

Interplay between the morphometry of the lungs and the mode of locomotion in birds and mammals

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ABSTRACT

We studied the lung diffusion parameters of two species of birds and two species of mammals to explore how structural and functional features may be paralleled by differences in life style or phylogenetic origin. We used two fast-flying species (one mammal and one bird), one running mammal and one bird species that flies only occasionally as models. The harmonic mean thickness of the air-blood barrier was very thin in the species we studied. An exception was the Chilean tinamou *Notoprocta perdicaria*, which only flies occasionally. It showed an air-blood barrier as thick as that of flightless Galliformes. We found that the respiratory surface density was significantly greater in flying species compared to running species. The estimated values for the oxygen diffusion capacity, D_{lO_2} follow the same pattern: the highest values were obtained in the flying species, the bat and the eared dove. The lowest value was in *N. perdicaria*. Our findings suggest that the studied species show refinements in their morphometric lung parameters commensurate to their energetic requirements as dictated by their mode of locomotion, rather than their phylogenetic origin. The air-blood barrier appears to be thin in most birds and small mammals, except those with low energetic requirements such as the Chilean tinamou. In the species we studied, the respiratory surface density appears to be the factor most responsive to the energetic requirements of flight.

Key terms: diffusing capacity, lungs, flight.

INTRODUCTION

The morphology and the efficiency of respiratory organs correspond with the oxygen requirements of animals. Flying animals, such as birds, insects and bats, which have high aerobic capacities, appear to have the most efficient respiratory organs (Maina, 2000a; Maina and King, 1984; Maina et al., 1991).

Among extant vertebrates, flight evolved independently in birds and bats. According to their phylogenetic and morphological differences, the strategies to accomplish this energetically expensive form of locomotion appear to be different (Maina, 1998). Birds exchange gas through a

process in which a wide range of pulmonary and extrapulmonary factors are integrated, operating sub-maximally (a broad-based low-keyed strategy); bats, however, appear to have a restricted number of factors operating close to their maximal levels (a narrow-based high-keyed strategy) (*sensu* Maina, 1998), probably related to the mammalian lung design that they possess.

Of all components of the respiratory chain, pulmonary diffusion is the only variable that exclusively depends upon morphometric intrapulmonary parameters (Weibel et al., 1991), providing an excellent model to study of the structural optimization of the lung. Oxygen flow is

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directly related to the oxygen partial pressure gradient between the alveoli and blood capillaries, and the oxygen diffusion capacity (D_{O_2}) (Weibel, 1970/71; Maina, 2002a). The latter is determined by the ratio between the respiratory surface area (AA) and the harmonic mean thickness of the air-blood barrier (τ_h) (Weibel, 1970/71; Gehr et al., 1981; Maina et al., 1982; Maina and King, 1984; Maina et al., 1991; Maina and Nathaniel, 2001; Maina, 2002a). Since AA may be expressed as the product of the volume of the exchange lung tissue (V_{LE}), the respiratory surface density (AS_d), and Krogh's permeation coefficient (κ) (Gehr et al., 1981; Maina, 2002a) the oxygen conductance (D_{O_2}) may only be modified by changes in V_{LE} , AS_d

$$\text{or } \tau_h: D_{O_2} = \kappa \frac{AS_d \cdot V_{LE}}{\tau_h} .$$

There is evidence that birds and bats have different methods to optimize oxygen flow. For example, the respiratory surface density of bats appears to be only slightly lower than that of non-flying mammals, but much lower than that of birds. Also, the harmonic mean thickness of the blood-gas barrier in bats is slightly thinner than that of non-flying mammals, yet thicker than that of birds (Maina, 2000b). In birds, the lung volume is lower than that of non-flying mammals, while bat lungs show the opposite trend. Although a high respiratory surface density and a thin air-blood barrier have been reported in several small bird species, such as *Colibri coruscans* (Dubach, 1981), most birds have similar values of respiratory surface density and thickness of the blood-gas barrier (Maina, 2002a). However, the Orders containing non-flying birds or birds that fly occasionally (e.g., Galliformes, Struthioformes and Sphenisciformes), appear to have a thicker blood-gas barrier (Maina, 2002a).

