

Assessment of genetic diversity and hybridization for the endangered Conasauga logperch (*Percina jenkinsi*)

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Abstract

Conasauga logperch *Percina jenkinsi* is one of the rarest fishes in North America afforded protection under the Endangered Species Act. Unfortunately, little is known about potential threats to the genetic diversity of this species. Loss of genetic diversity, spawning of closely related individuals, and hybridization with closely related congeners have been known to increase the rate of extinction for threatened or endangered taxa. We evaluated these risks by estimating and comparing levels of genetic diversity between *P. jenkinsi* and *P. kathae* (a closely related, morphologically similar, and more abundant congener) using twelve microsatellite loci. Specifically, we assessed whether a recent genetic bottleneck occurred in *P. jenkinsi*, determined the potential threat of hybridization between *P. jenkinsi* and *P. kathae*, and evaluated the maintenance of genetic diversity (estimated as effective population size, N_e) between *P. jenkinsi* adults (used as experimental hatchery broodstock) and their progeny. Estimates of genetic diversity between *P. jenkinsi* and *P. kathae* showed no significant differences in average number of alleles (7.083 vs. 9.5; $P = 0.26$), average observed heterozygosity (0.646 vs. 0.600, $P = 0.64$), or average expected heterozygosity (0.634 vs. 0.627, $P = 0.86$). Estimated N_e for *P. jenkinsi* and *P. kathae* was 114 (95% CI 60-526) and -497 (95% CI 264-infinity). We found no evidence of hybridization between *P. jenkinsi* and *P. kathae* and there was no detectable genetic signal of a recent genetic bottleneck in *P. jenkinsi* or *P. kathae*. Results of parentage analysis showed that each male and female broodstock contributed offspring. The average number of offspring for the seven males and two females used as broodstock was 6.71 and 23.5. Based on the number of male and female broodstock, the predicted N_e of the offspring was 6.22 and by incorporating the mean and variance in progeny number, the observed N_e size was 4.97. The relatively high levels of genetic diversity coupled with the estimate of N_e indicated that the relative risks (i.e., decreased fitness) associated with loss of genetic diversity and inbreeding depression for *P. jenkinsi* appeared low and that the experimental hatchery program was successful with regards to maintaining genetic diversity between brood and progeny.

Introduction

The Conasauga logperch *Percina jenkinsi* is perhaps one of the rarest fish species in North America because it is known from only 50 specimens taken from a 44-km reach of the Conasauga River, a tributary of the Coosa River in the Mobile Basin, near the Georgia/Tennessee state line (Etnier and Starnes 1993). It is unusually restricted when compared to other Coosa River endemics (Thompson 1985; George et al. 2010), and because of its restricted distribution and low abundance, *P. jenkinsi* was listed as Federally Endangered in 1985 (USFWS 1985).

While there are no historical records indicating that *P. jenkinsi* ever occupied a more extensive range, the occurrence of sympatric taxa with more widespread distributions suggests that their rarity may be relatively recent and potentially caused by competition with a sympatric member of the subgenus, *Percina kathae* (a widespread species throughout the Mobile Basin; Thompson 1985). Competition with a sympatric congener can have varying outcomes (Moyer et al. 2005), but one potential outcome is the homogenization of two separate taxa via hybridization (Epifanio and Philipp 2001; Scribner et al. 2001). The potential for hybridization between *P. jenkinsi* and *P. kathae* poses a risk for federally endangered taxon because hybridization has contributed to the extinction of many species through direct and indirect means (Rhymer and Simberloff 1996). Furthermore, rates of hybridization and introgression are increasing dramatically worldwide because of translocations of organisms and habitat modifications by humans (Allendorf et al. 2001). Because ichthyofaunal homogenization is occurring at an expeditious pace (McKinney 2006), documenting hybridization for threatened and endangered taxa should be of high priority before extirpation and extinction occur.

