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Outline approach: Identification of wild and insectarium tsetse fly vectors of trypanosomes

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Abstract

The outline approach of geometric morphometry was used to distinguish tsetse species, vectors of the trypanosomes responsible for trypanosomosis in sub-Saharan Africa. Tsetse were captured and collected in Côte d'Ivoire (Tiessou and Sinématiali) and at the CIRDES insectarium (Bobo-Dioulasso, Burkina-Faso). At Tiessou, 61 individuals (40 males and 40 females) of *Glossina tachinoides* were collected, while at Sinématiali, 60 individuals (39 females and 21 males) of *G. palpalis gambiensis* were collected. Sampling or collection was carried out using 'Vavoua' traps. At the CIRDES insectarium, we collected 80 *G. morsitans submorsitans* aged 8 to 10 days, including 40 females and 40 males. The condition of the wings of the male and female tsetse flies from CIRDES was satisfactory. They were isolated, scan at the same resolution and then digitised by outline using CLIC 98 software. The three tsetse species were correctly discriminated based on of the size estimated by the perimeter of the contour. The known sexual dimorphism in tsetse flies was confirmed by this approach, with a significant difference ($p<0.05$). Discriminant analysis enabled three species to be distinguished on the basis of their shape. In addition, this method facilitated the precise reclassification of species in their original group with a high percentage or score (98.31% for females and 97.56% for males). Thus, the use of the contour approach to discriminate between species in the absence of homologous landmarks is efficient.

Keywords: Morphometric; Outlines; Sexual Dimorphism; Tsetse Species

1. Introduction

Tsetse flies are vectors of the trypanosomes responsible for Human African Trypanosomiasis (HAT) and African Animal Trypanosomiasis (AAT). Rural communities engaged in farming, fishing, animal husbandry and hunting, are the most vulnerable to HAT. For the period 2016-2020, it is estimated that 55 million people are at risk of contracting the disease, but only 3 million are at moderate or high risk [1]. Even if new therapeutic combinations exist, the lack of new molecules to treat HAT remains an obstacle to its elimination [2]. Furthermore, due to the resurgence of resistance to trypanocides and the failure to control the animal reservoir, vector control appears to be one of the most effective methods [3, 4]. Implementing this control requires not only a better understanding of the behaviour of these vectors. The implementation of this control requires not only a better understanding of the behaviour of these vectors [5, 6], but also their precise identification, especially as not all species are vectors of pathogenic trypanosomes. To find an alternative to the traditional method of tsetse fly species identification, we suggest examining the ability of geometric morphometry to distinguish tsetse species using the point-contour approach.

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2. Materials and methods

2.1 Study area

This research is based on samples of wild tsetse collected in two separate localities, Sinématiali and Tiessou (Figure 1). Tsetse was also collected at the CIRDES insectarium. The first site is in the department of Sinématiali, in the north of Côte d'Ivoire. It has an annual rainfall of 1258 mm. The trapping site, along the Bandama river, is located at 9°36'0'' north latitude and 5°19'25'' west longitude. The climate is tropical, with an average annual temperature of 26.6°C. From December to January, the climate is characterised by the harmattan and its procession of violent winds from the Sahara. In this area, the edge of the Bandama is generally unspoilt, with small forest reserves dotted with teak woods. The second capture zone is in the north of Côte d'Ivoire, in the Gbêkê region. Tiessou is located in the sub-prefecture of Ando-Kekrénou and the department of Béoumi. The climate is tropical and Baoulean, with a temperature of 26.5°C and annual rainfall of 1,057 mm (see Figure 1). It has four seasons, with two rainy periods (March-June and September-October) and two dry periods (August-September and November-February). The climate is characterised by the harmattan and the boreal or continental trade winds, which bring in hot, dry air from the north-east. The flora includes wooded savannah and pre-forest savannah with gallery forests. Located in the northern part of Côte d'Ivoire, Sinématiali and Tiessou are home to cases of AAT. This disease is a major obstacle to livestock farming in the country [7, 8]. These areas were selected because of the presence of *Glossina palpalis gambiensis* and *G. morsitans submorsitans*, vectors of the trypanosome pathogens that cause AAT, and *G. tachinoides*, vectors of the trypanosome pathogens that cause HAT and AAT. The laboratory tsetse flies came from the CIRDES insectarium (temperature = 25°C and a relative humidity=80%) of Bobo-Dioulasso in Burkina-Faso a center carring out advanced rearch on the health of domestic animals.

