

Axes of differentiation in the bower-building cichlids of Lake Malawi

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Abstract

The 500–1000 cichlid species endemic to Lake Malawi constitute one of the most rapid and extensive radiations of vertebrates known. There is a growing debate over the role natural and sexual selection have played in creating this remarkable assemblage of species. Phylogenetic analysis of the Lake Malawi species flock has been confounded by the lack of appropriate morphological characters and an exceptional rate of speciation, which has allowed ancestral molecular polymorphisms to persist within species. To overcome this problem we used amplified fragment length polymorphism (AFLP) to reconstruct the evolution of species within three genera of Lake Malawi sand-dwelling cichlids that construct elaborate male display platforms, or bowers. Sister taxa with distinct bower morphologies, and that exist in discrete leks separated by only 1–2 m of depth, are divergent in both sexually selected and ecological traits. Our phylogeny suggests that the forces of sexual and ecological selection are intertwined during the speciation of this group and that specific bower characteristics and trophic morphologies have evolved repeatedly. These results suggest that trophic morphology and bower form may be inappropriate characters for delineating taxonomic lineages. Specifically the morphological characters used to describe the genera *Lethrinops* and *Tramitichromis* do not define monophyletic clades. Using a combination of behavioural and genetic characters, we were able to identify several cryptic cichlid species on a single beach, which suggests that sand dweller species richness has been severely underestimated.

Keywords: amplified fragment length polymorphism, bower, ecological speciation, pharyngeal jaws, sexual selection

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Traditional allopatric models of speciation begin with the disruption of gene flow by geographic barriers, which allows separate populations to be pushed into novel evolutionary trajectories (Mayr 1963). Recent discussion has focused on the relative importance of natural vs. sexual selection during population divergence (van Doorn *et al.* 1998; Danley & Kocher 2001; Streelman & Danley 2003; Arnegard & Kondrashov 2004; Coyne & Orr 2004; Kirkpatrick & Nuismer 2004). In ecological models, resource competition leads to genetic divergence through disruptive natural selection (Orr & Smith 1998; Rundle & Nosil 2005). If the populations are allopatric, then reproductive isolation is established as a by-product of

ecological character divergence, or by genetic drift. Sexual selection can increase the rate of divergence among such populations (West-Eberhard 1983). Several models suggest that speciation can be driven by sexual selection alone (Lande 1981; Wu 1985; Turner & Burrows 1995; Higashi *et al.* 1999; Lande *et al.* 2001). Since sexual selection acts on mate recognition systems, it has the potential to create reproductive isolation without ecological differentiation (Panhuis *et al.* 2001). Divergence of courtship signals is a characteristic common to many species-rich lineages (Barraclough *et al.* 1995; Boughman 2002; Streelman & Danley 2003). However, some authors contend that without subsequent niche divergence, competitive exclusion would lead to the extinction of one or both sister taxa during secondary contact (van Doorn *et al.* 1998; Arnegard & Kondrashov 2004).

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The extraordinary radiation of Lake Malawi's estimated 1000 species of endemic cichlid fishes has become a model system for studying adaptive radiation (Kornfield & Smith 2000) and gives us an opportunity to identify the forces shaping recently evolved sister taxa. Reconstructing the phylogenetic history of this rapid radiation has proved challenging, but the analysis of large numbers of independent genetic loci using amplified fragment length polymorphism (AFLP) has allowed significant progress to be made within the clade of rock-dwelling cichlids (Albertson *et al.* 1999; Allender *et al.* 2003). The patterns of divergence revealed by these studies encouraged Danley & Kocher (2001) to suggest that natural and sexual selection have acted in a sequential pattern of three stages during the diversification of this species rich lineage. The initial stage of radiation is characterized by adaptation to distinct macrohabitats and produced the major lineages that contain most of the current species diversity in the lake (the rock-dwelling *mbuna* and the sand-dwelling *utaka* and *chisawasawa*). The reciprocal monophyly of the rock- and sand-dwelling lineages has been confirmed by data from mtDNA (Moran *et al.* 1994; Kocher *et al.* 1995; Salzburger *et al.* 2002) and short interspersed repetitive elements (SINE) insertions (Takahashi *et al.* 2001). The second stage of radiation was driven by competition for trophic resources. AFLP data confirm the monophyly of rock-dwelling genera (Albertson *et al.* 1999) that were originally defined largely by their feeding morphology (Regan 1922; Trewavas 1935). The third stage of radiation in the rock-dwelling species is characterized by the divergence of secondary male colour patterns without dramatic changes in trophic morphology (Danley & Kocher 2001). Closely related species often differ in colour pattern (Allender *et al.* 2003), which has been shown to be an important component of conspecific mate recognition (Seehausen & van Alphen 1998; Kidd *et al.* in press). While this radiation-in-stages model was initially proposed to explain diversification of the *mbuna*, it has been extended to explain species diversity in other well-characterized vertebrate radiations (Streelman & Danley 2003).

Sister group comparisons are one of the best ways to identify the factors involved in the evolution of diversity (Barraclough *et al.* 1998) and the sand-dwelling lineage of Lake Malawi cichlids affords us the opportunity for such a comparison. Like their *mbuna* cousins, the sand-dwelling genera are defined primarily by trophic morphology (Eccles & Trewavas 1989). Unlike the *mbuna*, the nuptial coloration of the sand-dwelling males is relatively inconspicuous (Turner 1996; McKaye *et al.* 2001). However, they do construct elaborate bowers of sand and gravel from which they display to potential mates (McKaye 1991; McKaye *et al.* 2001). These bowers are constructed in heterospecific leks in which each species builds a stylized bower with a species-specific shape (McKaye 1983). All endemic Lake Malawi cichlids are maternal mouthbrooders, so these

bowers are used for male display and egg-laying purposes only. After spawning, the female leaves the male's territory with the fertilized eggs in her buccal cavity. McKaye (1991) described 10 categories of qualitatively different bower form in Lake Malawi cichlids, including pits, mounds and volcanoes. Bowlers within each category vary quantitatively, ranging in size from a few centimetres to 3 m across (Stauffer *et al.* 1995; McKaye *et al.* 2001). If a male takes over a heterospecific bower, he will initially display over the existing bower while gradually altering its form and structure to his species-specific style (McKaye 1984). Bower shape is so species-specific that Stauffer *et al.* (1993) used it as a taxonomic character to distinguish three new species (*Copadichromis conophoros*, *Copadichromis cyclicos*, and *Copadichromis thinos*).

The development of robust phylogenies for the sand-dwelling lineage has been problematic (Kornfield & Smith 2000; Turner *et al.* 2001). Phylogenies based on morphology are limited by the lack of appropriate morphological synapomorphies (Stiassny 1991). Convergence of morphological characters is seen among lineages within and between rift valley lakes (Kocher *et al.* 1993; Reinthal & Meyer 1997). The construction of robust phylogenies from most DNA sequence data has been confounded by the extraordinary tempo of cichlid evolution and has allowed the retention of genetic polymorphisms (Moran & Kornfield 1993; Parker & Kornfield 1997). The problem of incomplete lineage sorting can be overcome by surveying thousands of independent restriction sites spread across the nuclear genome using AFLP (Albertson *et al.* 1999; Allender *et al.* 2003; Seehausen *et al.* 2003). Here we used AFLP markers to reconstruct the evolutionary history of three bower-building genera: *Copadichromis*, *Protomelas* and *Lethrinops* (including *Tramitichromis*). Our goal was to identify groups of recently evolved sister taxa which might provide information about the mechanisms of speciation in this group. We also wanted to evaluate instances of morphological convergence by comparing the current morphology-based taxonomy to an independent molecular phylogeny. Finally, we wished to discover patterns in the evolution of bower shape and melanin pigmentation patterns within the context of a robust phylogeny for members of the sand-dwelling clade in Lake Malawi.

Methods

Taxa studied

Individuals of the genera *Copadichromis*, *Protomelas* and *Lethrinops* were collected for this study (Table 1). The genus *Copadichromis* consists of species with elongated premaxillary pedicels that are extended forward to form a protrusible mouth. Additionally, these species have weak jaws and teeth that are recurved simple or bicuspid (Eccles & Trewavas

Genus	Species	Collection site	Bower type
<i>Copadichromis</i>	<i>eucinostomus</i>	Chembe Beach	Volcano
<i>Copadichromis</i>	<i>likomae</i>	Otter Point	Pit
<i>Copadichromis</i>	<i>jacksoni</i>	Otter Point	Vertical rock surface
<i>Copadichromis</i>	<i>borleyi</i>	Otter Point	Horizontal rock surface
<i>Protomelas</i>	<i>similis</i>	Otter Point	Pit
<i>Protomelas</i>	<i>taeniolatus</i>	Otter Point	Horizontal rock surface
<i>Protomelas</i>	'black rock'	Black Rock Beach	Volcano
<i>Tramitichromis</i>	<i>variabilis</i>	Otter Point	Pit-Volcano
<i>Tramitichromis</i>	'microstoma-type'	Otter Point	Volcano
<i>Tramitichromis</i>	'black rock'	Black Rock Beach	Volcano
<i>Tramitichromis</i>	'deep'	Chembe Beach	Volcano
<i>Tramitichromis</i>	'shallow'	Chembe Beach	Volcano
<i>Tramitichromis</i>	'yellow gular'	Chembe Beach	Volcano
<i>Tramitichromis</i>	'pink gular'	Chembe Beach	Volcano
<i>Lethrinops</i>	<i>auritus</i>	Chembe Beach	Mounds
<i>Lethrinops</i>	<i>auritus</i>	Songwe Hill	Mounds
<i>Lethrinops</i>	<i>rock display</i>	Otter Point	Horizontal rock surface
<i>Lethrinops</i>	<i>lethrinus</i>	Chembe Beach	Pit with mounds
<i>Lethrinops</i>	<i>parvidens</i>	Songwe Hill	Volcano
<i>Taeniolethrinops</i>	<i>praeorbitalis</i>	Chembe Beach	Pit
Outgroups			
<i>Metriaclima</i>	<i>zebra</i>	Mumbo Island	Cave
<i>Metriaclima</i>	<i>zebra</i>	Domwe Island	Cave
<i>Rhamphochromis</i> sp.		Chembe Beach	None reported
<i>Tropheus</i>	<i>duboisii</i>	Lake Tanganyika	Horizontal rock surface

Table 1 Taxa of bower-building cichlids sampled for AFLP fingerprint analysis

1989). Eccles & Trewavas (1989) described the genus *Protomelas* without defining any synapomorphies common to the group. Species of this genus lack any of the synapomorphies used to define other sand-dwelling genera, but do share one or two longitudinal dark bands that may be overlaid on vertical bars. The genus *Lethrinops*, as established by Regan (1922) and revised by Trewavas (1931), is separated from other haplochromine genera based on oral jaw dentition. Eccles & Trewavas (1989) described two new genera (*Tramitichromis* and *Taeniolethrinops*), which share the *Lethrinops* dentition.

