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RESEARCH ARTICLE

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Population structure in Banggai cardinalfish, *Pterapogon kauderni*, a coral reef species lacking a pelagic larval phase

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Abstract Previous studies on two reef fish lacking a pelagic larval phase (*Acanthochromis polyacanthus* and *Embiotoca jacksoni*) revealed features that may be characteristic of their lifestyle: (1) low levels of gene flow, (2) frequent population bottlenecks, and (3) strong phylogeographic breaks, all within their over 1,000 km coastal geographic ranges. The present study tested the predictive nature of these three characteristics in another species lacking a pelagic larval stage, but with a very restricted distribution ($<10{,}000$ km^2). The Banggai cardinalfish, *Pterapogon kauderni* Koumans, 1933, is a mouthbrooding species occurring in the Banggai Archipelago (eastern Indonesia). Fish were captured in January and February (2001, 2002). The mitochondrial control region of 122 individuals from 22 locations was sequenced. *P. kauderni* individuals clustered in two reciprocally monophyletic clades corresponding to a southwestern population (restricted to the southwest of Bangkulu Island) and all northern and eastern populations, which included all the remaining samples. Data were compatible with reduced gene flow and the presence of severe bottlenecks; however, small sample sizes and limited genetic variability in *P. kauderni* prevented a definitive conclusion. Further studies using larger samples and more rapidly evolving molecular markers may provide enough power to conclusively test our hypotheses.

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Introduction

The great diversity of fish species found on coral reefs is often considered a paradox (Shulman 1998). Indeed, the vast majority of reef fishes exhibit a bipartite life history that includes a dispersive pelagic larval phase, which in turn results in high levels of gene flow (Doherty et al. 1985; Leis 1991; Palumbi 1992, 1994; Leis and McCormick 2002; Taylor and Hellberg 2003). Such high gene flow is presumed to counteract population genetic drift and ultimately speciation (Scheltema 1986; Palumbi 1992, 1994); yet, coral reefs are rich in species, and very poor in species that lack a pelagic larval stage. Thus, there must be high evolutionary or ecological costs in lacking a pelagic larval stage, such as increased vulnerability to environmental changes. Among the 335 species of damselfishes (Pomacentridae), only 3 species are known to lack a pelagic larval phase: *Acanthochromis polyacanthus*, *Altrichthys azurelineatus*, and *Altrichthys curatus* (Robertson 1973; Allen 1999). Among the 250 species of cardinalfishes (Apogonidae) (Nelson 1994; Allen and Morrison 1996), only 1 species mouthbroods its young until settlement, the Banggai cardinalfish (*Pterapogon kauderni*) (Allen and Steene 1995; Vagelli 1999; Allen 2000). In temperate reefs, all 24 members of the surfperch family (Embiotocidae) give birth to fully developed young. Other examples are few and in some cases questionable (Leis 1991; Leis and McCormick 2002). In-depth genetic studies have focused on only two of these species, the spiny damselfish *A. polyacanthus* (Doherty et al. 1994; Planes and Doherty 1997a, 1997b; Planes et al. 2001) and the black surfperch *Embiotoca jacksoni* (Bernardi 2000). These two species share the following characteristics: (1) low levels of gene flow, (2) a propensity for population fluctuations resulting in frequent bottlenecks, and (3) the presence of strong phylogeographic breaks. *Acanthochromis polyacanthus* and *E. jacksoni* have very large geographic ranges, which cover $>1{,}000$ km of coastline. In contrast, the two damselfish species in the genus *Altrichthys* and the