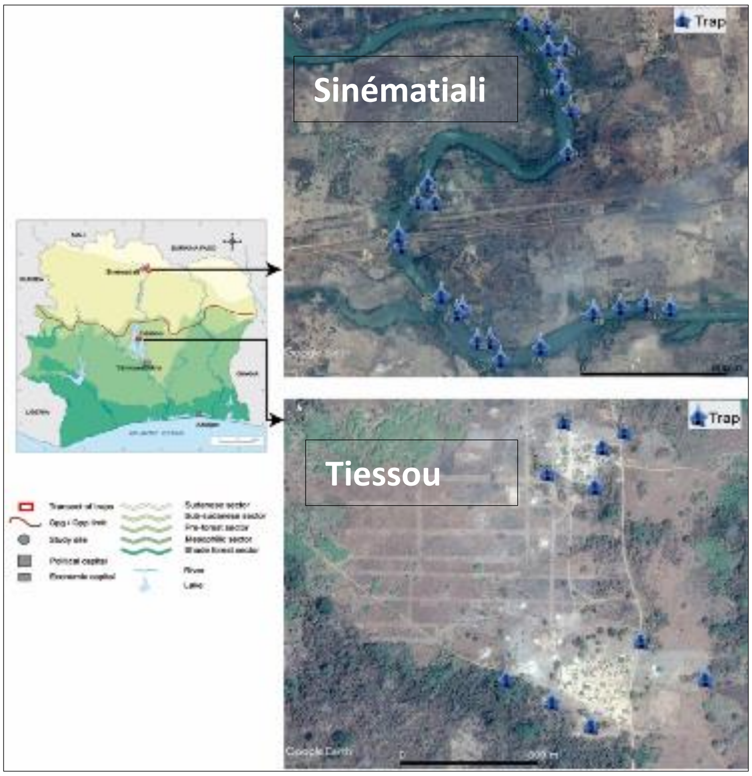

Figure 1 Study area

2.2 Biological material

Three species of tsetse belonging to two taxonomic groups constituted the biological material. The *palpalis* group includes *G. tachinoides* (Figure 2) and *G. palpalis gambiensis*, while the morsitans group includes *G. m. submorsitans*.

Figure 2 Glossina (https://www.alamyimages.fr/la-mouche-tse-tse-glossina-morsitans-morsitans-cette-mouche-africaine-est-responsable-de-l-infecter-les-humains-par-la-maladie-du-sommeil-et-les-bovins-avec-nagana-image334789551.html?imageid=88C4E978-10BB-4FBF-A2B5-E172470C3790&p=1779752&pn=1&searchId=d14a9b19e785b6472bbd2adfb6bc4c09&searchtype=0,23/01/2025: 18 :53)

2.3 Methods

In Sinématiali, a total of 60 individuals (39 females and 21 males) were identified using an identification key as *G. p. gambiensis*. In May 2016, these individuals were collected using 24 'Vavoua' traps (Figure 3). Sampling took place over a period of two successive days (48 hours) along the River Bandama. The traps were separated by an interval of approximately 100 metres.

Figure 3 ''Vavoua'' trap set on the banks of the Bandama river

In September 2017, in Tiessou, 61 individuals (40 females and 21 males) were also identified as belonging to the *G. tachinoides* species. These were collected using 10 'Vavoua' traps. They were caught over two consecutive days (Figure 1).

In July 2016, at the CIRDES insectarium, tsetse fly collection took place. We recovered 80 young tsetse flies, 8 to 10 days old (*G. m. submorsitans*), comprising 40 females and 40 males.

2.3.1 Mounting and scanning the wings

The wings of some tsetse fly species were collected in the field, while others were sampled at the insectarium and then dry-mounted between slides. They were then scanned using a binocular magnifier (GX 0.7) mounted on a camera (Dino-Eye - AM7023B(R4)/ www.dino-lite.eu), with a resolution of 2048×1536 pixels, all connected to a computer. Finally, each wing was recorded in the image format of a mixed group of photography experts (JPEG).

2.3.2 Collecting outlines data

The cell used for digitisation is called the 'D' cell. It is a distinctive cell that is homologous with all tsetse fly wings. The points were collected using CLIC 98 software (Figure 4) and the 'Collection of Coordinates' module from[9]. The points placed automatically produce two-dimensional coordinates, x and y (Figure 5).

