Foot shape in arboreal birds: two morphological patterns for the same pincer-like tool

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Abstract

The feet are the only contact between the body and the substrate in limbed animals and as such they provide a crucial interface between the animal and its environment. This is especially true for bipedal and arboreal species living in a complex three-dimensional environment that likely induces strong selection on foot morphology. In birds, foot morphology is highly variable, with different orientations of the toes, making it a good model for the study of the role of functional, developmental, and phylogenetic constraints in the evolution of phenotypic diversity. Our data on the proportions of the phalanges analyzed in a phylogenetic context show that two different morphological patterns exist that depend mainly on habitat and toe orientation. In the anisodactyl foot, the hallux is the only backward-oriented toe and is enlarged in climbing species and reduced in terrestrial ones. Moreover, a proximo-distal gradient in phalanx size is observed depending on the degree of terrestriality. In the two other cases (heterodactyl and zygodactyl) that have two toes that point backward, the hallux is rather small in contrast to the other backward-pointing toe, which is enlarged. The first pattern is convergent and common among tetrapods and follows rules of skeletal development. The second pattern is unique for the clade and under muscle–morphogenetic control. In all cases, the functional result is the same tool, a pincer-like foot.

Key words: feet; functional morphology; grasping; osteology.

Introduction

Extant birds live in different environments. Among them, the tree canopy is an environment that is characterized by a complex geometry, the size and the orientation of the supports changing radically from one branch to the next (Cartmill, 1972, 1992). The feet have adapted to these complex functional demands with the evolution of foot morphologies that allow birds to obtain a secure grip. The bipedal nature of humans and birds ensures that only two feet assure the contact between the substrate and the body, and consequently the hind feet have to assure gripping and balance in bipeds. Humans, being primates, belong to an essentially arboreal clade, but the foot has become secondarily specialized to bipedal terrestrial locomotion. Birds originate from bipedal, terrestrial, and cursorial theropods,

and the adaptation for perching comprises modifications of the proportions of the feet (Hopson, 2001; Kavanagh et al. 2013; Backus et al. 2015) and a new orientation of the toes (Bock & Milner, 1959; see Sustaita et al. 2013 for a review). By analyzing the morphology of the foot in perching birds, our aim was to identify the characteristics of this specialization in birds. The juxtaposition of the avian features with those observed in primates and other arboreal clades may provide insights into the functional response to this specific habitat in tetrapods.

In general, the feet of birds have four toes. Toe I (hallux) was oriented forward in non-avian theropods, but it reversed and is oriented backward in birds (Middleton, 2001; Botelho et al. 2015). The number of toes varies between three and four, except in the cursorial ostrich, in which toes I and II are missing. Toe I has a variable morphology as it can be placed distally or more proximally on the tarsometatarsus (Raikow, 1985). It is always present in passerine birds, but its size is variable and it disappears in 15% of the non-passerine birds (Raikow, 1985). The phalangeal composition is described by the formula 2-3-4-5, representing the number of phalanges of toes I to IV. This is the same phalangeal formula as observed in primitive amniotes (Romer, 1956). Whereas in most taxa this

organization is preserved, the morphology of the feet is highly variable and has been considered 'a text book example of the adaptive variation' (Romer, 1956). We consider three main configurations corresponding only to the orientation of the toes: anisodactyl, with toe I pointing backward and toes II, III and IV pointing forward; heterodactyl, with toes I and II pointing backward and toes III and IV pointing forward; and zygodactyl, with toes I and IV pointing backward and toes II and III pointing forward. These configurations do not occur at the same frequency, since 88% of the bird species are anisodactylous, including all the passerine birds, as are 56% of the non-passerines (Raikow, 1985). Among the non-passerine species, 8% are zygodactylous. The heterodactylous configuration is the rarest and is represented by only one order, the Trogoniformes. The zygodactylous configuration has arisen independently in different lineages and has long been recognized as a convergence in arboreal birds (Bock & Milner, 1959). A modification of the orientation of the toes is observed in most arboreal clades. To grasp efficiently, the foot needs to be able to exert two opposing forces on the branch and hold it in a vice-like grip (Backus et al. 2015). Depending on their orientation, the toes will participate in one or the other part of the vice and the mechanical demands on the toes will thus be different.

The morphological patterns of phalangeal proportions in tetrapods are constrained by developmental rules (Kavanagh et al. 2013). In general, the developmental regulation of the phalanges leads to a proximo-distal gradient of large to small. However, elongated distal phalanges convergently evolved in different lineages under selection for grasping, as in arboreal birds (Hopson, 2001). These patterns are determined early during the development, at the time of the initial cell condensation. The orientation of the toes in birds is under the control of another developmental mechanism in which the backward orientation of the toes arises during the development of a muscle, inducing an asymmetrical force on the toe that pulls it backward (Botelho et al. 2014). The shape of the feet is thus controlled by two developmental mechanisms: the mechanism that drives the length of the phalanges and sets up their proportions, and the mechanism that drives the development of the muscles that determines the orientation of the toes. However, it is not known how these mechanisms interact in response to the functional demands associated with the diversity of uses of the feet in birds.

As the feet are a crucial functional trait allowing the exchange of forces between an animal and its environment, we performed a shape analysis of the foot in different species of birds that display different toe orientations (Fig. 1). We described the variation in the phalangeal proportion and test (i) whether the phalangeal proportions of the four toes and the three-dimensional (3D) orientation of the metatarsal trochlea are different in zygodactylous and anisodactylous birds; and (ii) whether locomotion and/or

substrate size and orientation are correlated with the shape of the foot. To do so we employed phylogenetically informed analyses. Finally, we assessed the contribution of developmental, functional, and phylogenetic constraints to the evolution of toe shape in birds.

Methods

Specimens

Our study was based on 28 bird species, representing 19 families and 13 orders, obtained from the wet collections of the Museu Nacional (Anatomia), Rio de Janeiro, Brazil (MNA) and the Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo (AZUSP; listed in Table 1). The specimens were all adults, with the exception of *Spheniscus magellanicus*, which was a sub-adult.