We studied the lung diffusion parameters of two species of birds and two species of mammals to explore how structural and functional features may be paralleled by differences in life style (running or flying) or phylogenetic origin (mammal or bird). To do this, two fast-

lying species (one mammal and one bird), one running mammal and one bird species that flies only occasionally were used as models.

MATERIAL AND METHODS

We used the Leaf-eared mouse *Phyllotis darwini* (Rodentia: Muridae) as our running mammal model ($n = 3$; $M_b = 75.36 \pm 4.96$ g where M_b is the body mass: $\bar{x} \pm 1sd$), a rodent dwelling in grasslands, scrub, open forest and rocky areas. Our flying mammal model was the Mouse-eared bat *Myotis chiloensis* (Chiroptera: Vesperilionidae) ($n = 3$; $M_b = 6.05 \pm 0.10$ g), a small bat inhabiting thickets near watercourses. The bird models were 1) the Eared dove *Zenaida auriculata* (Columbiformes: Columbidae) ($n = 3$; 141.1 ± 1.55 g), a common fast-flying bird which inhabits farm lands and urban zones from the coast to the Andes foothills; and 2) the Chilean tinamou *Nothoprocta perdicaria* ($n = 3$; 398.33 ± 11.70 g), an endemic species with a chubby body and short wings dwelling in grasslands, thickets and farmland. This species usually runs between shrubs and only occasionally makes short flights.

Three adult individuals of each species were captured with mist nets or Shermann traps in central Chile during March, 2004. After capture, animals were weighed, killed by an overdose of anesthetic by intraperitoneal injection, dissected and the trachea were exteriorized and cannulated. In the case of birds we avoided injection into the air sacs. A quantity of 2.3% glutaraldehyde in phosphate buffer (ph 7.4) was introduced in the trachea (Maina 2002a). When the fixative solution exceeded the level of the trachea, we closed it and tied it to the cannula. Immediately following this procedure, the lungs were removed and immersed in the same fixative at 4°C for a minimum of 2 hours. Next, tissues (right and left lungs) were processed for routine electronic transmission microscopy. Briefly, two pieces of 1-2 mm thickness were obtained from each lobe in mammals and four randomly chosen pieces were obtained from each lung of birds. The

pieces were washed with buffer and post-fixed with 1% osmium tetroxide for 1 hour at 4°C. Tissues were then dehydrated in graded concentrations of alcohol and infiltrated and embedded in epoxy resin constructing cubes of 2-3 mm³, obtaining semi-thin VUR sections of 1µm. Tissue samples were stained with 1% toluidine blue. Ultrathin sections of 60-90 nm of thickness were made and mounted on copper mesh grids. These sections were contrasted with lead citrate. Sections were studied through optical and transmission electron microscopy (JEOL/JEM 100SX). Eight to twelve sections were photographed, digitalized and twelve semi-thin and twelve ultra-thin sections per individual were analyzed using Scion Image Software.

The respiratory surface density (AS_d) was estimated by means of line-intersection stereologic method (Weibel, 1970/71):

$$AS_d = \frac{2N}{1/2 \cdot P_T \cdot Z}, \text{ where } N \text{ is the number}$$

of intersections between line probes of length Z with the respiratory surface and P_T is the number of test points (Figure 1).

The harmonic mean thickness of the air-blood barrier (τ_h) was estimated by a stereologic method in a square lattice grid as suggested by Weibel (1970/71) and

$$\text{Maina (2002a): } \frac{1}{\tau_h} = \frac{3}{2} \cdot \frac{\sum_{j=1}^m f_j \cdot \frac{1}{l_j}}{\sum_{j=1}^m f_j}, \text{ where } l_j$$

is the mid-value of intercept length of linear probes, f_j the frequency of class j and m the number of classes (Figure 2).

