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Parallel evolution of facial stripe patterns in the *Neolamprologus brichardi/pulcher* species complex endemic to Lake Tanganyika

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Abstract

Colour pattern diversity can be due to random processes or to natural or sexual selection. Consequently, similarities in colour patterns are not always correlated with common ancestry, but may result from convergent evolution under shared selection pressures or drift. *Neolamprologus brichardi* and *Neolamprologus pulcher* have been described as two distinct species based on differences in the arrangement of two dark bars on the operculum. Our study uses DNA sequences of the mitochondrial control region to show that relatedness of haplotypes disagrees with species assignment based on head colour pattern. This suggests repeated parallel evolution of particular stripe patterns. The complete lack of shared haplotypes between populations of the same or different phenotypes reflects strong philopatric behaviour, possibly induced by the cooperative breeding mode in which offspring remain in their natal territory and serve as helpers until they disperse to nearby territories or take over a breeding position. Concordant phylogeographic patterns between *N. brichardi/N. pulcher* populations and other rock-dwelling cichlids suggest that the same colonization routes have been taken by sympatric species and that these routes were affected by lake level fluctuations in the past.
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1. Introduction

Colour pattern diversity within or between populations is often not merely due to random genetic variation but rather generated and maintained by selection. Consequently, colour pattern similarities are not always correlated with common ancestry, but may result from convergent evolution under shared selection pressures (Meyer, 2006). For example, adaptive camouflage to minimize predation risk evolved repeatedly in beach mice (Hoekstra et al., 2006), and the convergent evolution of cryptic colour patterns in anurans inhabiting similar habitats has been attributed to selection by visual predators (Hoffman and Blouin, 2000; Thorpe and Stenson, 2003). Convergent aposematic colouration, signalling inedibility to potential predators, may represent Müllerian mimicry (Brower, 2006; Chiari et al., 2004; Jiggins et al., 2006; Joron et al., 2006; Sanders et al., 2006). Parallel evolution of colour patterns can also be driven by sexual selection in an ecological context (Boughman et al., 2005), such as when recurrent habitat differences or receiver biases favour alternative nuptial colours (Allender et al., 2003). The genetic mechanisms underlying colour pattern convergence are not well understood. In some cases, genetic modifications of homologous genes result in the same phenotype

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of distantly related species, whereas in other instances different genetic mechanisms may lead to the same pattern elements (Brower, 2006; Hoekstra et al., 2006; Joron et al., 2006; Prud'homme et al., 2006).

Different facial stripe patterns and associated blue and/or yellow colour markings on the head distinguish the two endemic cichlid species *Neolamprologus brichardi* and *Neolamprologus pulcher*, both rock-dwelling members of the tribe Lamprologini in Lake Tanganyika. *Neolamprologus brichardi* and *N. pulcher* are cooperative breeders where 1–14 offspring of both sexes continue to stay as helpers in the territory of the dominant breeding pair to take part in territory defence and brood care (Balshine et al., 2001; Taborsky, 1984, 1994; Taborsky and Limberger, 1981). The two taxa were originally described as subspecies of *Neolamprologus savoryi* (Trewavas and Poll, 1952) but were later elevated to species status (Poll, 1974). The main difference between *N. brichardi* and *N. pulcher* lies in their operculum markings: two black bars arranged in the shape of a horizontal 'T' in *N. brichardi* and arranged as two vertical curved bars in *N. pulcher* (Fig. 1). The distribution of the two species along the lake shore is discontinuous, with *N. brichardi* in the northern half of the lake as well as in several stretches of coastline in the south, and *N. pulcher* exclusively in the south (Fig. 1; Konings, 1998). They are never found sympatrically, but may occur in close vicinity to each other, e.g. on either side of a habitat barrier such as a river estuary. At one location in the south (Chaitika), the phenotype of the resident population has been described as intermediate between *N. brichardi* and *N. pulcher* (Konings, 1998).

