Parallel cases of ring speciation within a rapid adaptive radiation of cichlid fishes

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ABSTRACT

Ring species provide a valuable framework for studying intermediate steps of speciation resulting in two new species. Classic examples of ring species have involved large spatial and temporal scales – and this is partly why few idealised cases exist. In contrast, we focus on two species complexes of cichlid fishes from Southern Lake Victoria that evolved rapidly within the last 15,000 years across relatively small geographic space. We reconstruct the path to speciation between two forms of *Pundamilia* and *Neochromis* species that exist in a ring-like distribution around deep water, which acts as a dispersal barrier for these shallow water adapted fishes. We test key predictions of ring-like biogeography, population connectivity, terminal overlap and differentiation in mating and ecological traits**.** Our results indicate that both species complexes show a gradual change in craniofacial morphology along a ring-like distribution, corresponding to diet shifts. This phenotypic change is correlated with geographical distance in both rings, although they appear to have spatially different evolutionary trajectories. Sympatric species at the ends of both rings exhibit signs of convergent and divergent ecological character displacement in several craniofacial traits, potentially explaining their coexistence. In contrast to craniofacial morphology, male nuptial colouration changes abruptly in both rings, with the change occurring at the end of the ring in *Pundamilia* and early in *Neochromis*. We discuss the influence of environmental factors on the observed phenotypic variation and how resistance to connectivity might alter the isolation-by-distance pattern. Our study provides compelling evidence of how concerted changes in mating and ecological traits during isolation by distance can lead to new species and highlights the importance of parapatric speciation in adaptive radiation.

Key words: parapatric speciation, isolation by distance, ecological character displacement, craniofacial shape, male nuptial colour

INTRODUCTION

Unravelling the processes through which new species arise is essential to understand global biodiversity patterns (Blackburn and Gaston, 2003; de Aguiar *et al.*, 2009). Parapatric speciation, where populations diverge despite geographical connectivity and gene flow, can shed light on the intermediate steps of speciation (Hendry *et al.*, 2009), thus providing a valuable framework to study the speciation continuum. A special case of parapatric speciation called 'ring species' occurs in ring-like geographical distributions, where two terminal reproductively isolated populations are connected by a chain of intergrading populations around a barrier (Jordan, 1905; Mayr, 1942; Cain, 1954; Irwin and Wake, 2016). This 'stepping stones' mode of speciation follows a pattern of isolation

by distance and generates intermediate and terminal populations from an ancestral population within a single temporal window, allowing for the study of ecological and sexual changes across populations. Cases of ring speciation have been reported across the plant and animal kingdoms, with famous examples being in greenish warblers (Irwin *et al.*, 2001 a), *Larus* seagulls (Liebers *et al.*, 2004), *Ensatina* salamanders (Highton, 1998) and *Acacia* trees of South Africa (Brain, 1989; Ward, 2011). However, no examples have been demonstrated in freshwater fish, one of the most species rich groups on Earth.

Ernst Mayr (1963) set four criteria characterising an idealised ring species: (1) the presence of two terminal, sympatric populations that are reproductively isolated from one another, (2) each of the other populations that make up the ring must be currently, and have been historically, connected to their nearest neighbours through gene flow and this pattern must be the result of range expansion around a barrier, (3) all populations must be geographically connected forming a complete ring and (4) the two terminal forms must be connected by gradual differences (often described as subspecies) around the ring. These criteria imply a continuity in the historical biogeography of the species, and a gradual divergence strong enough to lead to the reproductive isolation of terminal populations. A vast majority of the potential cases of ring species investigated lack at least one of the criteria (reviewed in Irwin *et al.*, 2001 b). The most frequent infringements of Mayr's criteria are breaks in the historical geographical continuity of the ring, causing populations to diverge in allopatry before coming into secondary contact (Highton, 1998; Liebers *et al.*, 2004; Joseph *et al.*, 2008), and/or the existence of viable offspring resulting from the hybridization of the terminal populations (Päckert *et al.*, 2005). Mayr (1942, p. 182) stated that "overlapping rings (species) are disturbing to the orderly mind of the cataloguing systematist, but they are welcome to the student of speciation". Whether idealised rings exist or not is not so relevant anymore as even broken rings have provided important insights into the processes of speciation and microevolution (Wolf, 2021; Bouzid *et al.*, 2022; Amir Afzali *et al.*, 2024; Monjaraz-Ruedas *et al.*, 2024).

Repeated breaches in the continuity of ring species may be attributed to the spatiotemporal scales that their distribution and evolution spans. Classical ring species consider distributions around especially large geographical barriers such as chains of mountains (Highton, 1998; Irwin *et al.*, 2005), oceans (Liebers *et al.*, 2004), or even the entire globe (Bowen *et al.*, 2001), with divergence times spanning thousands to millions of years. In contrast, modelling approaches on the distribution and stability of ring species indicate that such species complexes are very unstable over time and break up into separate species shortly after sympatric reunion (Ashlock *et al.*, 2010; Martins *et al.*, 2013). Simulations on the much-scrutinised case of greenish warblers predict a rapid breakup of the ring in 10,000 to 50,000 years, with the existing ring being no older than 8,000 years (Martins *et al.*, 2013). Based on this, diversification events on smaller spatial scales could provide a more suitable framework for investigating complete ring species.

Thirty years ago, Seehausen (1996) described two complexes of rocky-shore cichlid fishes in the genera *Pundamilia* and *Neochromis* in the Southern part of Lake Victoria within which subspecies (or populations) displayed gradual ecomorphological and male nuptial coloration in a "chain-like" distribution around deep water, which acts as a dispersal barrier for these shallow water specialised fishes. These species complexes, like the entire Lake Victoria species-flock, evolved in the last 15,000 years (Turner *et al.*, 2001; Seehausen, 2002; Sturmbauer *et al.*, 2011). *Neochromis* and *Pundamilia* comprise populations with gradual differences in male nuptial coloration across patches, which is known to be a major driver of speciation through assortative mating in this radiation (Seehausen, 1997; Seehausen *et al.*, 1998, 2008; Seehausen and Schluter, 2004; Magalhaes *et al.*, 2010), along with convergent and divergent shifts in trophic ecology (Seehausen, 1996; Seehausen and Bouton, 1996; Bouton *et al.*, 1998; Bouton *et al.*, 1999)(Fig. 1a). For each genus, the two most differentiated ecomorphs occur in sympatry at one location (Juma island) and are connected by intermediate colour morphs with variable diet preferences in a parallel circular distribution around the Mwanza Gulf in Tanzania (Fig. 1b). These two two species complexes have not been studied in detail

to establish if they indeed meet the conditions for ring species. Here we aim to describe the morphological differences associated with mating and trophic ecology across the distributions of *Neochromis* and *Pundamilia* complexes to scrutinise their potential as cases of ring speciation.