The factor effects (taxa and locomotion type) on τ_h and AS_d were analyzed with a two-way repeated measures ANOVA. Multiple comparisons were performed with Tukey's test.

RESULTS

The respiratory surface density was different between mammals and birds (F_{6,3} = 123,2, p < 0.01) and between flying

species and running or occasionally flying species (F_{6,3} = 114,9, p < 0.01). The interaction between taxa and locomotion type was not significant (F_{6,3} = 6.59). The *a posteriori* Tukey analyses showed that these differences were sustained by differences between the flying bird *Z. auriculata* and the running mammal *P. darwini*. The former has a higher AS_d, therefore, the difference between birds and mammals appears to be only a consequence of the high AS_d of *Z. auriculata*. However, differences among locomotion types appear to be well sustained (Table 1 and Figure 3).

The harmonic mean thickness also showed a significant effect of taxa (F_{6,3} = 19.15, p < 0.01), locomotion type (F_{6,3} = 26.89, p < 0.01) and interaction (F_{6,3} = 29.52; p < 0.01). However, multiple comparisons revealed that these differences were only sustained by the thicker air-blood barrier observed in *N. perdicaria* (Figure 4).

DISCUSSION

The oxygen diffusion capacity (D_{O2}) may be estimated from morphometric information as a theoretical capacity determined by the exchange surface and the tissue barrier (Weibel et al., 1991). Regarding only the tissue component, the tissue oxygen diffusion capacity (D_{tO2}) from air to plasma is determined by the harmonic mean thickness of the air-blood barrier (τ_{ht}), the most appropriate estimator of the conductance (Weibel and Knight, 1964; Weibel, 1970/71), the respiratory surface density (AS_d) and the lung volume (V_L). In this study we only analyzed τ_{ht} and AS_d.

Lungs of small birds with high mass-specific metabolisms (Novoa 2003) exhibit different morphometric refinements (Maina 2000a). For instance, the order Passeriformes, a group that includes 60% of extant bird species, possess more efficient lungs compared to non-passerine species. In contrast, soaring and gliding birds, need less energy during flight and appear to have relatively less efficient lungs (Vitali and Richardson, 1998; Maina, 2000a; 2002a).

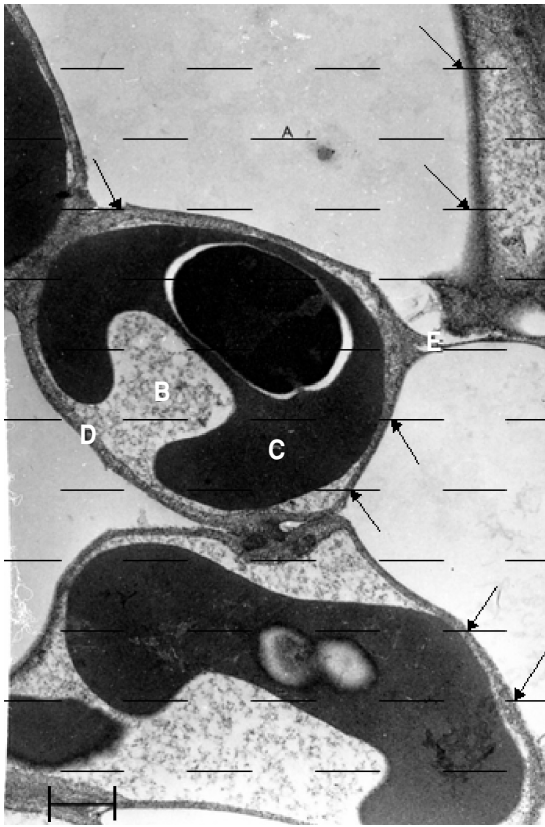


Fig. 1: Electron micrograph of gas exchange tissue of *Zenaida auriculata*. 3000X with a test line system to estimate respiratory surface

density (AS_d): $AS_d = \frac{2N}{1/2 \cdot P_T \cdot Z}$, where N is the

number of intersections between line probes of length Z with the respiratory surface and P_T is the number of test points. Each line with two test points, thus $1/2 P_T Z$ represents the length of the total test line system. Arrows mark some intersections. A: Air capillaries. B: Blood capillaries. C: Erythrocytes. D: Air-blood barrier. E: Tissue not involved in gas exchange. Scale bar, 0.5 μm .

