Body Mass, Phylogeny and Diet Composition Affects Kidney Morphology in Passerine Birds

Gonzalo Barceló,[1]* Jonathan Salinas,[1] and Pablo Sabat[1,2]

[1]*Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile*
[2]*Center for Advanced Studies in Ecology and Biodiversity, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile*

ABSTRACT   We studied the renal morphology of 16 species of passerines to assess whether the composition of the diet of birds have been paralleled by differences in the characteristics of the kidneys. We determined the number and length of the medullary cones and the percentage of renal medulla in birds with contrasting dietary habits and then correlated these features with the percentage of nitrogen and proportion of invertebrates in the diet. To examine the correlation between kidney and diet variables, we first used standard correlation and least square regression, and correlation and regression on phylogenetically independent data. We found that the mass of the medullary portion of the kidney, and the medullary cone length were negatively correlated with the percentage of invertebrates present in the diet. We hypothesized that the further development of the renal medulla observed in granivorous birds may be correlated with the small amount of water present in the seeds. Our results suggest that the availability of water in different foods is probably one of the main factors that have led to structural and probably functional changes of the kidney in the studied species. J. Morphol. 273:842–849, 2012.   © 2012 Wiley Periodicals, Inc.

KEY WORDS: dietary habits; kidney; passeriformes; phylogeny; water

INTRODUCTION

Dietary habits are influenced by both environmental food availability as well as quality, and by the animal's physiological ability to forage, digest and metabolize different nutrients, electrolytes, secondary compounds, and water (Klasing, 1998; Karasov and Martinez del Río, 2007). The selection of a particular food item, such as insects, fruit, nectar and seeds might have consequences for energy and water balance. For instance, consumption of fruit and nectar, which are rich in water and sugars, requires water disposal capacity. In contrast, the consumption of a high protein diets, such as insects, requires a high capability to dispose of nitrogenous waste (Schondube et al., 2001; Singer, 2003). Thus, the particular composition of water, electrolytes and proteins in ingested foods determines the amount of nitrogenous waste, electrolytes, and water that animals must process. In terrestrial birds, this function is accomplished primarily by the kidney (Yokota et al., 1985; Roxburgh and Pinshow, 2002; Braun, 2003). Consequently, the dietary chemical composition may be a selective force that modulates the morphology and renal function in birds.

Kidney function and morphology has been studied both at inter and intraspecific level in birds (see Braun, 1993; McNab, 2002; Bicudo et al., 2010). Comparative studies have revealed that both intrinsic and ecological factors seem to play a role in the osmoregulatory capacity. For instance, it has been reported that variations in renal function are affected by body mass (Goldstein and Braun, 1989), salt load (Sabat et al., 2006a) and the habitat aridity (Casotti and Braun, 2000). At an interspecific level, it has been suggested that functional changes in the kidney of birds are shown by differences in the renal anatomy (Casotti and Braun, 2000).

The fundamental mechanisms driving the ability to concentrate urine in birds and mammals is a countercurrent multiplier system producing an osmolarity gradient in the renal medulla (Goldstein and Skadhauge, 2000; Bozinovic and Gallardo, 2006). One of the basics of the countercurrent multiplier principle in the kidney of birds is that the longer the loop of Henle, present in the mammalian-type nephrons, the greater the multiplier effect. Therefore, longer cones (with longer loops of Henle) are able to retain more water through a more hyperosmotic urine production (Casotti et al., 2000). As noted by Goldstein and

Braun (1989) and McNab (2002), there is not a simple relationship between structural indicators and the ability to concentrate urine on a broad interspecific comparison of birds (Goldstein and Braun, 1989). However, when the comparison is made between related birds a significant relationship between the structure and bird's life modes seem to be present (Casotti and Braun, 2000). In this vane, the proportion of kidney mass found in the medulla increases with the proportion of mammalian-type nephrons (see McNab, 2002 and references therein). Accordingly, the ability to produce hyperosmotic urine (maximum urine concentrating ability), and hence to cope with aridity and with diets with high salt loads, is correlated with both the relative size of the kidney, with the fraction of the kidney owed to medullary tissue, and with the relative number of medullary cones (Goldstein and Braun, 1989; Sabat et al., 2004a; Hill, 2004). Thus, high renal concentrating ability improves the efficiency of renal water conservation, and therefore, species with the highest proportion of renal medulla must be highly effective in water conservation (Warui, 1989).

