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Ecology, conservation, and phylogenetic position of the Madagascar Jacana *Actophilornis albinucha*

Josephine D'Urban Jackson^{1,2*} , Sama Zefania³ , Sebastien Moehy³, Andrew J Bamford⁴ , Michael W Bruford^{2,5} 
and Tamás Székely¹ 

¹ Milner Centre for Evolution, Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom

² Organisms and Environment Division, School of Biosciences, Cardiff University, Cardiff, United Kingdom

³ Institut d'Enseignement Supérieur de Menabe, Port Morondava, Madagascar

⁴ Wildfowl and Wetlands Trust, Slimbridge, Gloucestershire, United Kingdom

⁵ Sustainable Places Research Institute, Cardiff University, Cardiff, United Kingdom

* Corresponding author, email: josiedjackson@gmail.com

The Madagascar Jacana *Actophilornis albinucha* (Jacanidae) is an endemic shorebird found in the threatened wetlands of western Madagascar. This species is presumed to exhibit classical polyandry; however, few data are available to support that assumption. More generally, a lack of basic understanding of this species hinders conservation efforts. We conducted the most extensive study of the Madagascar Jacana to date, and report on its: 1) distribution, population size and density; 2) degree of sexual size dimorphism; and 3) phylogenetic position. The surveys were conducted at 54 lakes, between January and October in 2016. Madagascar Jacana were found at 22 lakes, and within these were distributed at a mean density of 3.5 ± 0.74 [SE] individuals per hectare of surveyed habitat. We estimate the global population size to be between 975 and 2 064 individuals, and habitat destruction appears to be the main threat to the species. Females were significantly larger than males, consistent with reports for other Jacanidae species. Using a mitochondrial DNA fragment, we expanded the Jacanidae genetic phylogeny, and confirmed that Madagascar Jacana is the sister species to the African Jacana *Actophilornis africanus*. Further studies are urgently needed to thoroughly re-assess the threat status and population trend of the Madagascar Jacana.

Ecologie et conservation du Jacana malgache *Actophilornis albinucha*

Le Jacana Malgache *Actophilornis albinucha* (famille Jacanidae) est un oiseau de rivage endémique trouvé dans les zones humides menacées de l'ouest de Madagascar. Cette espèce est présumée une polyandrie classique, cependant, peu de données sont disponibles pour soutenir ces hypothèses. Plus généralement, un manque de compréhension de base de cette espèce entrave les efforts de conservation. À ce jour, nous avons mené une étude plus extensive sur le Jacana Malgache pour mettre à jour et rapporter sa: 1) répartition, taille de la population et sa densité; 2) le degré de dimorphisme sexuel; et 3) la position phylogénétique. Des recensements ont été menés dans 54 lacs entre janvier et octobre 2016. Le Jacana Malgache a été trouvé dans 22 lacs auxquels il était réparti à une densité de 3.5 ± 0.74 [SE] par hectare de l'habitat inventorié. Nous avons estimé la taille de la population globale entre 975 et 2 064 individus et la destruction de l'habitat semble être la principale menace pour cette espèce. Les femelles étaient significativement plus grandes que les mâles, cette observation concorde avec les déclarations d'autres espèces de Jacana. À l'aide d'un fragment d'ADN mitochondrial, nous avons élargi la phylogénie génétique des Jacanidae et confirmé que le Jacana Malgache est l'espèce sœur du Jacana Africain *Actophilornis africanus*. D'autres études sont nécessaires d'urgence pour réévaluer en profondeur l'état de la menace et les tendances de la population du Jacana malgache.

Keywords: African wetlands, Jacanidae, mtDNA, phylogeny, population density, sexual size dimorphism, shorebirds, visual survey

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Introduction

In the biodiversity hotspot of Madagascar (Myers et al. 2000), freshwater wetlands are considered a highly threatened habitat owing to land-use changes, specifically conversion to rice paddies (Benstead et al. 2003; Kull 2012; Bamford et al. 2017). It is predicted that the severe degradation of Madagascar's wetlands will have a catastrophic impact on biodiversity within these ecosystems, and currently there

are few areas offering protection for wetland biodiversity (Bamford et al. 2017). As highlighted by the most-thorough assessment of the freshwater biodiversity of Madagascar to date, 43% of the species inhabiting this ecosystem are currently considered threatened (Máiz-Tomé et al. 2018).

The Madagascar Jacana *Actophilornis albinucha* is one of 22 wetland bird species endemic to Madagascar

(Young 2003). Its population size is roughly estimated as 670–6 700 mature individuals; however, the quality of the data for this prediction has been considered poor (BirdLife International 2018). The distribution of the Madagascar Jacana stretches almost the entire length of the western wetland region (Safford 2013) (Figure 1); however, the predicted range is based on records that are now out of date (some are from more than 100 years ago), and may therefore be inaccurate. Furthermore, in recent years, Madagascar's wetland habitat has been reduced by 60%, largely owing to conversion to rice paddies (Kull 2012); this situation may additionally have affected the species' distribution owing to reduction in the availability of suitable habitat. Although the species was previously common in western Madagascar (Safford 2013; BirdLife International 2018), recent anecdotal evidence of reduced sightings suggests this is unlikely to still be the case (BirdLife International 2018). Consequently, in 2016, its threat status was elevated from Least Concern to Near Threatened because of a declining population trend as a result of hunting, habitat loss and wetland degradation (BirdLife International 2018). The species currently receives no level of protection and, to our knowledge, as with many wetland species that are understudied (Darwall et al. 2011), there has been no study conducted focusing only on its population status. The lack of basic knowledge of this species is hindering further research and conservation efforts, which require up-to-date estimates of distribution and population size (IUCN 2001; Buckland et al. 2008).

Jacanas are highly adapted to freshwater aquatic environments (Emlen and Wrege 2004). The eight extant species have all colonised tropical and subtropical wetlands (Jenni 1996), but, despite sharing ecological similarities, have shown a mixed response to anthropogenic land-use changes (Okes et al. 2008; Pierluissi 2010). For example, the African Jacana *Actophilornis africanus* is known to take advantage of manmade wetlands such as sewage works (B Taylor pers. comm.), have adapted to invasive species (Jenni 1996), and has shown signs of population expansion (Okes et al. 2008). In contrast, the Lesser Jacana *Microparra capensis* is thought to have suffered recent declines in population size (Okes et al. 2008).

