Isotopic niches support the resource breadth hypothesis

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Summary

1. Because a broad spectrum of resource use allows species to persist in a wide range of habitat types, and thus permits them to occupy large geographical areas, and because broadly distributed species have access to more diverse resource bases, the resource breadth hypothesis posits that the diversity of resources used by organisms should be positively related with the extent of their geographic ranges.

2. We investigated isotopic niche width in a small radiation of South American birds in the genus *Cinclodes*. We analysed feathers of 12 species of *Cinclodes* to test the isotopic version of the resource breadth hypothesis and to examine the correlation between isotopic niche breadth and morphology.

3. We found a positive correlation between the widths of hydrogen and oxygen isotopic niches (which estimate breadth of elevational range) and widths of the carbon and nitrogen isotopic niches (which estimates the diversity of resources consumed, and hence of habitats used). We also found a positive correlation between broad isotopic niches and wing morphology.

4. Our study not only supports the resource breadth hypothesis but it also highlights the usefulness of stable isotope analyses as tools in the exploration of ecological niches. It is an example of a macroecological application of stable isotopes. It also illustrates the importance of scientific collections in ecological studies.

Key-words: *Cinclodes*, Furnariidae, isotopic niche, niche evolution, resource breadth hypothesis, stable isotope analysis

Introduction

The concept of the ecological niche has many complementary guises, each of which attempts to quantitatively depict different features of an organism's ecological characteristics (Schoener 2009). For example, in its contemporary form, the Grinnellian niche (Grinnell 1917; Soberón 2007) focuses on the environmental variables measured at broad spatial scales that shape a species' geographical distribution (Soberón & Peterson 2005). This niche delineation is dependent on what Peterson *et al.* (2011) have christened non-interactive (or non-linked) variables, which are not consumed and hence are not subject to competition (Peterson *et al.* 2011). Conversely, the Eltonian

(Elton 1927; Soberón 2007) niche emphasizes variables that are consumed, and hence can be dynamically linked with an organism's population density, and with those of other organisms with which it interacts (Chase & Leibold 2003). In contrast with the Grinnellian niche, which is a tool for the study of species distributions (Peterson *et al.* 2011), the Eltonian niche is well suited to study ecological interactions, including trophic relationships (Chase & Leibold 2003). Brown (1984) hypothesized that Grinnellian and Eltonian niches are positively related. He argued that species with broad Eltonian niches should be geographically widespread, and hence have broad Grinnellian niches (Gaston 2003).

The resource breadth hypothesis has two complementary explanations. First, a broad Eltonian niche allows a species to persist in a wide range of habitat types, and

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thus permits it to occupy large geographical areas that are associated with a wide variety of environmental conditions, i.e. a broad Grinnellian niche. In contrast, narrow Eltonian niches restrict species to the few places where their requirements are met (Williams *et al.* 2006). Second, a positive relationship between Eltonian niches and broad areas of distribution could arise from a sampling effect. Broadly distributed species are likely to have access to a more diverse resource base (Gaston 2003). Therefore, measurements of the Eltonian niche breadth should be broader in these species than in geographically restricted ones. We used stable isotopes, and the idea of the 'isotopic niche', to test the resource breadth hypothesis in *Cinclodes* ovenbirds, an ecologically diverse group of South American birds (Remsen *et al.* 2003; Chesser 2004).

The term isotopic niche was coined by Bearhop *et al.* (2004). In coarse analogy with Hutchinson's (1957) *n*-dimensional niches, the isotopic niche is a subset of a multivariate space. The axes of this space are defined by the isotopic values of different elements (most commonly carbon, nitrogen, hydrogen and oxygen) in an organism's tissues (Newsome *et al.* 2007). Newsome *et al.* (2007) noted that variation in the carbon and nitrogen isotope values in consumer tissues are primarily driven by photosynthetic pathways at the base of food webs and trophic level respectively (see also, Smith & Epstein 1971), and thus reflect a combination of habitat and resource use. Hence, the isotopic analyses of these two elements can provide insights into the variables that comprise Eltonian niches, including the habitats that animals use. The isotope values of hydrogen and oxygen vary geographically, often in concert with latitude and elevation (Poage & Chamberlain 2001; Bowen, Wassenaar & Hobson 2005), and therefore can inform variables of Grinnellian niches. We used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values in the feathers of museum specimens to characterize the Eltonian isotopic niches of *Cinclodes* species, and hydrogen (δ^2H) and oxygen ($\delta^{18}O$) isotope values to characterize their Grinnellian niches. We used the area of standard ellipses in $\delta^{13}C$ vs. $\delta^{15}N$ and δ^2H vs. $\delta^{18}O$ isotopic spaces to quantify the breadth of isotopic Eltonian and Grinnellian niches respectively (Jackson *et al.* 2011). The analysis of stable isotopes on tissues of museum bird specimens represents an opportunity to characterize aspects of the ecological niche in organisms that, although difficult to study in the wild because they inhabit remote areas, such as many *Cinclodes* species, are relatively well represented in museums (see Edwards *et al.* 2002).

