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Colour polymorphism in owls is linked to light variability

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Abstract

Owls show an astonishing variation in their degree of colour polymorphism, although the exact mechanisms driving such variation remain controversial. Here we address this fundamental question by considering information on all extant owls and recent advances in comparative methods in the frame of three mutually non-exclusive evolutionary scenarios. In addition, we study for the first time whether the evolution of influential ecological characters facilitated the evolution of colour polymorphism (or vice versa). In agreement with the niche divergence hypothesis, we found that species living under more variable luminal conditions, i.e., species with diurnal and crepuscular habits and those inhabiting in a mixture of open and closed habitats, were more likely to show colour polymorphism. Correlated evolution analyses revealed that a change in the luminal niche might be a fundamental requisite for the evolution of colour polymorphism. Moreover, polymorphism was more frequent among owl species occupying lower trophic levels, which could be explained by a particularly high selection for crypsis on small predator owls. Our results, thus, provide support for the idea that colour polymorphism in owls is an adaptive character likely maintained by the selective advantage of morphs under different environmental conditions via disruptive selection mechanisms.

Keywords Activity rhythm · Comparative methods · Disruptive selection · Luminosity · Owls polymorphism

Introduction

Colour polymorphism is widespread in several animal taxa including invertebrates and vertebrates (McLean and Stuart-Fox 2014), and long attracted evolutionary biologists interested in the understanding of evolution and maintenance of genetic and phenotypic diversity (Darwin 1859; Ford 1945; Huxley 1955; Milstead et al. 1974; Bond 2007; McKinnon and Pierotti 2010). Following the definition proposed by Roulin (2004), a species is polymorphic "when in a population individuals of the same age and the same sex display one of several colouration variants that are genetically inherited and for which the expression is sensitive neither to environment nor to body condition". Hence, polymorphism occurs when morphs occupy the same area at the same time, excluding geographical races and seasonal forms (White and Kemp 2016).

Colour polymorphism reaches high incidences in birds, being reported in 61% of avian orders (Roulin 2004). After Huxley (1955) acknowledged the relevance of birds for the study of polymorphism, this group has been the target of large body of empirical and comparative tests about the adaptive significance of colour polymorphism and the mechanisms facilitating its maintenance (e.g., Roulin 2004; Roulin et al. 2008; Roulin and Wink 2004; Galeotti et al. 2003; Galeotti and Rubolini 2004; Fowlie and Krüger 2003). However, the exact mechanisms promoting inter-specific variation in avian colour polymorphism are still under discussion.

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So far, two main adaptive hypotheses for the evolution of bird colour polymorphism have been proposed (reviewed in Galeotti et al. 2003; Roulin 2004). The apostatic selection hypothesis has been invoked to explain colour polymorphism in avian predators (Ford 1945; Paulson 1973; Arnason 1978; Caldwell 1986) and brood parasites (Payne 1967), and it is based on negative frequency-dependent selection arising when rarity confers a selective advantage (see White and Kemp 2016). The hypothesis states that it would be advantageous for a predator to evolve a different, less frequent colour phenotype to result less recognizable to its prey and hence acquiring hunting benefits. Under this scenario, prey is the selective agent and predators would be more likely to evolve colour polymorphism (Rohwer and Paulson 1987). Accordingly, this hypothesis would predict that top predators show higher occurrence of polymorphism than species occupying lower levels in the food web (i.e., small predators). In the same vein, the apostatic selection hypothesis would predict that species feeding more frequently on prey species able to form an avoidance image (i.e., birds and mammals, which have good memory and learning skills (e.g., Fowlie and Krüger 2003; Galeotti and Rubolini 2004)) are more likely polymorphic. Finally, Fowlie and Krüger (2003) also suggested that species displaying less frequent colour variants may attain hunting benefits during migration because their prey would not be able to form rapidly an avoidance image, hence predicting higher polymorphism in migratory than sedentary owls.

On the other hand, the niche divergence hypothesis is thought to lead to stable colour polymorphism through a mechanism of disruptive selection. It considers habitat heterogeneity experienced by individuals as a key prerequisite for the evolution of colour polymorphism, and states that species occupying heterogeneous environments will benefit by exhibiting different morphs because in that way individuals can better locally adapt to different ecological niches (Dreiss et al. 2012). Heterogeneity may involve various aspects of environmental complexity as climate and/or light conditions (Tate et al. 2016), both in space and time (Galeotti et al. 2003). Enhanced crypsis in different habitats within a population (i.e., resemblance with the habitat background) achieved through polymorphism may provide protection against predators by reducing prey detectability (Baker and Parker 1979) and, at the same time, by avoiding detection by prey (Götmark 1987) or competitors (Spear and Ainley 1993). In a comparative framework, this hypothesis would predict that species with broader ecological niches are more polymorphic than species occupying narrow niches (Van Valen 1965). Consequently, with regard to the light niche, the niche divergence hypothesis would predict that species living under more heterogeneous luminal conditions are more likely to evolve colour polymorphism than those inhabiting in more homogenous luminal conditions.

Finally, it has also been suggested that colour polymorphism may be simply a neutral trait, without an adaptive value (Fowlie and Krüger 2003). In this sense, colour polymorphism may represent a transient occurrence of certain alleles that are more likely expressed in large populations because they have a more diversified and wider gene pool promoting the occurrence of variations (Ford 1953; Galeotti et al. 2003; Roulin and Wink 2004). This hypothesis would predict that species with larger population sizes are more likely polymorphic than those with smaller population sizes.

