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Sexual Wing Shape Dimorphism in *Piophila casei* (Linnaeus, 1758 Diptera: Piophilidae)

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ABSTRACT

The Piophilidae are synanthropic flies that grow and develop on cheese, fish, cured meat, among others substrates. Some species represents an important forensic indicator associated to carcasses, because the adults are common during the bloat stage, and the larvae are associated to the advanced to dry stages. Recently, the geometric morphometrics has been used to quantified the sexual shape dimorphism in medical important insects. We proposed to evaluate the shape sexual dimorphism in *Piophila casei* through the wing geometric morphometrics. Were collected 34 *P. casei* specimens and sorted by sex (17 females and 17 males), later the wing was dissected and slide mounted for digitalizing eight landmarks. The x,y coordinates were aligned by Generalized Procrustes Analysis to extract the matrix configurations and centroid size. The sexual shape dimorphism differences were evaluated by means a Discriminant Analysis (DA), and the size with non-parametric ANOVA. We didn't found differences in centroid size between females and males; however, the shape was significantly different between sex. In the cross-validation DA, 82% specimens were correctly classified into females and 88% males. Our study represents the first investigation that quantifies the sexual shape and size dimorphism in *Piophila casei*, and this could be a basis for further studies that combines geometric morphometrics tools and forensic entomology.

Keywords: *Phiophilinae, Procrustes analysis, Geometric morphometrics, Forensic entomology.*

INTRODUCTION

The Piophilidae commonly know as "cheese skippers", most species are scavengers and synanthropic flies that grow and develop on cheese, fish, cured meat, human excreta, household garbage, among others substrates [1,2]. In the stored food industry are an economically important pests [3,4], also some species has been reported to cause human myiasis [5,6,7].

From a forensic point of view, some Piophilidae species represents an important indicator associated with carcasses, because the adults are common during the bloat stage, and the larvae are associated to the advanced and dry/remain stages [4,8,9]. *Piophila casei* (Linnaeus, 1758) adults are attracted by the carrion in early stages[10]; however, some investigations suggested that the presence of adult does not necessarily is associated to oviposition on the carcass; and it has been assumed that immature stages do not develop in a corpse until 3-6 months after death when fatty acids and caseic products are present [3]. Nevertheless, *P. casei* has had little forensic importance and is unnoticed in most cases because of its small size (2-5 mm) and as referred to secondary colonizer. The immature stages of some Piophilidae species are still unknown, and the correct identification is a crucial factor for the Postmortem interval estimation [11,12]. Due to this, some studies are focused on determined the life cycle and life statistical parameters in different temperature [2,13]; also the sexual dimorphism is an interesting source of phenotypic variation, and several Piophilidae species showed sex differences among head, foreleg, and wings

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[14, 15]. Geometric morphometrics is a technique that quantifies phenotypic differences among groups [16], and recently demonstrated its importance for blowfly adult and immature taxonomic identification [17,18]. In our investigation, we proposed to evaluate the shape sexual dimorphism in *P. casei* through the wing geometric morphometrics.

MATERIAL AND METHOD

Specimens source and data acquisition: *Piophila casei* specimens were obtained from collections in an urban area of Valencia (10º13'78'' north Latitude and 68º00'32'' west Longitude) between February and May 2016. The 34 specimens were sorted by sex (17 females and 17 males), and the right wing was dissected, slide mounted and photographed. Were selected eight anatomical points (Figure 1) type I [19] and morphological definitions of McAlpine [20]: Intersection between subcostal vein and costal (LM1), intersection between radial (R_1) and wing margin (LM2), intersection between radial (R_{2+3}) and wing margin (LM3), intersection between radial (R_{4+5}) and wing margin (LM4), intersection between medial (M_1) and wing margin (LM5), intersection between medial and transversal discal medial-cubital (LM6), intersection between radial (R_{4+5}) and transversal radio-median (LM7), and medial with transversal radio-median (LM8).

Morphometric analysis: From 34 configurations matrix, the coordinates were aligned by Generalized Procrustes Analysis using MorphoJ software [21] to extract the matrix configurations (Partial Warps = PW) and centroid size (CS). Later, the PW matrix was used to perform a Discriminant Analysis (DA) for testing the group (sex) membership significance with Hotelling's test. Also was estimated the group configuration differences with TwoGroup software [22], by means a Goodall test. The F test compares the difference in mean shape between two samples relative to the shape variation found within the samples [17,23]. For statistical significance, we perform an F test based on a Bootstrap analysis on 1000 random data permutations. Finally, CS differences were analyzed with PAST software [24] by means the non-parametric Kruskal-Wallis test (P 0.05) with Bonferroni correction.

RESULTS

Centroid size: Were not found significant differences ($\chi 2 = 0.003$, df 1, P 0.958) among the wing centroid size between sex; the females specimens shows similar size (mean 2.60 mm, 0.52 standard deviation) than males (2.66 ± 0.44).

Wing conformation: Figure 2 shows the DA results for 34 *P. casei* specimens: histogram with values of the discriminant scores for sex (Figure 2A) and the thin-plate spline deformation grid with the differences between females and males (Figure 2B). The main sex distinction occurs in two landmarks: intersection between medial and transversal discal medial-cubital (LM6) moved to the posterior wing margin, and intersection between medial with transversal radio-median (LM8) displacement to wing base. The DA Hotelling and Goodall tests show significant differences between female and male conformations: $T^2 = 110.74$; $P < 0.001$ and $F = 3.21$; df = 12,384; $P < 0.0001$, respectively. Finally, in the cross-validation DA, 82% specimens were correctly classified into females and 88% males.