Sexual size dimorphism in jacanas

Seven of the eight extant jacana species are thought to have a polyandrous breeding system, although no study has yet investigated the breeding system of the Madagascar Jacana. Classical polyandry (i.e. a reduction, or complete lack of, female parental care, combined with rapid multi-clutching) (Emlen and Oring 1977; Graul 1977; Andersson et al. 1994) is a rare breeding system among birds, although some of the best-studied polyandrous species include shorebirds (Andersson 1994; Butchart 2000; Mace 2000; Emlen and Wrege 2004; Lipshutz 2017). However, the Madagascar Jacana has so far been neglected, and classical polyandry has been inferred from only a few observations of the species (Jenni 1996; Safford 2013). Sexual size dimorphism (SSD) can be predictive of mating systems in shorebirds, with larger females relative to the males often found in polyandrous species

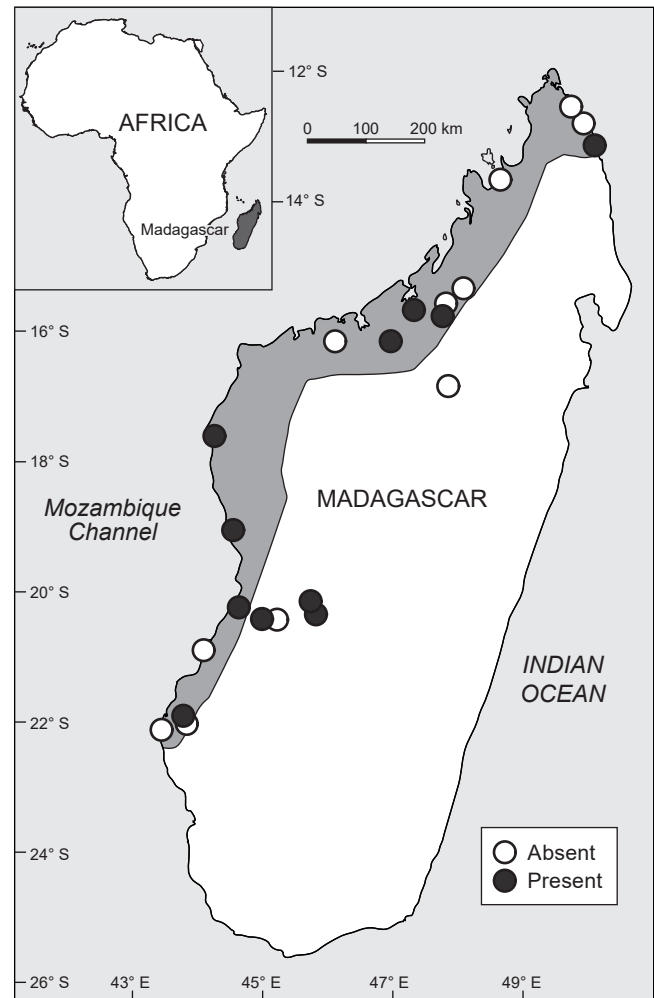


Figure 1: Map of areas surveyed, between January and October in 2016, for the Madagascar Jacana *Actophilornis albinucha*. Solid circles = locations lakes where jacanas were found; open circles = locations of lakes where jacanas were absent. Lakes within close proximity of each other are combined as one area for visual simplicity. Grey shading shows the species range according to BirdLife International (2018)

(Székely et al. 2000). Supporting this, female polyandrous jacanas are markedly larger than males in their body size and mass, and have a greater tarsus length, and within species with them, facial shield size and wing-spur length (Jenni and Collier 1972; Johnsgard 1981; Tarboton 1995; Jenni 1996; Butchart 2000; Mace 2000; Emlen and Wrege 2004; Lipshutz 2017). The female-to-male mass ratio for African Jacana is 1.68:1 (Tarboton 1995), which is similar to the reversed SSD ratios of other polyandrous jacana species, which range between 1.50:1 and 1.85:1 (Jenni 1996; Butchart 2000; Mace 2000; Emlen and Wrege 2004; Lipshutz 2017). However, published morphometric data for the Madagascar Jacana are based on just a few individuals that were not molecularly sexed (Safford 2013). Therefore, to confirm SSD, additional morphometric measurements, combined with a genetic-sexing approach is required.

Field-study challenges

Evaluating the basic ecology and conservation status of Madagascar Jacana is difficult for at least four reasons. First, jacanas live and breed in areas with poor accessibility, especially in Madagascar, where wetlands are often inaccessible during the rainy season, which is when the species breeds (Safford 2013). Second, jacanas frequently disperse in response to changing environmental conditions (Jenni 1996; Tarboton 1995); therefore, assessing population size and distribution is difficult due to haphazard records (Runge et al. 2015). Third, Madagascar Jacanas lead an inconspicuous life-style, foraging and breeding in dense vegetation, which complicates ecological and behavioural data collection. Fourth, Madagascar Jacana often share their habitat with species such as the Nile Crocodile *Crocodilus niloticus* and waterborne disease-causing parasites of genus *Schistosoma* (WHO 2012), constraining fieldwork efforts.

Phylogenetic relationships

Whittingham et al. (2000) carried out the most comprehensive phylogenetic analyses of Jacanidae yet did not include the Madagascar Jacana. Understanding the evolutionary relationships between Jacanidae species can provide insight into the origin of Madagascar's endemic avian diversity (Reddy et al. 2012). Furthermore, phylogenetic information can improve conservation planning by identifying areas of high biodiversity value (Buerki et al. 2015; Chaudhary et al. 2018). There is a particular urgency for well-informed conservation management in Madagascar, given its status as a biodiversity hotspot that is facing severe anthropogenic threats (Brooks et al. 2006; Kremen et al. 2008; Jantz et al. 2015; Chaudhary et al. 2018).

Study objectives

Given the logistical, life-history and accessibility constraints of this elusive species, the aim of our study was to conduct as thorough a conservation and ecological assessment of the Madagascar Jacana as was feasible. Our objectives were to: 1) assess the species' distribution, population size and density, as well as trends in these; 2) determine the degree of SSD displayed in this species; and 3) provide the first molecular phylogeny that includes all eight Jacanidae species. Overall, we hope to provide more data on the Madagascar Jacana to benefit a thorough re-assessment of the conservation status of this species.