Conversely, several studies have shown that dietary composition can affect kidney structure and function in birds (McNabb et al., 1972; Ward et al., 1975a,b; Singer, 2003; Sabat et al., 2006a,b). Experimental studies demonstrated that acclimation to high protein diets usually produces an increase of kidney mass, thickening of the ascending segment of the nephron and medulla hypertrophy associated with an increase in the amount of nitrogenous waste produced (Goldstein et al., 2001; Sabat et al., 2004b; Aldea and Sabat, 2007). Although those studies have verified the significant ability to express phenotypic plasticity (i.e., environmental effect) by renal systems in birds, comparative studies that relate dietary diversity with kidney morphology in an evolutionary scale (i.e., genetic effect) have been largely ignored (see Sabat et al., 2006a; Casotti et al., 2006). In this regard, it is expected that adaptive changes observed by short-term changes in diet are also expressed on an evolutionary scale. Interspecific physiological differences among ecologically separate species can provide insight into the evolutionary processes that allow species to cope with different dietary composition. Specifically, we predicted that birds specialized in consuming animal prey might possess relatively larger kidneys and longer Henle's loops to concentrate nitrogenous wastes and thus conserve water.

In this study, we use an explicit phylogenetic context to establish whether dietary composition of birds have been paralleled by differences in kidney features. As a biological model, we used species of the order Passeriformes from central Chile. We focused our study on passerine birds because they are one of the most diverse groups of terrestrial birds (Raikow, 1986; Sibley and Ahlquist, 1990). Our study included species that feed on invertebrates, seeds, fruit, or are omnivores (Lopez-Calleja, 1995; Sabat et al., 1998; Jaksic, 2001). This large dietary spectrum provides an opportunity to investigate the influence of diet on renal traits.

Most studies that have attempted to relate bird physiology with dietary habits are based on discrete categorization of animal diets. In some cases, this has led to apparent mismatches between dietary categorization and physiological variables (McNab, 1982; Elgar and Harvey, 1987). McNab (1992) noted that in some cases, these contrasting results could be the result of a misallocation of species to a particular dietary habit. This highlights the importance of reliable trophic assignment of species. This is especially true for species and populations that may exhibit temporary dietary variation (Klasing, 1998). To test the hypothesis that dietary patterns affect the kidney structure, we investigated possible relationships between kidney features and the percentage of nitrogen, and the percentage of prey items in the gut content of each species.

MATERIALS AND METHODS
Collection of Individuals

Sixteen species of songbirds (see Table 1) were captured in Central Chile (33°23′S, 70°30′W). This area has a Mediterranean climate with cool and wet winters and relatively dry and hot summers. Individuals were captured with mist nets from November 2008 to March 2009. We captured birds only in late spring and summer, to avoid the putative seasonal variation in physiological parameters. Following capture, we transported individuals to the laboratory in Santiago, Chile.

Kidney Morphology

After the capture, animals were weighed, sacrificed by CO_2 exposure and dissected to remove the organs. Kidneys were removed from the synsacrum, weighed (± 0.001 g) and preserved in paraformaldehyde glutaraldehyde (4%). Medullary cones were dissected from the left kidney using a dissecting microscope and the collection was counted, weighted (± 0.0001 g) and measured (± 0.01 mm). The percentage of renal medulla volume was determined as the mass of medullary tissue divided by the total mass of the kidney section, assuming a tissue density of 1 (Sabat and Martinez Del Río, 2002).