The species were chosen in accordance with the main types of arrangement of the toes (e.g. anisodactylous, zygodactylous, and heterodactylous) according to Bock & Milner (1959) and Raikow (1985), so as to represent variation in the use of the feet for locomotion (walking, hopping, climbing, swimming, and posture only) and other uses such as manipulation and grasping. The characteristics of the support on which they move was also noted as wide (ground or cliff) or thin (branches), and as horizontal or vertical (del Hoyo et al. 1992–2002, 2006, 2016; Fig. 1).

Data acquisition

A micro-CT (Skyscan 1176) system was employed to analyze the bony elements in the feet of 23 museum specimens. The microtomographies (resolution between 9 and 18 μm) were segmented with Avizo and imported in Meshlab to visualize the skeleton. The IDAV landmark software was used to obtain the 3D coordinates of 65 landmarks (Fig. 2). The left foot was measured in all species. However, only the right foot was available for *Galbula albirostris* and *Glyphorynchus spirurus* and thus these two species were not used for the 3D analysis of the trochlea. Six other specimens were scanned but used only for comparative purposes.

The 3D coordinates of the landmarks were used to calculate the width and depth of the toes at the level of the joints, the length of the phalanges, and the depth of the ungual tubercle (Fig. 2). The sum of the lengths of the phalanges was used as a proxy for the length of the toe. Since it was not possible to compare the proportions of the phalanges in toes with different phalangeal composition, we first compared the proportions between birds having the same phalangeal composition, excluding three species. This analysis showed that the third phalanx of the toe IV was less variable than the phalanges. Consequently, we decided to run all analyses without the measurements of the third phalanx of the toe IV but including all species. The results of both analyses showed exactly the same trends, but here we present only the analysis that included all 23 species but excluded the third phalanx of the toe IV.

Thus the dataset for the toe shape analysis includes the linear dimensions of the length, depth, and width of the phalanges (plus the height of the flexor tubercle) calculated from the 3D coordinates of the landmarks. However, the height of the flexor tubercle of the ungual phalanx of the toes strongly impacted the variability described by the first two PC axes (Supporting Information Figs S1 and S2). The loadings of these variables on PC1 and PC2 were so high that the importance of the measurements of the phalangeal
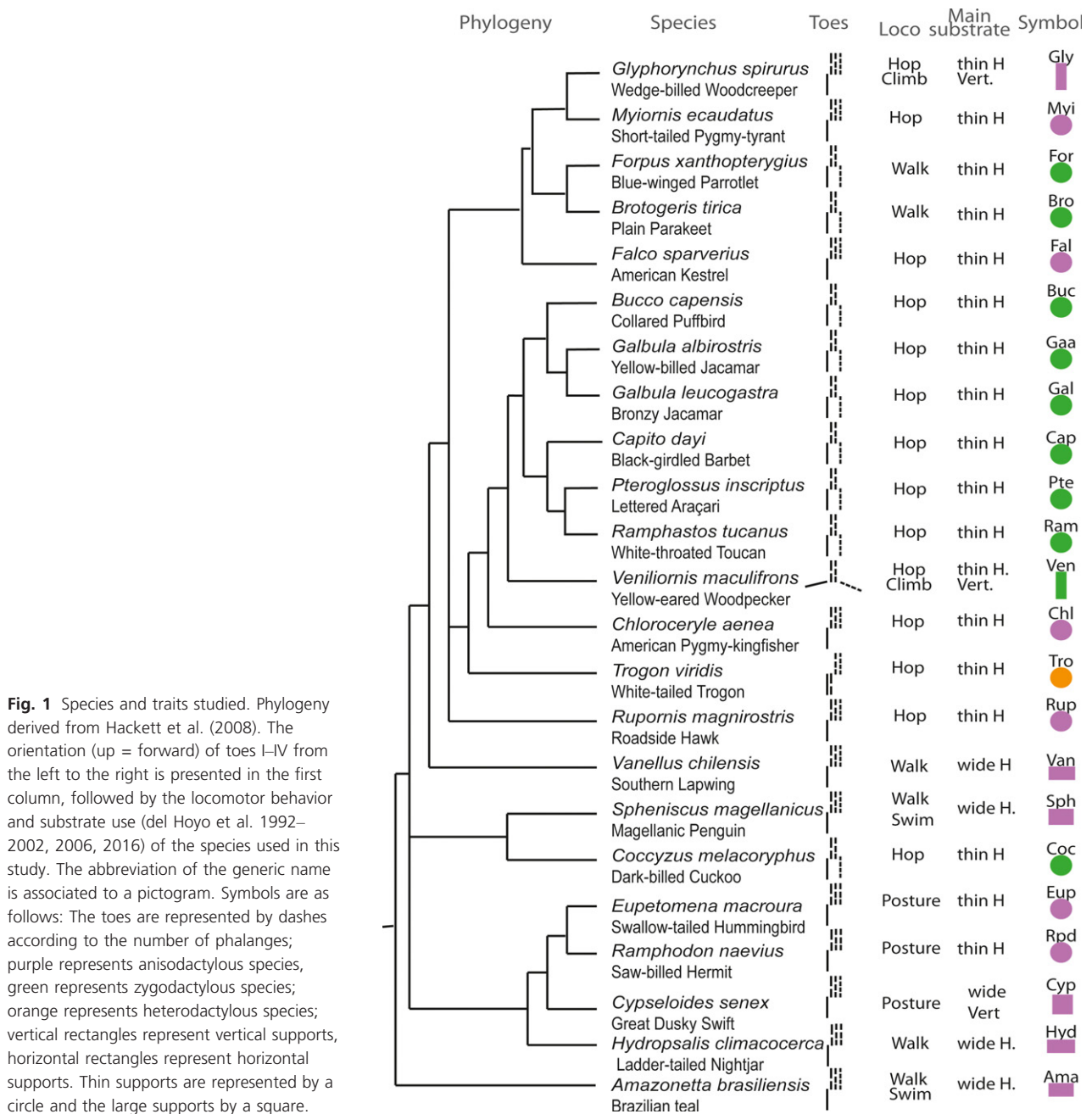
Fig. 1 Species and traits studied. Phylogeny derived from Hackett et al. (2008). The orientation (up = forward) of toes I–IV from the left to the right is presented in the first column, followed by the locomotor behavior and substrate use (del Hoyo et al. 1992–2002, 2006, 2016) of the species used in this study. The abbreviation of the generic name is associated to a pictogram. Symbols are as follows: The toes are represented by dashes according to the number of phalanges; purple represents anisodactylous species, green represents zygodactylous species; orange represents heterodactylous species; vertical rectangles represent vertical supports, horizontal rectangles represent horizontal supports. Thin supports are represented by a circle and the large supports by a square.

