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AVIAN BROOD PARASITISM AND ECTOPARASITE RICHNESS–SCALE-DEPENDENT DIVERSITY INTERACTIONS IN A THREE-LEVEL HOST–PARASITE SYSTEM

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Brood parasitic birds, their foster species and their ectoparasites form a complex coevolving system composed of three hierarchical levels. However, effects of hosts' brood parasitic life-style on the evolution of their louse (Phthiraptera: Amblycera, Ischnocera) lineages have never been tested. We present two phylogenetic analyses of ectoparasite richness of brood parasitic clades. Our hypothesis was that brood parasitic life-style affects louse richness negatively across all avian clades due to the lack of vertical transmission routes. Then, narrowing our scope to brood parasitic cuckoos, we explored macroevolutionary factors responsible for the variability of their louse richness. Our results show that taxonomic richness of lice is lower on brood parasitic clades than on their nonparasitic sister clades. However, we found a positive covariation between the richness of cuckoos' Ischnoceran lice and the number of their foster species, possibly due to the complex and dynamic subpopulation structure of cuckoo species that utilize several host species. We documented diversity interactions across a three-level host parasite system and we found evidence that brood parasitism has opposing effects on louse richness at two slightly differing macroevolutionary scales, namely the species richness and the genera richness.

KEY WORDS: Independent contrasts, host–parasite evolution, macroevolution, parasitism, parasite transmission, Phthiraptera.

Understanding the emergence and maintenance of biological diversity is a major task of evolutionary biology. A relatively large proportion of animals live a parasitic way of life (Poulin and Morand 2004), however, zoologists scarcely address the causes and consequences of their diversity and the authors often conclude their studies with general and over simplified verdicts like "parasite diversity covaries with host diversity" (see, e.g. Hechinger and Lafferty 2005; Vas et al. 2012). Below we aim to search for some more specific macroevolutionary patterns. How do lineages in historical associations with more than two levels (such as host–parasite–hyperparasite) affect each others' diversity? Do

host life-history traits affect their parasite richness at different macroevolutionary scales in a similar way? Do different parasite taxa exhibit similar responses to similar changes in host life history? To address these questions, we analyze diversity measures of parasitic lice (Phthiraptera) hosted by brood parasitic birds (Aves), as brood parasitic birds, their foster species and their ectoparasites create a complex coevolving system built of three hierarchical levels.

Obligate interspecific brood parasites exploit the parental investment of another species so as to obtain nutrition and care for their offspring. This behavioral strategy apparently

emerged in seven independent cases during the evolution of birds (Rothstein 1990; Payne 1997, 2005): once in the Black-headed Duck (*Heteronetta atricapilla*), three times in the family of cuckoos (Cuculidae), once in the family of honeyguides (Indicatoridae), and two times among passerines (Icteridae: *Molothrus* spp., and Viduidae).

Interspecific brood parasitism is an intensively studied phenomenon in ornithology; numerous studies address its evolution, ecology, and the emergent coevolutionary arms-race between brood parasites and their foster species (see, e.g., Ortega 1998; Rothstein and Robinson 1998; Davies 2000; Payne 2005 for reviews). Despite this huge interest and research effort, only a few papers have dealt with the ectoparasites of brood parasitic birds (Clayton et al. 2003). The phenomenon that the chicks of brood parasites never come into physical contact with their own parents raises the questions how do their highly host-specific parasites such as parasitic lice (Phthiraptera) maintain their populations on obligate brood parasitic host species, and how do hosts' brood parasitic life-style affect the evolution of their louse lineages. Lice are wingless obligate ectoparasitic insects that complete their entire life-cycle on the body of their hosts. Several authors showed that lice affect both life expectancy (Booth et al. 1993; Brown et al. 1995; Barbosa et al. 2002; Pap et al. 2005) and reproductive success (Clayton 1990; Kose and Møller 1999; Kose et al. 1999) of their hosts, making them excellent model organisms for studying the evolutionary ecology of contagious pathogens.