Data Analysis

Diet compositions for each of the sixteen species were taken from Ramírez-Otarola et al. (2011). Ramírez-Otarola et al. (2011) determined the volumetric percentage of invertebrates found in each bird's gastrointestinal tract and also analyzed the percentage of nitrogen and carbon in food for the same time and localities of capture of our sample animals. In addition, each species was categorized as insectivore, granivore, or omnivore according to the available literature (Jaksic and Feinsinger, 1991; Estades, 1997; Reid and Armesto, 2011). When appropriate, we arcsin square root transformed percentage data before analyses to satisfy normality assumptions of parametric statistical tests (Zar, 1996).

To examine the correlation between kidney structure and diet, we first used standard correlation and least squares

TABLE 1. Body mass, kidney features, and dietary habits of 16 Chilean passerine species used in this study

Species	Diet	n	Mb (g)	Kidney Mass (g)	Medullary Cones[a] Length (mm)	Medullary Cones[a] Number	Medulla[a] Mass (mg)	Medulla[a] %
Asthenes humicola	I/S	7	20.50 ± 2.09	0.26 ± 0.06	2.02 ± 0.2	38.4 ± 5.5	7.90 ± 3.36	5.64 ± 1.7
Leptasthenura aegithaloides	I	6	7.83 ± 0.58	0.12 ± 0.03	1.51 ± 0.3	26.3 ± 7.2	1.80 ± 0.52	3.58 ± 0.6
Pteroptochos megapodius	I/S	3	135.4 ± 10.4	1.22 ± 0.30	2.30 ± 0.4	78.3 ± 29.7	16.18 ± 6.4	2.95 ± 0.9
Xolmis pyrope	I	3	32.55 ± 2.5	0.36 ± 0.07	1.77 ± 0.8	41.3 ± 6.0	4.93 ± 1.07	3.64 ± 1.0
Anairetes palurus	I	5	5.43 ± 0.56	0.08 ± 0.03	1.22 ± 0.4	29.8 ± 6.7	1.03 ± 0.15	4.52 ± 1.6
Elaenia albiceps	I/F	14	13.52 ± 1.91	0.18 ± 0.04	1.63 ± 0.4	34.5 ± 6.8	3.37 ± 2.08	4.38 ± 1.4
Mimus thenca	S/F/I	8	65.97 ± 6.25	0.92 ± 0.08	2.40 ± 0.3	80.4 ± 17.1	27.59 ± 8.1	5.90 ± 2.5
Turdus falcklandii	F/I	11	70.50 ± 11.14	0.91 ± 0.24	1.67 ± 0.3	66.5 ± 19.0	8.73 ± 4.40	2.16 ± 0.8
Troglodytes aedon	I	11	8.92 ± 0.88	0.14 ± 0.02	1.56 ± 0.3	35.4 ± 5.7	2.01 ± 0.78	3.08 ± 0.7
Carduelis barbata	S	7	14.30 ± 2.22	0.18 ± 0.03	1.94 ± 0.2	39.7 ± 6.1	5.23 ± 2.23	6.71 ± 2.3
Curaeus curaeus	I/F/S	6	90.09 ± 10.26	1.06 ± 0.15	2.27 ± 0.4	67.8 ± 11.7	14.68 ± 8.0	3.03 ± 1.3
Sturnella loyca	S/I	5	88.53 ± 11.52	0.87 ± 0.14	2.51 ± 0.3	56.2 ± 2.9	16.56 ± 8.9	4.23 ± 2.7
Zonotrichia capensis	S/I/F	4	19.29 ± 3.30	0.28 ± 0.05	1.54 ± 0.3	31.5 ± 7.0	4.35 ± 2.47	3.26 ± 1.7
Sicalis luteola	S	6	15.47 ± 1.02	0.16 ± 0.04	1.89 ± 0.2	31.7 ± 3.7	4.37 ± 0.88	5.67 ± 1.2
Diuca diuca	S	7	34.63 ± 3.28	0.40 ± 0.08	1.97 ± 0.5	39.9 ± 12.7	8.67 ± 5.27	4.20 ± 1.7
Phrygilus fruticeti	S	2	39.11 ± 1.01	0.41 ± 0.11	1.96 ± 0.1	58.5 ± 2.1	5.50 ± 0.28	2.50 ± 0.2