proportions (length, depth and width) was reduced. Removing the tubercle height values from the analysis permitted a better understanding of the distribution of the phalangeal proportions. Therefore, we analyzed the morphospace of the feet omitting tubercle values. The dataset for the tarsometatarsal shape included the 3D trochlear landmark coordinates.

Shape analysis

Analysis of linear measurements using log-shape ratios

Mosiman's geometric framework (Mosimann, 1970; Mosimann & James, 1979) was used to estimate the form (size and shape) of the foot. It provides an indicator of size, isometric size (the mean of all

logarithmic variables for each specimen), and shape parameters, the log-shape ratio, corresponding for each individual to the subtraction of its size value from the set of its logged distances. Log-shape ratios (Mosimann & James, 1979) were calculated based on the raw $\log_{10}$-transformed linear dimensions of the foot. Principal component analyses (PCA) were run on the log-shape ratios and used to visualize differences in foot shape. We analyzed the loadings of the original variables on the first three principal components, representing 75% of the total variance, in order to evaluate which morphological traits structure the variability. Shape co-variation between the phalanges and the trochlea was tested using an RV test (Escoufier, 1973; Klingenberg, 2009; Goswami & Polly, 2010) in R using the ade4 library (Dray & Dufour, 2007). The RV coefficient

Table 1 List of the specimens.

Family	Species	Collection number
Anatidae	*Amazonetta brasiliensis* (Gmelin, 1789)	MNA 1067*
Spheniscidae	*Spheniscus magellanicus* (Forster, 1781)	MNA s/n°*
Charadriidae	*Vanellus chilensis* (Molina, 1782)	MNA 1580*
Falconidae	*Falco sparverius* (Linnaeus, 1758)	MNA 3251*
Accipitridae	*Rupornis magnirostris* (Gmelin, 1788)	MNA 2896*
Cuculidae	*Coccyzus melancoryphus* (Vieillot, 1817)	MNA 8179*
Psittacidae	*Forpus xanthopterygius* (von Spix, 1824)	MNA 6626*
Psittacidae	*Brotogeris tirica* (Gmelin, 1788)	MNA 1516*
Caprimulgidae	*Hydropsalis climacocerca* (Tschudi, 1844)	AZUSP 405*
Caprimulgidae	*Hydropsalis torquata* (Gmelin, 1789)	AZUSP 319
Caprimulgidae	*Nyctipolus nigrescens* (Cabanis, 1848)	AZUSP 190, AZUSP 193
Apodidae	*Cypseloides senex* (Temminck, 1826)	MNA 6638*
Trochilidae	*Ramphodon naevius* (Dumont, 1818)	MNA 5061*
Trochilidae	*Eupetomena macroura* (Gmelin, 1788)	MNA 4934*
Trochilidae	*Phaethornis superciliosus* (Linnaeus, 1766)	MNA 7475
Trochilidae	*Aphantochroa cirrochloris* (Vieillot, 1818)	MNA 4656
Trochilidae	*Thalurania glaucopis* (Gmelin, 1788)	MNA 4220
Trogonidae	*Trogon viridis* (Linnaeus, 1766)	MNA 7876*
Alcedinidae	*Chloroceryle aenea* (Pallas, 1764)	MNA 7770*
Bucconidae	*Bucco capensis* (Linnaeus, 1766)	MNA 7714*
Galbulidae	*Galbula albirostris* (Latham, 1790)	MNA 7764*
Galbulidae	*Galbula leucogastra* (Vieillot, 1817)	MNA 7817*
Capitonidae	*Capito dayi* (Cherrie, 1916)	AZUSP 446*
Ramphastidae	*Ramphastos tucanus* (Linnaeus, 1758)	AZUSP 732*
Ramphastidae	*Pteroglossus inscriptus* (Swainson, 1822)	AZUSP 632*
Picidae	*Veniliornis maculifrons* (Spix, 1824)	MNA 4720*
Dendrocolaptidae	*Glyphorynchus spirurus* (Vieillot, 1819)	MNA 5662*
Tyrannidae	*Myiornis ecaudatus* (d'Orbigny & Lafresnaye, 1837)	AZUSP 401*

*Specimens for 3D reconstruction.

provides a metric of the strength of co-variation between two structures and returns a value ranging between 0 and 1. This coefficient is analogous to a correlation coefficient. Its calculation mainly involves the division of the covariance between the two sets of variables by the variance of each dataset. When the RV coefficient is 0, no co-variation between structures is present; when the RV coefficient is 1, the two structures are perfectly integrated. We compared the measured RV coefficient with the one estimated based on 100 permutations. A P-value is associated and allows one to assess the significance level of the observed signal. The P_{95}-value is calculated by comparison of the observed RV coefficient with those obtained after re-sampling. If the RV coefficient was higher than those obtained from permutated blocks, then its associated P_{95}-value is considered significant.

Analysis of trochlear shape using geometric morphometrics

Shape variation of the trochlea was assessed using a geometric morphometric approach allowing analysis of size and shape component independently (Zelditch, 2004). Generalized Procrustes superimposition (Rohlf & Slice, 1990) was performed on the point coordinates using the package Rmorph (Baylac, 2012) in the program R (R Core Team, 2014). During the Procrustes superimposition, all configurations are scaled to a unit centroid size (i.e. the square root of the sum of the squared distances from each landmark to the centroid

of the configuration) by dividing all coordinates by the corresponding centroid size. Next the centroids (center of gravity) of the configurations are superimposed through translation, and the configurations are rotated around their centroids so as to minimize the sum squared distance between homologous landmarks to optimize the superimposition. The new coordinates (Procrustes coordinates) are used as shape variables.