The species in *Cinclodes* are well suited to conduct a test of the isotopic version of the resource breadth hypothesis because (i) they vary in the extent of their geographical distribution; (ii) they are ecologically diverse; and (iii) the natural history of several species has been relatively well investigated (Remsen *et al.* 2003; Chesser 2004). The 16 species in the genus inhabit a wide variety of ecological habitats throughout extensive latitudinal (from c. 10° N to c. 50° S, (Remsen *et al.* 2003; Chesser 2004) and elevational ranges (from 0 to over 5000 m; Chesser 2004; Fig. 1a). *Cinclodes* includes a species that is only found on islands (*C. antarcticus*; Remsen *et al.* 2003 and references therein; Fig. 1a), species that are associated exclusively with intertidal marine environments (*C. nigrofumosus* and *C. taczanowskii*; Sabat *et al.* 2006a; Fig. 1a), and species that appear to migrate seasonally between low and high elevation sites (*C. oustaleti* and *C. patagonicus*; Martínez del Rio *et al.* 2009). Following Brown's (1984) resource breadth hypothesis, we predicted that species that occupy wider geographical and elevational ranges, and hence wider areas of δ^2H vs. $\delta^{18}O$ space, would also rely on prey derived from more diverse food webs, and therefore occupy wider areas of $\delta^{13}C$ vs. $\delta^{15}N$ space. In addition to being ecologically diverse, the evolutionary relationships among *Cinclodes* species are relatively well understood (Chesser 2004; Sanín *et al.* 2009; Derryberry *et al.* 2011). The existence of a well-resolved phylogeny for the genus allowed us to explore the resource breadth hypothesis in an evolutionary context. Specifically, we used phylogenetically informed analyses to ask whether the isotopic niche breadth in δ^2H vs. $\delta^{18}O$ space was positively correlated with the isotopic niche breadth in $\delta^{13}C$ vs. $\delta^{15}N$ space.

In addition to the diverse ecological characteristics of *Cinclodes*, recent evidence shows that the genus is also morphologically diverse. Rader *et al.* (2015) found that species in the genus have diversified in body size (species range in body mass from 21 to 104 grams) and wing and foot morphology. In addition to testing the resource breadth hypothesis, we examined the relationship between niche width and morphology. We hypothesized an association between broad Grinnellian isotopic niches (i.e. large areas in δ^2H vs. $\delta^{18}O$ space) and longer and more pointed wings, which confer aerodynamic advantages to species that undertake elevational movements, and hence potentially have evolved to occupy larger elevational ranges.

Materials and methods

Details of methods are presented in Appendix S1, Supporting Information. Briefly, we collected contour feathers for isotopic analysis from 254 museum study skins. Specimens had been collected from 1890 to 1950. Our sample included 12 of the 16 *Cinclodes* species (see Appendix S2). We used standard stable isotopic analyses to measure the δ^2H, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ values of feather samples. As a result of anthropogenic CO_2 inputs, $\delta^{13}C$ atmospheric values have been declining at an accelerating pace over the last 200 years (Verburg 2007). Because the change in $\delta^{13}C$ from 1890 to 1950 is only about $-0.3‰$ (Verburg 2007), we decided not to correct for temporal variation in carbon isotopes.

We used standard ellipse areas (SEAs) estimated using a Bayesian approach as estimates of isotopic niche (Jackson *et al.* 2011). We also estimated the hypervolume of the δ^2H vs. $\delta^{18}O$ vs. $\delta^{13}C$ vs. $\delta^{15}N$ space using the supermajor and superminor axes derived from each species' pair of ellipses as:
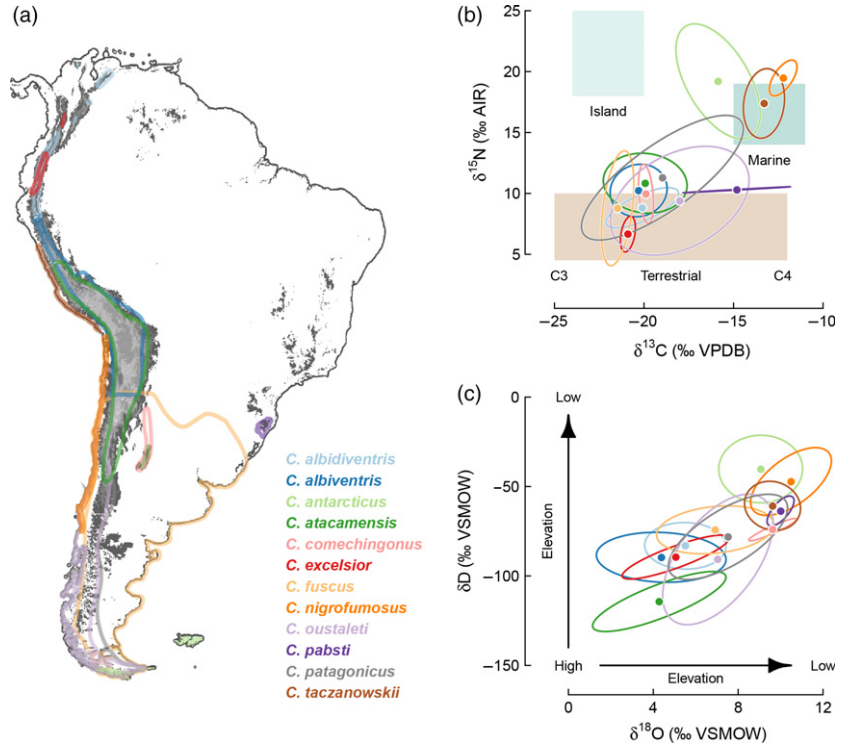
Fig. 1. (a) Geographic ranges of *Cinclodes*, redrawn from (Remsen *et al.* 2003). (b) Carbon and nitrogen isotopic niches, and (c) Oxygen and hydrogen isotopic niches of *Cinclodes* are depicted as standard ellipses. We used the area of these ellipses as a measure of niche breadth. For descriptive purposes, we outlined the $\delta^{13}C$ and $\delta^{15}N$ isotopic space occupied by the food webs that *Cinclodes* can potentially use. The island space represents only island vegetation fertilized by seabird guano.

