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2013

SEDAR58-RD07

30 January 2018

North American Journal of Aquaculture

ISSN: 1522-2055 (Print) 1548-8454 (Online) Journal homepage: http://www.tandfonline.com/loi/unaj20

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To cite this article: John R. Gold , Melissa M. Giresi , Mark A. Renshaw & Jin-Chywan Gwo (2013) Population Genetic Comparisons among Cobia from the Northern Gulf of Mexico, U.S. Western Atlantic, and Southeast Asia, North American Journal of Aquaculture, 75:1, 57-63, DOI: 10.1080/15222055.2012.713899

To link to this article: http://dx.doi.org/10.1080/15222055.2012.713899

North American Journal of Aquaculture 75:57–63, 2013

ISSN: 1522-2055 print / 1548-8454 online
DOI: 10.1080/15222055.2012.713899

COMMUNICATION

Population Genetic Comparisons among Cobia from the Northern Gulf of Mexico, U.S. Western Atlantic, and Southeast Asia

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Abstract

Nuclear-encoded microsatellites and mitochondrial DNA (mtDNA) sequences were assayed from Cobias *Rachycentron canadum* sampled in waters offshore of Virginia (U.S. Atlantic), Mississippi and Louisiana (Gulf of Mexico), and Taiwan (Southeast Asia). Global exact tests and analysis of molecular variance revealed that Cobias from U.S. waters were homogeneous for alleles and genotype distributions at 27 nuclear-encoded microsatellites and were homogeneous in mtDNA haplotype distribution, whereas both genetic markers in Cobias from Taiwan differed significantly from those of Cobias in U.S. waters. Based on these genetic differences, use of Cobia broodstock from Southeast Asia in U.S. aquaculture facilities is not recommended. Results are compatible with the use of Cobia broodstock from either the U.S. Atlantic or the Gulf of Mexico for aquaculture at U.S. facilities; caveats to the exchange of broodstock between these two regions are discussed.

The Cobia *Rachycentron canadum* is a large, coastal pelagic fish that is widely distributed in tropical, subtropical, and warm-temperate seas except for the eastern Pacific (Shaffer and Nakamura 1989). Cobias in the western Atlantic are distributed from Massachusetts and Bermuda to Argentina (Briggs 1958), but are most common in the Gulf of Mexico (Shaffer and Nakamura 1989). Cobias are prized sport fish because of their large size and food quality; the majority of Cobias caught in U.S. waters are caught by recreational fishers (Shaffer and Nakamura 1989; Franks et al. 1999). Aquaculture of Cobias has been expanding rapidly worldwide, especially in Southeast Asia, as roughly 80% of the world's Cobia production occurs in Taiwan and China (FAO 2007). Currently, facilities for Cobia aquaculture exist in several U.S. Atlantic and Gulf coast states (Benetti et al. 2008). Cobias exhibit many desirable attributes for culture, including ease of spawning and larval rearing, rapid growth, high survival through the first year, and low feed conversion ratios (Benetti and Orhun 2002).

A priority of the Draft Aquaculture Policy of the U.S. National Oceanic and Atmospheric Administration (NOAA 2011) is to ensure the protection of wild species. This concern relates in part to matching the genetic profiles of cultured fish to those of local wild stocks of the same species, thereby mitigating the potential negative genetic impacts caused by escapees or by hatchery fish that are released for restoration programs (Triantafyllidis et al. 2007). Data on the stock structure of Cobias in U.S. waters are sparse. Franks et al. (1991) and Hammond (2001) reported movement of Cobias between the Gulf of Mexico (hereafter, "Gulf") and the U.S. Atlantic coast (hereafter, "U.S. Atlantic"), and Biesiot et al. (1993, cited by Garber et al 2002) could not distinguish between mitochondrial DNA (mtDNA) haplotypes of Cobias that were sampled from localities in the Gulf and the U.S. Atlantic. However, there are differences in growth rate, adult size, and longevity between Cobias in the Gulf and those in the U.S. Atlantic (Burns et al. 1998).

