

## Preliminary genetic analysis of koaro (*Galaxias brevipinnis*) in New Zealand lakes: Evidence for allopatric differentiation among lakes but little population subdivision within lakes

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**Abstract** Lake systems are often notable for the diversification of freshwater fish species. This has yet to be documented in New Zealand, although a possible case has been described in *Galaxias brevipinnis* from Lake Okareka, where lake- and stream-resident fish show morphological and ecological differences. Samples of *G. brevipinnis* collected from three New Zealand lakes (Wakatipu, Wanaka, and Okareka) were examined using microsatellite DNA analysis to look for evidence of genetic structuring among samples within lakes (possibly indicative of incipient sympatric speciation or natal stream loyalty). Comparison among lakes was made to provide a context for genetic structuring within lakes. There was evidence of genetic differentiation among lake populations, reflecting original founder effect and subsequent genetic drift. However, there was little evidence of restricted gene flow among samples from different parts of lakes and their tributaries. Namely, there is no genetic evidence of incipient speciation in Lake Okareka, and little or no evidence of stream loyalty in Lake Wakatipu. However, the situation in Lake Okareka warrants further analysis using more microsatellite loci and wider sampling.

**Keywords** *Galaxias brevipinnis*; galaxiid; genetics; lake; microsatellites; speciation

### INTRODUCTION

Lake systems are noted for radiations of freshwater fish, a prime example being the species complexes of cichlid fishes in the African rift lakes (Echelle & Kornfield 1984). In temperate regions, there are many examples of formation of species pairs, typically in postglacial lakes (Taylor 1999). The three-spined stickleback (*Gasterosteus aculeatus*) has undergone rapid radiation into benthic and limnetic morphs in lakes of British Columbia (McPhail 1993). Salmonid species often form species pairs, such as brook charr *Salvelinus fontinalis* (McLaughlin 2001) and arctic charr *Salvelinus alpinus* (Jonsson & Skulason 2000) within American and European lakes. The osmerid fish *Osmerus mordax* has evolved sympatric dwarf and normal forms in North American lakes (Taylor & Bentzen 1993). Many of these examples provide evidence for sympatric speciation, as opposed to speciation that occurs as the result of geographical isolation (Tregenza & Butlin 1999).

One of the driving forces of speciation in postglacial lakes appears to be that the fish fauna is often depauperate, with many unexploited ecological niches (Taylor 1999). Sympatric

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**Fig. 1** New Zealand lakes from which *Galaxias brevipinnis* were sampled. Lake Wakatipu tributary samples are indicated by numbers as follows: 1, Kingston; 2, Wye Creek; 3, 12 Mile Stream; 4, Little Stony Creek; 5, Turner Creek.

speciation results as a consequence of exploitation of these niches (Schluter 1996). The speed of ecological specialisation in lakes may be fast, with reports of reproductive isolation between different ecotypes of *Oncorhynchus nerka* within 13 generations (Hendry et al. 2000).

The speciation processes observed in Northern Hemisphere lakes postdate the Pleistocene glaciation (10 000–12 000 years ago), approximately the same time that many New Zealand lakes, including Lakes Wakatipu and Wanaka (Fig. 1), were formed (Suggate et al. 1978). It is possible that genetic differentiation of this type may be taking place within New Zealand lakes.

The Galaxiidae is a family of osmeriform fishes that might be predicted to undergo radiation in New Zealand. Galaxiids are found in temperate regions of the Southern Hemisphere and comprise approximately 50 species in 6 genera (Waters et al. 2000a). In New Zealand, the Galaxiidae is the dominant freshwater fish family, represented by at least 20 species in 2 genera (McDowall 2000). Many galaxiids are restricted to freshwater but five New Zealand species are diadromous, with a regular (although not obligate) juvenile marine phase which returns to streams as tiny transparent “whitebait” in the spring (McDowall 1990). Landlocked populations of diadromous species have whitebait-like pelagic larvae that enter lakes soon after hatching. Juveniles return to lake tributaries where they spend the rest of their lives (McDowall 1990). Some adult populations have been reported from lakes, although often in low densities (McDowall 1970; Young 2002).