A nested set of outgroups was also included in this study. *Metriaclima zebra* is a member of the rock-dwelling clade that is sister to the sand dwellers. *Rhamphochromis* is a more basal lineage in the Lake Malawi radiation (Moran *et al.* 1994; Kocher *et al.* 1995). *Tropheus duboisii* is part of Lake Tanganyika's haplochromine lineage, which is sister to both the Lake Malawi and Lake Victoria species flocks (Meyer 1993).

Sample collection and field work

All samples were taken from live fish collected in the field, with the exception of *Tropheus duboisii*, which were purchased through the aquarium trade. Field collections were made in Lake Malawi National Park on the Nankumba Peninsula (Fig. 1a) during July and August 2001. Chembe beach is

a 4-km sandy beach that stretches from Domwe Island to Otter Point (Lewis *et al.* 1986). The substrate alternates between fine sand, gravel, and flocculent mud. Beds of *Vallisneria* and *Potamogeton* punctuate areas of sand. Black Rock Beach is a narrow (5 m long) beach of fine sand wedged between continuous rocky coastlines. The sand/rock interface is very shallow throughout this area (about 1 m) and underwater the narrow beach opens to a wide expanse of open sand punctuated with beds of weeds. The Songwe Hill collection site is on the eastern side of the Nankumba Peninsula and is described in Ribbink *et al.* (1983).

Initial underwater surveys were performed to identify populations that differed in bower form (Fig. 2a–d). The following measurements were taken for each individual bower ($n = 5–30$): platform diameter, base diameter, total height, lip height, and slope (Fig. 2e). Bower measurements were recorded from every population sampled with the exception of *Tramitichromis* 'microstoma-type.' Populations that differed in bower design were treated as isolated breeding populations, regardless of overlapping morphological characters. Prior to collection, each male was observed to verify that he was actively maintaining and defending the bower he occupied. Individual males were surrounded with a monofilament net, which was slowly drawn closed while maintaining visual contact with the specimen. This ensured that the appropriate male was captured for each

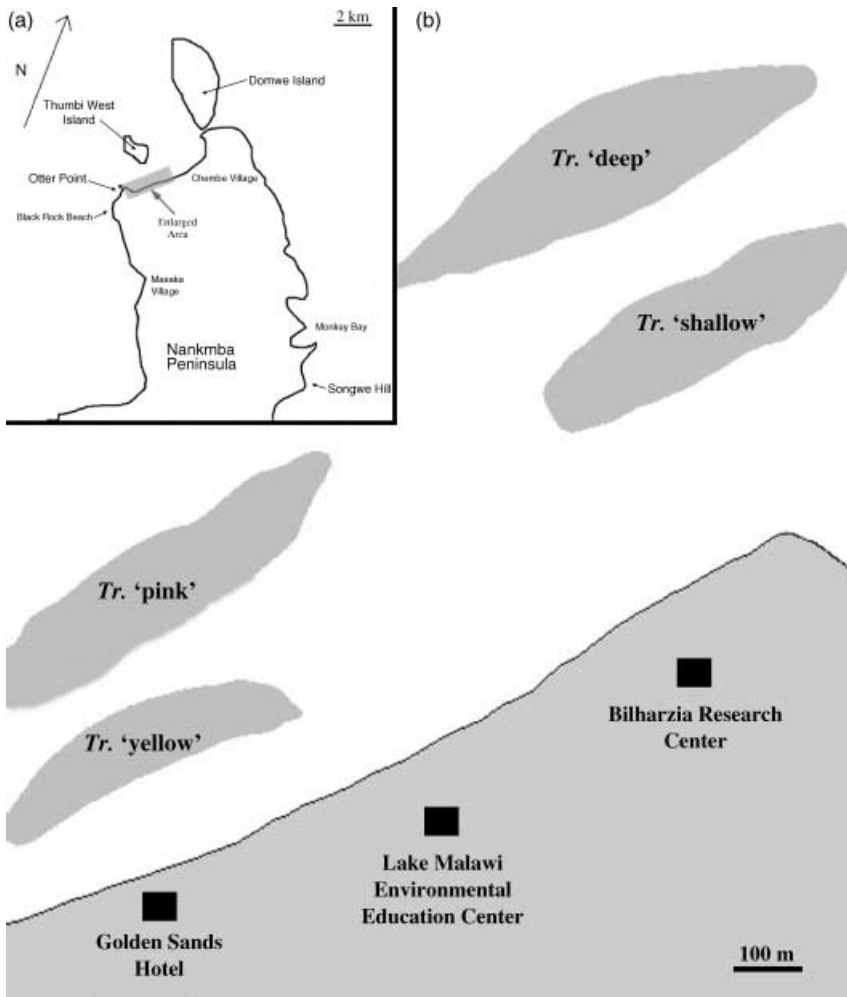


Fig. 1 Sample locations along the Nankumba Peninsula in southern Lake Malawi. (a) Bower-building taxa were collected from Black Rock Beach, Otter Point, Chembe Beach, and Songwe Hill. (b) Enlarged section of Chembe Beach displaying the distribution of *Tramitichromis* bowers. Sister taxa occupied parallel leks with a very narrow depth distribution (separated by 1–1.5 m of depth). While these leks overlapped with *Copadichromis eucinostomus*, they did not overlap with other *Tramitichromis* or *Lethrinops* species. No *Tramitichromis* or *Lethrinops* bowers were found in the intervening areas.

bower type. Fin clips were taken from the right pectoral fin of each specimen and stored in 95% EtOH. Three to five voucher specimens were preserved in 10% formalin and later transferred to 70% ethanol for long-term storage.

Morphology

Additional specimens (3–50) were collected and dried on site. These were later disarticulated in the laboratory using a colony of dermestid beetles. Digital photographs of the lower pharyngeal and oral jaws were taken with a SPOT digital camera (Diagnostic Instruments, Inc.) mounted on a Zeiss SV11 dissecting scope. The photographs were imported into NIH Image 1.59 and analysed following the methods of Albertson & Kocher (2001). All specimens were examined for the haplochromine vs. *Lethrinops* oral dentition and the presence of the 'keel' on the lower pharyngeal jaw. Length and width of the lower pharyngeal bone and the muscular process (horn width) were measured following Barel *et al.* (1977) and Hoogerhoud (1984).

The tooth area and proportion of molariform teeth was calculated following Barel *et al.* (1977).

Molecular techniques

Genomic DNA was extracted from the fin clips of three individuals of each taxa (two for *Protomelas similis* and *Lethrinops parvidens*) using a standard phenol–chloroform extraction (Wang *et al.* 2003). AFLP is a DNA fingerprinting technique that characterizes thousands of restriction polymorphisms spread throughout the genome (Vos *et al.* 1995). In this method, genomic DNA is first double-digested using two restriction enzymes (in this case *EcoRI* and *MseI*). Double-stranded adapters are then ligated onto the 'sticky' ends of the fragments. An initial 'preselective' polymerase chain reaction (PCR) amplifies a subset of fragments that match adapter primers containing an additional nucleotide (*EcoRI*-A and *MseI*-C). The product of this preselective amplification was then used as the template for 11 different 'selective' amplifications performed with primers containing

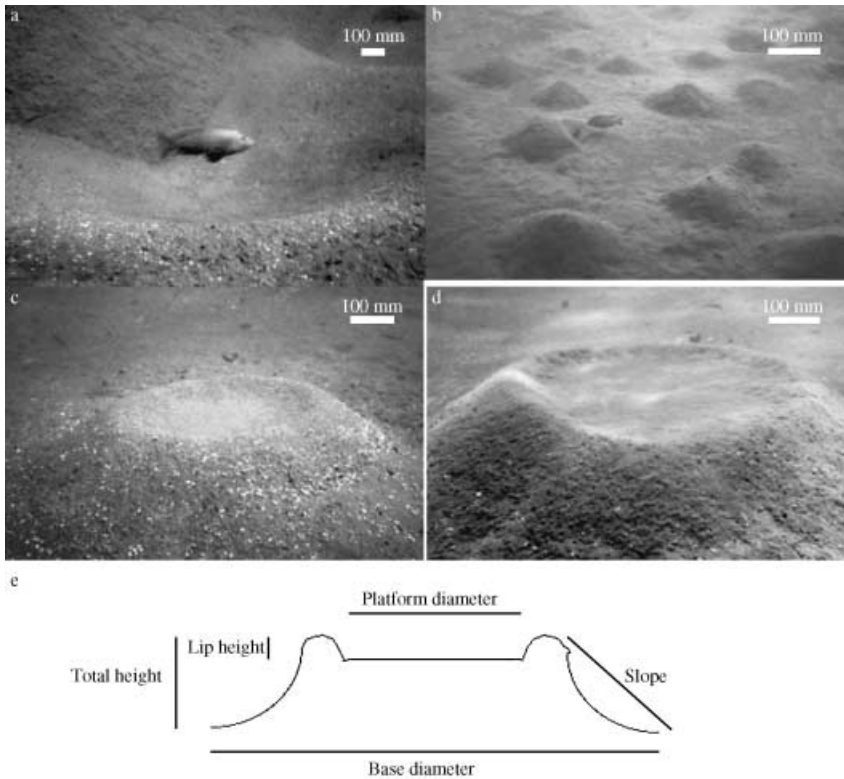


Fig. 2 Basic bower forms built by the cichlid fishes of Lake Malawi. (a) Typical pit bower constructed by *Dimidiochromis kiwinge*. (b) Display sites built by *Lethrinops auritus* consist of a central mound surrounded by smaller satellite mounds. (c) *Protomelas* 'Black Rock' builds a typical volcano (or cone) type bower. Egg deposition occurs in a cup-like depression against the lip of the raised platform. All of the bowers built by this population were covered with snail shells (*Melanoides* sp.). (d) *Tramitichromis* sp. 'yellow throat' also builds a volcano-style bower. Several *Tramitichromis* spp. build one or more raised sections on the platform lip (backsplashes) typically near the site of egg deposition. (e) Sketch of a volcano bower indicating the measurements used to identify populations that differed in bower form (after Kellogg *et al.* 2000). Minimum and maximum values of each measurement were used to quantify bower shape.

an additional two nucleotide extension (E-AGG, M-CTT, M-CTG; E-ACT, M-CTA, M-CAG, MCAT; E-ACA, M-CAA, M-CAG; E-AGC, M-CAG, M-CAT; E-ACC, M-CAA, M-CAC). The result of these amplifications is a PCR product containing a small subset of the genomic fragments produced by the initial restriction. The detailed protocols can be viewed at (<http://hogs.unh.edu/protocol/basic>).

