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Effect of Fungi Presence on Wing Morphometry and Flight Behaviour in the Common Pipistrelle Bats (*Pipistrellus pipistrellus*)

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Abstract

Bats play an important role in ecosystem function, serving as a biocontrol agent of insects and pollination in plants, hence the need to understand the factors affecting their health and performance. Infestation of fungi has been identified as a potential threat to populations of mammals, especially bats, yet their specific effects on wing morphology and flight behavior remain understudied. In this study, swabs of *Pipistrellus pipistrellus* bat wings were taken from bat carers around the United Kingdom. For identification, samples were exposed to conventional PCR using fungal-specific primers to amplify the ribosomal DNA's internal transcribed spacer (ITS). The wind morphometric parameters such as wing angles (maximum, minimum, mean, and amplitude) and wing frequency were determined for both wings with fungi infestation and without fungi infestation. A rain cloud distribution plot with a box plot was used to determine the distribution of flight parameters of the wings. A principal component analysis biplot was used to determine the relationship between the flight parameters in terms of wings with infestation by fungi and without infestation. The findings in this study are important to bat biology, ecology, and conservation and give insights into the need for wildlife management and conservation, ultimately contributing to the ongoing efforts to preserve bat populations and support ecological balance in diverse ecosystems.

Keywords: Bats, Fungi infestation, Wing morphometry, Flight behavior.

Introduction

Bats are mammals belonging to the order *Chiroptera* and comprise about 20% of mammalian diversity [1-3]. Bats play a crucial role in the ecosystem such as seed dispersion, pollination, and control of insect populations [4-7]. Bats also help to maintain ecosystem stability [7].

In the realm of mammals, bats stand out as the sole creatures endowed with the ability of powered flight [2- 3]. This distinctive trait merits exploration in its own right and serves as the foundation for further discussion. Regarding flying behaviour, bats usually fly at night to forage for food [7]. Regarding flight membranes in bats, which are called patagia, they comprise 85% of the body surface area of a bat [8]. The membranes are made up of 4 sections; the first section is called the protopatagium, which spreads from the bat's shoulder to the thumb; the second section is called chiropatagium (or dactylopatagium), which is the skin between fingers 1 and 5; the third section is called the plagiopatagium, which is the skin between the fifth

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fingers and the body trunk, [9-11]. The fourth section involves a tail membrane between the hind limbs, found in various species of bat and called the uropatagium [10, 11]. The bat wings have an important role in critical for powering flight, as all those sections have a function in bat flight [2, 12]. However, the large and thin wing membranes make them particularly susceptible to injuries, holes, and tears [8, 13].

As with many animals, bats face some threats during their lives; those threats could be related to anthropogenic activities such as light pollution, noise, collisions with man-made structures, and habitat loss [7, 14-16]. On the other hand, bats could face other natural threats, such as weather, fungal infections, and cat predation [17- 21]. Some of those threats affect the bat wing, thus affecting bats' flight behaviour.

One of the critical fungal infections in bats is caused by a fungus called keratin-digesting fungus (*Pseudogymnoascus destructans*), which infects the bat's skin and causes a disease called White-Nose Syndrome (WNS) [18- 23]. That fungus grows quickly through the winter during the hibernation season for bats [24]. Through the hibernation season, bats experience some factors that permit the fungus to grow and spread commonly among the bats' populations, such as deficiency of food and water, a reduction in metabolism, immune function, and body temperature, so bats commonly avoid losing moisture by find humid or heavily populated areas for hibernation [18-20, 22].

This infection by WNS kills millions of bats and threatens some bat species in the USA and Canada [22-23]. Moreover, the WNS-affected Little Brown Bats (*Myotis lucifugus*) during the winter, the wings of the infestation bats seemed crumpled due to losing their elasticity and they were no longer strong so they would tear easily [19, 24]. Additionally, the microscopic analysis of the affected wings by *P. destructans* infestation, exposed damage to some glands as a result of the skin corrosion as the fungus digests the wing skin, then which would influence all structures in the wing such as connective tissues and blood vessels [19, 24]. The *P. destructans* not only infects the skin, but it could interrupt the physiological homeostasis which causes bat mortality [19].

The *P. destructans* fungus has been identified in European hibernating bats [25, 26]. Moreover, it was identified by molecular analysis in several European countries, including the United Kingdom [26].

In this study, we aim to 1) investigate the fungal presence in the wing of Common Pipistrelle bats (*Pipistrellus pipistrellus*) in the United Kingdom and detect the fungal species; 2) identify the effect of fungal presence on bat flying behavior in those bats.

Materials and Methods

Collected samples:

Swabs of *P. pipistrellus* bat wings were collected from bat carers around the United Kingdom. The total number of samples collected was 72. All the collected swabs were stored at -20°C before genetic analysis.

Fungal DNA

The internal transcribed spacer (ITS) region of ribosomal DNA from fungi was amplified by standard PCR on the materials using primers specific to fungi [27, 28]. In a total volume of 20 µL, the following reagents are used in the DNA amplification reactions: 0.5 µM ITS1 forward primer (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Invitrogen, Thermo Fisher Scientific), 0.5 µM ITS2 reverse primer (5'-GCTGCGTTCTTCATCGATGC-3') (Invitrogen, Thermo Fisher Scientific), 1x MyTaq Red Mix

(Bioline, UK), and 2 µL of extracted DNA. The following parameters were used to amplify the samples: 10 minutes for the first denaturation at 95°C; 30 cycles on a Prime heat cycler (Techne, UK) at 95°C for 60 seconds, 55°C for 60 seconds, and 72°C for 90 seconds, followed by a final extension at 72°C for 10 minutes.

Saccharomyces cerevisiae (Purchased strain, NCYC 87) cultivated on agar plates provided the DNA for the positive control. Using a sterile loop to select one colony, 500 µL of molecular biology grade water (Fisher Scientific, UK) was added to a 1.5 mL sterile Eppendorf tube, vortexed, and incubated for five minutes at 100°C. The supernatant was then centrifuged for five minutes at 6000 xg and transferred to a new sterile Eppendorf tube. There were negative controls that lacked template DNA.

Agarose gel electrophoresis

The amplified PCR products were performed on a 1% agarose gel in 1x TBE to evaluate the PCR results. The PCR results were placed onto the gel in an aliquot of 10 µL along with 5 µL of a DNA size ladder (HyperLadder™ 100 bp, Bionline, UK). Gels were made in accordance with the procedure outlined in section 5.2.4.

Sanger sequencing

Sanger sequencing was applied to samples that tested positive for fungal DNA. After adding 4 µL of ExoSAP-IT™ reagent (Express PCR Product Clean-up, Applied Biosystems, UK) to 10 µL of the PCR products, gently vortexing and briefly spinning down, the PCR products were cleaned up. The samples were snap-cooled on ice after four minutes of incubation at 37°C and one minute at 80°C.

Following sample cleaning, 6 µL of the PCR product was combined with either 0.5 µM of the ITS1 forward or ITS2 reverse primer, and the entire amount was brought up to 10 µL using molecular biology-grade water to prepare the samples for sequencing.

Phylogenetic tree

To find the relationship between the fungal DNA sequences, a phylogenetic tree was created using MEGA7 software (version 7.0.26) (https://www.megasoftware.net) and aligning sequences using the Maximum Likelihood method [30].

Flight behaviour analysis

From all the bat wing swabs that were received for genetic analysis, 12 *P. pipistrellus* bats have been filmed over 3 summers around the United Kingdom. The bats were in good condition without any wing injuries. From all specimens, 7 bats did not reveal any fungal infection, while five bats obtained fungal infection. The filming protocol that was used was mentioned in Khayat et al. (2020) [31].