Phylogenetic signal

Because species share their evolutionary history, they cannot be considered independent data points (Felsenstein, 1985). Consequently, phylogenetic comparative analyses need to be performed to test for differences between groups. We pruned the phylogenetic tree of Hackett et al. (2008) and used it in our analyses. As no specific branch lengths are available they were all set to unit length. To estimate the phylogenetic signal in foot morphology, we used a randomization test (Blomberg et al. 2003) and the extended methods of Adams (Adams, 2014). A multivariate K-statistic was calculated based on the first three principal components of the log-shape ratio dataset (accounting for more than 75% of the variance) and on Procrustes coordinates of the trochlea data set using the 'geomorph' library (Adams & Otarola-Castillo, 2013) in R (R Core Team, 2014). Next, a univariate K was calculated for the first three principal components of the shape of the feet and trochlea representing more
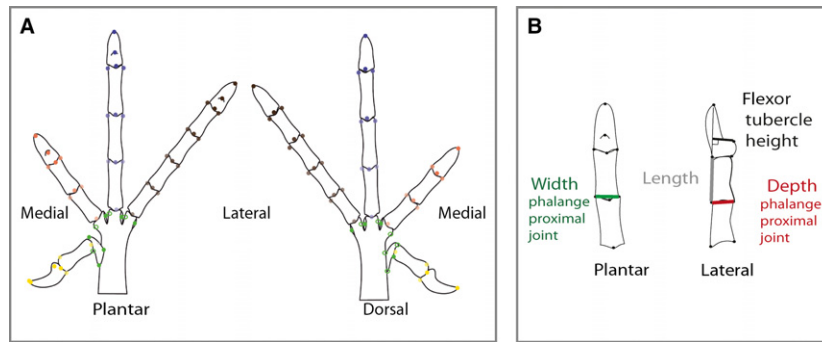
Fig. 2 Measurements: (A) 3D schematic representation of the 3D landmarks on the specimens: Distal metatarsus. Digit I: on the proximal end, proximal and dorsal points and on the middle of the distal side of the trochlea, lateral and medial points dorsally. Digits II, III IV: lateral and medial point. For all the toes, all the phalanges except the ungual were measured in the same way: base – middle of the ventral face and middle of the dorsal face; head of the phalanx – lateral point and – medial point. Ungual phalanx: base – middle of the ventral face and – middle of the dorsal face – top of the tubercle – apex of the phalanx. (B) Linear measurements on the phalanges, calculated from the 3D landmarks. The color code is the same as in the Fig. 4. Hallux (toe I), yellow; toe II, orange; toe III, purple; toe IV, brown. For each toe, a gradient of color is used going from a lighter color for the first phalanx to a darker color for the ungual phalanx. The width, measured from the proximal joint is in green, the depth at the proximal joint in red, and the length of the phalanx is in gray. The height of the flexor turbercle, in black, is the perpendicular distance between the most plantar point on the tubercle and the line between the apex of the phalanx and the basis at the middle of the dorsal face and the apex of the ungula phalanx.

than 75% of the variation using the 'Kcalc' function in the 'picante' library (Kembel et al. 2010) in R (R Core Team, 2014).

We used the first three principal components describing the shape of the feet (log shape ratios) and trochlea (geometric morphometrics) to test quantitatively for differences in shape between groups. We used phylogenetic multivariate analysis of variance (MANOVAS) coupled to univariate analysis of variance (ANOVA; Garland et al. 1993) in R (R Core Team, 2014). We used the aov.phylo function in the 'geiger' library (Harmon et al. 2008) in R (R Core Team, 2014) for our analysis to test whether animals with different locomotor habitats, phalanx orientations, and size and orientation of the substrates differed in shape after accounting for phylogeny.

Results

Phalangeal formula

Toes I, II, III, and IV of birds have, respectively, two, three, four, and five phalanges (Fig. 2), but the Caprimulgidae *Hydropsalis climacocerca*, *H. torquata* and *Nyctipolus*

nigrescens have four phalanges in toe IV. Among the Trochilidae, the two species measured, *Eupetomena macroura* and *Ramphodon naevius*, and *Aphantrochroa cirrochloris* and *Thalurania glaucopis*, which we also observed, have four phalanges in toe IV, whereas *Phaethornis superciliosus* has five phalanges.

Length of the toes

The general pattern is an increase in size from toe I to II to IV to III. However, in *Myionis ecaudatus* toe I is longer than toes II and IV; in *Veniliornis maculifrons* toe IV is the longest, and in *Hydropsalys climacocerca* toe III is proportionally long (Fig. 3).

Morphospace of the feet of the birds

We first analyzed the loadings of the variables on the first three principal components (Fig. 4). Next, we compared the
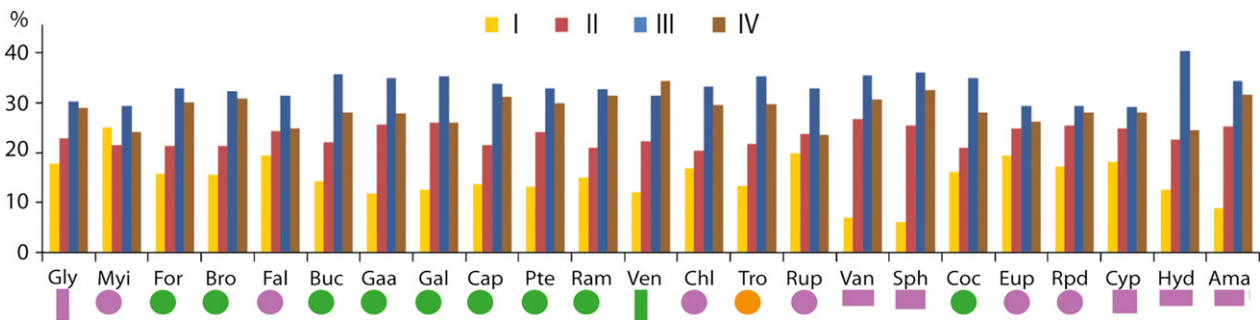