Fragments were separated using a Beckman Coulter CEQ 8000 capillary sequencer. Since only the *EcoRI* selective primers were fluorescently labelled, only those fragments containing an *EcoRI* site were detected. Peaks were scored using a quartic model with a slope threshold of 2.0% and relative peak height of 5.0%. Bands were scored as present/absent using Beckman Coulter's Fragment Analysis Module. The presence of each fragment was confirmed manually. Fragments between 70 and 600 bp in size were binned (1 nucleotide bin width) using Beckman Coulter's AFLP ANALYSIS software. The binary output was imported to an Excel spreadsheet and formatted for PAUP 4.0b8 (Swofford 2001).

Phylogenetic analysis

Matrices were generated by calculating Nei & Li's (1979) genetic distance. The phylogram was constructed from 1000 bootstrap replicates using a neighbour-joining algorithm in PAUP version 4.0b8 (Swofford 2001). The data were tested for hierarchical structure by analysing the frequency

and distribution of tree lengths for 1 000 000 randomly generated trees (Hillis & Huelsenbeck 1992). Kishino-Hasegawa tests (KH) were used to assess the validity of alternate topological hypotheses (Kishino & Hasegawa 1989). We tested the reciprocal monophyly of specific sister taxa pairs, by constructing a suite of constraint trees in PAUP version 4.0b8 which sequentially forced the exclusion of each individual from the clade. Using the same parameters described above, PAUP identified the best tree that included the constraint and a KH test was used to compare the constrained and unconstrained trees ($n = 12-36$ permutations for each clade). The maximum P value is reported for each node.

Because hybridization may occur between taxa, the traditional phylogenetic view of a bifurcating tree may not be an accurate representation of the evolutionary history during rapid adaptive radiations (Posada & Crandall 2001; Seehausen 2004). Two methods were employed to detect the effect of reticulation during the diversification of these bower-building taxa. The first method was the homoplasy excess test described by Seehausen (2004) and utilized by Schliewen & Klee (2004). Since hybrids are intermediate to both parents, hybrid taxa will increase the amount of homoplasy within the data set, which can produce identifiable conflict within the clades contributing to the hybridization. The removal of excess homoplasy in the data set (by removing hybrid taxa) will result in a substantial

increase in the resolution of the parental clades. Following Schlieven & Klee (2004), each taxon was sequentially removed and the phylogeny reconstructed using the same parameters described above. Bootstrap values for each node of each pruned tree were recorded and compared. Additionally, the pairwise distance matrix created in PAUP was imported into SPLITSTREE version 2.4 (Hudson 1998). We used split decomposition (Bandelt & Dress 1992) to produce a 'splits graph' which displays conflicting topologies as a network. For ideal data, this method will produce a standard bifurcating tree; however, less ideal data give rise to a tree-like network. Cassens *et al.* (2003) found that split decomposition revealed network topology for a data set that yielded unambiguous topology when analysed with either statistical parsimony or median-joining algorithms.

Results

AFLP phylogeny

Eleven primer pair combinations generated 4355 characters ($\bar{X} = 397.8$ per primer pair). Of these, 3171 were polymorphic ($\bar{X} = 288.3$ per primer pair). A plot of the length of 1 000 000 random trees demonstrated significant nonrandom structure to the data set ($g_1 = -0.536$, 69 samples, $P < 0.01$). These data were used to construct a distance tree (Fig. 3) with all but one node resolved above a 50% bootstrap value. The entire tree has a mean bootstrap value of 86.7% (Fig. 4). Replicate samples from the same population cluster together with a mean bootstrap value of 97.3%. Species collected from multiple locations (*Lethrinops auritus*, *Metriaclima zebra*) also cluster together consistently.

This tree recovers the expected relationship of all outgroup taxa. *Tropheops duboisi* from Lake Tanganyika was used to root the tree. The genus *Rhamphochromis* emerges as basal to all other Lake Malawi samples, which corresponds to previous mtDNA-based (Kocher *et al.* 1993; Meyer 1993) and AFLP-based (Albertson *et al.* 1999; Allender *et al.* 2003; Seehausen *et al.* 2003) phylogenies. The *mbuna* clade, represented by *Metriaclima zebra*, is sister to all of the sand dweller taxa sampled.

The species of *Copadichromis* sampled in this study form a monophyletic lineage (KH $P < 0.0002$). *Protomelas* 'black rock', *Protomelas taeniolatus* and *Protomelas similis* also form a monophyletic clade (KH $P = 0.022$). The genera *Copadichromis*, *Protomelas* and *Taeniolethrinops* are embedded within a clade that also contains multiple representatives of *Lethrinops* and *Tramitichromis*. Neither the *Lethrinops* oral dentition, nor the *Tramitichromis* pharyngeal keel, appears to define monophyletic clades (KH $P < 0.0001$ for both alternatives).

Both tests used to detect the presence of conflicting phylogenetic signal attributed to hybridization yielded similar results. The removal of both populations of *Lethrinops auri-*

tus substantially increased the bootstrap support value for the node uniting *Lethrinops lethrinus* and *Lethrinops parvidens* (Fig. 5a). The removal of all other taxa failed to produce any outlying improvement for the bootstrap support value of any other node. Similarly, split decomposition also indicated potential reticulation within this lineage. There was no evidence of network-like relationships when all taxa were used to construct the splits graph. However, subdividing the data set to focus on specific lineages (*Copadichromis*, *Protomelas*, *Lethrinops* and *Tramitichromis*) revealed that a conflicting relationship exists between *L. lethrinus* and *L. auritus* of Songwe Hill (Fig. 5b).

Copadichromis

When erecting the genus *Copadichromis*, Eccles & Trewavas (1989) considered oral jaw structure to be more important than melanin pattern. Species with different colour patterns (barred, three-spot, and mid-lateral striped) were unified by a synapomorphic protractile mouth. While several authors have suggested that this genus is polyphyletic (Konings 1990; Stauffer 1993), the populations of *Copadichromis* we sampled clearly form a distinct and monophyletic lineage. Konings (1990) suggested the placement of the volcano building and vertically barred *Copadichromis eucinostomus*, *Copadichromis prostoma*, and *Copadichromis boadzulu* within the genus *Nyassachromis*. While the bootstrap values strongly suggest that *Copadichromis borleyi* and *Copadichromis jacksoni* form a lineage that is sister to *C. eucinostomus* and *Copadichromis likomae*, we are unable to rule out an alternate topology where *C. eucinostomus* is basal to the other three species (KH $P = 0.111$).

The four taxa of *Copadichromis* collected in this study make qualitatively different bowers. Two taxa display over bare rock: *C. borleyi* were observed defending territories over horizontal rock surfaces and *C. jacksoni* defended vertical rock surfaces. *C. likomae* builds a large pit that may develop a short lip (Table 2). *C. eucinostomus* makes a symmetrical volcano bower in huge heterospecific leks (Table 3).

Protomelas

While Eccles & Trewavas (1989) erected the genus *Protomelas* without defining a shared synapomorphy, the taxa sampled in this study are strongly united. Within this clade, *P.* 'black rock' and *P. taeniolatus* cluster together to the exclusion of *P. similis* (KH $P = 0.017$). All three taxa of this lineage share longitudinal dark bands. However, Eccles and Trewavas did not consider this trait to be a synapomorphy and often gave more weight to trophic characters. The exclusion of the horizontally striped *Lethrinops lethrinus* and *L.* 'rock display' from this clade supports Eccles & Trewavas's (1989) assertion that this colour pattern does not define a monophyletic group.