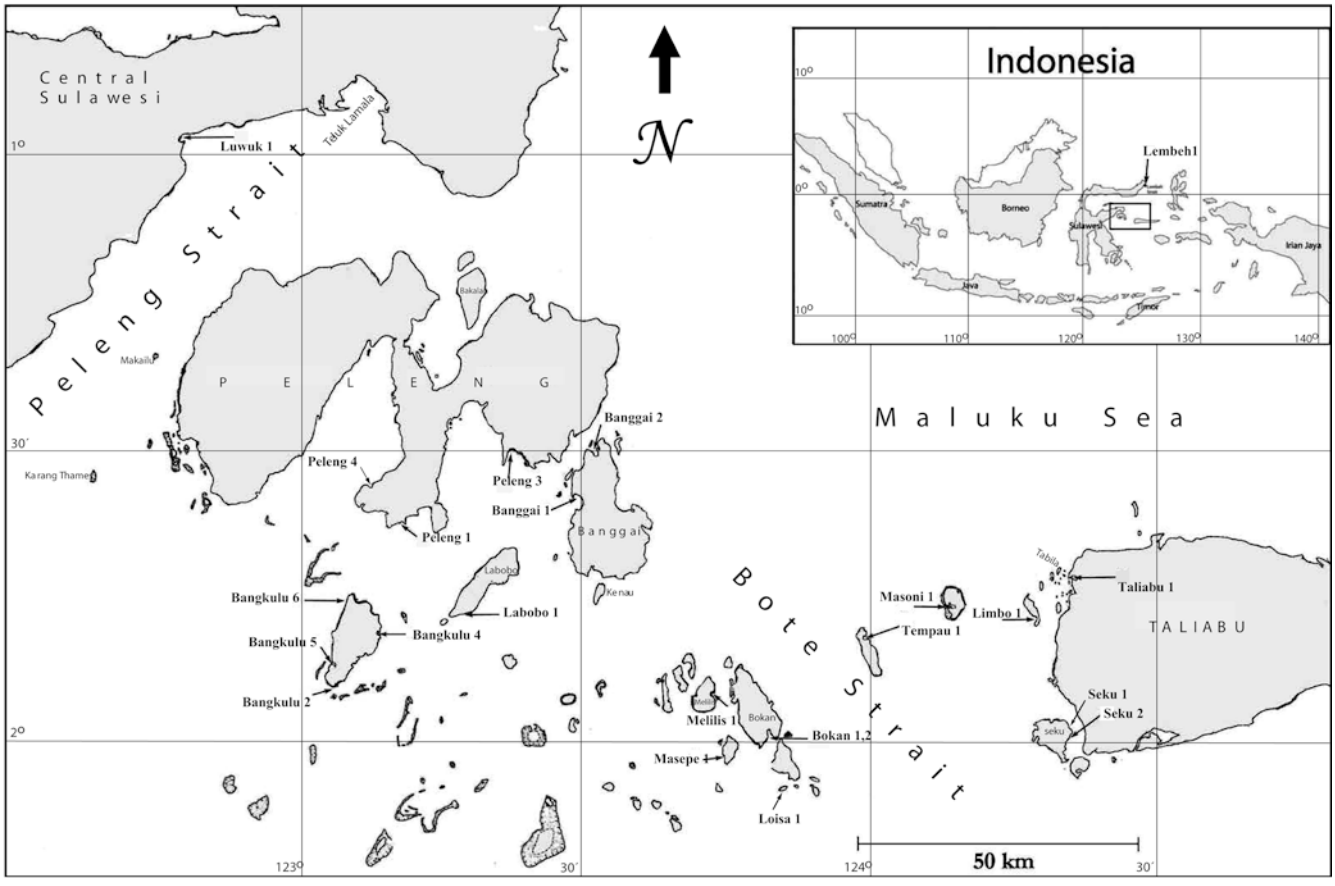

Fig. 1 *Pterapogon kauderni*. Distribution maps and sampling locations

Banggai cardinalfish have very restricted distributions. *Altrichthys* species are found in a restricted region of the Philippines, the Calamian Island group, in the northern Palawan province (Allen 1999). The focus of the present study, the Banggai cardinalfish *Pterapogon kauderni*, also has an extremely limited geographic range, namely $< 10{,}000$ km^2 within the Banggai Archipelago (eastern Indonesia). Its highly endemic distribution encompasses the shallows of only 20 islands (see Table 1; Fig. 1). Also, a small population inhabits Luwuk harbor in central Sulawesi, a very small enclosure separated from the Banggai Island group by the Peleng Strait. In addition, a small (but growing), recently introduced population inhabits the Lembeh Strait (North Sulawesi), ~400 km northwest of the Banggai Archipelago (Erdmann and Vagelli 2001; Vagelli 2002; Vagelli and Erdmann 2002).

Pterapogon kauderni exhibits direct development and lacks a dispersive larval phase. This species has very low fecundity, and the male broods a clutch of ~50 embryos for about 20 days (Vagelli 1999). After hatching, the embryos remain in the parental oral cavity for about 10 days until completion of development. On release, the juveniles take shelter among benthic substrates in shallow waters without leaving the parental habitat (Vagelli 1999; Vagelli and Erdmann 2002). The combination of biological parameters (lack of pelagic dispersal phase, benthic adult habits, low fecundity) and particular oceanographic and physiographic characteristics of the Banggai region have likely contributed to the extreme philopatry (for a marine tropical fish) of *P. kauderni*. In addition, the sedentary nature of the adults and their attachment to shallow substrates in coral reefs and seagrass beds, probably preclude *P. kauderni* from effectively dispersing to nearby islands only a few kilometers away.