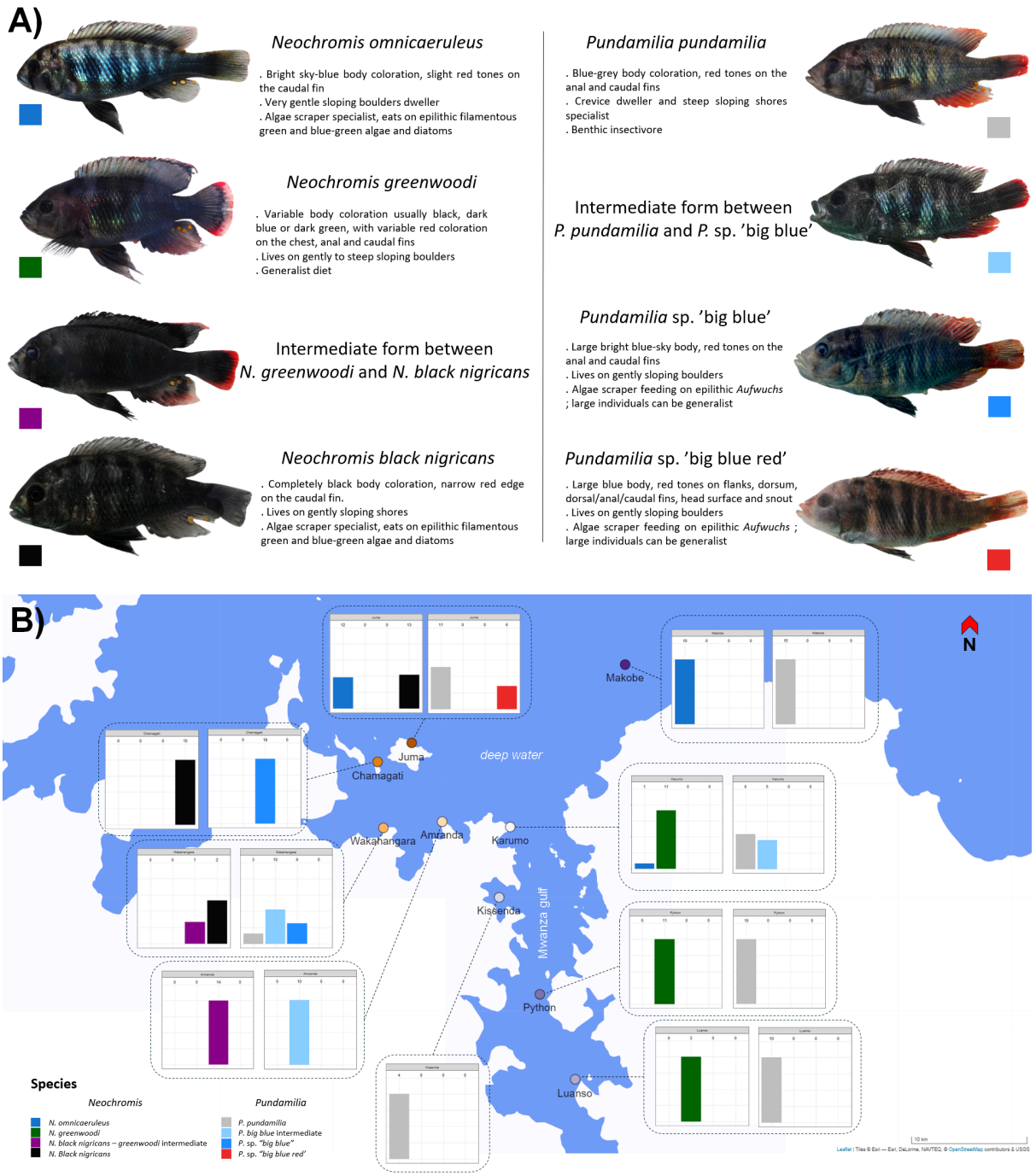

Figure 1: A) Overview of parallel ring speciation in *Pundamilia* and *Neochromis* species complexes in Lake Victoria., Details of male nuptial coloration, habitat characteristics, and diet are based on Seehausen 1996. Note that fish coloration rapidly degrades after sampling, therefore photos show faded colours compared to in situ conditions. **B)** Proportions of individuals per population across the total number of samples measured for each location, for *Neochromis* and *Pundamilia* genera (left and right respectively; no fish from Kissenda island was used for the study in *Neochromis*). Numbers above the bars indicate the number of fish for each species at one location. Relative proportions of species for each purported ring are consistent with the compositions observed in

the field (Seehausen and Bouton, 1996). Kissenda, Karumo, Amranda, Wakhangara, Chamagati and Juma locations are part of the Sengerema region. Python and Luanso islands are in the Southern Mwanza gulf and Makobe island is outside the Mwanza gulf.

MATERIALS AND METHODS

Fish sampling and linear measurements

Specimens used in this study come from a larger collection sampled in 2022 by P. Singh, O. Seehausen, and A. Mahulu in Southern Lake Victoria around the Mwanza Gulf and Sengerema region (Tanzania). The Sengerema region comprises Kissenda, Karumo, Amranda, Wakhangara, Chamagati and Juma locations, while Python and Luanso islands are in the Southern Mwanza gulf. Makobe island is located outside the gulf, five kilometres northwards the mainland. Additional specimens collected during field trips led by O. Seehausen in 1996 and 2014 were also used to increase sample sizes for Makobe and Python islands. Collection took place in specific locations of rocky shores along mainland and islands' coasts following the distribution pattern described by Seehausen (1996) ensuring a representative sampling of the populations of interest. Fish were collected using gillnets and angling and were sacrificed humanely using clove oil anaesthetic in accordance with the Tanzanian and Swiss animal welfare regulations. Samples were fixed in formalin solution before being preserved in ethanol. For the *Pundamilia* complex, we used 114 male individuals belonging to the populations (or intermediate forms) of interest: *Pundamilia pundamilia*, *Pundamilia* sp. "big blue", and *Pundamilia* sp. "big blue red". For the *Neochromis* complex, we used 93 males from the populations of interest: *Neochromis omnicaeruleus*, *Neochromis greenwoodi*, and *Neochromis black nigricans*. For both complexes only males were analysed, as females' head morphology undergoes important trade-off between foraging and mouthbrooding resulting in a strongly marked sexual dimorphism (Tkint *et al.*, 2012; Ronco *et al.*, 2019).

