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The Head of *Fannia pusio* (Fanniidae: Diptera) as A Novel Source of Morphometric Data for Assessing of Variation Along Geographic and Biological Lines

Yolanda Bravo-Pena[1,*], José Herrera-Russert[1,2], Elena Romera[1], and José Galián[1,3]

[1]*Department of Zoology and Physical Anthropology, University of Murcia, Campus Mare Nostrum, 30100, Murcia, Spain.*
**Correspondence: E-mail: yolanda.bravo@um.es (Bravo-Pena)*
E-mail: eromera@um.es (Romera)
[2]*Department of Insect Biotechnology, Institute of Insect Biotechnology. Heinrich-Buff-Ring 26-3,35392, Gießen, Germany.*
E-mail: jose.herrera-russert@agrar.uni-giessen.de (Herrera-Russert)
[3]*Arthropotech SL, Arthropod Biotechnology, Nave Apícola, Granja Veterinaria UMU, Avenida de la Libertad, s/n, Guadalupe, 30071, Murcia, Spain.*
E-mail: jgalian@um.es (Galián)

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Fannia Robineau-Desvoidy, 1830 is the most diverse genus in the family Fanniidae (Diptera), with 288 species, many of which are include many of sanitary, economic and legal interest. The morphological homogeneity within the genus often makes species determination difficult. The best option for correct identification is to combine molecular and morphological analyses. The variation in the shape of a selection of body characters can be assessed by Geometric Morphometrics using the head as an innovative structure. Sex must be accounted for as a key covariate in this kind of study, since *Fannia*, as many other Diptera, has a sexually dimorphic head structure, with holoptic males and dicoptic females. Firstly, we analysed a set of *Fannia* sp. specimens sampled across the Iberian Peninsula (2012–2015), of which *Fannia pusio* (Wiedemann, 1830) was found to be the most abundant species. Our analyses provide significant morphological information. *Fannia pusio* exhibits clear intraspecific morphometric variation along an Iberian-wide East-West axis. A similar pattern emerged when comparing a laboratory-bred colony and wild samples.

Key words: Biological Variability, Geographical Variability, Geometric Morphometrics, Head landmarks, Iberian Peninsula.

BACKGROUND

Insects are one of the groups that have received most attention in the application of Geometric Morphometrics (GM) (Dujardín et al. 2014; Gerard et al. 2015), particularly the Diptera (Espra et al. 2015; Grzywacz et al. 2017; Macedo 2017; Mikery et al. 2019; Szpila et al. 2019). Morphology, in terms of shape and dimensions of the exoskeleton, gives us information about an animal's lifestyle (Menes-Hernández 2004).

Wings are an ideal biological structure for this type of analysis due to the taxonomic information they provide (Grzywacz et al. 2017; Sontingun et al. 2017), but other structures such as the head have also received attention (Baylac et al. 2003; Khamis et al. 2012; De Souza et al. 2015; Godoy et al. 2018). These types of studies allow us to establish the degree of diversity at both inter (Fuentes-López 2018) and intra-populational levels (Menes-Hernández 2004), and to determine the history of the species establishing the processes that

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explain the evolutionary patterns observed in organisms (Bustamante et al. 2004).

Fannia Robineau-Desvoidy, 1830 is the most diverse genus in the family Fanniidae (Diptera), with 288 species. The morphological similarities among its species become a significant hurdle for reliable identification. *Fannia* spp. share the following characteristics (Al Gazi et al. 2004): i) small size; ii) dark integument; iii) predominantly yellow abdomen (Rozkosný et al. 1997); iv) dorsal submedian seta on the hind tibia; v) short vein CuA+1A as an extension of vein 2A of the margin of the wing (Pont 1977).

Fannia pusio (Wiedemann, 1830), commonly known as the "chicken dung fly" because of its common appearance in laying hen farms, is a species of great sanitary, economic and legal interest. The originally Nearctic species (Couri and Sousa 2019) is currently found worldwide thanks to the transportation of livestock.

Part of the species' economic and sanitary interest stems from the female's common role as the phoretic host of the eggs of *Dermatobia hominis*, the human botfly, which causes myasis in humans and other animals (Gomes et al. 2002). Females can be easily identified due to the sexuality dimorphic eye arrangement in *F. pusio*: they are dichoptic with the eyes well separated by the frons (Domínguez and Pont 2014). On the other hand, the legal interest refers to the forensic field, as this species exploits decaying organic matter, both animal and human (De Souza et al. 2008; Grzywacz and Prado e Castro 2012; Vasconcelos and Araujo 2012).

For all these reasons, a correct identification is essential. The best option is to use molecular analysis with morphological tools like Geometric Morphometrics, which use the shape variability of body characters (Bookstein 1982). Landmark-based morphological analysis has been successful in examining the morphological variations in different animal groups, including the cranial morphology of rodents (Vallejo et al. 2017) and carapaces of zooplankton (Wong et al. 2018; Hethke and Weeks 2020). This tool has not yet been used as an identification methodology in the genus *Fannia*, so the present study is the first in its field. However, Grzywacz et al. (2017) used GM as an alternative to the classical morphology in Muscidae due to the fact that the identification of adults is considered difficult. In that paper, the wings were chosen as the study structure, and it was concluded that this method facilitates identification compared to more difficult and time-consuming approaches, with a very high success rate in terms of results.

