend to be more concave, but some relatively narrow bursae with
convex right sides exist (specimens on right and down on the plot). As the distribution of dots tapers towards the right on the plot, there is more width variation in bursae with concave right sides than in those with convex right sides. The variation in the third relative warp is predominantly due to the degree of concavity of the left side of the bursa, the character that should have diagnostic value in identification (Fig. 11B). According to the analysis, however, the variation in this feature is relatively small and continuous.

### Discussion

Taxonomic studies usually produce widely accepted solutions, regardless of the methods used. Problems arise when dealing with a difficult species complex examined with inappropriate methods. *Euxoa tritici* is a typical example of a species split into several sibling species on the basis of minor, subjective morphological differences. There is no reason to assume that the *E. tritici* complex forms an exception among other difficult species groups. Many taxonomic revisions are based on subjective comparisons of minor genitalic or other morphological differences, with limited material (in many cases the number of examined specimens is not even mentioned). It is very possible that in many such cases, taxonomic conclusions have been unjustified. For example, when studied quantitatively and genetically, it was shown that the debated sibling beetles *Agabus bipustulatus* (Linnaeus, 1767) and *A. solieri* Aubé 1837 had no discontinuity in their morphologies or genomes (Drotz et al., 2001). Due to increased knowledge and advanced preparation methods, particularly for artificial male vesica eversion, systematic papers based partly or predominantly on vesica characteristics are published frequently (e.g. Lafontaine et al., 1987; Andersen, 1988; Bolte, 1990; Staddon & Ahmad, 1995; Zilli, 2000; Simonsen, 2005). Surprisingly often, species splitting is based not on quantitative analyses on morphology, but only on subjective visual comparisons.

This study demonstrates difficulties that may arise when variable taxa are split into morphologically similar species without quantitative morphometric or genetic support. As shown here, all treated genitalic structures showed continuous patterns of variation, such that the variation in 'diagnostic characters' was distributed normally. The conclusion of a single variable species was supported by traditional...
morphometrics as well as modern geometric morphometrics. Moreover, no support for the existence of several morphologically distinguishable species was found using ‘experienced eyes and brains’, as four specialists were unable to classify the study samples unambiguously. According to all analyses performed here, the complex does not consist of several morphologically distinct species in the study area. Similarly, despite the restricted study area, coexistence of several sibling species in a larger area in Europe appears unjustified as the total genitalic variation within the study area greatly exceeds the total variation among the named species. In any case, demonstrating the existence of distinct taxa of *E. tritici* requires thorough genetic or morphometric analyses. The possibility of several

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**Fig. 7.** Male internal genitalia of three named species of the *Euxoa tritici* complex, drawn after photographs presented by Fibiger (1997).

**Fig. 8.** Graphical presentation of the results of the principal component analysis of traditional morphometric measurements of the male (A) and female (B) study specimens.
species in *E. tritici* is also questioned by the finding that the coefficients of variation of genitalic traits within the *E. tritici* complex are essentially no larger than in close relatives *E. obelisca* (Denis & Schiffermüller, 1766) and *E. cursoria* (Hufnagel, 1766) (M. Mutanen, A. Kaitala & M. Mönkkönen, unpublished). They are both always treated as variable but single species. It has also been argued that the European species of the *E. tritici* complex might have differences in wing patterns (Fibiger, 1990, 1997; Baungaard & Fibiger, 2002). Wing patterns vary substantially and are commonly susceptible to both geographical and local variation. This is the case, for example, in the close relative *E. cursoria*. Comparisons of large series of photographed adult specimens of species of the *E. tritici* complex (Fibiger, 1990) give an impression that there is much overlap in external features between named species.

The methods of geometric morphometrics are very applicable to taxonomic studies as they enable both statistical treatments and illustrative visual presentations of shape differences. Although a statistical approach is not necessary in most taxonomic conclusions (because most species have qualitatively very distinct characters), it would be a useful tool in minimizing the risk of type I error in critical cases. Particularly in difficult cases, morphometric statistics should be used to test the reliability of a given character. Geometric morphometric methodology is used increasingly in different fields of biological sciences (Adams *et al.*, 2004), but is still surprisingly seldom applied in insect taxonomy, perhaps because of its quite recent introduction (Bookstein, 1991). The effectiveness in taxonomic identifications was explored recently using the water mites of the genus *Torrenticola* as a test case (Becerra & Valdecasas, 2004), in which identification was simpler with geometric morphometric methods. The same method was also used successfully in resolving the taxonomy of *Trichogramma pretiosum* Riley, a parasitoid wasp, which was shown to consist of several morphologically distinct host-specific groups (Querino *et al.*, 2002).

Although this study has shown that the three north European species of the *E. tritici* complex are hardly identifiable either with quantitative morphometrics or nonquantitative methodology, there is, of course, a possibility that distinguishing morphological characters not treated in this study exist. In addition to the three species dealt with in this study, the *E. tritici* complex comprises three additional species occurring in central and eastern Europe. These species, *E. montivaga* Fibiger, 1997, *E. segnilis* (Duponchel, 1837) and *E. diaphora* Boursin, 1928, should, according to Fibiger (1997), be distinguishable by genital characteristics. *Euxoa montivaga* was considered as a close relative to *E. obelisca* by Fibiger (1997). However, its morphological similarity with the members of the *E. tritici* complex suggests that it rather belongs to the *E. tritici* complex. Obviously the European *E. tritici* complex should be revised using explicit and objective methodology, and probably the same applies to many other European Noctuidae as well as many groups of sibling species. Several, if not all, species of the *E. tritici* complex probably need to be synonymized.

Fig. 9. Variation among study specimens in male subbasal diverticulum (A) and female bursa copulatrix (B) landmark and semilandmark configurations after superimposition. For landmarks, see Figs 3 and 4.
The variety of species concepts and lack of consensus in applying them bring theoretical problems for taxonomy that are hard to solve with morphometrics or any other method (see Wheeler & Meier, 2000). Taxonomists should be well aware of the theoretical background of different species concepts, especially when dealing with taxonomy at the specific level. Taxonomists seldom mention which species concept is applied in their works. A good example is a recent division of two allopatric populations of *Entephria multivagata* (Hulst) into different species on the basis of minor morphological, mainly genitalic, differences (Troubridge, 1997), without mention of the species concept applied. As a consequence, taxonomists do not always delimit species according to the same criteria. Implicitly, most taxonomists define species as reproductively isolated, genetically distinct units of nature and use morphology as indirect evidence of biological or genetic relationships of organisms. This is especially true for genitalic morphology that has been considered superior in taxonomic decisions (Lafontaine & Mikkola, 1987; Mikkola, 1992), relying on the assumption of genital mismatch causing mechanical isolation, although the evidence for this theory is weak (Eberhard, 1985; Arnqvist, 1998; Hosken & Stockley, 2004).

**Supplementary material**

The following material is available from: http://www.blackwellpublishing.com/products/suppmat/SEN/SEN296/SEN296sm/htm

**Appendix S1.** A list of the identification results of 142 study specimens by four independent specialists.

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Fig. 11. Graphical presentation of the first (x-axis) and second (y-axis) (A) and the first (x-axis) and third (y-axis) (B) relative warps of the female bursa copulatrix. Changes in genital structure along both axes are shown by landmark configurations in thin-plate spline deformation grids.

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