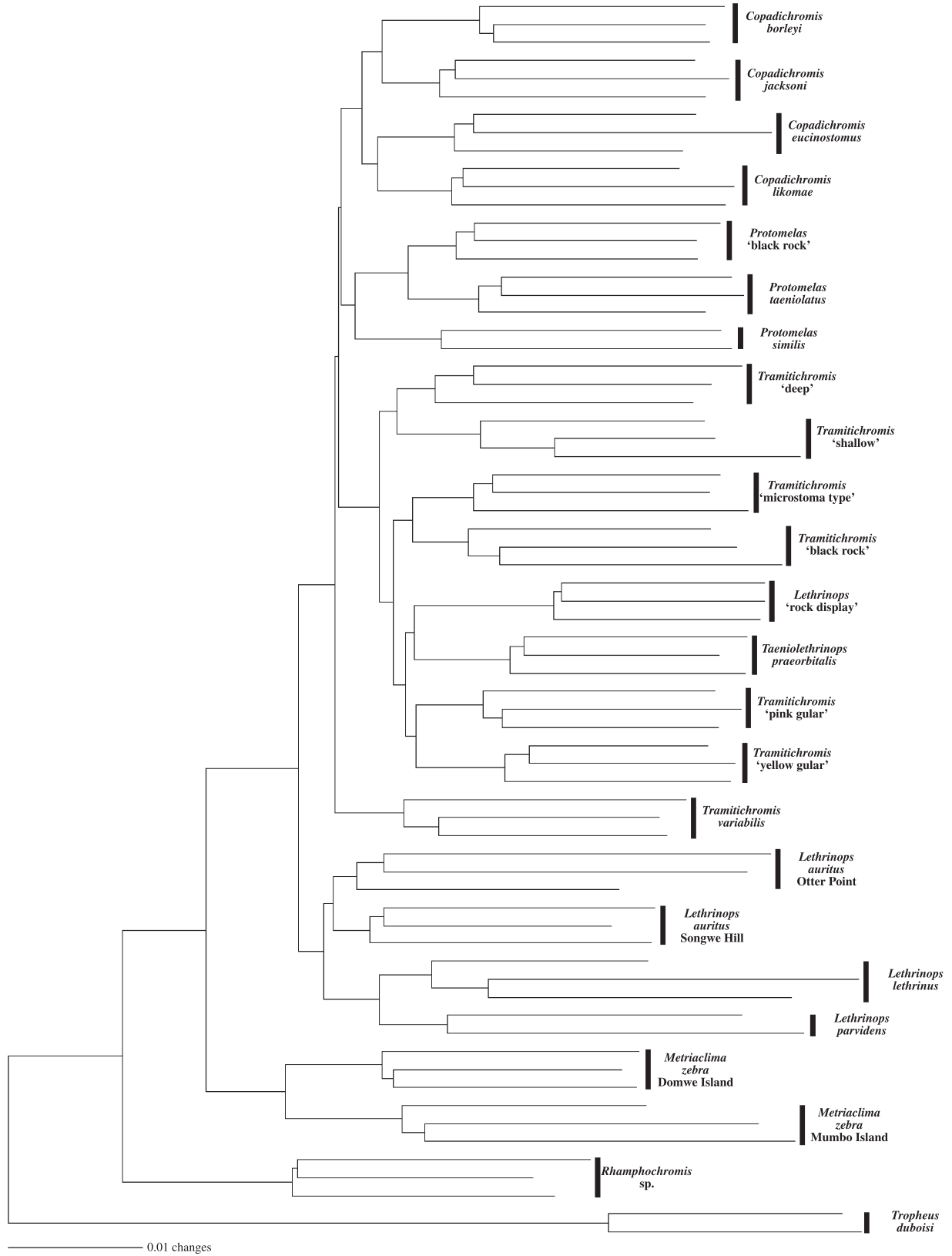


Fig. 3 Dendrogram of the bower building cichlids of Lake Malawi based on Nei & Li's genetic distance and constructed using the neighbour-joining algorithm.

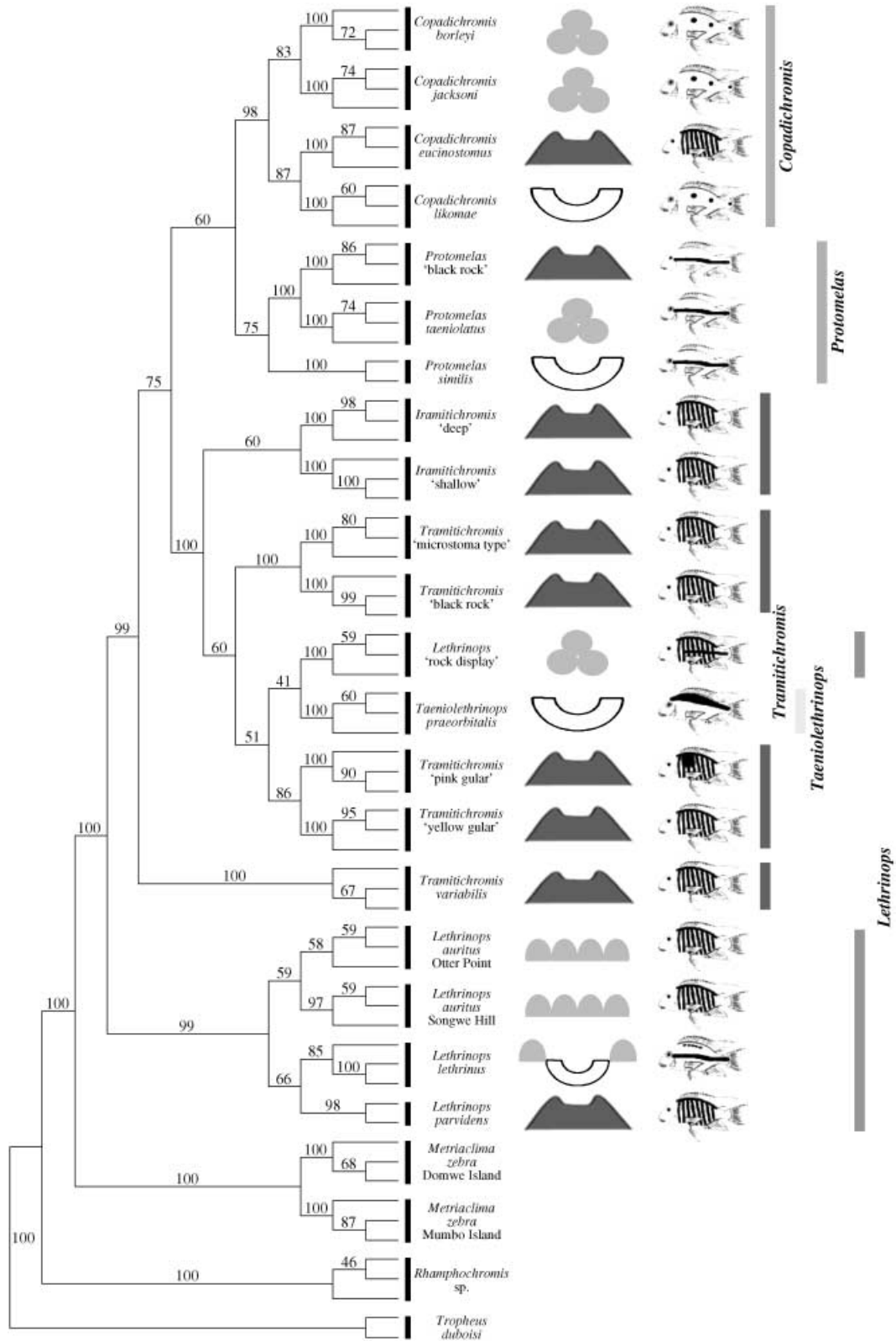


Fig. 4 Phylogeny of bower building cichlids of Lake Malawi based on Nei & Li's genetic distance and constructed using the neighbour-joining algorithm. Numbers at each node indicate the bootstrap values (from 1000 replicates) for that node. All but one node is resolved above a 50% bootstrap value. Symbols indicate the general bower type (pit, volcano, rock and mound) constructed by each population. The cichlid diagrams illustrate the melanin colour pattern of each population. Bars on the right indicate the current generic assignment of each taxon.

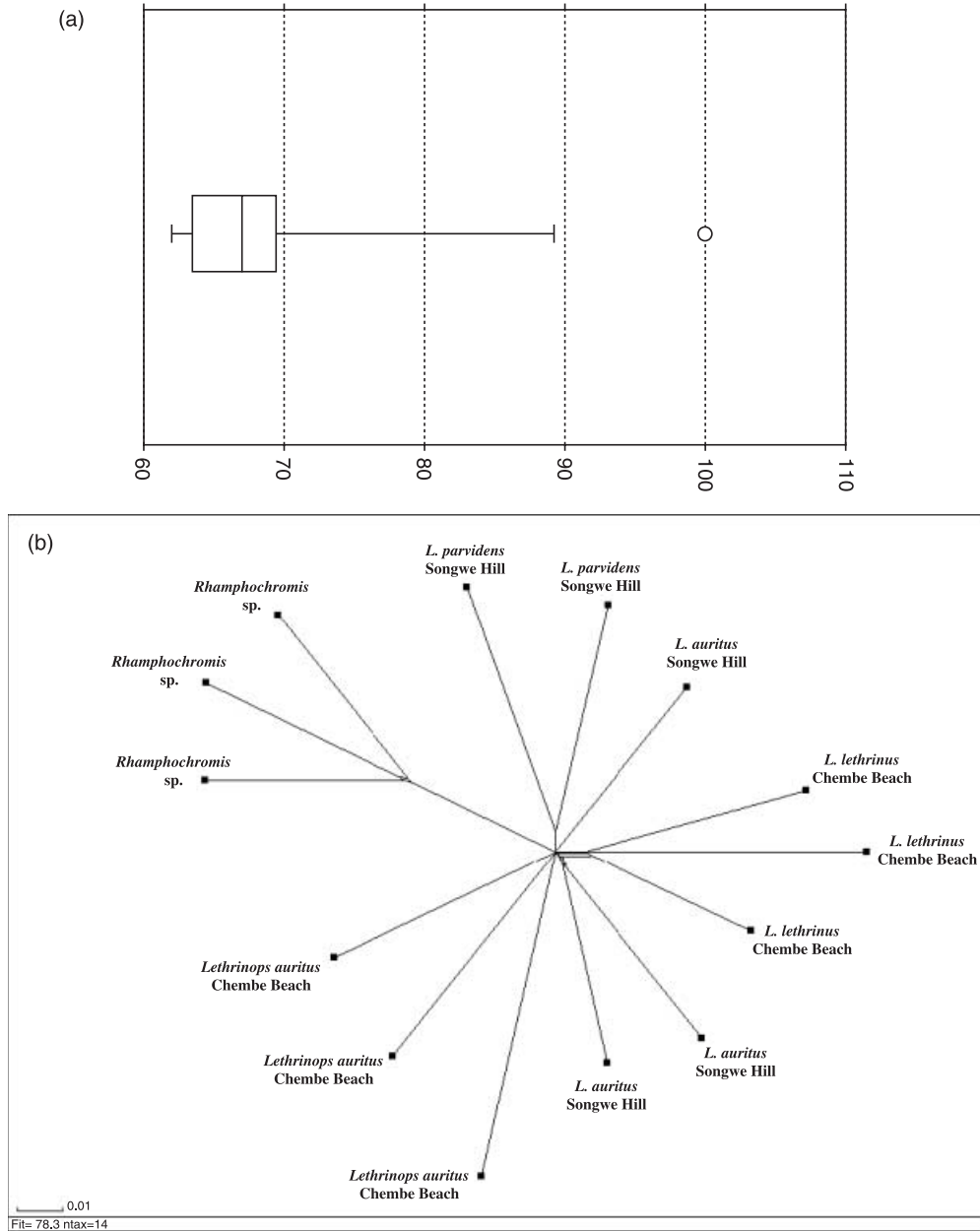


Fig. 5 The box plot of the distribution of bootstrap support values (%) for the node uniting *Lethrinops lethrinus* and *Lethrinops parvidens* during the sequential removal of each taxa (a). Only the removal of both populations of *Lethrinops auritus* (open circle) resulted in a substantial increase in bootstrap support (100%). Additionally the graphical representation (splits graph) of the split decomposition of the distance matrix (b) suggests that instead of a bifurcating tree, a network better explains the relationship between *L. auritus*, *L. lethrinus*, and *L. parvidens*.