Hypervolume $= 1/2\pi^2(ABCD)$

where A and B represent the lengths of the supermajor ellipse axes and C and D are the lengths of the superminor ellipse axes (Bowman 1953). We based our comparative analyses on a phylogenetic reconstruction of the family Furnariidae (Derryberry *et al.* 2011), pruned to include only *Cinclodes* species, and including associated estimated branch lengths and divergence times. We used phylogenetically controlled generalized least squares regression (PGLS; Martins & Hansen 1997; Freckleton, Harvey & Pagel 2002; Blomberg *et al.* 2012) to ask if SEAs in $\delta^{13}C$ vs. $\delta^{15}N$ space were correlated with SEAs in $\delta^{2}H$ vs. $\delta^{18}O$ space using the picante and caper packages in R (R Development Core Team 2013). To estimate the effect of elevation and latitude on $\delta^{2}H$ and $\delta^{18}O$, we used PGLS models that included latitude, elevation and their interaction as independent variables, in addition to an ordinary least squares model (OLS). We used the sample size-corrected Akaike information criterion (AICc) to choose among models including combinations of these variables. Finally, we used PGLS to ask whether niche width, as estimated by SEAs, was correlated with two principal component (PC) axes previously derived from morphological measurements (Rader *et al.* 2015). These explained 56·7% (PC1) and 7·9% (PC2) of the variance respectively. PC1 was a good index of body mass, whereas PC2 described variation in hallux length and wingtip pointedness (Rader *et al.* 2015). In all our analyses, we assessed the effect of phylogenetic signal, as measured by Pagel's λ, and assessed the effects of kappa (κ) and delta (δ) branch length transformations on our inferences (Blomberg *et al.* 2012). We also estimated the maximum likelihood optimized values of lambda, as well as κ and δ branch length transformations simultaneously. Briefly described, a kappa transformation investigates the influence of tree topology by raising the branch lengths to the power of κ. Therefore, $\kappa = 1$ results in no branch length

transformation, while a $\kappa = 0$ forces all branch lengths equal to 1 (O'Meara 2012). Delta transformations stretch either early ($\delta < 1$) or later ($\delta > 1$) branches, demonstrating whether trait evolution occurred early and deep within the tree, or more recently near the species tips respectively (O'Meara 2012). The PGLS analyses were conducted using the picante and caper package in the R statistical computing environment (R Development Core Team 2013).

Results

ISOTOPES AND NICHES

The models relating $\delta^{2}H$ and $\delta^{18}O$ with elevation and latitude that were best supported by the data included these two variables and their interaction (ΔAIC >5, Table 1). Pagel's λ was optimized at 0·0 in all models. Hence, using OLS was more appropriate than using PGLS for these relationships. $\delta^{2}H$ and $\delta^{18}O$ decreased with elevation and increased with both latitude and the interaction between elevation and latitude (Table 1). However, these relationships had a large amount of variation ($R^2 = 0·28$ and 0·36 for $\delta^{2}H$ and $\delta^{18}O$ respectively; Fig. 2). This variation was especially large for specimens collected at low elevations (0–500 m). For specimens collected at sea level, $\delta^{2}H$ values ranged from −150‰ to −25‰ and that of $\delta^{18}O$ ranged from 0 to 15‰ (Fig. 2).