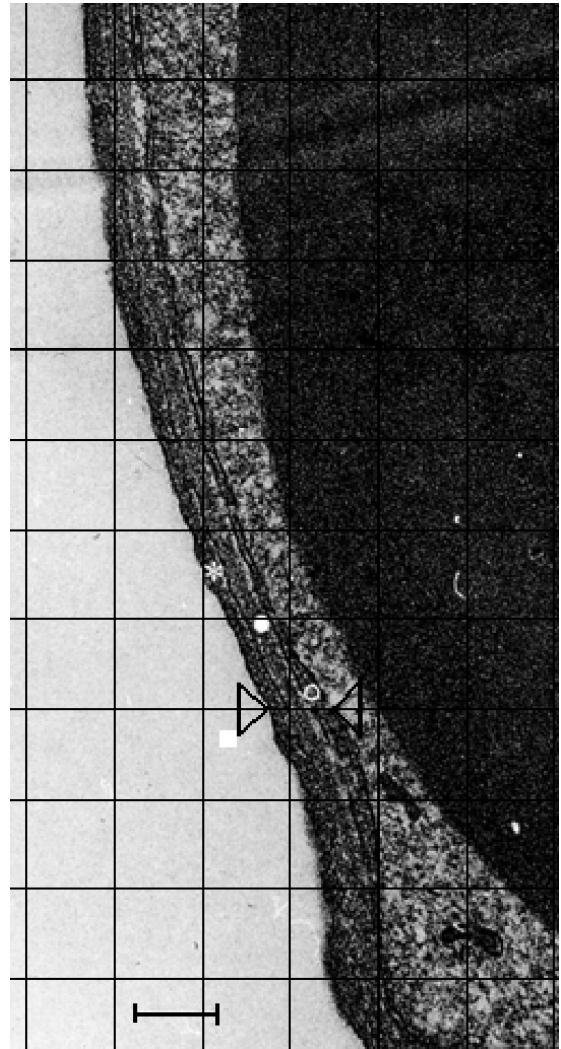


Fig. 2: Air-blood barrier of lung of *Phyllotis darwini*. With a square lattice grid to estimate the harmonic mean thickness of the air-blood barrier (τ_{ht}). Arrowheads mark an example of the points between which the thickness of the air-blood barrier was measured.

*: Epithelium, O: Basement membrane, •: Endothelium. 20000X. Scale bar, 0.23 μm .

TABLE 1

Respiratory surface density (AS_d) and harmonic mean thickness of the air-blood barrier (τ_{ht}) in the studied species. All measurements are presented as average \pm 1 standard deviation

	AS_d (mm^{-1})	τ_{ht} (μm)
<i>Z. auriculata</i>	394.99 \pm 27.3	0.171 \pm 0.026
<i>N. perdicaria</i>	230.57 \pm 2.57	0.469 \pm 0.019
<i>M. chiloensis</i>	257.18 \pm 11.18	0.219 \pm 0.015
<i>P. darwini</i>	145.19 \pm 9.2	0.223 \pm 0.033