In order of importance: I = Invertebrates; F = Flushy Fruits and S = Seed. Values are means ± SD.
[a]Both the number of medullary cones and the mass of medulla was estimated from the left kidney.

regression. Next, we performed correlation and regression analyses on phylogenetically independent data (Felsenstein, 1985). To test the possible effect of phylogenetic inertia on body mass and kidney morphology we calculated K-statistics (Blomberg et al., 2003). Because most variables were strongly affected by body mass, we transformed, when appropriate, the data using the method described by Blomberg et al. (2003) before calculating the phylogenetic signal for mass-independent trait values. Briefly, we first log-transformed the data and calculated the phylogenetically independent contrasts (PICs) for each response variable. Then, we performed a conventional least squares regression (CLSR) through the origin. The slope of this model was used to standardize the original variable (log[variable/mass^{slope}]). We used this standardized variable to calculate the phylogenetic inertia using K-statistics.

PIC data were calculated using the dendrogram of genetic distances (Fig. 1) based on the Sibley and Ahlquist (1990) hypothesis. Even though more recent avian phylogenies have been proposed (Fain and Houde, 2004; Barker et al., 2004; Hackett et al., 2008), the resulting topologies for the species studied here are virtually identical. In addition, we opted to use the dendrogram proposed by Sibley and Alquist (1990) to maintain consistency with previous work (Ramírez-Otarola et al., 2011). When species of interest were not listed by Sibley and Alquist (1990), substitute species within the same genus were used to determine phylogenetic branch lengths. We estimated the phylogenetic distance between *Asthenes humicola* and *Leptasthenura aegithaloides* from Moyle et al. (2009). The distance between *Xolmis pyrope* and the rest of the family Tyranidae was based on data published by Ohlson et al (2008). The distance between *Sturnella loyca* and *Curaeus curaeus* was obtained from the phylogeny proposed by Lanyon and Omland (1999), and the distance between *Zonotrichia capensis* and genus *Phrygilus* was estimated from Patten and Fugate (1998).

To perform the regressions, kidney morphological variables and body mass were log transformed and phylogenetic independent contrasts (PIC) were calculated for all variables (i.e., mass, dietary, and kidney traits). When traits were positively correlated with body mass, we removed its effect, instead calculating the residuals (mass-independent data) from least-squares linear regressions through the origin both in conventional and PICs regressions (see Garland et al., 1992). These residuals, independent of mass and phylogeny were correlated with the independent contrasts of dietary variables. All phylogenetic analysis was performed using Independent Contrasts v2.18 programs (Withers, P., pers. comm.)

RESULTS

All mass-corrected kidney features (excepting the length of medullary cones) and body mass showed a significant phylogenetic signal (i.e., a value of $K > 1$). Thus, indication that these traits are more similar between related species than would be expected assuming a Brownian motion model of evolution. Based on 1000 permutations, we found the following values: body mass $K = 3.41$, $P < 0.01$; kidney mass $K = 5.38$, $P < 0.01$; number of medullary cones $K = 5.32$, $P < 0.01$; length of medullary cones $K = 0.55$, $P < 0.01$; medullary mass ($K = 4.04$, $P < 0.01$ and percentage of renal medulla $K = 2.84$, $P < 0.01$. The presence of a significant phylogenetic signal for the majority of traits justifies the use of phylogenetically based statistical methods.

