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Ecology and morphological variations in wings of *Phlebotomus ariasi* (Diptera: Psychodidae) in the region of Roquedur (Gard, France): a geometric morphometrics approach

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Abstract

Background: *Phlebotomus ariasi* Tonnoir, 1921, is the predominant sand fly species in the Cevennes region and a proven vector of *Leishmania infantum*, which is the main pathogen of visceral and canine leishmaniasis in the south of France. Even if this species is widely present in Western Mediterranean countries, its biology and ecology remain poorly known. The main goals of this work are to investigate the phenotypic variation of *P. ariasi* at a local scale in a region characterized by climatic and environmental fluctuations, and to determine if slope and altitude could affect the sand fly phenotypes.

Results: Sand flies were captured along a 14 km-long transect in 2011 from May to October. At the same time, environmental data such as altitude and slope were also collected. Morphological analysis of *P. ariasi* wings was performed by a geometric morphometrics approach. We found morphological variation among local populations of *P. ariasi*. Strong shape and size variations were observed in the course of the season (particularly in June and July) for both genders. During June, we highlighted differences in wing phenotypes according to altitude for both sexes and to slope and station for females.

Conclusions: The phenotypic variations observed in *P. ariasi* along the studied transect indicated these populations are subjected to environmental pressures. Nevertheless, it seems that sand flies are more sensitive to extrinsic factors in June and July, suggesting a phenotypic plasticity.

Keywords: Sand fly, Southern France, Geometric morphometry, *Phlebotomus ariasi*, Phenotypic plasticity

Background

Phlebotomus ariasi T , 1921 e ed a a d ec e e Ce e e e [1] a d e e e ec e a a ca ed b *Leishmania infantum* e F a ce, *Phlebotomus perniciosus* Ne ead, 1911 [2]. T e e e *P. ariasi* a e d e , a a ed , ca e a d e a ("ba baca e"), ea ad a e. T ec e ac e d d a d , e e d e e e a e e , ab da e - ba a d a e e a d e e c e a a d d e c a a a [3]. T ec e de e e We e Med e a ea c e [4–6].

C e , b a d ec *P. ariasi* e a . D e a 10 ea , e e e e ce e-e e e ce e a a a c ea ed F a ce [7]. T e c e e a e d b d ea e de e e eed

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c ea e ed e ec . H e e, e de a e bee e ed de a d e a- c e ec e a d e e e ba ed e a a c c a d ca b [8], e e [9], a d a ed c DNA [10], e e ce e c d a c c e *b* e e [5] a d c a- e e a e [11]. U , d a e e- c e c a ac a bee d e e a e e c a a *P. ariasi* F a ce. S ca e c a e a a e e d e a d ec e a - a a Med e a ea e , c e a d a c e e ece , c a *P. papatasi* [12, 13] a d *P. sergenti* [14, 15]. T e e de ed e e e ce ca e c a a ed e e a ac .

T e a a e e e a e e c a a *P. ariasi* a c ec ed a a ca ca e a e c a ac e ed b c a c a d e e a c a , a d de e e e a d a de c d a ec a d e e .

Methods

Study area

T e ed d a e ed e F a ce, e "e a de 'O e e e" ca ed be ee a e : "H a " (Ga e , H a) a d "A e" (Le V a , Ga d). Sa d e e e c ec ed a a 14 a ec be ee Sa J e de a N a d e V a , c d R ed -e-Ha (a 601 ab e ea e e) (F . 1). T e a e e e ec ed a a - ec (Tab e 1 a d F . 1). T e b ec e Med e a ea b- d c a e [16] a d c a ac e- ed b e e e ce "Ga e" ec e c a *Quercus ilex* a d *Quercus pubescens*.

T e a ca a ea be a e de c a ea a a d ca e e a a ca ed b *L. infantum* [1]. Va d e c a a a a e e a a d e e e e e e a ec , c a c c e , ee , d c , ee e, e , abb , ca a d d . F e e, e a e e ca ed e - a a ea a d a d a a c d be a a d e [17].

T e e ec e e a ac e a d e a d a e, e a e e a ed acc d e e a d a de. T e e, a- e e ed e : S ea (SE) a d N e (NW), a d e a d a : 0 (100–200), 1 (200–300), 2 (300–400), 3 (400–500), 4 (> 500) (Tab e 2).

Sand fly collection and identification

Sa d c ec e e ed be ee Ma a d N e be 2011 CDC a e a (J W. H c C . FL, USA) a d e ce - a (20 20 c e a e c e ca)