From the data obtained, we formulated two main question: i) is the head of *F. pusio* a structure of enough taxonomic resolving power? and furthermore, ii) does the documented distribution of head landmarks match the geographical and biological differences in *F. pusio*?

MATERIALS AND METHODS

Sampling

The individuals analyzed in this work were collected over the course of several collection trips made throughout the Iberian Peninsula from 2012 to 2015 (sampling design detailed in Fuentes-López 2018). Eighty-one specimens were keyed to the family level using the keys provided by Szpila (2012) and identified as Fanniidae.

The geographical information on the samples analyzed in this study is presented in table 1. We collected the following samples: *F. aequilineata* (N = 2), *F. canicularis* (N = 5), *F. lepida* (N = 2), *F. leucosticta* (N = 4), *F. monilis* (N = 1), *F. pusio* (N = 65) and *Hydrotaea floccosa* (N = 2); *F. pusio* was by far the most common species recorded.

In addition to the field sampling, a colony of *F. pusio* was stabilized under controlled laboratory conditions in the Laboratory of Necrophagous Diptera at the University of Murcia (Spain): 25°C, 65% relative humidity and a 12:12 cycle. Adults were given water and sugar *ad libitum*, supplemented with canned cat food to induce oviposition (Couri 1991). The choice of this product for obtaining eggs was a consequence

Table 1. Description of the landmarks used in the analysis

Landmarks	Description of the landmarks
1	Right eye upper margin
2	Left eye upper margin
3	Right eye lateral margin
4	Left eye lateral margin
5	Right eye lower margin
6	Left eye lower margin
7	Lower right margin of the mouth
8	Lower left margin of the mouth
9	Lower margin of the clypeus
10	Interior angle of right eye
11	Interior angle of left eye
12	Flagellum base of the right antennae
13	Flagellum base of the left antennae
14	Flagellum apex of the right antennae
15	Flagellum apex of the left antennae
16	Upper margin of the frontal suture
17	Upper final of right orbital bristles line
18	Upper final of left orbital bristles line

of the poor success of other substrates—*e.g.*, dog and human faeces and chicken and pig liver (D'Almeida 1994).

The samples considered here were from the 165th generation, which was obtained after about four years of laboratory breeding of the same lineage. Initially, 30 individuals were sampled for a study on the morpho-geometric differences between lab-raised individuals (domestic) versus wild-captured specimens (wild type). These samples were also preserved, although four of the lab-raised specimens were removed from the study due to excessive damage. Finally, 26 domestic samples were used together with 39 wild type samples collected in the sampling.

Molecular analysis

To verify the identification at the species level, we performed a molecular analysis of the *cox1* gene of the mitochondrial genome, which was described elsewhere (Bravo-Pena et al., under revision). Briefly, DNA extraction was performed using the CCDB Glass Fiber Plate DNA extraction protocol (Ivanova et al. 2006). Amplification of the *cox1* barcode region was performed on a 2720 Thermal Cycler (Applied Biosystems, Foster City, USA) using a PCR kit from KAPA BIOSYSTEMS (Wilmington, USA) (Folmer et al. 1994). Finally, the samples were sequenced at Macrogen (Amsterdam, Netherlands). The software GENEIOUS 7.1.3 was used to manually edit the sequences (Kearse et al. 2012) and the alignment was performed using MUSCLE (Edgar 2004). The sequences were uploaded to GenBank under reference codes MT527094–MT527174.

Data analysis

As stated in the introduction, wings are generally the best insect structure for this type of study. In our particular case, *Fannia* has a small body size, which compounded the poor state of conservation of some samples, making the wings impossible to use. We therefore decided to use the better conserved heads of the 81 samples (Fig. 1) to search for useful landmarks (described in Table 2).

The strongly dimorphic sexual character of *Fannia* (Fig. 1) made it a straightforward choice to focus only on one sex for a meaningful analysis. We focused our analysis on the more frequently caught females, which have a stronger tendency to enter the traps in their search for moist and nutritious substrate to deposit their eggs on (Domínguez and Pont 2014).

To examine the variation in head shapes in the samples studied, data files were generated with a

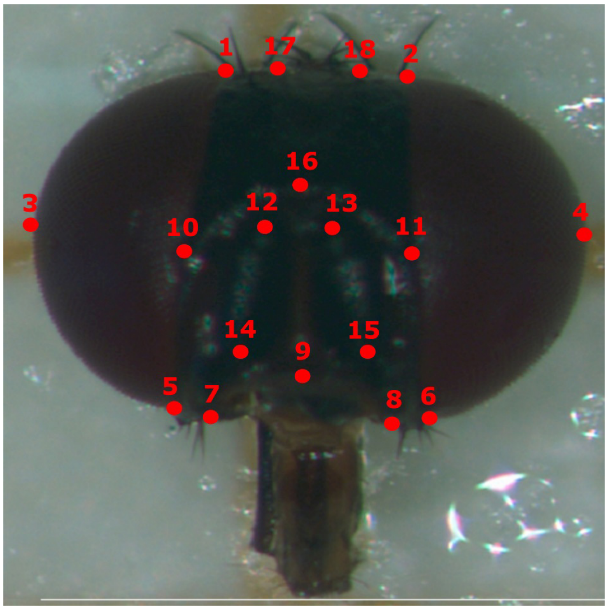

Fig. 1. Head of female *Fannia pusio.* The numbered points indicate the location of the 18 landmarks used for head measurements.