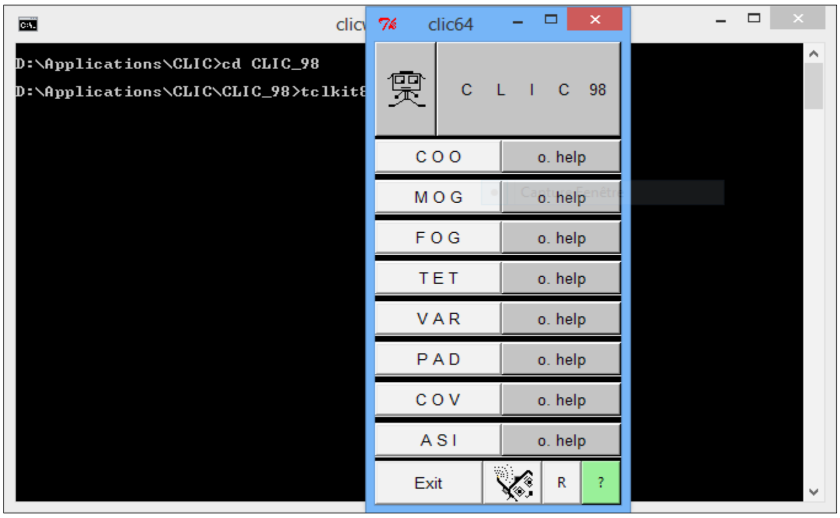

Figure 4 CLIC 98 software

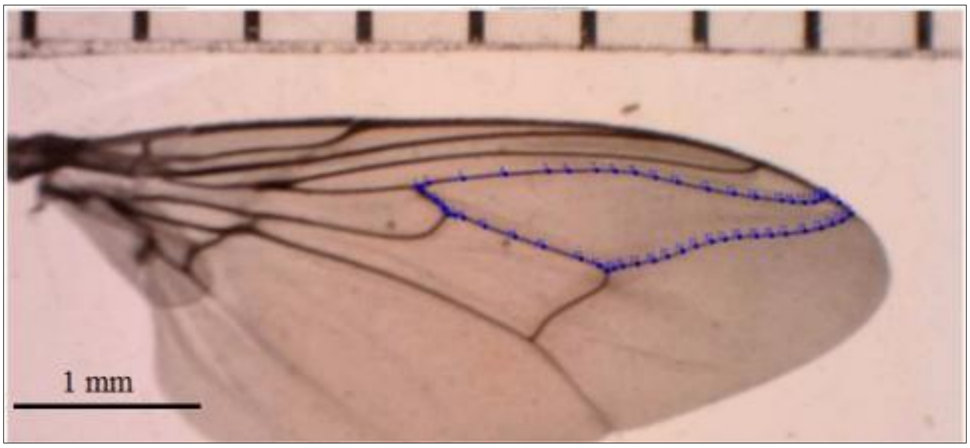

Figure 5 Digitisation of the 'D' cell of a tsetse fly wing using outlines

2.4 Data analysis

The online program XYOM [10] was used for analysis and can be accessed at: https://xyom.io/me. Mean values of wing ''D'' cell contour perimeter between species were examined and non-parametric permutation tests (1000 rounds) were performed to estimate significant differences between means, followed by Bonferroni correction at $p < 0.05$ [11]. Using FOURIER's elliptical statistical analysis (est outline based approach) shape variables were obtained [12]. Discriminant analysis was used to measure the disparity in wing shape between each species, as demonstrated by a factorial map. Non-parametric tests, using permutations of 1000 turns, were carried out on Mahalanobis distances [13]. The Bonferroni correction is significant when p<0.05 [11].

3. Results

3.1 Variation in size estimated by the perimeter of the contour of the 'D' cell of the wings

A study of the variation in size estimated by the perimeter of the outline of the wings of the 'D' cell revealed a recognised disparity in size between the different tsetse species, for both females and males. The species with the largest size among the three species were *G. m. submorsitans*, *G. p. gambiensis* and *G. tachinoides* respectively (Figure 6). In all species, the centroid size of female wings was generally greater than male wings, with a significant disparity of p<0.05 (Table 1). In general, the wings of females exceeded those of males. This finding shows that this method confirms the sexual dimorphism in size observed in tsetse flies (Table 1).

Table 1 Overall size (pixels) of the species as a function of wing size between male and female groups in three species.