Vertical transmission (i.e., from parent to offspring) is apparently the most common transmission route of lice (Clayton and Tompkins 1994); however, brood parasitic birds, which lack physical contact between parents and their offsprings, still harbor several host-specific louse species (Price et al. 2003). Lice specific to brood parasitic birds must adapt to infest new host individuals via horizontal transmission, either as fledglings or adults (Marshall 1981; de Brooke 2010). Sexual transmission is known to occur in some species of avian lice (Hillgarth 1996). In addition, body-to-body contact between brood parasites and foster parents constitute a natural experiment of interspecific louse dispersal, potentially initiating host switches among different host species. Previous studies on lice of brood parasitic birds mainly focused on louse dispersal between brood parasitic chicks and their foster parents. Juvenile cuckoos (Lindholm et al. 1998), cowbirds (Hahn et al. 2000), and indigobirds (Balakrishnan and Sorenson 2007) tend to acquire lice specific to their foster species. Nevertheless, it seems that foster-borne infestations cannot establish viable populations on adult brood parasites (Clayton and Johnson 2001; Clayton et al. 2003). In a more focused study on viduid finches, Balakrishnan and Sorenson (2007) found that the successful colonization of brood parasitic finches was constrained by host-specific adaptations (but not dispersal abilities) of louse species specific to estrildid finch foster species.

It is reasonable to suspect that brood parasitic clades may harbor less diverse louse faunas than their nonparasitic sister clades. Take cuckoos as an example; 7 of 10 louse genera infesting Culid birds do not occur on brood parasitic cuckoos, although some of the louse genera infesting nonparasitic cuckoos are restricted only to small subclades of hosts (Price et al. 2003). In addition, Balakrishnan and Sorenson (2007) found that *Myrsidea* lice (Amblycera) were entirely absent from brood parasitic indigobirds, although they are common on estrildid finches, which are both their typical foster species and closest nonparasitic sister clade. However, effects of hosts' brood parasitic life-style on the evolution of their louse lineages have never been tested. A phylogenetic analysis comparing brood parasitic avian clades to their nonparasitic sister clades is needed to test whether or not these cases represent a general trend.

In this article we present two separate analyses (A1, A2) to investigate the effect of hosts' brood parasitic life-style on louse richness at two different macroevolutionary scales. First, we test the hypothesis that louse richness is reduced on brood parasitic host clades compared to nonparasitic sister clades (see sister clades in Fig. 1), considering all the seven independent origins of avian brood parasitism (A1). We assume that brood parasitic life-style affects louse richness negatively due to the lack of vertical transmission routes.

Then, narrowing our scope to the oldest (Hackett et al. 2008) taxa of brood parasites, the cuckoos, we explore which factors shape the richness of their lice communities (A2). Host traits such as body mass, geographic range, and habitat diversity may affect cuckoos' louse richness, so too may traits related to brood parasitic life-style such as the number of foster species a brood parasitic cuckoo utilizes. It is reasonable to suspect that foster species-related traits may affect the louse richness of brood parasites as their chicks are exposed to louse transmission from the foster parents. The variance in the number of foster species among cuckoos is remarkable (Payne 2005); hence certain cuckoo species might interact with more diverse foster-borne ectoparasite fauna than others during their evolution.