Table 2. Information on the geographic data of the sampling of each species and the number (N) of individuals collected (see also Fig. 1)

Species	N	Collection date	Country	Region	Latitude	Longitude	Elevation
F. aequilineata	2	28-09-2015	Portugal	Lisbon	38.75818	-9.15804	79 m
F. canicularis	5	28-09-2015	Portugal	Lisbon	38.75818	-9.15804	79 m
F. lepida	2	28-09-2015	Portugal	Lisbon	38.75818	-9.15804	79 m
F. leucosticta	3	28-09-2015	Portugal	Lisbon	38.75818	-9.15804	79 m
	1	22-07-2012	Spain	Valencian Community	38.45880	-0.77851	403 m
F. monilis	1	28-09-2015	Portugal	Lisbon	38.75818	-9.15804	79 m
F. pusio	28	4-09-2012	Spain	Region of Murcia	38.02773	-1.17556	150 m
	30	28-09-2015	Portugal	Lisbon	38.75818	-9.15804	79 m
	3	23-06-2012	Spain	La Rioja	42.09369	-2.56187	1265 m
	4	19-07-2014	Spain	Valencian Community	38.45880	-0.77851	403 m

STEMI-200-C stereoscopic (Fisher Scientific, Madrid, Spain) calibrated with the SPOT 4.6 AdvancedTM program. TpsDig2 v.2.31 and tpsUtil32 v.1.73 were used to digitize the landmarks (Fig. 1). The resulting numerical data were analyzed with MorphoJ statistical software (Klingenberg 2013). Based on the results of Fuentes-López (2018) on three species of *Lucilia*, we selected 18 landmarks. It should be noted that landmarks 12–15 (Table 2) are antennal and thus mobile relative to the head capsule, so these landmarks were analyzed separately to not introduce any artifacts.

The plot of the two first relative warps shows the scores of each specimen in that shape space, as well as the shape changes explained by each axis. Principal Component Analysis (PCA) was performed to reduce the dimensionality of the data and to determine the variables chosen for this study (Dujardín et al. 2014). We followed up with a Canonical Variate Analysis (CVA) to explore the differences among groups (Zelditch et al. 2004). Transformation grids and wireframes representing the PCA and CVA shape changes, showing variations for the relative location of each landmark, were presented. Finally, a reclassification with Cross-validation (Refaeilzadeh et al. 2009) and Mahalanobis distance (McLachlan 1999) with permutation for pairwise was made to verify our results (Wink-da-Silva et al. 2018).

RESULTS

Three sets analyses were performed. The first concerned the species of the genus *Fannia* present in the initial sample (N = 81) and was intended to test if the head is a suitable structure for this type of analysis and if the chosen landmarks are adequate. *Hydrotaea floccosa* (Muscidae) was used as an outgroup, allowing the effectiveness of the analysis to be tested. Identification at the species level was achieved through molecular analysis of DNA sequences (*cox1*), which are now available on GenBank.

The other two analyses were performed only on a sub-sampling of *F. pusio* (N = 65). One was geography based, to interpret the morpho-geometric differences according to the sampling locations (Table 1). The other was based on the biological of the species to test for differences between our domestic lineage and the samples collected in the wild.

Search for suitable head landmarks in the genus *Fannia*

As previously mentioned, for the comparative study of the *Fannia* species present in the sampling, we recovered 81 individuals (Table 1). First, the Principal Component Analysis (PCA) showed great differences in the landmarks located in the parafacial zone. This area is where the antennae and interocular space are located. Specifically, the landmarks that show this difference ordered by degree of variation are: 10, 11, 13, 12, 14 and 15. These differences can be observed in the transformation grid and the wireframe (Fig. 2). The projection of the geometric configuration of the landmarks in the tangent space is shown in figure 3.

On the other hand, according to the Canonical Variate Analysis (CVA), the landmarks that show most

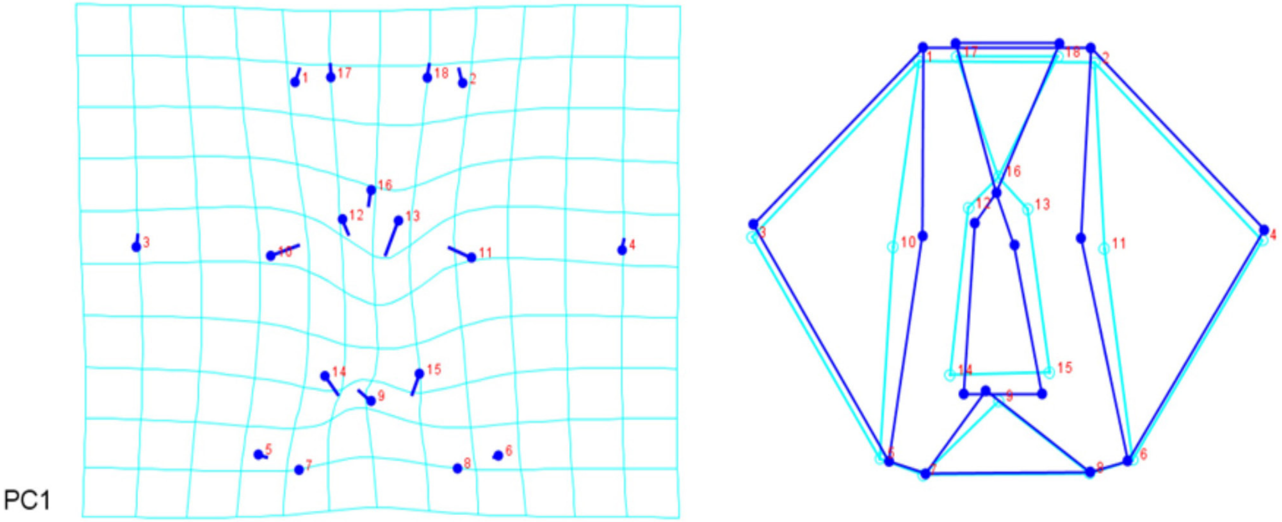

Fig. 2. Transformation Grid (left) and Wireframe (right) representation of shape variations between *Fannia* species based on Principal Component Analysis. *In the wireframe the turquoise outline characterizes the position of consensus landmarks, while the blue outline represents landmarks configurations.