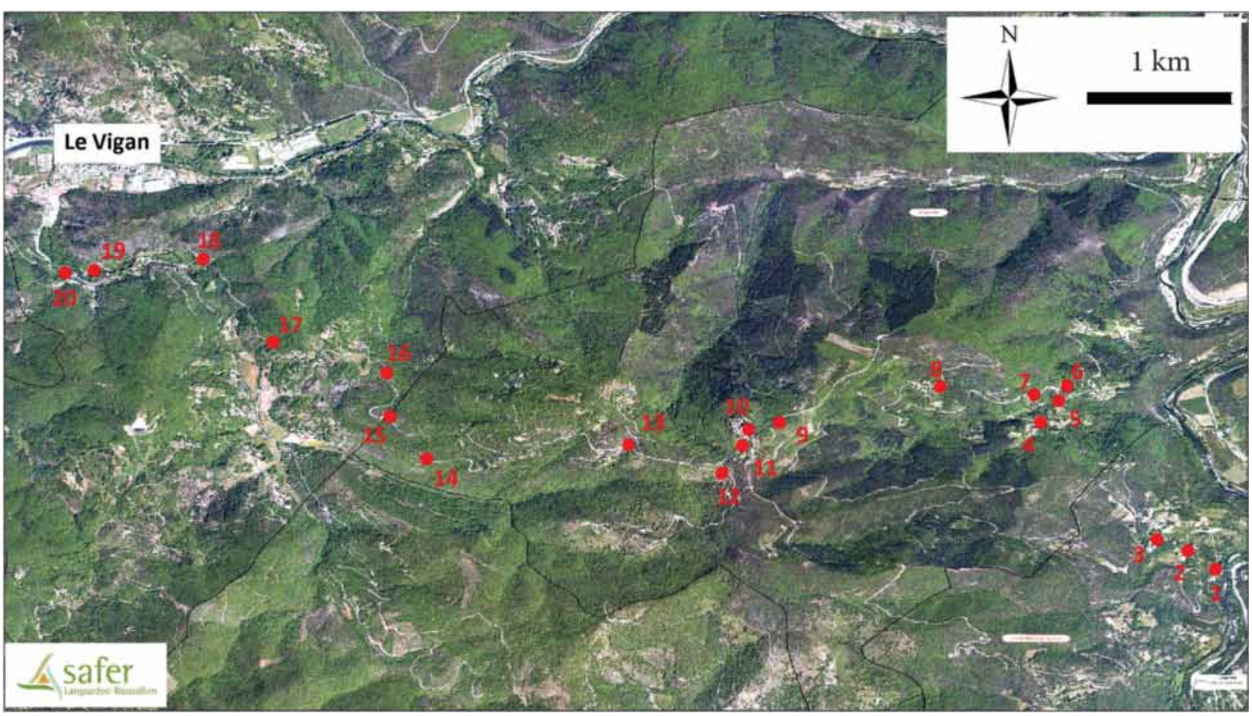

Fig. 1 Map of the study area showing the main sites sampled. Red circles indicate the sampling localities

Table 1 Sampling stations in the study area

Station number	Coordinates		Altitude (m)	Biotope	Traps
	North	East			
ST01	43.96548	3.686828	175	Hamlet	CDC + ST
ST02	43.96663	3.685075	228	Hamlet	ST
ST03	43.96733	3.683149	244	Hamlet	CDC + ST
ST04	43.97462	3.675871	321	Hamlet/Hutch	CDC + ST
ST05	43.97595	3.677038	322	Rural	ST
ST06	43.97687	3.677551	341	Kennel	CDC + ST
ST07	43.97632	3.675457	354	Hamlet	CDC + ST
ST08	43.97683	3.669611	443	Hamlet	ST
ST09	43.9746	3.659549	586	Poultry	CDC
ST10	43.97416	3.657616	606	Poultry	CDC
ST11	43.97317	3.657214	603	Hamlet	CDC + ST
ST12	43.97144	3.655944	573	Rural	ST
ST13	43.97321	3.650088	539	Rural	CDC + ST
ST14	43.97235	3.637441	417	Rural	ST
ST15	43.97495	3.635153	397	Rural	CDC + ST
ST16	43.97765	3.634949	362	Rural	ST
ST17	43.97961	3.627845	343	Rural	CDC + ST
ST18	43.98478	3.623463	282	Rural	CDC + ST
ST19	43.98409	3.616661	255	Hamlet	CDC + ST
ST20	43.98392	3.614822	245	Hamlet/Sheep barn	CDC + ST

CDC CDC miniature light traps, *ST* Sticky Traps

[18]. A a ec, 20 a e e e ec ed. I 14 a e (Tab e 1), e a e e e (de a d/ de e, a a ba , e c.), e a be ee 18:00 a d 08:00 a . A a 105 a e e e d 210 a . I 17 a (Tab e 1), a a 3,589 c a e e ed a d a ea 189 c a b a e e aced a b e, de a d a d a d e a d a a , c e e e e a a d c e ce e a . T e c a e e e ed e a e 2 c - ec e da .

A e e d e a e d, c ec ed ec e e e a e ed d d a 1.5 E e d be 96% e a a d abe ed acc d . P , ead , e a a a d e a d e e e e ed. T e ead a d e a a e e c ea ed Ma c-A d (c a d a e/ace c ac d) a d ed c a [3]. S ec e de ca a d d a e ed ba ed e e a e a d/ e a e e a a e a e e a- ecae, e e Ab e c [3], Le [6] a d K c -Ke d c e a . [19]. F e de ca , e e ec ed e e *P. ariasi* ec e , c e e e a a e.

Wing preparation

A a 374 ec e *P. ariasi* e e ed e e e c e c a a (186 a e a d 188 e a e) (Tab e 2). R e a . [20] e e de e a a b a d a e b a be ee e de . I d , ec e e e ed. T e e e a ed e e d e de c bed P d e e a . [13] a d e , ed E a a abe ed de . T e de e e a ed a Le ca Z16 APOA e e c c d ec- c c e DFC 425 d a ca e a e , d ed, a d a c ed.

Morphometric analysis

P c e e e e e ed -U 1.60 [21]. T e , 16 a d a e e ed e a a e e d R e a . [20] DIG2 2.18 a e [22] (F . 2). La d a a e ca ed a e e ec e e a a d a e e ec- c e a e (F . 2). T e e c a a e a e a a ca e e e ed a d e e CLIC ac a e [23]. T e ce d e e e a a ed a a e e a a a a e c W c -Ma -W e e K a -Wa e ed b a *post-hoc* e Ma -W e e B e c ec , e a ca ac a e R, e 3.1.2 [24]. Ce d e e a e e a ed d a ce a e a d a e ce d, .e. e a e e e a a ce e a d a ab a ce d - a d - d ec [25]. T e a d a c a e e ca ed, a a ed, a d a ed a a e c e c a b e GLS P c - e e e d [25–28] de d ce a e a abe (a a a , PW). T e c a c e (ba ed e a a a) [25] e e ed c a e a a e . T a e e de ee a be ee a , a e Ma a a b d a ce be ee a e e ca c a ed CLIC a e [23] a d e ed b a a e c e - a e 1,000 e a eac . T e e d a ce e e a ed e a e ec a ca e eac d d a . T e e ce a e c ec a ed d d a e c e d a a e ed. F a , e d a a e (c b e a e) a e a ed b a a e e e PW e.