Materials and methods

Madagascar Jacana 2016 surveys

To collect data on the behaviour, current distribution, population size, and sexual size dimorphism (SSD) of Madagascar Jacana, we conducted surveys between January and October in 2016. However, remote identification of freshwater wetland habitat is difficult to conduct without major field investment (Judah and Hu 2019); furthermore, due to logistical restrictions, including inaccessibility, we were unable to systematically survey all freshwater wetlands within the species' range. Therefore, we focused our surveys on lakes that were chosen using two methods. First, we used literature searches to indicate

wetlands with previous jacana records; second, we used unpublished surveys of wetland birds (pers. comm.: Durrell Wildlife Conservation Trust, and The Peregrine Fund; Safford 2013) and through conversations with local people in freshwater wetland areas we established where they had seen Madagascar Jacana (Supplementary Table S1). Wetlands/lakes that were surveyed are presented in Figure 1 and Table 1.

Wetland vegetation varies within the year and from site to site; therefore, we conducted a preliminary survey of each lake to make an initial assessment of habitat suitability, prior to conducting more thorough surveys focused on lakes with floating and/or boundary vegetation (Safford 2013). We conducted thorough visual surveys, once at each lake, starting in the morning (at approximately 08:00), for 1–3 h depending on the lake size. We used two canoes to complete the surveys, with one person in each canoe surveying and counting Madagascar Jacanas, using 10 × 8 binoculars. We minimised double-counting by verbal communication between the two observers. Where possible, we surveyed the entire perimeter of the lake; however, in large lakes (e.g. Lake Bemamba) we only visited locations with suitable habitat. We calculated the total area surveyed at each lake (rather than the total size), in sections, by multiplying length by width, using a GPS (Garmin GPSMAP62st). These sections were summed to provide one area of surveyed habitat per lake. Annual rains and changes to fringe vegetation lead to large fluctuations in the area of suitable habitat for wetland birds in Madagascar (Young et al. 2005) and the area that can be surveyed; therefore, we did not collect more-exact measurements at these single time points.

We visually noted the number of adults (identified by full adult plumage: Safford 2013) and immatures (including intermediate plumage: Safford 2013) (Supplementary Figure S1) at each lake. We also recorded habitat characteristics, such as whether a lake showed evidence of human or livestock disturbance, vegetation dominated by the invasive Water Hyacinth *Eichhornia crassipes*, and the presence of other wetland bird species. Where Madagascar Jacana were found foraging, we observed their behaviour for 10–20 min before setting up mist nets in an arc shape above the water surface, around the foraging area. We enticed jacanas into mist nets by paddling slowly towards them in the direction of the net. We also searched for nests on the periphery of the lakes, and, when found, we measured the width and length of any eggs with sliding callipers. We recorded morphometric measurements for each bird caught, following de Beer et al. (2001). We measured wing length and tarsus length with a wing ruler, whereas shield width and length, head length, and beak length were measured with sliding callipers. We measured body mass using a Pesola® spring scale (capacity 500 g), or a digital scale (accuracy 0.01 g) when the spring scale was not available. We took photographs of the head (including shield), neck and body, with the bird placed on a grey card (Supplementary Figure S1), and we recorded the body condition (e.g. presence of parasites, any injuries, feather damage, and moult stage) for all jacanas caught. For genetic sexing and mitochondrial (mt)DNA sequencing, we collected 25 µl of blood (stored in Queen's Lysis

Table 1: The location of lakes in western Madagascar occupied by Madagascar Jacana *Actophilornis albinucha* during surveys conducted between January and October in 2016. The surveyed area within each lake is presented in addition to the total number of jacana observed at each; density was calculated by dividing the total number of individuals observed by the area surveyed

Site	Longitude (E)	Latitude (S)	Area surveyed (ha)	Density (individuals ha ⁻¹)	Total no. of individuals (immatures, adults)
Lake Anosy	49.90	13.14	0.39	5.13	2 (0, 2)
Lake Ambinagny	49.96	13.13	0.17	5.88	1 (0, 1)
Lake Matsaborilava	47.56	15.76	1.00	3.00	3 (0, 3)
Lake Tsinjomitondraka South	47.12	15.67	10.37	1.25	13 (5, 8)
Lake Madiromilomboka	46.77	16.15	8.77	0.46	4 (1, 3)
Lake Ampisarah	46.76	16.14	1.44	1.39	2 (0, 2)
Lake Marogoaky	46.77	16.14	6.79	1.18	8 (3, 5)
Lake Bejio Est	44.12	17.57	2.27	3.09	7 (0, 7)
Lake Bejio Ouest	44.11	17.57	1.03	2.92	3 (0, 3)
Lake Ampiliravao	44.05	17.55	2.87	0.35	1 (0, 1)
Lake Mokotobe	44.06	17.55	1.63	3.06	5 (0, 5)
Lake Nosin'omby	44.07	17.55	1.00	8.00	8 (0, 8)
Lake Betakilotra	44.04	17.54	34.32	0.29	10 (1, 9)
Lake Bemamba	44.36	18.84	8.00	1.50	12 (1, 11)
Lake Belinta	44.43	19.05	2.00	5.00	10 (1, 9)
Lake Besitera	44.35	19.04	0.60	6.67	4 (3, 1)
Lake Ranovorindagory	45.54	20.13	1.75	1.71	3 (1, 2)
Lake Ambariratibe	44.79	20.42	0.72	5.56	4 (2, 2)
Lake Berano	44.79	20.41	0.99	2.02	2 (1, 1)
Lake Belalitra	45.62	20.34	1.00	8.00	8 (1, 7)
Lake Allée de Baobab	44.41	20.26	0.50	4.00	2 (1, 1)
Lake Andramagnokely	43.59	21.89	3.62	6.35	23 (6, 17)
Total			91.22	1.48	135 (27, 108)

Buffer) (Seutin et al. 1991) from each bird by puncturing the brachial vein with a 25G needle (Owen 2011). Finally, we fitted a unique colour ($n = 3$) and metal ring ($n = 1$) combination to the tibiotarsus (two on each leg) for future population monitoring.

