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Outline-based morphometrics, an overlooked method in arthropod studies?

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ABSTRACT

Modern methods allow a geometric representation of forms, separating size and shape. In entomology, as well as in many other fields involving arthropod studies, shape variation has proved useful for species identification and population characterization. In medical entomology, it has been applied to very specific questions such as population structure, reinfestation of insecticide-treated areas and cryptic species recognition. For shape comparisons, great importance is given to the quality of landmarks in terms of comparability. Two conceptually and statistically separate approaches are: (i) landmark-based morphometrics, based on the relative position of a few anatomical "true" or "traditional" landmarks, and (ii) outline-based morphometrics, which captures the contour of forms through a sequence of close "pseudo-landmarks".

Most of the studies on insects of medical, veterinary or economic importance make use of the landmark approach. The present survey makes a case for the outline method, here based on elliptic Fourier analysis. The collection of pseudo-landmarks may require the manual digitization of many points and, for this reason, might appear less attractive. It, however, has the ability to compare homologous organs or structures having no landmarks at all. This strength offers the possibility to study a wider range of anatomical structures and thus, a larger range of arthropods.

We present a few examples highlighting its interest for separating close or cryptic species, or characterizing conspecific geographic populations, in a series of different vector organisms. In this simple application, i.e. the recognition of close or cryptic forms, the outline approach provided similar scores as those obtained by the landmark-based approach.

1. Introduction

Contrary to classical morphology, the main objective of morphometrics is not to describe organisms, but to compare them. Because of the geometric constraints of modern morphometrics, data are generally collected on a non-articulated part, often a single organ (but see David et al. (1996) and Adams (1999)). A few characters are enough, provided that they are homologous.

Insect species studies generally made use of the wings because these structures are almost bidimensional and relatively rigid, reducing digitizing error; also, importantly, because the wings of many groups of insects provide a large number of landmarks. Not only are they many, they also are of good quality, called "true" (or "anatomical", or "traditional") landmarks. True landmarks are considered homologous. Homology here refers to the positional equivalence of a small biological structure, as small as a point at the requisite scale. The level of homology of a landmark is governed by the precision with which it can be localized from one organism to another. Thus, true, anatomical landmarks are homologous in the sense that they are relocatable points, and according to this criterion various levels of homology have been recognized (see type I, II and III landmarks, Bookstein (1991)). A special development of type III landmarks, called "semi-landmarks" (also "sliding semi-landmarks") allows the description of curved lines between two classical landmarks (Bookstein, 1997).

Anatomical, true landmarks are opposed to "pseudo-landmarks" used in the outline-based approach. Pseudo-landmarks describing contours or boundary outlines do not depend on the presence of true anatomical landmarks, they can exist with no

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anatomical landmark at all, or can include one or more of them. Obviously, pseudo-landmarks are of another nature than true landmarks: comparability is not expected from them separately, but from the structure they describe. Thus, if carefully chosen, like the mandible of a beetle, the genital paramere of a sandfly or an internal cell of the wing, a contour represents an homologous structure allowing interindividual and/or intergroup comparisons.

It is quite possible to develop both approaches on the same organ (Baylac and Frieß, 2005). One can see the wings of an insect as a configuration of anatomical landmarks, but the wing can be seen also as an assemblage of closed contours, i.e. the internal cells designated by the intersecting veins (Francoy et al., 2012). Moreover, other anatomical parts, because they are generally deficient in true landmarks, are only amenable to an outline analysis. For instance, many insect species, including very close species, are separated by the shape of some genital pieces. Such parts often lack anatomical landmarks, like the paramere of sandflies, the pronotum of Coleoptera (Faille et al., 2007), the genital leaflet of *Culex neavei* (Boussès et al., 2013; Garros and Dujardin, 2013) or the mandible parts of beetles (Tatsuta et al., 2009). Eggs and juvenile instars which also provide important taxonomic characters generally lack acceptable configurations of true landmarks: they can be considered through outline analyzes. Also good or natural candidates for the outline approach are the many wingless forms of insects, as for instance fleas, lice, ants, many of them of great veterinary, medical or agronomic importance. Finally, arthropods other than insects may present poorly defined landmarks but a particular anatomy which can be submitted to outline analyzes, as for instance the rounded body of ticks. In sum, the outline approach offers the possibility to study a wider range of organs, along with a wider range of arthropods.

The question is not limited however to technical considerations about presence or absence of landmarks (Rohlf, 1986), it also concerns the biological relevance of outlines. For entomologists, the primary needs are to distinguish species, especially cryptic species, and to detect them where they are not expected. Conspecific, but geographically or ecologically separated populations, are of interest too, in order to design relevant control strategies (Kaba et al., in press) or to detect the origin of reinfestation after treatment (Feliciangeli et al., 2007; Hernández et al., 2013; Gaspe et al., 2013). Does the outline approach distinguish species and populations as well as the landmark approach? We present here a few examples showing no significant difference between the two methods when it is about discriminating between close species or between conspecific populations.