Results

Sexual dimorphism

T e a e ed a ca ca d e - e ce be ee a e a d e a e (F . 3). Ma a a b d a ce e e ca d e e be ee b e e

Table 2 Number of *Phlebotomus ariasi* wings used by station for the geometric morphometric analysis

Station	Slope	Altitude groups	Wing number by month											
			May		June		July		August		September		Total	
			♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
ST01	SE	0[a]	0	0	0	0	0	0	0	0	0	0	0	0
ST02	SE	0	0	0	0	0	0	0	0	0	0	0	0	0
ST03	SE	1[b]	0	0	4	4	2	6	0	0	0	0	6	10
ST04	SE	2[c]	0	0	1	14	6	0	0	0	0	0	7	14
ST05	SE	2	0	0	8	0	0	0	0	0	0	0	8	0
ST06	SE	2	0	0	6	14	4	16	8	0	0	1	18	31
ST07	SE	3[d]	0	0	0	0	0	0	0	0	0	0	0	0
ST08	SE	4[e]	0	0	0	0	0	0	0	0	0	0	0	0
ST09	SE	4	0	0	9	9	2	5	0	0	0	0	11	14
ST10	SE	4	0	0	0	0	0	2	0	1	0	0	0	3
ST11	NW	4	1	0	9	23	3	19	5	2	3	0	21	44
ST12	NW	4	4	0	6	3	0	0	0	0	0	0	10	3
ST13	NW	4	0	0	7	0	0	1	1	1	4	1	12	3
ST14	NW	3	3	0	12	0	2	0	0	0	2	0	19	0
ST15	NW	2	0	0	5	0	2	0	1	0	0	0	8	0
ST16	NW	2	1	0	12	0	0	0	0	0	0	0	13	0
ST17	NW	2	0	0	0	2	0	4	0	0	0	0	0	6
ST18	NW	1	0	0	8	4	5	9	2	0	1	0	16	13
ST19	NW	1	0	0	0	4	8	4	0	0	0	0	8	8
ST20	NW	1	0	0	27	32	2	7	0	0	0	0	29	39
Total			9	0	114	109	36	73	17	4	10	2	186	188

Abbreviations: *SE* southeast, *NW* northwest, ♂ male, ♀ female
[a]100–200 m altitude
[b]200–300 m altitude
[c]300–400 m altitude
[d]400–500 m altitude
[e] > 500 m altitude

(Ad ed *P*- a e < 0.0001). M e e, e ec a - ca c e e e e e e e a 96% a e a d 93% e a e, e b e ed d e e - a be ee e . Ce d e e e ed a a ea e e e a e d e ce a a . T e e ed e e de a d be ca d e e (K a-Wa e : χ^2 = 253.4024, df = 1, P < 0.0001), e a e d a a e a a e (F . 4). T e c b e a e d e e a a 72% (F . 5).

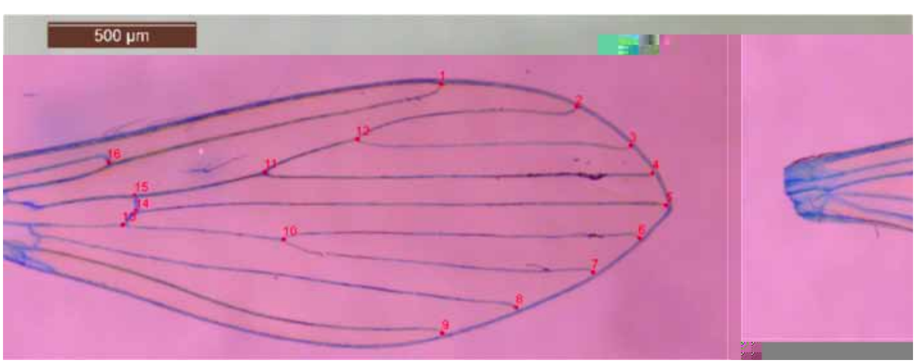

Fig. 2 Location of the 16 wing landmarks used in the morphometric analysis of *Phlebotomus ariasi*

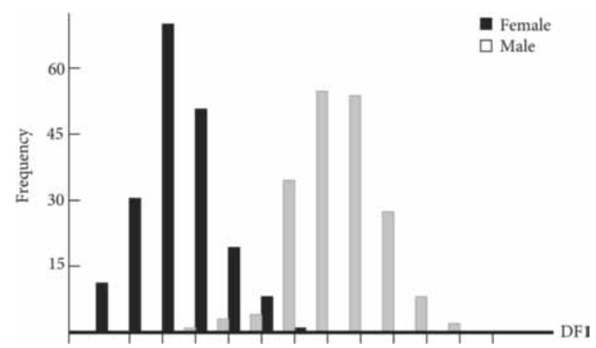

Fig. 3 Distribution of individuals along the first discriminant factor (DF1) of shape analysis by gender

S ce a e c d e e ce be ee a e a d e- a e a b e ed, e b e e a a e e e e- ed e a a e eac e .

Differentiation by month

Ma a a b d a ce , ed e a e a e a a , e e ca d e e be ee e ; be ee J e-J e a e (A ed *P*- a e < 0.0001) a d be ee J e-J a d J -A a e (A ed *P*- a e < 0.05 a e B e c ec- ; e c e , 85.53% a a e a a ce) (F . 6). F e a e , a b e e a e d e e a e e d e e a be ec e (Tab e 2). I deed, e d c - a a a e , e a e a ac ; e be c a c e d e a e a e a a ce e da a e . M e e , e ec a ca c e e e e a e a e e a 73.6% a e (58–88%) a d 81% e a e (80–82%), e d e e a b e ed be ee a e .