In addition, most morphological variables examined exhibited a positive association with body mass. The allometric equations for the kidney features using conventional least square regression were: kidney mass = $0.02 \times$ mb$^{0.88}$ ($r^2 = 0.98$, $P < 0.01$, $F_{(1,14)} = 721.6$); number of medullary cones = $14.4 \times$ mb$^{0.34}$ ($r^2 = 0.83$, $P < 0.01$, $F_{(1,14)} = 70.25$), length of the medullary cones = $1.1 \times$ mb$^{0.16}$ ($r^2 = 0.66$, $P < 0.01$, $F_{(1,14)} = 27.19$), and mass of medullary cones = $3.7 \times 10^{-4} \times$ mb$^{0.84}$ ($r^2 = 0.84$, $P < 0.01$ $F_{(1,14)} = 73.76$). The allometric equations described by the regressions based on PIC were: kidney mass = $1.02 \times$ mb$^{0.88}$ ($r^2 = 0.98$, $P < 0.01$, $F_{(1,13)} = 806.8$), number of medullary cones = $1.02 \times$ mb$^{0.32}$ ($r^2 = 0.83$, $P < 0.01$, $F_{(1,13)} = 64.46$), length of the medullary cones = 0.99 mb$^{0.17}$ ($r^2 = 0.67$, $P < 0.01$, $F_{(1,13)} = 26.59$) and mass of medullary cones = $1.01 \times$ mb$^{0.85}$ ($r^2 = 0.82$, $P < 0.01$ $F_{(1,13)} = 60.76$). When we analyzed the mass-standardized number of medullary cones,
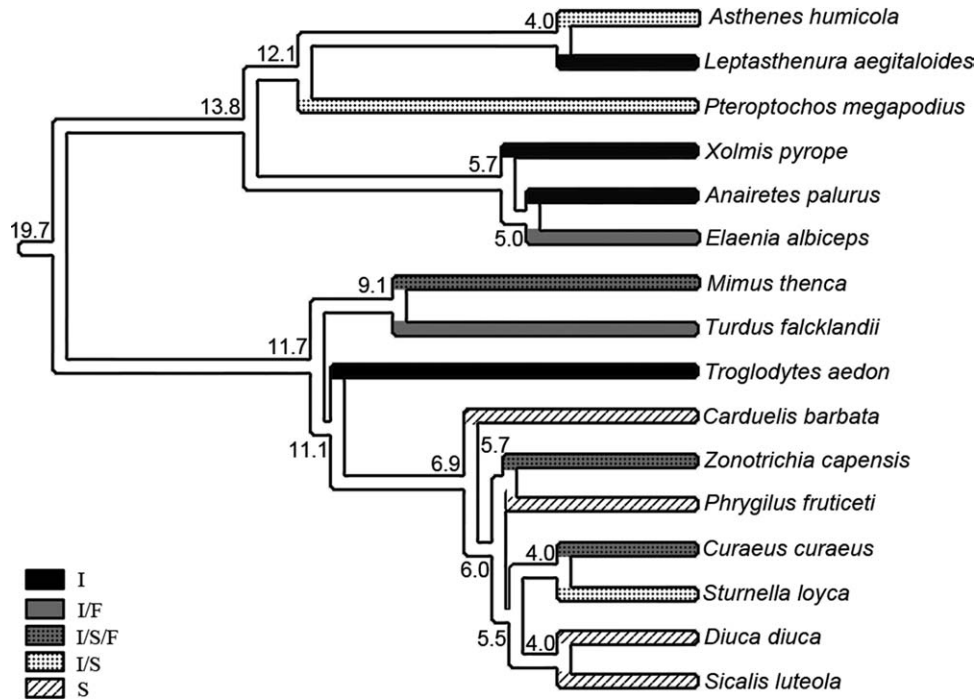
Fig. 1. Dendrogram of genetic distance under the method of hybridization of DNA / DNA. Based on the hypothesis of Sibley and Ahlquist (1990). Numbers on nodes indicate the distances between species. The patterns of the branches suggest diet (I: invertebrates; F: fruits; S: seeds).

we found an inverse association with body mass; the allometric equations described by the conventional regression (Fig. 2) were mass-standardized number of medullary cones = $1274 \times \text{mb}^{-0.52}$ (r^2 = 0.93, $P < 0.01$, $F_{(1,14)}$ = 201.3), and mass-standardized number of medullary cones = $1.0 \times \text{mb}^{-0.53}$ (r^2 = 0.94, $P < 0.01$, $F_{(1, 13)}$ = 200.3) for the PIC. The percentage of renal medulla was the only morphological variable independent of body mass both using CLSR and PIC (r^2 = 0.11, P = 0.19, $F_{(1,14)}$ = 1.81 and r^2 = 0.05, P = 0.4 $F_{(1,13)}$ = 0.76, respectively).