The goal of the present study was to evaluate the predictive power of the three characteristics of fishes lacking a pelagic stage (i.e. low levels of gene flow, evidence of bottlenecks, and phylogeographic breaks) in the Banggai cardinalfish, *P. kauderni*. To test these predictions, we used DNA sequences of the rapidly evolving mitochondrial control region in fish collected over a representative part of the species range.

Materials and methods

Fish collections

During two consecutive expeditions (January and February 2001 and February 2002) *Pterapogon kauderni* Koumans, 1933 specimens were collected from 22 locations (21 natural and 1 introduced) throughout its geo-

Table 1 *Pterapogon kauderni*. Collection localities (west to east). Number of individuals included in the study (n), haplotype number, haplotype diversity, and pairwise genetic divergence (Kimura-2 distance) are given. Number of haplotypes, number of variable sites, and mean sequence divergence were calculated using DNAsp (Rozas and Rozas 1999) for samples with $n > 3$. Diversity was calculated according to the equation: $h = (1 - Sx_i^2)n/(n-1)$, where x_i is the frequency of the ith mtDNA type (Nei 1987)

Population	Code	n	Number of haplotypes	Haplotype diversity	Mean percent seq. diverg.	Date of collection
Luwuk 1	LUW1	10	5	0.82	0.37	6 Feb 2002
Peleng 1	PEL1	4	1	0.00	0.00	10 Feb 2001
Peleng 3	PEL3	5	2	0.40	0.20	14 Feb 2002
Peleng 4	PEL4	2	1			19 Feb 2002
Bangkulu 2	BAK2	8	3	0.61	0.18	10 Feb 2001
Bangkulu 4	BAK4	5	3	0.80	0.35	17 Feb 2002
Bangkulu 5	BAK5	5	1	0.00	0.00	18 Feb 2002
Bangkulu 6	BAK6	5	1	0.00	0.00	18 Feb 2002
Labobo 1	LAB1	4	1	0.00	0.00	5 Feb 2001
Banggai 1	BAN1	5	2	0.40	0.20	29 Jan 2001
Banggai 2	BAN2	3	2			12 Feb 2001
Melilis 1	MEL1	4	1	0.00	0.00	15 Feb 2002
Masepe 1	MAP1	5	1	0.00	0.00	16 Feb 2002
Bokan 1	BOK1	5	1	0.00	0.00	31 Jan 2001
Bokan 2	BOK2	8	2	0.43	0.11	15 Feb 2002
Loisa 1	LOI1	7	2	0.28	0.07	31 Jan 2001
Tempau 1	TEM1	3	1			3 Feb 2001
Masoni 1	MAS1	4	1	0.00	0.00	11 Feb 2002
Limbo 1	LIM1	4	1	0.00	0.00	2 Feb 2001
Seku 1	SEK1	10	1	0.00	0.00	1 Feb 2001
Seku 2	SEK2	3	2			8 Feb 2002
Taliabu 1	TAL1	7	1	0.00	0.00	12 Feb 2002
Lembeh 1	LEM1	6	1	0.00	0.00	18 Feb 2001
BAK 2, 4, 5		18	4	0.54	0.16	
Main clade		104	10	0.40	0.18	
Total		122	14	0.47	0.53	

graphical range (Fig. 1; Table 1). Individuals were preserved in 95% ethanol, and tissue samples (fin clips, liver, heart, or muscle) were later dissected from whole individuals, which were kept as voucher specimens (New Jersey Aquarium). Tissues were digested overnight at 55°C in 500 µl of extraction buffer (NaCl 400 mM, Tris 10 mM, EDTA 2 mM, SDS 1%). We purified the DNA by standard chloroform extraction and isopropanol precipitation (Sambrook et al. 1989).