differences between species were: 5, 6 and 16. These landmarks cover the lower margins of the eyes and the ptilinal suture respectively (Fig. 4). As portrayed in figure 5, these results allow us to differentiate most of the species. However, the samples of *F. lepida* overlap with *F. leucosticta*.

As observed in table 2, the statistics contradict the graphic results obtained. Statistically significant differences of *p*-value < 0.05 were observed between *F. pusio* and all other species, except for *F. canicularis* and *F. leucosticta*. However, the cross validation obtained was higher than 75% in all comparisons between *F. pusio* and the other species.

Regarding the differences among other species comparisons, no statistical significance was obtained with a $p > 0.05$. However, the pairs *F. aequilineata* – *F. leucosticta* and *F. leucosticta* – *F. monilis* showed a percentage higher than 75% in the cross validation (Table 3).

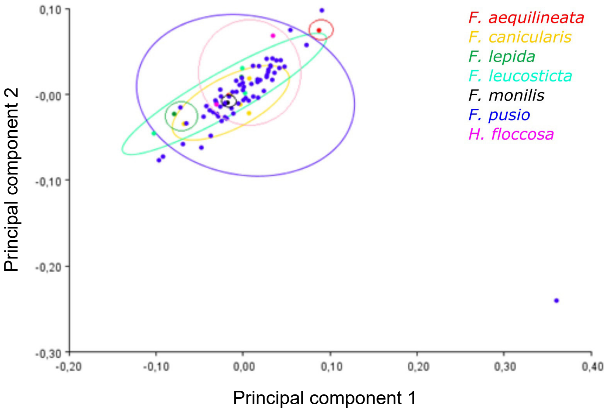

Fig. 3. Discrimination of *Fannia* species with a *Hydrotaea floccosa* as outgroup based on Principal Component Analysis.

Most of our samples belonged to *F. pusio* and it is in this species where statistically significant differences are actually observed. This led us to carry out two further analyses in this species, the first according to the locations where *F. pusio* was collected (Table 1) to evaluate whether geography has an explanatory role in the morpho-geometric differences found in the species. In the second analysis, we evaluated whether environmental fluctuations affected the GM parameters of the species. To this end, we compared the previously considered individuals of *F. pusio* with a set of lab-reared flies originating from a colony kept under constant laboratory conditions for several years.

Geographical differences among *F. pusio* populations

The PCA shows that the landmarks with the greatest difference in order of variation are: 13, 10, 11, 9, 14, 15 and 12. All of them are points arranged along the parafacial area, where the antennae and interocular space are located. A further visualization of these differences is offered in figure 2. The projection of the geometric landmark configuration onto the tangent space is shown in figure 6.

According to the CVA, the landmarks that bear the most differences between species are: 9, 14, 12 and 15. These points also reflect the parafacial area (Fig. 7). As

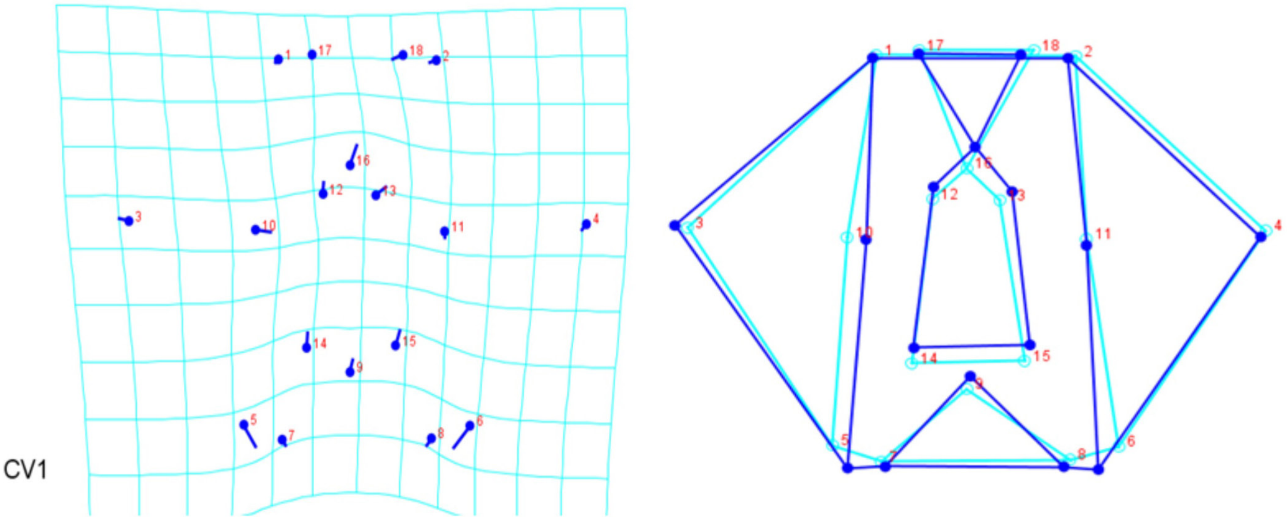

Fig. 4. Transformation Grid (left) and Wireframe (right) representation of shape variations between *Fannia* species based on Canonical Variate Analysis. *In the wireframe the turquoise outline characterizes the position of consensus landmarks, while the blue outline represents landmarks configurations.

can be gleaned from figure 8, these results are sufficient to differentiate between the sites where *F. pusio* was found. In the case of the individuals from the Region of Murcia and the Valencian Community, there is extensive overlap.