Methods

Since Felsenstein (1985) it had been widely accepted that comparative studies have to take evolutionary history into account, as traits of related taxa are statistically nonindependent. In addition, comparative studies focused on parasite diversity among host taxa can also be confounded by uneven sampling effort of parasites (Walther et al. 1995; Krasnov et al. 2005; Poulin 2007); hence researchers need to control both for phylogenetic effects and uneven sampling to recognize relevant macroevolutionary patterns of parasite richness and distribution.
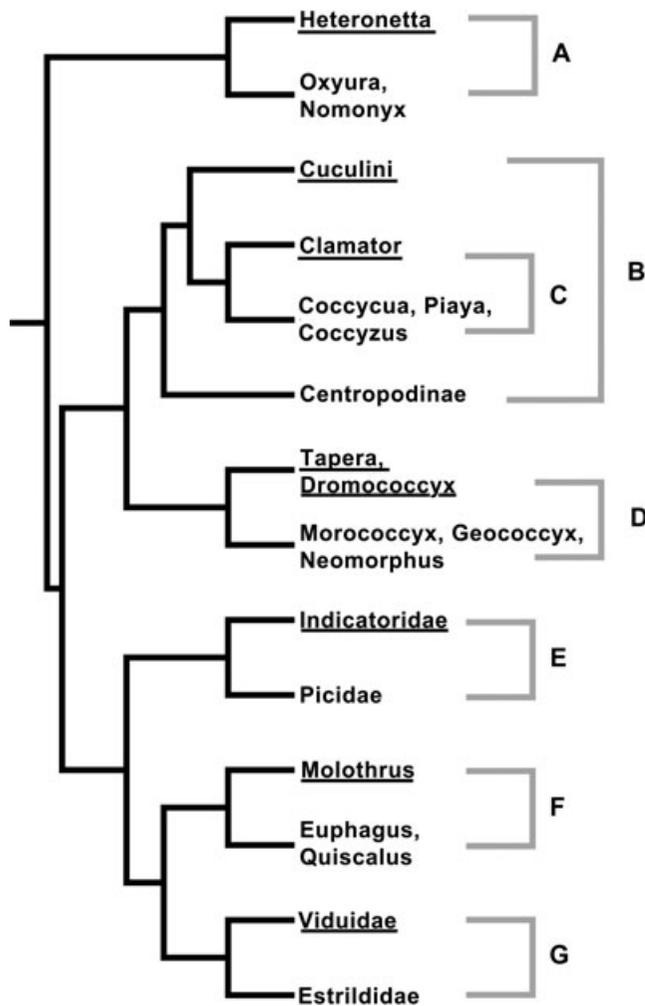
Figure 1. Phylogeny of the seven avian brood parasitic clades (underlined) and their nonparasitic sister clades (see text for the sources). Grey lines connect the brood parasitic and nonparasitic sister clade pairs (A to G) compared in subsequent analyses (note that the phylogeny is arbitrarily ultrametricized).

DIVERSITY MEASURES AND HOST–PARASITE ASSOCIATIONS

We applied three different measures to describe the diversity of brood parasites, their foster species and their lice, namely genera richness (GR), species richness (SR), and the taxonomic distinctness index (TDI) developed by Warwick and Clarke (1995). These measures are clearly not independent of each other; however, they capture different features of diversity. Patterns of GR and of SR may reflect different scales of evolution. TDI calculates the mean number of steps up the Linnaean taxonomic hierarchy to reach the common ancestor of two given parasite species, computed across all species pairs within a group. In this sense, TDI focuses on the taxonomic structure (i.e., closely related or distinct parasite lineages) of a group rather than the pure number of its associates; hence this index is less dependent on research

effort (Clarke and Warwick 1998; Poulin and Mouillot 2003). Furthermore, taxonomists often use different species concepts to describe louse faunas (Mey 2003) making SR a less reliable measure than GR. The biological interpretation of louse GR is quite straightforward as louse genera exhibit distinct body size and body shape according to the specific microhabitats they occupy on hosts (Johnson and Clayton 2003). Therefore, different louse genera can be roughly interpreted as different ecological guilds utilizing different environmental resources (such as topographic refugia to avoid host preening).

We obtained GR and SR data of lice and birds from comprehensive checklists (Dickinson 2003; Price et al. 2003). TDI was calculated with TaxoBiodiv2 software (Poulin and Mouillot 2005). Given that parasitic lice (Phthiraptera) is not a monophyletic group (Jonson et al. 2004; Murrell and Barker 2005; Smith et al. 2011), we collected data separately for Amblyceran and Ischnoceran lice to explore potential differences in their adaptive responses to brood parasitism.

We emphasize here that comparing avian sister clades (rather than sister taxa) is a method that inherently ensures the identical age of compared units (Fig. 1). Therefore, we need not control for the potential age differences between the compared units. Differences in the louse richness of natural sister clades arose along the independent evolution of the sister lineages. In this sense we counted every louse genera and species once—regardless how many host species they infest in a given clade. Characterizing the louse richness of natural sister clades as a whole is unbiased by arbitrary taxonomic decisions such as the uncertain genus-level classification of cuckoos (Dickinson 2003; Payne 2005). In this case confounding effects may arise from factors such as the different diversity of the compared host clades—the effect known as Eichler's rule (Eichler 1942; Vas et al. 2012), or the different body masses. Hence our model included separate variables describing host clade diversity (SR, GR, and TDI), and average body mass to control for their potential effect.