We found variation in both the position and area of standard ellipses. *Cinclodes nigrofumosus* and *C. taczanowskii*, the two closely related marine species, occupied areas in $\delta^{13}C$ vs. $\delta^{15}N$ and $\delta^{2}H$ vs. $\delta^{18}O$ space that were distinctly enriched (Fig. 1b and c). In a similar fashion, the species

Table 1. Top ranked models (in bold) of δD and δ^{18}O variation included elevation, latitude, and their interaction as independent variables

Model	AICc
δ^{2}H = −61·59 − 0·01 × Elev + 0·37 × Lat + 0·0002 × [(Lat + 20·03) × (Elev − 1889·06)]	**1468·10**
δ^{2}H = −62·69 − 0·008 × Elev + 0·14 × Lat	1478·25
δ^{2}H = −68·32 − 0·007 × Elev	1485·37
δ^{2}H = −88·08 − 0·35 × Lat	1498·71
δ^{18}O = 9·95 − 0·002 × Elev + 0·08 × Lat + 0·00002 × [(Lat + 20·03) × (Elev − 1888·04)]	**744·8**
δ^{18}O = 9·83 − 0·002 × Elev + 0·06 × Lat	749·80
δ^{18}O = 7·36 − 0·001 × Elev	769·05
δ^{18}O = 5·06 − 0·03 × Lat	803·15
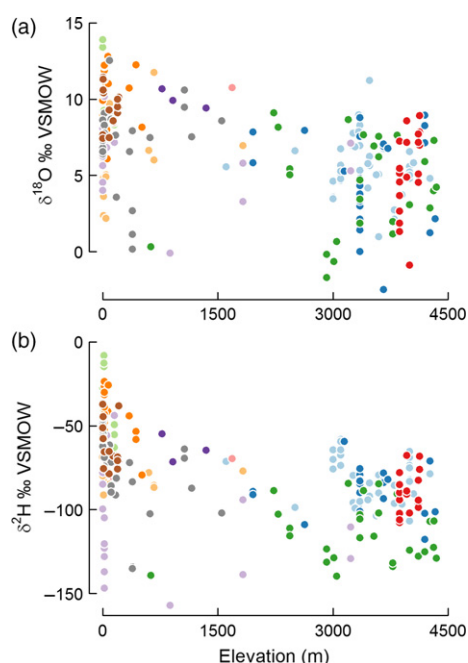
Fig. 2. (a) Oxygen (δ^{18}O) and (b) hydrogen (δ^{2}H) isotopic values as functions of estimated elevation (m) of specimen collection site. The relative abundance of heavy isotopes (δ^{2}H and δ^{18}O) in *Cinclodes* feathers decreased with increased elevation (Table 1). Species colours as in Fig. 1.

that are found exclusively at high elevations (*C. atacamensis* and *C. excelsior*) had lower isotope values (Fig. 1b and c). *Cinclodes pabsti*, the only species that inhabits areas with a significant contribution from primary producers using C$_4$ photosynthesis (Still *et al.* 2003), had a broad range of δ^{13}C values, but our sample size for this species was small (*n* = 5). Species often considered to have broad elevational ranges (*C. oustaleti* and *C. patagonicus*) occupied the mid-range of values in both δ^{13}C vs. δ^{15}N and δ^{2}H vs. δ^{18}O spaces and had higher SEAs, and hence broader isotopic niche widths.

Ellipse areas in δ^{13}C vs. δ^{15}N and δ^{2}H vs. δ^{18}O isotopic space were highly correlated, and this correlation was robust to the value of Pagel's λ, and hence, to the assumption of phylogenetic relatedness. When λ was nearly 0 (the value optimized in our analyses), and hence when the model assumed little or no phylogenetic signal, the ordinary least squares was highly significant (*r*^{2} = 0·84, *P* < 0·001; Fig. 3b). For λ = 1, the phylogenetically independent contrasts also yielded a highly significant correlation (*r*^{2} = 0·67, *P* < 0·001); note that when λ = 1 PGLS converges with Felsenstein's phylogenetic independent contrasts (Blomberg *et al.* 2012). Thus, in *Cinclodes*, the width of the isotopic niche in δ^{13}C vs. δ^{15}N space and that in δ^{2}H vs. δ^{18}O isotopic space niche were correlated, as predicted by the resource breadth hypothesis. To assess whether the two species with the broadest niches on all isotopic axes, *C. oustaleti* and *C. patagonicus*, could potentially have a disproportionate effect on the relationship between SEAs of δ^{13}C vs. δ^{15}N and those of δ^{2}H vs. δ^{18}O, we repeated analyses using robust regression (package 'robust' in R, Wang *et al.* 2014). We found a slight drop in the significance of the relationship between δ^{13}C vs. δ^{15}N and those of δ^{2}H vs. δ^{18}O SEAs, but the trend remained statistically significant. Additionally, because the data set for *C. comechingonus* is small, we conducted all analyses with and without this species and found no difference in the statistical significance of trends. Here we report only results including *C. comechingonus*.