Polymerase chain reaction (PCR) amplification

Amplification of the mitochondrial control region was accomplished using the primers CR-A and CR-E (Lee et al. 1995). All amplifications (25 µl) contained 10–100 ng of DNA, 10 mM Tris HCL (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 U of *Taq* DNA Polymerase (Perkin-Elmer, Norwalk, Conn.), 150 mM of each dNTP, and 0.3 mM of each primer, and used a cycling profile of 45 s at 94°C, 45 s at 48°C, 1 min at 72°C, for 35 cycles. Automated sequencing was performed in both directions with the primers used in the amplification, using an ABI 3100 automated sequencer (Applied Biosystems, Foster City, Calif.).

Molecular analyses

We used the computer program Clustal V implemented by Sequence Navigator (Applied Biosystems) to align the mitochondrial sequences. Phylogenetic relationships were assessed using the neighbor-joining (NJ, Kimura-2 distances) method and the maximum-parsimony (MP) method implemented by the software package PAUP (phylogenetic analyses using parsimony, version 4.0; Swofford 1998). Topological confidence was evaluated with 1,000 bootstrap replicates (Felsenstein 1985). Both population structure statistics used here, pairwise F_{st} and analysis of molecular variance (AMOVA), were estimated using the software package Arlequin (Schneider et al. 1997). Haplotype diversity (H_d) was calculated using the software package DNAsp version 3 (Rozas and Rozas 1999).

Results

In the present study 116 samples of *Pterapogon kauderni* were collected from 21 sampling sites from 14 islands in the natural distribution range of the species (Table 1; Fig. 1). The 14 islands support approximately 90% of the total *P. kauderni* population, which is estimated at about 1.7 million individuals (Vagelli, unpublished data). Specimens from Bandang, Bakakang, Banko, Buangbuang, Kembongan, and Mangoa were not included in the survey. Additionally, we analyzed six fish from an introduced population in the Lembeh Strait, which is outside the natural range of the species (Fig. 1). Sample numbers per locality are given in Table 1.

A 385-bp portion of the mitochondrial control region was sequenced for all individuals. No insertions or deletions (indels) were observed. Out of the 385 base pairs, 13 were variable and 12 were phylogenetically

informative, resulting in 14 haplotypes. As expected, transitions were more frequent than transversions (ratio = 3.8), confirming that substitutions had not reached saturation. Thus, transitions and transversions were equally weighed in our phylogenetic analysis.

Phylogenetic analysis

Samples grouped in two well-separated clades (Fig. 2). The average pairwise sequence divergence between individuals belonging to these two clades was 1.6% (Kimura-2 distance). Their separation was strongly supported by bootstrap analysis (89% NJ and 86% MP bootstrap replicates). One clade consisted of all the samples collected at the three southwesternmost sites from the island of Bangkulu: Bangkulu 2, 4, and 5 (18 individuals). Extensive search south of Bangkulu has not uncovered any *P. kauderni*, so this clade corresponds to the southwesternmost population of the species. The other clade included samples collected from the northern site on Bangkulu Island (Bangkulu 6), as well as all the remaining samples. This clade included the most common haplotype, which was shared by 98 individuals (~85% of all sampled individuals) from 17 sites. In addition, within this clade, five groups were found: (1) all Peleng 3 individuals, (2) all Bangkulu 6 individuals and two Banggai 2 individuals, (3) seven of the Luwuk 1 individuals (out of ten) and one Seku 2 individual, (4) one Banggai 1 individual and one Loisa 1 individual, and (5) one Luwuk 1 individual and six Bokan 2 samples (out of eight) that grouped basally. Bootstrap support for these groups, however, was relatively low, due to the low number of synapomorphies characterizing each subclade. The six introduced individuals collected in the Lembeh Strait grouped in the main cluster with the 98 individuals described above.