Species	**F**	**M**	**p**	**F + M**
Gpg	8.11	7.29	0.001	7.7
Gms	8.3	7.42	0.001	7.86
Gt	7.46	7.24	0.04	7.36

Gms: *G.m. submorsitans*; Gpg: *G. p. gambiensis*; Gt: *G. tachinoides;* F: Females M: Males p: p_value (p<0.05)

3.2 Variation in wing shape

Thanks to the global analysis of Procrustes and the superposition of the contour of the 'D' cells, an imperfect superposition of the shapes of the groups analysed was observed (Figure 7). A difference in shape between the species can be observed by observing these imperfect superpositions.

Analysis Discriminant (AD) revealed a difference between male and female species. Different coloured polygons symbolise the three species: *G. m. submorsitans* (blue), *G. p. gambiensis* (red) and *G. tachinoides* (green), as shown in Figure 7.

The Mahalanobis Distances (MD) calculated between species in pairs, and subjected to the Bonferroni correction after the 1000-turn permutation test (distances significant at a 5% risk if p<0.01667), are large in females (5.24 to 5.45) and males (3.39 to 4.65) and significant between all species (Table 2). This analysis shows that the groups of species are morphologically different from each other, with a significant difference of p<0.05 (Table 2).

Table 2 Mahalanobis distances (DM) between pairwise male groups and pairwise female groups, and Bonferroni correction for contour points, p<0.05

Groups/OTL	**DM between females**	**Test** p<0.01667**	**DM between males**	**Test** p<0.01667**
Gpg and Gt	5.24	0.000001	3.39	0.000001
Gpg and Gms	3.12	0.000001	2.82	0.000001
Gt and Gms	5.45	0.000001	4.65	0.000001

** Bonferroni correction using the 1000-turn permutation test. The test is significant at 5% risk if p < 0.01667.
Gms : *G.m. submorsitans* ; Gpg : *G. p. gambiensis* ; Gt : *G. tachinoides*
OTL: Outline

The results of the reclassification show that all the female and male individuals were accurately reclassified in their original group with very high scores of between 95 and 98%. For females, the overall reclassification score was 98.31%, while for males it was 97.56% (Table 3).

Table 3 Percentage of reclassification of the three tsetse species using the contour point approach

Species	Sex (F/M)	%F (OTL)	%M (OTL)
Gms	40/40	40/40 (100%)	38/40 (95%)
Gpg	39/21	39/39 (100%)	21/21 (100%)
Gt	40/21	38/40 (95%)	21/21 (100%)
Total	119/82	117/119 (98,31%)	80/82 (97,56%)

Legend: Gms: *G. m. submarines*; Gpg : *G. p. gambiensis* ; Gt : *G. tachinoides*
F/M: Females/Males
OTL: Outline