The wing angles were measured in degrees to calculate the flight parameters, which are maximum angle (Max. angle), minimum angle (Min. angle), mean angle (Mean. angle), and the Root Mean Square Amplitude (RMS). Also, the wing beat per second was determined in Hertz to calculate the wing frequency (FREQ). The raw data were subjected to Leven's test of homogeneity of variance and the Shapiro-Wilk test for normality before other analyses. A rain cloud distribution plot with a box plot was used to determine the distribution of flight parameters of the wings. A principal component analysis biplot was used to determine the relationship between the flight parameters in terms of wings with infestation by fungi and without infestation. R statistics software for Windows v.4.0.3 and SPSS v.23 were used for the analysis.

Results

Fungal analysis

The bat swab samples were analyzed and showed the presence of fungus in 24 samples out of all samples (33.33% of cases). The gel results exposed PCR products in 24 samples ranging from 265bp to 297bp (Fi. 1), which is expected for the ITS1 and ITS2 regions [32]. The positive control also exposed a PCR product at 450 bp, also within the expected size range of *S. cerevisiae* [32, 33]. Moreover, the sequencing results validate *S. cerevisiae spp.*, with a 90% similarity match. The negative control was pure, which represented no contamination.

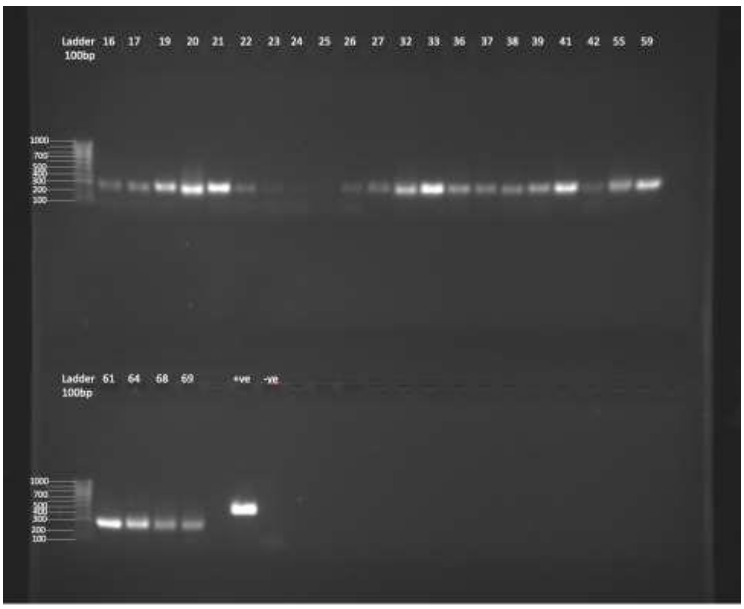

Figure 1: Photo shows the agarose gel electrophoresis of the PRC products of the amplified fungal DNA. The numbers above each lane refer to the number of bat swabs, +ve is the positive control, and -ve is the negative control.

DNA sequencing of these samples showed the presence of 12 different fungal species (Fig. 2, 3), which are usually found in the environment, like water, soil, air, and plants. The most common species in a quarter of the samples was *Cladosporium* (Fig. 3). The phylogenetic tree shows the presence of 8 different families, which are *Sydowia, Cladosporium, Penicillium, Trichoderma, Aureobasidium, Coniochaeta, Talaromyces, Septoria* (Fi. 3).

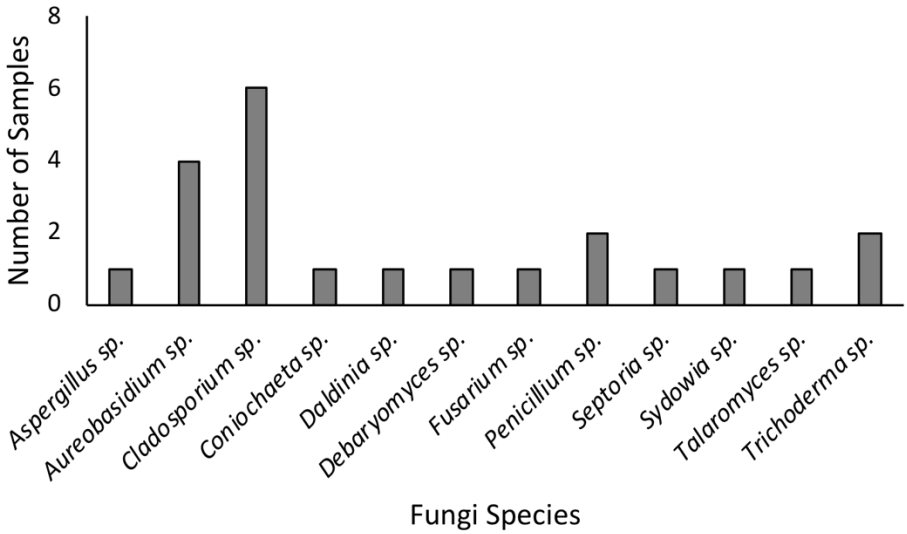

Figure 2: The 12 different fungal species found on *P. pipistrellus* bat wing.

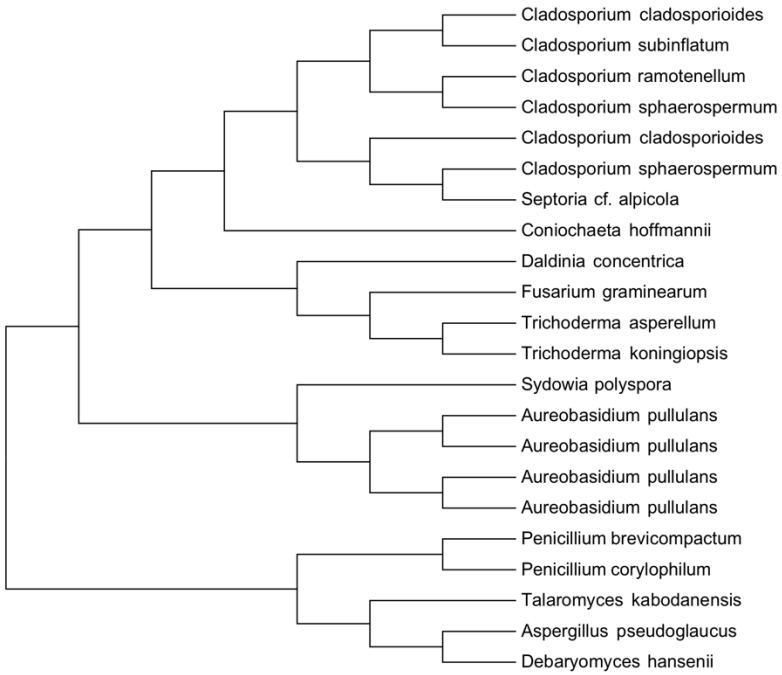

Figure 3: Molecular Phylogenetic Tree analysis by the Maximum Likelihood method

Bat Wing Morphometry

Fig.4 is the distribution and rain cloud plot for bat wing morphometry for bat wings with fungi and wings with no presence of fungi for the left (Fig. 4a) and right (Fig. 4b) wings. In this plot, the max. angle morphometry is more distributed in both the left and right bat wings with no fungi infestation. However, higher distributions were recorded at the final count for both max. the angle of the left and right wings. The wing without fungi infestation still recorded more distribution in terms of the max. angle. Additionally, the initial counts for both the right and left wings in terms of no fungi and fungi infestation were sparse, while the max. The angle distribution count was higher in the right-wing than in the left (Fig. 4b).