Table 2 Means and standard deviations recorded for all measurements of pit-type bower dimensions. The pit shape was significantly different (ANOVA, $P < 0.001$) for all measurements

Species (<i>n</i>)	Pit depth (mm) Max. (SD)	Pit diameter (mm) Max. (SD)	Min. (SD)	# Satellite mounds (SD)	Water depth m (SD)
<i>Copadichromis likomae</i> (6)	228.0 (77.9)	742.0 (170.4)	742.0 (170)	0	NR
<i>Protomelas similis</i> (7)	80.0 (28.9)	317.1 (68.2)	276.0 (38.2)	0	NR ~1.2
<i>Taeniolethrinops praeorbitalis</i> (7)	442.5 (220.3)	1583.0 (167)	NR	0	5.1 (0.7)
<i>Lethrinops lethrinus</i> (7)	116.7 (16.3)	NR	NR	8.1 (3.7)	4.1 (0.16)

Table 3 Means and standard deviations of measurements taken from 10 populations that build volcano-type bowers. Bower dimensions differed significantly (ANOVA, $P < 0.001$ for all measurements) between taxa

Species (n)	Total height (mm)		Lip height (mm)		Base diameter (mm)		Platform diameter (mm)		Slope (mm)		Water depth (m) (SD)
	Max. (SD)	Min. (SD)	Max. (SD)	Min. (SD)	Max. (SD)	Min. (SD)	Max. (SD)	Min. (SD)	Max. (SD)		
<i>Copadichromis eucinostomus</i> (6)	83.3 (21.6)	83.0 (22.0)	53.3 (13.6)	53.3 (13.6)	475.0 (88.9)	475.0 (88.9)	230.0 (30.3)	230.0 (30.3)	NR	NR	NR
<i>Protomelas</i> sp. 'black rock' (6)	271.7 (43.1)	271.7 (43.1)	49.2 (10.2)	45.0 (17.3)	1253.3 (131.6)	1253.3 (131.6)	481.7 (47.9)	481.7 (47.9)	445.0 (97.5)	445.0 (97.5)	6.8 (0.36)
<i>Tramitichromis</i> 'yellow gular' (11)	253.6 (67.3)	211.0 (93)	61.8 (22.7)	17.4 (10.7)	1263.6 (253.7)	1263.6 (253.7)	412.3 (56.5)	412.3 (56.5)	478.2 (156.0)	478.2 (156.0)	2.5 (0.17)
<i>Tramitichromis</i> 'pink gular' (7)	159.2 (47.2)	123.0 (48)	68.3 (15.7)	40.0 (13)	1096.7 (305.7)	1018.0 (237)	515.0 (87.3)	485.0 (74)	259.0 (79)	259.0 (79)	3.9 (0.23)
<i>Tramitichromis</i> 'deep' (10)	291.0 (80.8)	291.0 (80.8)	68.0 (25.6)	22.0 (7.9)	1379.0 (276.5)	1262.0 (247)	456.0 (105.5)	375.0 (47.4)	470.0 (118.1)	470.0 (118.1)	4.9 (0.21)
<i>Tramitichromis</i> 'shallow' (7)	182.9 (54.7)	182.9 (54.7)	65.0 (24.7)	57.9 (23.4)	1172.9 (74.1)	1133.0 (81.8)	540.0 (60.8)	491.0 (108)	310.0 (46.2)	310.0 (46.2)	3.7 (0.12)
<i>Tramitichromis</i> 'microstoma-type' (0)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR-11
<i>Tramitichromis</i> 'black rock' (5)	310.0 (59.6)	290.0 (39)	32.0 (13.5)	30.0 (11.7)	1182.0 (174.6)	1182.0 (174.6)	364.0 (39.1)	364.0 (39.1)	498.0 (102.8)	498.0 (102.8)	2.7 (0.0)
<i>Tramitichromis</i> 'variables' (8)	174.4 (51.6)	161.0 (66)	96.3 (43.7)	79.4 (23.1)	1036.9 (158.9)	1036.9 (158.9)	698.1 (113.3)	698.1 (113.3)	233.3 (110.15)	233.3 (110.15)	6.8 (2.41)
<i>Lethrinops parvidens</i> (7)	62.9 (20.6)	61.0 (19)	52.1 (12.9)	40.0 (5.8)	474.3 (91.3)	474.3 (91.3)	317.1 (52.5)	317.1 (52.5)	94.3 (29.9)	94.3 (29.9)	4.9 (0.12)

Protomelas taeniolatus defended territories over horizontal rock surfaces. Small aggregations of males (5–10 individuals) were seen displaying over small rings of sand and gravel placed on large rock boulders. These rings have the appearance of a squat volcano, but the platform surface is bare rock. *Protomelas similis* builds a pit bower with a sloped edge within beds of *Vallisneria* (Table 2). The undescribed species of *Protomelas* encountered at Black Rock makes a very tall and symmetrical volcano bower (Table 3). The sloping surface of these bowers were covered with hundreds of snail shells of the *Melanoides* genus (Fig. 2c). Interestingly, the pharyngeal jaws of *P.* 'Black Rock' have papilliform teeth, suggesting that this species does not prey upon snails.

While pharyngeal jaw dentition was similar for all *Protomelas* taxa, *P. taeniolatus* and *P.* 'Black Rock' possess different oral dentitions. As described by Eccles & Trewavas (1989), the lower jaws of the observed *P. taeniolatus* have teeth arranged in a four to five series with the outer bicuspid and inner tricuspid teeth. Conversely, the teeth of the lower jaw of *P.* 'Black Rock' are in a three to four series with strongly recurved outer unicuspid teeth (some weakly bicuspid posterior) and bicuspid teeth in the inner series.

Lethrinops

The genus *Lethrinops* (including *Tramitichromis* and *Taeniolethrinops*) is characterized by a unique lower jaw dentition (Fig. 6) in which the outer tooth row curves inward to terminate behind the inner row (Regan 1922). This distinguishes these genera from other haplochromines in which the outer tooth row continues backward in a single series. Our phylogeny indicates that the *Lethrinops* clade is paraphyletic: the genera *Copadichromis* and *Protomelas* are embedded within this lineage. This would suggest that there has been parallel evolution of the haplochromine-type dentition within Lake Malawi. Furthermore, the *Lethrinops*-type dentition does not define a monophyletic lineage.

The taxa *L. auritus*, *L. lethrinus*, and *L. parvidens* form a monophyletic lineage (KH $P = 0.024$). Within this clade, the mound-building *L. auritus* and *L. lethrinus* do not cluster together (KH $P = 0.037$). As predicted by Eccles & Lewis (1979), and reaffirmed by Ngatunga & Snoeks (2003), *L. auritus* and *L. parvidens* share a close affinity. The placement of *L. lethrinus* within this clade is surprising since Eccles & Lewis (1978) suggested that *L. lethrinus*, *L. argentea*, *L. leptodon*, and *L. lunaris* constitute a distinct lineage within *Lethrinops*. As more *Lethrinops* spp. are added, the specific lineages with this old genus may be resolved.

Lethrinops lethrinus builds a pit bower with steep sides (Table 2), which is surrounded by four or more ($\bar{X} = 8.1 \pm SE = 3.7$) satellite mounds constructed of flocculent mud. A few *L. lethrinus* individuals were observed with two pits within their territory. *L. auritus* builds a bower consisting