Determination of population density and size

We calculated population density of Madagascar Jacana for each lake by dividing the area surveyed by the total number of birds that were visually observed during the survey. We then estimated population size by calculating the total surface area of wetlands in the species' range, using data from Bamford et al. (2017). During our surveys, we found Madagascar Jacana up to 113 km beyond its current recorded range (Figure 1); therefore, we increased the total surface area of wetlands to reflect a buffer of 113 km around the range (Supplementary Figure S2). This increased the total land area of wetlands from 876 km² (within the current range) to 1 124 km² (including a 113-km buffer around the edge). Jacanas occupy areas of wetlands with floating vegetation (Safford 2013), commonly found on the periphery of lakes; to account for this we first calculated the total area of suitable habitat as a 10-m border inside each wetland or lake. However, as Madagascar Jacana do not occur on all wetlands within their range, we reduced this total area. The most recent unbiased survey of wetlands in the jacana's range, conducted in 2004 (Young et al. 2014), found them at 5 of 34 lakes surveyed (15%). Therefore, to provide a total population size estimation, we randomly selected 15% of the lakes within the buffered range and extrapolated the overall population density of occupied

lakes (mean 3.5 ha⁻¹; see Results), across the randomly selected area. Random selection of sites was necessary because predicting suitable wetland vegetation from satellite images is unreliable, with only patchy presence data available for model training. This random-selection procedure was then repeated 1 000 times to give a range of population estimates. Data are provided as means and standard errors, unless otherwise stated.

Estimation of population trends

To provide trend estimates of the Madagascar Jacana population we extracted records of this species from wetland bird surveys conducted within 11 lakes between 2001–2016 (Supplementary Tables S2, S3). The wetland bird survey data were obtained from the Durrell Wildlife Conservation Trust (Richard Lewis pers. comms.) and from The Peregrine Fund (Lily-Arison Rene de Roland pers. comm.; Razafimanjato et al. 2015), and we also included the records from the present study. For two lakes, we present trend data from the New Protected Area of Mandrozo (Mandrozo NAP, including lakes Mandrozo and Andranovaobe: The Peregrine Fund) and Lake Bemamba (Durrell Wildlife Conservation Trust), as data for both wetlands were available for more than three consecutive years (Supplementary Figure S3). Details of the visual survey methods used for the Mandrozo NAP surveys are described in Razafimanjato et al. (2015). Waterbirds at Lake Bemamba were counted by visual surveys, at defined locations around the lake, by a consistent team of staff from the Durrell Wildlife Conservation Trust. Data collected for the remaining areas are presented in Supplementary Table S3.

Molecular sexing

To infer SSD, we molecularly sexed all of the Madagascar Jacanas caught. We purified DNA from the blood samples using ammonium acetate extraction (Bruford et al. 1998). The sexes were determined by amplifying the Chromo Helicase DNA-binding gene (CHD) using the 2550 (5'-GTTACTGATTCGTCTACGAGA-3') and 2718 (5'-ATTGAAATGATCCAGTGCTTG-3') primer pair (Fridolfsson and Ellegren 1999). Within a final reaction volume of 10 µl we included: 2 µl of 5× Green GoTaq® Flexi Buffer, 0.8 µl of 25 mM MgCl₂, 0.5 µl of each primer at 10 pmol/µl, 0.2 µl of 10 µM dNTPs, 0.25 units (0.05 µl) of GoTaq® G2 Flexi DNA Polymerase, 1 µl of DNA (approximate concentration 5–20 ng µl⁻¹), and 4.95 µl of double distilled H₂O. The PCR reaction used the following thermal profile: initial denaturation at 94°C for 2 min, 40 cycles of 94°C (45 sec), 48°C (45 sec) and 72°C (45 sec), and a final extension step for 5 min at 72°C. For failed PCRs, a second primer pair for the CHD gene was used: CHD1F (5'-TATCGTCAGTTTCCTTTTCAGGT-3') and CHD1R (5'-CCTTTTATTGATCCATCAAGCCT-3') (Lee et al. 2010). We visualised all PCR products on a 2% agarose gel electrophoresed for 1 h at 100 V. A single band represented a male and two bands represented a female (Fridolfsson and Ellegren 1999; Lee et al. 2010).

Calculating sexual size dimorphism

We compared sex differences in tarsus length and wing length of adult Madagascar Jacana using data from 13 females and 22 males. To prevent inconsistencies in calculating the sexual size dimorphism (SSD) of body mass as a result of using two weighing methods (see above), we restricted the mass SSD measurement to birds weighed with the spring scale, which provided the most-consistent measurements between observers, as compared with measurements made with the electronic scale; this measurement included eight males and four females. We also compared the shield length (including bill) and width (widest point) between adult males and females (Supplementary Figure S4). We calculated SSD index using $\log(\text{male}/\text{female})$ (Székely et al. 2007). In addition, to compare size between Madagascar Jacana and African Jacana, we obtained measurements of tarsus length, mass, and wing length (using consistent methods) from 35 females and 29 males of African Jacanas, variously caught in Mozambique, Botswana and Zimbabwe, logged in the Southern African Ringing Scheme database (G Cumming pers. comm.).

Data in each category of measurement (tarsus length, wing length and mass) of both sexes and the two species was assessed for normal distribution using a Shapiro–Wilk test. Next, we performed parametric *t*-tests or nonparametric unpaired Wilcoxon rank-sum tests (when the distribution was not normal) to assess the significance between pairwise comparisons. All statistical analyses were performed with R v3.5.1 (R Development Core Team 2019), unless otherwise stated.

Determining phylogenetic relationships

We amplified a 412-bp region of the cytochrome b gene (CytB) in two Madagascar Jacana individuals. The primer

pair for this region was designed by aligning Jacanidae CytB sequences obtained from GenBank (accession numbers: EF373117.1 – *Actophilornis africanus*; EU166999.1 – *Jacana jacana*; DQ485894.1 – *Jacana spinosa*; EF373135.1 – *Hydrophasianus chirurgus*; EF373144.1 – *Microparra capensis*; EF373137.1 – *Irediparra gallinacea*; KF289833.1 – *Metopidius indicus*) (Whittingham et al. 2000) and selecting primers to amplify the area of overlap in all species. The primers we designed were JacCytBF 5'-TCCTCCTTCTAACACTCATAGCA-3' and JacCytBR 5'-TGCTGTTAGGGCTAATACGC-3'. For a final reaction volume of 20 µl, we included 4 µl of 5× Green GoTaq® Reaction Buffer, 2 µl of MgCl₂, 0.5 µl of dNTPs (10 mM), 0.5 µl of each primer (10 pmol µl⁻¹), 0.50 units (0.1 µl) of GoTaq® G2 Flexi DNA Polymerase, 11.4 µl of double distilled H₂O, and 1 µl of DNA. We used the following thermal profile: 94°C for 1 min 30 sec initial denaturation, 36 cycles of 94°C for 40 sec, 64°C for 40 sec, 72°C for 1 min, and a final extension for 5 min at 74°C. We included negative controls for all PCR reactions and visualised with the same gel conditions as stated above. PCR reactions were conducted in a Veriti™ 96-well thermal cycler (Applied Biosystems™). The PCR products were sequenced by Eurofins Genomics Sequencing Services on an AB1 platform. We trimmed sequences in Sequencher® v5.1 (GeneCodes Corporation), and aligned all Jacanidae CytB sequences with the ClustalW aligner method BioEdit v7.1.11 (Hall 1999). DNAsp v5 (Rozas et al. 2003; Librado and Rozas 2009) was used to produce diversity statistics and estimate sequence divergence.