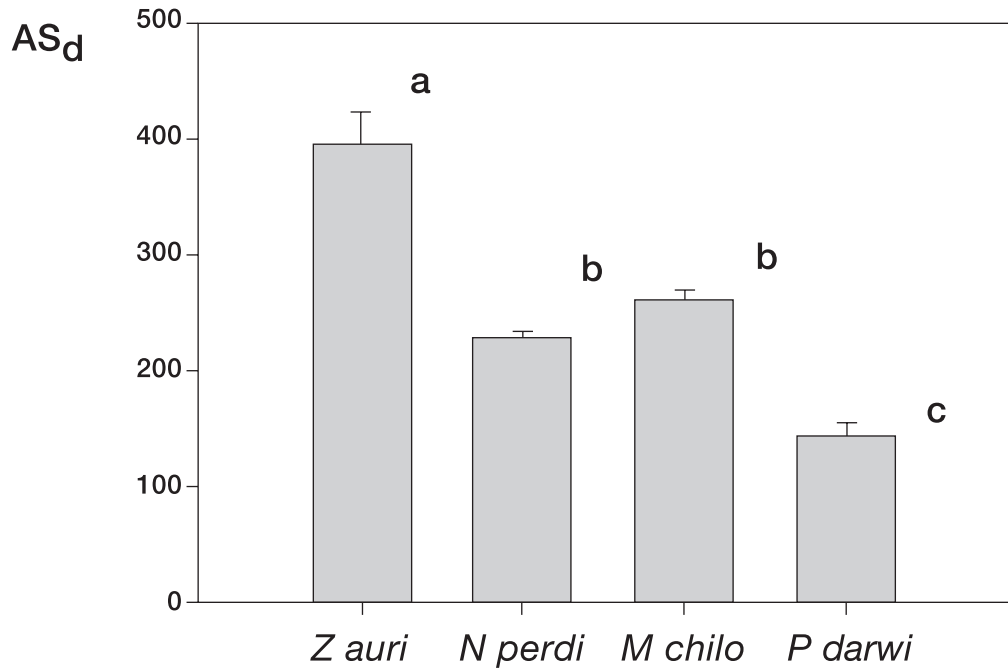


Fig. 3: Respiratory surface density (AS_d) in the studied species. All measurements are presented as average ± 1 standard deviation. Different letters indicate significant differences among species from the Tukey test.

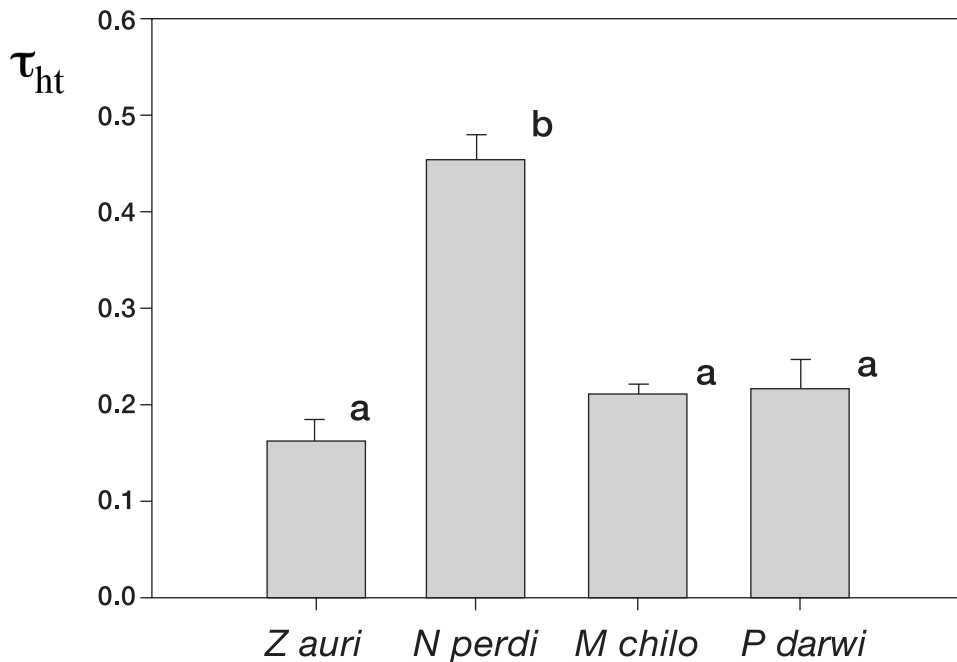


Fig 4: Harmonic mean thickness of the air-blood barrier (τ_{ht}) in the studied species. All measurements are presented as average ± 1 standard deviation. Different letters indicate significant differences among species from the Tukey test.