Despite intensive research into avian brood parasitism, an exhaustive list of brood parasitic birds' foster species is still incomplete (Payne 2005). Here we rely on Lowther's (2011) list to quantify the species richness and TDI of foster species. We deleted all records marked as "most probably erroneous," "questionable" or "misidentified," and all presumed brood parasites without any known foster species. The classification and nomenclature were revised according to Dickinson (2003). Data on host–parasite associations of birds and lice were obtained from Price et al. (2003).

BODY MASS, DISTRIBUTION, AND HABITAT DIVERSITY OF HOSTS

We obtained avian body mass data from the literature (Perrins 2003; Payne 2005; Boerner and Krüger 2008; Dunning 2008).

Average body mass data for each host species or clade were log transformed in subsequent analyses. We used the distribution maps of Payne (2005) to estimate the extent of the breeding areas. Initially we georeferenced the maps using QGIS (Quantum GIS Development Team 2011), then calculated the area in square kilometers and log transformed the value. We also estimated a rough but standard measure of habitat diversity for each cuckoo species from BirdLife International (2011). BirdLife Data Zone lists standard habitat categories (e.g., temperate grassland, dry savanna) used by each species, making the sum of these habitat categories a reliably comparable measure of habitat diversity.

CONTROL FOR RESEARCH EFFORT

The research effort focused on each brood parasite species is likely to affect both the number of known foster and the number of known parasite species (Walther et al. 1995; Payne 2005). Nevertheless, GR is a less sampling biased measure than SR (i.e., a larger proportion of louse species awaits description than louse genera). Similarly, TDI is less sensitive to sampling bias (Walther et al. 1995; Clarke and Warwick 1998), as the discovery of new parasite or foster species—as far as they are congeneric to the formerly known species—will not affect the value.

To control for potential bias caused by uneven sampling, we calculated louse sampling effort measure for brood parasitic clades and their nonparasitic sister clades (A1). We estimated sampling effort as the number of host species known to be associated with lice in a host clade divided by the total number of species in that clade. Then we applied linear regression with each louse richness measure as response and sampling effort rate as an explanatory variable. We obtained the residuals from these linear regression models and used them in the subsequent analyses. This is a common method in comparative studies to control for confounding variables (Garland et al. 1992; Poulin 1992), even though it may sometimes cause bias (Freckleton 2009), particularly when the explanatory variables are strongly correlated (Freckleton 2002). However, as we compared the louse richness of brood parasitic clades and their nonparasitic sister clades, this method was the only possible way to consider the effect of uneven sampling.

When investigating traits that potentially covary with cuckoo louse richness (A2), we used a separate variable describing research effort and analyzed it together with other explanatory variables in a multiple regression model, as suggested by Freckleton (2009). We estimated research effort focused on each cuckoo species with the (log transformed) raw number of the results that Google Scholar returned by searching for their scientific name (e.g., "clamator jacobinus," accessed 18 August 2011. The search terms were not refined as we needed a general measure of research effort on cuckoos covering both the research effort of their ectoparasites and that of their foster species. Including this variable in a multivariate model equally controls for the relationship

between study intensity and louse richness and also for the relationship between study intensity and foster species richness in a statistically unbiased way (Poulin 1992; Freckleton 2002, 2009).

PHYLOGENETIC RELATIONSHIPS

We constructed a clade-level phylogeny of the seven independent brood parasitic lineages and their nonparasitic sister lineages (regarding A1) following the rule that branches connecting brood parasitic clades to their sister clades should not overlap along the phylogeny. This criterion ensures the phylogenetic independence of comparisons (Purvis and Rambaut 1995; Maddison 2000; Felsenstein 2004). Tree topology and the recognition of appropriate sister clades (Fig. 1, pairs A to G) were based on published phylogenies (Johnson and Lanyon 1999; McCracken et al. 1999; Barker et al. 2004; Sorenson and Payne 2005; Hackett et al. 2008). The species-level phylogeny of cuckoos (attending to A2) was based on Sorenson and Payne (2005). Phylogenies were constructed using Mesquite (Maddison and Maddison 2011). Each tree was subsequently analyzed by using two different arbitrary branch length transformations. Branch length values were either derived from the tree topology as described by Nee (Purvis 1995), or were set to 1. Our results were qualitatively identical by using any of these transformations; however, only the latter setting provided perfect fit according to model diagnostic methods (Garland et al. 1992). Hence, we report only the analyses based on the tree with branch lengths set to 1.