Owls are a particularly suitable group of birds to test predictions about the apostatic selection hypothesis, given huge inter-specific variation in the degree of colour polymorphism (Mikkola 2014) and in their predator role, ranging from large top predators feeding on mammals and birds (i.e., prey that are assumed to have good learning capabilities and thus able to form avoidance-images) to small insectivorous species. Moreover, owls also show extraordinary variation in their degree of niche specialization with some species being restricted to very specific habitats and/or environmental conditions, as *Megascops* species in center and south of America, while others, as the Barn owl *Tyto alba*, being world-wide distributed (König and Weick 2008; Mikkola 2014). This also makes owls a suitable group to test how niche diversification may have driven the evolution of polymorphisms (niche divergence hypothesis). Non-surprisingly, owls have been the target of several comparative studies aiming to test the functional basis of colour polymorphism (Fowlie and Krüger 2003; Galeotti and Rubolini 2004), although such researches considered small subsamples of species, which could have determined that the conclusions are still controversial. Indeed, in a first comparative study based on 57 owl species, Fowlie and Krüger (2003) did not find support for the apostatic selection hypothesis, and interpreted a phylogenetically corrected correlation between the number of plumage morphs (including intra- and inter-population morphs) and the range size of species as evidence that polymorphism simply arises more likely in larger populations. In a second study, Galeotti and Rubolini (2004) compared ecological and behavioural traits of 31 closely related pairs of monomorphic and polymorphic species of owls. They found that number of used habitats was larger, and the distributional range wider in polymorphic than in monomorphic species, which would agree with expectations from the niche divergence hypothesis. These results, however, may be flawed as they vanished when correction for multiple testing was applied (Galeotti and Rubolini 2004), and given that pair species comparisons are not an appropriate approach to deal with phylogenetic relatedness (Harvey and Pagel 1991). Moreover, the results of these two studies were based on a limited number of species, and did not quantify and account for the tendency for related species to resemble each other (i.e., phylogenetic signal) (Freckleton

et al. 2002; Revell et al. 2008), which impedes any strong inference about the evolution of polymorphism in owls.

Here we revisited the study of the functional basis of colour polymorphism in owls by retrieving data on polymorphism on all extant owls. We relied on a fully resolved avian phylogeny and benefited of recent advances in comparative methods to evaluate the association between colour polymorphism and predictors of the three above described mutually non-exclusive evolutionary scenarios. This approach is useful in identifying key correlations, but it does not inform about the evolutionary sequence of changes giving rise to colour polymorphism over time. Therefore, in a second set of analyses we reconstruct the ancestral state of colour polymorphism in owls, and used analyses of correlated evolution (Pagel 1994) to estimate transition rates from and to colour polymorphism under a range of different character states. All together, these analyses will allow identifying the main drivers of colour polymorphism in owls and whether the evolution of identified influential characters facilitates the evolution of colour polymorphism (or vice versa).

Materials and methods

Data collection

We collected data on colour polymorphism and behavioural and ecological predictors on 206 owl species by consulting the most complete literature currently available on this order (del Hoyo Elliott and Sargatal 1999; König and Weick 2008; Mikkola 2014) (see Table S1). These 206 species are the entire owl number of species considered in the most updated birds' phylogeny of Jetz et al. (2012). We considered as polymorphism only the occurrence of two or more colouration patterns in all individuals of both sexes and at any age within a given population (i.e., White and Kemp 2016), such as reported in König and Weick (2008). Therefore, our study deals with variation in sympatric colour polymorphism and excludes allopatric polymorphism that could be affected by different evolutionary processes (Bolton et al. 2015). We, however, considered *Tyto capensis* and *Tyto longimembris* as polymorphic rather than monomorphic as reported in König and Weick (2008) based on personal observation of A. Roulin. In a first step, we quantified the number of plumage morphs described for each species (see Table S1). However, the number of plumage morphs show very low variation in owls as most polymorphic species only display two (72.5% of species), or three (26.1% of species) morphs (see Table S1 and results). Furthermore, the number of morphs cannot be considered as a good proxy of colour polymorphism because in many species colour variation is in fact continuous rather than discrete. Therefore, polymorphism was classified here as a binary character with 1

indicating the presence of polymorphism and 0 its absence. The following 12 predictors were initially considered. As a proxy of biometrical measures, we gathered information on (1) body weight.

Because the apostatic selection hypothesis predicts that polymorphism would be more frequent among top predators, owls were classified regarding their (2) trophic level. We classified each species into one of three categories based on their diet and size relative to other species in the community. Accordingly, species included in the genera *Bubo*, *Scotopelia*, *Ketupa* and some species of genera *Strix* and *Ninox* were scored as top predators; medium-sized species were categorized as mesopredators as they can act as prey and predators in their communities; and small-sized species, which mainly prey on invertebrates and/or amphibians and reptiles, were considered as small predators (Table S1). In addition, we also classified each species regarding (3) the capability of its main prey to memorize and create an "avoidance image" as species feeding on invertebrates and amphibians and reptiles versus those feeding on other vertebrates (i.e., birds and mammals) (Table 1).

Niche breadth (4) was estimated as the number of different habitats used by a species (see Table 1 for a description of considered habitats);

Diet breadth (5) was estimated as the number of different prey a given species consumes (see Table 1 for a description of considered prey categories);

Migratory behaviour (6) species were classified as resident (i.e., species that live all the year in the same place; but also those showing nomadic or erratic short-distance non-reproductive movements in relation to prey availability), partially migratory (i.e., species with both migrant and resident population) and migratory (i.e., species that show regular migration). Moreover, following (del Hoyo et al. 1999; König and Weick 2008), we also classified owls regarding their (7) activity rhythm as strictly nocturnal species versus those that are also active during the day or at twilight.