Linear measurements were conducted on twelve traits characterising head shape and preo-orbital features, known to retain large amounts of variation across cichlid species and diets (Barel *et al.*, 1976; Seehausen, 1996; Hulsey and León, 2005; Cooper *et al.*, 2010; Hulsey *et al.*, 2010; Magalhaes *et al.*, 2010): body depth (BD), head length (HL), head width (HW), lower jaw length (LJL), lower jaw width (LJW), snout length (SnL), snout width (SnW), eye length (EyL), eye depth (EyD), cheek depth (ChD), pre-orbital depth (POD), and inter-orbital width (IOW). All morphometric distances were measured using digital callipers and following classical cichlid measurement protocol (Barel *et al.*, 1976)(Fig. 2). We accounted for fish size heterogeneity by performing a log/log regression against the fish standard length (SL) for all traits (Fleming and Gross, 1994; Magalhaes *et al.*, 2009; Magalhaes *et al.*, 2010).

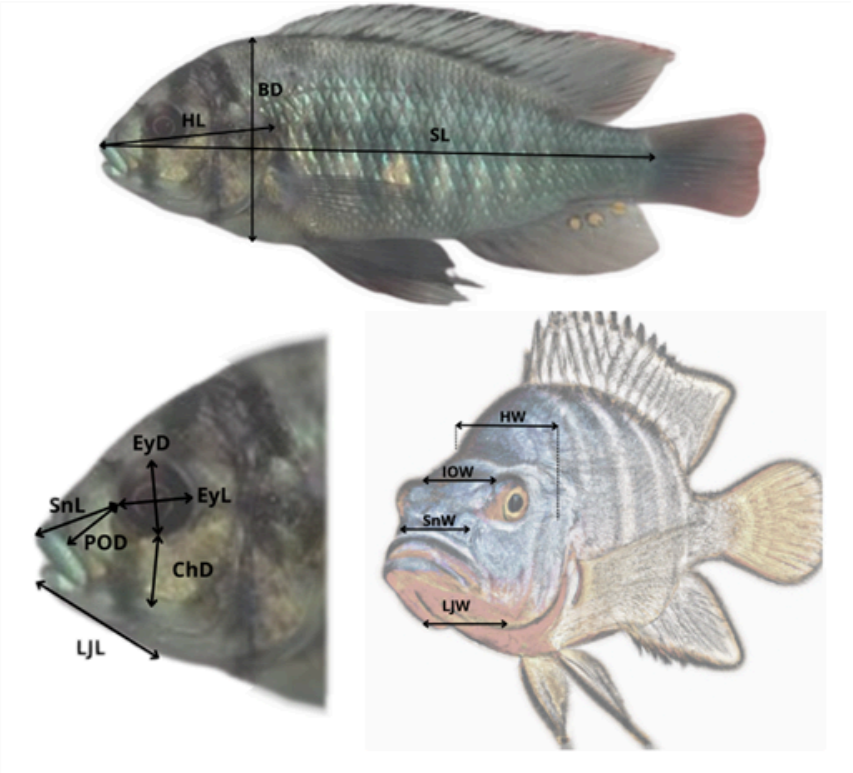

Figure 2: Linear measurements used for the morphological analysis of *Pundamilia* and *Neochromis* populations, based on Barel *et al.* 1976. SL: Standard length; BD: Body depth; HL: Head length; EyL: Eye length; EyD: Eye depth; ChD: Cheek depth; POD: Pre-orbital depth; SnL: Snout length; LJL: Lower jaw length; SnW: Snout width; LJW: Lower jaw width; IOW: Inter-orbital width; HW: Head width.

Morphological analysis

For all analyses we used *R* programming software version 4.3.2. (R Core Team, 2023) in *RStudio* 2023.12.1 (Posit team, 2024) and analysed the potential rings –*Pundamilia* and *Neochromis*– separately.

Principal components analysis (PCA)

Average morphology at each sampled location and main axes of variation were scrutinised by reducing variables to principal components: we used the *PCA* function from the *FactoMineR* package (Husson *et al.*, 2024) on log-transformed residuals of traits measurements and represented mean PC scores along axes of variation using the *factoextra* package (Kassambara and Mundt, 2020).

Ring delimitation and isolation-by-distance pattern

N. omnicaeruleus and *P. pundamilia* are considered the basal species of each species complex , given their overall distribution in southern Lake Victoria (Seehausen, 1996). In this regard, Makobe

island is thought to be the starting point of both rings as only the two basal species are to be found along its shores. As the exact distribution path of ring speciation is not known, four distinct ring path scenarios were tested, differing by the latitude at which the fish were expected to cross the gulf where water is deeper: at the level of Karumo bay (northernmost point of crossing), Gabalema islands, Python islands, or Luanso island (southernmost point of crossing) (Fig. S1). For each scenario, distances around the ring were computed as the geographical distance between each location and Makobe island following the shoreline and the scenario of crossing, and excluding islands southwards the point of crossing, using the *geosphere* package (Hijmans *et al.*, 2022). We then performed a Mantel correlation test (1000 permutations) between pairwise geographical distance and Euclidean distance between PC1 scores to compare geographical and morphological distances for each scenario. A strong correlation would indicate a convincing fit to an isolation-by-distance (IBD) pattern and support the hypothesis that Makobe island is the starting point of the ring.

Morphological variation around the ring

We used polynomial regressions to visually describe the variation of PC scores across distance for the four first dimensions obtained in the PCA. The best polynomial fit for each regression was selected based on the amount of variation explained by the model (using r-squared and *p* values) and a visual assessment of the standard deviation around the fitted curve. To focus only on the gradual evolution across species, regressions included all individuals from the locations forming the retained ring-scenario, except for the basal species found at the terminal location (*N. omnicaeruleus* and *P. pundamilia* individuals from Juma island).

Comparison of terminal species and ecological character displacement

We compared mean values for all traits in terminal species between sympatric and allopatric location using Dunn's tests to search for divergence in traits that could facilitate coexistence. Such phenomenon of divergence in sympatry, called ecological character displacement (ECD), occurs when species have similar ecologies or resource use. Divergent displacement of characters associated with resource use reduces competition between sympatric species and facilitates coexistence (Brown and Wilson, 1956). In *Pundamilia*, we compared *P. pundamilia* and *P.* sp. 'big blue red' found in sympatry on Juma island with allopatric populations of *P. pundamilia* and *P.* sp. "big blue" found on Makobe and Chamagati islands respectively. In *Neochromis*, we compared *N. omnicaeruleus* and *N. black nigricans* found in sympatry on Juma island with allopatric populations of *N. omnicaeruleus* and *N. black nigricans* found on Makobe and Chamagati islands respectively. . Note that *P.* sp. 'big blue' from Chamagati island was considered as an allopatric equivalent of *P.* sp. 'big blue red' from Juma island given their highly similar ecologies and diets (Seehausen, 1996).