One purpose of the present study was to determine whether the operculum markings used for species distinction are useful systematic characters, or whether parallel evolution repeatedly created the two alternative phenotypes. If the current classification of *N. brichardi* and *N. pulcher* as two distinct species is correct, we expect the populations to segregate according to phenotype in a phylogenetic reconstruction, provided that the divergence time is sufficiently long for lineage sorting to be completed. If the alternative phenotypes do not represent phylogenetic clusters, multiple events of convergent evolution of the supposedly diagnostic traits render the taxonomic status of the two taxa uncertain. The second aim of this study was to unravel the phylogeographic pattern of populations of both taxa. Recent studies showed that the extant phylogeography and population structure of many of the over 200 endemic cichlid species in Lake Tanganyika was shaped by two main factors, lake level fluctuations and habitat barriers, and that major phylogeographic patterns agree across ecologically diverse species (Baric et al., 2003; Duftner et al., 2006; Koblmüller et al., 2007; Rüber et al., 2001; Sefc et al., 2007; Sturmbauer et al., 2001, 2005; Taylor et al., 2001). Notably, the majority of these studies has focussed exclusively on mouthbrooders that are characterized by a markedly different reproductive ecology compared to the substrate-brooding Lamprologini. In contrast to the large species flocks of Lakes Malawi and Victoria, which exclu-

sively comprise mouthbrooding cichlids, substrate-spawners make up almost half the number of cichlid species in Lake Tanganyika. Here, we compare the genetic structure within and between populations of the two *Neolamprologus* taxa with that of other rock-dwelling cichlids in order to unravel phylogeographic similarities shared by species that display the same or a different breeding strategy and live in the same habitat.

2. Materials and methods

2.1. Sampling and DNA extraction

A total of 81 individuals of *N. brichardi* and *N. pulcher* were included in our study (Table 1), comprising three previously published sequences that were downloaded from GenBank (AF400721, AF438785, AF438787). Samples were collected during field expeditions to Lake Tanganyika in 2000, 2001 and 2002. *Variabilichromis moorii*, which occupies an ancestral branch within the lamprologine phylogeny (Colombe and Allgayer, 1985; Poll, 1986; Sturmbauer et al., 1994), was used as outgroup taxon (AF438776). Tissue samples were preserved in 96% ethanol and stored at room temperature until further processing. Genomic DNA was extracted by applying proteinase *K* digestion followed by ethanol precipitation.

2.2. Amplification and sequencing of the mitochondrial DNA control region

Partial sequences of the mitochondrial DNA control region were amplified with the primers THR-2 (Kocher et al., 1989) and TDK-D (Lee et al., 1995). The total reaction volume of 17 µl contained 0.085 µl of Taq DNA polymerase (BioTherm™), 1.7 µl of each primer (10 µM), 1.7 µl 10× dNTP solution, 1.7 µl 10× MgCL$_2$ buffer, 1.62 µl enzyme diluent (Idaho Technology), 6.8 µl water, and 1.7 µl genomic DNA. PCR-amplification was performed on an Air-Thermo Cycler (Idaho Technology) under the following conditions: an initial denaturation at 94 °C for 15 s was followed by 40 cycles with denaturation at 94 °C for 0 s, primer annealing at 52 °C for 0 s, and extension at 72 °C for 15 s. The PCR-products were purified in an enzymatic reaction with ExoSAP-IT (Exonuclease I and Shrimp Alkaline Phosphatase in buffer). Chain termination sequencing followed the protocol of Koblmüller et al. (2004) by using the forward primer THR-2 (Kocher et al., 1989). DNA fragments were purified by applying a standard ethanol precipitation and subsequently analyzed with an ABI 373 Automatic DNA Sequencer (Applied Biosystems). Sequences were deposited in GenBank under the Accession Nos. EF137273–EF137353.

2.3. Phylogenetic and phylogeographic analysis

Sequencing yielded 55 haplotypes that were individually screened in Sequence Navigator (Applied Biosystems), and

Fig. 1. Map of Lake Tanganyika with sampling sites and facial stripe patterns of *Neolamprologus brichardi* and *N. pulcher*. Squares along the lake shore with lower case letters and corresponding pictures of phenotypes refer to populations that have been photographed by Ad Konings (1998). Differences in hue are mainly due to variations in light conditions and light exposure. For this study, only the orientation of facial stripe patterns is crucial. Sampling sites are indicated by locality name and either roman number of clade to which the mtDNA haplotypes of the population were assigned, or an asterisk for haplotypes that could not be assigned to any cluster in the TCS-network analysis (Fig. 2). When there is an overlap between our and Konings' sampling sites, pictures of phenotypes are framed. Full squares indicate the occurrence of *N. brichardi*-, open squares of *N. pulcher*-, and shaded squares of intermediate phenotypes. (A) *N. brichardi* at Loasi River North, (B) *N. pulcher* at Loasi River South (photographs provided by H. Büscher) as examples of two very distinct phenotypes in close geographic proximity that have been included in our study.