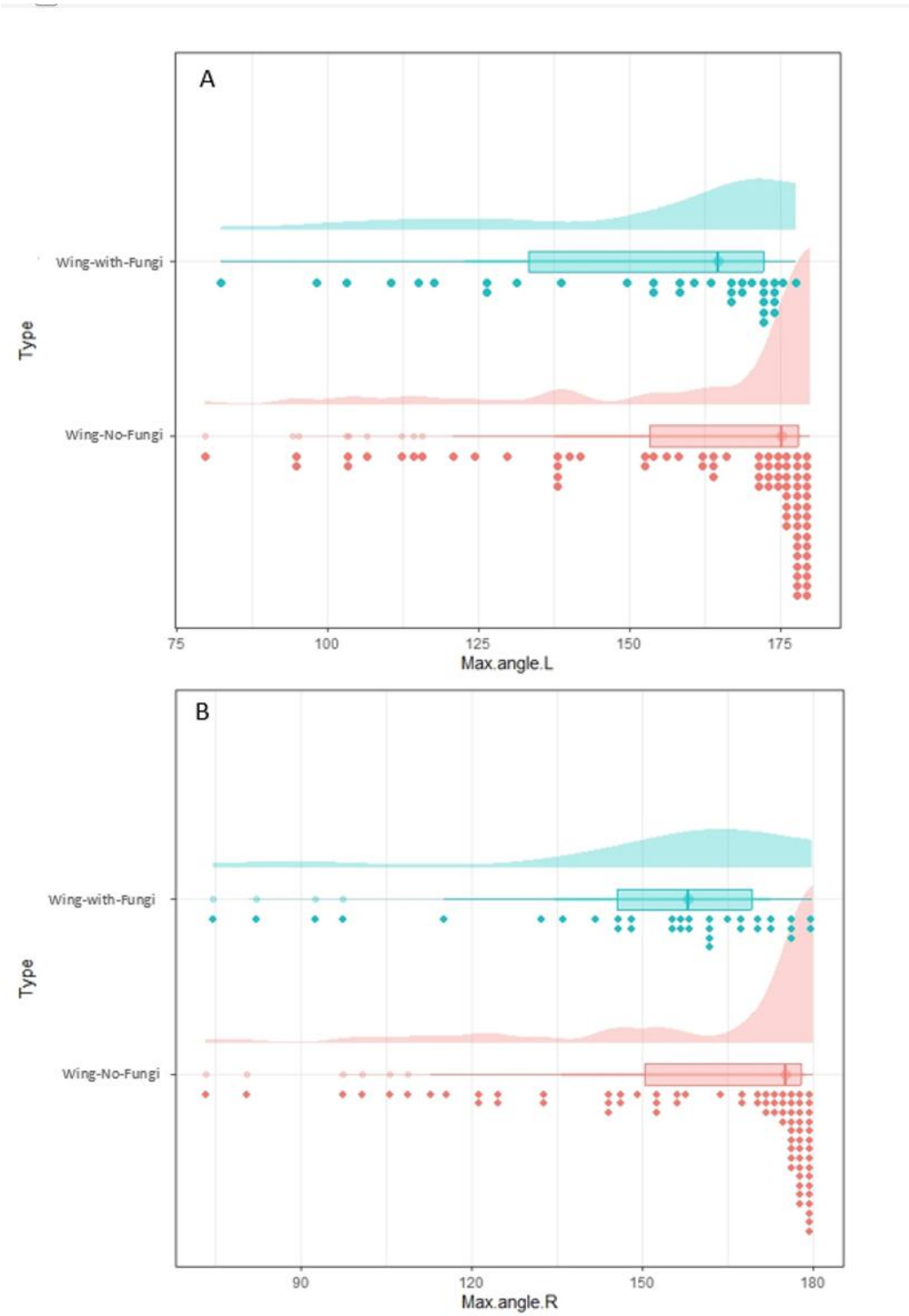

Fig. 4 Distribution of max. angle a) Left b) Right as a morphometric for bats' wings with fungi and wings with no presence of fungi.

The distribution and rain cloud plot for bat wing morphometry for bats wings with fungi and wings with no presence of fungi for the left using min. angle measurement of the left and right wings is shown in (Fig.s 5a and b). In this plot, the min. angle morphometry is more distributed in both the left and right bat wings with no fungi infestation, even though sparse distribution was recorded in the left min. angle of the wing with no fungi infestation, and right min. angle of the wing with fungi infestation. For both left and right min. angle, higher counts were recorded at the initial count, and as the count increases, the distribution becomes sparse (Figs. 5a and b).

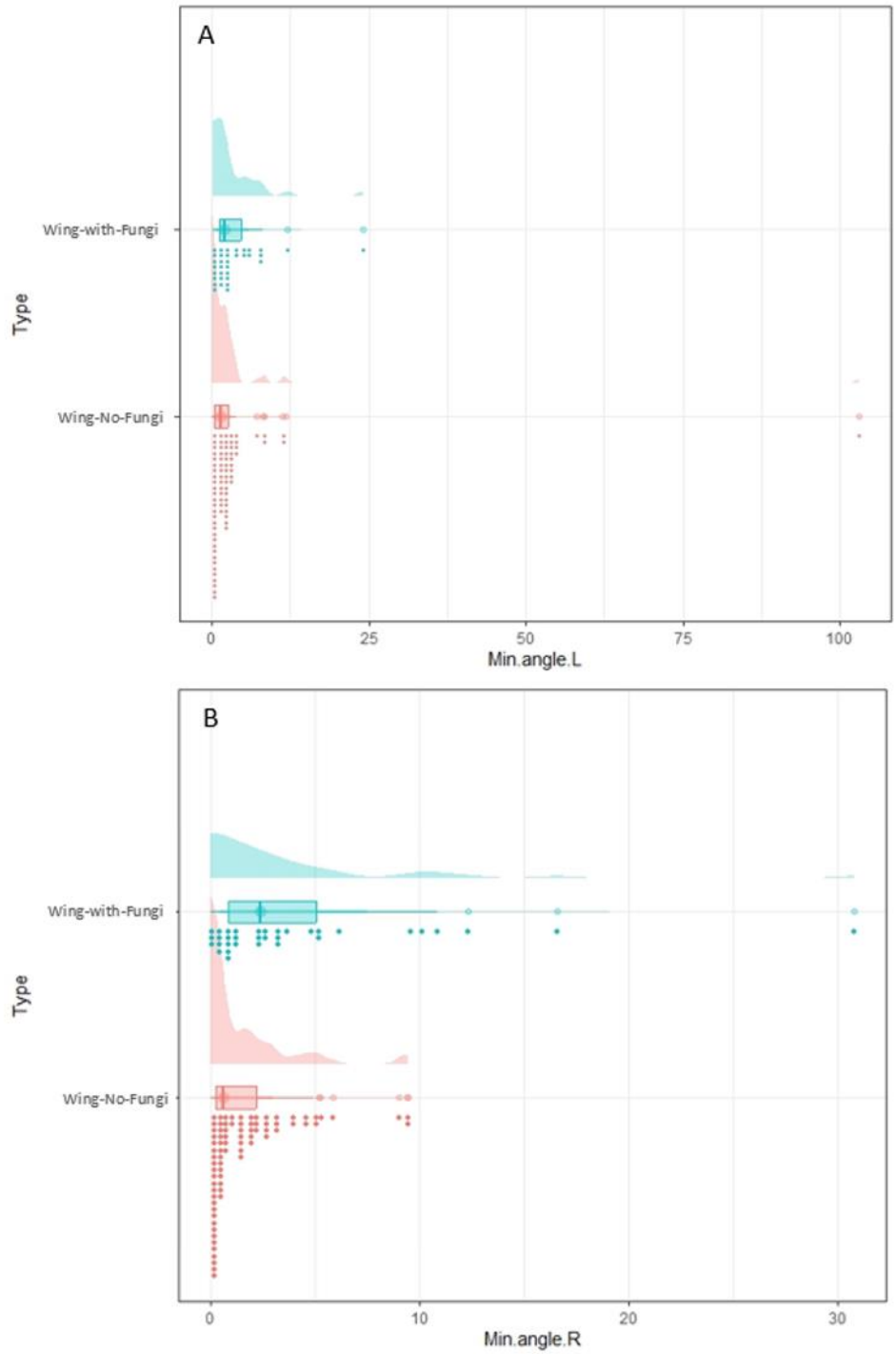

Fig. 5 Distribution of min. angle a) Left b) Right as a morphometric for bat wings with fungi and wings with no presence of fungi.