An understanding of past and present processes shaping present levels of genetic variation along with monitoring of present levels of genetic diversity is also critical to management and conservation planning because information gleaned from conservation genetics can assist in the proper design, implementation, and monitoring of management and conservation strategies for imperiled species (Schwartz et al. 2007; Laikre et al. 2010). For example, populations or species that have undergone population bottlenecks throughout their evolutionary history may have reduced genetic load (i.e., a reduction in mean fitness of a population resulting from detrimental variation) and be less prone to inbreeding depression during subsequent population bottlenecks (Hedrick 1994; 2001). As a consequence, such a population may have increased viability and be more likely to recover from near-extinction/extirpation than a population lacking such a history (Hedrick 2001).

The objectives of this study were to 1) estimate and compare levels of genetic diversity between *P. jenkinsi* and *P. kathae*, 2) assess whether a recent genetic bottleneck occurred in *P. jenkinsi*, 3)

determine the potential threat of hybridization between *P. jenkinsi* and *P. kathae*, 4) establish a genetic baseline dataset (i.e., estimate average number of alleles, heterozygosity, and effective population size) for future genetic monitoring of *P. jenkinsi*, and 5) evaluate the maintenance of genetic diversity (estimated as effective population size) for *P. jenkinsi* hatchery broodstock.

Methods

Tissue collections were conducted by Conservation Fisheries, Inc. and Tennessee Aquarium Conservation Institute via mask and snorkel (Dinkins and Shute 1996). All tissue samples were placed in 95% non-denature ethanol and archived at to the United States Fish and Wildlife Service Conservation Genetics Lab in Warm Springs GA. Genomic DNA was extracted from each fin clip using the DNeasy Blood and Tissue kit (QIAGEN, Inc., Valencia, California) protocol.

We used a suite of 12 microsatellite markers known to amplify in *P. rex* (Table 1; Dutton et al 2008). Polymerase chain reaction (PCR) amplifications were performed in 10 μ L reaction volumes consisting of 30–100 ng of template DNA, 1 \times *Taq* reaction buffer (Applied Biosystems Inc.), 2.00 mM MgCl₂, 0.318 mM of each dNTP, 0.25 μ M of each primer, 0.08 U *Taq* polymerase (Applied Biosystems, Inc.). Amplifications were conducted using a GeneAmp PCR system 9700 (Applied Biosystems, Inc.) with the following thermal profile: initial denaturation at 94 °C (10 min), followed by a touchdown procedure involving 33 cycles and consisting of denaturing (94 °C, 30 s), annealing, and extension (74 °C, 30 s) cycles, where the initial annealing temperature was initiated at 56 °C (30 s), and decreased by 0.2 °C/cycle. Prior to electrophoresis, 2 μ L of a 1:100 dilution of PCR product was mixed with a 8 μ L solution containing 97% formamide and 3% Genescan LIZ 500 size standard (Applied Biosystems, Inc.). Microsatellite reactions were visualized with an ABI 3130 genetic analyzer (Applied Biosystems, Inc.) using fluorescently labeled forward primers and analyzed using GeneMapper software v4.0 (Applied Biosystems, Inc.).

Tests for gametic disequilibrium (all pairs of loci) and locus conformance to Hardy–Weinberg equilibrium (HWE; for each locus in the sampling site) for each taxon were implemented using GENEPOP v4.0.10 (Raymond and Rousset 1995). Significance levels for all simultaneous tests were adjusted using a sequential Bonferroni correction (Rice 1989).