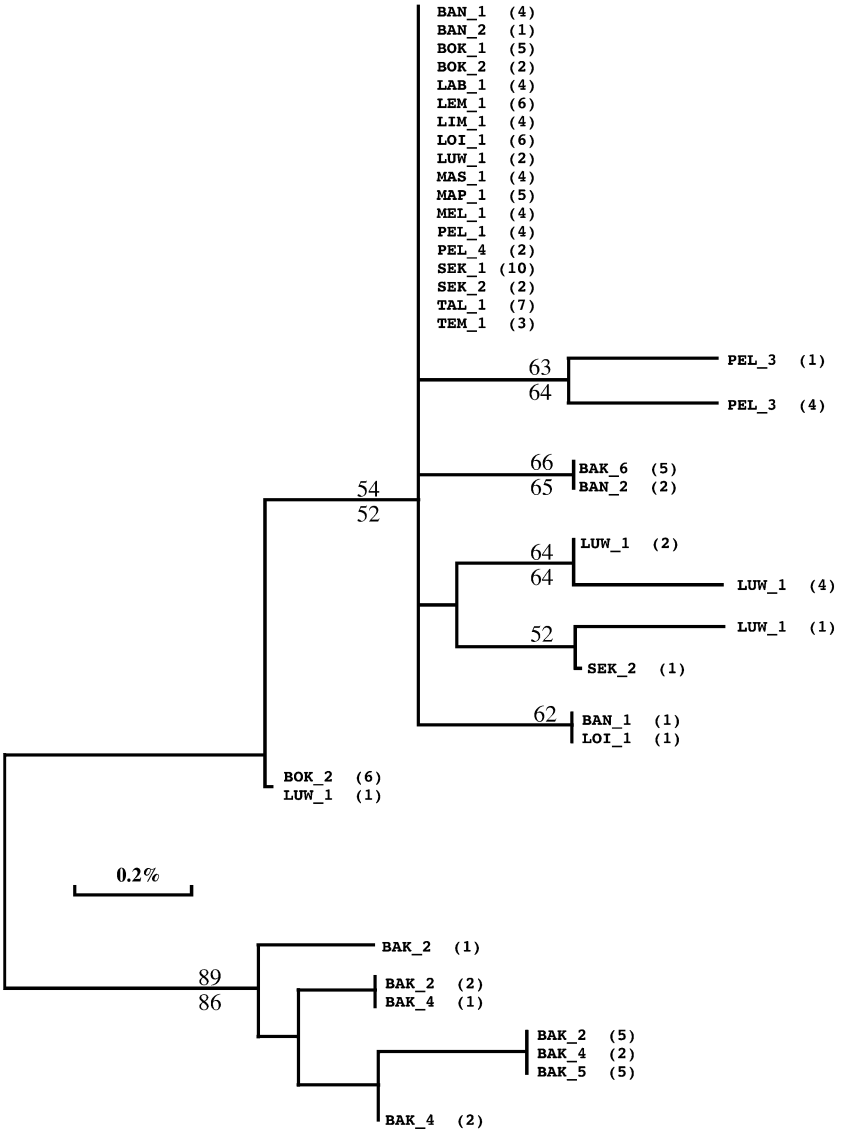

Fig. 2 *Pterapogon kauderni.* Molecular phylogeny based on the mitochondrial control region marker using the neighbor-joining method. Bootstrap support is shown when > 50%: neighbor-joining bootstrap support *above the nodes* and maximum-parsimony bootstrap support *below the nodes*. *Scale bar*: 0.2% Kimura-2 sequence divergence

Genetic diversity among populations

The two clades described above were unequal in size; yet, the haplotype diversity (H_d) of the smaller southern Bangkulu clade (populations 2, 4, 5) was higher than the haplotype diversity of the main clade. In 18 individuals from southern Bangkulu, there were four haplotypes ($H_d = 0.54$), while the main clade had only ten haplotypes for 104 individuals ($H_d = 0.40$). The Bangkulu 4 population had three distinct haplotypes (5 individuals). The eastern edge locality of Taliabu showed, as expected, very low haplotype diversity (7 individuals, 1 haplotype, $H_d = 0.00$). In contrast, the northwestern edge locality of Luwuk showed the highest haplotype diversity (10 individuals, 5 haplotypes, $H_d = 0.82$).

Gene flow among populations

There was no evidence for gene flow between the southern Bangkulu group and the other populations, as individuals from these regions grouped into two reciprocally monophyletic clades. An AMOVA showed that this genetic break was responsible for 87% of the total variance present in our data. Gene flow was also low within clades. Out of 174 "within-clade" pairwise population comparisons, 40% of the F_{st} values (69 comparisons) were > 0.33. At genetic equilibrium this would correspond to the exchange of one migrant per generation ($N_m < 1$), a basis for independent evolutionary development of populations (Avise 1994). In addition, ~50% (83) of the comparisons corresponded to exchanges of fewer than ten migrants per generation (Table 2).