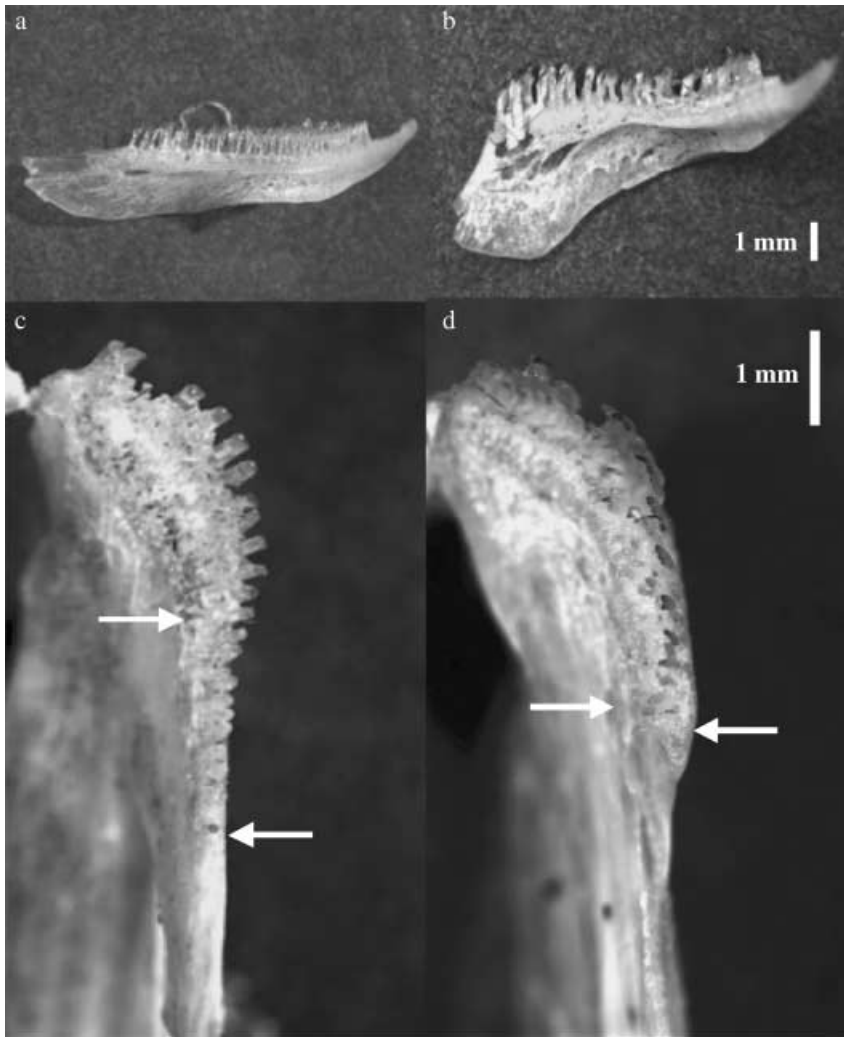


Fig. 6 Pharyngeal and oral jaw morphology used to classify Lake Malawi cichlid taxa. The upper pair of photos displays a lateral view of the lower pharyngeal jaw of (a) *Taeniolethrinops praeorbitalis* and (b) *Tramitichromis variabilis*. The ventral inclination of the anterior blade distinguishes members of the genus *Tramitichromis* from *Lethrinops* and *Taeniolethrinops*. The lower pair of photos displays a dorsal view of the lower oral jaws of (c) *Protomelas similis* and (d) *Taeniolethrinops praeorbitalis*. Arrows to the left and right of the jaws indicate the termination of the inner and outer (respectively) rows of teeth. Typical 'haplochromine' dentition (c) is characterized by the outer row of oral dentition continuing posteriorly after the termination of the inner row. In contrast, *Lethrinops* dentition (d) is defined by the outer row of teeth curving inward and terminating immediately behind the inner row of teeth.

of a large central mound (Fig. 2b) which is surrounded by 8–19 smaller satellite mounds ($\bar{X} = 12.9 \pm \text{SE} = 3.9$) arranged in concentric circles. *L. parvidens* built the smallest volcano bower observed in this study (Table 3).

Tramitichromis

Eccles & Trewavas (1989) erected the new genus *Tramitichromis* to include species that had the *Lethrinops*-style dentition, but also have an anterior blade of the lower pharyngeal jaw that is directed downward instead of straight out (Fig. 6). The anterior blade of the pharyngeal jaw is the origin for the pharyngocleithralis externus muscle which inserts into the cleithrum, and the pharyngo-hyoideus muscle which inserts into the hyoid (Liem & Kaufman 1984). However, this morphological character does not define a monophyletic lineage (KH $P < 0.0001$). Both pharyngeal jaw dentition and gular coloration were highly variable within this lineage (Table 4). The position

Table 4 Mean proportion of molariform teeth and gular colour for all *Tramitichromis* taxa sampled

<i>Tramitichromis</i> spp.	Mean proportion molariform teeth	Gular colour (pink/yellow)
<i>T.</i> 'pink gular'	0.21	pink
<i>T.</i> 'yellow gular'	0.00	yellow
<i>T.</i> 'deep'	0.17	yellow
<i>T.</i> 'shallow'	0.18	yellow
<i>T.</i> 'black rock'	0.00	yellow
<i>T.</i> 'microstoma-type'	0.00	yellow
<i>T. variabilis</i>	0.00	polymorphic

of *Tramitichromis variabilis* makes *Tramitichromis* paraphyletic as *Copadichromis*, *Protomelas*, and *Taeniolethrinops* are contained within this lineage.

One population within this lineage was found displaying over rock at Otter Point. These fish courted females

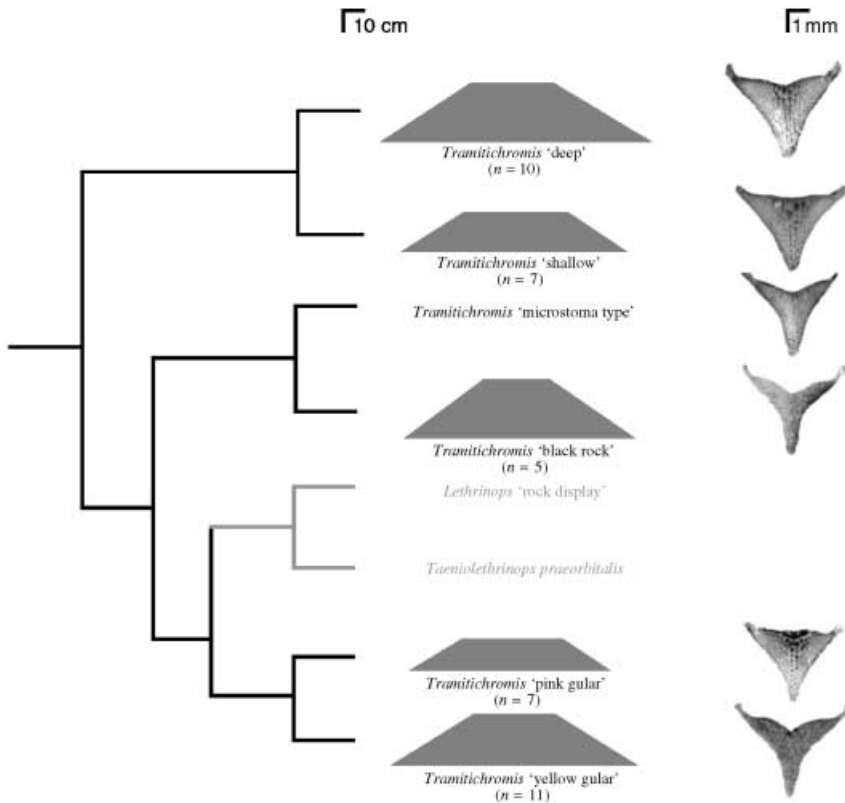


Fig. 7 The *Tramitichromis* lineage demonstrates parallel evolution of bower form and pharyngeal jaw dentition. While each pair of sister taxa show replicated evolution of tall/narrow vs. short/wide volcano forms, these species exhibit different degrees of trophic differentiation. *Tramitichromis* 'deep' and *T.* 'shallow' possess lower pharyngeal-jaws that share a similar molariform dentition. The pharyngeal jaws of both *T.* 'black rock' and *T.* 'microstoma type' have papilliform dentition. In contrast, *T.* 'yellow gular' and *T.* 'pink gular' have divergent pharyngeal tooth morphology.

from bare rock near the sand/rock interface. Like *P. taeniolatus*, they moved small amounts of sand up to the rock and fashioned it in a ring. While we designated this population as *Lethrinops* 'rock display', its taxonomic status is uncertain. As is typical for *Lethrinops*, the symphysis of the lower oral jaw expands to form a horizontal platform for the teeth (Trewavas 1931), yet the outer row of teeth extends back in a single series (haplochromine tooth form). While these fish have the 'haplochromine' oral dentition, they are strongly embedded within the *Tramitichromis* lineage (KH, $P < 0.001$). While this finding is initially surprising, *Lethrinops* 'rock display' is in the same clade as *Taeniolethrinops praeorbitalis*. Eccles & Trewavas (1989) have reported that specimens of another *Taeniolethrinops* species, *Taeniolethrinops cyrtonotus*, also possess haplochromine-type oral dentition.

While all of the observed species of *Tramitichromis* built a volcano-style bower, there was a great deal of quantitative variation (Table 3). Bowers built by *Tramitichromis* were significantly different for all measurements (ANOVA, $P < 0.05$) except base diameter (ANOVA, $P = 0.26$). This difference in bower size (bower volume) was not correlated with the mean standard length of the population (Pearson, $r = 0.019$, $P = 0.67$). With the exception of *T. variabilis*, all *Tramitichromis* bowers were constructed from fine sand with no perceptible difference in substrate consistency.

Even though *T. variabilis* constructed a volcano composed of pea-sized gravel, the platform (often below the level of the substrate) was made of fine sand. Pairs of sister taxa within this lineage displayed repeated divergence of short-wide and tall-narrow volcano forms (Fig. 7).

Tramitichromis 'black rock' and *T.* 'microstoma-type' are reciprocally monophyletic sister taxa (KH $P = 0.017$). *Tramitichromis* 'black rock' was confined to a small lek of five tall, narrow bowers (Table 3) at Black Rock Beach. These bowers abutted the shallow edge of a *Vallisneria* bed at 3 m (\pm SE = 0) of depth. Even though similar plant beds are located on Chembe Beach, no additional leks of *T.* 'black rock' were found. The population at Black Rock Beach was morphologically similar to *Tramitichromis* 'microstoma-type', which was collected on volcano bowers in 35 ft (10.745 m) deep water off the shore of Otter Point. Unfortunately, time did not permit quantification of the bowers at Otter Point. In general, the *T.* 'black rock' bowers were taller and narrower than those of *T.* 'microstoma-type'. Specimens from both populations possessed 100% papilliform pharyngeal dentition (Table 4).