Potential trait convergences resulting from different environmental conditions between sympatric and allopatric locations can mask a net increase of differentiation stemming from ECD (Goldberg and Lande, 2006). To account for it, we computed pairwise Euclidean distances for each trait between terminal species living in allopatry and compared it to the distances between species in sympatry using non-parametric Mann-Whitney tests.

RESULTS

PCA

Principal axes of variation of the two rings showed different compositions. The first four PC dimensions in *Pundamilia* were associated with clear morphological domains (Fig. 3.1B). First dimension was a proxy to head length with a positive loading of *head length* and *snout length*. Second dimension was a proxy to head width and depth with a positive loading of *body depth*, *inter-orbital width*, and *head width*. Third dimension represented eye size with a negative loading of *eye length* and *eye depth*, and the fourth dimension consisted of pre-orbital width with a positive loading of *lower jaw width* and *snout width*. PC dimensions in *Neochromis* were less characterised, with the first dimension being loaded almost equally by all traits while retaining nearly 40% of all variation between individuals (Fig. 3.2B). The second and third dimensions can be interpreted as proxy to head length and head depth/width respectively, although some traits that are not related to these morphological domains have heavy loadings on these dimensions (e.g. *inter-orbital width* in dim. 2 and *eye length* in dim. 3).

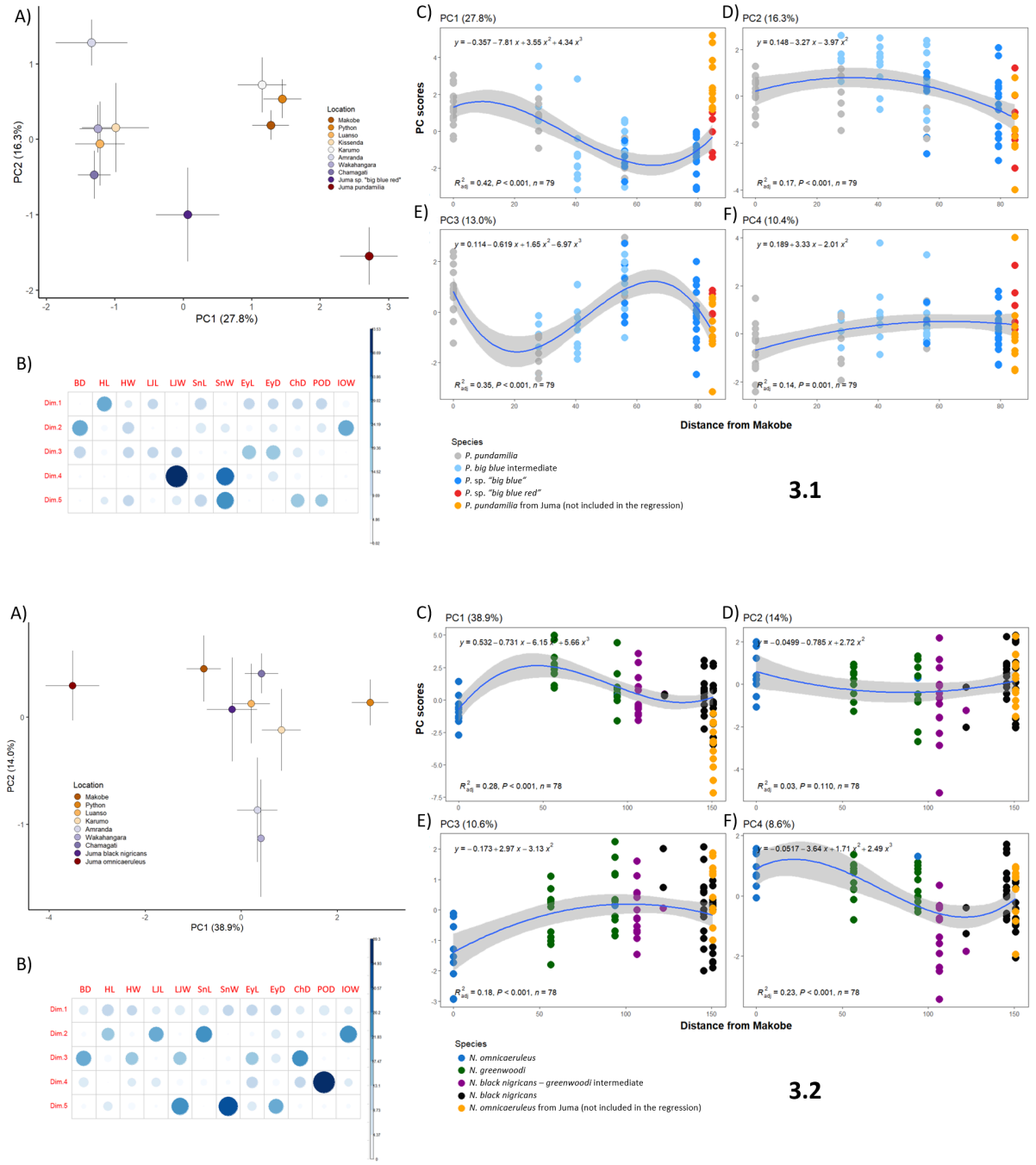

Figure 3: 3.1/ Potential ring speciation in *Pundamilia* (n = 114): **A)** PC plot of all locations of sampling on the first two dimensions, with distinction between terminal species in Juma island (lines represent standard deviation); **B)** Contributions of each trait to the five principal axis of variation following PCA (BD: Body depth; HL: Head length; HW: Head width; LJL: Lower jaw length; LJW: Lower jaw width; SnL: Snout length; SnW: Snout width; EyL: Eye length; EyD: Eye depth; ChD: Cheek depth; POD: Pre-orbital depth; IOW: Inter-orbital width); Polynomial regressions of **C)** PC1, **D)** PC2, **E)** PC3, and **F)** PC4 scores against distance from Makobe island in the scenario of a crossing point at Karumo bay's level (Python, Luanso, and Kissenda island are removed). **3.2/** Same results are plotted for the potential ring in *Neochromis* (n = 93); distances for the regressions **C) to F)** have been determined in the scenario of a crossing point at Python islands' level (Luanso island is removed).

Ring delimitation and morphological variation

Spatial extent of the ring was found to be different in *Pundamilia* and *Neochromis.* Mantel's tests between geographical and morphological distances based on PC1 scores indicated stronger correlation for scenarios of crossing in the northern region of the gulf in *Pundamilia* while a crossing point in the southern region of the gulf led to better correlation index in *Neochromis* (Tab. 1). The distribution of locations' centroid on the PC plot in *Pundamilia* (Fig. 3.1A) shows an important resemblance between individuals of Makobe and Karumo based on the first two dimensions, which corroborates the scenario of a crossing at gulf entry level.

Table 1: Results of the Mantel correlation tests between pairwise geographical distance between islands and PC1 scores for different scenarios of crossing points across the Mwanza gulf. Note that islands outside the ring in each scenario are excluded from the correlation.