We scored the extension of the distribution area of each species, i.e., their (8) distributional range, following Mikkola (2014)' maps, which were built as in Galeotti and Rubolini (2004). Species were classified as 1 = isolated; 2 = small and fragmented; 3 = small and continuous; 4 = wide and fragmented; and 5 = wide and continuous. A distributional area was considered "small" if it occupied less than 30% of the biogeographical region in which a given species inhabits, and "wide" if it occupied more than 30% of the biogeographical region. In addition, previous comparative work has shown that owl plumage colouration is related to latitude and hemisphere (Roulin et al. 2011). Therefore, based on Mikkola (2014)' maps, we calculated (9) latitude (in degrees) as the average between the most northern and southern latitude using Google Maps. Moreover, we divided species according to the (10) hemisphere in which they breed. We

Table 1 Definition of variables and details of scoring methods used to study colour polymorphism in owls

Variables	Description and categorization
Polymorphism	0 = monomorphic species
	1 = polymorphic species
Body weight	Average body weight of each species
Trophic level	1 = top predators
	2 = mesopredators
	3 = small predators
Prey capability	0 = species mainly feeding on prey thought to be unable to form an avoidance image
	1 = species feeding on prey thought to be able to form an avoidance image
Niche breadth	Sum of the following categories:
	1 = tropical forest, cloud forest, rain forest
	2 = sub-tropical forest, temperate forest, forest
	3 = woodland, cultivated areas, gardens, open country with scattered trees, bushy country
	4 = freshwater habitats, wetlands, mangroves, coastlines, gallery forest
	5 = savannah
	6 = grassland, prairies, alpine meadows
	7 = rocky areas
	8 = semi-desert and desert habitats
Diet breadth	Sum of the different prey consumed by a species:
	1 = Arthropods (including crustaceans, spiders, scorpions and insects)
	2 = other invertebrates (snails, mollusks and worms)
	3 = Amphibians
	4 = Reptiles
	5 = Fish
	6 = Birds
	7 = Mammals
	8 = carrions
	9 = fruit
Migratory behaviour	0 = resident species
	1 = partially migratory species (including species presenting both resident and migratory populations)
	2 = migratory species (including species showing regular migratory movements)
Activity rhythm	0 = strictly nocturnal species
	1 = crepuscular species and species active also during the day
Distributional range	Extension of the distribution area:
	1 = isolated
	2 = small and fragmented
	3 = small and continuous
	4 = wide and fragmented
	5 = wide and continuous
Latitude	Average latitude between the most northern and southern latitude (Google Maps, degrees)
	Calculation based on distributional maps in Mikkola 2014
Hemisphere	0 = species breeding in the Northern hemisphere
	1 = species breeding in both hemispheres or cosmopolitan species
	2 = species breeding in the Southern hemisphere
Vegetation cover	0 = species using stable light environments (closed or open habitats)
	1 = species using unstable light environments (both closed and open habitats or intermediate habitats)
Study effort	Number of studies dealing with a specific species in Web of Science database

consider three categories: 0 = species breeding in the Northern hemisphere, 1 = species breeding in both hemispheres and 2 = species breeding only in the Southern hemisphere.

We also gathered information on (11) vegetation cover based on descriptions of the used habitats for each species. Briefly, vegetation cover was classed as "closed" when species only inhabited habitats with dense canopy cover (including tropical, sub-tropical, cloud, rain, temperate and gallery forests and mangroves), or "open" when species only inhabited habitats without canopy cover or with a very scarce canopy (including wetlands, marshes, coastline, grasslands, prairies, meadows, rocky areas, deserts or semi-deserts). Vegetation cover was classed as "intermediate" for species either inhabiting habitats without a complete canopy cover (including savannah, woodlands, cultivated areas, bushy country with shuttered trees) and for species reported to use both "closed" and "open" habitats. Previous studies have relied on vegetation cover as an estimate of the luminal niche width potentially influencing the evolution of bird colour polymorphism (Galeotti et al. 2003). Open and closed habitats differ in the amount of light received because light is filtered by the canopy in closed habitats, but not in open ones. Therefore, luminosity is lower in closed than in open habitats (Martin 2017). Additionally, it is known that in open habitats light is rich in almost all wavelengths because most light comes from the sun and open sky, whereas in closed habitats light is rich in middle wavelengths (Endler 1993). Therefore, species living in "intermediate" conditions regarding vegetation cover are likely to experience a wider range of luminal conditions than those exclusively inhabiting "open" or "closed" habitats. Finally, since probability of reporting polymorphism is likely to differ among well-known and very rare species, we included study effort (12), calculated as the number of studies (log-transformed) for each species in Web of Science database in our comparative study (Table 1).

Phylogeny

The phylogenetic hypothesis was based on the most recent comprehensive time-calibrated set of complete phylogenies of extant bird species (Jetz et al. 2012). This phylogeny relies on the bird genome-based Hackett et al.'s (2008) phylogenies as a backbone for their phylogenetic reconstructions. We tested the sensitivity of our results to the phylogenetic hypothesis using 1000 randomly extracted phylogenetic trees including all the considered species in our study from the site http://birdtree.org (Online file 1).

Ancestral character reconstruction

We reconstructed ancestral state of owl colour polymorphism on a majority-rule consensus tree, created on 1000

trees through the function consensus edges in PHYTOOLS (Revell 2012) using the R function rerooting method in PHYTOOLS package. This approach allows estimating the marginal ancestral state for each internal node of the tree using likelihood and comparing the performance of various models of evolution. Specifically, we contrasted two different models: (1) the "Equal Rates" model (ER hereafter), which assumes colour polymorphism is lost or acquired at a similar rate over time; and (2) the "All Rates Different" model (ARD hereafter), which allows for differences in the rate of gain and loss of polymorphism. We used AIC (Akaike Information Criterion) to compare the models and select the best one, considering as support to our choice a difference of 4 or greater between models (Burnham and Anderson 2003).