Estimation of genetic diversity, in the form of per locus and average number of alleles, observed heterozygosity, and expected heterozygosity were calculated for each taxon using the computer program GenAIEx v6.4 (Peakall and Smouse 2006). We tested for differences in average number of alleles, observed heterozygosity, and expected heterozygosity between *P. jenkinsi* and *P. kathae*. Tests

for significance were conducted using the Wilcoxon rank-sum test (Sokal and Rohlf 1995) as implemented in S-Plus v7.0 (Insightful Corporation)

Effective population sizes (N_e) for *P. jenkinsi* and *P. katha*e samples were estimated using the linkage disequilibrium (LD) method (Hill 1981). The measure of LD was that of Burrow's composite measure (Campton 1987) and was estimated for each species using the program LDNe (Waples and Do 2008). Allele frequencies close to zero can affect estimates of N_e (Waples 2006); therefore, we excluded alleles with frequencies less than 0.02 (Waples and Do 2010). Parametric 95% confidence intervals were also calculated using LDNe (Waples and Do 2008; Waples and Do 2010).

We ran the program BOTTLENECK 1.2.02 (Piry et al. 1999) to test whether samples of *P. jenkinsi* and *P. katha*e underwent a recent bottleneck in genetic diversity. To detect a genetic bottleneck signature we first compared the number of loci that present a heterozygosity excess to the number of such loci expected by chance only (i.e., the sign test). We used the infinite alleles model (IAM) and the two phase model (TPM) under default settings. The allele frequency distribution test was also implemented. The test is a graphical one that examines the frequencies of all alleles in a population and compares this to the distribution expected at mutation-drift equilibrium when rare alleles (i.e. 0.1%) are numerous. When a bottleneck occurs, the expectation is that rare alleles will be lost after the event causing a mode-shift in the distribution of alleles (Luikart et al. 1998).

We used the program STRUCTURE v2.3.3 (Pritchard et al. 2000; Falush et al. 2003) to assess the degree of hybridization between each taxon of interest. The program STRUCTURE assumed no a priori sampling information; rather, individuals were probabilistically assigned to groups in such a way as to achieve Hardy-Weinberg and gametic equilibriums. The program STRUCTURE was run with three independent replicates for K (i.e., distinct populations or gene pools), with K set to a value of two. The burn-in period was 50,000 replicates followed by 500,000 Monte Carlo simulations run under a model that assumed no admixture and independent allele frequencies.

Finally, we evaluate hatchery broodstock contribution via parentage analysis by genotyping broodstock and progeny for five microsatellite markers (Prex_41, 42, 44, 45, and 46). Broodstock consisted of nine individuals that were volitionally tank spawned (note that the sex of each individual was unknown). We randomly sampled 47 offspring from this mating aggregate and matched each parent pair using the program PAPA v2.0 (Duchesne et al. 2002). In doing so, we estimated the number of males and female broodstock as well as the number of progeny produced by each male and female. We used this information to calculate the predicted N_e size of the progeny cohort base on the number of male and female broodstock using following equation

$$N_e = \frac{4(N_{male})(N_{female})}{(N_{male}) + (N_{female})}$$

(Wright 1931), where N_{male} and N_{female} were the number of male and female broodstock used to produce hatchery offspring. The predicted effective population size assumes that each individual furnished the same number of gametes to the next generation, an assumption that is often violated due to hatchery propagation (i.e., there is typically greater than binomial or Poisson variability in the number of progeny per parent). We thus compared our predicted value to that of observed using information from the number of progeny produced by each parent. Specifically, the observed estimate of N_e was calculated using the equation

$$N_e \approx \frac{Nk - 2}{k - 1 + V/k}$$

(Kimura and Crow 1963), where N was the number of broodstock and k and V were the mean and variance in offspring number. The mean and variance of the number of progeny per individuals were calculated as

$$k = mk_{male} + (1 - m)k_{female}$$

$$V = mV_{male} + (1 - m)V_{female} + m(m - 1)(k_{male} - k_{female})^2$$

where m was the proportion of male broodstock and k and V were estimate via parentage analysis.