Of the four species studied in this work, the thinnest air-blood barrier (see Table 1) was found in the flying bird *Z. auriculata*. Its air-blood barrier was almost as thin as the Rock dove *Columbia livia* (0.172 μm) (Maina, 2002a). The air-blood barrier of the bat *M. chiloensis* was also very thin (0.219 \pm 0.01 μm), similar to other Vespertilionidae, such as *Minopterus minor* (0.216 μm), and was in the range of several other bats, which have values between 0.202 μm and 0.282 μm (Maina et al., 1982; Maina and King, 1984). However, the rodent *P. darwini* also showed a thin air-blood barrier (0.223 \pm 0.03 μm). This value is surprising, because it is similar to the smallest known value in mammals, which is found in the shrew *Suncus ethruscus* (0.23 μm), and similar to that of *Mus musculus* (0.29 μm) (Gehr et al., 1981). Since *P. darwini* is not a very small mammal and probably does not have particularly high energetic requirements compared to other mammals (see Nespolo et al. 2001), we considered the possibility that the values measured were artificially diminished by the methods. In this case, the fixative and buffer used in this study would have produced a thickness barrier 6-24% thinner than the combination used by Gehr et al. (1981, for small mammals (Maina, 2002a)). Even if we applied a correction to our value, we would obtain 0.236-0.277 μm , which is still a very small value range. Perhaps the air-blood barrier observed in *P. darwini* is a consequence of its high Andean origin, which supposes the existence of higher energy requirements for thermoregulation and hence the possession of an optimized air-blood barrier, in spite of the fact that its current energy requirements are lower since its present range is a stable Mediterranean habitat (Engel et al. 1998)

The highest value of τ_{ht} was found in the tinamou *N. perdicaria* (0.469 \pm 0.02 μm). This is the first report to date of measurements of the lung parameters for any Tinamiform, so we cannot compare our results with those from closely related species. However, *N. perdicaria* has a thicker air-blood barrier than most bird species studied to date. Most birds have a lower τ_{ht} , in general ranging between 0.099

and 0.385 μm . Only *Struthio camelus* and *Spheniscus humboldti* have τ_{ht} of roughly 0.5 μm (Maina, 2002a). The value of τ_{ht} in *N. perdicaria* approaches those of Galliformes species, which range from 0.318-0.346 μm in the domestic fowl *Gallus gallus* to 0.385 μm in *Meleagris gallopavo*. The flightless domestic galliform species have relatively thicker air-blood barriers, lower respiratory surface areas and pulmonary diffusing capacities compared to species with higher energetic requirements (Maina, 2002a; 2002b). This may be also the case for *N. perdicaria*. Other members of Ratites have dissimilar thicknesses of the air-blood barrier, 0.530 μm in *Struthio camelus* (Struthioformes) and 0.232 μm in *Dromaius novaehollandiae* (Casuariiformes), suggesting that phylogenetic origin is not responsible.

The respiratory surface density showed strong differences between flying and "non-flying" species. The highest values were observed in the dove *Z. auriculata* (310.29 \pm 17.5 mm^{-1}) and the bat *M. chiloensis* (202.03 \pm 7.1 mm^{-1}). *Zenaida auriculata* exhibits values in the range of other fast-flying birds, such as *Columba livia* (302 mm^{-1}) and several Passeriformes (\approx 300 mm^{-1}) (Maina, 2002a). The respiratory surface density of the bat *M. chiloensis* was higher than those of the small shrews, 121 mm^{-1} on average (Gehr et al., 1981), and those of several bats spp. (see Maina et al., 1982). The running mammal *P. darwini* and the occasionally flying *N. perdicaria* showed low respiratory surface density. The former has values similar to other non-flying mammals, but *N. perdicaria* has a low value only comparable to the domestic Galliformes and great non-flying birds such as *Struthio camelus* and *Dromaius novaehollandiae*.