subsequently aligned in SE-AL (Rambaut, 1996). The final alignment comprised 82 sequences with a total length of

443 base pairs (bp). Phylogenetic trees based on neighbour joining (NJ) as well as Bayesian Inference (BI) were calcu-

Table 1
Sampling localities, sample sizes, geographic coordinates and species assignment according to phenotype

Locality	Sample size	Latitude	Longitude	Species assignment
Burundi	1	?	?	*N. brichardi*
Kabogo	1	5° 67′	29° 77′	*N. brichardi*
Kalo	1	7° 45′	30° 30′	*N. brichardi*
Kapampa	2	7° 30′	30° 12′	*N. brichardi*
Kigoma	3	4° 88′	29° 67′	*N. brichardi*
Loasi River North	9	8° 17′	31° 00′	*N. brichardi*
Malasa Island	5	8° 12′	30° 56′	*N. brichardi*
Ujiji	1	4° 54′	29° 40′	*N. brichardi*
Wapembe	1	8° 00′	30° 53′	*N. brichardi*
Chaitika	9	8° 34′	30° 47′	*N. brichardi/N. pulcher*
Cape Kaku South	4	8° 41′	30° 54′	*N. pulcher*
Kantalamba	1	8° 33′	31° 10′	*N. pulcher* "Daffodil"
Kipili	1	7° 43′	30° 60′	*N. pulcher*
Loasi River South	13	8° 19′	31° 02′	*N. pulcher*
Mbita Island	1	8° 46′	31° 06′	*N. pulcher*
Misepa/ Kapembwa	24	8° 36′	30° 49′	*N. pulcher*
Mpulungu	2	8° 46′	31° 06′	*N. pulcher*
Mtondwe Island	1	8° 42′	31° 07′	*N. pulcher*
Tongwa	1	8° 40′	30° 53′	*N. pulcher*

lated in PAUP∗ (Swofford, 2000) and MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001), respectively, with *Variabilichromis moorii* used as outgroup species. A hierarchical likelihood ratio test in Modeltest version 3.7 (Posada and Crandall, 1998) suggested the Hasegawa–Kishino–Yano model (Hasegawa et al., 1985) HKY + I + G as the best fitting substitution model for the mitochondrial control region data set (gamma shape distribution parameter, 0.4947; proportion of invariable sites, 0.4320; base frequencies, A, 0.3504; C, 0.2282; G, 0.1403; T, 0.2811). For BI, 10 Markov chains (chain temperature 0.2) were run for two million generations, where a tree was sampled every 100th generation. The burn-in value was set to 200 trees, and the remaining 19,800 trees were used to calculate posterior probabilities for the resulting tree topology. Uncertainties of phylogeny estimates in NJ were assessed by bootstrap analysis (1000 pseudo-replications). Alternative tree topologies were tested against the Bayesian consensus tree by means of a Kishino–Hasegawa test (Hasegawa and Kishino, 1989; Kishino and Hasegawa, 1989) in PAUP∗. For this test, topologies were constrained for monophyly of *N. brichardi* and *N. pulcher* phenotypes regardless of geographic origin, and various constraints were enforced for the phenotypically intermediate population from Chaitika (Konings, 1998). The likelihood scores of the resulting evolutionary hypotheses were statistically compared to the unconstrained Bayesian tree. All taxa were collapsed to the level of phenotype: (1) ((*N. brichardi*)((*N. pulcher*)(Chaiti-

ka))); (2) ((*N. pulcher*)((*N. brichardi*)(Chaitika))); (3) ((Chaitika)((*N. brichardi*)(*N. pulcher*))); (4) ((*N. pulcher* and Chaitika)(*N. brichardi*)); (5) (*N. brichardi* and Chaitika)(*N. pulcher*)). The facial stripe patterns of the two phenotypes *N. brichardi* and *N. pulcher* as well as the intermediate phenotype from Chaitika were mapped onto the Bayesian phylogeny in MacClade (Maddison and Maddison, 1992). The phylogeographic relationships among haplotypes were visualized by constructing a network based on statistical parsimony with the TCS software package (Clement et al., 2000), which is based on an approach developed by Templeton et al. (1992).