Phylogenetic signal

We estimated the phylogenetic signal of colour polymorphism using the phylo.d function in CAPER package in R (Orme 2013), that specifically allows to compute it for binary variables. This approach is based on calculation of the statistic D (Fritz and Purvis 2010), whose value ranges continuously from 0 to 1. D values close to 1 are indicative that trait variation is random regarding the phylogeny. Instead, a D value next to 0 indicates the trait variation in the phylogeny is clumped and thus that the character evolved under a Brownian model. We calculated the phylogenetic signal for all 1000 phylogenies and then we verified distribution of values to determine the departure from both 0 and 1.

Phylogenetic logistic regression

We ran a Phylogenetic logistic regression, using the function binaryPGLMM in APE package in R (Paradis et al. 2004) to control for the possible effects of common ancestry on the relationship between colour polymorphism and the above predictors. As our aim was assessing the relative importance of the three proposed evolutionary hypotheses in the evolution of polymorphism, this analysis was based on the subset of 196 species for which we gathered complete information (see Table S1). Before performing the logistic regression analyses, we determined the degree of multi-collinearity among predictors through estimation of VIF (Variance Inflation Factor) using the CAR package in R (Fox et al. 2010). Only body size and trophic level shared a large amount of variance (VIFs for log weight and trophic level 5.47 and 5.41, respectively). Therefore, we opted to retain trophic level instead of body mass as a predictor to avoid multi-collinearity, and, given that we have a clear-cut prediction regarding the role of trophic level on the evolution of colour polymorphism (see introduction).

Testing correlated evolution between characters

We used the programme BayesTraits (Pagel and Meade 2013) to investigate whether colour polymorphism in owls correlated and coevolved with activity rhythm, trophic level and vegetation cover. As the method can only be applied to binary variables, we transform trophic level, initially categorized in three levels (see table S1), as upper tropic level including top- and mesopredator species versus lower trophic level including small predators. We used a Bayesian approach based on a Markov Chain Monte Carlo (MCMC) sampling algorithm and a Reversible-Jump (RJ) procedure which allow taking into account both uncertainty of the model estimates and phylogeny. This approach is based on the observation of transition rate of character' states under two contrasting models. The dependent model assumes that the two traits coevolved, and, thus the rate of change in one character is contingent on the state of the other character; conversely, under the independent model the transition from a state to another in one character is independent of the state of the other character. Each MCMC chain was run three times for all 1000 phylogenies for 1 010 000 iterations sampled every 1000 with the first 10,000 excluded as burn-in period after visually confirming that convergence had been reached. We used a uniform prior for both the independent and the dependent model, which were compared by means of the Bayes factor (BF) based on the harmonic means of the model likelihoods. By convention, a value > 2 is taken as a positive evidence for hypothesized relationship and values > 5 as a strong evidence (Pagel and Meade 2006).

After detecting coevolution between colour polymorphism and any of the predictors, we estimated the distribution of posterior probabilities of the values of the parameters, also referred to as z scores, using the RJ MCMC procedure using an hyperprior (0, 100; 0, 100). The z scores provide a means of analyzing the probability that the true value of the transition parameter between two character states is nonzero (i.e., the transition does not occur) based on the proportion of models visited by the Markov chain. The most likely evolutionary path from the hypothetic ancestral state to derived state of two discrete traits thus, can be inferred from the posterior probability distributions of the transition parameters in the model of evolution (Pagel and Meade 2006).

Results

Species coverage

Colour polymorphism was found in 69 species belonging to 9 genera, whereas 137 species were classed as monomorphic (Table S1). Among the 69 polymorphic species, 50 showed 2 morphs (rufous and greyish), 18 showed 3 morphs (rufous, brown and greyish) and one species showed 5 morphs (Table S1 Appendix).

Ancestral character reconstruction and phylogenetic signal

The model "ARD" fitted the data considerably better than the model "ER" (ΔAIC = 28.90, see also Fig. 1S). However, the potential of the ARD model to infer ancestral states is limited as it invariably assigned an equal likelihood for presence or absence of colour polymorphism for all internal nodes in the owl phylogeny (Fig. 1).

The average D phylogenetic signal calculated on the 1000 trees was 0.63, and differed significantly from 0 and 1 (Fig. S2)

Correlates of colour polymorphism

Phylogenetic logistic regression revealed that colour polymorphism was influenced by activity rhythm, trophic level and vegetation cover (Table 2). Specifically, species with a more diurnal activity rhythm were more likely to display colour polymorphism (Fig. 2a). In addition, colour polymorphism was relatively more frequent among small predators than among top- or mesopredators (Fig. 2b). Finally, species inhabiting in mixed habitats regarding vegetation cover were more likely polymorphic than those inhabiting exclusively open or closed habitats (Fig. 2c).

Coevolution between colour polymorphism and predictors

The evolutionary model in which colour polymorphism and activity rhythm evolved dependently was more likely than the model in which the two characters evolved independently (Bayes factor = 18.57). Comparison between z scores at the transition $q2,4$ and at transition $q1,3$ revealed that there is a tendency of polymorphism to evolve more easily in species with both crepuscular and diurnal habits than in strictly nocturnal species (z score value of $q1,3 = 0.000$ versus z score value of $q2,4 = 0.997$, Fig. 3). In addition, it is relevant to question which changed first: owl colouration followed by activity rhythm or vice versa. Comparison of these z scores at the transition $q1,2$ with the alternative $q1,3$ suggests that both transitions are possible (Fig. 3a), and therefore that the first evolutionary step could not be unambiguously established. The analysis also shows that the transition probability from polymorphism to monomorphism was similar in species with crepuscular and diurnal habits (z score at the transition $q3,1 = 0.000$ versus z score at the transition $q4,2 = 0.000$, Fig. 3a).