Discussion

The absence of pelagic larval stages in marine organisms is an evolutionary strategy that is uncommon because the life expectancy of the species is generally short (Hansen 1980, 1982; Valentine and Jablonski 1983; Doherty et al. 1985). In addition, limited dispersal prevents colonization over large geographic ranges (Hellberg 1995, 1996), making the species vulnerable to catastrophic events resulting in local or complete extinction. Recent evidence has also linked small geographic range with reduced speciation rates (Gavrilets et al. 2000; Gaston 2003; Jablonski and Roy 2003; Mora et al. 2003). Thus, *Pterapogon kauderni* may be unique in its reproductive strategy and very restricted geographic range. The goal of this project was to evaluate the predictive nature of three characteristics found in *Acanthochromis polyacanthus* and *Embiotoca jacksoni*, two other reef fish lacking a pelagic phase but with wide distributions. We found that our data could not conclusively determine if *P. kauderni* populations: (1) have experienced bottlenecks and (2) show a low level of gene flow, although the data are compatible with these characteristics. Larger sample sizes and more variable molecular markers would help answer these questions.

Our data did show a very strong phylogeographic break (1.6% divergence) between the southwesternmost populations and the remaining populations at the island of Bangkulu. The island of Bangkulu itself is not large (with a perimeter of only 60 km); yet, there was no evidence of gene flow between the southern populations (Bangkulu 2, 4, and 5) and the northern population (Bangkulu 6). Some limited exchange between these populations may occur, but it was not uncovered by our small sample size for the island (23 individuals). Further sampling at these sites, together with some extensive sampling at the boundary region between the northern and southern populations on this island, is crucial to completely describe the situation there. The cause for such a large genetic gap to occur within the island of Bangkulu may be artificial. Indeed, one possibility that may confuse the situation is that population 6, which clusters with the "main" clade (the Bangkulu 6 haplotype is shared with one Banggai 2 haplotype), could be an invasive population that originated (like the Lembeh population) from an accidental introduction by fish traders. The homogeneity of the Bangkulu 6 samples (only one haplotype) may be due to the fact that the northern Bangkulu site may have originally lacked *P. kauderni*. In the absence of human introduction, the alternative explanation for the genetic gap is almost certainly historical, as the genetic divergence between the two clades is large (1.6%). Using generally accepted molecular clocks for fish mitochondrial control regions (10–2% per million years, McMillan and Palumbi 1997; Terry et al. 2000; Bargelloni et al. 2003), the genetic separation between the two main clades of *P. kauderni* occurred 800,000–160,000 years ago, consistent with low sea level periods (Voris 2000). During these low sea level periods, Bangkulu was connected with the rest of the Banggai Archipelago. Thus, low water levels did not divide southern Bangkulu from the rest of the species range. Yet, lowered sea levels, possibly combined with tectonic activity and water currents, could have separated fish populations, which may later have experienced secondary contact on the island of Bangkulu. Southern Bangkulu individuals would therefore be a remnant of an older population. The high genetic diversity in this small group is consistent with an older origin.

The island of Bangkulu is relatively small; yet, in the case of *Acanthochromis polyacanthus*, a large genetic (and coloration pattern) break also occurs within one small island, Hyde Reef (perimeter ~12 km), on the central Great Barrier Reef (Planes and Doherty 1997a, 1997b; Planes et al. 2001). For *A. polyacanthus*, changes in sea water level have been proposed for the physical separation of populations, which was followed by secondary contact. In order to maintain the genetic break over ecological time scales, assortative mating (between color morphs) was proposed for *A. polyacanthus*. We did not detect any morphological differences or mating

Table 2 *Pterapogon kauderni*. Pairwise comparisons of 23 samples (122 individuals). Sample codes from Table 1. Number of migrants per generation (N_m)*above the diagonal*; F_{st} values *below the diagonal* (statistical significance is indicated by +) (*inf*. infinity)