C ce e, a K a -Wa e e- ea ed a ca e ec e ce d e ($\chi^2 = 11.6239$, $df = 4$, $P = 0.02038$) e a e . T e *post-hoc* e ed ca d e e ce

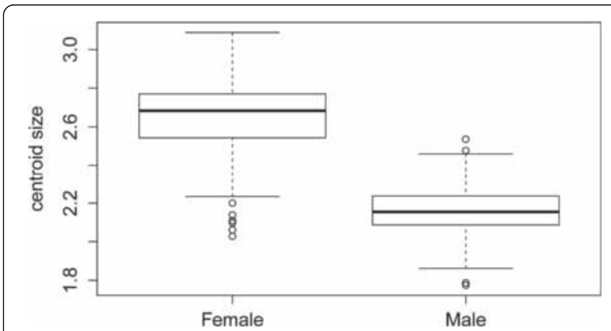

Fig. 4 Mean, standard deviation and error of centroid wing sizes for each sex

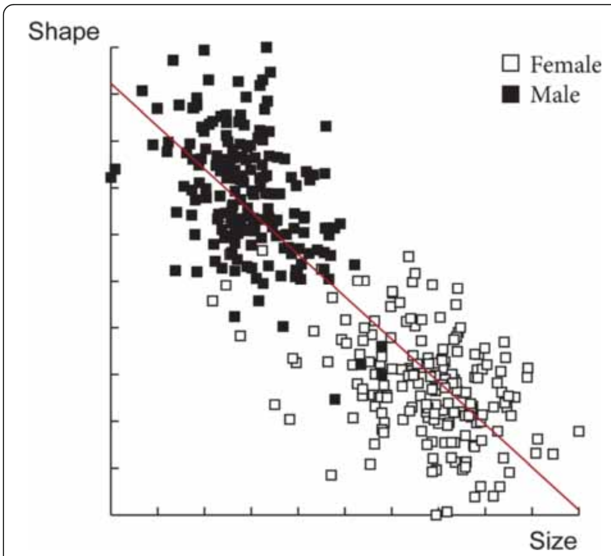

Fig. 5 Regression of first discriminant factor on centroid size. Vertical axis: first discriminant factor, representing 100% of the total discrimination; Horizontal axis: centroid size of the wing. The analysis was based on the partial warps. Regression line is shown

Fig. 6 Distribution of the individuals along the discriminant factors of shape analysis in June and July. **a** Distribution of the female individuals along the discriminant factor. **b** Distribution of the male individuals along the first two discriminant factors. The analysis was based on the partial warps. *Abbreviations*: CV1, discriminant factor 1; CV2, discriminant factor 2 be ee J e a d J ($P = 0.03741$), be ee J e a d Se e be ($P = 0.02588$) a d be ee J a d Se e be ($P = 0.03678$) (F . 7a). F a e , e dffe e ce be ee a a ce a ed ($\chi^2 = 8.5591$, $df = 4$, $P = 0.07312$) (F . 7b). T e c b f e a e dffe e a a 0% f fe a e a d f a e 43% a d 2% (D c a fac 1 a d 2, e ec e) (F . 8).

T e e e ed a e c c be ee , a c a be ee J e a d J f b e e . S ce a e c dffe e ce be ee e e a a b e ed, e f a a e e f ed de e e e b e ffec f e, a de a d a e e ea ed f fe a e a d a e J e a d J e a a e . I a be e f e e d e a a be f ec - e (Tab e 2).

Differentiation by slope

A de a ed ab e de e e e b e ffec f e *P. ariasi* e e, e e f ed a a c de eac e a d e , J e a d J , e a a e (ee Tab e 2).

Ma a a b d a ce d a e e e f ca dffe e be ee e e (A ed P- a e < 0.01) f fe a e J e (F . 9). M e- e , e ec a f ca c e e e e e a e a e e a 95% (80–90%), e b e ed dffe e a be ee a e . H e e , f ca dffe e ce a b e ed be ee e e f e fe a e J (A ed P- a e > 0.05) a d f e a e f a (A ed P- a e > 0.05; 13 c - e , 96.5% f a a e a a ce) (da a).

F e e, e K a -Wa e d e e ea ed a f ca ffec f e J e a d J ($\chi^2 = 1.0064$, $df = 1$, $P = 0.3158$; $\chi^2 = 0.4636$, $df = 1$, $P = 0.4959$, e ec e) f fe a e a d a f a e ($\chi^2 = 0.4915$, $df = 1$, $P = 0.4833$; $\chi^2 = 0.6771$, $df = 1$, $P = 0.4106$) (da a).

T e c b f e a e dffe e a a 0% f fe a e f b a d 0 a d 32% f a e J e a d J , e ec e (F . 10).

Differentiation by altitude

T e ffec f a de *P. ariasi* e e a e ed f fe a e a d a e J e a d J e a a e (ee Tab e 2). I J e, Ma a a b d a ce ed d a e e e f ca dffe e be ee e a d a 1 a d 2 f fe a e (A ed P- a e $<$ 0.01667) a d be ee 3 a d a e e f a e (A ed P- a e < 0.00833, 11 c e , 93.2% f a a e a a ce) (F . 11). M e e , e ec a f ca c e e a d a e e a e a e e a 73% (65–80%) f fe a e a d 53% (43–75%) f a e , e b e ed dffe - e a be ee a e . H e e , J , f - ca dffe e ce a b e ed be ee e f fe a e a d a e (A ed P- a e > 0.01667, 19 c - e , 99.2% f a a e a a ce a d A ed P- a e > 0.01667, 4 c e , 71.3% f a a e a - a ce, e ec e) (da a). T ea e e a a- e a e J , e 4 ad be e ed beca e f a be f ec e ($n = 2$).