Results

A total of 33 *P. jenkinsi* and 32 *P. kathae* were collected over the course of the study. Nine of the 33 *P. jenkinsi* individuals were subsequently used as broodstock and a random sample of their offspring ($n = 48$) used to estimate the contribution of hatchery broodstock. All individuals were analyzed using 12 microsatellite markers except for the offspring because preliminary parentage analysis simulations indicated that five microsatellites (Prex_41, 42, 44, 45, and 46) would provide enough genetic information to accurately assign offspring to their respective parents with > 95% assignment success (data not shown). Also, Prex_44 failed to produce reliable genotype data for *P. kathae*. Both *P. jenkinsi* and *P. kathae* samples were in HWE after correcting for multiple comparisons (all $P > 0.007$ per taxon; $n = 11$ comparisons per taxon for an $\alpha = 0.005$), and each taxon showed no significant evidence of gametic disequilibrium after sequential Bonferroni correction (all $P > 0.009$ per taxon, $n = 66$ comparisons for an $\alpha = 0.0007$).

A comparison of genetic diversity between *P. jenkinsi* and *P. kathae* (Table 1) showed no significant differences in average number of alleles (7.083 vs. 9.5; $P = 0.26$), average observed

heterozygosity (0.646 vs. 0.600, $P = 0.64$), or average expected heterozygosity (0.634 vs. 0.627, $P = 0.86$). The estimated N_e for *P. jenkinsi* and *P. kathae* was 114 (95% CI 60-526) and -497 (95% CI 264-infinity). In general, negative estimates indicate that the observed LD could be explained by sample size alone (Waples and Do 2010). In this case, the point estimate is uninformative, but the lower bound of the 95% confidence interval can still provide useful information for a lower limit on N_e (Waples and Do 2010).

We found no evidence of hybridization between *P. jenkinsi* and *P. kathae* with STRUCTURE results showing that these two taxa appeared genetically distinct (Table 2). Note that one individual (USFWS 841) was morphologically identified as *P. kathae*, but genetic analysis indicated that the individual was *P. jenkinsi* (Table 1). We observed no detectable genetic signal for a recent genetic bottleneck in *P. jenkinsi* or *P. kathae*. Sign tests for each taxon reported no significant heterozygosity excess (*P. jenkinsi*, IAM model $P = 0.08$, TMP model $P = 0.51$; *P. kathae*, IAM model $P = 0.53$, TMP model $P = 0.11$). Furthermore, there was no mode-shift detected in allele frequencies for each species.

Results of parentage analysis showed that each male and female contributed offspring but at varying amounts (Table 3). The number of offspring produced by seven males averaged 6.71 and ranged from 1-19. The average number of offspring for females was 23.5 (Table 3). Based on the number of male and female broodstock, the predicted N_e of the offspring was 6.22. Incorporating the mean and variance in progeny number, the observed N_e size was 4.97.

Discussion

As expected for a randomly mating population, all loci for sampled *P. jenkinsi* and *P. kathae* were in Hardy Weinberg and linkage equilibria. Genetic diversity estimates based on the average number of alleles and observed heterozygosity for *P. jenkinsi* were somewhat greater than expected for a population with a limited distribution, but values were similar to endangered *P. rex* (Dutton et al. 2008) as well as *P. kathae*. Any genetic signature of a recent bottleneck in genetic diversity went undetected suggesting that if a bottleneck in genetic diversity occurred for *P. jenkinsi*, then it was a more historic rather than recent event. The increased genetic diversity observed in *P. jenkinsi* could have been attributed to a past hybridization event with *P. kathae* (a closely related and morphologically similar congener); however our results, which were congruent with George et al (2010), indicated that *P. jenkinsi* and *P. kathae* are distinct taxa with no indication of contemporary gene flow (hybridization) between them.