Fig. 3 Proportions of the toes as percentages of toe length. Anisodactylous species are in purple, zygodactylous ones in green, and heterodactylous ones in orange. Abbreviations are explained in Fig. 1. The general pattern is an increase in size from toe I to II to IV to III. However in *Myionis ecaudatus* toe I is longer than toes II and IV; in *Veniliornis maculifrons* toe IV is the longest, and in *Hydropsalys climacocerca* toe III is proportionally long.
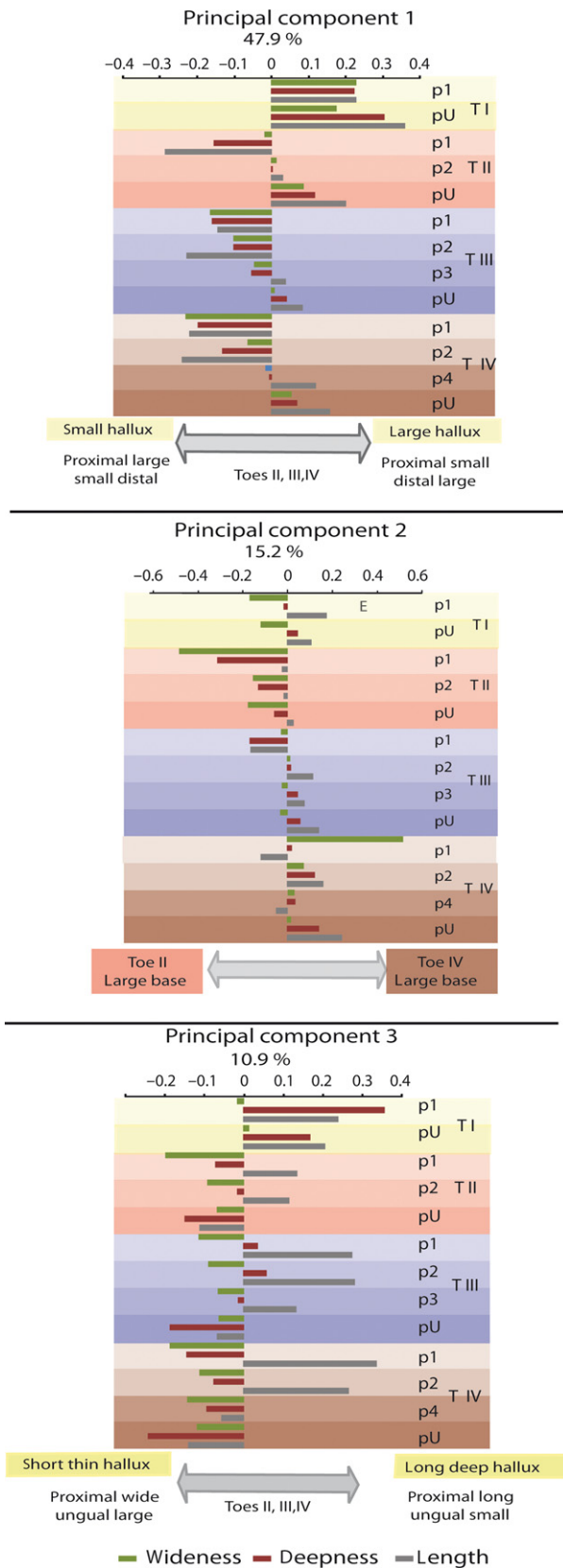
Principal component 1
47.9 %

Principal component 2
15.2 %

Principal component 3
10.9 %

— Wideness — Deepness — Length

Fig. 4 Shape of the phalanges. Loadings of the principal components on the first three axes. Each color represents one toe (T): toe I, yellow; toe II, orange; toe III, purple; toe IV, brown. For each toe, a gradient of color is used going from a lighter color for the first phalanx (p1) to a darker color for the ungual phalanx (pU). For each phalanx, from top to bottom, the first value is the width at the proximal joint (green), the second value is the depth at the proximal joint (red), and the third value is the length of the phalanx (gray). Values on the PCA are presented horizontally, with the negative values on the left and the positive values on the right.
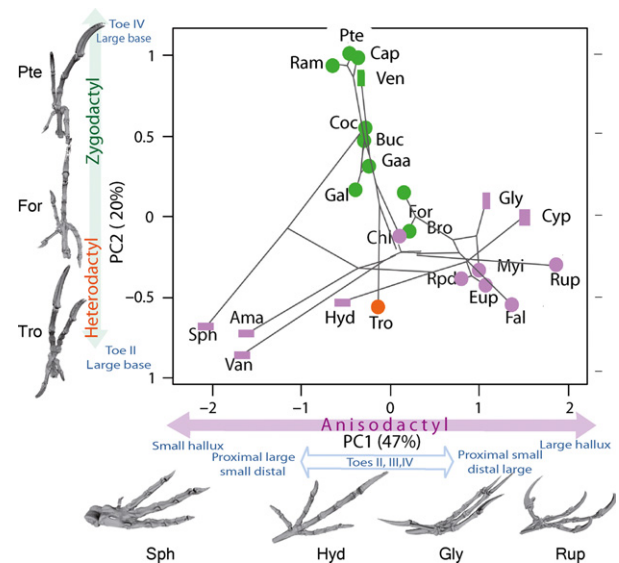

Fig. 5 Feet of the species. Morphospace of the first two principal component axes representing over 67% of the variation in the linear dimension dataset. The arrows near the axes illustrate the distribution of the species with different feet (anisodactyl, zygodactyl, hetero-dactyl). The overall distribution of the loading is reported near the axes in blue. The phylogeny has been plotted in the morphospace. Abbreviations and symbols are explained in Fig. 1 and Table S1.

distributions of the species in the morphospace (Fig. 5) to determine the influence of the orientation of the toes, loco-motion, substrate, and phylogeny on the morphology of the toes.