STATISTICAL ANALYSIS

We used the method of independent contrasts to control for phylogenetic nonindependence (Felsenstein 1985). Analyses were carried out by the "caper" package (Orme et al. 2011) in R 2.14.0 (R Development Core Team 2011). We used *brunch* (A1) and multivariate *crunch* (A2) functions implemented in this package, originally presented by CAIC (Purvis and Rambaut 1995). Character evolution was simulated under a Brownian motion model (Felsenstein 1985). Although this model may not represent perfectly the process of evolutionary changes, several authors showed that even with errors in branch lengths and deviations from Brownian motion the method of independent contrasts is still robust and reliable (Díaz-Uriarte and Garland 1996, 1998). Louse GR and SR were log transformed (value $+ 1$) in the *crunch* model to achieve a better fit. The distributional assumptions of all statistical tests were checked graphically (e.g., quantile-comparsion plot), and all tests were two-tailed. Multicollinearity in multivariate models was checked by calculating the variance inflation factor (VIF) using the R package "faraway" (Reiczigel et al. 2007; Faraway 2011). Our datasets (regarding A1 and A2) are available in Dryad Digital Repository.

Table 1. Results of *brunch* function (direction of subtraction by contrast calculating: brood parasitic–nonparasitic; $n = 7$; df $= 6$).

	Slope	R^2	t	P
Contrasts between sister clades in:				
(Residual) Amblyceran GR	−0.84	0.73	−4.50	0.004
(Residual) Amblyceran SR	−1.94	0.30	−1.99	0.093
(Residual) Amblyceran TDI	−0.42	0.73	−4.52	0.004
(Residual) Ischnoceran GR	−0.75	0.40	−2.36	0.056
(Residual) Ischnoceran SR	−2.26	< 0.01	−1.02	0.348
(Residual) Ischnoceran TDI	−0.33	0.44	−2.56	0.043
(Log) body mass	−0.23	0.34	−2.15	0.076
Host clade GR	−4.63	0.11	−1.36	0.223
Host clade SR	−31.29	0.15	−1.50	0.183
Host clade TDI	−0.15	< 0.01	−1.22	0.267

Results

Our hypothesis that louse richness is reduced on brood parasites as compared to their nonparasitic sister clades (A1) was supported (Table 1, Fig. 2). Using the *brunch* function of the independent contrast method we found that Amblyceran GR and TDI of brood parasitic clades were significantly lower than those of their nonparasitic sister clades. This effect was weaker in the case of Ischnocerans, where TDI was significantly lower on brood parasitic clades than on nonparasitic sister clades, whereas GR was only marginally significantly ($P = 0.056$) reduced on brood parasitic clades. Neither Amblyceran nor Ischnoceran SR differed significantly between avian sister clades. The relationship we found between the emergence of brood parasitic life-style and the reduction of louse richness was not significantly influenced by confounding factors such as host clade diversity (so-called Eichler's rule) or body mass (Table 1).