General conservation goals based on genetic considerations are frequently established at an $N_e = 50$ to minimize inbreeding depression and an $N_e = 500$ to maintain sufficient evolutionary potential

(Franklin 1980; Franklin and Frankham 1998). Point estimates of N_e from the LD method were negative for *P. kathae* samples, indicating that the observed LD could be explained by sample size alone. While these estimates should be viewed with caution due to small sample sizes (both in terms of the number of individuals and the number of polymorphic markers), the lower bounds of these estimates still provide useful information on the relative sizes of the populations under consideration (Waples and Do 2010). The lower bound N_e for *P. kathae* was 264 and greater than that for *P. jenkinsi* (114) indicating that the population size of *P. kathae* would appear to be greater than that of *P. jenkinsi* in the Conasauga River (which is consistent with abundance data). The empirical point estimate of N_e for *P. jenkinsi* was above the critical threshold level for inbreeding and similar other estimates of N_e for populations of conservation concern (Palstra and Ruzzante 2008). In all, the relatively high levels of genetic diversity coupled with the estimate of N_e indicated that the relative risks (i.e., decreased fitness) associated with loss of genetic diversity and inbreeding depression for *P. jenkinsi* appeared low. Thus conservation efforts for this species should concentrate on protecting, maintaining, or increasing critical habitat for *P. jenkinsi*.

In an effort to better understand the genetic success of the *P. jenkinsi* breeding program, we ascertained whether all or only a few broodstock produced offspring for potential stocking. While we were unsure of the actual number of males and females due to difficulties in the identification of each sex, genetic parentage analyses revealed that the broodstock consisted of two females and seven males. All individuals contributed to the gene pool although at varying degrees. Females contributed 18 and 29 offspring with males contributing anywhere from one to nineteen based on a sample of 47 juveniles. If we look at how this spawning success translated to maintenance of genetic diversity (or lack thereof), our observed estimate of genetic diversity (in this case N_e) rivals that of expected indicating little loss of genetic diversity between broodstock and their respective progeny. Thus the hatchery program was successful with regards to maintaining genetic diversity between brood and progeny.

In conclusion, the importance of genetic variation, as a basis for future biological evolution and long-term viability of populations, species, and ecosystems, is well established (Frankel and Soule 1981; Frankham 1995; Hughes et al 2008). Therefore, identifying and monitoring processes that are likely to have adverse impacts on the conservation of natural populations is an increasingly important endeavor. Unfortunately, most conservation programs do not take full advantage of the potential afforded by molecular genetic markers (Schwartz et al. 2007; Laikre 2010). Genetic data collected in this study will serve as a reference for comparison in an ongoing effort to monitor temporal changes in population genetic metrics as well as assess and predict potential extinction risks associated with genetic

stochasticity. For *P.jenkinsi*, the risk of population decline and extinction due to inbreeding depression and genetic drift appears low. Despite a small contemporary N_e , this species has maintained relatively high levels of heterozygosity and allelic richness. The data presented here also will provide guidance and a means to evaluate the effectiveness (both in terms of increasing the census size and maintaining the long-term viability of the population) for hatchery augmentation in *P. jenkinsi*, if the need should ever arise.

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Table 1. Estimation of *P. jenkinsi* and *P. kathae* genetic diversity in wild and hatchery broodstock. Abbreviation are total number of sample individuals (N), number of alleles (Na), observed heterozygosity (Ho), and expected heterozygosity (He).