Further statistical analysis was applied for full validation (Table 4). They reflect statistical significance in shape difference between the Lisbon – Region of Murcia ($p < 0.01$), while cross-validation shows us a considerable percentage in all of them (> 60%), except between the Region of Murcia – Valencian Community.

Variations between a laboratory bred colony versus wild samples of *F. pusio*

Regarding the ecology of the species, some differences were observed; The PCA shows variation in the same landmarks as in the geographical comparison: 13, 10, 11, 9, 14, 15 and 12 (points of the parafacial area) that can be observed in the transformation grid and the wireframe (Fig. 2). However, the projection on the scatter diagram varies (Fig. 9).

On the other hand, in the CVA the landmarks

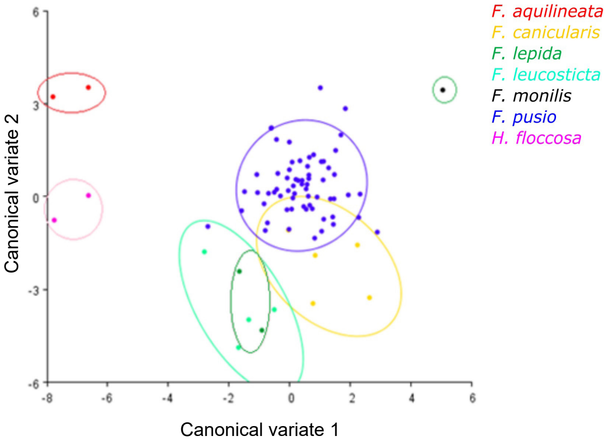

Fig. 5. Discrimination of *Fannia* species with a *Hydrotaea floccosa* as outgroup based on Canonical Variate Analysis.

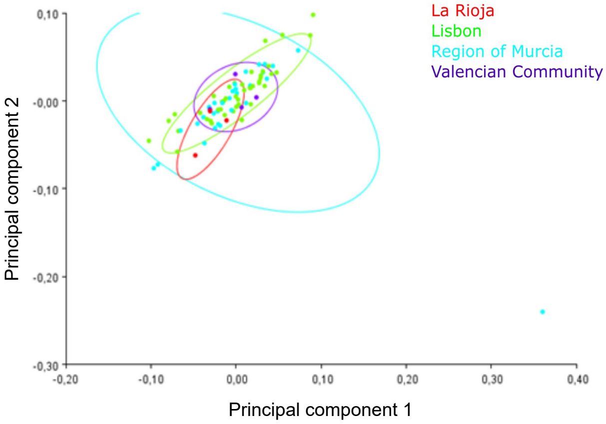

Fig. 6. Geographical differences among *Fannia pusio* populations based on Principal Component Analysis.

Table 3. Results of Mahalanobis distances (*p*-value) and Cross-validation (%) between different *Fannia* species

Species	Mahalanobis distance	Cross-validation
F. aequilineata – F. canicularis	*p*-value > 0.05	71.43%
F. aequilineata – H. floccose	*p*-value > 0.05	25%
F. aequilineata – F. lepida	*p*-value > 0.05	50%
F. aequilineata – F. leucosticta	*p*-value > 0.05	83.33%
F. aequilineata – F. monilis	*p*-value > 0.05	33.33%
F. aequilineata – F. pusio	*p*-value < 0.01**	97.01%
F. canicularis – H. floccosa	*p*-value > 0.05	57.14%
F. canicularis – F. lepida	*p*-value > 0.05	57.14%
F. canicularis – F. leucosticta	*p*-value > 0.05	44.44%
F. canicularis – F. monilis	*p*-value > 0.05	50%
F. canicularis – F. pusio	*p*-value > 0.05	75.71%
H. floccosa – F. lepida	*p*-value > 0.05	50%
H. floccosa – F. leucosticta	*p*-value > 0.05	16.67%
H. floccosa – F. monilis	*p*-value > 0.05	33.33%
H. floccosa – F. pusio	*p*-value < 0.01**	94.03%
F. lepida – F. leucosticta	*p*-value > 0.05	16.67%
F. lepida – F. monilis	*p*-value > 0.05	33.33%
F. lepida – F. pusio	*p*-value < 0.05*	97.01%
F. leucosticta – F. monilis	*p*-value > 0.05	80%
F. leucosticta – F. pusio	*p*-value > 0.05	84.06%
F. monilis – F. pusio	*p*-value > 0.05	98.48%