We created a Bayesian phylogenetic tree in MrBayes v3.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) using the GTR + gamma substitution model, which was identified as most suitable by the Smart Model Selection tool in PhyML (Lefort et al. 2017). For consistency with the previous phylogeny (Whittingham et al. 2000), we used Greater Painted Snipe *Rostratula benghalensis* as an outgroup (GenBank accession no. AF146623.1; Whittingham et al. 2000). The parameters used were as follows: 5 000 000 MCMC generations, sampling every 500 repeats, burnin and sumpt burnin = 1 250 000. The tree was visualised in FigTree 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>). Sequences are deposited in GenBank. Divergence time was calculated based on the '2% rule' for mtDNA, meaning that 2% sequenced divergence occurs per million years (cf. Lovette 2004).

Results

Species distribution and population trends

In 2016, we surveyed 54 lakes for Madagascar Jacana, across their entire known range, in addition to adjacent areas of suitable habitat (Figure 1). A total of 135 Madagascar Jacana (adults and immatures) were found in 22 of the lakes, representing 91 ha and 41% of the lakes surveyed (Tables 1, S1). Four of the 22 lakes with Madagascar Jacana were outside the species' known range (BirdLife International 2018), with one lake situated 113 km east of the range extent (Figure 1). In the lakes with Madagascar Jacana, the number of individuals ranged between 1 and 23, with an average of 6.1 ± 1.3 individuals,

and increased with the area surveyed (Supplementary Figure S5). The mean density of Madagascar Jacana in occupied lakes was 3.5 ± 0.74 per hectare (ha) of area surveyed, and the mean density combining all occupied lakes together was 1.48 per ha (Table 1). The total surface area of wetlands in the Madagascar Jacana range, including those 113 km to the east, was 1 154 km², with the periphery of these wetlands amounting to 43 km². Following the survey by Young et al. (2014) in 2004, in which the jacana were found at only 15% of sites, and using a density of 3.5 birds per ha, we predict a population in the range of 975–2 064 individuals, with a median estimate of 1 423 individuals.

Records of the Madagascar Jacana in wetland bird surveys have indicated large within- and among-year population-size variations (Young et al. 2005; Young et al. 2014; Bamford et al. 2017; Durrell Wildlife Conservation Trust pers. comm.; The Peregrine Fund pers. comm.) (Supplementary Figure S3). Of the two best-monitored populations (i.e. at Mandrozo NAP and Lake Bemamba), we found a declining trend for the species at Lake Bemamba (Figure S3a) and a consistently large population in the Mandrozo NAP (Supplementary Figure S3b). However, the available data across wetlands were patchy, with large temporal gaps and some estimates without a date associated with them. For these reasons, we are unable to provide reliable estimates of overall population trends with these data, but we present preliminary estimates in Supplementary Figure S3.

Threats

Human disturbance (e.g. washing, fishing, agriculture) was found at all sites with Madagascar Jacana present. Unless directly disturbed, jacanas appear tolerant of people. We did not witness hunting during our surveys; however, local people confirmed that the hunting of waterbirds was common. During our surveys we witnessed extensive and rapid destruction of natural wetlands for conversion to agricultural land. For example, the Lake Sahaka region (including lakes Anosy and Ambinagny) in northern Madagascar was previously described as a permanent site for ~66 Madagascar Jacana year-round (Safford 2013); however, in our surveys we found only three individuals in this area. Consistent with our findings, local people and birding tour guides described a steep decline in the number of Madagascar Jacanas that they had observed in recent years. Twelve of the surveyed lakes were overgrown with water hyacinth (Supplementary Table S1); we found Madagascar Jacana in seven of these lakes, and at one lake (Lake Belinta) we also found a Madagascar Jacana nest within the hyacinth vegetation.

Local people and NGO staff reported that many lakes only provide temporarily suitable habitat for Madagascar Jacana owing to seasonal weather fluctuations and agricultural practices (e.g. changes in the rice-growing season; FAO 2016). For example, at Lake Bemamba, local people reported that following the rice-growing season (January to April), the grasses and reeds provide dense cover for nest sites and chick-rearing, and during this time immature Madagascar Jacana can be seen. After the rice harvest, we witnessed piles of rice-reed debris that provided

foraging areas for adult birds. However, as these findings are based on anecdotal reports, we are unable to assess their accuracy pending more-thorough investigations into the specific threats to Madagascar Jacana.

Sexual size dimorphism

We captured a total of 55 Madagascar Jacana, comprising 35 adults (22 males, 13 females) and 20 immatures (11 males, 9 females). Females were significantly larger and heavier than males (Figure 2; Supplementary Table S4), and their shield length (but not width) was also significantly greater (Supplementary Figure S4). Both sexes of Madagascar Jacana were significantly larger than African Jacana in terms of body mass and wing length (mass: $t = 3.08$, $df = 37$, $p < 0.01$; wing length: $t = 8.45$, $df = 35.83$, $p < 0.01$) but not tarsus length ($t = 0.73$, $df = 46$, $p = 0.464$) (Figure 2). The female-to-male ratios of Madagascar Jacana were tarsus length 1.09:1, wing length 1.15:1, and body mass 1.60:1. SAFRING morphometric data from 35 females and 29 males of African Jacana showed consistent SSD measurements of 1.10 for tarsus length, 1.13 for wing length, and 1.61 for body mass.