C ce e, J e, a f ca ffec f a de ce d e a b e ed f fe a e ($\chi^2 = 7.0282$, $df = 2$, $P = 0.0298$), e *post-hoc* e

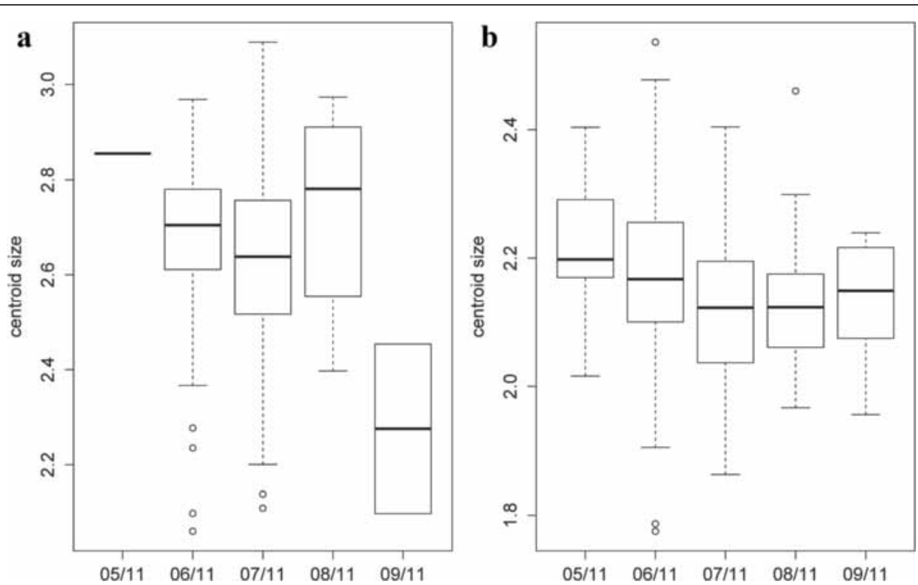

Fig. 7 Mean, standard deviation and error of centroid wing sizes by month. **a** Females. **b** Males

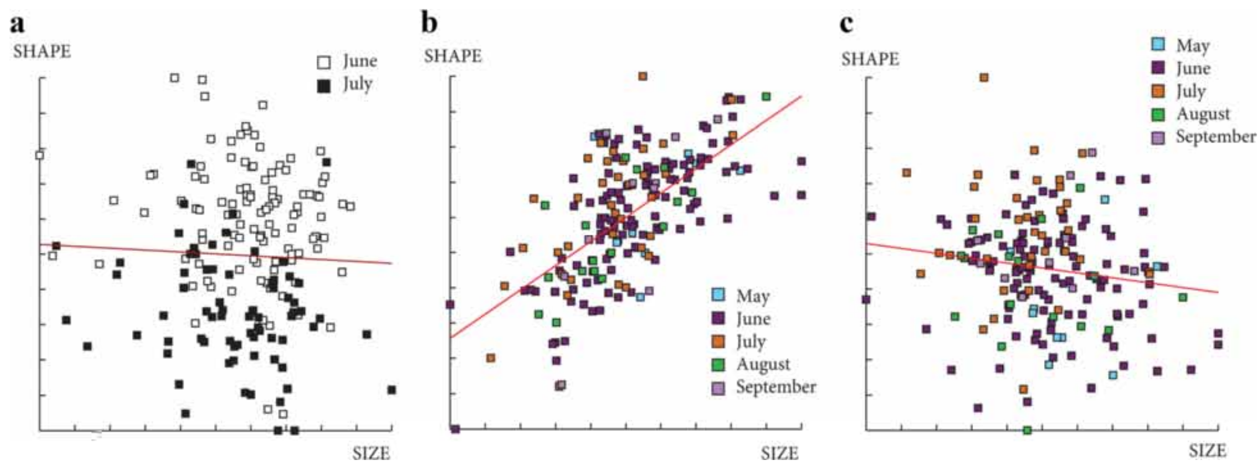

Fig. 8 Regression of the discriminant factors on centroid size by months. **a** Regression of the first discriminant factor for females in June and July. **b**, **c** Regression of the two first discriminant factors for males by month. Horizontal axis: centroid size of the wing; Vertical axis (**a**): discriminant factor 1, representing 100% of the total discrimination; Vertical axis (**b** and **c**): discriminant factors 1 and 2, representing 24% and 17% of the total discrimination, respectively. This regression was based on the partial warps. Regression line is shown. Squares indicate individual sand flies

ed fi ca d ffe e ce be ee 1 a d 2 (P = 0.022). F a e , e d ffe e ce a fi ca be ee 1 a d 3 (χ^2 = 12.4331, df = 3, P = 0.006) a P- a e fi 0.0076 fi e *post-hoc* e (F . 12). C e e , fi ca e ffec fi a - de a fi d e ce d e J fi b e e (χ^2 = 4.9144, df = 2, P = 0.0857; χ^2 = 0.4848, df = 3, P = 0.9222, fi fe a e a d a e e ec e) (da a).

T e c b fi e a e d ffe e a a 7 a d 0% fi fe a e a d 48 a d 1% fi a e J e (F . 13) a d 37 a d 14% fi fe a e a d 36 a d 0% fi a e J (da a).

Phenotypic differentiation by station

I de b e e a b e ffec fi a e e, e e a a a e e e ea ed b e J e a d J . D e be fi d d a ce a a , e e fi ed a e a fi d c a a a e c de fi a e a a d e e c d a a be fi d d a de - c ea e e be fi c a c e a d e a a ce e a ed (Tab e 3).

F fe a e J e, Ma a b d a ce e e fi ca d ffe e be ee a fi e fi a a- ba ed e 3 c a c e c ded (da a), b fi ca be ee ST19 a d ST20 fi e ec d a a ba ed 8 c a c e (Tab e 4 a d F . 14). I J , e e fi Ca ca a a e a a (CVA) ed e b a (da a), Ma a b d a ce e e fi - ca d ffe e be ee a fi a a a e (Tab e 4). F a e , fi ca d ffe e ce a b e ed be ee a fi J e a d J (da a). T e e fi e a e a a e a d e c b fi e a e d ffe e a a e e ed Tab e 4.

K a -Wa e ed d e e- ea ed fi ca e ffec fi a ce d e J e (χ^2 = 12.4043, df = 9, P = 0.1915; χ^2 = 18.3754, df = 11, P = 0.07327) a d J (χ^2 = 12.5229, df = 8, P = 0.1294; χ^2 = 11.6, df = 9, P = 0.2368) fi fe a e a d a e , e ec e .