Crossing point	*Pundamilia*	
	rho	*p*
Karumo	0.270	**<0.001** (n=3081)
Gabalema	0.265	**<0.001** (n=3081)
Python	0.240	**<0.001** (n=4278)
Luanso	0.193	**<0.001** (n=5253)
	Neochromis	
	rho	*p*
Karumo	0.029	0.181 (n=2211)
Gabalema	0.030	0.227 (n=2211)
Python	0.104	**0.007** (n=3003)
Luanso	0.117	**0.008** (n=3240)

Therefore, we chose the scenario of a crossing point at Karumo bay's level as the spatial delimitation of the ring in *Pundamilia*. For *Neochromis,* we chose to limit the ring to Python islands' level and exclude Luanso island because *N. greenwoodi* individuals at this location display strong ecomorphological differences due to specific environmental conditions such as high turbidity (Seehausen, 1996, 1997). Removing individuals from Luanso for further analysis limits the impact of this particular ecologically driven differentiation that could impede the characterisation of the ring.

Polynomial regressions of PC scores against locations' distance from Makobe for the retained scenarios all showed significant correlations in both rings (Fig 3.1C to F; Fig. 3.2C to F). In *Pundamilia*, fish display a shorter and broader head at intermediate distance, where *P.* sp. 'big blue'

occurs, compared to the beginning and the end of the ring (Fig. 3.1C and D), with a strong correlation coefficient (adjusted R^2 = 0.42). We observe a gradual increase of pre-orbital width characters along the ring and across the continuum of species, from *P. pundamilia* to *P.* sp. 'big blue red' (Fig. 3.1F). We also observe larger eyes in fish that live near the gulf entry, between 10 and 40 kilometres from Makobe (Fig. 3.1E). In *Neochromis,* fish morphology also correlates with distance, but differences are less marked and correlation coefficients are lower (adjusted R^2 between 0.03 and 0.28). We observe a pattern of differentiation peaking in intermediate distances for PC1 and PC4 scores (Fig. 3.2C and F). Variations in head length (PC2) and width/depth (PC3) also seem to peak in *N. greenwoodi* populations, the latter having slightly shorter and broader heads compared to *N. omnicaeruleus* and *N. black nigricans* (Fig. 3.2D and E).

Ecological character displacement in terminal species

Comparison of mean traits values in terminal species showed only a few cases where populations were significantly different in sympatry but not in allopatry. In *Pundamilia,* difference between populations was conserved in sympatry for *head length* and was increased in sympatry for *eye length* and *cheek depth* (Fig 4.A). In *Neochromis*, difference increased in sympatry for *head length*, *eye length*, and *eye depth* (Fig. 4.B). On the contrary, significant differences in allopatry were lost in sympatry for *head width*, *lower jaw length*, *snout length*, and *inter-orbital width* in *Pundamilia* and for *body depth* in *Neochromis*.

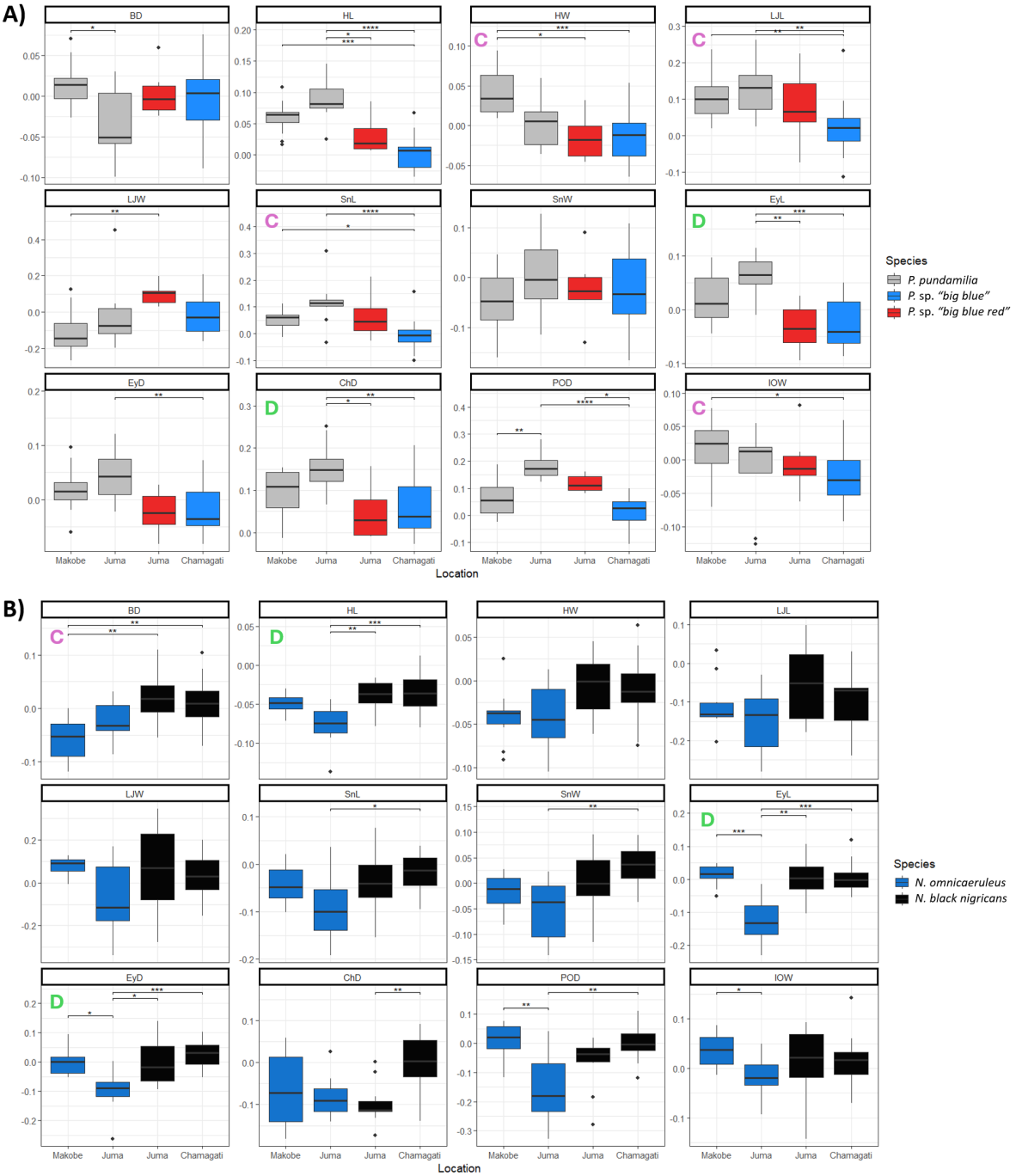

Figure 4: A) Comparison of terminal species of the *Pundamilia* ring (n = 50) living in allopatry (Makobe and Chamagati islands) and sympatry (Juma island). Significance is indicated for the result of a Dunn's test with Bonferroni correction for each pair. Letters in colours indicate the direction of a potential character displacement in relevant traits based on these comparisons: C = convergent displacement; D = divergent displacement. **B)** Comparison of terminal species for the *Neochromis* ring (n = 50). BD: Body depth; HL: Head length; HW: Head width; LJL: Lower jaw length; LJW: Lower jaw width; SnL: Snout length; SnW: Snout width; EyL: Eye length; EyD: Eye depth; ChD: Cheek depth; POD: Pre-orbital depth; IOW: Inter-orbital width.