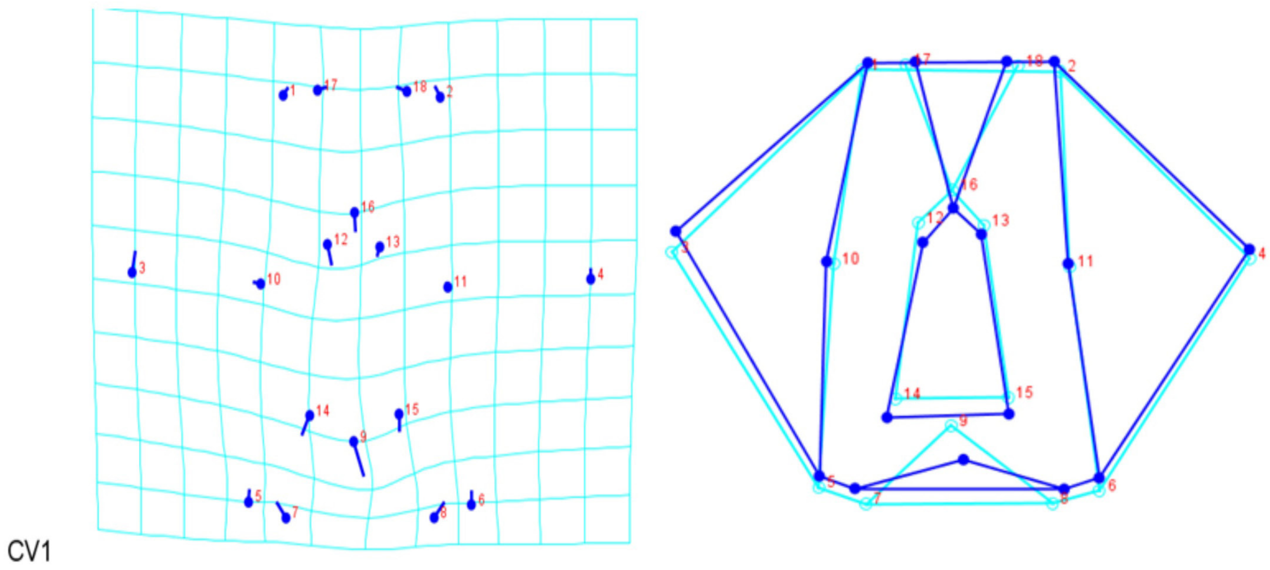

Fig. 7. Transformation Grid (left) and Wireframe (right) representation of shape variations among *Fannia pusio* populations in terms of geographical distribution based on Canonical Variate Analysis. *In the wireframe the turquoise outline characterizes the position of consensus landmarks, while the blue outline represents landmarks configurations.

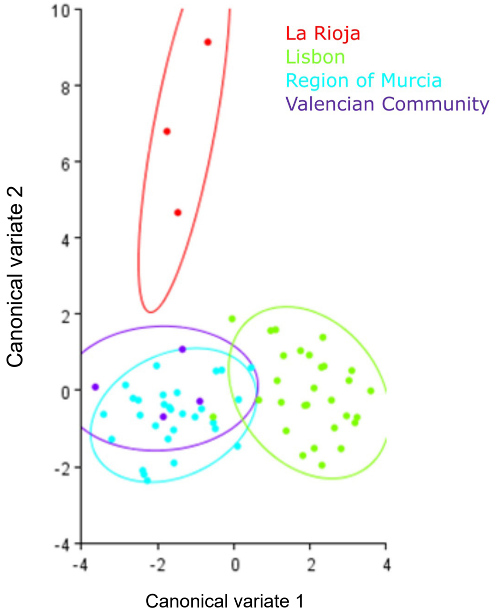

Fig. 8. Geographical differences among *Fannia pusio* populations based on Canonical Variate Analysis.

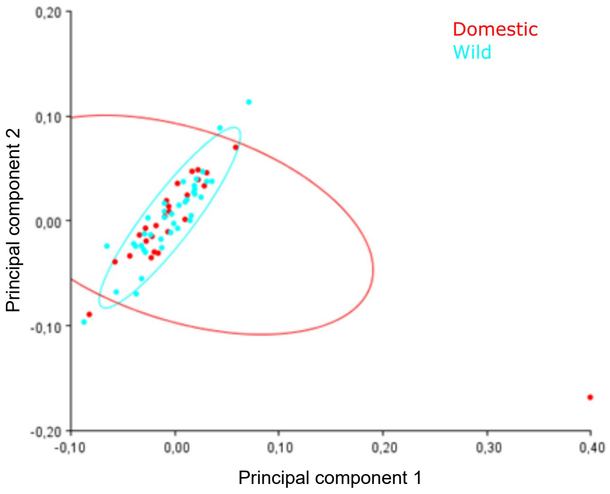

Fig. 9. Variation between a laboratory bred colony *versus* wild sample of *Fannia pusio* based on Principal Component Analysis.

Table 4. Results of the Mahalanobis distances (*p*-value) and Cross-validation (%) among *F. pusio* populations in terms of geographical distribution

	Lisbon La Rioja	Lisbon R. Murcia	Lisbon Valencian C.	La Rioja R. Murcia	La Rioja Valencian C.	R. Murcia Valencian C.
Mahalanobis	$p > 0.05$	$p < 0.01$**	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Cross-validation	71.86%	69.49%	62.5%	78.79%	66.67%	39.39%

present another order of variation: 9, 10, 15 and 13, also from the parafacial area, as shown in figure 10. Figure 11 shows the alterations that exist between the subspace projections of individual landmark configurations according to environmental and life-history differences.