We found a wide variation among species in the morphology of the kidney (Table 1). The number of medullary cones among species ranged from ca. 26 to 78, whereas the percentage of kidney devoted to medullary tissue ranged from 2.1% in the omnivore *Turdus falklandii* to 6.7% in the granivore *Carduelis barbata*. In the standard correlation, the percentage of invertebrates in gut did not affect any of the kidney variables. The length of medullary cones, the medullary mass and the percentage of renal medulla were negatively correlated with dietary percent nitrogen content (Fig. 3). This was supported by the independent contrasts analyses, which revealed a significant and negative correlation between the percentage of nitrogen in the diet and the percentage of renal medulla, as well as with the length of the medullary cones and the medullary mass. Surprisingly, when the phylogenetic history was accounted for

(i.e., from PIC analyses), the percentage of medullary tissue and the medullar mass were negatively correlated with the percentage of invertebrates in diet (see statistics in Table 2).

DISCUSSION
Evolutionary Aspects

In an evolutionary sense, bird phenotypes have been modified by physical and chemical composi-
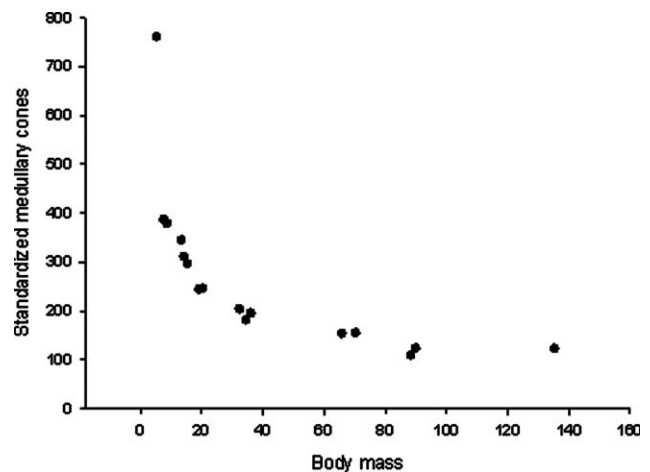

Fig. 2. Relation between body mass and the mass-standardized number of medullary cones for sixteen Chilean passerine species.
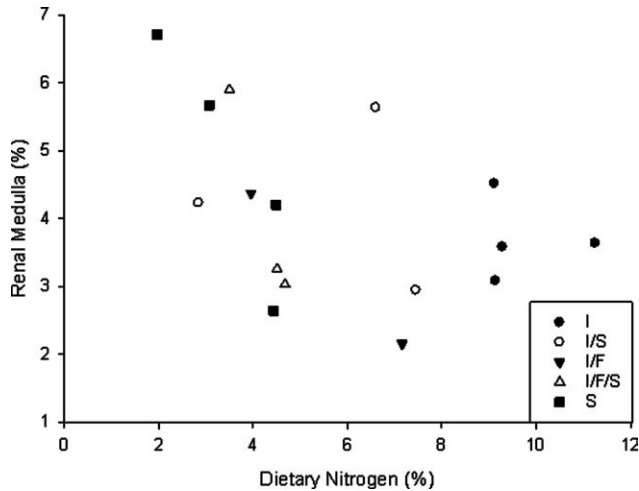
Fig. 3. Conventional correlation between the percentages of renal medulla and the percentage of nitrogen in the diet for the different diet categories. Symbols denote species food habits (S seeds, I invertebrates, F fruits).

tion of natural diets (Klasing, 1998; Korzun et al., 2008; Karasov et al., 2011). However, in some cases, the adaptive value of these modifications has been called into question. This concern is, in part, caused by the absence of explicit analysis that includes the possible effect that the phylogenetic history would have on the phenotype. Indeed, in several examples in the literature, the correlation between phenotype and environment disappears when the effect of phylogeny is incorporated (eg. Cruz-Neto et al., 2001; Álvarez et al., 2011). In this study, it is noteworthy that we found strong coincidences between phenotype and diet using both conventional and phyllogenetically constrained analyses. This demonstrates a consistent effect between dietary habits and renal structure in passerine birds.