Early isozyme work found little genetic structuring within diadromous galaxiid species (Barker & Lambert 1988; Allibone & Wallis 1993). The latter study found high levels of

differentiation among populations of the non-migratory galaxiids, including *G. vulgaris*. Allibone & Wallis (1993) speculated that this structuring could indicate speciation within *G. vulgaris* sens. lat.; this was confirmed by further morphological and isozyme studies (Allibone et al. 1996). Subsequent morphological analyses led to the identification of four new species and the redescription of another (McDowall & Wallis 1996; McDowall 1997; McDowall & Chadderton 1999). Four additional divergent lineages have been identified but are yet to be described, bringing the total number of *G. vulgaris* sens. lat. lineages to 10 (Waters & Wallis 2000, 2001a,b). The sister group of the *G. vulgaris* complex is the morphologically similar diadromous koaro, *G. brevipinnis*. MtDNA analysis has suggested that the *G. vulgaris* complex is polyphyletic and implies three convergent losses of diadromy (Waters & Wallis 2001b). The *G. brevipinnis*-derived radiation probably dates to the Pliocene/late Miocene (McDowall & Pole 1997), with a possible link to the vast lake systems of Central Otago of that time (Waters & Wallis 2001a).

In addition to these divergent older non-migratory derivatives, koaro have many landlocked populations formed both naturally (postglacial, volcanic) and anthropogenically (hydroelectric impoundments). A case of possible within-lake differentiation has been identified in the volcanic Lake Okareka with morphological and ecological differences detected between lake-resident and stream-resident fish (Young 2002). We predict that these differences may represent a case of incipient sympatric speciation, which is also proposed to have occurred in Australian *Paragalaxias* (McDowall 1998).

Philopatry is a term used to describe the tendency of individuals to return to or stay in a particular area, commonly the birthplace. In many Northern Hemisphere fish, an extreme form of philopatry, natal river loyalty, has been documented by tagging and inferred from genetic analysis (Carlsson & Nilsson 2000; Waters et al. 2000b; Bentzen et al. 2001; Miller et al. 2001; Quinn et al. 2001). River loyalty in these fish involves repeated return to the natal watercourse for the purpose of breeding (anadromy), probably using olfactory cues (Hasler & Scholz 1983; Ditman & Quinn 1996). The galaxiids are different in that there is only a single migration to and from the sea (or lake in this case) as a small juvenile. Their return to the stream is not associated with breeding, and there is little growth or development in the larger body of water (McDowall 1988). Little information has been collected concerning the possibility of stream loyalty in galaxiids (McDowall & Eldon 1980; Barker & Lambert 1988; Allibone & Wallis 1993), but Waters et al. (2000c) found no evidence of stream loyalty in diadromous *G. maculatus* along the length of New Zealand. Lake populations have yet to be examined for stream loyalty or genetic structuring.

This study uses microsatellite DNA analysis to (1) look for evidence of genetic structure within lake populations of koaro, possibly indicative of incipient speciation or natal river loyalty, and (2) look for evidence of genetic structure among lake populations of koaro, which may be indicative of allopatric speciation.

## MATERIALS AND METHODS

### Sampling

Adult koaro were collected using a combination of electric fishing and hand netting from five sites around Lake Wakatipu (Fig. 1; Table 1). Sites were selected from streams with previously recorded koaro populations and chosen to give a broad geographical spread over the lake. The fish were sedated (2-phenoxyethanol) before fin clip samples were taken from caudal or pectoral fins. Samples were preserved in 70% ethanol. At one site (Wye Creek), only juvenile fish were caught. These fish were exposed to a lethal dose of 2-phenoxyethanol (Iwama & Ackerman 1997) and stored in 70% ethanol. All samples were stored at  $-20^{\circ}\text{C}$ .

Koaro from Lake Okareka were caught by Fyke net or electrofishing. Fin clips or large pieces of tissue were preserved in 95% ethanol.

Genomic DNA was extracted following a 5% Chelex 100 protocol (Walsh et al. 1991) and stored at  $-20^{\circ}\text{C}$ . PCR amplifications for the microsatellite loci (*Gvu4*, *Gvu5*, and *Gvu7*) were performed following Waters et al. (1999). PCR products were run on 8–9% non-denaturing polyacrylamide gels (19:1 acrylamide:bis-acrylamide) and visualised by SYBR GREEN 1 (Roche) staining. Allele sizes were determined with comparison to a 20 base pair size ladder standard (BIORAD). Subpopulations from Lake Wanaka were pooled for all analyses.