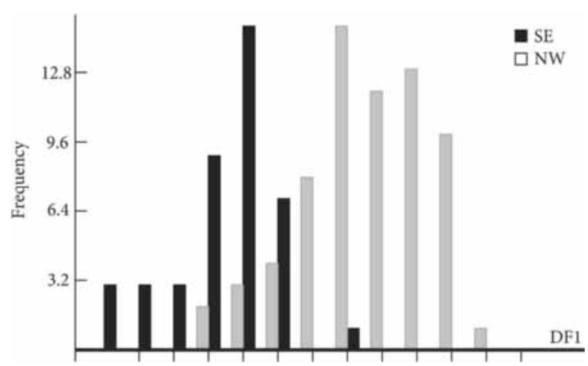

Fig. 9 Distribution of female individuals along the first discriminant factor of shape analysis according to slopes. This distribution was based on the partial warps in June. *Abbreviations*: SE, southeast slope; NW, northwest slope

Discussion

Sexual dimorphism

O e ed fi ca d ffe e ce a e a d e be ee a e a d fe a e ; fe a e a e a a e . Se a d de b e ed a ec c a *Mansonia* [29] a d *Aedes* e [30], *Anopheles*, *Culex* a d *Ochlerotatus* e [31], *Drosophila melanogaster* [32] a d *D. subobscura*

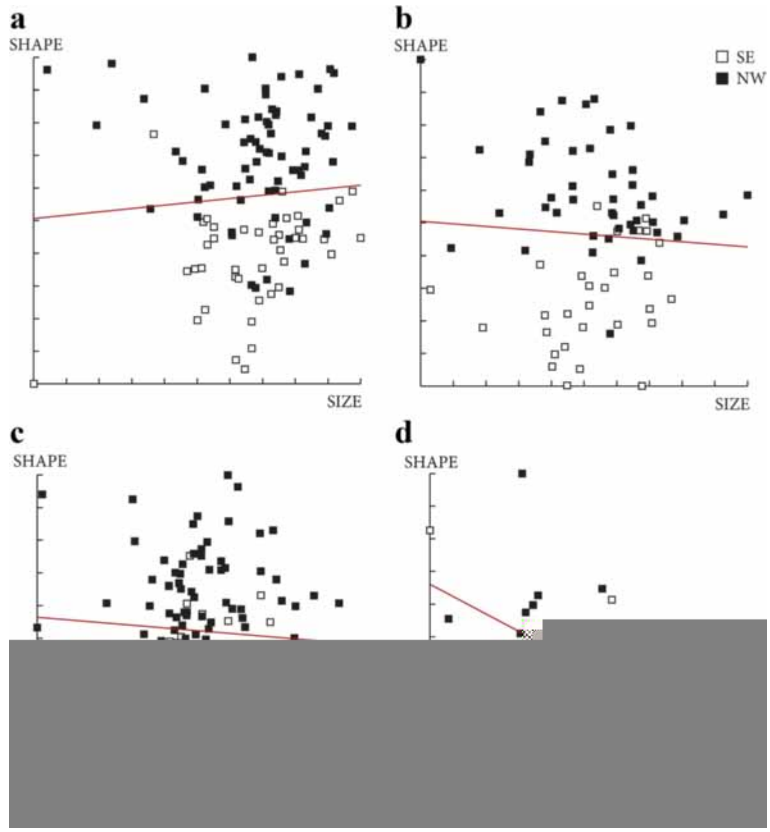

Fig. 10 Regression of first discriminant factor of shape analysis on centroid size from females (**a**, **b**) and males (**c**, **d**). Horizontal axis: centroid size of the wing; Vertical axis: first discriminant factor, representing 100% of the total discrimination. This regression was based on the partial warps in June (a, c) and July (b, d). Regression line is shown. Signs indicate each individual. *Abbreviations*: SE, southeast slope; NW, northwest slope

[33]. T - a e e de a a a e ed a eed a ea e ca ac c a ed a e . e de a a e e e ed a a T c d e a e d e e ce be ee e . e . T e ed a e ec A e d ba ed a a - e ea e- eca e a a e e a e ca ab [34]. e d ed a *P. ariasi* e a e e e a d e- Beca e b d ea ece , e e a e a d e ab e a 1 [35]. I d be

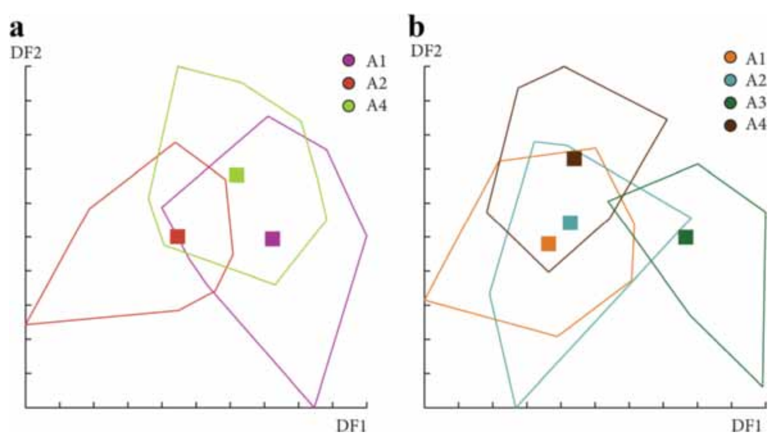

Fig. 11 Distribution of the individuals along the first two discriminant factors of shape analysis by altitude groups for females (**a**) and males (**b**). This distribution was based on the partial warps in June. Horizontal axis: discriminant factor 1; Vertical axis: discriminant factor 2. Altitude groups: 1 (200–300 m), 2 (300–400 m), 3 (400–500 m), 4 (> 500)