Tramitichromis liturus species group

Members of the *Tramitichromis liturus* species group (McKaye *et al.* 1993; Stauffer *et al.* 2002) formed two pairs of parallel

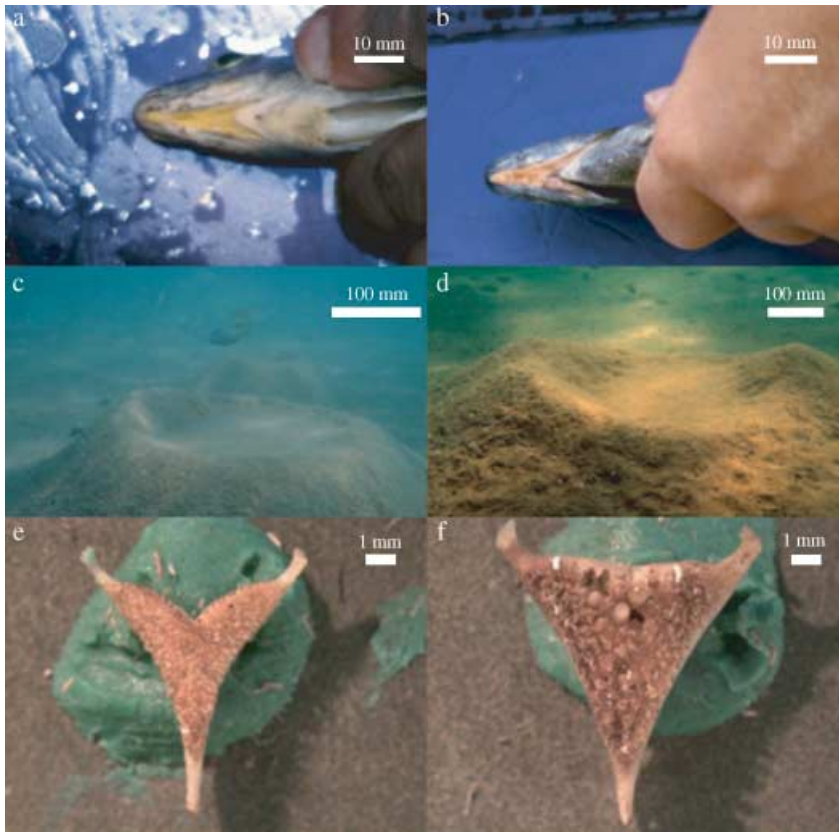


Fig. 8 Morphological and behavioural differentiation of *Tramitichromis liturus* 'yellow gular' (a) and *Tramitichromis liturus* 'pink gular' (b). Despite being separated by less than 1.5 m in depth, these sister taxa are clearly divergent in gular colour, bower form and trophic morphology. *Tramitichromis* 'yellow gular' (c) constructs a tall narrow bower with a level platform and one back-splash (raised platform lip). In contrast, *T.* 'pink gular' (d) constructs a shorter and wider bower (ANOVA, $P < 0.001$) with a concave or slanted platform and 2–3 back-splashes. The lower pharyngeal jaw of *T.* 'yellow gular' has fine papilliform teeth (e), while *T.* 'pink gular' has a larger proportion of molariform teeth (f) (t -test, $P < 0.0001$).

leks with distinct depth distributions (Fig. 1b). No *Tramitichromis* or *Lethrinops* bowers were present in the intervening depths between these four distinct leks. The two populations in each pair are reciprocally monophyletic sister taxa (KH $P < 0.01$).

In the first pair, individuals found in the shallow lek (2.0–3.0 m $\bar{X} = 2.47 \text{ m} \pm \text{SE} = 0.16$) constructed a highly symmetrical tall narrow bower with sharp crisp edges. The platform was generally level and made of very finely packed sand (Fig. 8c). The lip of the volcano was typically raised on one end to form a 'backsplash'. The platform area under this backsplash is where egg deposition occurs and is made up of finely sifted sand. All males caught over these tall, narrow bowers possessed a yellow gular (Fig. 8a). In contrast, all males caught in the parallel, deeper lek (3.5–4.5 m, $\bar{X} = 3.9 \text{ m} \pm \text{SE} = 0.22$) had pink-orange gulars (Fig. 8b). *Tramitichromis* 'pink gular' constructed short, wide bowers with a curved or poorly defined platform that occasionally sloped directly into the lake floor/substrate. The platform lip had rounded edges and two to three raised sections (Fig. 8d). *Tramitichromis* 'pink gular' and *T.* 'yellow gular' bowers were significantly different for every measurement (Table 3, ANOVA, $P < 0.001$ for all measurements), except base diameter (ANOVA, $P = 0.24$) and lip height (ANOVA, $P = 0.54$). Surprisingly, the slightly smaller (ANOVA, $P =$

0.058) *T.* 'yellow gular' built the larger bower. While superficially similar, *T.* 'pink gular' and *T.* 'yellow gular' possessed significantly different pharyngeal dentition (Table 4, t -test, $P < 0.0001$). The lower pharyngeal jaw of *T.* 'yellow gular' had narrow posterior horns, and small papilliform teeth (Fig. 8e). Conversely, *T.* 'pink gular' had robust posterior horns and several large molariform teeth (Fig. 8f).

Approximately 200 m east, a second pair of parallel *Tramitichromis* leks were found. These populations also appear to be reciprocally monophyletic sister taxa (KH $P = 0.013$) and were designated *T.* 'deep' and *T.* 'shallow'. Males occupying the shallow lek (3.5–4.0 m $\bar{X} = 3.69 \text{ m} \pm \text{SE} = 0.16$) constructed short, wide bowers with crisp lip edges and flat platforms (Table 3). Males in the deeper lek (4.5–5.5 m $\bar{X} = 4.89 \text{ m} \pm \text{SE} = 0.21$) built tall, narrow bowers with rounded lip edges and slanted platforms, which were often oblong or triangular. *Tramitichromis* 'deep' and *T.* 'shallow' bowers were significantly different for every measurement ($P < 0.001$ for all measurements), except base diameter (ANOVA, $P = 0.07$) and lip height (ANOVA, $P = 0.81$), but there was no discernable difference in each population's standard length (ANOVA, $P = 0.28$). This pair of sister taxa also have similar pharyngeal dentition (Table 4, t -test, $P = 0.39$) and their gulars were a similar shade of yellow.

Discussion

Speciation — ecology vs. sexual selection

Our results show that both ecologically and sexually selected traits are differentiated among the most closely related species of bower-building cichlids, lending credence to the idea that an interaction of natural and sexual selection is responsible for the rapid speciation of this group of fishes. The 3-stage model proposed by Danley & Kocher (2001) would suggest that sexual selection is the dominant force of divergence during recent speciation events. Every pair of sister taxa in this study differed in sexually selected characteristics and several sister taxa constructed significantly different bowers without apparent trophic differentiation. Species within the genus *Copadichromis* construct a wide range of bower types, but are all zooplanktivores (Turner 1996). The sister taxa *Tramitichromis* 'deep' and *T.* 'shallow' share similar oral and pharyngeal dentitions and have leks that are separated by only 1 m of depth, yet they build bowers that are significantly different in size. Similarly, both *T.* 'black rock' and *T.* 'microstoma-type' share similar trophic morphology, but build their bowers in different habitats. These taxa are congruent with models that propose that sexual selection can drive speciation without ecological differentiation (Wu 1985; Turner & Burrows 1995; Higashi *et al.* 1999; Takimoto *et al.* 2000; Lande *et al.* 2001). Intrageneric comparisons of other rift valley cichlid species have also generated empirical support for these models. Mate choice experiments have demonstrated that colour morphs of the Lake Victorian cichlid, *Haplochromis nyererei*, are reproductively isolated even though they are ecologically indistinguishable in the wild (Seehausen & van Alphen 1998). Similarly, species within the Lake Malawi genus *Metriaclima* display a dramatic diversity of male colour pattern yet show little ecological divergence (Danley & Kocher 2001).

While all pairs of sister taxa constructed different bowers, several taxa were also trophically differentiated. *Tramitichromis* 'pink gular' and *T.* 'yellow gular' possess different pharyngeal jaw morphologies, in addition to differences in gular colour and bower form (Fig. 8). The robust muscular process and large molariform teeth of *T.* 'pink gular' is indicative of a diet based on gastropods or shelled insects (Barel *et al.* 1977; Greenwood 1984; Trapani 2003). In contrast, the slender muscular process and narrow papilliform teeth of *T.* 'yellow gular' are associated with a diet of softer benthic invertebrates. Louda *et al.* (1983) found that maximum gastropod densities occur over a very narrow depth range (1.5–4.5 m), suggesting that competition for trophic resources may exist over restricted spatial scales. Within this same lineage, *Lethrinops* 'rock display' also possess molariform pharyngeal teeth while *Taeniolethrinops praeorbitalis* has papilliform dentition.

While more populations need to be sampled to ascertain the relationship between these two taxa, it is clear that there has been repeated trophic divergence within this lineage (Fig. 7). Additionally, ecological divergence (inferred from morphology) has been reported for species within the sand-dwelling genera *Trematocranus* (Eccles & Trewavas 1989), *Otopharynx* (Arnegard & Snoeks 2001) and *Protomelas* (Stauffer 1993).

While this pattern of differentiation may be specific to the sand-dwelling lineage, ecological divergence appears to be common in many other cichlid lineages. Trophic differentiation has been reported for species within Lake Malawi's rock-dwelling genus *Melanochromis* (Bower & Stauffer 1997) and subgenus *Pseudotropheus tropheops* (Genner *et al.* 1999; Albertson 2002). The oral dentition of the Eretmodini tribe in Lake Tanganyika exhibits considerable morphological differentiation (Poll 1986). Species of Lake Natron and Lake Magadi tilapiine flocks have divergent oral dentition (Tichy & Seegers 1999). Trophic specialization has also been important during the formation of the West African (Schliewen *et al.* 2001) and Neotropical (Barluenga & Meyer 2004) crater-lake species flocks.

The divergence of sister taxa by both natural and sexual selection is not uncommon among cichlid lineages. In addition to those taxa observed in this study, many closely related cichlid species differ in pharyngeal jaw morphology (Eccles & Trewavas 1989; Smits *et al.* 1996; McKaye *et al.* 2002; Barluenga & Meyer 2004). Unlike the morphological characters important during stickleback (Rundle *et al.* 2000) or *Anolis* trophic differentiation, it is difficult to envision direct female choice for divergent pharyngeal jaw dentition. In Darwin's finches male courtship song is strongly influenced by beak morphology (Podos 2001). Similarly, several sand-dwelling cichlids also make sound during courtship (Lobel 1998). However, while stridulation of the pharyngeal apparatus produces these sounds, they appear to be genus-specific rather than species-specific. Although the pharyngeal jaw activity of *Tramitichromis intermedius* appears to be sexually dimorphic (Rice & Lobel 2002), there is not yet evidence of female preference for any sound characteristic. Additionally, there is no obvious direct connection between pharyngeal jaw morphology and either gular colour or bower form. While the genetic architecture underlying these traits is currently unknown, quantitative trait loci (QTL) studies may give us a better understanding of how each character is shaped by both natural and sexual selection (Albertson *et al.* 2003).