### Data analysis

GENEPOP (version 3.1b; Raymond & Rousset 1995) was used to perform exact tests for Hardy-Weinberg equilibrium and population differentiation among pairs of populations. GENEPOP also calculates estimates of population parameters including allelic frequencies, isolation by distance, and  $F$  statistics. Gene diversity ( $H_e$ ) was measured for each population at each locus using an unbiased estimator (Nei 1987) by FSTAT 2 (Goudet 1995).

$F_{IS}$ ,  $F_{ST}$ , and  $F_{IT}$  were estimated for all populations across all loci by FSTAT 2 (Goudet 1995).  $F_{IS}$ ,  $F_{ST}$ , and  $F_{IT}$  represent correlation of alleles in individuals within subpopulations, among subpopulations, and within total, respectively. A fourth hierarchical level (lake) was introduced permitting partitioning within and among lakes.  $F_{IL}$ ,  $F_{LT}$ , and  $F_{IT}$  partition variation among lakes (ignoring subpopulations), and  $F_{IS(WAK)}$ ,  $F_{SL(WAK)}$ , and  $F_{IL(WAK)}$ ;  $F_{IS(WAN)}$ ,  $F_{SL(WAN)}$ , and  $F_{IL(WAN)}$ ;  $F_{IS(OKA)}$ ,  $F_{SL(OKA)}$ , and  $F_{IL(OKA)}$  represent values within and among subpopulations for each lake (WAK, Lake Wakatipu; WAN, Lake Wanaka; OKA, Lake Okareka).

GENETIX was used to perform factorial correspondence analysis (AFC) of allelic data (Belkhir et al. 1999). This technique is used to project individuals into multidimensional space on the basis of allelic information, with each allele analysed as an independent variable.

PRIMER 5 (Clarke & Gorley 2001) was used for multidimensional scaling (MDS) of  $F_{ST}$  estimates produced by GENEPOP. MDS matrices represent samples as points such that the relative distances between points are in the same rank order as the relative dissimilarities of the samples.

**Table 1** Details of koaro collections.  $N$  = number of fish. Grid references are from NZMS 260 map series.

Site name	Lake	$N$	Year collected	Grid reference
Kingston	Wakatipu	36	2001	F42/768348
Wye Creek	Wakatipu	48	2001	F42/761547
12 Mile Stream	Wakatipu	41	2001	E41/588616
12 Mile Stream	Wakatipu	37	1991	E41/588616
Little Stony Creek	Wakatipu	34	2001	E41/477794
Turner Creek	Wakatipu	35	2001	E40/412904
Bradys Creek	Wanaka	5	1991	G38/313624
Camerons Creek	Wanaka	5	1991	G38/331667
Glendhu Bay Creek	Wanaka	5	1991	F40/933076
Lake Okareka	Okareka	12	2001	U16/043324
Okareka stream mouth	Okareka	40	2001	U16/037324
Okareka stream mid	Okareka	5	2001	U16/034328

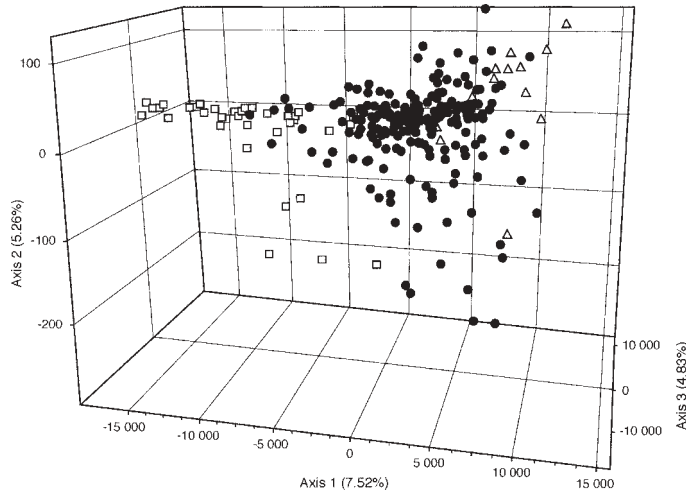
## RESULTS

Three microsatellite loci (*Gvu4*, *Gvu5*, and *Gvu7*) were found to be highly polymorphic, with 6, 13, and 10 alleles, respectively. Allele frequencies were found to be highly variable among lakes. Most striking were differences observed for *Gvu7* between South Island and North Island populations (Table 2). *Gvu7* was monomorphic in Lake Okareka samples (*Gvu7*–78) but segregated 5–9 alleles in samples from Lakes Wakatipu and Wanaka.