Loading of the variables on the principal components
The first principal component axis corresponds to the differ-ent proportions of the hallucal phalanges (Fig. 4), with rela-tively small hallucal phalanges on the negative part of the axis and relatively large ones on the positive part of the axis. The other toes (II, III, and IV), present the opposite pat-tern, with the proximal phalanges being relatively smaller whereas the distal ones are relatively larger on the positive part of the axis, and conversely on the negative part of the axis. The second principal component corresponds to a rela-tively large proximal part of toe IV on the positive part of

the axis, and to large proximal part of toe II on the negative part of the axis. On the third principal component axis, the length and depth of the hallux are the variables with the strongest loading. The pattern for the other toes is similar, with the proximal phalanges being longer than the ungual on the positive part, and conversely so on the negative part.

Distribution of the species on the morphospace

The positive side of the first principal component axis is occupied by birds of prey (such as *Rupornis* and *Falco*), the swift (*Cypseloides*), and perching birds. They display a long hallux, short proximal phalanges, and long distal phalanges (Fig. 5). The other side of the axis is occupied by birds that walk on land such as the teal (*Amazonetta*), the plover (*Vanellus*), and the penguin (*Spheniscus*) which are characterized by a short hallux and longer proximal phalanges. The nightjar (*Hydropsalis*), which does not walk but stands on land, displays an intermediate morphology. Zygodactylous birds are positioned near the middle of the first axis. In all zygodactylous birds the hallux has a similar mean shape and no proximo-distal gradient in phalanx length is observed. The zygodactylous birds are spread along the positive part of the second axis, which is most strongly determined by the width of the proximal part of the first phalanx of toe IV and the relative smaller size of toe II. The heterodactylous birds are found towards the negative part of the second axis. This is driven by the width of the proximal part of the first phalanx of toe II and the relatively smaller size of the toe IV. Thus the second axis reflects the enlargement of the base of the toe with a backward orientation. The shape of toe III is not affected by the orientation of the toes and it is always large (Fig. 2). However, the other forward-oriented toe has a rather thin base.

Phylogenetic signal

The multivariate K, calculated based on the linear dimensions, is not significant ($K_{mult} = 0.25$, $P = 0.053$). However, univariate K statistics show a significant phylogenetic signal on the second ($K = 0.45$, $P = 0.002$) and third axes ($K = 0.42$, $P = 0.001$). Aniso/zygodactyly influences locomotion, size, and the orientation of the substrate on the proportions of the phalanges.

Multivariate analysis of variance performed on the first three principal components show a significant difference on the third component in the phalangeal proportions depending on whether birds are zygodactylous or anisodactylous (Wilks' $\lambda = 0.2$, $F = 7.42$, $P = 0.001$). Moreover, birds that show different types of locomotion (Wilks' $\lambda = 0.3$, $F = 2.8$, $P = 0.01$) and birds that use differently sized substrates (Wilks' $\lambda = 0.47$, $F = 7$, $P = 0.002$) also differ. However, these results are no longer significant when the phylogeny is taken into account.

Subsequent univariate ANOVAS showed that birds that use different substrate orientations are different on the first axis, but only when the phylogeny is taken into account ($F = 1.8$, $P = 0.04$). Thus, the traits determining this axis (proximo-distal gradient in phalanx length and the size of the hallux) are convergent and depend on substrate use. The results of the non-phylogenetic univariate ANOVAS testing for differences between anisodactylous and zygodactylous configurations ($F = 25$; $P = 0.001$), locomotion ($F = 3.2$; $P = 0.04$), and the size of the substrate ($F = 7.5$; $P = 0.01$) are significant only for the third axis. However, when taking into account the phylogeny, these groups are no longer significant (Supporting Information Table S1, Table S2).

The 3D shape of the metatarsal trochlea

Morphospace of the trochlea

The distribution of the species in the morphospace clearly separates birds with anisodactylous, heterodactylous, and zygodactylous feet. This is to be expected, as the position of the metatarsal trochlea corresponds to the place where the toes articulate with the dorsal or plantar face of the leg (Fig. 6).

Phylogeny

The result of the multivariate K performed on the Procrustes coordinates of the trochlea is not significant. Nevertheless, there is a significant phylogenetic signal on the third principal component ($K = 0.32$, $P = 0.03$; Supporting Information Table S3, Table S4).
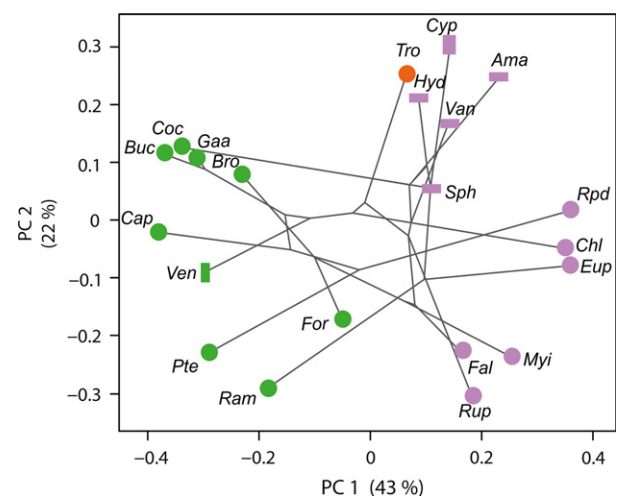

Fig. 6 3D shape of the metatarsal trochleas. Scatterplots of the first two principal components of the analysis performed on the trochlea representing over 64% of the variation in the 3D landmarks dataset. The phylogeny has been plotted in the morphospace. The distribution tends to separate species depending on the position of their toes. See Fig. 1 and Table S1 for definition of abbreviations and symbols.