We analyzed which life-history traits of brood parasitic hosts affect louse richness at species level (A2) by examining cuckoos, the most intensively studied group of avian brood parasites. Using the *crunch* function of the independent contrast method we found that different factors covary with Amblyceran and Ischnoceran richness of brood parasitic cuckoo species (Table 2). The only significant predictor of all Amblyceran richness measures was the research effort focused on hosts. Contrarily, however, both cuckoo body mass and the number of foster species (but not their TDI) covaried significantly with each Ischnoceran richness measure (Table 2). There was no multicollinearity between the
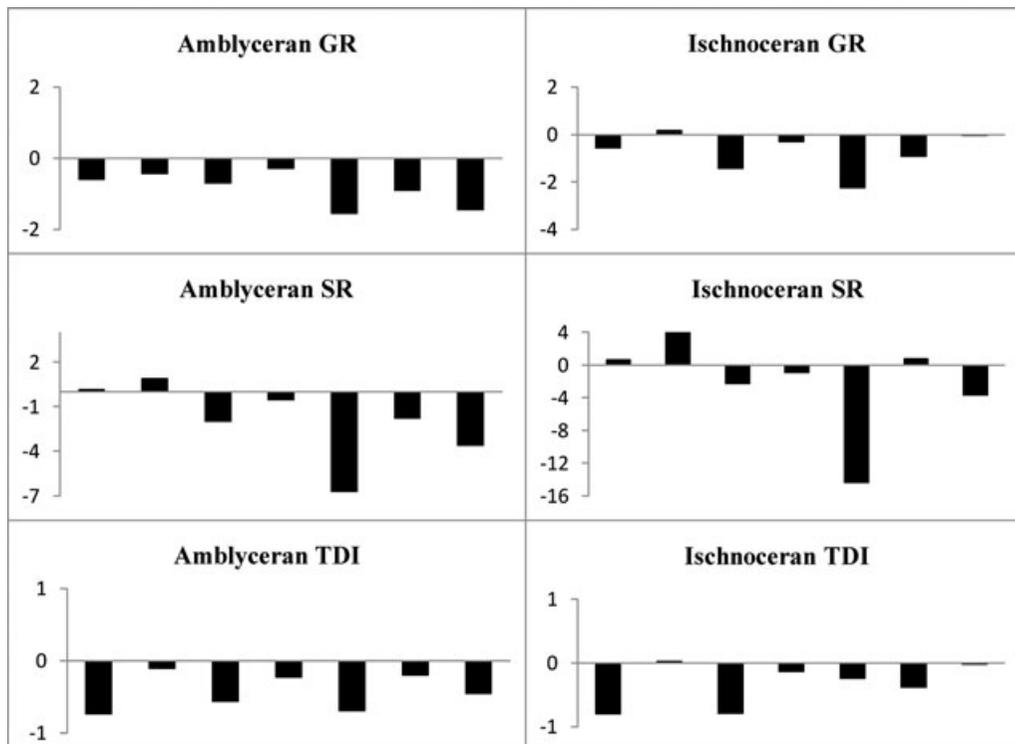
Figure 2. Standardized contrasts of (residual) louse richness measures between brood parasitic and nonparasitic sister clades. Contrasts on the horizontal axis follow the order (A to G) of Fig. 1 (direction of subtraction by contrast calculation: brood parasitic–nonparasitic, hence negative contrast values indicates lower richness on brood parasitic clades than on nonparasitic sister clades; GR, generic richness; SR, species richness; TDI, taxonomic distinctness index).

Table 2. Results of *crunch* function: louse richness measures as response variables, and their significant explanatory variables (in italics); louse GR and SR (value + 1) were log transformed. Nonsignificant explanatory variables (TDI of foster species, cuckoo breeding area size, cuckoo habitat diversity) are not listed.

	Slope	P	F	R^2
Amblyceran GR ($n = 45$; df $= 41$)				
(log) research effort	0.12	0.002	10.58	0.19
Amblyceran SR ($n = 45$; df $= 41$)				
(log) research effort	0.13	< 0.001	13.87	0.23
Amblyceran TDI ($n = 45$; df $= 41$)				
(log) research effort	0.38	0.002	10.58	0.19
Ischnoceran GR ($n = 45$; df $= 40$)				
(log) no. of foster species	0.11	0.017	9.04	0.28
(log) body mass	0.34	0.004		
Ischnoceran SR ($n = 45$; df $= 40$)				
(log) no. of foster species	0.12	0.015	10.58	0.31
(log) body mass	0.43	0.001		
Ischnoceran TDI ($n = 45$; df $= 40$)				
(log) no. of foster species	0.42	0.013	8.98	0.26
(log) body mass	1.25	0.006		

explanatory variables (VIF value = 1.021). Neither the size of the breeding area nor the habitat diversity correlated significantly with louse richness measures (Table 2).

Discussion
EFFECTS OF BROOD PARASITIC LIFE-STYLE
We found solid evidence that the brood parasitic life-style reduced GR and TDI of lice as compared to their nonparasitic sister clades (Table 1, Fig. 2). Apparently, only a few louse lineages were able to adapt to the limited transmission possibilities while certain louse taxa became extinct on brood parasites. Louse taxa specific to obligate brood parasites have to rely exclusively on horizontal transmission routes to infest conspecific birds during the short and scarce events of direct physical contact among adult birds such as copulation, aggression or during communal roosting (Marshall 1981; de Brooke and Nakamura 1998). There is some evidence that both Amblyceran and Ischnoceran lice can transmit during the copulation of birds (Hillgarth 1996). However, our results indicate that several louse lineages went extinct when the vertical transmission route is lacking. It is worth noting that brood parasitic life-style of the hosts may also act as a barrier of successful louse host-switching from other (nonparasitic) bird species.