Two significant deviations from Hardy-Weinberg equilibrium ( $P < 0.05$ ) were recorded at *Gvu4* that are attributable to the presence of a rare homozygous individual in each case. *Gvu5* showed heterozygote deficits in all samples, possibly indicating a null allele. Wanaka showed heterozygote excess for *Gvu7* ( $P < 0.05$ ).

**Table 2** Allele frequencies, expected heterozygosity and sample size ( $n$  = number of alleles scored) for three microsatellite loci in 10 collections of *Galaxias brevipinnis*.

Locus	Allele	Population									
		Kingston	Wye Creek	12 Mile Stream 2001	12 Mile Stream 1991	Little Stony Creek	Turner Creek	Lake Wanaka	Lake Okareka	Okareka Stream mid	Okareka Stream mouth
<i>Gvu4</i>	132	0.083	0.032	0.073	0.054	0.104	–	–	–	–	–
	140	–	0.011	–	–	–	–	–	–	–	
	144	0.056	0.011	0.037	0.081	0.090	0.043	–	0.583	0.300	0.575
	148	0.333	0.347	0.390	0.270	0.403	0.343	0.100	0.417	0.700	0.425
	152	0.431	0.474	0.354	0.446	0.299	0.543	0.767	–	–	–
	153	0.097	0.105	0.146	0.149	0.104	0.071	0.133	–	–	–
	156	–	0.021	–	–	–	–	–	–	–	–
<i>n</i>	72	95	82	74	67	70	30	24	10	80	
<i>Gvu5</i>	129	–	0.010	–	–	–	–	–	–	–	–
	132	0.086	0.031	0.085	0.027	0.029	0.057	0.033	–	–	–
	135	–	–	–	–	–	–	–	0.100	–	0.103
	140	0.086	0.021	0.110	0.122	0.015	0.057	0.067	–	–	–
	141	0.043	0.052	0.037	0.081	0.074	0.086	0.033	0.100	0.100	0.064
	144	0.114	0.188	0.122	0.162	0.029	0.129	0.167	0.300	0.200	0.064
	147	0.143	0.167	0.183	0.270	0.250	0.229	0.400	0.150	0.300	0.179
	150	0.086	0.229	0.207	0.054	0.147	0.057	0.167	–	–	0.013
	153	0.043	0.021	0.049	0.014	0.044	–	–	0.050	–	0.038
	156	0.029	0.031	0.024	0.054	–	0.071	–	0.150	0.100	0.244
159	0.329	0.229	0.171	0.203	0.324	0.271	0.033	0.150	0.300	0.295	
165	–	–	–	–	0.029	–	–	–	–	–	
171	0.043	0.021	0.012	0.014	0.059	0.043	0.100	–	–	–	
<i>n</i>	70	96	82	74	68	70	30	20	10	78	
<i>Gvu7</i>	78	0.671	0.585	0.563	0.716	0.522	0.621	0.357	1.000	1.000	1.000
	80	–	0.043	0.038	–	0.030	0.030	0.107	–	–	–
	82	–	–	–	–	0.015	0.015	–	–	–	–
	84	0.071	0.128	0.150	0.095	0.149	0.136	0.036	–	–	–
	88	0.157	0.128	0.087	0.095	0.104	0.061	0.357	–	–	–
	90	0.014	0.011	0.025	–	0.030	0.015	0.036	–	–	–
	94	0.071	0.096	0.125	0.081	0.134	0.076	–	–	–	–
	96	–	–	0.013	–	–	–	–	–	–	–
	98	–	–	–	–	0.015	0.030	–	–	–	–
	100	0.014	–	–	–	–	–	0.107	–	–	–
102	–	0.011	–	0.014	–	0.015	–	–	–	–	
<i>n</i>	70	94	80	74	67	66	28	24	10	79	
$H_e$		0.686	0.699	0.737	0.673	0.737	0.682	0.649	0.461	0.425	0.436



**Fig. 2** Multivariate (AFC) analysis of *Galaxias brevipinnis* based on allelic data from three microsatellite loci. Subpopulations from each lake were pooled to form total lake populations. Squares, Lake Okareka; circles, Lake Wakatipu; triangles, Lake Wanaka.