Examples from the literature show that differences in morphology and physiology among species may be produced by genetic and/or environmental effects (including developmental plasticity, sensu Piersma and Drent, 2003) or their interaction (Garland and Adolph, 1991). However, in all of these studies, the effect of exposure to different

diets on medullary size was relatively small (between 17 and 61%, see Casotti, 2001; Goldstein et al., 2001; Aldea and Sabat, 2007; Sabat et al., 2009) and smaller than the differences found among the extremes for our species (>200%). Moreover, the number of medullary cones in passerines exposed to different osmoregulatory demands (e.g., salt load, and protein consumption) did not differ in adult birds (e.g., Sabat et al., 2004a,b; Aldea and Sabat, 2007). These studies suggest that phenotypic flexibility in adult birds is unlikely to explain all the variation in renal traits observed among the passerine species.

Kidney Structure and Dietary Habits

We found a strong association between the gross morphology of the kidney and diet among Chilean passerine species. Our working hypothesis predicted the existence of a functional relationship between the development of renal structure and the percentage of invertebrates and the amount of nitrogen present in the diet. However, the mass of the medullary portion of the kidney (as a percentage of the kidney or residuals in relation to body mass), and the length of the medullary cones were negatively correlated with the percentage of nitrogen in the diet. In other words, we found an apparent higher development of medullary structure in granivorous birds than in insectivorous and omnivorous birds. This result might appear contradictory to what was observed in birds at an intraspecific level. Indeed, experimental studies have documented that the consumption of high protein diets by sparrows increased the medullary tissue and size of medullary cones. These changes were associated with changes of urinary flux and the elimination of nitrogen wastes (Goldstein et al., 2001; Sabat al, 2004b). Furthermore, interspecific studies in vertebrates including birds (Richardson et al., 1991; Schondube et al., 2001; Casotti et al., 2006) reported that species whose diets have higher protein content, also exhibits a high development of the renal medulla.

What could explain the difference between the patterns seen in the literature to that found in the

TABLE 2. Correlations of residuals obtained after plotting kidney features against body mass with dietary variables for 16 species of passerine birds, using conventional and phyllogenetically corrected statistics

	PIC				Conventional correlation			
	Invertebrates (%)		Diet nitrogen (%)		Invertebrates (%)		Diet nitrogen (%)	
	R	p	R	p	R	P	R	p
Kidney mass	0.10	0.73	−0.01	0.97	0.12	0.67	0.08	0.51
Total cones	−0.09	0.74	−0.24	0.37	0.16	0.54	0.04	0.87
Cones length	−0.51	0.05	−0.62	**0.01**	−0.44	0.09	−0.56	**0.02**
Medullary mass	−0.62	**0.01**	−0.73	**0.01**	−0.41	0.11	−0.58	**0.02**
% Medulla	−0.53	**0.04**	−0.67	**0.01**	−0.38	0.14	−0.57	**0.04**

Values in bold represent values statistically significant (p < 0.05).

present study? In general terms, the primary consumers (nonfaunivores birds) used in the aforementioned studies were frugivores and nectarivores. On the contrary, the non-faunivores passerines used in this study were mainly granivores. These two dietary components differ in both protein content and in preformed water (Klasing, 1998). In fact, seeds can be very dry (between 8 and 14% of water, see Hart et al., 1962), particularly in the dry season. In our case, the percentage of nitrogen and the amount of water present in the diet are positively correlated because seeds have both the lowest water content and low nitrogen (about 30% of protein content, see Barclay and Earle, 1974), while invertebrates have higher nitrogen and water (between 54 and 77% of protein in the dry mass and between 62 and 80% of water, see Redford and Dorea, 1984; Robel et al., 1995; Withers and Dickman, 1995; Díaz, 1996). Thus, the negative correlation exhibited between kidney morphology and dietary nitrogen may be spurious and caused by the water/nitrogen ratio. Conversely, fruits have a high amount of preformed water, and the lowest protein content (between 1.7 and 8% of protein content and about 71% of water, see Johnson et al., 1985; Bosque et al., 1995). Among our studied species, five omnivorous species seemed to incorporate at least some fruit into their diet (Estades, 1997; Reid and Armesto, 2011). Because such diets should have a high proportion of water and a low percentage of nitrogen, it would be expected that these species would also have a lower demand for renal function. Accordingly, we found that *Turdus falckandii* is the species with the lowest percentage of renal medulla, while *C. curaeus* and *Z. capensis* are among those with the lowest proportion of medullary tissue.