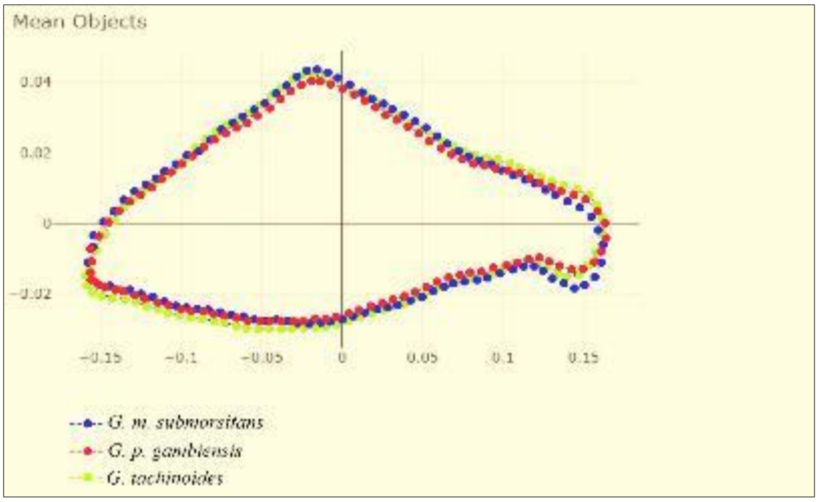

Figure 6 Mean Objects of cell ''D '' of tsetse species

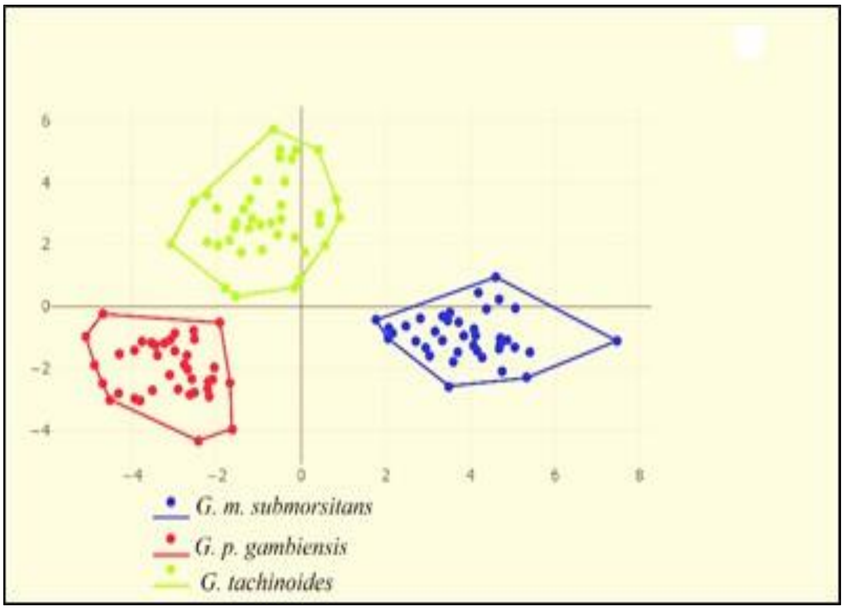

Figure 7 Factor map for discriminant analysis of tsetse species

4. Discussion

This search made it possible to identify the three tsetse species. However, during the various analyses, the year effect of *G. p. gambiensis* 'captured in May 2016' and *G. tachinoides* 'captured in September 2017' was not examined to differentiate the species between them or between sexes. Indeed, the year effect could influence tsetse species discrimination.

The perimeter contour study revealed that *G. m. submorsitans* had the largest size (7.86), followed by *G. p. gambiensis* (7.7) and finally *G. tachinoides* (7.36), which corresponds to the actual size difference between the three species [14]. In all species, the centroid size of female wings is generally greater than male wings, with a significant difference (p<0.05).

This approach confirmed the known sexual dimorphism in size in favour of females, which characterises tsetse flies [15–18]. Females were systematically better discriminated than males on the basis of shape. This result is similar to that of [14]. This performance of the contour could be linked to the effective capture of the shape of the ''D'' cell, characteristic of the tsetse wing, and whose capture power was 0.997.

The study of the Mahalanobis distance between the different groups made it possible to distinguish between groups of species. This result is consistent with the research conducted by [14, 19–21]. The discriminant analysis study allowed effective discrimination between the different species. *G. m. submorsitans* is a Savannah-dwelling species belonging to the subgenus *Glossina* and the morsitans group, which lives in habitats with little or no moisture. It can be distinguished from the other species (*G. tachinoides* and *G. p. gambiensis*), which belong to the same subgenus nemorhina and the same *palpalis* group.

The species *G. p. gambiensis* is found in gallery forests and dry and humid savannahs, making it a riparian species. The discrimination of species groups by the outlines approach is similar to other studies and the morphological classification of tsetse species [15, 16, 19, 20]. In females, the average reclassification score was higher than in males (98.31% for females and 97.56% for males). These results are consistent with the work of [21, 22].

5. Conclusion

This research evaluated the performance of the outline's method. This technique was used to distinguish three tsetse species from two distinct categories. The individuals were correctly grouped in their group of origin, obtaining remarkable scores (98.31% for females and 97.56% for males). Thus, the contour point approach can be used to identify species or also be used in the absence of landmarks or insufficient landmarks on a biological structure. The results of this research should be studied in greater depth to understand the annual impact on different groups of tsetse species.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] WHO. Control and surveillance of human African trypanosomiasis: report of a WHO expert committee. World Health Organization, https://books.google.com/books?hl=fr&lr=&id=sbQXDAAAQBAJ&oi=fnd&pg=PP1&dq=Who+(2013)+Control+and+Surveillance+of+Human+African+Trypanosomiasis:+Report+of+a+Who+Expert+Committee.+World+Health+Organization+249p.&ots=LclcNybYzT&sig=UxBeNx0RsMI5YXP7GIN0LaPpUD0 (2013, accessed 7 February 2025).