Meyer (1987) suggested that phenotypic plasticity could explain the majority of morphological variation observed within the African species flocks. However, this was predicted on the assumption that New and Old World cichlids displayed a similar magnitude of plasticity. Meyer further noted that the results of Witte (1984) suggested that the

effect of plasticity was less pronounced in Old World lineages, a result further corroborated by Smits *et al.* (1996) and Huysseune (1995). While both studies demonstrated that diet induced morphological plasticity of the lower pharyngeal jaw, this variation is not sufficient to fully explain the magnitude of interspecific variation observed in this study. Further there are reports of strong genic control of pharyngeal jaw dentition in both New (Trapani 2003) and Old World (Smits *et al.* 1996) lineages. Kornfield & Taylor (1983) suggest that pharyngeal dentition (molariform vs. papilliform) is controlled by a single locus, with papilliform dominant to the molariform dentition. Even if the morphological variation observed in this study is the result of phenotypic plasticity, it would still indicate that some sister taxa are trophically divergent regardless of the mechanism of morphological differentiation (Trapani 2003).

Bower evolution

Bower form is more evolutionarily labile than previously expected. McKaye (1991) was the first to suggest that species with similar bower forms have arisen from a common ancestor. Stauffer *et al.* (2002) further suggested that general bower design could be used as a genus defining synapomorphy, implying phylogenetic constraint. However, our AFLP phylogeny indicates that multiple bower forms are found within the same lineage. Furthermore, both the pit and volcano bower forms are found in each major lineage, indicating repeated convergence on these forms. While mound building is found within only one major lineage, it does not appear to define a monophyletic group of species. These results are in sharp contrast to bowerbirds in which the two major bower forms (avenue and maypole) form distinct clades (Kusmiński *et al.* 1993, 1997).

While bower form is not a reliable character for defining genera, it does appear to be species specific. AFLP markers clearly demonstrate that populations which differ qualitatively or quantitatively in bower forms are genetically distinct species. Each population that we initially identified by bower type, even those separated by only a metre in depth, appears to be a genetically distinct species. For example, *Lethrinops* populations from opposite sides of the Nankumba Peninsula, but which make the same bower, are more closely related to each other than are *Tramitichromis* populations that make slightly different bowers only a metre apart. This result tends to support the use of bower shape in the definition of species (e.g. Stauffer *et al.* 1993).

Even though bower form appears to be species specific, we must be cautious using bower form as a taxonomic character due to our lack of knowledge concerning the relative importance of genetics and environment on bower form. Certainly, the bowers of some sister taxa differ by discrete changes. The *Copadichromis* lineage appears to be

made of two distinct clades: rock- and sand-based bower types. *Protomelas taeniolatus* carry sand up to the rock surface to form rings that are reminiscent of shallow volcanoes and are more closely related to the volcano-building species than they are to the pit-building *Protomelas similis*. Many sister taxa within the *Tramitichromis* lineage show replicated divergence (Fig. 7) of volcano size (tall narrow/short wide), location (depth, weed bed), and lip/crown construction (defined/undefined). However, while McKaye *et al.* (1993) suggested that divergence in bower form was correlated with genetic distance, there is no direct evidence that bower form is heritable. Only one species in this study was sampled at multiple locations (*Lethrinops auritus*), so the magnitude of intraspecific bower variation is unknown. Stauffer & Sato (2002) suggest that the newly described *Copadichromis atripinnis* may be closely related to *Copadichromis geertsi*, and *Copadichromis trewavasae* due to the shared incorporation of a rock in their bower. However, Lake Tanganyika's *Cyathopharynx furcifer* exhibits geographic variation in rock use during bower construction (Rossiter & Yamagishi 1997). Further geographic sampling and rigorous laboratory experimentation will be necessary to tease apart the relative effects of genetics and environment on bower construction.

While the interplay of forces shaping cichlid bowers are still poorly understood, it has been suggested that bower evolution is driven by intense sexual selection via female choice (McKaye *et al.* 1990, 1993; Stauffer & Kellogg 1996). McKaye (1991) has suggested that the wide range of bower forms utilized by Lake Malawi cichlids implies that there is no adaptive 'optimum' bower form. The great variation we observe in bower form may be driven entirely by sexual selection. Even though males provide no resources or parental care, many studies have demonstrated female mating preferences within the lek (McKaye *et al.* 1990; McKaye 1991; Stauffer & Kellogg 1996; Kellogg *et al.* 2000). Female *Copadichromis eucinostomus* preferentially visited and circled with males over taller bowers (McKaye *et al.* 1990). Similarly, Kellogg *et al.* (2000) were able to correlate bower height and location with female visitation in *Lethrinops parvidens*. However, they were unable to find any bower characteristic that was correlated to the later stages of the spawning sequence: circling and spawning. Female *Otopharynx argyrosoma* choose centrally located bowers, regardless of height (McKaye 1991). However, it is still uncertain if aspects of bower shape or location provide a direct selective advantage to the parents through increased protection against egg predation by hetero- and conspecifics (McKaye 1984).

Most discussions of bower evolution have bypassed the questions regarding the role of natural selection and bower heritability, and instead have focused on which model of sexual selection is appropriate. McKaye (1991) proposed that bower divergence is driven by Fisherian runaway

selection as modelled by Lande (1981, 1982). While variation in the male character is well documented (McKaye 1984, 1991; McKaye *et al.* 1990; Kellogg *et al.* 2000) variation in female preference is assumed from variable male success (McKaye 1991). However, this ignores the possibility that females utilize multiple cues during male assessment. Additionally, no data concerning the heritability of either bower form or female preference have ever been presented. Taylor *et al.* (1998) found a negative correlation between parasite load and male reproductive success, as well as a positive correlation between parasite load and bower height skew (ratio of minimum and maximum bower height) in *C. eucinostomus*. While this suggests that bower shape may be an honest indicator of a male parasite resistance, Taylor *et al.* (1998) did not find any correlation between bower height skew and female preference. Seehausen & Schluter (2004) have recently suggested that character displacement due to male–male aggression has driven the diversification of Lake Victoria's rock-dwelling cichlids. Males possessing a novel colour pattern would receive less aggression from nearby conspecifics and have a selective advantage during territory acquisition. If applied to Lake Malawi's bower-building cichlids, this model would predict that bowers of recently evolved sister taxa would be interdigitated on the same area. Our results indicated that while leks of different species often overlap, recently evolved sister taxa are found in mutually exclusive leks (Fig. 1b). Undoubtedly, individual bower characters have different or multiple discrete functions (conspecific recognition, male/male competition, intra-specific female choice, ecological constraint, reduction of egg predation). Rigorous experimental manipulation will be needed to tease apart the various aspects of individual character function.

Sand dweller evolution and diversity

This study has identified several cryptic species within the genus *Tramitichromis*, suggesting that sand dweller species richness has been substantially underestimated. Genner *et al.* (2004) contend that estimates of Lake Malawi species richness have been exaggerated due to the incorporation of putative allopatric species. They achieved a 'more realistic' estimate of species richness by collapsing these estimates and limiting alpha taxa to species, or colour variants, that can be found in sympatry. However, the presence of cryptic species makes estimating alpha diversity very difficult. McKaye *et al.* (1993) recognized five distinct taxa within what they referred to as the *Tramitichromis liturus* species group; however, they were unable to differentiate between populations using allozyme markers. Turner (1996) agreed that *Tramitichromis liturus* was poorly defined and was likely a complex of related species. On Chembe Beach, we have identified seven separate species belonging to the

genus *Tramitichromis*. Of the five species currently recognized (Eccles & Trewavas 1989), only two descriptions correspond with the species we surveyed at Chembe Beach. Continued sampling of additional beaches and taxa may uncover more cryptic species and result in a startling increase in the magnitude of species richness in Lake Malawi cichlids.

Populations of sand dwellers demonstrate a higher degree of population substructuring than previously expected. In contrast to the stenotypic *mbuna*, species within the sand-dwelling lineage were thought to have large effective population sizes and wider geographic distributions (Taylor & Verheyen 2001; Turner 2002). This impression has been bolstered by recent studies that found no significant population substructuring in pelagic *Diplotaxon* species (Shaw *et al.* 2000) or populations of the sand-dwelling *P. similis* (Pereyra *et al.* 2004). However, Taylor & Verheyen (2001) were able to detect weak, but significant structure between widely dispersed *Copadichromis* 'virginialis kajose' and the lithophilic *Protomelas taeniolatus*. Our AFLP data clearly resolved the populations of *Lethrinops auritus* collected from opposite sides of the Nankumba Peninsula. Additional collections will be needed to assess the effect of migration on the evolution of these diverse sand-dwelling communities and its potential impact on fisheries conservation.

The stunning diversity of Lake Malawi cichlids will require rigorous molecular studies to identify distinct species and identify appropriate units of conservation (Soltis & Gitzendanner 1999). Sand-dwelling cichlids make up a vital component of the Malawian diet (Munthali 1997; Ribbink *et al.* 2001) and are heavily fished. *Copadichromis* and *Lethrinops* species are a significant portion of both artisanal and commercial harvests (Turner 1994; Duponchelle & Ribbink 2000). However, despite their economic importance, our knowledge of sand dweller taxonomy is extremely limited. At least 30–50% of the species lack formal description (Turner 1996) and many of the current species descriptions are based on a single museum specimen or lack a type locality (Eccles & Trewavas 1989). Systematic collections of sand-dwelling cichlids were traditionally performed using demersal trawling, which would fail to perceive the cryptic diversity identified in this study. Barlow (2002) has indicated that the technology is available for long-term storage of behavioural data. Coupled with improving database technology for molecular data (Hong & Chuah 2003), the holistic holotype necessary to describe species in this incredibly diverse system is within our grasp.

Conclusions

The ever-increasing complexity of the Lake Malawi system continues to challenge our ability to model the forces behind cichlid diversification. Our results suggest that the forces of natural and sexual selection work both independently and synergistically during the formation of

new species within this natural laboratory of evolution. The parallel evolution of colour pattern, trophic morphology and bower form diminish their effectiveness as taxonomic characters. The co-occurrence of sister taxa separated by only 1–2 m of depth suggests strong isolating mechanisms and repeated displacement of depth preference during sympatric speciation or colonization by allopatric taxa during lake level fluctuations. The presence of cryptic species suggests a severe underestimation of the species diversity within the sand-dwelling cichlid lineage and will complicate fisheries and conservation policy.

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