The morphometry of the wing was highly magnified in terms of the distribution following almost normal distribution of the count for the mean angle of both the left and right wings, irrespective of whether they had been infected with fungi (Figs. 6a and b). However, the distribution count was higher in the mean angle of the wing without fungi infestation for both left and right. It is important to note that for the mean angle of the left wing, even though the count was higher in the wing with no infestation, sparse distribution was recorded at the highest count of mean angle morphometry. For the right-wing mean angle, the distribution was normal in the wing without fungi infestation than what was observed in the wing with fungi infestation, even though a negligible sparse distribution was observed in the right mean angle of the wing without fungi infestation (Fig. 6b).

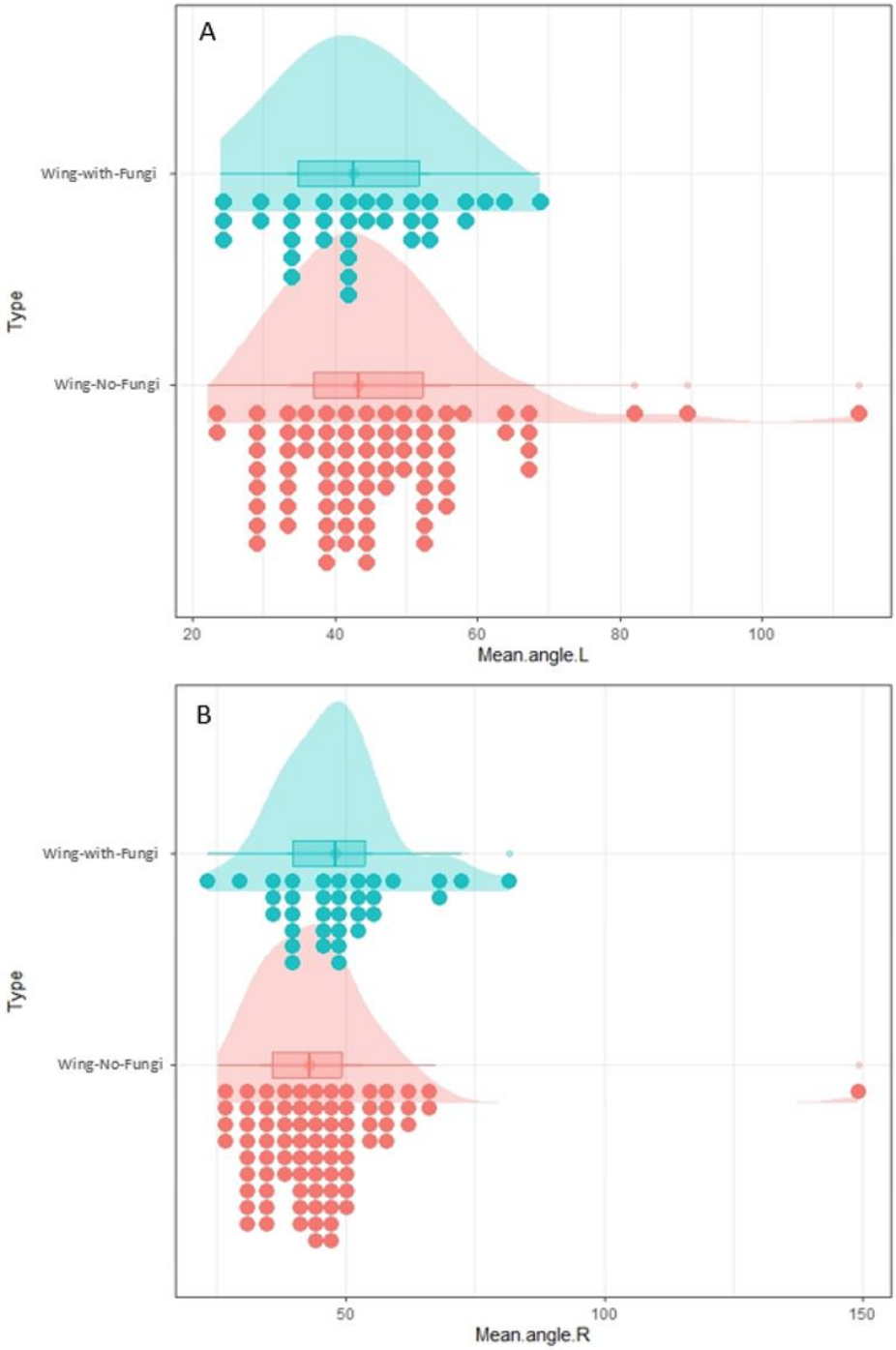

Fig. 6 Distribution of mean. angle a) Left b) Right as a morphometric for bats' wings with fungi and wings with no presence of fungi.

The RMS results revealed the wing's morphometry to be highly magnified in terms of the distribution, with the count for left-wing RMS for the wing with fungi infestation following an almost normal distribution (Fig.s 7a and b). For the RMS of the left wing, the distribution count was higher in the wing without fungi infestation, although the distribution for both the wings with infestation and not was sparse at the initial count of the RMS (Fig. 7a). In addition, for the right wing, even though RMS count was higher in the wing without fungi infestation, the count for the RMS was almost normally distributed than the count in the wing without fungi infestation (Fig. 7b)