Ellipse areas in δ^{13}C vs. δ^{15}N and δ^{2}H vs. δ^{18}O isotopic space, and total niche hypervolume were not correlated with PC1 (an index of body size), independently of the value of λ (*r*^{2} < 0·12, *P* > 0·29). The total niche hypervolume, however, was strongly and significantly correlated with PC2 (an axis of wingtip pointedness and toe length; Fig. 4a) when λ was optimized (λ$_{opt}$ = 0·99, *r*^{2} = 0·42, *P* < 0·03). This relationship was strengthened when λ, κ and δ were optimized simultaneously (*r*^{2} = 0·70, *P* < 0·001, λ$_{opt}$ = 0·98, κ$_{opt}$ = 2·05, δ$_{opt}$ = 2·02). This result was strongly dependent on the assumption that λ was equal to 1, or nearly so (i.e. a strong phylogenetic signal in the residuals of the regression). We also found significant relationships between PC2 and δ^{13}C vs. δ^{15}N and δ^{2}H vs. δ^{18}O standard ellipse areas. The statistical significance of these relationships was also strongly dependent on the assumptions of the model chosen. The strongest correlation was between the SEAs in δ^{2}H vs. δ^{18}O isotopic space (*r*^{2} = 0·86, *P* < 0·001, λ$_{opt}$ = 1·0, κ$_{opt}$ = 2·07, δ$_{opt}$ = 1·69; Fig. 4b). Similar to our results with niche hypervolume, the concurrent optimization of λ, κ and δ
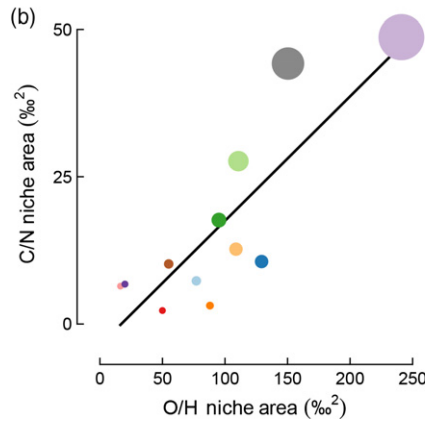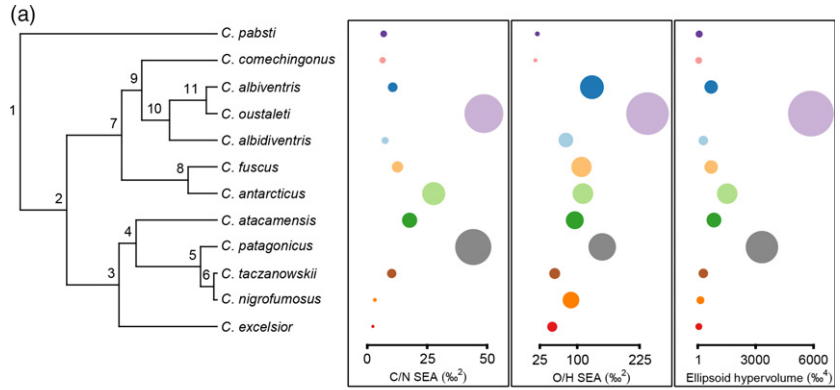
Fig. 3. (a) Isotopic niche width varied among *Cinclodes* species, with *C. patagonicus* and *C. oustaleti* displaying the broadest niches. Points are scaled relative to niche width values. 95% confidence intervals are contained within the points. (b) PGLS analyses indicate that $\delta^{13}C$ vs. $\delta^{15}N$ SEAs among *Cinclodes* are correlated with δ^2H vs. $\delta^{18}O$ SEAs. This result is robust to the assumption of phylogenetic covariance. Species colours as in Fig. 1.
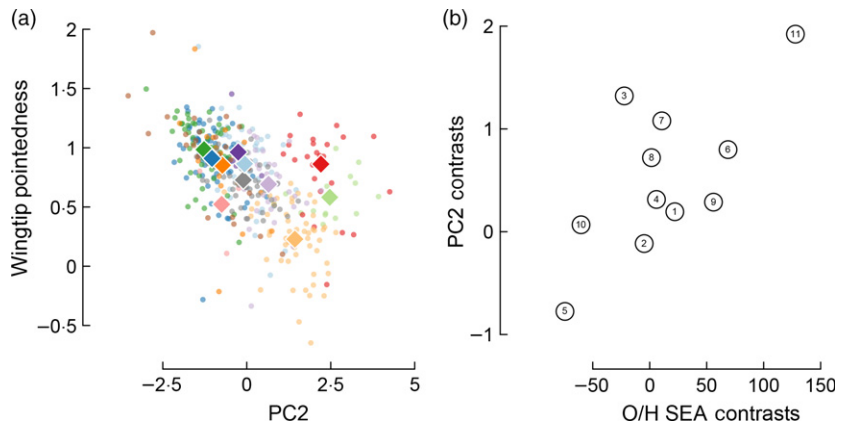

Fig. 4. (a) The index of wingtip pointedness (Lockwood, Swaddle & Rayner 1998) was negatively related with PC2 (Rader *et al.* 2015). (b) PC2 was correlated with isotopic niche width (represented in the figure by the width of the δ^2H and $\delta^{18}O$ niche). Because optimized Pagel's $\lambda \approx 1$, we used phylogenetically independent contrasts to depict this relationship. Numbers represent the contrast/comparison (panel a in Fig. 3).