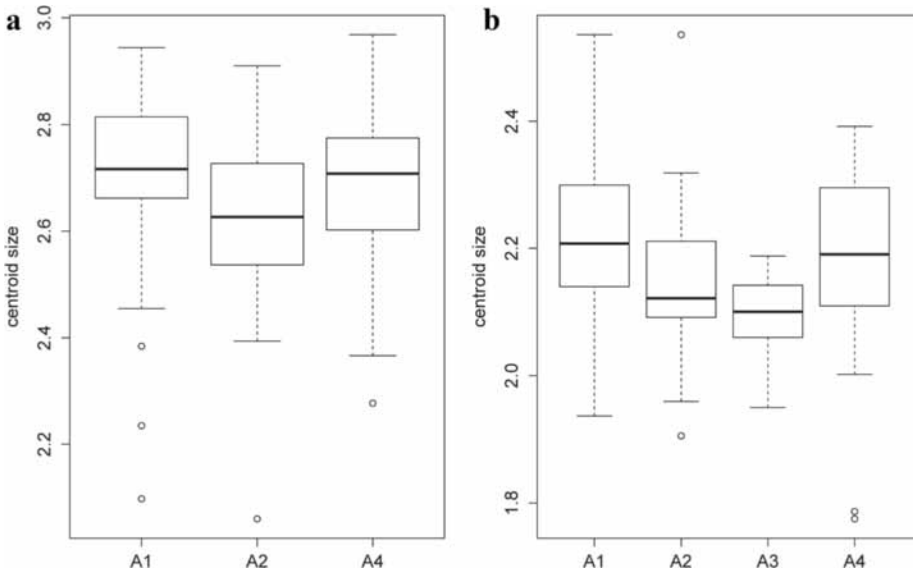

Fig. 12 Mean, standard deviation, and error of centroid wing sizes by altitude groups in June for females (**a**) and males (**b**). Altitude groups: 1 (200–300 m), 2 (300–400 m), 3 (400–500 m), 4 (> 500 m)

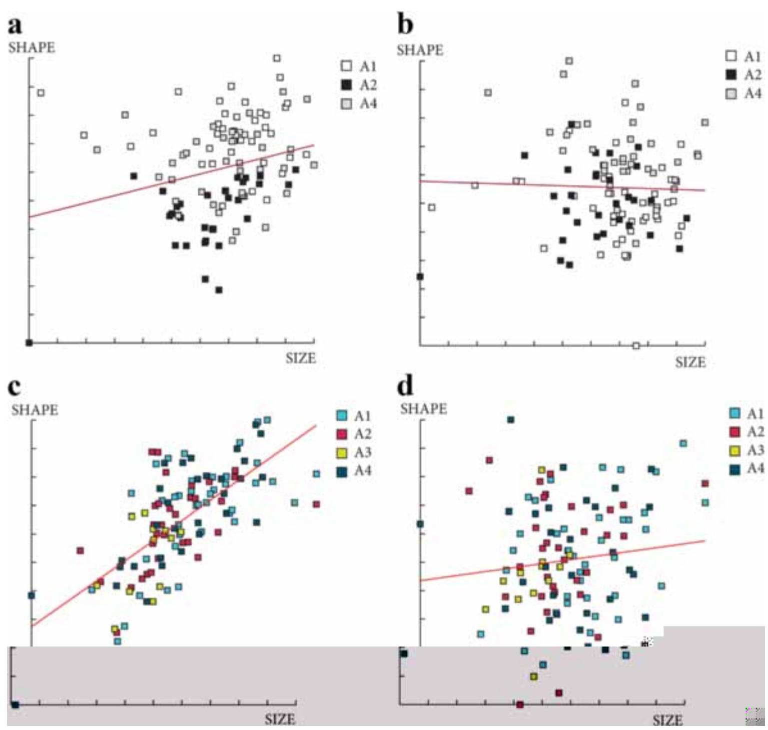

Fig. 13 Regression of first discriminant factor on centroid size by altitude groups in June for females (**a**, **b**) and males, (**c**, **d**). Horizontal axis: centroid size of the wing. Vertical axis: discriminant factor 1 (**a**, **c**) or discriminant factor 2 (**b**, **d**). The regression was based on the partial warps. Regression line is shown. Squares indicate individual sand flies. Altitude groups: 1 (200–300 m), 2 (300–400 m), 3 (400–500 m), 4 (> 500)

Table 3 Number of *Phlebotomus ariasi* wings used by station for the geometric morphometric analysis

	Females in June		Females in July			Males in June			Males in July		
NC	3	8	3	4	5	3	4	5	2	3	4
V (%)	59.29	87.67	55.32	64.88	70.04	54.91	63.67	71.24	54.65	67.69	75.66
ST03	4	–	6	6	6	4	–	–	–	–	–
ST04	14	14	–	–	–	–	–	–	6	6	6
ST05	–	–	–	–	–	8	8	8	4	4	–
ST06	14	14	16	16	16	6	6	6	–	–	–
ST09	9	9	5	5	–	9	9	9	–	–	–
ST10	–	–	–	–	–	–	–	–	–	–	–
ST11	23	23	19	19	19	9	9	9	3	–	–
ST12	–	–	–	–	–	6	6	6	–	–	–
ST13	–	–	–	–	–	7	7	7	–	–	–
ST14	–	–	–	–	–	12	12	12	–	–	–
ST15	–	–	–	–	–	5	5	–	–	–	–
ST16	–	–	–	–	–	12	12	12	–	–	–
ST17	–	–	4	–	–	–	–	–	–	–	–
ST18	4	–	9	9	9	8	8	8	5	5	5
ST19	4	–	4	–	–	–	–	–	8	8	8
ST20	32	32	7	7	7	27	27	27	–	–	–

Abbreviations: *NC* number of components included in the analysis, *ST* station, *V* percentage of shape variance explained by the components included in the analysis

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Wing phenotype differentiation by month

We b e ed a dffe e ce ff a e be ee J e a d J ff b e e a d a dffe e ce ff e ff ffe a e (a e J). I a be a e a