Influence of anisodactylous vs. a zygodactylous configuration, locomotion, and the size and the orientation of the substrate on the 3D shape of the metatarsal trochlea
Multivariate analysis of variance performed on the first three principal components shows a significant difference in the 3D shape of the metatarsal trochlea, depending on whether birds are zygodactylous or anisodactylous (Wilks' $\lambda = 0.3$, $F = 7.42$, $P = 0.002$). Furthermore, birds that use differently sized substrates (Wilks' $\lambda = 4.6$, $F = 7$, $P = 0.01$) also differ in the shape of the trochlea. However, these results are not significant when the phylogeny is taken into account. The univariate ANOVAS showed different trochlear shapes can be observed on the first ($F = 6.1$, $P = 0.008$) and third axis ($F = 4.7$, $P = 0.02$) between anisodactylous and zygodactylus birds. Furthermore, birds that use substrates of different sizes have a different trochlear shape on the second axis ($F = 10.31$, $P = 0.004$) with birds moving on a wide support separated from the others (*post-hoc* test: $P = 0.01$). However, as for the multivariate analyses, these results are no longer significant when the phylogeny is taken into account. The swift *Cypseloides* and the nightjar *Hydropsalis*, both of which do not use their feet for walking but rather for posture, group with the walking birds (the penguin, the plovers, and the teal). Finally, an RV test showed that there is no co-variation ($P > 0.05$) between the orientation of the trochlea and the dimensions of the phalanges.

Discussion

Most birds have the same phalangeal formula as the one observed in primitive amniotes, i.e. 2-3-4-5 (Romer, 1956). In birds with four toes this formula is usually conserved, although the morphology of feet is highly variable. The exceptions are found among the Caprimulgidae and Trochilidae, where toe IV has only four phalanges as reported previously (e.g. Beddard, 1898; Livesey & Zusi, 2006). According to Zusi (2013), a fusion (synostosis) between phalanges 3 and 4 occurs in the Trochilidae. This is considered a derived state within hummingbirds, and has evolved in parallel repeatedly within the family, as well in some Apodidae (Zusi, 2013).

The height of the ungual flexor tubercle, an distinctive trait

The most striking morphological trait of the toes of birds is the height of the flexor tubercle of the ungual phalanx. This trait is expressed similarly in each toe, as previously documented by Mosto & Tambussi (2014) for birds of prey. Its functional significance was explained by Livesey & Zusi (2006) as a raptorial adaptation. In fact, a large flexor tubercle is also found on the ungual phalanx of birds that hang like *Cypseloides* or *Glyphorynchus*. Backus et al. (2015) demonstrated that the action of the tendon

inserted on the distal phalanx is determinant in grasping or carrying. The height of the tubercle is likely developed in response to the tension needed to grip with higher tubercles providing greater moment arms. In walking birds, a flat tubercle could be advantageous, as it limits friction (Livesey & Zusi, 2006). Interestingly, this highly functional trait follows a pattern that is different from that of the phalangeal proportions, as shown by its strong and divergent loading when analyzed together with the phalangeal proportions.

Morphological patterns and developmental pathways

The proportions of the phalanges and the orientation of the metatarsal trochlea clearly distinguish two morphological patterns depending on the orientation of the toes: anisodactylous vs. zygo- or heterodactylous. The proportions of the phalanges of the anisodactylous birds show a morphological gradient from long distal/short proximal to short distal/long proximal phalanges. Kavanagh et al. (2013) found a similar morphological pattern for a large sample of anisodactylous birds and other tetrapods. This pattern is explained by the developmental pathways that control phalanx segmentation (Sanz-Ezquerro & Tickle, 2003; Kavanagh et al. 2013). The main differences in the phalanges of the zygodactylous and heterodactylous feet are the width at the base of the toe that is orientated backward: toe II of the heterodactylous and toe IV of the zygodactylous species are, respectively, the widest. Neither the proportions of the phalanges nor the size of the hallux expressed on the first axis are different in these two groups. The developmental pattern that regulates the length of the phalanges in anisodactylous birds is unlikely to be modified. The toes that are reoriented, i.e. toe II in heterodactylous and toe IV in zygodactylous birds, are reinforced. This is probably due to the mechanical stress imposed by the embryonic muscular activity that reorients the toes in a backward direction.

The 3D shape of the metatarsal trochleae is different in anisodatylous and zygodactylous birds and depends on the orientation of the toes. Moreover, there is no co-variation in the shape of the metatarsal trochlea and the length of the phalanges. This may be explained by the two different modules of the skeleto-morphogenetic pathway that control the tarsometatarsus growth and the phalangeal growth (Kavanagh et al. 2013). Thus, three developmental processes participate. There are two modules of skeletal morphogenesis, one at the level of the phalanges and one at the level of the tarsometatarsus, as described by Kavanagh et al. (2013). The other module is the result of embryonic muscular activity (Botelho et al. 2014, 2015), which likely participates in re-orientating the metatarsal trochleae and toes and likely causes the enlargement of reoriented toes.

Morphological patterns and phylogeny

The orientation of the substrate is the only significant difference in the phalanx proportions remaining when including the phylogeny in our analysis. The distribution of species on the first axis of the PCA indicates that the birds walking on the horizontal substrates (ground) are counterposed to those moving on vertical substrates (tree trunks and cliffs). This difference persists when the phylogeny is included in the analysis. The reduction of the size of the hallux is known to be a typical adaptation in walking birds, and the hallux can even disappear in non-passerine birds (Raikow, 1985). The differences in the size of the hallux, being smaller in ground-dwelling species as the penguin *Spheniscus,* the teal *Amazonetta,* and the Lapwing *Vanellus* and being larger in climbers such as the woodcreeper *Glyphorynchus* and the swift *Cypseloides*, are convergent. However, the woodpecker *Veniliornis* is in a neutral position on the first axis. In zygodactylous birds, the hallux size may be not affected by the orientation of the substrate because it is the other opposite toe (II) that is enlarged. Its enlargement in climbing species has an obvious functional meaning, as this provides a large and strong support. The other traits were not different when taking into account the phylogeny. This may be due to the strong link between the history of the species, the behavioural and environmental traits, and the shape of the feet.