2. Materials and methods

2.1. Material

2.1.1. *Rhodnius prolixus* and *Rhodnius robustus*

R. prolixus Stal, 1859 and *R. robustus* Larrousse, 1927 are morphologically very close species with a long history of controversy among taxonomists (Bargues et al., 2010). *R. prolixus* is a major vector of Chagas disease in the northern part of Latin America, recently eradicated from Central America (Hashimoto and Schofield, 2012) but still active in Venezuela and Colombia (Gorla et al., 2010). The two species generally are found in different ecological conditions: in domestic and peridomestic structures for *R. prolixus*, in the crown of palm trees for *R. robustus*. Because of its silvatic habit preferences, *R. robustus* is not considered as an important vector of Chagas disease, but it is involved in some local transmission (Feliciangeli et al., 2002). The overall morphology of the two species is very similar, although striking size differences are often observed, with *R. robustus* being generally the larger species. In the field, there is frequently some size overlap, so specimens collected from trees are assumed to be *R. robustus*, and those collected from houses are assumed to be *R. prolixus*. Our material contains three groups previously identified by molecular tools (Monteiro et al., 2003): one is *R. prolixus* from houses in the Pampanito village (State of Trujillo, Venezuela), the second one is *R. prolixus* from palm trees collected in another locality, San José Tiznados (State of Guárico, Venezuela), and the last one is *R. robustus* from two localities of Venezuela: Pampanito and Candelaria (State of Trujillo).

We performed analyzes both combining the two *R. prolixus* habitats, as well as the two *R. robustus* origins, into single samples and considering them as separated groups. A total of 7 landmarks could be used, thus excluding the eighth used in Feliciangeli et al. (2007) because of the inconsistency of the clavum position on the pictures. The outline submitted to the elliptical Fourier analysis (EFA, see Section 2.2.2) contained 5 of them, as well as the external boundary of the membranous part of the wing (Fig. 1).

2.1.2. *Glossina palpalis palpalis* and *Glossina palpalis gambiensis*

G. p. palpalis (Robineau-Desvoidy), 1830 and *G. p. gambiensis* Vanderplank 1949 (Diptera: Glossinidae) are important vectors of sleeping sickness in West Africa. Both species hybridize readily in the laboratory but hybridized females produce fewer offspring and hybrid males are sterile (Gooding, 1988). These two subspecies are difficult to separate on morphological ground. Although males show consistent differences in the terminal dilatation of inferior claspers of their genitalia (Machado, 1954), morphological differentiation of female flies is not conclusive. The material we used here is from Ivory Coast and was collected in 2007. Male and female specimens of *G. p. palpalis* were collected in Aniassue, South of Ivory Coast. The *G. p. gambiensis* specimens came from Ganse, North of Ivory Coast. The subspecies identity of all the female and male specimens of the present study had no morphological nor molecular confirmation.

Ten landmarks were used as in Kaba et al. (in press). The EFA method was applied to the comparison of the central cell of the wing which includes five landmarks (Fig. 2). This cell is considered as having an important taxonomic significance for tsetse flies (De la Rocque et al., 2002).

2.1.3. *Anopheles strodei* and *Anopheles oswaldoi*

A. oswaldoi (Peryassú) has an unclear taxonomic and vectorial status in South America (Ruiz-Lopez et al., 2013), and *A. strodei* Root shows a high levels of morphological polymorphism (Bourke et al., 2013). Both species are difficult to distinguish based on existing dichotomous keys (Faran, 1980; Faran and Linthicum, 1981; Gonzales and Carrejo, 2007). Females were collected from the municipality of Puerto Asis, department of Putumayo, Colombia. They were induced to oviposit in the laboratory, where the male genitalia and associated immature stages of offspring were used for taxonomic identification. *A. oswaldoi* specimens were confirmed by PCR-RFLP of the rDNAmarker ITS-2 (Ruiz et al., 2005). From *A. oswaldoi*, 30 adult F1 females and from *A. strodei*, 32 adult F1 females were randomly chosen for the morphometric studies.

Eleven anatomical landmarks were selected on the crossing of wing veins, while the EFA method used the contour of a small cell at the internal base of the wing, a contour which did not include any of the eleven landmarks (Fig. 3).

2.1.4. *Ornithodoros maritimus*

O. maritimus is a soft tick (Family Argasidae) and part of the widespread species complex, *Ornithodoros capensis* sensu lato, exploiting colonial seabirds in tropical and sub-tropical areas of the world (Dietrich et al., 2011). The different members of this complex are known vectors of several viruses and bacteria,

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