Table 4 Synthesis of the Mahalonobis distances results

Analysis	NC	V (%)	Ajusted *P*-value	Allometry[a] (%)	
				1	2
Females in June	3	59.29	> 0.00179	26	1
	8	87.67	< 0.00500[b]	25	22
Females in July	3	55.32	> 0.00179	38	13
	4	64.88	> 0.00333	41	15
	5	70.04	> 0.00500	33	23
Males in June	3	54.91	> 0.00076	48	1
	4	63.67	> 0.00091	49	0
	5	71.24	> 0.00111	48	1
Males in July	2	54.65	> 0.00500	36	0
	3	67.69	> 0.00833	35	1
	4	75.66	> 0.01667	26	8

Abbreviations: *NC* number of components included in the analysis, *V* percentage of shape variance explained by the components included in the analysis

[a]Percentage of size contribution to wing shape differentiation for the first component (1) and the second one (2)

[b]Significant effects

c c ff e e (Ma , A a d Se e be) d e e be ff d d a ca ed. T e e dffe e ce b e ed be ee J e a d J a be d e e e a a a a a b e ed e d [36]. T e d d a ca ed J e a d J a e ce a e e ed a e e d ff Ma a d J e, e ec e . Ma e ea e a d e a e d dffe e ce e e b e ed be ee e e 2 . M e e, e e ea e ff c a be ee a d da a e ea e Ma a J e J . E e ff e e ac c d a e e , e c a c a a e e ff e ce e e d a a e a d e e a d a e a ae de e e ff ec [37]. *Phlebotomus ariasi*, a ff e ec e ce , e e a e d ff d a a e (a e ff - a a a a e) de e e e e a d e e e d c ff a ab e c d [38]. T e c a c dffe e ce be ee , a e e d a d/ a e be ff e a d ff ea a be e b e ff dffe e ce e de e e ff e a ae be ff e a d/ a ff e e d a a e [39]. I deed, e dffe e ce a ae de e e c d ac e d a a a e a d/ ad e e.

Environmental factors and phenotypic variation in June

T e da a a a e e ea ed a e a d e dffe e ce b a de ff a e a d ff e a e J e e dffe e a e e a a d c a c e e acc d a de. P e de ed e ac ff a de a d

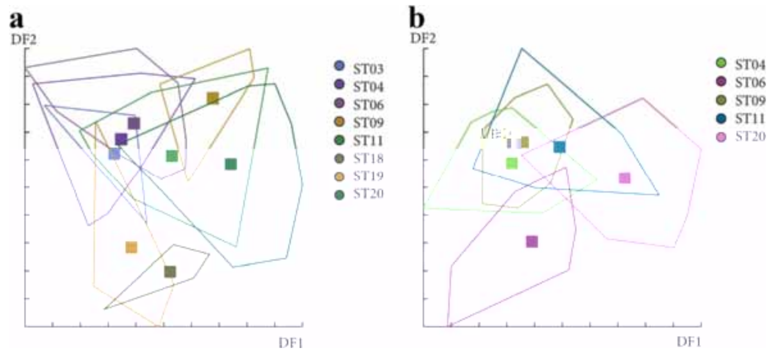

Fig. 14 Distribution of the individuals along the first two discriminant factors of shape analysis by station. The analysis was based on the partial warps on female individuals in June with 3 (**a**) and 8 components (**b**). Horizontal axis: discriminant factor 1; Vertical axis: discriminant factor 2

e ea e(c ea ed a a ee) a d b [12, 40, 41]. T e e ad a e e ced b e ea e, a e ad d a e ea e a d a e ad a e ea e [12]. I a a ae de e e e ced b e ea e c - d [12, 42]; a e ea e d ce a a d de- e e a d a d d a . A a e ec e e ec e , ce d e d e- d c a e d d a a e e ea e a d a e a de [43]. H e e, e e d e e ce b e ed b a d a d d ca a e a e a de . T c ea a be b e ed d beca e a a d a d e e ce a d e ea e c a be ee a e .

We a d a ca a a d e e a J e e a e b e a d a . T e ab e ce a a d e e a b e ed a e, c e e e a e c d be a c e e ce e e - ec c e ec e e c a e a a ab b d ea e a ed a . T e e a d- b e ed a e e e ce e- ec c e ec e e (ee ab e). Ne e e e , e ea d e e a a e a d e be ee e e a e bee e e ed [44].

Conclusions

I c c , d ed e c a a : a ca a P. *ariasi* d e e e : e a d , a e a d e a a be ee (J e a d J a c a) a d d e e e e acc d a de b e e a d b e a d a e a e .

T e de ee e c a a b e ed *P. ariasi* a ee ec e ca e - e a c a e c a c b a b c c a ac e c c e e a a e b ec ed. T e e da a de e e a c a d e ca ac ada a e e ec e e a a a c e e .

T e e a a a a e a ec e b *P. ariasi* e d e , e a d a a e c a *Leishmania*. I deed, e ec e e ece de a ed e ec c a e a be ee a e a d e d c - e de *Lysiphlebus fabarum* (H e e a) [45] e a ce e e a ce b e e c a a a e D a [46]. F e d e a e ece a e a e e ac a - a a d b ca a d ec ca a .

Abbreviations

CDC: Communicable Disease Center miniature light trap; CVA: canonical variate analysis; NC: number of components included in the analysis; NW: northwest; SE: southeast; ST: sticky trap; V: percentage of shape variance explained by the components included in the analysis

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Availability of data and material

The data supporting the conclusions of this article are included within the article.

Authors' contributions

JP was the main investigator of the study, he was involved in sand fly capture and identification, wing preparation for geometric morphometrics, analysis and interpretation of data, drafting of the manuscript. CC and CT contributed to sand fly capture and identification, and wing preparation for geometric morphometrics. NR contributed to sand fly capture, dissection and identification. MH, and BV contributed to sand flies capture. JPD contributed to wing geometric morphometrics analysis. BA was involved in conception and design of the study, sand fly capture, interpretation of data and manuscript revising. DS and ALB were involved in conception and design of the study, sand fly capture, interpretation of data, critical revising of the manuscript and acquisition of funding. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
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Ethics approval and consent to participate
Not applicable.

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