The silent trait

Interestingly, toe III never emerges from the analyses as being discriminating and its proportions do not exhibit global modifications. It is always the longest toe and is oriented forward except in the case of a specialization of this toe for climbing, for example in the Picidae such as *Veniliornis,* where it can move laterally. Furthermore, this toe is never lost in archosaur evolution, either in the fore- or the hind limb (de Bakker et al. 2013) and it is likely the most conservative ray of the autopod.

Function and shape of the feet

Not only the orientation of the toes but also the type of locomotion (hopping/walking) and the width of the substrate have a significant impact on phalangeal proportions and on the 3D shape of the trochleas. These are related to the degree of terrestriality, since hopping birds are often also arboreal and as such move on narrow substrates as compared with terrestrial walking birds. Indeed, the metatarsal trochlea, spreading toe II medially and toe IV laterally in terrestrial anisodactylous birds, offers an advantage for support and stability on wide substrates. In contrast, arboreal anisodactylous birds have their toes closer together, with the trochlea and the proximal phalanges confined in a common skin envelope (Zusi, 2013; pers. obs.). We also observed the presence of tubercles on the tarsometatarsus near the trochlea in zygodactylous and heterodactylous feet that guide the tendons of the flexor muscles at the base of the toes.

The pincer-like foot

An arboreal lifestyle constrains all animals with the same functional demand: to move on cylindrical substrates that differ in flexibility depending on the diameter and spatial orientation. Even though tetrapods have developed many anatomical ways to adapt to this habitat, a pincer-like foot is a useful tool to move on this type of substrates (Backus et al. 2015). To make this pincer, birds developed two morphological patterns: in anisodactylous birds the backward part of the pincer is made up by a strong hallux and the three other toes are joined to form the forward part. In zygodactylous and heterodactylous birds the hallux is small and the backward part of the foot is mainly composed of the enlargement of the reversed toe. In these birds, the forward part of the pincer is mainly formed by toe III assisted by the other forward-pointing toe. However, the tarsometatarsus shape changes depends on the substrate. The orientation of the trochlea is correlated with the orientation of the toes, and is dependent on the functional demands such as the width of the substrate. Some crests on the trochleae reorient the directions of the muscular tendons (Zusi, 2013; pers. obs.) and as such modify the mechanical system by controlling the moment arm or the direction of the force and by adjusting the shape of the bone to the mechanical demands.

The pincer-like foot in tetrapods

A pincer-like foot is a useful tool for grasping and its occurrence is spread across the tetrapod clade, being present in lissamphibians, lepidosaurians, and mammals. This convergent feature often involves an opposable hallux (Sustaita et al. 2013). The most specialized clades, apart from birds, are chameleons and primates. In the chameleon hind foot, toes I and II are always opposed to toes III and IV (Anderson & Higham, 2014; Diaz & Trainor, 2015), as is observed in the heterodactylous bird foot (note that birds do not exhibit a fifth toe). However, in chameleons, the splitting of the autopodial element appears early in their development, implying a different skeletal morphogenesis (Diaz & Trainor, 2015), and always involves the same toes. The radiation of chameleons was accompanied by modifications in the number of metatarsal elements that change the flexibility of the foot (Herrel et al. 2013). This process is different from the mechanical process induced by the embryonic muscular activity that causes the backward rotation of the bird toes (Botelho et al. 2014, 2015) and which underlies the functional diversity of bird feet.

In the primate foot, the hallux is not permanently opposed to the other toes. The pincer is achieved by an adduction–abduction of the hallux. As in anisodactylous birds, species living on thin branches have a larger hallux compared with those walking on the ground or on wider branches (Preuschoft et al. 1993). In humans, the only strictly terrestrial bipedal primate, the hallux is no longer opposable but is oriented forward. This condition is functionally convergent with the hallux loss in some terrestrial birds. The modifications of the proportions of the foot in primates are primarily those of the tarsal bones (Gebo, 2014). As in anisodactylous birds, the proportions of the phalanges do not differ markedly between the toes. The modification of the digits that are found in the hands, such as the thin third finger in the Aye-aye (*Daubentonia madagascariensis*) or the very short second finger in the slow lorises, *Loris* and *Nycticebus* (Gebo, 2014), are not found in the feet. Thus, even if the primate hallux is not permanently reversed as in birds, the same morphological pattern is found as in anisodactylous birds.

The two main morphological patterns led to a pincer-like foot in vertebrates: the opposition of the hallux only or the opposition of the hallux plus another toe. The third toe is not involved in this process and is the most conserved digit. Developmental rules guide the proportions of the phalanges, but their robustness may be reinforced by skeletal plasticity. The widening of the phalanges can be, like the orientation of the toes, a response of the bone tissue to mechanical demands. However, the overall proportions stay within a limited morphospace that depends on both the plesiomorphic osteogenetic rules and the functional demands in anisodactylous species, and yet are constrained by function and phylogeny in zygodactylous species.

Conclusion

The feet are a crucial functional trait allowing the exchange of forces between an animal and its environment. Our comparative study of the feet in birds shows that two morphological patterns exist which permit the adaptation of the foot to an arboreal life. The backward orientation of the hallux is shared with primates and is convergent and evolved independently; the other one, the orientation of another toe (II or IV), is unique and linked to the evolutionary history of the clade. The impact of mechanical constraints is different in both patterns. However, both morphological patterns result in the same function: a strong pincer-like foot essential to grasp the 3D complex substrate.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Results of the MANOVAs and phylogenetic MANOVAs on the proportions of the phalanges.

Table S2. Results of the ANOVAs and phylogenetic ANOVAs on the proportions of the phalanges.

Table S3. Results of the MANOVAs and phylogenetic MANOVAs on the 3D shape of the trochleae.

Table S4. Results of the ANOVAs and phylogenetic ANOVAs on the 3D shape of the trochleae.

Fig. S1. (A) Projection of the vectors on the first PC plane. (B) Details of the eigenvalues on the first axis. (C) Details of the eigenvalues on the second axis of the PCA.

Fig. S2. Projection of the individuals on the first PCA plane.