DISCUSSION

In general, insects females are larger than males, due to adaptive advantages such as greater fecundity and better parental care. Sexual dimorphism is of interest in entomological studies since frequently the differences between sexes are not obvious or the individuals are very small [25]; Piophilidae females are easy to recognize by the weakly telescopic piercing ovipositor [20].

Many studies has been reported the wing utility for dimorphism description in medical entomology important species. In Triatominae, the transition to different ecotopes (sylvatic, peridomestic and domestic habitats) are associated to size variations: the body is larger for specimens found in natural conditions, versus those reared in the laboratory or collected in domiciliary habitats [26]. In general, Triatominae sylvatic females are larger than males, but in colonies (or domestic habitats) some species showed strongly reduced sexual dimorphism, becoming the same size [26, 27]. Virginio *et al*. [28] explored the wing sexual shape and size variation in ten Culicidae species, found significant sexual dimorphism in all the species studied, indicating that phenotypic expression of wing shape in mosquitoes is indeed sex-specific.

Bonduriansk and Rowe [14] conduced arena experiments in male-male combats of *Prochyliza* *xanthostoma* Walker, 1849 (Piophilidae), and encountered that the first interaction with a new opponent selected for large body size but reduced head elongation, whereas multiple interactions with the same opponent favored large body size only. In contrast, male-female interactions, females preferred males with relatively elongated heads. Thus, both male-male and male-female interactions favored large male body size, while male head shape appeared to be subject to conflicting sexual selection. Later, Bonduriansk [29] studied the dimorphism patterns in *P. xanthostoma*, and stated that these differences may have evolved in response to sexual selection on male body shape, suggesting a shared pattern of covariation among traits (head, thorax and wing) may have conduced the evolution of sexually body elongation. Recently, Oudin *et al*. [15] reported the dimorphism in *Protopiophila litigata* Bonduriansky (1995) reared under different larval diets. The sex variation did not increase with diet quality within any trait (head, thorax, tibia and wing), among traits the extent of dimorphism was positively associated with the strength of condition dependence in males but not females.

Our study represents the first investigation that quantifies the sexual shape and size dimorphism in *Piophila casei*, and this could be a basis for further studies that combines geometric morphometrics tools and forensic entomology.

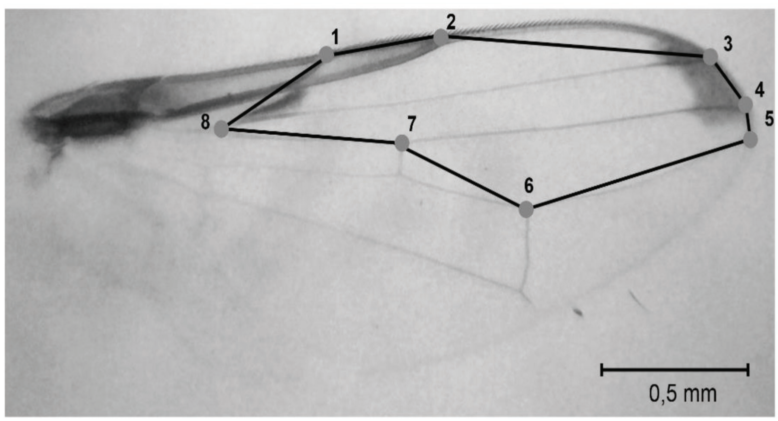

Figure 1. Wing of *Piophila casei* showing the landmarks (1-8) disposition. The polygon enclosed by the points conform the configurations analyzed.

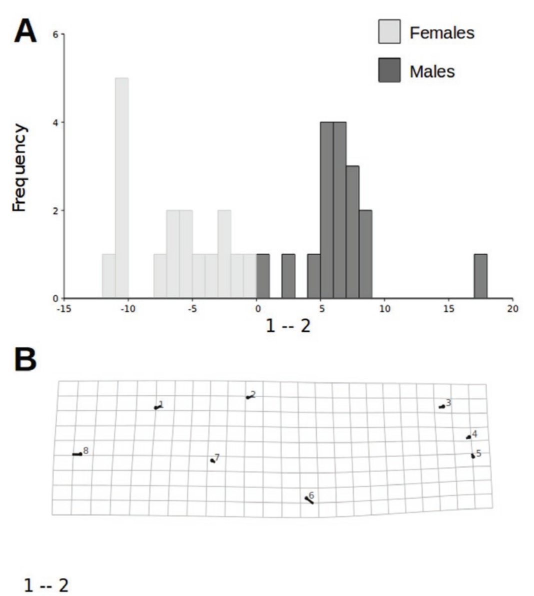

Figure 2. Discriminant analysis for 34 *Piophila casei* specimens: A) histogram with the values of the discriminant scores for groups: 1 = females and 2 = males, and B) the thin-plate spline deformation grid with the differences between females and males.

Ethical Clearance. Was not required.

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Conflict of Interest. We declare that no conflict of interest.

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