Furthermore, in the same manner as with the previous analyses, statistical testing was performed. Mahalanobis distances returned a very high statistical significance ($p < 0.01$) and cross-validation with a considerably high percentage (72.3%).

DISCUSSION

The present work provides a case study of the application of GM on the species *F. pusio*, and alleviates the scarcity of information in the scientific literature on this important Dipteran family (Szpila et al. 2019). The samples used were collected all throughout the Iberian Peninsula.

Despite the fact that the wings are the Diptera structure from which the most taxonomic information regarding morphometric applications has been obtained (Grzywacz et al. 2017), other structures may also hold promise, and in this work, we assess the usefulness of the head (De Souza et al. 2015; Fuentes-López 2018). We reported for the first time the application of GM to aid in the differentiation of *Fannia* species morphology and assess the intraspecific variability of *F. pusio* from head landmarks, focusing on the parafacial area.

As mentioned above, most *Fannia* are morphologically very similar and GM may deliver an important tool for defining some ambiguously or hither to unidentifiable specimens (Dobigny et al. 2002). The possibility to identify them with GM suggests that shape is more relevant than size (Sumruayphol and Chaiphongpachara 2019). Results of our data analysis showed a clear differentiation among species, except for the pairs *F. pusio – F. canicularis and F. pusio – F. leucosticta* ($p > 0.05$). In the latter case, we know that the lack of differences between the species may also be associated with close phylogenetic relationship—within the same subgroup (pusio-group)—which is classified in higher canicularis-group described by Chilcott (Wang et al. 2016). However, members of the pair *F. pusio – F. canicularis* are phylogenetically separate taxa and yet offer no clearly differentiated results. It is possible that, in this case, the inability of the analysis to reflect differences between them could result from the low number of specimens of (N = 5) available in our sampling.

However, comparison between species belonging to different families, such as *H. floccosa* (Muscidae) – *F. pusio* (Fanniidae), even though we found a low number of the former (N = 2), allowed us to demonstrate clear differentiation with a $p < 0.01$ and a reclassification with cross validation of 94.03%. We view the following alternative conclusions as the most likely: i) the structures and landmarks chosen are efficient for differentiating at the family level (De Souza et al. 2015), but not as good at the genus level. Alternatively, ii) it could be inferred that the species that show this overlap ($p > 0.05$) have a close phylogenetic relationship (Dos Santos et al. 2003) and therefore present smaller

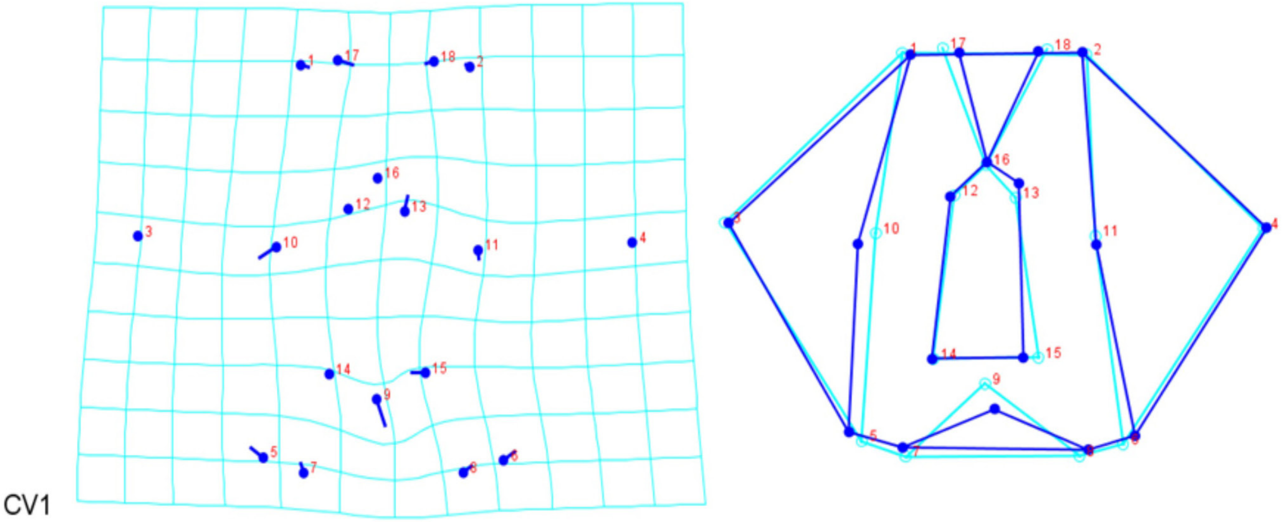

Fig. 10. Transformation Grid (left) and Wireframe (right) representation of shape variations between a laboratory bred colony *versus* wild samples of *Fannia pusio* based on Canonical Variate Analysis. *In the wireframe the turquoise outline characterizes the position of consensus landmarks, while the blue outline represents landmarks configurations.

measurable morphometric differences.

The *Fannia* species from which the greatest number of samples could be analyzed in this work was *F. pusio*. The sampling was carried out from June to September (Table 1), with the bulk of specimens collected in September. This was been observed in the samplings carried out in other studies (De Carvalho et al. 2003; Carles-Tolrá 2006; Grzywacz and Prado e Castro 2012; Monteiro et al. 2014), confirming that this *Fannia* species predominates in autumn (Smith 1986; Bélo et al. 1998). Based on the samples available, we focused on intraspecific variations according to geographic location and differences in lifestyle, comparing wild caught samples to a laboratory population.