Nest and parental care

We found one nest containing three eggs; the length and width of the eggs were 33.4 ± 0.3 [SD] and 25.2 ± 0.2 [SD] mm, respectively. Molecular sexing of the brooding adult indicated it was a male. In the only other instance of an adult observed with (three) chicks, the adult was molecularly sexed as a female.

Sister-group relationship

Phylogenetic analysis using CytB confirmed the Madagascar Jacana is a sister species of the African Jacana, and together they represent a monophyletic clade (Figure 3). There were no sequence differences between the two Madagascar Jacana individuals. Of the 421-bp region sequenced, four polymorphic sites were found between the Madagascar Jacana and the African Jacana.

Discussion

This study provides the first detailed account of Madagascar Jacana, an understudied endemic shorebird restricted to freshwater wetlands of Madagascar.

Distribution and population-density size and trends

Our findings largely support the estimated range of the Madagascar Jacana; however, we suggest the current range should be extended eastwards. Based on our findings, we encourage future surveys of the Madagascar Jacana to include a buffer zone of at least 100 km eastwards and using this data a new range polygon could be drawn. Shorebird range changes can be attributed to multiple interacting factors, including food availability (e.g. Verkuil et al. 2012) and climate change (e.g. Bart et al. 2007). Alternatively, the African Jacana has extended its range in recent years, by 28%, by taking advantage of manmade wetlands (Okes et al. 2008). However, as there is little known about that species, it is likely that the range predicted by BirdLife International (2018) was simply too restrictive, rather

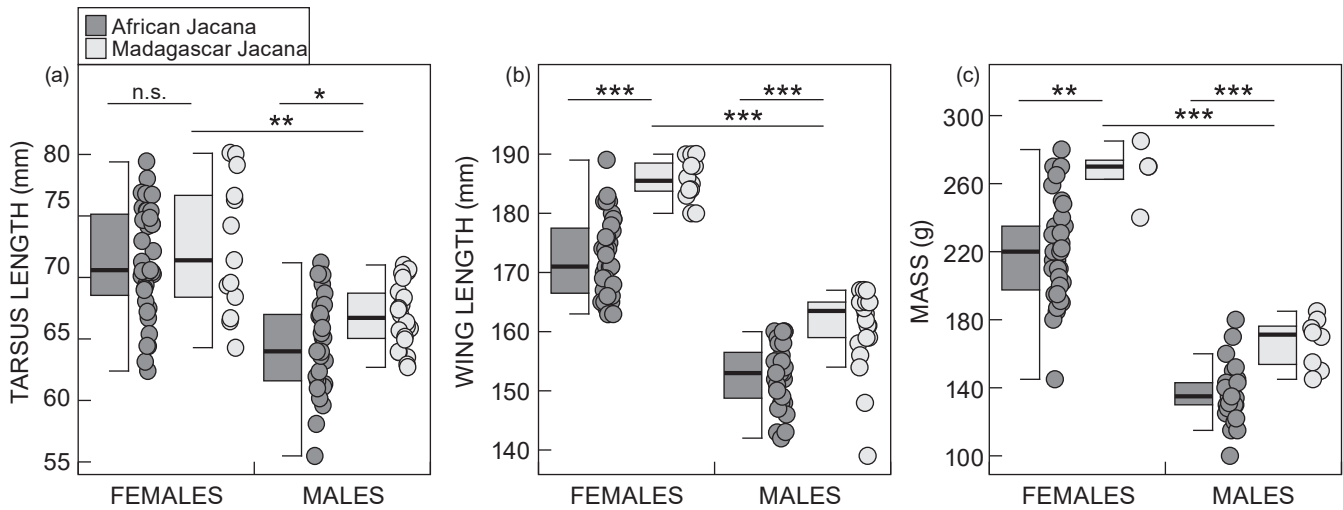


Figure 2: Morphometric measurements of adults of African Jacana *Actophilornis africanus* (dark grey) and Madagascar Jacana *Actophilornis albinucha* (pale grey): (a) tarsus length, (b) wing length, and (c) body mass. Comparisons of tarsus length and wing length included 35 Madagascar Jacana (13 females, 22 males) and 64 African Jacana (35 females, 29 males); sample sizes for body mass included 12 Madagascar Jacana (8 males, 4 females) and 64 African Jacana (35 females, 29 males). n.s. = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

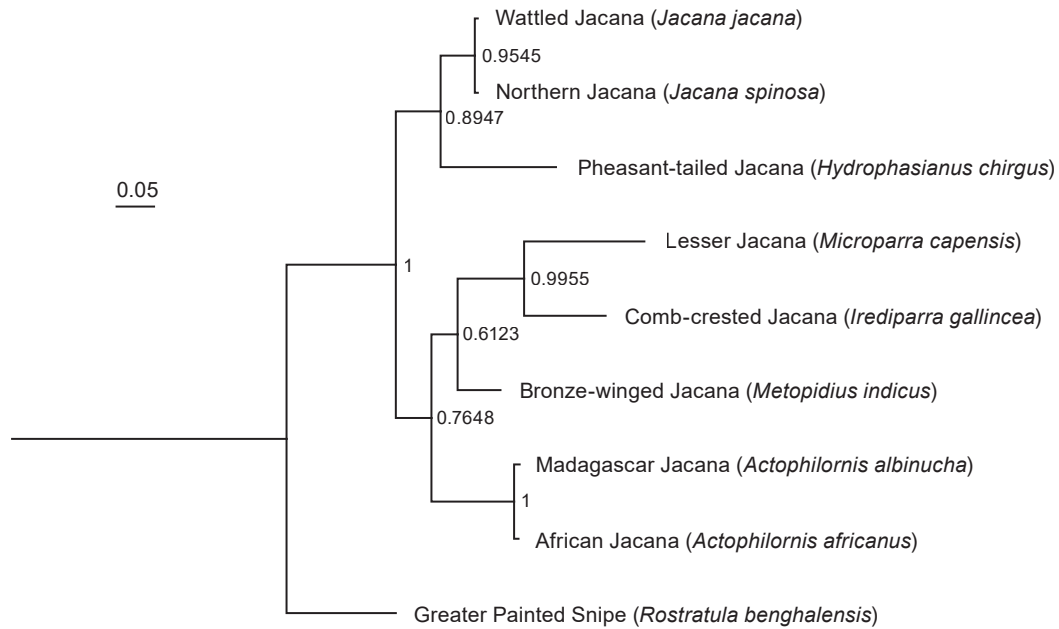


Figure 3: Phylogenetic relationships of all eight extant species of the family Jacanidae, created with a Bayesian inference method using 421 bp of the cytochrome b mitochondrial DNA gene. Posterior probabilities are shown for each node; one sequence of each species was included to create the tree. Outgroup = Greater Painted Snipe *Rostratula benghalensis*