The evolutionary model in which colour polymorphism and trophic level evolved dependently did not significantly improve the fit of the model in which these two characters evolved independently (Bayes factor = − 13.73) (Fig. 3b).
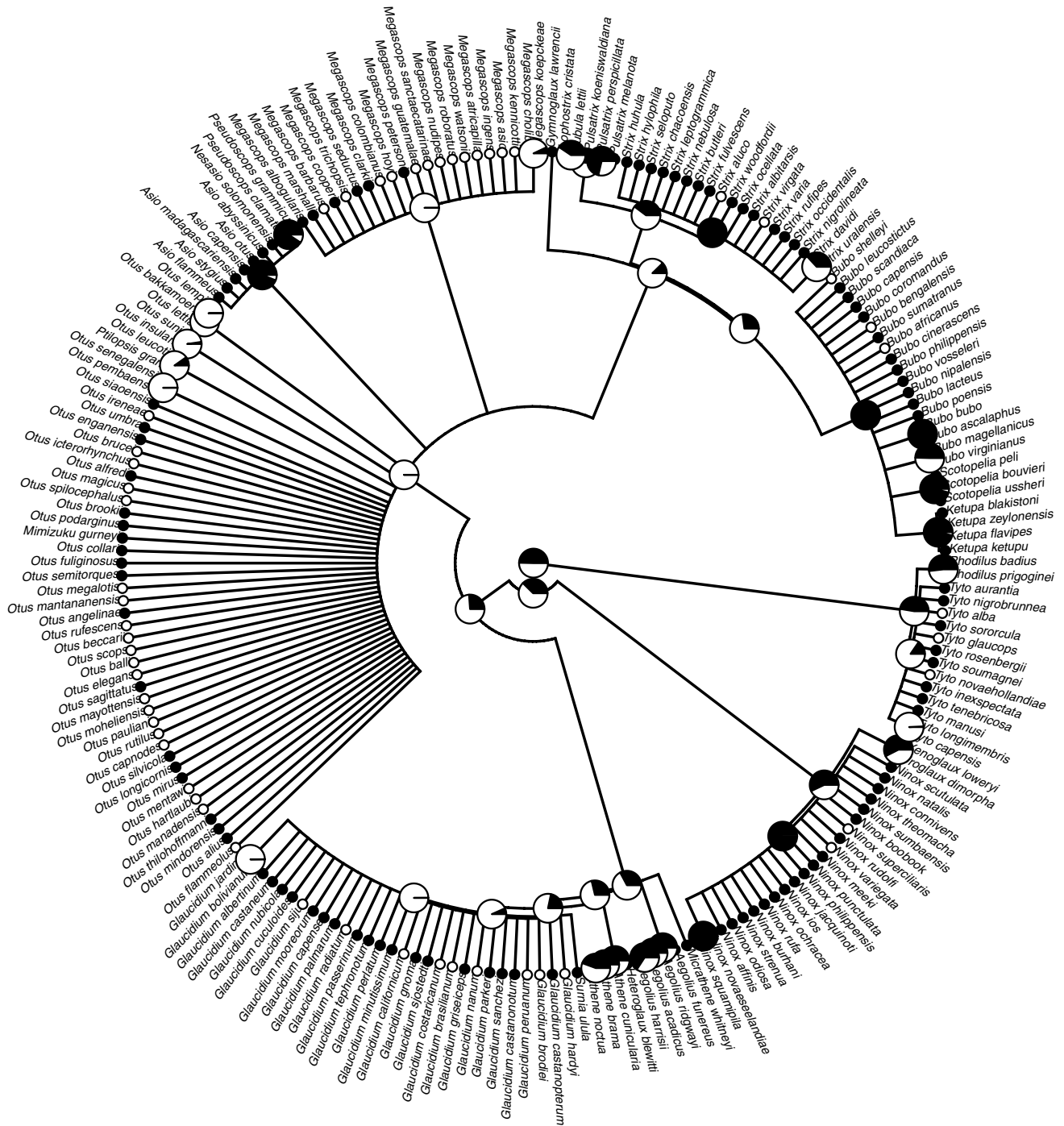
Fig. 1 Ancestral state reconstruction of the binary character colour polymorphism across a majority-rule consensus tree created on 1000 phylogenies using the ARD model. Pie charts at the nodes represent proportional maximum-likelihood support for the monomorphic (black) and polymorphic (white) character states from the ancestral state reconstruction

We found strong evidence of correlated evolution between colour polymorphism and vegetation cover (Bayes factor $= 7.30$, Fig. 3c). Comparison of the z scores at the transition $q2,4$ with that of the alternative transition $q1,3$ suggests that polymorphism is more likely to evolve in intermediate than in open or closed habitats(z score at $q2,4 = 0.033$ versus

z score at $q1,3 = 0.842$) (Fig. 3c). In addition, the analysis shows that the transition probability from polymorphism to monomorphism was similar in intermediate versus open and closed habitats (z score at the transition $q3,1 = 0.093$ versus z score at the transition $q4,2 = 0.000$, Fig. 3c). Finally, comparison of z scores at $q1,2$ with $q1,3$ provides strong

Table 2 Results of the phylogenetic logistic regression to study colour polymorphism in owls

	Coefficient	Standard error	Z score	P value
(Intercept)	− 7.64	1.86	− 4.10	< 0.001
Study effort	0.51	0.35	1.46	0.143
Trophic level	**1.89**	**0.48**	**3.96**	**< 0.001**
Diet breadth	0.05	0.19	0.24	0.807
Niche breadth	− 0.20	0.23	− 0.84	0.401
Prey capability	0.19	0.82	0.23	0.816
Activity rhythm	**1.10**	**0.45**	**2.45**	**0.014**
Migratory behaviour	− 0.50	0.56	− 0.89	0.376
Distribution range	0.20	0.17	1.17	0.240
Latitude	0.00	0.02	0.13	0.893
Vegetation cover	**0.83**	**0.41**	**2.02**	**0.044**
Hemisphere	0.39	0.38	1.03	0.305

Significant results are reported in bold

$N = 196$ species with complete information for all predictors

evidence that changes in owl colouration only occurred after a change in habitat use (z score at $q1,2 = 0.000$ versus z score at $q1,3 = 0.842$) (Fig. 3c).