The thickness of the air-blood barrier and the respiratory surface density do not determine uniquely the oxygen diffusion capacity. Estimating lung volume from allometric relationships, considering 90 and 46% of lung volume as parenchymal volume in mammals and birds respectively (Maina, 2002a) and using a constant value for the Krogh's diffusion coefficient $\kappa = 4.1 \cdot 10^{-8} \text{ cm}^2 / \text{s} \cdot \text{Pa}$ because this is not greatly

influenced by temperature (Maina 2002a), we estimated the mass-specific oxygen diffusion capacity (D_tO_2/M_b) for the studied species (Table 2). According to these estimations, the bat and the dove have much greater diffusion capacity than the other species.

During flight, the energetic requirements of bats are comparable to that of birds (Jurgens et al., 1981). Birds satisfy these requirements by means of morphological specializations, such as fine subdivision of exchange tissue and thinner air-blood barrier, among others. The respiratory surface density, a variable that denotes the relative size of the terminal gas exchange components, has been reported as substantially greater in the avian lung than in the lungs of bats and the non-flying mammals (Maina 2000a; 2002b). However, the respiratory surface density of the species studied in this work appears to be high in good flyers, the bat and dove spp., but not in the occasionally flying bird *N. perdicaria*. This finding suggests a differential morphometry in response to energetic demands.

Since bats achieve high oxygen consumption during flight (Norberg, 1987; Thomas, 1987) without cross-current or counter-current exchange systems, their

lungs show other structural adaptations such as: 1) exceptionally large lungs (and hearts) (Jurgens et al., 1981; Canals et al., 2005b), 2) relatively extensive mass specific surface area and 3) thin air-blood barrier, among others (Maina et al., 1982). The studied bat *M. chiloensis* has a large lung, about $0.058 \text{ cm}^3/\text{g}$ (Canals et al., 2005b,c), while shrews and hummingbirds, the most metabolically active in their respective vertebrate classes, have average values of $0.03 \text{ cm}^3/\text{g}$ (Gehr et al., 1981) and $0.043 \text{ cm}^3/\text{g}$ (Maina et al., 1982), respectively. The estimated mass specific respiratory surface area of the bat was 2.8 times that of the dove, 4.1 times that of *P. darwini* and 4.8 times that of *N. perdicaria*. It was larger than the value reported for the small hummingbird *Colibri coruscans*, $87 \text{ cm}^2/\text{g}$ (Maina, 2002a), but smaller than that of the small bat *Epomophorus wahlbergi*, $138 \text{ cm}^2/\text{g}$ (Maina et al., 1991).

The estimated values for the oxygen diffusion capacity, D_tO_2 , confirm the same pattern: the highest values in the good fliers, the bat and the Eared dove, and the lowest value in the Chilean tinamou. The D_tO_2 of *M. chiloensis* was greater than that of *Pipistrellus pipistrellus* ($12.8 \times 10^{-6} \text{ mlO}_2/\text{Pa s g}$) and close to that of *E. wahlbergi* ($23.5 \times 10^{-6} \text{ mlO}_2/\text{Pa s g}$), these are two

TABLE 2

Pulmonary diffusion parameters of studied species: AS_d = Respiratory surface density; τ_{ht} = Harmonic mean thickness of the air-blood (tissue) barrier; M_b = Body mass; V_L = Lung volume estimated by allometry; RA = Respiratory area ($RA = SA_d * V_{LE}$) where V_{LE} is the exchange lung volume estimated as 90% of V_L in mammals and 46% of V_L in birds (see Maina, 2002a); ADF is an anatomic diffusing factor ($ADF = AS_d/\tau_{ht}$); D_{tO_2} = Pulmonary morphometric diffusion capacity of the air-blood barrier ($D_{tO_2} = RA * K / \tau_{ht}$). The Krogh's coefficient was considered as a constant $\kappa = 4.1 \cdot 10^{-8} \text{ cm}^2 / \text{s} \cdot \text{Pa}$. All measurements and estimations are presented as average \pm 1 standard deviation.