than a range expansion. Due to the patchy distribution of Madagascar’s wetlands and the ongoing transformation of the island’s landscape (Benstead et al. 2003; Kull et al. 2012; Bamford et al. 2017), we hypothesise that the range of the Madagascar Jacana is not continuous, and that the area of occupancy is likely to be far smaller than the current range suggests. Our estimate, even though an approximation, was of just 43 km². For wetland specialist

species, this discontinuous habitat distribution should be considered for conservation management by focusing on creating connected networks of wetlands (Haig et al. 1998; Amezaga et al. 2002). Water hyacinth is a problematic invasive species for freshwater wetlands in Madagascar (Lammers et al. 2015); nonetheless, our findings suggest Madagascar Jacana co-exist with this free-floating plant and use the vegetation for nest-building. However, we caution

against assuming that this invasive aquatic plant does not affect Madagascar Jacana, because indirect effects such as insectivore abundance were not examined, and waterbird community responses to water hyacinth are likely to be species-specific (Villamagna et al. 2012).

Our findings suggest that the Madagascar Jacana is increasingly vulnerable to extinction. However, estimating a reliable population size and trend is challenging for species of highly dispersive birds (Runge et al. 2015), especially for understudied species like the Madagascar Jacana. Our estimate of 975–2 064 individuals does not strictly represent a decline from the existing estimate of ~1 000–10 000 individuals (BirdLife International 2018) but suggests an estimate at the lower end of that range. Furthermore, given a lack of reliable data on population trends, it is difficult to reassess the status of the Madagascar Jacana for IUCN Red List criteria (IUCN 2001). Nevertheless, based simply on the population size there are implications for re-evaluating the species' threat status as possibly Vulnerable or even Endangered.

We emphasise that the methodology used here to estimate population size has limitations which are likely to have led to an inflated estimate. We calculated the density of Madagascar Jacana using the number of individuals found within an estimated survey-area size. This could have increased the estimated density of the jacana for two reasons: first, the survey areas were not systematically selected, since we visited lakes with a higher likelihood of finding Madagascar Jacana and visited only accessible sites; second, within lakes, we surveyed only areas of suitable habitat (i.e. lake margins and areas with floating vegetation). To account for these methodological limitations in our estimate of the current population size across Madagascar, the size of each wetland was calculated by including only an area that represented a border of 10 m from the wetland perimeter, and we accounted for the occupancy of jacana within these wetlands by randomly selecting only 15% of the wetlands, based on the most recent survey of Madagascar wetlands (Young et al. 2014). However, that survey was conducted in 2004–10 years prior to its publication and may not reflect current circumstances. Furthermore, our own field surveys were conducted across a large time period, which included one extreme weather event (Siderius et al. 2018); therefore, our estimates are likely to be incomplete. We strongly encourage a more systematic approach to the estimation to Madagascar Jacana presence/absence in the future, to obtain a more accurate estimate of population size. Such estimates are a high priority for future studies of the Madagascar Jacana, and these should include wetlands to the east of the current range boundaries.

In addition, we strongly support the use of repeated surveys of wetland birds in Madagascar, with the aim of calculating reliable population trends. The methods used in future, repeated surveys should be consistent between researchers and organisations, to allow the results to be adequately compared (Buckland et al. 2008). However, long-term monitoring of this sort is logistically challenging in Madagascar, meaning that population trends are uncertain even for well-known species (e.g. Murphy et al. 2017).

Repeated surveys in the Lake Mandrozo area (17.56° S, 44.08° E), conducted by The Peregrine Fund, suggest this

site represents an important permanent habitat for Madagascar Jacana, with 78–127 individuals recorded each visit between 2009 and 2015 (Razafimanjato et al. 2015; Supplementary Table S2). Additionally, Bamford et al. (2017) found 80 Madagascar Jacana in the Ambonara wetlands (17.03° S, 45.52° E) in 2016 and 64 individuals in 2017, along with a high diversity of wetland avifauna. This suggests that the Ambonara wetlands require long-term monitoring and urgent protection against land conversion.

There are at least three explanations for the small number of Madagascar Jacana found during the 2016 surveys. First, extreme departures from normal weather conditions caused by one of the strongest recorded El Niño events on record (Siderius et al. 2018) may have made previously suitable areas unsuitable during this year. Jacanas are known to disperse in response to wetland availability (Tarboton 1995; Safford 2013), and rainfall is highly variable in Madagascar (Tadross 2008; Macron et al. 2016). Therefore, the sites we visited in 2016 may not have provided optimal habitat at that time. Other jacana species occur at naturally low densities (Jenni 1996; Dostine and Morton 2000; Changder et al. 2015), which can make population surveys difficult, especially in areas of low accessibility.

Second, an absence or low densities in areas with suitable habitat may be a result of interspecific competition or predator avoidance, as Madagascar Jacanas were rarely observed alongside species with a similar ecological niche, such as the Common Moorhen *Gallinula chloropus* and Allen's Gallinule *Porphyrio alleni*. In addition, we also witnessed aggressive displays from Madagascar Jacanas towards moorhens and gallinules. This supports the observation of aggressive interactions recorded between the Northern Jacana *Jacana spinosa* and the Purple Gallinule *Porphyryula martini*, which are known to prey on the eggs of Northern Jacana (Stephens 1984).

Third, the low density recorded could be a result of a genuine population decline, as predicted from the most recent IUCN Red List assessment (BirdLife International 2018). Unfortunately, the survey data we compiled was not suitable to draw reliable estimates of population trends, although these are urgently needed to re-assess the Madagascar Jacana's threat status. During our surveys, anecdotal observations by local people as well as birding field guides suggested a downwards population trend of the Madagascar Jacana, similar to that of other Madagascan wetland bird species (e.g. White-backed Duck *Thalassornis leuconotus insularis* and Madagascar Grebe *Tachybaptus pelzelinii*), among which many are classified as threatened (Bamford et al. 2017; IUCN 2018). Several factors are likely to be responsible for these population declines, most notably extensive wetland habitat destruction from conversion to agricultural land, particularly rice paddies (so far estimated to have caused the loss of 60–82% of marsh habitat: Kull 2012; Bamford et al. 2017). The low number of individuals found in large lakes also used for growing rice (e.g. lakes Bemamba and Sahaka) suggests that rice paddies provide a suboptimal habitat for this species, yet one which they can utilise for some parts of the year. The seasonal use of rice paddies by breeding and foraging waterbirds (including other jacana species) is common around the world (reviewed by Pierluissi et al.