3. Results

3.1. Multiple origins of the "brichardi" phenotype

Phylogenetic analysis identified four main lineages, which correspond to the major clades identified from statistical parsimony (clades I–IV; see below) (Fig. 2). Statistical support for the main clusters ranged from 64 to 98 in NJ-bootstrapping, and amounted to 100 for Bayesian posterior probabilities. Within clades, the arrangement of taxa differed slightly depending on the algorithm employed and was not well supported. However, clearly, the phylogenetic reconstructions never reflected the current taxonomic separation of *N. brichardi* and *N. pulcher*, nor did they illustrate a genetic pattern tightly linked to current geographic distribution along the lakeshore. Haplotypes were not shared between populations. Three out of the four main clades comprised both phenotypes. Mapping the two different types of facial stripe patterns onto the Bayesian tree suggests that, within clade I, the "brichardi" phenotype evolved from "pulcher"-type ancestors three times. Clade II exclusively comprises "pulcher"-type populations from three locations within about 13 km of shoreline. Clade III groups one "pulcher"-type with one "brichardi"-type individual (haplotype divergence 1.6%), without uncovering the putative ancestral facial pattern. In clade IV, the "brichardi" phenotype apparently evolved from "pulcher"-type ancestors three times, while the ancestral state of the remaining "brichardi"-type individuals could not be reconstructed. This may be due to geographically less dense taxon sampling compared to those instances where ancestral states were inferred.
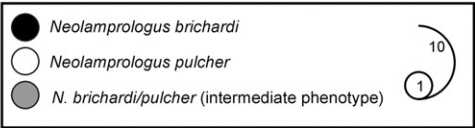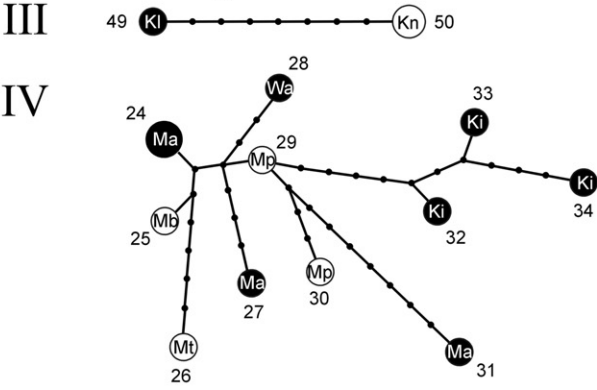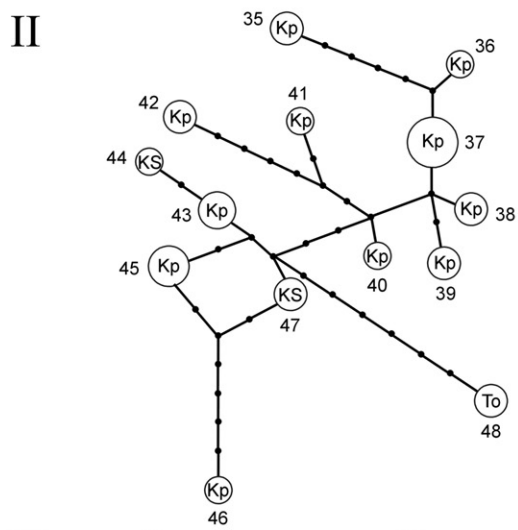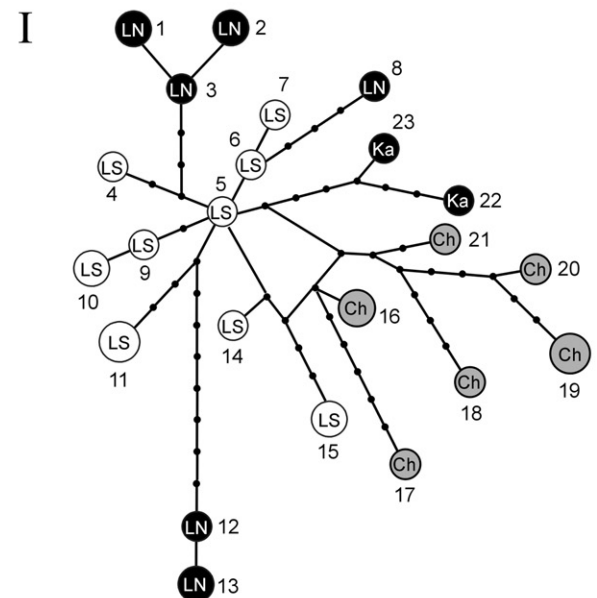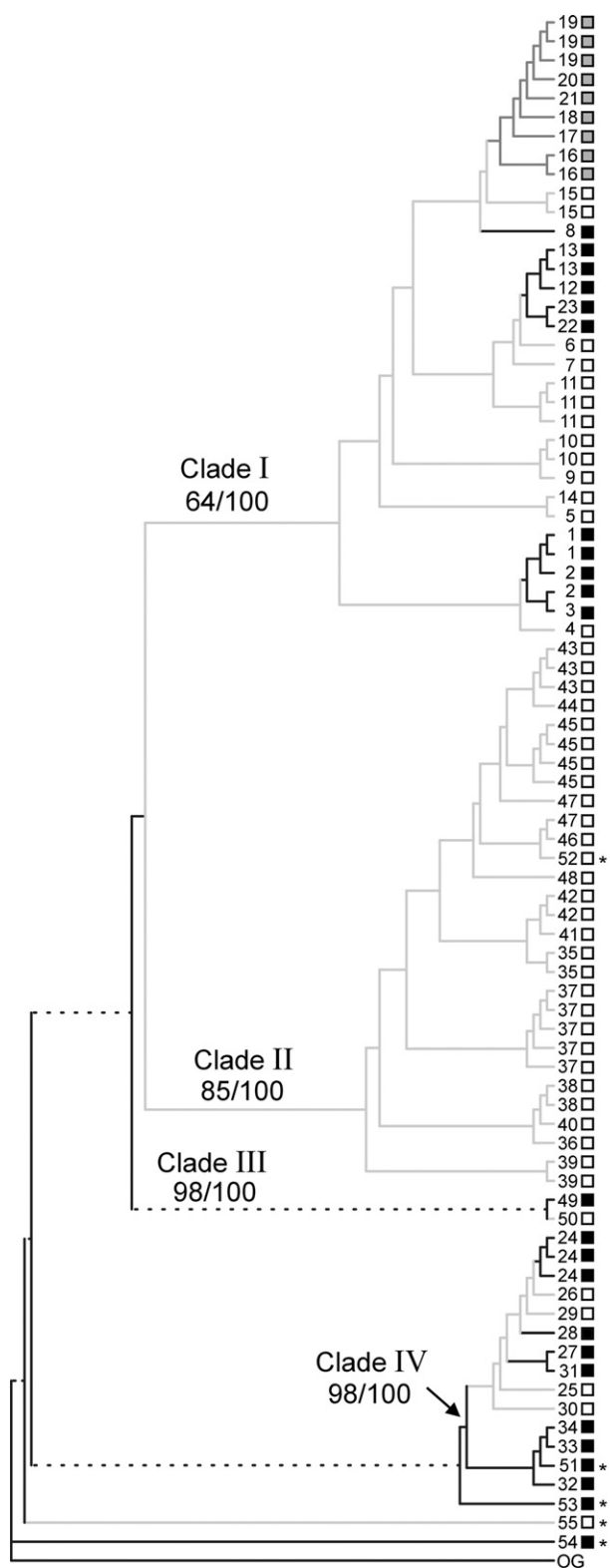
Alternative tree topologies with reciprocal monophyly of *N. brichardi* and *N. pulcher* and different treatments of the intermediate Chaitika phenotype were rejected by the Kishino–Hasegawa test at the significance level $p < 0.001$.