Amblyceran richness (GR and TDI) appears to be reduced more than Ischnoceran richness (Table 1). This phenomenon may be explained by the difference in Amblyceran and Ischnoceran transmission strategies. Amblycerans may tend to transmit from parents to the chicks earlier and possibly at a greater extent than Iscnocerans (Darolová et al. 2001; de Brooke 2010). Amblyc-erans partially feed on dead or living parts of the skin, blood,

and other excretions (Johnson and Clayton 2003; Mey et al. 2007), whereas Ischnoceran feed largely on feathers (Johnson and Clayton 2003). For this reason, Amblycerans may be less depen-dent on chick plumage development than Ischnocerans; hence they are able to infest younger chicks. As nestlings possess un-developed and hence more-or-less ineffective defenses against parasites, selection may favor early vertical transmission in Am-blycerans. Thus, it seems conceivable that vertical transmission has a greater importance for Amblycerans, and consequently, they may be less capable of relying on the horizontal transmis-sion routes exclusively. Ischnoceran lice—unlike Amblycerans—also often rely on phoretic transmission using Hippoboscid flies (Harbison et al. 2009) as means of vertical transmission; a behav-ior known to occur in cuckoo-specific Ischnocerans as well (Clay and Meinertzhagen 1943; Keirans 1975).

Previous studies on factors shaping louse richness found that several host traits affected Amblycerans, but not Ischnocerans (see, e.g., Whiteman and Parker 2004; Møller and Rózsa 2005; Whiteman et al. 2006; Møller et al. 2010; Vas et al. 2011). To our best knowledge, only two former studies found any signifi-cant correlates of Ischnoceran richness (Hughes and Page 2007; Vas et al. 2012). Thus, our results indicating that brood para-sitic life-style affects both Amblyceran and Ischnoceran richness (at least TDI of the latter) contribute significantly to the poorly understood Ischnoceran macroecology.

LOUSE RICHNESS OF BROOD PARASITIC CUCKOOS
Despite GR and TDI were reduced both in Amblycerans and to a lesser extent also in Ischnocerans, SR of lice showed no significant difference between brood parasitic clades and their nonparasitic

sister clades in either case (Table 1). This correlational evidence suggests relatively rapid speciation events in the louse lineages adapted to brood parasitic hosts. By narrowing our scope to a species-level phylogeny of brood parasitic cuckoos we identified certain cuckoo traits that significantly covaried with their louse richness measures. First, research effort focused on cuckoos predicted Amblyceran richness; this is a well-known phenomenon in parasite ecology (Clayton and Walther 2001; Poulin and Morand 2004; Poulin 2007; Krasnov 2008). More importantly, host body mass and the number of foster species covaried positively with the species richness of Ischnoceran lice on brood parasitic cuckoos (Table 2). Contrarily, cuckoo habitat diversity and geographic range had no effect on the richness measures of their lice (Table 2).

The fact that host body size affects Ischnoceran, but not Amblyceran richness, may possibly be explained by the differences in habitat use between the two louse suborders. Because Ischnocerans live on the surface of feathers and avoid preening by hiding in topographic refugia of the plumage, they depend more on feather size and surface topology of feathers than Amblycerans do. The narrow and elongated body of Ischnoceran "wing lice" is an evolutionary consequence of their strict key-lock mechanism with feather barb size (Johnson and Clayton 2003; Johnson et al. 2005), and this mechanism may prevent successful host switching (Clayton et al. 2003). Johnson et al. (2005) found that both body and feather size of pigeon species correlated positively with the size of their Ischnoceran "wing lice," but not with the size of their Ischnoceran "body lice." They suggested that "body lice" may depend less on host body size, and depend more on microhabitat structures not predicted by overall host body size. The majority of Amblyceran lice also possesses an oval-shaped body and lives partially on the skin and on downy feathers (Johnson and Clayton 2003). Therefore, they may not exhibit a high-level "key-lock" matching with host feather size. Our results concerning the influence of host body mass on parasite taxonomic richness are consistent with previous studies yielding contradictory results (Poulin and Morand 2004), suggesting that host body mass may not necessarily act as a general predictor of parasite diversity (Krasnov et al. 2004, 2008).