However, when comparing allopatry and sympatry using pairwise morphological distances between species, an important proportion of traits revealed increased differences in sympatry (Tab. 2). In *Neochromis*, differences between species increased in sympatry for all traits except *body depth* and *cheek depth* (reduced difference), and *head width* (no significant variation of difference). This analysis suggests that *N. omnicaeruleus* has a smaller head overall relative to *N. black nigricans* when in sympatry. In *Pundamilia*, five traits from various morphological domains showed significant increase of differences in sympatry, and one trait showed a reduced difference compared to allopatry.

Table 2: Results of Mann-Whitney tests comparing sympatry and allopatry for pairwise distances between species for each trait, in *Pundamilia* (n = 50) and *Neochromis* (n = 50). Direction of the difference is indicated as D (divergent) or C (convergent) for increased or reduced difference in sympatry respectively. BD: Body depth; HL: Head length; HW: Head width; LJL: Lower jaw length; LJW: Lower jaw width; SnL: Snout length; SnW: Snout width; EyL: Eye length; EyD: Eye depth; ChD: Cheek depth; POD: Pre-orbital depth; IOW: Inter-orbital width.

	Pundamilia		*Neochromis*	
	p-value	Direction	*p*-value	Direction
BD	**0.016**	**D**	**<0.001**	**C**
HL	0.53	ns	**<0.001**	**D**
HW	**<0.001**	**C**	0.70	ns
LJL	0.56	ns	**<0.001**	**D**
LJW	**0.007**	**D**	**<0.001**	**D**
SnL	**0.03**	**D**	**<0.001**	**D**
SnW	0.74	ns	**<0.001**	**D**
EyL	**<0.001**	**D**	**<0.001**	**D**
EyD	0.15	ns	**<0.001**	**D**
ChD	**<0.001**	**D**	**<0.001**	**C**
POD	0.32	ns	**<0.001**	**D**
IOW	0.35	ns	**0.011**	**D**

Overall, the pairwise distances method corroborated the results obtained with mean values comparisons or revealed undetected increase/ decrease in difference between species, except for *snout length* in *Pundamilia* where the two methods lead to different interpretations.

DISCUSSION

The search for idealised ring species has resulted in many cases of broken ring species (Irwin *et al.*, 2001 b). The reason for this is that most cases of ring speciation have focused on large spatial scales spanning entire countries or even the globe, and deep evolutionary timescales of millions of years, even though simulations suggest that ring species are unstable beyond a few thousand years (Ashlock *et al.*, 2010; Martins *et al.*, 2013). Here we report two potential cases of ring species in the *Pundamilia* and *Neochromis* cichlid fishes from Lake Victoria that diverged within the last 10,000 - 15,000 years over an area of less than 1000 km². We show evidence of divergence in mating and ecological traits along the ring, resulting in eco-morphologically differentiated terminal species in sympatry. We find that the *Pundamilia* and *Neochromis* parallel rings adhere to three out of the four criteria of Mayr (1942) – the fourth criterion requires genetic data to be tested.

Ecomorphological and male colouration divergence around the rings

***Pundamilia* ring:** our results from the morphological analysis of craniofacial shape obtained for the potential ring in *Pundamilia* are consistent with a shift in trophic ecology (Seehausen 1996) from insectivory to algae grazing. Insectivorous populations of *P. pundamilia* from Makobe island, have a longer and narrower head compared to *P*. 'big blue red' and *P*. 'big blue' algae grazers living on the west side of the gulf in the Sengerema region. Such oblong head morphology facilitates foraging in deep crevices where *P. pundamilia* preys on benthic larvae and insects, while short and broad head and jaws in *P.* 'big blue' and *P.* 'big blue red' generate more strength to efficiently scrape off epilithic algae (RÜber and Adams, 2001; DeLorenzo *et al.*, 2022). The increase in head width and depth is maximal in the *P.* 'big blue' populations, while the terminal *P.* 'big blue red' individuals converge towards a shorter head despite having the same diet as *P.* 'big blue'. This morphological difference could be partly explained by the habitat conditions found in Juma island, where the lakeshore is made of steeply sloping boulders compared to the gently sloping shores of Chamagati island (Seehausen, 1996). Such environmental constraints would result in a longer head shape to manoeuvre into narrower crevices, a trade-off with larger jaws that facilitates algae grazing. Our results also indicate that fish from the first third of the ring (distances from 0 to 50 kilometres, spanning the Mwanza gulf) have larger eyes. While this variation in eye size could reflect a need for larger eyes in insectivores, it most likely correlates with the turbidity gradient of the Mwanza gulf (Seehausen, 1997) as cichlid fish living in turbid and light deficient environment develop larger eyes to improve light acquisition and vision (Tiarks *et al.*, 2024).

***Neochromis* ring:** the gradual change in diet in the *Neochromis* ring from *N. omnicaeruleus* to *N. greenwoodi* to *N. black nigricans* (algae browser to generalist, and back to algae browser) is supported by the morphological variation found in the populations around the *Neochromis* ring. Algae browsers have similar head morphology based on PC scores, while the generalist *N. greenwoodi* and kindred intermediate morphs slightly differ with shorter and broader heads. Despite this spatial variation being consistent with a pattern of parapatric speciation with trophic shift, the ecological gradient mentioned above -larger and steeper boulders at starting and ending point of the ring- could still influence our results. Yet, differences in morphology observed along the cline remain weak, which qualifies the importance of the ecological gradient.