3.2. Phylogeographic relationships between mtDNA haplotypes

The majority of mtDNA control region sequences (50 out of 55 haplotypes) of *N. brichardi/N. pulcher* populations were resolved as four independent networks in TCS, while five haplotypes could not be connected to

any cluster (Fig. 2). Clade I comprised *N. brichardi* and *N. pulcher* samples from opposite locations at the south-

ern basin, Kapampa and Loasi River North (*N. brichardi*), Loasi River South (*N. pulcher*), as well as the

Fig. legend:
- Neolamprologus brichardi
- Neolamprologus pulcher
- N. brichardi/pulcher (intermediate phenotype)

intermediate population from Chaitika. The second network, clade II, exclusively comprised haplotypes of *N. pulcher* from three nearby locations, Misepa/Kapembwa, Tongwa and Cape Kaku South. In the third network, two haplotypes from two remote locations were connected to each other by eight mutation steps: *N. brichardi* from Kalo (haplotype 29) and *N. pulcher* from Kantalamba (haplotype 50). The phenotype of the latter is also known as *N. pulcher* ''Daffodil'', which is characterised by *N. pulcher*-like facial stripe patterns and yellow unpaired fins, and occurs only at a very small section of the southern Tanzanian coast (Konings, 1998). Clade IV grouped *N. pulcher* and *N. brichardi* from distinct locations across the lake, and consisted of haplotypes from Malasa Island, Wapembe, and Kigoma (*N. brichardi*) as well as from Mbita Island, Mtondwe Island, and Mpulungu (*N. pulcher*). Each network contained long branches with only few reticulations and several missing haplotypes, maybe in part as a consequence of the moderate within-population sample sizes. It is remarkable that the mean sequence divergence in a clade of haplotypes from nearby locations (clade II, 1.5%) is almost as high as that within clades that span almost the entire lakeshore (clade IV, 1.6%; clade I, 1.9%).

Five haplotypes remained unconnected to any of the networks. These comprise one haplotype from Cape Kaku South (haplotype 52) with an 11 base pair deletion, which was placed with other samples from the same region in the phylogeny; two samples from the northern part of the Lake, Kabogo (Tanzania; haplotype 53) and an unknown location in Burundi (haplotype 51), which were resolved in the clade comprising other northern samples in the phylogeny; and two further haplotypes, one from the northern basin (Ujiji; haplotype 54) and one from the southern basin (Kipili), which split at the base of the phylogenetic tree.

4. Discussion

4.1. Parallel evolution of facial stripe patterns

Our data do not support reciprocal monophyly of the Tanganyikan cichlids *N. brichardi* and *N. pulcher*. In contrast, the dark T-shaped gill-cover markings with associated blue and/or yellow patterns that identify *N. brichardi* evolved several times independently from pulcher-type ancestors. The intermediate phenotypes of *N. pulcher* at Chaitika (Konings, 1998) might represent a transitional state in stripe pattern evolution or, alternatively, are the result of previous hybridization events between the two phenotypes. However, given that our analysis is based on a single stretch of mtDNA our results need to be treated with some caution. As in birds (Hoekstra and Price, 2004) colour evolution is weakly constrained in East African cichlids (Seehausen et al., 1999), which seems particularly true for stripe patterns not associated with ecology such as orbital stripes, the lachrymal stripe and the preopercular stripe. While there is some evidence for hue to evolve under the force of sexual selection, hitherto no such associations have been found for the evolution of stripe patterns (Seehausen et al., 1999).

Within the Lake Tanganyika cichlid tribe Lamprologini, similar stripe patterns are sometimes found in species from different phylogenetic lineages (Salzburger et al., 2002). For instance, the two phylogenetically distinct species *N. pulcher* and *Neolamprologus olivaceous* share gill cover markings with two slightly curved vertical bars, and have even been regarded as one species based on morphological data (Snoeks, pers. comm.). *Neolamprologus savoryi*, *Neolamprologus splendens* and *Neolamprologus helianthus* all have facial stripes arranged in a V-shaped pattern.

Conspicuous stripes on the head might play a major role in the differentiation between species and the individual identification of conspecifics within populations (Seehausen et al., 1999). For cooperative breeders such as *N. brichardi*/ *N. pulcher* it is crucial to differentiate between familiar animals and strangers. When allowed to use visual cues only, members of *N. brichardi* breeding pairs identified their own helpers, and they could also distinguish unfamiliar helpers from non-helpers (Hert, 1985). Even when presented with video images, they individually identified conspecifics and fought strangers but courted mates (Balshine-Earn and Lotem, 1998). While it is yet unclear which individual signatures afford visual recognition among members of a social group, the colour patterns on the head including the black stripes are very plausible features for this accomplishment. Furthermore, *Neolamprologus* head and operculum markings may function in a way similar to ''badges of status'' as found in other taxa (Ferns and Hinsley, 2004; Rohwer, 1975; Setchell and Wickings, 2005; Tibbetts, 2006) and advertise the social status as helper, non-helper, and dominant breeder. Inter- and intraspecific discrimination through vision plays a crucial role in many cichlids (Carleton et al., 2005; Seehausen and van Alphen, 1998), but other sensory