Therefore, the greater renal medulla development observed in granivorous may be correlated with the small amount of water present in the seeds. Aldea and Sabat (2007) documented that dehydration in *Z. capensis* has a greater effect on kidney structure than dietary protein content. MacMillen (1990) also highlighted that seed – eating birds have better resistance to dehydration. Several studies (e.g., Poulson, 1965; Johnson and Ohmart, 1973a,b; Johnson and Skadhauge, 1975; Skadhauge, 1976; Goldstein and Braun, 1986; Casotti et al., 1998), suggest that granivores might also exhibit a higher urine concentrating ability, and has been demonstrated that this ability is related to differences in renal structure (see Johnson, 1972; Goldstein and Braun, 1989). This is consistent with the idea that seed-eaters sparrows live in an antidiuretic or a more dehydrated condition. In this vane, our results suggests that amount of water intake could be the variable that explains the kidney morphology observed in our data set. Although few studies have documented changes in renal anatomy in birds consuming different diets, previous studies of osmoregulatory parameters in field birds support this view. For example, Sabat et al. (2009) reported that for the omnivore *Z. capensis*, position in the food web (as inferred from nitrogen isotopic signatures) corresponded with urine osmolality without any changes in kidney features along a latitudinal gradient. In contrast, for *Z. capenis*, the kidney size and proportion of kidney devoted to medullary tissue differed between arid and mesic localities.

It is reasonable to assume that water balance might depend upon water intake including drunk and preformed water of food. In this vane, the birds might compensate the lower content of preformed water intake in food by drinking free water. However, studied birds inhabit a Mediterranean semiarid habitat characterized by hot, dry summers and cold, rainy winters (Di Castri and Hajek, 1976). Annual mean precipitation in this area is 367 mm, but during the austral summer (December–March), this area only receives 3% of the annual mean precipitation. Thus, at least for summer months, birds probably rely almost exclusively on the water content of food. Accordingly some granivorous birds have the ability to survive on a dry-seed diet, without drinking water (Bartholomew, 1972).

In short, we found that the studied characteristics of kidney morphology exhibited an association with diet in passerines. However, according to our results, the amount of protein (or nitrogen) does not appear to be the main dietary component that has modulated the renal system throughout evolutionary history in passerines. Our results suggest that water availability in the different food items is one of the main factors that have led to the structural and functional modifications of the kidney (see Sabat et al., 2006a, for an example of adaptive radiation in renal function mediated by salt consumption). Our findings do, however, require more detailed analysis with respect to: i) dietary composition, and ii) the way in which other aspects related to the water balance (e.g., evapotranspiration rate, consumption of free water) affect or have affected the evolution of kidney attributes. For example, it has been documented that some seeds and buds have high contents of secondary metabolites (Swain, 1977; Wink, 1988; Díaz, 1996). Foley et al. (1995) demonstrated that the processes of biotransformation and excretion of absorbed secondary metabolites in birds result in the need to process and to excrete large amounts of organic acids. We hypothesize that differences in kidney structure (and probably function) could also be explained by differences in the amounts of secondary compounds ingested by bird species. Besides, further efforts are needed to asses the renal function, such as the ability to concentrate urine or the urine/plasma osmolality ratio, because

they provide information on the end product of kidney excretion. Finally, our model species do not represent the whole avian class. Indeed, our data set only represents the lower spectrum of body mass distribution in birds and a sample of all bird's dietary habits. This observation does not invalidate our comparative analyses, but limits the inferences that we can derive from them.

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