	LUW1	PEL1	PEL3	PEL4	BAK2	BAK4	BAK5	BAK6	LAB1	BAN1	BAN2	MEL1	MAP1	BOK1	BOK2	LOI1	TEM1	MAS1	LIM1	SEK1	SEK2	TAL1	LEM1
LUW1		0.93	0.28	2.17	0.09	0.12	0.08	0.32	1.08	1.00	1.33	1.08	0.93	0.50	0.93	0.83	1.36	1.08	1.08	0.87	1.72	0.74	0.82
PEL1	0.35+		0.13	inf.	0.04	0.05	0.00	0.00	inf.	inf.	0.56	inf.	inf.	0.26	inf.	−9.54	inf.	inf.	inf.	8.50	0.60	inf.	inf.
PEL3	0.64+	0.80+		0.22	0.05	0.07	0.03	0.08	0.14	0.19	0.26	0.14	0.13	0.13	0.13	0.15	0.17	0.14	0.14	0.25	0.36	0.10	0.11
PEL4	0.18	0.00	0.69+		0.06	0.08	0.00	0.00	inf.	−2.22	inf.	inf.	inf.	0.42	inf.	−2.10	inf.	inf.	inf.	0.14	inf.	inf.	inf.
BAK2	0.84+	0.92+	0.90+	0.89+		8.11	27.88	0.03	0.04	0.06	0.07	0.04	0.04	0.06	0.04	0.05	0.05	0.04	0.04	0.07	0.13	0.03	0.04
BAK4	0.80+	0.90+	0.88+	0.85+	0.05		2.52	0.04	0.06	0.07	0.10	0.06	0.05	0.08	0.05	0.06	0.07	0.06	0.06	0.11	0.17	0.04	0.04
BAK5	0.86+	1.00+	0.95+	1.00+	0.01	0.16		0.00	0.00	0.02	0.02	0.00	0.00	0.03	0.00	0.01	0.00	0.00	0.00	0.01	0.07	0.00	0.00
BAK6	0.61+	1.00+	0.86+	1.00+	0.93+	0.92+	1.00+		0.00	0.10	0.56	0.00	0.00	0.09	0.00	0.09	0.00	0.00	0.00	0.09	0.25	0.00	0.00
LAB1	0.31	0.00	0.77+	0.00	0.91+	0.89+	1.00+	1.00+		−10.00	0.80	inf.	inf.	0.30	inf.	−5.60	inf.	inf.	inf.	inf.	4.75	inf.	inf.
BAN1	0.33+	0.00+	0.72+	−0.29	0.89+	0.87+	0.96+	0.83+	−0.05		2.40	−10.00	inf.	0.39	inf.	−3.09	−4.29	−10.00	−10.00	13.00	0.70	6.36	12.00
BAN2	0.27	0.47	0.66+	0.00	0.88+	0.83+	0.96+	0.47	0.38	0.17		0.80	0.56	0.38	0.56	1.33	1.50	0.80	0.80	15.20	0.75	0.35	0.43
MEL1	0.31	0.00	0.7+	0.00	0.91+	0.89+	1.00+	1.00+	0.00	−0.05	0.38		inf.	0.30	inf.	−5.60	inf.	inf.	inf.	0.14	inf.	inf.	inf.
MAP1	0.35+	0.00	0.80+	0.00	0.92+	0.90+	1.00+	1.00+	0.00	0.00+	0.47	0.00		0.26	inf.	−9.54	inf.	inf.	inf.	0.14	inf.	inf.	inf.
BOK1	0.49+	0.65+	0.79+	0.54	0.88+	0.86+	0.94+	0.84+	0.62	0.56+	0.56+	0.62	0.65+		0.26	0.34	0.34	0.30	0.30	0.51	0.52	0.22	0.24
BOK2	0.35+	0.00	0.80+	0.00	0.92+	0.90+	1.00+	1.00+	0.00	0.00+	0.47	0.00	0.00	0.65+		−9.54	inf.	inf.	inf.	0.62	inf.	inf.	inf.
LOI1	0.37+	−0.05	0.76+	−0.31	0.91+	0.89+	0.97+	0.85+	−0.09	−0.19	0.27	−0.09	−0.05	0.59+	−0.05		−3.50	−5.60	−5.60	0.52	inf.	inf.	−21.00
TEM1	0.26+	0.00	0.74+	0.00	0.90+	0.87+	1.00+	1.00+	0.00	−0.13	0.25	0.00	0.00	0.59	0.00	−0.16		inf.	inf.	4.00	0.50	inf.	inf.
MAS1	0.31+	0.00	0.77+	0.00	0.91+	0.89+	1.00+	1.00+	0.00	−0.05	0.38	0.00	0.00	0.62	0.00	−0.09	0.00		inf.	4.00	0.50	inf.	inf.
LIM1	0.31+	0.00	0.77+	0.00	0.91+	0.89+	1.00+	1.00+	0.00	−0.05	0.38	0.00	0.00	0.62+	0.00	−0.09	0.00	0.00		4.00	0.50	inf.	inf.
SEK1	0.46+	0.06	0.66+	0.78	0.87+	0.82+	0.97+	0.85+	−0.01	0.04	0.03	0.78	0.78	0.68+	−0.09	0.49	0.11	0.11	0.11		0.95	inf.	2.50
SEK2	0.22	0.45	0.58+	0.00	0.79+	0.74+	0.85+	0.66+	0.09	0.41	0.40	0.00	0.00	0.49+	0.00	−0.18	0.50	0.50	0.50	0.34		2.25	2.50
TAL1	0.40+	0.00	0.83+	0.00	0.93+	0.92+	1.00+	1.00+	0.00	0.07+	0.58	0.00	0.00	0.69+	0.00	0.00	0.00	0.00	0.00	−0.01	0.18		inf.
LEM1	0.37+	0.00	0.81+	0.00	0.93+	0.91+	1.00+	1.00+	0.00	0.04	0.53+	0.00	0.00	0.67+	0.00	−0.02	0.00	0.00	0.00	0.17	0.00	0.00	