Discussion

Our results show that colour polymorphism is an evolutionary labile trait in owls, with a trend to show similar values among related species, but that cannot be fully explained by the phylogenetic relatedness. Indeed, we were able to discriminate among the three evolutionary scenarios here considered to explain the appearance of colour polymorphism in owls, i.e., niche divergence hypothesis, apostatic selection hypothesis, and polymorphism by chance.

Scenario 1: the niche divergence hypothesis

Our results provide support for a role of ecology, namely activity rhythm and vegetation cover, in explaining colour polymorphism in owls. Specifically, species with diurnal and crepuscular habits and those inhabiting in a mixture of open and closed habitats are more likely polymorphic than strictly nocturnal species or those inhabiting only closed or open habitats. The niche divergence hypothesis would predict that species living under heterogeneous luminal conditions are more likely to evolve colour polymorphism than those inhabiting in homogenous luminal conditions. Compared to strictly nocturnal species, owls active at dawn and dusk experience highly variable luminal conditions (Martin 1990). In the same vein, species inhabiting in a mixture of open and closed habitats experience more variable luminal conditions due to the different structure of vegetation, daily
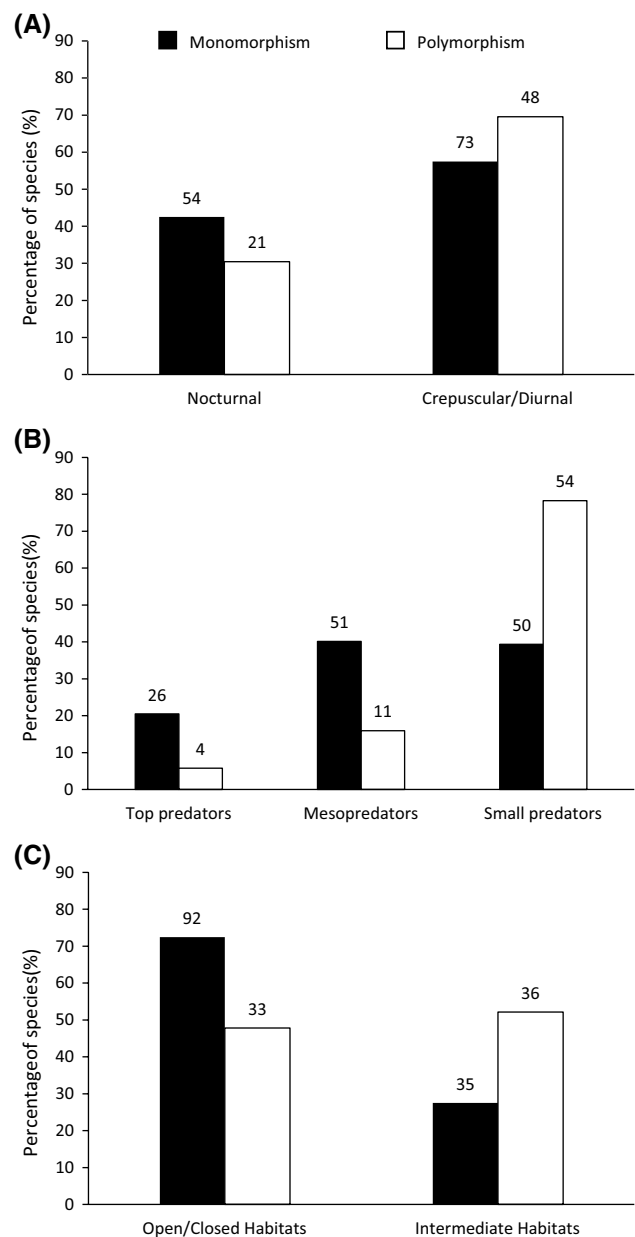
Fig. 2 Percentage of owl species exhibiting colour polymorphism and monomorphism in relation to activity rhythm (**a**), trophic level (**b**) and vegetation cover (**c**). Number of species is shown above bars

variation in the incidence of sun light and weather conditions which affect propagation of coloured signals (Endler 1993). Therefore, our results suggest that the selection pressure due to detectability in different light conditions may be a key predictor of colour polymorphism, and thus that polymorphism in owls might be primarily driven by disruptive selection.