These two approaches analyzed through GM will help better understand the variability that this species carries (Webster and Sheets 2017). This goal has medical, economic and forensic importance. First, *F. pusio* is a vector of myasis in humans and cattle; furthermore, it is a useful indicator species in forensic entomology, since it is known to be present in animal remains and human corpses (Grzywacz et al. 2017; Szpila et al. 2019). Furthermore, Nuñez-Rodriguez and Liria (2017) already observed that GM provides the means to differentiate ecological conditions and geographic range in forensic entomology, thus helping in criminal investigations.

Regarding the geographic range of *F. pusio*, different phenomena are observed. First, the specimens collected in the Valencian Community and the Region of Murcia do not show GM differentiation ($p > 0.05$; cross-validation of 39.39%) apart from indicating overlap (Fig. 8). The explanation could be the small sample size obtained in the Valencian Community; however, a low number of samples was also obtained in La Rioja and the analysis does in this case show a great difference to the rest of the locations. Therefore, we understand that there is a close relationship between the samples of the Valencian Community and the Region of Murcia, which is plausible since they are geographically closely located in the East of the Iberian Peninsula where environmental conditions are very similar.

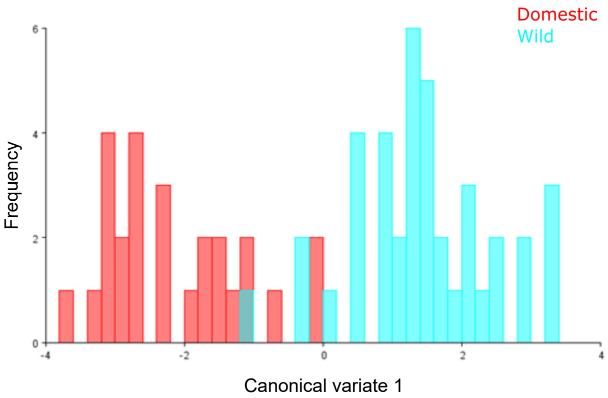

Fig. 11. Geometric morphometrics variation of *Fannia pusio* head landmarks based on Canonical Variable Analysis. Individuals from the colony reared under laboratory conditions are compared against wild-caught individuals.

Consequently, the morpho-geometric differentiation along the observed Iberian locations ranges among the Northeast (La Rioja), Southeast (Valencian Community + Region of Murcia) and Southwest (Lisbon), giving a cross validation > 60% among all (Table 3). Nevertheless, at statistically significant difference is found between Lisbon - Murcia Region with a p-value in the Mahalanobis distance < 0.01 (Table 4). Intraspecific differentiation is a clear example of how abiotic factors affect individual development (Pacheco et al. 2017; Sumruayphol and Chaiphongpachara 2019); and GM represents the best option to analyze population segregation (Mikery et al. 2019).

From these data it can be inferred that the environmental conditions shaped by the Atlantic Ocean in Lisbon and the Mediterranean Sea in the Region of Murcia may result in morpho-geometric changes to the species. The areas bordering the Atlantic are colder and less humid, while the Mediterranean areas are much warmer and wetter. These climatic variations provide morphometric alterations among populations of the same species (Hajd et al. 2014; Espra et al. 2015; Fuentes-López 2018).

Regarding the comparison between samples of lab-raised *F. pusio* under constant conditions and those collected in the wild, we see that there is no overlap between the domestic and the wild samples (Fig. 11). This is a fairly noticeable component within the same species, with statistically significant differences among individuals' flies ($p < 0.01$; cross validation > 70%) (Table 4), indicating that environmental fluctuations also affect the morphology of the species. Although all samples belong to the same species, the variation in shape could be affected by environmental conditions instead of genetic drift and evolutionary divergences (Arias et al. 2017). Another aspect to take into account is the high inbreeding of the colony since it is a 165th generation. Alternatively, the fact that domestic individuals are raised ad libitum and with the absence of predators might provide advantageous conditions for their development (Riaño et al. 2008).

In the different analyzes carried out in this paper, all the results show the variation in the parafacial and fronto-orbital zones. As previously mentioned, the landmarks fixed at the apices and base of the antennaes study the differences between them and not with the rest of the structure of the head. This should be clear since they are two mobile organs and can change their position. These results differ from those obtained by Godoy et al. (2018) where it shows differences in landmarks fixed in the contour of the head instead of in internal areas.

It should be noted that this work is innovative, with a relatively scarce bibliographical background to draw form, especially regarding the Fanniidae. As mentioned by Szpila et al. (2019), more studies should be performed on the Fanniidae using GM to improve our knowledge of the family. This study has provided some proof that previously unused structures, such as the head, are useful to discriminate between species and even to find differences within the same species, along geographic and ecological axes of variation (Garzón and Schweigmann 2018).

CONCLUSIONS

In conclusion, the analyses carried out in the present work using GM showed that head landmarks provide valid and significant morphological information, with the most variable structures in all the analyzes performed being found along the parafacial and fronto-orbital zones.

Fannia pusio has only recently been introduced to Europe from South American; this has yielded a clear and rapid intraspecific differentiation with respect to the East and West of the Iberian Peninsula. This diversification could be attributable to varying environmental conditions. However, the distinction from a laboratory-bred colony to a wild-caught sample of *F. pusio* may be due to both stable environmental conditions and the high inbreeding of the colony

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