While changes in craniofacial shape are gradual, both *Pundamilia* and *Neochromis* rings show abrupt shifts in male nuptial colouration. This evolutionary pattern may shed light on the genetic architecture of these traits. The genetic background for gradual changes in craniofacial morphology in cichlids has received mixed interpretation, whether governed by the accumulation of alleles of small effect (Henning *et al.,* 2017) or by a few major alleles with strong effect (Albertson *et al.*, 2003). The

thin-scale changes in craniofacial shape we observe are more consistent with the small-effects accumulation hypothesis. Sudden changes in male nuptial colouration are more likely to be the result of one or few large effect alleles, governing pigmentation in males (O'quin *et al.*, 2012) or mate choice in females (Ding *et al.*, 2014). Mapping the genetic basis of trait evolution along the *Pundamilia* and *Neochromis* rings could identify the evolutionary forces that maintain ecological and mating traits during parapatric speciation such as interchromosomal linkage disequilibrium (Hench *et al.*, 2019; Singh *et al.*, in prep). In *Neochromis* the colour shift from blue bodied males to black bodied males occurs in the early phases of the ring, while in *Pundamilia* the shift from blue bodied males to reddish-blue males occurs at the end of the ring, where the terminal species co-exist in sympatry. Taken together, trophic and mating trait evolution along the rings is suggestive of convergent ecological by-product speciation in *Neochromis* and speciation through the emergence of reproductive isolation via assortative mating in *Pundamilia.*

Ecological character displacement between terminal species: diet and colour

For both the *Neochromis* and *Pundamilia* rings, the craniofacial shape described by PC1 scores was strongly differentiated between terminal species (Fig. 3.1C and 3.2C). In addition, populations of the parental species (*P. pundamilia* and *N. omnicaeruleus*) differ between Makobe and Juma islands in each ring . The comparison of 12 linear traits that make-up the craniofacial shape shed light on possible ecological character displacement (ECD) in the terminal end of rings. We found evidence of convergent and divergent ECD, depending on the trait.

In the *Pundamilia* ring, the craniofacial shape of sympatric populations is narrower and longer compared to allopatric populations (Fig. 4). This convergence can be explained by differences in habitat conditions between allopatric and sympatric locations in Juma, Chamagati, and Makobe islands discussed in detail above. Such phenomenon of convergence dependent on niche conditions is more common than generally thought, as coexisting species tend to differentiate only on specific ranges of the niche characteristics, while converging on major environmental and ecological factors (McPeek, 2019). In this context, competing species can show a morphological convergence as constraints for optimal foraging prevail over processes like competitive exclusion (Vadas, 1990). By performing a pairwise comparison for species difference between allopatric and sympatric populations the habitat-driven convergence was reduced and more traits appeared to have increased difference in sympatry (Tab. 2). The insectivorous *P. pundamilia* from Juma island have a shallower body, narrower jaws, bigger eyes, and deeper cheeks relative to *P.* 'big blue red' compared to allopatric populations (*P. pundamilia* in Makobe and *P.* 'big blue' in Chamagati). All these body and craniofacial traits reflect a specialisation towards insectivory and efficient foraging in crevices for evasive prey (Hulsey and León, 2005).

In the *Neochromis* ring, the ECD analysis revealed a more pronounced displacement, with terminal species having increased divergence in sympatry in most traits based on pairwise distance comparisons. Both *Neochromis* terminal species are algae browsers and competing for the same resources, however *N. omnicaeruleus* have a smaller head, which could facilitate access to foraging patches that the longer headed *N. black nigricans* cannot reach. This steady pattern of divergence is most likely an adaptation reducing competition between these species as they share the same resources (Brown and Wilson, 1956).

While these diet-related character displacements probably facilitate coexistence of terminal species at Juma island, the importance of the displacement in male nuptial coloration is not to be neglected. The colour shift between *P.* 'big blue' and *P.* 'big blue red', for example, is a critical feature for sympatric coexistence with *P. pundamilia* as both *P. pundamilia* and *P.* 'big blue' have similar blue coloration.

Assortative mating through male nuptial coloration preference acts as a prezygotic barrier to reproduction, which prevents sympatric species from hybridising especially in the context of parapatric or sympatric speciation with gene flow (Seehausen *et al.*, 1998; Magalhaes *et al.*, 2010; Servedio, 2016; Kopp *et al.*, 2018; van Rijssel *et al.*, 2018; Svensson *et al.*, 2024). Modelling approaches found that cases of ECD are even more likely to occur on traits associated with mate choice than traits related to resource acquisition (Goldberg and Lande, 2006). The gradual shift along the ring from blue to red in *Pundamilia* and blue to black in *Neochromis* are therefore essential components in the terminal species coexistence in Juma island and prevent the rings from collapsing because of hybridization.

Current and historical delimitation of ring species

The isolation-by-distance (IBD) analysis for the *Pundamilia* and *Neochromis* species complexes excluded locations in the upstream part of the Mwanza gulf due to local environmental factors. Specifically, turbidity-driven adaptation in head morphology and hybridization between *P. pundamilia* and *P. nyererei* in the southern part of the gulf (Seehausen, 1996; Magalhaes *et al.*, 2009; Meier *et al.*, 2017) likely disrupted the expected ring-like distribution pattern.

While the *Neochromis* and *Pundamilia* rings exist geographically in parallel, we found that they crossed the Mwanza gulf at different locations: *Pundamilia* may have crossed anywhere in the Mwanza gulf and *Neochromis* crossed in the southern part of the gulf. This could explain the evolutionary timing of the emergence of the two rings. *Neochromis* and *Pundamilia* cichlids evolved between 15ky and 12,4ky ago (Johnson *et al.*, 1996; Galis and Metz, 1998; Turner *et al.*, 2001; Sturmbauer *et al.*, 2011) when Lake Victoria water level was nearly 10 metres below the modern level that was reached 11ky to 10ky ago (Wienhues *et al.*, 2024). This means that most of the Mwanza gulf was dry when Pundamilia emerged, limiting the species distribution to the gulf entry, i.e., Karumo bay. With the increase in lake levels, the *Pundamilia* ring may have shifted from an ancestral ring limited to Northern Mwanza gulf to a modern ring extending to the south of the gulf. This would explain the strong pattern of IBD observed for all points of crossing (Tab. 1). In contrast, our data suggest that the *Neochromis* ring expanded after the *Pundamilia* ring, as the crossing point extends deep into the Mwanza gulf.

Isolation by distance, environment, or resistance?

In most cases of ring speciation, progressive divergence of intermediate forms along the barrier can be attributed to ecological, behavioural, or geographical processes (Irwin *et al.*, 2001 b). One of the major drivers of speciation in Lake Victoria cichlids is assortative mating based on male nuptial coloration, which rapidly raises reproductive isolation between colour morphs even in closely related species (Seehausen, 1997; Magalhaes *et al.*, 2010; Meier *et al.*, 2017). While high colour polymorphism seems to be selected for in cichlids, many correlations between coloration and environmental parameters such as turbidity or habitat type (rocks, open water, vegetation) suggest that male visual attributes, thus speciation, could be constrained by the environment (Seehausen *et al.*, 1999; Seehausen *et al.*, 2008) even in the closely related species of the *Pundamilia* genus (Carleton *et al.*, 2005). Another driver of cichlid diversification is the fine-scale partitioning of niches, which relies on a high diversity of trophic specialisations and subsequent morphological adaptations (Ford *et al.*, 2016). Yet, cichlids' trophic apparatus has been found to be evolving rapidly in response to variable foraging environment (Parsons *et al.*, 2016; Conith *et al.*, 2020) and habitat or water conditions (Witte *et al.*, 2008; Gilbert *et al.*, 2020). Therefore, the environmental gradient along the

rings of this study might play an important role in shaping the speciation continuum we observe, both for coloration and diet-related morphology.