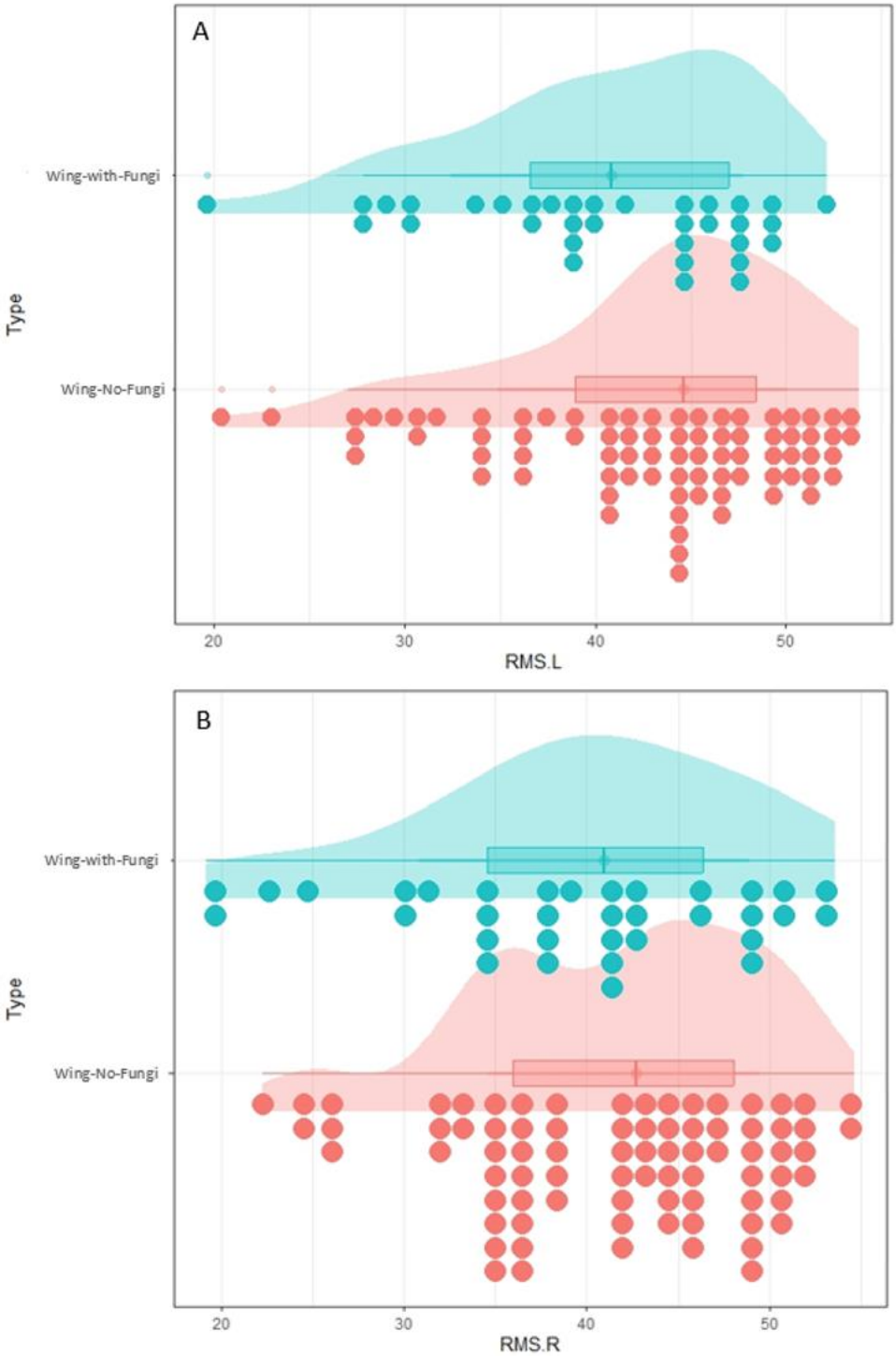

Fig. 7 Distribution of RMS a) Left b) Right as a morphometric for bat wings with fungi and wings with no presence of fungi.

Frequency as one of the variables used for the wing's morphometry was highly magnified in terms of the distribution, with the count for the left and right wings revealing the same patterns of distribution (Figs. 8a and b). However, a more normally distributed count was recorded for wings with no fungi infestation, while the highest peak count in terms of single value was recorded for wings with fungi infestation (Figs. 8a and b).

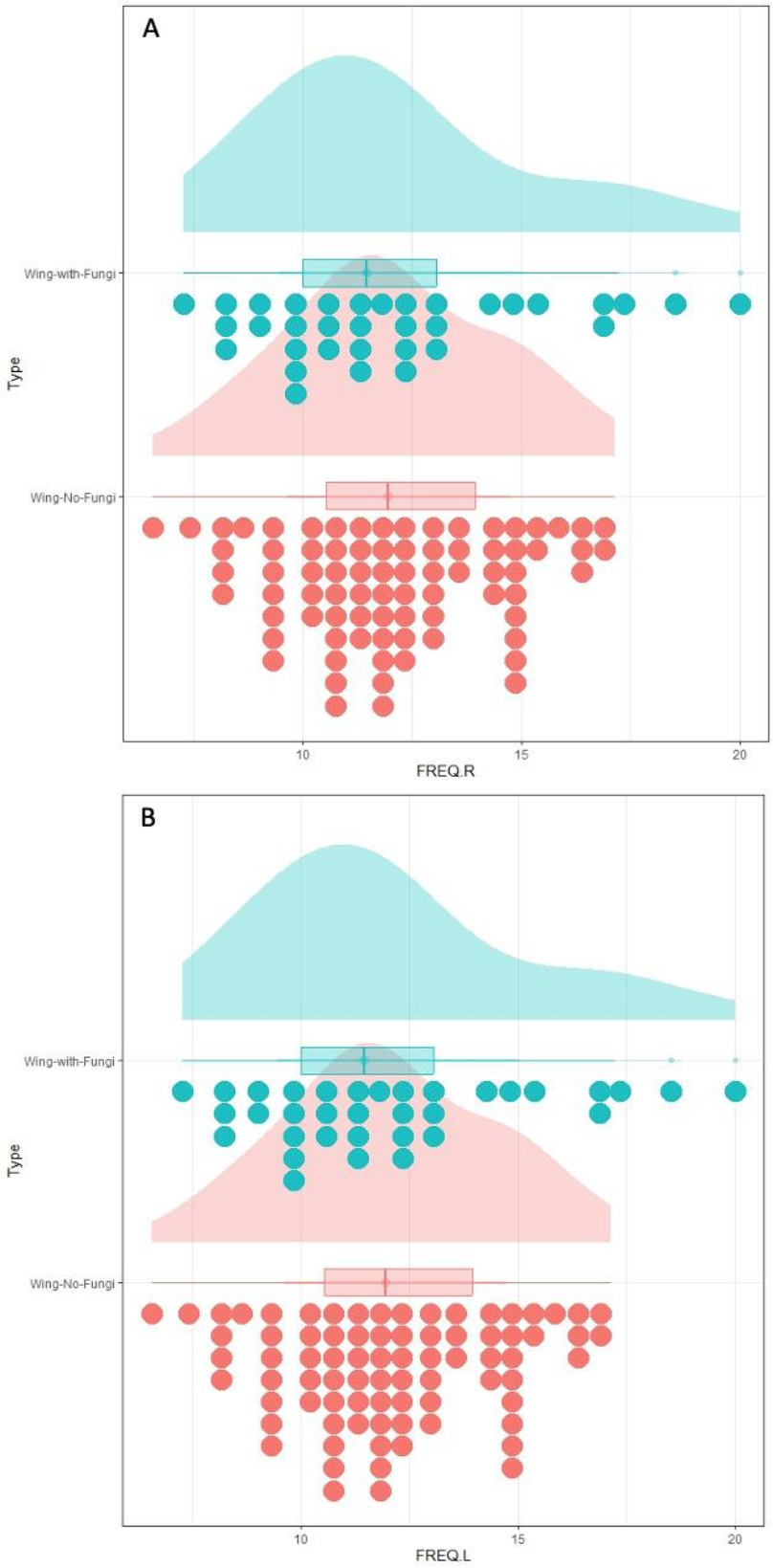

Fig. 8 Distribution of frequency a) Left b) Right as a morphometric for bat wings with fungi and wings with no presence of fungi.

Relationship Between Morphometric Parameters of Bat Wing

The group Principal Component Analysis (PCA) biplot for the relationship between Bat wing morphometric parameters in terms of the presence and absence of fungi infestation revealed components 1 and 2 accounted for a total variation of 49.9 % of the total variation (Fig. 9). In this result, a strong positive relationship exists between the min angle of the left and right-wing, the mean angle of the left and right-wing, and the RMS of the left and right-wing, max. angle of left and right wings, all in terms of the presence and absence of fungi infestation. The results from the plot revealed a strong association between the two groups as an influence on the relationship between the morphometric parameters (Fig. 9).

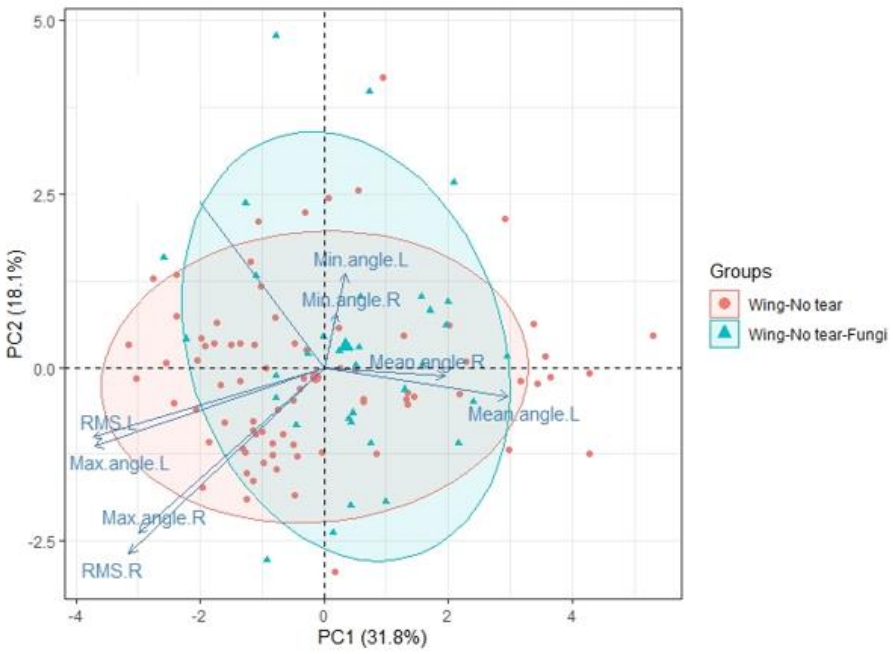

Fig. 9 Relationship between Bat wing morphometry in terms of presence and absence of fungi infestation