**Table 3**  $F$  statistics over all loci for all subpopulations of *Galaxias brevipinnis*. I, individuals; S, subpopulations; T, total.

Locus	$F_{IS}$	$F_{ST}$	$F_{IT}$
<i>Gvu4</i>	0.059	0.132	0.183
<i>Gvu5</i>	0.101	0.023	0.121
<i>Gvu7</i>	-0.019	0.085	0.067
Multilocus	0.058	0.076	0.129

**Table 4** Summary of  $F$  statistics within and among lake populations of *Galaxias brevipinnis*. IL, total within lakes; SL, among subpopulations within lakes; IS, within subpopulations in lake.

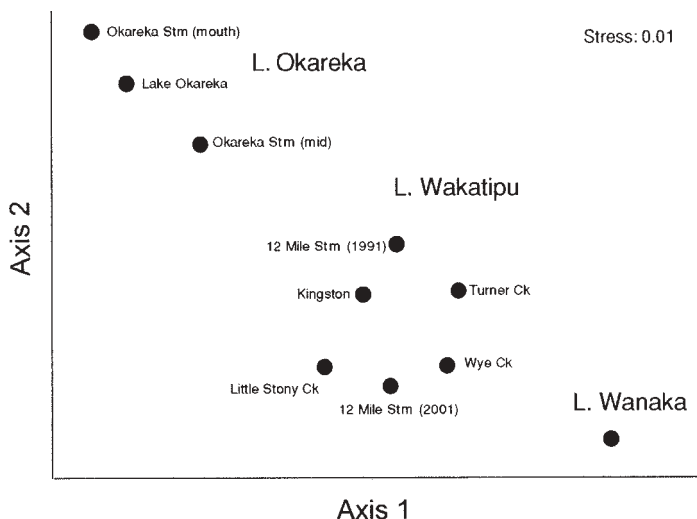
Lake	$F_{IS}$	$F_{SL}$	$F_{IL}$
Wakatipu	0.04	0.006	0.047
Wanaka	0.224	-0.037	0.196
Okareka	0.121	0.005	0.125

Significant values ( $P < 0.05$ ) were observed in five tests for population differentiation among subpopulations of Wakatipu, three of which included Little Stony Creek.  $P$  value estimates for population differentiation between lake-resident and stream-resident fish in Okareka yielded a non-significant value ( $P = 0.5578$ ).

Multivariate analysis (AFC) of microsatellite data yielded groups of individuals from the same lake in partly distinctive clusters (Fig. 2). The South Island lakes tended to group together away from Okareka. Multidimensional scaling (MDS) also produced distinctive groups of lake subpopulations (Fig. 3), with Wanaka again more similar to Wakatipu than Okareka. All lakes included in the study were found to be highly differentiated with highly significant results obtained for each comparison ( $P < 0.0001$ ). These results are evidence of genetic differentiation among Wakatipu, Wanaka, and Okareka, reflecting original founder effect and subsequent genetic drift.

***F* statistic analysis**

$F_{IS}$  values were close to zero for *Gvu4* and *Gvu7* but slightly higher for *Gvu5* (Table 3). A large  $F_{ST}$  value was observed for *Gvu4* which may be explained by the Okareka samples having only two alleles at this locus, lacking *Gvu4*-152. Overall variation in Wakatipu was



**Fig. 3** MDS similarity distance matrix of  $F_{ST}$  estimates for subpopulations of *Galaxias brevipinnis*. The subpopulations from the three lakes form distinct clusters. The low stress value indicates consistency of among-lake differentiation.

small ( $F_{IL(WAK)}$ ), with most of the variation explained by differences within subpopulations ( $F_{IS(WAK)}$ ) (Table 4). Variation in Wanaka ( $F_{IL(WAN)}$ ) was the highest of the three lakes, with differences within subpopulations ( $F_{IS(WAN)}$ ) being the major contributor (Table 4). However, the Wanaka collections comprised only 15 fish, so more samples need to be gathered before conclusions can be made on the importance of these values. The  $F_{IL(OKA)}$  value for Okareka is intermediate to the other two lakes, and once again almost totally explained by high  $F_{IS(OKA)}$  (Table 4).