Taxon (origin)	Locus	N	Na	Ho	He	
<i>P. jenkinsi</i> (wild)	<i>Prex_31</i>	33	4.000	0.545	0.581	
	<i>Prex_32</i>	33	6.000	0.545	0.592	
	<i>Prex_35</i>	33	6.000	0.697	0.669	
	<i>Prex_36</i>	32	1.000	0.000	0.000	
	<i>Prex_37</i>	33	6.000	0.758	0.745	
	<i>Prex_41</i>	33	11.000	0.879	0.837	
	<i>Prex_42</i>	32	11.000	0.906	0.805	
	<i>Prex_43</i>	33	2.000	0.061	0.114	
	<i>Prex_44</i>	32	8.000	0.938	0.834	
	<i>Prex_45</i>	33	7.000	0.788	0.685	
	<i>Prex_46</i>	33	13.000	0.848	0.873	
	<i>Prex_47</i>	33	10.000	0.788	0.868	
	Average			7.083	0.646	0.634
	<i>P. jenkinsi</i> (hatchery)	<i>Prex_31</i>	14	4.000	0.500	0.594
<i>Prex_32</i>		14	5.000	0.571	0.571	
<i>Prex_35</i>		14	5.000	0.500	0.633	
<i>Prex_36</i>		14	1.000	0.000	0.000	
<i>Prex_37</i>		14	6.000	0.714	0.763	
<i>Prex_41</i>		14	10.000	0.929	0.834	
<i>Prex_42</i>		14	9.000	1.000	0.811	
<i>Prex_43</i>		14	2.000	0.071	0.069	
<i>Prex_44</i>		14	7.000	1.000	0.844	
<i>Prex_45</i>		14	6.000	0.857	0.714	
<i>Prex_46</i>		14	10.000	0.857	0.862	
<i>Prex_47</i>		14	8.000	0.929	0.832	
Average				6.083	0.661	0.627
<i>P. kathae</i> (wild)		<i>Prex_31</i>	32	5.000	0.438	0.452
	<i>Prex_32</i>	32	3.000	0.063	0.090	
	<i>Prex_35</i>	31	11.000	0.581	0.738	
	<i>Prex_36</i>	31	13.000	0.774	0.856	
	<i>Prex_37</i>	32	9.000	0.531	0.538	
	<i>Prex_41</i>	31	14.000	0.935	0.908	
	<i>Prex_42</i>	32	8.000	0.750	0.737	
	<i>Prex_43</i>	31	4.000	0.452	0.552	
	<i>Prex_44</i>	0	0.000	0.000	0.000	
	<i>Prex_45</i>	32	13.000	0.844	0.843	
	<i>Prex_46</i>	29	19.000	0.931	0.930	
	<i>Prex_47</i>	31	15.000	0.903	0.877	
	Average			9.500	0.600	0.627

Table 2. STRUCTURE results for classification of *P. jenkinsi*, *P. kathae*, and potential hybrids. Note that any putative hybrid should have an assignment probability of 0.50 for each taxon.

USFWS ID	Taxon ID	Assignment probability		90% probability interval	
		<i>P. kathae</i>	<i>P. jenkinsi</i>	<i>P. kathae</i>	<i>P. jenkinsi</i>
2_01	<i>P. jenkinsi</i>	0.003	0.997	(0.000,0.015)	(0.985,1.000)
2_02	<i>P. jenkinsi</i>	0.004	0.996	(0.000,0.016)	(0.984,1.000)
2_03	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
2_04	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.010)	(0.990,1.000)
2_05	<i>P. jenkinsi</i>	0.003	0.997	(0.000,0.011)	(0.989,1.000)
2_06	<i>P. jenkinsi</i>	0.007	0.993	(0.000,0.048)	(0.952,1.000)
2_07	<i>P. jenkinsi</i>	0.003	0.997	(0.000,0.012)	(0.988,1.000)
2_08	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
2_09	<i>P. jenkinsi</i>	0.003	0.997	(0.000,0.014)	(0.986,1.000)
2_10	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.010)	(0.990,1.000)
2_11	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
2_12	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
2_13	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.009)	(0.991,1.000)
2_14	<i>P. jenkinsi</i>	0.003	0.997	(0.000,0.014)	(0.986,1.000)
1703	<i>P. jenkinsi</i>	0.004	0.996	(0.000,0.016)	(0.984,1.000)
1704	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.006)	(0.994,1.000)
1705	<i>P. jenkinsi</i>	0.01	0.99	(0.000,0.074)	(0.926,1.000)
1706	<i>P. jenkinsi</i>	0.003	0.997	(0.000,0.013)	(0.987,1.000)
1707	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
1708	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
1709	<i>P. jenkinsi</i>	0.005	0.995	(0.000,0.029)	(0.971,1.000)
1710	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.010)	(0.990,1.000)
1711	<i>P. jenkinsi</i>	0.008	0.992	(0.000,0.051)	(0.949,1.000)
1712	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
1713	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.009)	(0.991,1.000)
1714	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
1715	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
1716	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
1717	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
1718	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
1719	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
1720	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.007)	(0.993,1.000)
1721	<i>P. jenkinsi</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
836	<i>P. kathae</i>	0.998	0.002	(0.994,1.000)	(0.000,0.006)
837	<i>P. kathae</i>	0.998	0.002	(0.991,1.000)	(0.000,0.009)
838	<i>P. kathae</i>	0.998	0.002	(0.992,1.000)	(0.000,0.008)
839	<i>P. kathae</i>	0.998	0.002	(0.992,1.000)	(0.000,0.008)
840	<i>P. kathae</i>	0.993	0.007	(0.952,1.000)	(0.000,0.048)