Discussion

The results of the fungal analysis on bat swab samples present compelling evidence of a high incidence of fungal presence, with 96% of the samples showing the presence of fungus. Gel electrophoresis of PCR products, ranging from 265bp to 297bp, aligns with the expected size range for the ITS1 and ITS2 regions. The successful amplification in the positive control, targeting S. cerevisiae, and subsequent confirmation through sequencing further validate the reliability of our experimental procedures, with a 90% similarity match. The absence of contamination in the negative control underscores the robustness of our laboratory techniques. The subsequent DNA sequencing revealed the diversity of fungal species in the bat populations, with 12 different species identified. These species, commonly found in various environmental niches such as air, water, soil, food, and plants, highlight the intricate interplay between bats and their surrounding ecosystems.

The emergence of Cladosporium as the most prevalent species, present in 25% of the samples in this study, aligns with recent studies that emphasize the ubiquity of Cladosporium in environmental

samples. For instance, a study by Vanderwolf (2021) [34] reported a similar prevalence of Cladosporium in bat populations, suggesting a potential ecological association between bats and this fungal species. Additionally, the study by Meierhofer et al. (2023) [35] observed comparable results in their investigation of fungal diversity in bat populations, further supporting the notion that Cladosporium is a commonly encountered fungal species in bat environments.

As depicted in this study, the analysis of bat wing morphometry provides valuable insights into the impact of fungi infestation on the distribution of the maximum angle measurements for both left and right wings. Notably, the rain cloud plot reveals distinct patterns in the morphometric distributions under different conditions. The distribution and rain cloud plot for bat wing morphometry for wings with fungi and wings with no presence of fungi are presented for the left and right wings. Surprisingly, even in the presence of a fungi infestation, the maximum angle morphometry is more widely distributed in both left and right wings when there is no fungi infestation. This observation suggests that the absence of fungi may contribute to a broader range of wing morphological variations [36]. However, it is noteworthy that higher distributions were recorded at the final count for both left and right wings, indicating potential changes in wing morphology over time [37].

Interestingly, the wing without fungi infestation consistently exhibited a more extensive distribution in terms of the maximum angle. This finding aligns with a study, which demonstrated that fungal infestation in bat wings can lead to alterations in wing morphology [19]. Moreover, it was observed reduced wing flexibility and increased wing damage in bats with fungal infestation [19, 24, 38]. This supports our observation that wings without fungi exhibit a broader range of maximum angle measurements.

Furthermore, the initial counts for both right and left wings were sparse, categorized by the presence or absence of fungi infestation. This suggests there might be limited variability in wing morphometry at the outset. However, as the study progressed, the final count showed a higher distribution of maximum angle measurements, particularly in the right wing. This asymmetry in distribution between the right and left wings is consistent, where asymmetrical wing morphometry was observed in bat populations [39].

The observed patterns in the distribution of minimum angle measurements for both left and right wings are in line with recent findings in the literature. as it has been reported that, the bats with fungal infestation exhibited restricted wing flexibility, leading to altered wing angles [40]. Our results align with this, as we observed a more widespread distribution of minimum angles in wings without fungal infestation, indicating greater morphological variability.

Intriguingly, from this study, higher counts were recorded at the initial count for both left and right minimum angles, and as the count increased, the distribution became more sparse. This temporal pattern suggests that there might be initial uniformity or limited variability in minimum angle measurements, and as the study progressed, a divergence in wing morphometry became evident. Also, it has been documented that the dynamic changes in bat wing morphometry over time, support the idea that wing angles may undergo variations during specific phases of the study [41]. It is crucial to acknowledge that the observed patterns in minimum angle distribution are complex and may be influenced by various factors, including environmental conditions and individual bat behaviour. The comprehensive assessment of wing morphometry, considering fungi infestation, adds nuance to our understanding of bat wing adaptations.

The higher count distribution in the mean angle of wings without fungi infestation aligns with recent studies by Vanderwolf (2022), which demonstrated that bats with fungal infestation exhibited wing structure alterations, leading to wing angle changes [42]. The observed pattern may be attributed to the harmful effects of fungal infestation on wing flexibility and overall wing morphology, as reported by these studies [43].

An interesting finding emerged when analyzing the mean angle of the left wing, where, despite the higher count in the wing without infestation, a sparse distribution was recorded at the highest count of mean angle morphometry. This suggests that certain angles may be less prevalent or exhibit higher variability even in the absence of fungal infestation. This finding adds complexity to our understanding of mean wing angles and highlights potential asymmetries in the distribution of morphometric measurements [43]. For the right-wing mean angle, the distribution was more normal in wings without fungi infestation compared to those with infestation. This aligns with the notion that fungal infestation may lead to asymmetrical changes in wing morphology, as reported by recent studies such as Zepeda Mendoza et al. (2018) [44]. Although a negligible sparse distribution was observed in the right mean angle of wings without fungi infestation, this further emphasizes the potential impact of fungal infestation on the overall distribution of mean wing angles.

The RMS results reveal a highly magnified distribution for the left wing, with the count for the wing with fungi infestation following an almost normal distribution (Fig. 7a and b). However, the distribution count for the left-wing RMS was higher in wings without fungi infestation, indicating potential alterations in the wing surface structure due to fungal infestation. This observation aligns with recent studies by Federici et al. (2022) and Hathaway et al. (2023), which demonstrated significant changes in wing surface characteristics in bats with fungal infestation [45,46]. The sparse distribution at the initial count of RMS for both wings suggests that certain morphometric features may be less prevalent initially, regardless of the presence of fungi. The higher RMS count for right-wing in this study in wings without fungi infestation, and the count distribution was almost normally distributed compared to the wing with fungi infestation, even in the absence of fungal infestation, can be due to a typical distribution of morphological features across the wing surface. This finding is in line with the work of Bure and Moore (2019), who observed asymmetrical changes in wing surface morphology in bats with fungal infestation [47].

As a key variable in bat wing morphometrics determined in this study, frequency exhibited highly magnified distributions in both left and right wings, suggesting bilateral symmetry in response to considered variables [48]. Notably, wings without fungi infestation displayed a more normally distributed frequency count, while those with fungi infestation exhibited a pronounced deviation from normal distribution, marked by the highest peak count. This indicates a potential wing morphology disruption under the influence of fungi [19]. The findings underscore the importance of considering external stressors in bat wing studies, with implications for understanding adaptive mechanisms and vulnerabilities, as it was found the potential wing morphology change affects the wing movement during flight [31].

The correlation that exists between the minimum angle, mean angle, and RMS of both left and right wings, as well as the maximum angle of both wings, irrespective of fungi infestation, underscores the intricate nature of bat wing morphology. It suggests that alterations in one morphometric parameter may be mirrored in others. These findings align with Voigt (2013) and Forsythe et al. (2022) research, which also identified strong associations among

different morphometric parameters in bat wings [49, 50]. They emphasized the interdependence of wing angles, supporting our observation of a positive relationship between minimum, mean, and maximum angles [49, 50]. Elsewhere, Hedenström (2023) highlighted the influence of external factors, such as fungal infestation, on multiple morphometric parameters [51], corroborating our findings of an association between morphometric parameters and fungi infestation.