Fig. 2. Bayesian tree and haplotype networks based on a 443-bp long segment of the mtDNA control region. Major clades of the Bayesian tree were recovered in both the NJ and statistical parsimony analysis and are supported by high confidence values. NJ bootstrap values and posterior probabilities are given above the major branches. Ancestral state reconstructions for facial stripe patterns based on simple parsimony (MacClade) are mapped onto the molecular phylogeny (black, *N. brichardi*-phenotype; light grey/white, *N. pulcher*-phenotype; dark grey, intermediate phenotype; dashed line, equivocal). Numbers next to tip clades (1–55) correspond to haplotypes depicted in the networks next to the tree. *Variabilichromis moorii* was used as outgroup taxon (OG). Asterisks mark those haplotypes that could not be assigned to any cluster in the TCS-network analysis (51, Burundi; 52, Cape Kaku South; 53, Kabogo; 54; Ujiji; 55, Kipili). In the haplotype networks, each circle represents one haplotype, the size correlates with the number of individuals sharing the same haplotype. Each bar represents one mutation step, small filled circles indicate missing haplotypes. Ch, Chaitika; Ka, Kapampa; Ki, Kigoma; Kl, Kalo; Kn, Kantalamba; Kp, Misepa/Kapembwa; KS, Cape Kaku South; LN, Loasi River North; LS, Loasi River South; Ma, Malasa Island; Mb, Mbita Island; Mp, Mpulungu; Mt, Mtondwe Island; To, Tongwa.

modalities such as acoustic communication (Amorim et al., 2004; Lobel, 1998; Ripley et al., 2002) and olfaction (Cole and Stacey, 2006; Crapon de Caprona, 1980; Plenderleith et al., 2005) were also shown to mediate aspects of intra- and interspecific signalling.

There is no evidence suggesting that the parallel evolution of the two stripe patterns results from ecological selection. Also, given that *N. brichardi/N. pulcher* are sexually monomorphic and possess the characteristic facial pattern already in an immature state, it does not seem to be important in mate choice, and there are no signs pointing to an influence of intersexual selection. The divergent bar patterns are not utilised during social disputes such as threat displays, where instead the ubiquitous dark blotch on the operculum becomes prominent. Therefore, intrasexual selection is also unlikely to be a causal agent for the observed stripe patterns. Purely random variation is not responsible either, because intrapopulation variation seems to be low, although stringent tests are missing (Konings, 1998, pers. observ.). The facial stripe patterns in these lamprologine taxa may thus be mainly shaped by natural selection for performance as signals of identity at the level of intrapopulation and intrafamily communication. This type of social selection could afford some freedom for diversification between populations, perhaps influenced by covarying arbitrary sensory biases (Endler and Houde, 1995; Maan et al., 2006) and under the constraint of maintaining the communicative function. As described above, distinct head stripe patterns are found in several other *Neolamprologus* species, and there may be a predisposition for variability of this trait within the group. Crosses between the two phenotypes may help to determine the genetic mechanisms underlying the different facial stripe patterns.

4.2. Species assignment: N. brichardi and N. pulcher most likely represent one single species

According to current taxonomic classification based on morphological characters and colour patterns, *N. brichardi* and *N. pulcher* represent two independent species (Poll, 1974) with non-overlapping distribution ranges along the shores of Lake Tanganyika (Konings, 1998). However, new measurements of 13 morphological characters of *N. brichardi* and *N. pulcher* specimens from two populations sampled at the northern and southern end of Lake Tanganyika, respectively, questioned the current classification (E. Skubic, S. Balshine, A. Grantner and M. Taborsky, unpublished data). The only quantitative differences found concerned head length and body depth, and the latter was only significantly different in males indicating that this trait might have been subject to sexual selection.

Our analysis of the most variable part of the mitochondrial control region shows that *N. brichardi* and *N. pulcher* populations are genetically extremely diverse. Among the 81 individuals sequenced, 55 haplotypes were found. In contrast to traditional taxonomic expectations, *N. brichardi* and *N. pulcher* were consistently resolved in mixed clades, and a tree consistent with reciprocal monophyly of the two phenotypes was rejected by a Kishino–Hasegawa test ($p < 0.001$). Moreover, except for one clade (Clade II), none of the major lineages comprised solely haplotypes from populations sampled in close geographic proximity to each other. Instead, haplotypes were spread over large distances along the shore and were sometimes distributed on opposite sides of the lake. Samples from Chaitika that resemble intermediate forms between the two phenotypes (Konings, 1998) were resolved in a clade that contains both *N. pulcher* and *N. brichardi* phenotypes (Fig. 2). Given the high sequence diversity between scattered clades of brichardi-type samples and the overall phylogeographic structure it is highly unlikely that the species boundaries are obscured as a consequence of incomplete lineage sorting. On the other hand, we cannot completely rule out the possibility of mitochondrial introgression due to hybridization between two previously independently evolving population entities. In the light of our results, we suggest to treat *N. brichardi* and *N. pulcher* as one species in the future. Because *N. pulcher* was described in 1952 (Trewavas and Poll, 1952) while *N. brichardi* was described only 22 years later (Poll, 1974), *N. pulcher* has precedence as species designation.