strengthened the correlation beyond that found when only λ was optimized ($r^2 = 0.46$, $P < 0.02$, $\lambda_{opt} = 1.0$). The SEAs in $\delta^{13}C$ vs. $\delta^{15}N$ space were not correlated with PC2 when λ was optimized independently ($P > 0.05$, $\lambda_{opt} = 0.0$), but were significantly correlated when all three parameters were optimized simultaneously ($r^2 = 0.32$, $P = 0.037$, $\lambda_{opt} = 0.96$, $\kappa_{opt} = 1.70$, $\delta_{opt} = 2.64$). The correlations of δ^2H vs. $\delta^{18}O$ SEAs and niche hypervolume with PC2 rapidly lost statistical significance for values of Pagel's λ of less than ≈ 1.0. The strong dependence of these results on assuming that $\lambda \approx 1$, and hence on the use of phylogenetic independent contrasts, suggests that the positive relationship between PC2 and niche widths is not robust to varied assumptions of phylogenetic signal. In addition, the strength of these relationships

was strongly dependent on the weight of two points that represent differences in isotopic niche widths and PC2 within two clades (represented by contrasts 5 and 11 in Fig. 4b). Contrast 5 reflects the difference between the narrow-niched marine clade (including *C. taczanowskii* and *C. nigrofumosus*) and broad-niched *C. patagonicus* (Fig. 4b), and contrast 11 reflects the difference between *C. albiventris*, which has a relatively narrow isotopic niche, and *C. oustaleti*, which has a very broad one.

Discussion

Our analyses tested the isotopic version of the resource breadth hypothesis. We found that the estimated widths

of niches in δ^{13}C vs. δ^{15}N space were strongly and significantly correlated with niche widths in δ^2H vs. δ^{18}O space, which we interpret as evidence of broad elevational range. In *Cinclodes*, species that occupy broader elevational gradients also tend to feed in more diverse habitats and food webs. The broadest niches were occupied by species that obtain resources from both terrestrial and marine environments and that live in habitats ranging in elevation from sea level to >4000 m (Martínez del Rio *et al.* 2009), whereas the narrowest niches were occupied by species that feed exclusively on marine or terrestrial environments and are restricted to sites at either high or low elevation (Remsen *et al.* 2003; Chesser 2004). The results of our phylogenetic analyses suggest that this pattern is environmentally, rather than phylogenetically driven. We also found a correlation between isotopic niche width and morphology. Our results demonstrate that stable isotope analysis of museum specimens can inform not only many aspects of a species' niche but also of niche evolution. However, although stable isotope analyses are powerful tools for ecological research, they have limitations. Thus, after considering the implications of our results in the context of the resource breadth hypothesis, we consider the power and limitations of stable isotopes in our study and for the study of ecological niches and their evolution in general.

THE ISOTOPIC NICHE AND THE RESOURCE BREADTH HYPOTHESIS

The resource breadth hypothesis posits that organisms that use a greater array of resources can maintain viable populations within a wide variety of conditions, and hence occupy larger ranges (Boulangeat *et al.* 2012). Although the correlation between trophic niche breadth and geographical range size is intuitively appealing and has been investigated for almost 30 years, a general consensus on its validity has been slow to emerge. Slatyer, Hirst & Sexton (2013) conducted a meta-analysis of available studies and concluded that the resource breadth hypothesis is a general ecological pattern. They found a strong positive relationship between geographic range, and both environmental tolerance and breadth of habitat use. They also found a weaker, albeit statistically significant, positive relationship between range size and diet breadth (Slatyer, Hirst & Sexton 2013). We found a positive relationship between the width of the isotopic niche in δ^{13}C vs. δ^{15}N space and that in δ^2H vs. δ^{18}O space. This correlation suggests that species of *Cinclodes* that sample from a broad range of food webs found in different habitats also tend to occupy a broad elevational range, as indicated by their use of a variety of isotopically distinct water sources. Our results, therefore, support the isotopic version of the resource breadth hypothesis and hence, potentially, its generality.