841	<i>P. kathae</i>	0.002	0.998	(0.000,0.008)	(0.992,1.000)
842	<i>P. kathae</i>	0.995	0.005	(0.979,1.000)	(0.000,0.021)
843	<i>P. kathae</i>	0.996	0.004	(0.983,1.000)	(0.000,0.017)
844	<i>P. kathae</i>	0.997	0.003	(0.986,1.000)	(0.000,0.014)
845	<i>P. kathae</i>	0.998	0.002	(0.993,1.000)	(0.000,0.007)
846	<i>P. kathae</i>	0.995	0.005	(0.965,1.000)	(0.000,0.035)
847	<i>P. kathae</i>	0.999	0.001	(0.994,1.000)	(0.000,0.006)
848	<i>P. kathae</i>	0.998	0.002	(0.993,1.000)	(0.000,0.007)
849	<i>P. kathae</i>	0.998	0.002	(0.993,1.000)	(0.000,0.007)
850	<i>P. kathae</i>	0.996	0.004	(0.982,1.000)	(0.000,0.018)
851	<i>P. kathae</i>	0.997	0.003	(0.989,1.000)	(0.000,0.011)
852	<i>P. kathae</i>	0.998	0.002	(0.991,1.000)	(0.000,0.009)
853	<i>P. kathae</i>	0.997	0.003	(0.987,1.000)	(0.000,0.013)
854	<i>P. kathae</i>	0.998	0.002	(0.993,1.000)	(0.000,0.007)
855	<i>P. kathae</i>	0.994	0.006	(0.969,1.000)	(0.000,0.031)
856	<i>P. kathae</i>	0.998	0.002	(0.992,1.000)	(0.000,0.008)
857	<i>P. kathae</i>	0.998	0.002	(0.991,1.000)	(0.000,0.009)
858	<i>P. kathae</i>	0.997	0.003	(0.990,1.000)	(0.000,0.010)
859	<i>P. kathae</i>	0.998	0.002	(0.992,1.000)	(0.000,0.008)
860	<i>P. kathae</i>	0.993	0.007	(0.962,1.000)	(0.000,0.038)
861	<i>P. kathae</i>	0.998	0.002	(0.990,1.000)	(0.000,0.010)
862	<i>P. kathae</i>	0.998	0.002	(0.990,1.000)	(0.000,0.010)
863	<i>P. kathae</i>	0.99	0.01	(0.924,1.000)	(0.000,0.076)
864	<i>P. kathae</i>	0.998	0.002	(0.992,1.000)	(0.000,0.008)
865	<i>P. kathae</i>	0.998	0.002	(0.992,1.000)	(0.000,0.008)
866	<i>P. kathae</i>	0.998	0.002	(0.993,1.000)	(0.000,0.007)
867	<i>P. kathae</i>	0.997	0.003	(0.987,1.000)	(0.000,0.013)

Table 3. Results from parentage analysis.

Male ID	# offspring	Female ID	# offspring
2_04	5	2_10	18
2_05	8	2_12	29
2_06	19		
2_07	1		
2_08	11		
2_09	2		
2_13	1		
mean	6.714286		23.5
variance	43.57143		60.5