preferences in the laboratory among *P. kauderni* individuals. Another possible reason for the isolation is the lack of suitable habitat between the northern and southern parts of the island, making an effective ecological barrier to migration.

Phylogeographic patterns for three fish species lacking a pelagic larval phase have been described, *Pterapogon kauderni* (present study), *Embiotoca jacksoni*, and *Acanthochromis polyacanthus* (Bernardi 2000; Planes et al. 2001). Sample numbers for *P. kauderni*, *E. jacksoni*, and *A. polyacanthus* were 103,126, and 240, and in all three cases mitochondrial DNA sequences were used: the control region for *P. kauderni* and *E. jacksoni* and cytochrome *b* for *A. polyacanthus* (Bernardi 2000; Planes et al. 2001). Haplotype diversity of *E. jacksoni* and *A. polyacanthus* was very high ($H_d = 0.88$ and $H_d = 0.98$, respectively) compared to *P. kauderni* ($H_d = 0.38$). The low haplotype diversity in *P. kauderni* is likely due to the combined factors of a small geographic range and the population size (estimated at < 2 million individuals using an average density of 0.033 fish m^{-2}). Samples grouped in two (*P. kauderni*, *E. jacksoni*) or three (*A. polyacanthus*) major clades. An AMOVA showed that partitioning the data into these major clades was responsible for similar percentages of the total variance for the three species, 78%, 70%, and 71.8%, for *P. kauderni*, *E. jacksoni*, and *A. polyacanthus*, respectively. The sequence divergence between clades in *A. polyacanthus* (cytochrome *b*) was very high (7.6%). Sequence divergence between control regions of the two main clades of *P. kauderni* and *E. jacksoni* was essentially the same (1.6% and 1.5%, respectively), even though the distribution range of *E. jacksoni* is more than five times that of *P. kauderni*. The barrier to gene flow between the two main clades in *E. jacksoni* was proposed to result from historical factors and to be maintained by ecological factors, but not to be related to distance (Bernardi 2000). Similarly, gene flow between the two main clades of *P. kauderni* may have been caused by historical and oceanographic factors, maintained by ecological factors.

During the last 6–8 years *P. kauderni* has become a very popular aquarium fish, resulting in heavy trading in the Banggai region (Vagelli 2002; Kolm and Berglund 2003; Lunn and Moreau, personal communication). Artificial release has been documented in the Lembeh Strait, where a population introduced in September 2000 has been growing steadily ever since (Erdmann and Vagelli 2001). Six Lembeh individuals were included in our study, and, not surprisingly, all carried the most common haplotype.

The characteristics of the Luwuk site suggest that it could be a place where *P. kauderni* was introduced. This site is isolated from the rest of the range of the species by the deep and broad Peleng Strait, which makes any dispersal between the Banggai Islands and Sulawesi virtually impossible. Luwuk is a restricted, very polluted harbor, which was used by fish traders as a shipping point in the mid-1990s. Local people formerly involved in the fish trade at Luwuk report that it was common practice to release *P. kauderni* specimens from the Banggai Islands in the harbor (Lunn and Moreau, personal communication). Extensive search near Luwuk, but outside the harbor, did not uncover any fishes, suggesting that the Luwuk population is restricted to the harbor. Against our expectations, eight out of the ten Luwuk haplotypes were not shared with other samples, suggesting that the Luwuk population may be at least partly natural. This would support geological evidence of the structural unity of the Banggai Islands–Sula block with the eastern part of Sulawesi East arm (where Luwuk is localized, Hall 1996) and suggest that the Luwuk population is a paleoendemic relict that, at some point in the past, was geographically closer to the Banggai Islands.

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