INTERPRETATION OF POSITION AND WIDTH OF ISOTOPIC NICHES IN δ^{13}C VS. δ^{15}N AND δ^2H VS. δ^{18}O SPACES

Like many applications of stable isotopes to animal ecology, isotopic niches are 'near sighted' in that they do not, except in rare instances, identify to a high degree of taxonomic definition the species eaten by a consumer, nor the exact geographical position (latitude or elevation) where a tissue was synthesized (Martínez del Rio *et al.* 2009). Carbon isotope (δ^{13}C) values primarily reveal the metabolic pathways at the base of food webs (McKechnie 2004), whereas nitrogen (δ^{15}N) values reveal nitrogen inputs at the food web as well as trophic position (reviewed by Post 2002). As all species of *Cinclodes* feed on a wide range of invertebrates (Remsen *et al.* 2003), we can make the assumption that all *Cinclodes* feed at the same trophic level. Therefore, a parsimonious interpretation of a broad isotopic niche in δ^{13}C vs. δ^{15}N space in a *Cinclodes* species is that its members depend on a variety of food webs that differ in their carbon and nitrogen isotopic composition. Because the carbon and nitrogen values of food webs differ among habitats (e.g. marine vs. terrestrial, Sabat & Martinez del Rio 2002; Martínez del Rio *et al.* 2009), a wide niche in δ^{13}C vs. δ^{15}N space implies use of a diversity of habitats. It is important to note that a wide niche in δ^{13}C vs. δ^{15}N space need not be associated with a broader diet as estimated by the number of different prey species in an animal's diet (Sabat *et al.* 2006b; Newsome *et al.* 2015).

The relative positions of the isotope values in δ^{13}C vs. δ^{15}N space also inform the identity of these food webs. For example, the broad isotopic niche of *C. oustaleti* and *C. patagonicus* is a consequence of their use of both terrestrial and intertidal habitats (Fig. 1b, Sabat & Martinez del Rio 2002; Sabat *et al.* 2006a; Martínez del Rio *et al.* 2009; a). In a similar fashion, the orientation of the ellipse in *C. antarcticus* indicates this species' reliance on marine and ^{15}N-enriched island environments fertilized by seabirds (Fig. 1b, unpubl. data). The narrow niches of *C. nigrofumosus* and *C. taczanowskii* and the distinctly enriched values of δ^{13}C and δ^{15}N are evidence for the strict dependence of these two species on marine/intertidal environments (Fig. 1b, Sabat & Martinez del Rio 2002; Sabat *et al.* 2006a,b; Martínez del Rio *et al.* 2009).

The hydrogen (δ^2H) and oxygen (δ^{18}O) isotopic composition of animal tissues is often quite variable and depends on both ecological (Rubenstein & Hobson 2004) and physiological (Wolf *et al.* 2012) factors. Consequently, although the δ^2H and δ^{18}O values in animal tissues are broadly correlated with elevation and latitude (reviewed by Bowen, Wassenaar & Hobson 2005), they are not accurate estimators of the exact site at which the tissue was deposited (Ruyck *et al.* 2013). Like others (Hardesty & Fraser 2010; and references therein), we found the negative correlation between δ^2H and δ^{18}O values and elevation expected from geophysical principles (Poage &

Chamberlain 2001). However, we also found a large amount of variation in this relationship (see also Hardesty & Fraser 2010). The observed variation can be ascribed to several potential causes. First, the birds may not have been collected at the elevation where their feathers were moulted (Martínez del Rio *et al.* 2009). Second, *Cinclodes* species live and forage in or near streams, and depend on aquatic invertebrates (Remsen *et al.* 2003). Andean streams and rivers are often fed primarily by snowmelt from higher elevations (Ohlanders, Rodriguez & McPhee 2013). Stream water and invertebrate prey, and hence the tissues of birds feeding on them, might therefore represent the isotopic values of precipitation at higher elevations, rather than local values. A broad niche in δ^2H vs. $\delta^{18}O$ space implies reliance on multiple, isotopically distinct water sources. Because water sources vary isotopically in space (Hobson, Barnett-Johnson & Cerling 2010; Landwehr, Coplen & Stewart 2014), it is reasonable to infer that a wide niche in δ^2H vs. $\delta^{18}O$ space implies a broad range. This inference must be tempered by the existence of geographically large, but isotopically flat, isoscapes (e.g. as in large expanses of eastern South America; Bowen, Wassenaar & Hobson 2005). Throughout the main distribution of *Cinclodes* in the Andes, the primary spatial gradient in δ^2H and $\delta^{18}O$ is the outcome of variation in elevation, which can change along relatively short distances (Bowen, Wassenaar & Hobson 2005). In *Cinclodes*, the width in δ^2H vs. $\delta^{18}O$ space is correlated with the use of a variety of hydrological sources found along a broad elevational range.

In spite of these caveats, the interpretation of isotopic data in δ^2H vs. $\delta^{18}O$ space is clear: *C. oustaleti* and *C. patagonicus* have broad isotopic niches because they have broad elevational ranges (0–4000 m), whereas species that are found only at sea level (*C. nigrofumous*, *C. taczanowski* and *C. antarcticus* (Remsen *et al.* 2003; Chesser 2004) or exclusively at high elevation (*C. excelsior*, *C. atacamensis*, and *C. albiventris* (Remsen *et al.* 2003; Chesser 2004) have narrow isotopic niches characterized by δ^2H and $\delta^{18}O$ enriched or depleted values respectively (Fig. 1c).