Analysis of lakes without subpopulation specification showed that differences among lakes ( $F_{LT} = 0.152$ ) accounted for a large proportion of the total variation ( $F_{IT} = 0.204$ ).

## DISCUSSION

The microsatellite data show highly significant differentiation among all three lakes ( $F_{LT} = 0.152$ ), but little evidence for natal river loyalty or other genetic differentiation within lakes.

### Genetic differentiation within lakes

Morphological and ecological differences between stream-resident and lake-resident fish have been reported for Okareka (Young 2002). Lake fish from Okareka grew larger than stream-resident fish, but the latter were significantly heavier than lake fish of the same total length (Young 2002). Otolith growth rates in stream fish slowed after 3 years compared with lake fish in which steady growth of otoliths continued for at least 5 years (Young 2002). Spawning sites and times differed between lake and stream populations, suggesting that the life history traits are not interchangeable. However, our population differentiation tests between lake-resident and stream-resident fish based on three microsatellite loci yielded a non-significant result. A similar study used five microsatellite loci to test for genetic differentiation between two forms of brook charr (pelagic and littoral) in two Canadian lakes that date to approximately 15 000 years (Dynes et al. 1999). Genetic differentiation between the forms was seen in only one of the lakes despite clear morphological and ecological differences.

Koaro prefer rapid, tumbling rocky streams in native forest, and populations are effectively eliminated from deforested streams (McDowall 1970). Homing would allow adults to return to spawn in forested streams, and would thereby increase egg survival. However, we found little evidence to support natal river loyalty in koaro. Most variation within lakes was explained by differences within subpopulations, not among subpopulations (Table 4). Population differentiation comparisons between subpopulations of Wakatipu revealed only five significant results, with three involving Little Stony Creek. This could be explained by natural fluctuations in gene frequencies due to high female fecundity and high mortality at the larval stage, as observed in *G. maculatus* (McDowall & Eldon 1980) and used to explain population differentiation observed in that species (Barker & Lambert 1988). Alternatively, migration by juveniles from Little Stony Creek into Wakatipu may be hindered; consequently, Little Stony Creek may be more self-recruiting than other sampling sites in Wakatipu.

### **Genetic differentiation among lakes**

Lake populations of koaro have been landlocked and presumably reproductively isolated for 10 000–12 000 years (Suggate et al. 1978). Formation of such populations may have been aided by koaro's ability to climb steep rock faces to reach the stream and lake habitats above waterfalls. This allows koaro to penetrate much farther inland than other diadromous species (McDowall 1970). Climbing is assisted by the large ventral-facing pectoral and pelvic fins, which can grip damp surfaces (McDowall 1970). Consistent with their supposed isolation, distinct lake populations were found to be highly differentiated for microsatellite DNA. Much of the variation was attributable to differences among lakes, with relatively little structure within lakes (Table 4).

These results echo findings from other studies examining postglacial differentiation of fish populations. Murdoch & Hebert (1997) found evidence for genetic differentiation among populations of brown bullhead (*Ameiurus nebulosus*) from different lakes of the Great Lakes of North America. Analysis of populations of lake whitefish (*Coregonus clupeaformis*) also revealed large genetic differentiation among different lake populations (Bernatchez & Dobson 1990; Lu et al. 2001).

### **Future directions**

Clear evidence has been obtained that genetic differentiation caused by allopatric isolation and subsequent restriction of gene flow has occurred among different lake populations of koaro. However, the extent of genetic structure within lakes remains partly unanswered. The lake-resident and stream-resident fish of Lake Okareka are worthy of analysis using larger sample sizes and more microsatellite loci, as well as more detailed morphological and ecological studies. The relative abundance of lake-resident versus tributary populations of koaro, and their level of reproductive interaction, remains little understood. Closer observation of New Zealand lakes without exotic salmonids may reveal larger populations of lake-resident koaro. It may be that the two different ecotypes co-exist widely in lakes around New Zealand. MtDNA analysis of lake koaro could reveal the history of the colonisation of the lakes. If each lake were colonised once only, then each should contain a monophyletic group. This approach has been used to illustrate sympatric speciation in cichlid species pairs in African crater lakes (Schliewen et al. 1994).

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