The PCA biplot indicates a cohesive influence of fungi infestation on the relationship between morphometric parameters, portraying the two groups distinctly. it has been demonstrated how fungi and external factors, such as environmental stressors, can impact the overall wing morphology in bats [52]. Our results underscore the importance of considering fungi infestation as contributing to the integrated nature of bat wing morphometry.

Conclusion

In conclusion, our comprehensive investigation into both fungal infestation and bat wing morphometry has provided valuable insights into the intricate relationships governing bat adaptations. Fungal analysis revealed a high prevalence of fungi in bat swab samples, with Cladosporium being the most common species. The identification of 12 different fungal species, as confirmed by DNA sequencing, and the diverse phylogenetic tree underscore the environmental ubiquity of these fungi. The evaluation of bat wing morphometry using diverse parameters revealed nuanced responses to fungi infestation. Rain cloud plots effectively depicted the distribution patterns of wing angles, exposing heightened variability in wings unaffected by fungal infestation, a trend consistent with recent research on the impact of fungi on bat wing morphology. Furthermore, the RMS results underscored an amplified distribution of wing morphometry in reaction to fungal presence, emphasizing the intricate nature of these adaptations and suggesting potential implications for the flight behavior of the bats.

The Principal Component Analysis (PCA) biplot explained a strong positive relationship among morphometric parameters, irrespective of fungi infestation. Components 1 and 2 collectively explained a substantial portion of the total variation, emphasizing the interconnectedness of wing features. These findings contribute to the broader understanding of the dynamic interplay between fungi infestation and bat wing morphometry, providing a foundation for further ecological studies and conservation efforts. The observed associations underscore the need for a holistic approach when examining the ecological implications of fungal infections on bat populations.

Competing interests

The author declares that there is no conflict of interest.

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References

1. Teeling EC, Madsen O, Van Den Bussche RA, de Jong WW, Stanhope MJ, Springer MS. Microbat paraphyly and the convergent evolution of a key innovation in Old World rhinolophoid microbats. Proc National Acad Sci 2002;99(3):1431-6. Available from: https://doi.org/10.1073/pnas.022477199
2. Covey E, Neuweiler G. Biology of Bats. New York: Oxford University Press; 2000.
3. Baker M. Mammalia: chiroptera: immunology of bats. Adv Comp Immunol 2018:839-62.
4. Weier SM, Grass I, Linden VM, Tscharntke T, Taylor PJ. Natural vegetation and bug abundance promote insectivorous bat activity in macadamia orchards, South Africa. Biol Conserv 2018; 226:16-23.

Available from: https://doi.org/10.1016/j.biocon.2018.07.017

5. Russo D, Bosso L, Ancillotto L. Novel perspectives on bat insectivory highlight the value of this ecosystem service in farmland: Research frontiers and management implications. Agric Ecosyst Amp Environ 2018; 266:31-8. Available from: https://doi.org/10.1016/j.agee.2018.07.024
6. Kasso M, Balakrishnan M. Ecological and Economic Importance of Bats (Order Chiroptera). ISRN Biodivers 2013; 2013:1-9. Available from: https://doi.org/10.1155/2013/187415
7. Kunz TH, Braun de Torrez E, Bauer D, Lobova T, Fleming TH. Ecosystem services provided by bats. Ann New York Acad Sci 2011;1223(1):1-38. Available from: https://doi.org/10.1111/j.1749-6632.2011.06004.x
8. Makanya AN, Mortola JP. The structural design of the bat wing web and its possible role in gas exchange. J Anat 2007;211(6):687-97. Available from: https://doi.org/10.1111/j.1469-7580.2007.00817.x
9. Faure PA, Re DE, Clare EL. Wound Healing in the Flight Membranes of Big Brown Bats. J Mammal 2009;90(5):1148-56. Available from: https://doi.org/10.1644/08-mamm-a-332.1
10. Madej JP, Mikulová L, Gorošová A, Mikula Š, Řehák Z, Tichý F, Buchtová M. Skin structure and hair morphology of different body parts in the Common Pipistrelle (Pipistrellus pipistrellus). Acta Zool 2012;94(4):478-489. Available from: https://doi.org/10.1111/j.1463-6395.2012.00578.x
11. Pollock T, Moreno CR, Sánchez L, Ceballos-Vasquez A, Faure PA, Mora EC. Wound healing in the flight membranes of wild big brown bats. J Wildl Manag 2015;80(1):19-26. Available from: https://doi.org/10.1002/jwmg.997
12. Gardiner JD, Dimitriadis G, Codd JR, Nudds RL. A Potential Role for Bat Tail Membranes in Flight Control. PLoS ONE 2011;6(3): e18214. Available from: https://doi.org/10.1371/journal.pone.0018214
13. Ceballos-Vasquez A, Caldwell JR, Faure PA. Seasonal and reproductive effects on wound healing in the flight membranes of captive big brown bats. Biol Open 2014;4(1):95-103. Available from: https://doi.org/10.1242/bio.201410264
14. Baerwald EF, D'Amours GH, Klug BJ, Barclay RM. Barotrauma is a significant cause of bat fatalities at wind turbines. Curr Biol 2008;18(16): R695—R696. Available from: https://doi.org/10.1016/j.cub.2008.06.029
15. Cryan PM, Barclay RM. Causes of Bat Fatalities at Wind Turbines: Hypotheses and Predictions. J Mammal 2009;90(6):1330-40. Available from: https://doi.org/10.1644/09-mamm-s-076r1.1
16. Eiting TP, Gunnell GF. Global Completeness of the Bat Fossil Record. J Mamm Evol 2009;16(3):151-73. Available from: https://doi.org/10.1007/s10914-009-9118-x
17. Hedenstrom A, Johansson LC, Wolf M, von Busse R, Winter Y, Spedding GR. Bat Flight Generates Complex Aerodynamic Tracks. Science 2007;316(5826):894-7. Available from: https://doi.org/10.1126/science.1142281
18. Reichard JD, Kunz TH. White-Nose Syndrome Inflicts Lasting Injuries to the Wings of Little Brown Myotis (Myotis

lucifugus). Acta Chiropterologica 2009;11(2):457-64. Available from: https://doi.org/10.3161/150811009x485684

19. Cryan PM, Meteyer CU, Boyles JG, Blehert DS. Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. BMC Biol 2010;8(1). Available from: https://doi.org/10.1186/1741-7007-8-135
20. Fuller NW, Reichard JD, Nabhan ML, Fellows SR, Pepin LC, Kunz TH. Free-Ranging Little Brown Myotis (Myotis lucifugus) Heal from Wing Damage Associated with White-Nose Syndrome. EcoHealth 2011;8(2):154-62. Available from: https://doi.org/10.1007/s10393-011-0705-y
21. Khayat ROS, Shaw KJ, Dougill G, Melling LM, Ferris GR, Cooper G, Grant RA. Characterizing wing tears in common pipistrelles (Pipistrellus pipistrellus): investigating tear distribution, wing strength, and possible causes. Journal of Mammalogy 2019;100(4):1282-1294. Available from: https://doi.org/10.1093/jmammal/gyz081
22. Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-Zier BM, Buckles EL, et al. Bat White-Nose Syndrome: An Emerging Fungal Pathogen?. Science 2009;323(5911):227-227. Available from: doi: 10.1126/science.1163874
23. Lorch JM, Palmer JM, Lindner DL, Ballmann AE, George KG, Griffin K, Knowles S, Huckabee JR, Haman KH, Anderson CD, Becker PA, Buchanan JB, Foster JT, Blehert DS. First Detection of Bat White-Nose Syndrome in Western North America. mSphere 2016;1(4): e00148-00116. Available from: https://doi.org/10.1128/msphere.00148-16
24. Gargas A, Trest MT, Christensen M, Volk TJ, Blehert DS. *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. Mycotaxon 2009;108(1):147-54. Available from: https://doi.org/10.5248/108.147
25. Puechmaille SJ, Frick WF, Kunz TH, Racey PA, Voigt CC, Wibbelt G, Teeling EC. White-nose syndrome: is this emerging disease a threat to European bats? Trends Ecol Amp Evol 2011;26(11):570-6. Available from: https://doi.org/10.1016/j.tree.2011.06.013
26. Wibbelt G, Kurth A, Hellmann D, Weishaar M, Barlow A, Veith M, Prüger J, Görföl T, Grosche L, Bontadina F, Zöphel U, Seidl HP, Cryan PM, Blehert DS. White-Nose Syndrome Fungus (Geomyces destructans) in Bats, Europe. Emerg Infect Dis 2010;16(8):1237-43. Available from: https://doi.org/10.3201/eid1608.100002
27. Toju H, Tanabe AS, Yamamoto S, Sato H. High-Coverage ITS Primers for the DNA-Based Identification of Ascomycetes and Basidiomycetes in Environmental Samples. PLoS ONE 2012;7(7): e40863. Available from: https://doi.org/10.1371/journal.pone.0040863
28. Romanelli AM, Fu J, Herrera ML, Wickes BL. A universal DNA extraction and PCR amplification method for fungal rDNA sequence-based identification. Mycoses 2014;57(10):612-22. Available from: https://doi.org/10.1111/myc.12208
29. Hall, T. BioEdit [computer software]. Version 7.0.0. Ibis Therapeutics, a division of Isis Pharmaceuticals, Inc.
30. Hall BG. Building Phylogenetic Trees from Molecular Data with MEGA. Mol Biol Evol