	M_b (g)	RA (cm ²)	RA / M_b (cm ² /g)	V_L (cm ³)	V_L / M_b (cm ³ /g)	ADF (cm ²)(x10 ⁷)	D_{tO_2}/M_b (mlO ₂ s ⁻¹ Pa ⁻¹ g ⁻¹) (x10 ⁻⁶)
<i>Z. auriculata</i>	141 ± 1.55	6860.57±574.8	48.59±3.44	3.77±0.06	0.027±0.00005	23.43±3.58	11.81 ± 1.79
<i>N. perdicaria</i>	398 ± 11.7	12009.55±578.6	30.14±0.39	11.32±0.43	0.028±0.00006	4.92±0.25	2.64 ± 0.14
<i>M. chiloensis</i>	6 ± 0.10	833.33±38.2	138.8±6.17	0.36±0.01	0.06±0.00007	11.77±0.32	26.0 ± 0.69
<i>P. darwini</i>	75 ± 4.96	2514.13±470.6	33.17±3.51	1.91±0.17	0.026±0.00015	6.68±1.75	6.26 ± 1.68

small bats with very high D_tO_2 (Maina et al., 1982; Maina and King, 1984). *Z. auriculata* showed a D_tO_2 close to *C. livia* (10.0×10^{-6} mlO₂/Pa s g) and several other Columbiformes (Maina, 2002a). *P. darwini* showed a D_tO_2 close to *M. musculus* (4.2×10^{-6} mlO₂/Pa s g) (Gehr et al., 1981) and *N. perdicaria* showed the lowest D_tO_2 , which is close to the domestic fowl and the ostrich, both representatives of non-flying birds.

Considering the allometric relationships for the total oxygen diffusion capacity from air to red cells, D_{LO_2} : $D_{LO_2}(\text{birds}) = 7.19 \cdot M_b^{0.9198}$; $D_{LO_2}(\text{bats}) = 25.96 \cdot M_b^{0.6240}$ and $D_{LO_2}(\text{nonflyingmammals}) = 5.92 \cdot M_b^{0.9513}$, where M_b is in grams and D_{LO_2} in mm³O₂ min⁻¹mmHg⁻¹ and the observation that D_{LO_2} is about 1/10 of D_tO_2 (D_{LO_2}/D_tO_2 ratio = 0.092 ± 0.07 in birds and 0.10 ± 0.031 in bats) (Canals et al., 2005a), we did estimate the expected D_tO_2 value for the species we studied. These values were 6.06×10^{-6} mlO₂ s⁻¹Pa⁻¹g⁻¹ for the dove; 5.57×10^{-6} mlO₂ s⁻¹Pa⁻¹g⁻¹ for the Chilean tinamou; 16.69×10^{-6} mlO₂ s⁻¹Pa⁻¹g⁻¹ for the bat and 6.01×10^{-6} mlO₂ s⁻¹Pa⁻¹g⁻¹ for *P. darwini*. The values that we obtained represented 153.13%; 37.14%; 122.22% and 81.86% of those expected values respectively, reinforcing the idea that good flying species have greater oxygen diffusion capacities despite body mass differences.

In short, the studied species showed refinements in their morphometric lung parameters in response to energetic requirements associated mainly with their form of locomotion, rather than their phylogenetic origin. The air-blood barrier appears to be thin in most birds and small mammals, except those with low energetic requirements such as the Chilean tinamou. In the species we studied, the respiratory surface density appears to be the main factor responding to the energetic requirements of flight. However in the bats large lungs contribute to high oxygen diffusion capacity paralleling their high metabolic demand for oxygen. Since the context in which the interplay described in this work is allometric we would expect such interplay to hold true over a vast range of animals of different body weights.

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