[2] Manus J-. A new drug for sleeping sickness. Revue Francophone des Laboratoires 2018; 2018: 8.

[3] Solano P, Kaba D, Ravel S, et al. Population genetics as a tool to select tsetse control strategies: suppression or eradication of *Glossina palpalis gambiensis* in the Niayes of Senegal. PLoS Neglected Tropical Diseases 2010; 4: e692.

[4] Kuzoe FA, Schofield C. Strategic review of traps and targets for tsetse and African trypanosomiasis control, https://apps.who.int/iris/bitstream/handle/10665/69013/TDR_IDE_TRY_05.1.pdf (2005, accessed 7 February 2025).

[5] Courtin F, Sidibé I, Rouamba J, et al. Impact of demographic and climatic changes on the spatial distribution of humans, tsetse and trypanosomoses in West Africa. Parasite 2009; 16: 3–10.

[6] Courtin F, Rayaissé J-B, Tamboura I, et al. Updating the northern tsetse limit in Burkina Faso (1949–2009): impact of global change. International Journal of Environmental Research and Public Health 2010; 7: 1708–1719.

[7] Douati A, Küpper W, Kotia K, et al. Control of tsetse flies (Glossina: Diptera, Muscidae) using screens and traps (static methods): assessment of two years of control at Sirasso in northern Côte d'Ivoire. Revue d'élevage et de médecine vétérinaire des pays tropicaux 1986; 39: 213–219.

[8] Acapovi-Yao G, Allou K, Mavoungou JF, et al. Geographical distribution and infection of *Glossina palpalis palpalis* (Diptera, Glossinidae) by trypanosomes in forest relics in the city of Abidjan-Côte d'Ivoire. African Journal of Animal Health and Production 2013; 11: 37–42.

[9] Dujardin J-PA, Kaba D, Henry AB. The exchangeability of shape. BMC Res Notes 2010; 3: 266.

[10] Dujardin S, Dujardin J-P. Geometric morphometrics in the cloud. Infection, Genetics and Evolution 019; 70: 189–196.

[11] Dunn OJ. Multiple Comparisons among Means. Journal of the American Statistical Association 1961; 56: 52–64.

[12] Kuhl FP, Giardina CR. Elliptic Fourier features of a closed contour. Computer graphics and image processing 1982; 18: 236–258.

[13] Albrecht GH. Multivariate analysis and the study of form, with special reference to canonical variate analysis. American Zoologist 1980; 20: 679–693.

[14] Kaba D, Berté D, Ta BTD, et al. The wing venation patterns to identify single tsetse flies. Infection, Genetics and Evolution 2017; 47: 132–139.

[15] Borne F, Petiteau L, Geoffroy B, et al. Fly Picture Measurement, a new computer tool for the study of tsetse flies. Journal of animal husbandry and veterinary medicine in tropical countries 1999; 52: 19–21.

[16] De la Rocque S, Geoffroy B, Michel JF, et al. Tsetse fly wings, an identity card of the insect? Parasite (Paris, France) 2002; 9: 275–281.

[17] Sané B, Solano P, Garcia A, et al. Intraspecific variations in wing and thorax size in *Glossina palpalis palpalis* (Robineau-Desvoidy, 1830) in the forest zone of Côte d'Ivoire. Revue d'élevage et de médecine vétérinaire des pays tropicaux 2000; 53: 245–248.

[18] Camara M, Caro-Riano H, Ravel S, et al. Genetic and morphometric evidence for population isolation of *Glossina palpalis gambiensis* (Diptera: Glossinidae) on the Loos islands, Guinea. Journal of medical entomology 2006; 43: 853–860.

[19] Brunhes J, Cuisance D, Geoffroy B, et al. Tsetse flies, https://agritrop.cirad.fr/481347/ (1998, accessed 7 February 2025).

[20] Launois M, Charbonnier G, Laveissière G, et al. The pedagogical tsetse fly, https://agritrop.cirad.fr/523229 (2004, accessed 7 February 2025).

[21] Dujardin J-P, Kaba D, Solano P, et al. Outline-based morphometrics, an overlooked method in arthropod studies? Infection, Genetics and Evolution 2014; 28: 704–714.

[22] Chaiphongpachara T, Sriwichai P, Samung Y, et al. Geometric morphometrics approach towards discrimination of three member species of Maculatus group in Thailand. Acta tropica 2019; 192: 66–74.