The influence of habitat heterogeneity on speciation implies that the IBD pattern we detect could be insufficient to describe the correlation between distance and the morphological variations in the *Pundamilia* and *Neochromis* rings. Indeed, while the connection between the ring locations through stepping-stone migration is relatively low and constrained by distance (5 to 15 individuals per generation, Terai *et al.*, 2006), the habitat heterogeneity across our distributions may also decide where major morphological changes occur. The distribution of phenotypic variation we observe across our study area could then be better represented by a pattern of isolation-by-resistance (IBR) (McRae, 2006). This modelling approach of populations' divergence, merging the concepts of IBD and isolation-by-environment (IBE) (Wang and Bradburd, 2014), computes the environmental and spatial resistance to gene flow between locations. Distance, barriers, habitat suitability, and migration rates are considered to compute the resistance to connectivity and the isolation of each location. Such an approach is relevant for the study of ring species, as distance remains an important driver of species divergence while still accounting for a certain level of local adaptation.

The first case of ring speciation in freshwater fish?

The parallel cases of ring speciation we describe in our study are the first reported in freshwater fish and only the second case in fishes (trumpetfish, spanning tropical latitudes of the globe's oceans; Bowen *et al.*, 2001). This is surprising considering that fish represent more than half of all vertebrate species (Van Der Laan *et al.*, 2014). A reason for this scarcity of cases could be the lack of 'suitable' barriers. Ring speciation requires a continuous barrier of a reasonable scale around which mobility and gene flow are limited. However, in water bodies without major current variation fish face a homogenous resistance to mobility, meaning that divergence can only arise when habitat specificity leads to IBR along a barrier with heterogeneous habitat conditions. In *Pundamilia* and *Neochromis*, ring speciation is possible because they are highly specialised to live in shallow waters (<8m) along rocky substrates and rarely enter deeper waters (Seehausen, 1996). In the trumpetfish, a wide distribution of tropical populations separated by landmasses induces a reduced gene flow, maintained by the versatility of habitat preference displayed by larvae and some populations that migrate between oceans (Bowen *et al.*, 2001; Rosenblatt and Waples, 1986). Such appropriate conditions for ring speciation in aquatic environments are rare and not enough studied to reveal potential cases of ring species although a comprehensive description of suitable barriers has been made (Monahan *et al.*, 2012).

Pundamilia and *Neochromis* complexes appear to be idealised ring species, fulfilling three out of four of Mayr's criteria. Their recent emergence (<15,000 years ago) and the relative proximity between intermediate populations could explain the maintenance of gene flow and the absence of a break in the ring as observed in cases of former ring species and hypothesised over long evolutionary times (Martins *et al.*, 2013; Bouzid *et al.*, 2022; Amir Afzali *et al.*, 2024). In the future, genetic evidence demonstrating complete reproductive isolation between terminal species is necessary to establish the *Pundamilia* and *Neochromis* complexes as ring species. A comprehensive analysis of the genetic differentiation and gene flow between locations would better describe the connectivity across the distribution and help determine the status of these potential ring species. A continuous gene flow along the rings, as expected from the morphological results, would establish the *Pundamilia* and *Neochromis* complexes as idealised ring species.

Concluding remarks and future directions

From an evolutionary perspective, our results provide new insights into the rapid diversification of cichlid fishes. The process of parapatric speciation at small geographic scales presents itself as a driving force of diversification in this adaptive radiation. The parallel ring speciation we describe indicates that a single barrier with multiple habitat gradients could result in the evolution of several species complexes and thus may be a more common process driving speciation in East African cichlid adaptive radiations.

In conclusion, we provide compelling evidence for speciation in rings in rocky shore cichlids in Lake Victoria. We highlight the importance of ecological character displacement in trophic and mating traits as new species form and coexist in sympatry. While our study elucidates the ecological and morphological divergence in *Pundamilia* and *Neochromis* cichlids, in the future comprehensive genetic analysis is necessary to confirm their status as ring species.

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AUTHOR CONTRIBUTION

Grégoire Gautier: Morphological and statistical analysis, and manuscript writing.
Anna Mahulu: Sampling, linear measurements methods, and contribution to writing.
Ole Seehausen: Conception, sampling, and contribution to writing.
Pooja Singh: Conception, supervision, sampling, analysis, and contribution to writing.

DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article and in its online supplementary material.

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SUPPLEMENTARY

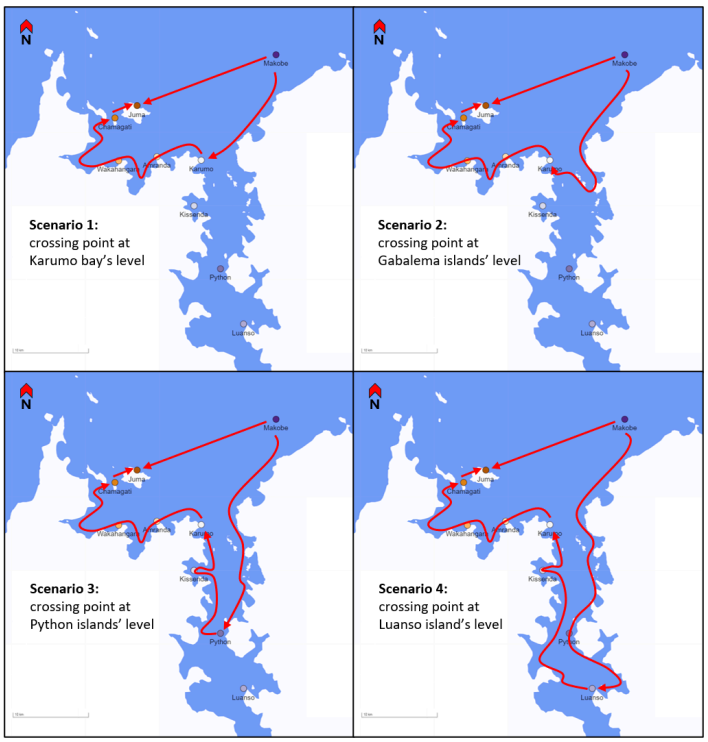

Figure S1: The four different scenarios of circular distribution, depending on the gulf crossing point.