Our results contrast with previous comparative studies in owls that found only weak (Fowlie and Krüger 2003; Galeotti and Rubolini 2004) support for the disruptive selection

Fig. 3 Flow diagram showing the most probably evolutionary pathway for the evolution of owl polymorphism in relation to **a** activity rhythm, **b** trophic level and **c** vegetation cover. Changes between monomorphism and polymorphism occurred independently of changes in trophic level, but polymorphism evolved jointly with activity rhythm and vegetation cover (see results). Likelihood of all transitions based on the *z* score (the higher the *z* score, the more unlikely the transition; see "Materials and Methods" for details) are shown close to arrows
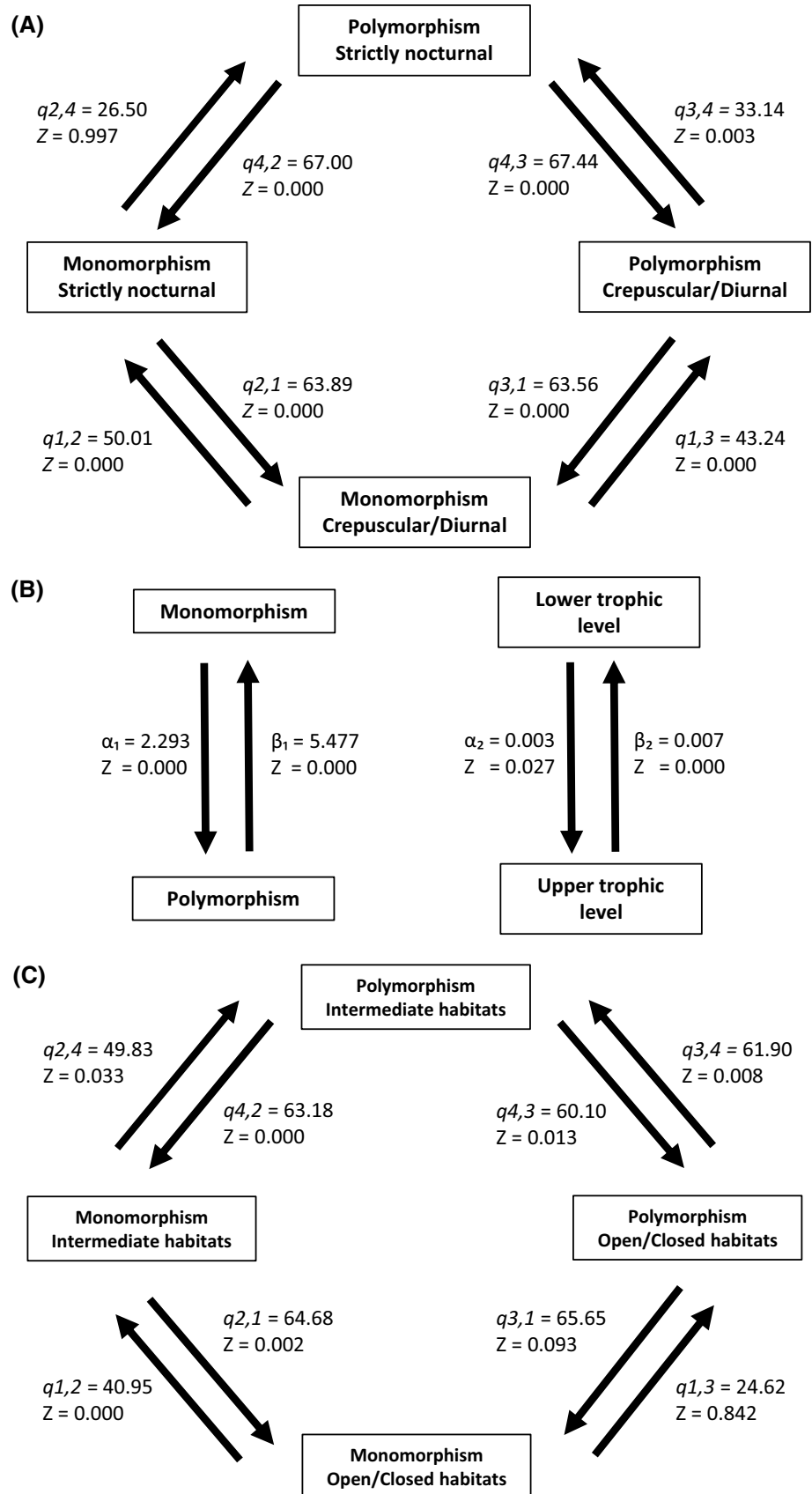
hypothesis. There are several differences between our study and earlier ones that could explain different results. First, these earlier studies considered about ¼ of all extant owl species. It could thus be argued that differences among studies might be due to non-intentional biases toward owls with non-representative ecological features in earlier studies. Second, and in contrast with our study, earlier analyses relied on a non-well resolved owl phylogeny. Finally, our study incorporates recent advances in comparative methods that allow for an appropriate treatment of binomial dependent variables as colour polymorphism in multiple-predictor models (Ives and Garland 2014). Our study, however, provides support for previous comparative studies with birds (Galeotti et al. 2003) and recent empirical evidence at the intra-specific level showing a role of light spectrum on promoting colour polymorphism (Tate et al. 2016). Individuals of the black morph in the Black sparrowhawk *Accipiter melanoleucus* provided more prey in lower light conditions whereas individuals of the light morph provided more prey in brighter conditions (Tate et al. 2016). Hence, the found relationships between polymorphism and activity rhythm and vegetation cover would suggest that morphs might function as an adaptation driven by light conditions to exploit varying niches in owls.

Alternatively, the found relationship between vegetation cover and polymorphism could be a side effect due to the fact that environmental conditions change across vegetation cover classes. A large body of empirical work has also shown that, in owls, different morphs may perform better under particular environmental conditions. For instance, in Eastern screech owl *Megascops asio*, redness was observed to be more frequent in climate presenting higher rainfall and humidity (Gehlbach 1994) and in closer habitats (Gehlbach and Gehlbach 2000). Similarly, the fluctuation of plumage redness in European scops owl *Otus scops* seems to reflect the increasing of temperature and precipitations (Galeotti et al. 2009). In their study about polymorphism in Tawny owl *Strix aluco*, Galeotti and Cesaris (1996) found a significantly higher mortality rate of red morphs during cool–dry years, which could be due to the fact that different colour morphs have different ability to thermoregulate (Dreiss et al. 2016). Additionally, Roulin et al. (2011) in a comparative study observed that frequency of plumage redness in owls increased near the equator. Finally, a recent study highlighted that rufous tawny owls have lower survival in winters with deep snow compared to grey ones (Karell et al. 2011).