We found a significant positive covariation between richness measures of Ischnoceran lice infesting cuckoos and the number (but not the TDI) of foster species these cuckoos utilize (Table 2). At least two alternative hypotheses might explain this pattern. First, generalist cuckoo species that exploit more foster species may be more exposed to colonization by foster-borne infestations creating a host switch. Recent evidence suggests that lice of foster species do occur on immature brood parasites (Lindholm et al. 1998; Hahn et al. 2000; Balakrishnan and Sorenson 2007). Their failure to establish viable populations on adult brood parasites does not exclude the possibility that there could have been successful host switches in the evolutionary past. However, the taxonomic composition of cuckoo lice communities falsifies this explanation. Lice infesting parasitic cuckoos form distinct lineages (*Cuculiphilus*, *Cuculicola*, *Cuculoecus*) that infest both parasitic and nonparasitic cuckoos, but do not occur on any other birds and, particularly, not on the typical foster species of brood parasitic cuckoos (Price et al. 2003). For this reason we exclude this hypothesis.

Alternatively, we propose that this covariation emerges due to the higher complexity of subpopulation structure of cuckoos with more foster species. The Common Cuckoo (*Cuculus canorus*) and other generalist cuckoo species tend to form host-specific races (called gentes) each adapted to different foster species (Payne 2005; Starling et al. 2006). Contrary to former beliefs (Marchetti et al. 1998), recent evidence suggests that not only females, but both sexes contribute to the evolution and maintenance of races (Fuisz and de Kort 2007; Fossøy et al. 2011). Hence the gentes are more-or-less separated in space and time (Møller et al. 2011) leading to a certain degree of genetic isolation. These gentes often exhibit quick evolutionary changes to abandon former foster species and to switch to new, naive foster species (Payne 2005). Consequently, both speciation and extinction rates are higher in parasitic than in nonparasitic cuckoos (Krüger et al. 2009).

Foster-opportunistic parasitic cuckoos are likely to have a more complex metapopulation structure built of a dynamically changing network of more or less isolated subpopulations (races, gentes; Møller et al. 2011). This metapopulation structure may affect the richness of their lice. On one hand, the evolution of lice is much faster than that of their hosts (Page et al. 1998), thus they may speciate rapidly on a network of cuckoo gentes. This process might result in higher louse richness on foster-generalist cuckoos as compared to foster species-specialist ones having a simpler metapopulation structure. On the other hand, complex metapopulation structures may also reduce the risk of louse extinction. Any parasite lineage going extinct from one particular host race—for example, due to a bottleneck in host population size (Kuris et al. 1980)—has a higher chance to get replaced by conspecific parasites if the host race is embedded into a complex network of populations. These conditions may promote the speciation of lice as well as reduce their extinction risk. This phenomenon may also be responsible for the higher Ischnoceran SR of the Cuculini clade than that of its closest nonparasitic sister clade (Fig. 2, pair B).

Briefly, we conclude that brood parasitic birds, their foster species, and their lice form a complex system with three ecological levels interacting with each other in a complex way. We found that brood parasitic life-style reduced the taxonomic richness of ectoparasitic lice in general, especially Amblyceran richness as compared to that of nonparasitic sister clades. This phenomenon may constitute an overlooked advantage of brood parasitic

life-style: it reduces their parasite richness as the vertical transmission of ectoparasites is impossible. Narrowing our scope to brood parasitic cuckoo lice we found that Ischnoceran richness covaried positively with the richness of utilized foster species, probably due to the complex and dynamic subpopulation structure of the foster-generalist cuckoos. Hence, we found evidence that the same macroecological factor (i.e., hosts' brood parasitic life-style) has opposing effects on louse richness at two slightly differing macroevolutionary scales, that is species richness versus genera richness. In addition, our results suggest that diversity interaction across more than two hierarchical levels may contribute considerably to the observed patterns, even when the association between these levels is not obvious, as the diversity interaction we found between the richness of brood parasitic cuckoo lice and that of foster species. Thus, it appears that other multilevel systems may offer a great possibility for future studies to understand how three or more associated lineages affect each others' diversity during their evolution and contribute to global biodiversity as a whole.

LITERATURE CITED

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