2013;30(5):1229-35. Available from: https://doi.org/10.1093/molbev/mst012

31. Khayat RO, Shaw KJ, Mead C, Ryan H, Dougill G, Melling LM, Grant RA. Measuring the effect of wing tears on flight in common pipistrelle bats (Pipistrellus pipistrellus). Mammal Commun 2020; 6:11-20. Available from: https://doi.org/10.59922/wlqr5749
32. Khodadadi H, Karimi L, Jalalizand N, Adin H, Mirhendi H. Utilization of size polymorphism in ITS1 and ITS2 regions for identification of pathogenic yeast species. J Med Microbiol 2017;66(2):126-33. Available from: https://doi.org/10.1099/jmm.0.000426
33. Arlorio M, Coïsson JD, Martelli A. Identification of Saccharomyces cerevisiae in bakery products by PCR amplification of the ITS region of ribosomal DNA. Eur Food Res Technol 1999;209(3-4):185-91. Available from: https://doi.org/10.1007/s002170050477
34. Vanderwolf KJ, McAlpine DF. Hibernacula microclimate and declines in overwintering bats during an outbreak of white-nose syndrome near the northern range limit of infection in North America. Ecol Evol 2021;11(5):2273-88. Available from: https://doi.org/10.1002/ece3.7195
35. Meierhofer MB, Johnson JS, Perez-Jimenez J, Ito F, Webela PW, Wiantoro S, Bernard E, Tanalgo KC, Hughes A, Cardoso P, Lilley T, Mammola S. Effective conservation of subterranean-roosting bats. Conserv Biol 2023: e14157. Available from: https://doi.org/10.1111/cobi.14157
36. Elliott TF, Jusino MA, Trappe JM, Lepp H, Ballard GA, Bruhl JJ, Vernes K. A global review of the ecological significance of symbiotic associations between birds and fungi. Fungal Divers 2019;98(1):161-94. Available from: https://doi.org/10.1007/s13225-019-00436-3
37. Le Roy C, Cornette R, L laurens V, Debat V. Effects of natural wing damage on flight performance in Morpho butterflies: what can it tell us about wing shape evolution? J Exp Biol 2019;222(16): jeb204057. Available from: https://doi.org/10.1242/jeb.204057
38. Ineson KM, Richardson CS, Looney CE, Fuller NW, Reichard JD. Patterns of post-hibernation wing damage healing in little brown bats (*Myotis lucifugus*) impacted by white-nose syndrome. J Mammal 2023;104(5):1072-1083. Available from: https://doi.org/10.1093/jmammal/gyad054
39. López-Aguirre C, Hand SJ, Koyabu D, Tu VT, Wilson LA. Prenatal Developmental Trajectories of Fluctuating Asymmetry in Bat Humeri. Front Cell Dev Biol 2021;9. Available from: https://doi.org/10.3389/fcell.2021.639522
40. Whiting JC, Doering B, Aho K, Rich J. Long-term patterns of cave-exiting activity of hibernating bats in western North America. Sci Rep 2021;11(1):8175. Available from: https://doi.org/10.1038/s41598-021-87605-0
41. Mushabati LM. Changes in the seasonal activity of bats occuring in the Kunene region, Namibia: Influence of environmental factors [Doctoral dissertation]. Namibia: University of Namibia; 2019.
42. Vanderwolf KJ, Campbell LJ, Goldberg TL, Blehert DS, Lorch JM. Skin fungal assemblages of bats vary based on

susceptibility to white-nose syndrome. ISME J 2021; 15:909-920. Available from: https://doi.org/10.1038/s41396-020-00821-w

43. Irschck DJ, Henningsen JP. Functional morphology: muscles, elastic mechanisms, and animal performance. Princeton Ecology Guide 2009: 27-37.

44. Zepeda Mendoza ML, Xiong Z, Escalera-Zamudio M, Runge AK, Thézé J, Streicker D, et al. Hologenomic adaptations underlying the evolution of sanguivory in the common vampire bat. Nat Ecol Amp Evol 2018;2(4):659-68. Available from https://doi.org/10.1038/s41559-018-0476-8

45. Federici L, Masulli M, De Laurenzi V, Allocati N. An overview of bats microbiota and its implication in transmissible diseases. Front Microbiol 2022;13; e.1012189. Available from: https://doi.org/10.3389/fmicb.2022.1012189

46. Hathaway JJ, Salazar-Hamm PS, Caimi NA, Natvig DO, Buecher DC, Northup DE. Comparison of Fungal and Bacterial Microbiomes of Bats and Their Cave Roosting Environments at El Malpais National Monument, New Mexico, USA. Geomicrobiol 2023:1-16. Available from: https://doi.org/10.1080/01490451.2023.2283427

47. Bure CM, Moore MS. White-nose syndrome: A fungal disease of North American hibernating bats. In: Encyclopedia of Caves. editors. William B. White, David C. Culver and Tanja Pipan. 3ed. Elsevier; 2019.; p. 1165-74. Available from: https://doi.org/10.1016/b978-0-12-814124-3.00136-9

48. Baumgart SL. Morphology and Evolution of the Avian Flight Apparatus in Relation to Ecology and Function. Chicago: The University of Chicago; 2021.

49. Voigt CC. Bat flight with bad wings: is flight metabolism affected by damaged wings? J Exp Biol 2013;216(8):1516-21. Available from: https://doi.org/10.1242/jeb.079509

50. Forsythe A, Fontaine N, Bissonnette J, Hayashi B, Insuk C, Ghosh S, Kam G, Wong A, Lausen C, Xu J, Cheeptham N. Microbial isolates with Anti-Pseudogymnoascus destructans activities from Western Canadian bat wings. Sci Rep 2022;12(1): e.9895. Available from: https://doi.org/10.1038/s41598-022-14223-9

51. Hedenström A. Effects of wing damage and moult gaps on vertebrate flight performance. J Exp Biol 2023;226(9). Available from: https://doi.org/10.1242/jeb.227355

52. Martínková N, Bačkor P, Bartonička T, Blažková P, Červený J, Falteisek L, et al. Increasing Incidence of Geomyces destructans Fungus in Bats from the Czech Republic and Slovakia. PLoS ONE 2010;5(11): e13853. Available from: https://doi.org/10.1371/journal.pone.0013853