Analyses of correlated evolution strengthened the suggested key role of luminal conditions for the evolution of colour polymorphism in owls. Colour polymorphism and activity rhythm have evolved in concert in owls and the transition from monomorphism to polymorphism was more frequent in diurnal and crepuscular species than in nocturnal species. In addition, polymorphism was more likely to evolve in "intermediate" habitats regarding vegetation cover, where species are likely to experience more heterogeneous luminal conditions. It was evident that changes in owl colouration were triggered by a previous change in the luminal niche of species, supporting the hypothesis that a change in the luminal niche probably was an important requisite for the evolution of colour polymorphism. Nonetheless, the ancestral state of polymorphism cannot be unambiguously established in our study, and losses and gains occurred in the owl phylogeny (Fig. 1). Moreover, we found that species living under heterogeneous lighting conditions were more likely to show colour polymorphism, which could be explained by a loss of colour polymorphism in species living in homogenous light conditions. Hence, a plausible alternative interpretation is a neutral scenario for the appearance of colour polymorphism, which is negatively selected in some situations.

Scenario 2: the apostatic selection hypothesis

Our results do not support the idea that detection by prey played a key role in the evolution of colour polymorphism in owls through apostatic selection. Indeed, contrary to expectations from the apostatic selection hypothesis, occurrence of polymorphism was not explained by the relative importance of prey capable to form an avoidance image or by migratory behaviour. This hypothesis also predicts that higher occurrence of polymorphism may occur in larger predators because they prey more frequently on birds and mammals, which are said to have good memory and learning skills. Contrary to this expectation, we found that polymorphism was more frequent among owl species occupying lower tropic levels in their food webs. Fowlie and Krüger (2003) also found a negative relationship between number of morphs and body size. The most likely explanation for this result is that colour polymorphism in small owls was driven by intraguild predation risk, i.e., escape from visual predators may favour the evolution of prey colour polymorphism (Bond and Kamil 2002, 2006). Intraguild predation is common in owls and: (a) larger owls often predates on smaller ones, the opposite being rare and (b) small owls are frequently the target of diurnal birds of prey (Mikkola 1976; Lourenço et al. 2014). Therefore, selection for crypsis due to intraguild predation is expected to be larger in smaller owls. Moreover, examination of niche breadth in relation to trophic level shows that small owls occupy a larger number of habitats than larger ones [PGLS with niche breadth as dependent variable: trophic level: coefficient (SE): − 0.39 (0.08), $t = − 4.34$, $P < 0.0001$], which suggest that the prerequisite of high habitat diversity is fulfilled.

This hypothesis was primarily proposed to explain polymorphism in diurnal raptors and other birds active during daytime (Ford 1945; Paulson 1973; Arnason 1978; Caldwell

1986; Roulin and Wink 2004). However, many owl species are nocturnal, and, given the low performance of a vertebrate eye in detecting colour differences at night (Kelber et al. 2003), it is unlikely that owl prey may form an avoidance image under low light conditions.

Contrarily to diurnal raptors, in which polymorphism seems to be closely tied to migration (Roulin and Wink 2004), there is no relationship between colour polymorphism and migration in owls. Owls are an almost completely sedentary group of birds, with very few species undertaking true migration and others being characterized by a discontinuous nomadic behaviour, associated to fluctuation of their main preys' population (König and Weick 2008), which minimizes the chance for selection of polymorphism based on apostatic selection.

Scenario 3: polymorphism by chance

Our results revealed that colour polymorphism is not associated with the distributional range of species, which likely reflect the size of the populations of each species (see Fowlie and Krüger 2003), and thus the chance that polymorphism may have evolved randomly. This finding, together with the found associations between polymorphism and predictors of the amplitude of the luminal niche, definitively undermines the possibility that colour polymorphism is a neutral trait without an adaptive value in owls (Fowlie and Krüger 2003).

Weakness of the used approach

This study, however, has several weaknesses worth mentioning here that may affect the strength of our conclusions. As above mentioned, polymorphism occurs when morphs occupy the same area at the same time, hence excluding geographical races and seasonal forms (White and Kemp 2016). Our measurement of polymorphism refers to variation within populations such as it would be required for testing the proposed evolutionary scenarios. However, predictor variables were collected from species descriptions and may include both inter- and intra-population variation that may potentially give rise to spurious relationships. Second, due to the absence of appropriate predictors measuring the intensity of sexual selection in owls, we have disregarded a potential role of sexual selection in driving colour polymorphism (Roulin and Bize 2007). However, most of owl species are long-lived species with a monogamous mating system (König and Weick 2008), which would suggest that the potential of sexual selection-based mechanisms to promote polymorphism might be particularly low in this group. Finally, the comparative method is in essence a correlative based approach in which inference about causality is established from statistical analyses in which multiple predictors are present (Bennett and Owens 2002).

Conclusions

This is the first comparative study simultaneously considering correlation and contingency analyses to test explicitly the three classic hypotheses on the evolution of colour polymorphism in owls. Our study provides evidence that variation in luminal conditions might be a key prerequisite promoting the evolution of colour polymorphism in this group of birds, in agreement with the niche divergence hypothesis. Our results, thus, provide support for the idea that colour polymorphism in owls is an adaptive character likely maintained by the selective advantage of camouflage under different light regimes or in terms of physiological adaptation to environmental conditions via disruptive selection mechanisms.

Author contribution statement AP, DP, VP and JMA conceived the study design. AP collected data. AP and JMA performed the analyses. AP, DP, VP and JMA wrote the manuscript.

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