4.3. Congruent phylogeographic patterns across rock-dwelling species

The strong genetic differentiation and the lack of shared haplotypes among neighbouring populations (Fig. 2) suggest that dispersal is confined to short distances. This might be mainly due to the social system of *N. brichardi* and *N. pulcher* (compare Painter et al., 2000; Peterson, 1992), where helpers usually remain in the natal territory and assist in defending the nest and taking care of the brood (Taborsky, 1984; Taborsky and Limberger, 1981) in exchange for increased survival (Heg et al., 2004; Taborsky, 1984), potential opportunities for their own reproduction (mainly males; Dierkes et al., in press, 1999; Heg et al., 2006; Taborsky, 1985), and territory inheritance (mainly females; Balshine-Earn et al., 1998; Dierkes et al., 2005). Helpers delay dispersal usually until long after reaching maturity and pay with their helping behaviour to be allowed to stay in the dominant's territory (Balshine-Earn et al., 1998; Bergmüller et al., 2005a,b; Bergmüller and Taborsky, 2005; Taborsky, 1985). The older the helpers get, the higher the likelihood that the breeders have been replaced by unrelated individuals, a transition which does not seem to affect helping behaviour (Taborsky, 1984). After leaving their natal territory, helpers may join non-reproductive aggregations, as was observed in *N. brichardi* populations in the north of Lake Tanganyika (Taborsky, 1984; Taborsky and Limberger, 1981) or alternatively, disperse and start to defend an own territory or join another group, as observed in *N. pulcher* in the south of the lake (Bergmüller et al., 2005b; Stiver et al., 2004). It is important to note here that mitochondrial haplotypes as used in this

study only cover the matrilineal history and might thus under- or overestimate the overall rate of migration and the extent of introgression between mtDNA-lineages. Indeed, Stiver et al. (2007, 2004) detected a tendency of large male-biased dispersal between subpopulations (i.e., aggregations of social groups separated from the neighbouring subpopulations by 10–100 m of unsuitable habitat), which goes hand in hand with a higher replacement rate of male breeders and consequently, a higher relatedness of helpers to the breeding female (Dierkes et al., 2005; Stiver et al., 2005, 2006).

The peculiar phylogenetic pattern where geographically distant populations clustered together while populations sampled in nearby locations were resolved in different clades has already been observed in other rock-dwelling cichlids in Lake Tanganyika (Baric et al., 2003; Rüber et al., 2001; Sturmbauer et al., 2005). In particular, the occurrence of closely related haplotypes on opposite sides of the lake was also found in several rock-dwelling cichlids of various life-styles such as the biparentally mouthbrooding Eretmodines (Rüber et al., 2001), the maternally mouthbrooding *Tropheus* spp. (Baric et al., 2003; Sturmbauer et al., 2005) and the substrate-spawning *Variabilichromis moorii* (Duftner et al., 2006). The congruence in phylogeographic patterns across highly divergent species (e.g. from the tribes Lamprologini and Tropheini) was attributed to shared environmental triggers such as geologically or climatologically induced lake level fluctuations (Sturmbauer et al., 2001, 2005). During periods of extremely low lake levels, cichlids confined to coastal regions may have crossed the lake along the major lake basin ridges (Baric et al., 2003; Rüber et al., 2001; Sturmbauer et al., 2005; Verheyen et al., 1996) or along the extending canyon of the Lufubu River, one of the few major tributaries to the lake (Duftner et al., 2006; Sturmbauer et al., 2005). By analyzing phylogeographic structures of currently widely distributed species adapted to different environmental conditions and by relating them to geological data on the history of the lake we will be able to further extend our knowledge on past colonization routes and speciation events in Tanganyikan cichlids. In addition to mitochondrial DNA, the application of nuclear markers will be necessary to comprehensively evaluate the extent of introgression among different populations and species.

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