Another important factor to consider when interpreting isotopic niches is the type of tissue analysed (Newsome *et al.* 2007; Martínez del Rio *et al.* 2009). We obtained estimates of isotopic niche breadth from the area of standard ellipses derived from measurements on a small number of contour feathers plucked from museum specimens. Feathers are deposited over a relatively short period from 10 to 20 days (assuming the growth rates estimated by De La Hera *et al.* 2011); hence, the isotopic values for each individual represent a snapshot of the food webs used and site (elevation/latitude) occupied by the individual at the time of feather deposition. In the case of *Cinclodes*, this is during the austral summer, shortly after the birds have bred. Therefore, our isotopic niche widths are likely underestimates of total/annual niche width. For example, a single feather does not capture seasonal variation in resource use, a phenomenon that has been documented in some *Cinclodes* species with the measurement of stable isotope values in several tissues (see Martínez del Rio *et al.* 2009).

THE EVOLUTION OF ISOTOPIC NICHES

Finally, we address the extent to which isotopic niches may be subject to evolution. Both the isotopic niches in $\delta^{15}N$ and $\delta^{15}C$, and δD and $\delta^{18}O$ spaces documented here are realized isotopic niches (*sensu* Hutchinson 1957). They represent a fraction of the isotopic space that a species can potentially occupy. Like other realized niches, they are shaped by a large number of factors including the presence of competitors and/or other negatively interacting species (Peterson *et al.* 2011). The fundamental isotopic niche is broader and can be defined as the set of all isotopic values that a species can acquire and thus correspond to all the food webs that a species can feed on and to all the geographical locations that it can occupy in the absence of competitors. Realized isotopic niches represent the outcome not only of the evolvable morphological and physiological traits that determine a species' tolerance for different types of foods and environmental conditions but also of the effect of other biotic factors (e.g. competition).

Interpreting isotopic niches requires associating isotopic values in delta space with food webs, trophic levels or geographical features such as elevation (Bearhop *et al.* 2004; Newsome *et al.* 2007; Martínez del Rio *et al.* 2009). Understanding the evolution of the characteristics of these realized isotopic niches requires complementing this correlative approach with mechanistic data on the (evolvable) physiological and morphological traits that contribute to shape them. We propose that the comparative analysis of realized isotopic niches is a step that permits the generation of testable hypotheses about the evolution of niche-shaping evolvable traits. A good example of this process is the evolution of concentrating ability in *Cinclodes* kidneys. Sabat *et al.* (2006a,b) documented a positive association between $\delta^{13}C$ and reliance on marine sources in the genus (see Fig. 2b). They hypothesized that this reliance on saline marine resources would be correlated with traits that permit the production of concentrated urine. A series of field and laboratory experiments verified this prediction (Sabat & Martinez del Rio 2002; Sabat *et al.* 2006a,b; Martínez del Rio *et al.* 2009). Sabat *et al.* (2004) also documented greater flexibility in renal morphology and function in broad-niched *C. oustaleti* than in marine specialist *C. nigrofumosus*, lending support to the idea that phenotypic plasticity can influence niche breadth, and through it, range size (Bozinovic, Calosi & Spicer 2011).

The association between wing morphology and isotopic niche width is another example of the complementary role that correlative and mechanistic approaches play in the interpretation of the evolution of isotopic niches. We hypothesize that the evolution of more pointed wings confers aerodynamic advantages to *Cinclodes* species that

conduct altitudinal migrations, and hence inhabit broad elevational ranges in the Andes (see O'Neill & Parker 1978). Although we caution against placing too much stock in this correlation between morphology and isotopic niche width, as it appears to be driven by two contrasts and is very dependent on the model of evolution assumed, we believe that it illustrates the potential of stable isotopes as tools in comparative analyses on the evolution of the correlates of ecological niche characteristics.

CONCLUDING REMARKS

The ecological niche is an elusive but useful construct. Each call to purge the ecological literature of its use (e.g., McInerny & Etienne 2012) is echoed by increased use and a richer body of work (Soberón 2014). Recently, niches have received renewed attention because the study of their dynamics in space and time promises to inform key urgent questions in ecology (Chase & Leibold 2003; Newsome *et al.* 2007), evolution (Rutschmann *et al.* 2011) and biogeography (Soberón & Peterson 2005; Peterson *et al.* 2011). The study of ecological niches has been traditionally fertilized by a variety of technological and conceptual advances. Our study on a small radiation of South American birds supports the idea that stable isotope analysis is a natural, and perhaps crucial, tool in contemporary studies of not only ecological niches, but also of their evolution. Stable isotopes have the advantage of allowing the exploration of many elements of ecological niches from tissue samples of specimens preserved in scientific collections.

Authors' contributions

J.A.R., P.S., S.D.N. and C.M.d.R. designed research. R.T.C. contributed to phylogenetic analyses, S.D.N. contributed to isotopic analyses. J.A.R., M.E.D. and C.M.d.R. conducted analyses and wrote the manuscript, incorporating revisions from co-authors.

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Data accessibility

The data presented herein are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.d866h (Rader *et al.* 2016), and available in the Supporting Information (Appendix S2).

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Supporting Information

Details of electronic Supporting Information are provided below.

Appendix S1. Detailed methods.

Appendix S2. Supplemental data.