2010; Marco-Méndez et al. 2015). However, the relative biodiversity value of these areas as compared with natural wetlands is largely unknown (Fasola and Ruiz 1996; Sundar and Subramanya 2010; Antunes Dias et al. 2014), but such research is urgently required in Madagascar. One consequence of agricultural expansion is the intensive use of pesticides (Parsons et al. 2010). Jacanas are insectivores (Jenni 1996) and pesticide use could affect their survival by reducing their food availability and/or have direct lethal effects (reviewed by Parsons et al. 2010). A population decline could also be caused, or exacerbated by, reduced habitat availability a result of global climate change causing extreme fluctuations in rainfall (Tadross 2008). Unlike for other habitats in Madagascar (e.g. Raxworthy et al. 2008), there have been no projections of how climate change will affect the island's freshwater biodiversity. This is despite a growing number of potential threats (Woodward et al. 2010) and the ecosystem's high level of vulnerability (Bamford et al. 2017; Máiz-Tomé et al. 2018). Finally, we did not observe any direct persecution of Madagascar Jacanas during our surveys, although previous reports suggest this is an additional threat to their persistence (BirdLife International 2018).

Reversed sexual size dimorphism

By combining genetic sexing with morphometric data we show that females are significantly larger than males, confirming previous findings of sexual size dimorphism (SSD) in Madagascar Jacana using a small sample size (Jenni 1996; Safford 2013). Our findings could suggest that, like the six other jacana species with reversed SSD, the Madagascar Jacana may display a classically polyandrous mating system (Vernon 1973; Thong-aree et al. 1995; Jenni 1996; Butchart 2000; Mace 2000; Emlen and Wrege 2004). However, without dedicated investigations of breeding behaviour and analyses of paternity to evaluate the generality of this observation, we are limited in the conclusions we can draw, as SSD is not always predictive of the mating system (Székely et al. 2004). Furthermore, we observed female uniparental care of three immature birds, which may indicate that this species has a flexible mating system.

Within Madagascar Jacana and African Jacana, the female-to-male morphometric ratios we describe here are remarkably similar, and the ratio of 1.61:1 for female-to-male African Jacana mass ratio is weaker than the 1.68:1 mass ratio previously reported (Tarboton 1995). These mass ratios lie within the range of values for other reversed SSD jacana species, including the Northern Jacana (1.67:1) and the Wattled Jacana (1.50:1) (Lipshutz 2017), but are less extreme than those for the Pheasant-tailed Jacana (1.85:1: Johnsgard 1981) and the Comb-crested Jacana (1.75:1: Mace 2000). We also found that the Madagascar Jacana is larger-sized than its mainland sister species, the African Jacana—supporting the 'island rule,' which predicts that island species evolve a larger body size than their mainland conspecifics (Foster 1964; Van Valen 1973).

Explanations for the island rule are complex and multifaceted, and they include foraging-preference shifts, character displacement, reduced resource competition, and reduced predation pressure (Clegg and Owens 2002;

Scott et al. 2003). The island rule for birds is inconsistently supported (Gaston and Blackburn 1995; Clegg and Owens 2002; Lomolino 2005; Olson et al. 2009; Lokatis and Jeschke 2018), and in mammals endemic to Madagascar there is little evidence supporting this pattern (Kappeler et al. 2019). Furthermore, Madagascar is considered by some as a subcontinent rather than an island due to its large land mass (de Wit 2003), such that the Madagascar Jacana may not constitute a true island endemic. In addition, the island rule is generally defined by smaller species increasing in size, while larger species evolve smaller body sizes (Foster 1964). Therefore, if jacanas represent a large-sized species (as do rails, ducks and ratites, according to Lomolino [2005]), the larger size of the Madagascar Jacana compared with the African Jacana is evidence against the island rule. The inconsistencies in findings between studies testing this rule could also be attributable to methodological differences and specific ecological traits that shift the strength of the rule (Clegg and Owens 2002; Lokatis and Jeschke 2018).

Phylogenetic relationships

As expected, based on phenotypic similarity and geographic proximity, the molecular phylogeny of all extant Jacanidae species indicated the Madagascar Jacana is a sister species of the African Jacana, extending the previous phylogeny (Whittingham et al. 2000). The observed sequence divergence of 1% corresponds to a time of divergence of 500 KYA based on the '2% rule' for mtDNA (cf. Lovette 2004). This indicates a more recent divergence of *Actophilornis* species in comparison with the divergence found between other Madagascan endemic avian species and their mainland Africa sister taxa, which reportedly ranges from 0.8 to 5 MYA (Bloomer and Crowe 1998; Groombridge et al. 2002; Woog et al. 2008; Melo et al. 2011; Arbabi et al. 2014; Fuchs et al. 2015). However, our estimate of divergence is based on a short section of CytB (412 bp) and a single representative sample for each species, conditions that can result in inaccurate estimates (Braun and Kimball 2002), and these data should be supplemented by more sequence data, preferably at the genome-scale.



Conclusions

Here, we present a baseline study of the Madagascar Jacana, aimed to improve our understanding of this endemic wetland bird. Many fundamental ecological and behavioural traits of the Madagascar Jacana remain unknown; therefore, we call for further investigation of this species to build upon our study. In addition, ongoing threats to Madagascar's wetlands and habitat destruction together suggest that this species requires a targeted reassessment to potentially up-list its threat status, possibly to Vulnerable, and efforts at direct monitoring of the population.

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ORCID

Josephine D'Urban Jackson  <https://orcid.org/0000-0001-8946-0772>
 Sama Zefania  <https://orcid.org/0000-0002-6260-1069>
 Andrew J Bamford  <https://orcid.org/0000-0003-4143-8087>
 Michael W Bruford  <https://orcid.org/0000-0001-6357-6080>
 Tamás Székely  <https://orcid.org/0000-0003-2093-0056>

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