Herein, we report tests of genetic homogeneity among Cobias sampled from offshore waters in the Gulf and the U.S. Atlantic. The study was designed in part to determine whether Cobias in the Gulf and U.S. Atlantic are genetically distinguishable, and if so, to identify genetic markers that could be used to genetically match aquaculture-produced fish with wild stocks in the same geographic region. The study was also prompted by queries from individuals and private companies interested in culturing Cobias in the Gulf; specifically, these parties had

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Received June 7, 2012; accepted July 16, 2012
Published online December 10, 2012

asked whether Cobia broodstock from Taiwan were compatible genetically with Cobias in U.S. waters.

METHODS

Fin clips were taken from 131 wild-caught Cobias that were sampled during the summers of 2010 and 2011 from localities offshore of Virginia ($n = 35$), Mississippi ($n = 46$), Louisiana ($n = 14$), and Taiwan ($n = 36$). Fin clips were fixed in 20% DMSO buffer (Seutin et al. 1991). Whole genomic DNA was extracted by using a Chelex resin (Bio-Rad) extraction protocol (Estoup et al. 1996), and all individuals were genotyped at 28 nuclear-encoded microsatellites. Polymerase chain reaction (PCR) primers, repeat motifs, and annealing conditions are described by Renshaw et al. (2005). Amplification products were electrophoresed by using an ABI 377 automated sequencer (Applied Biosystems, Inc. [ABI], Foster City, California). Gel images were analyzed with GeneScan version 3.1.2 (ABI), and alleles were scored with Genotyper version 2.5 (ABI). Genotypes at the 28 microsatellites for each assayed individual are available at http://agrilife.org/gold/doc/ under the file name "Cobia microsatellite genotypes" (J.R.G., unpublished data). In total, 352 bases from the cytochrome-*b* protein-coding mitochondrial gene were acquired from five individuals from each of the four sample localities. The PCR primers used were H15497 and L15080 (Finnerty and Block 1995). The PCR protocol was as follows: initial denaturation at 95°C for 2 min; 38 cycles of denaturation at 95°C for 30 s, annealing at 53°C for 45 s, and elongation at 72°C for 90 s; and final elongation at 72°C for 20 min. The PCR amplifications were electrophoresed on 2% agarose gels. Successful amplifications were band-cut and cleaned with QIAquick gel extraction kits (QIAGEN). Fragments were sequenced (both directions) by using the amplification primers and ABI BigDye Terminator version 1.1. Products were cleaned with Sephadex columns and were electrophoresed on an ABI 3100 automated DNA sequencer. Sequences were edited and aligned with Sequencher version 3.0 (Gene Codes Corp., Ann Arbor, Michigan).

GENEPOP version 4.0.10 (Raymond and Rousset 1995; Rousset 2008) was used to (1) test genotypes at each microsatellite for conformity to Hardy–Weinberg expectations and (2) execute tests of genotypic equilibrium between pairs of microsatellites. Exact probability tests employed a Markov-chain approach (Guo and Thompson 1992) with 10,000 dememorizations, 1,000 batches, and 10,000 iterations/batch. Sequential Bonferroni correction (Rice 1989) was employed to adjust for the simultaneous use of multiple tests. Occurrence of null alleles, stuttering, and large-allele dropout were evaluated with Micro-Checker version 2.2.3 (van Oosterhout et al. 2004). Estimates of allelic richness, gene diversity, and the inbreeding coefficient F_{IS} were generated by using F-stat version 2.9.3.2 (Goudet 2001). Homogeneity among samples in allelic richness and gene diversity was tested with Friedman rank tests as implemented in SYSTAT version 13 (Systat Software, Inc., Evanston, Illinois); tests between pairs of sample localities employed Wilcoxon's signed rank tests, which were also implemented in SYSTAT. Global tests for homogeneity of allele and genotype distributions (microsatellites) and haplotype distribution (mtDNA) employed (1) exact tests as implemented in GENEPOP and (2) analysis of molecular variance (AMOVA) as implemented in Arlequin version 3.5.1.3 (Excoffier and Lischer 2010). Exact probabilities were estimated by using the same Markov-chain approach as above; significance of the genetic differentiation index F_{ST} (from AMOVA) was assessed by permutation (10,000 replicates) for microsatellites and mtDNA. Exact tests also were used to determine homogeneity of allele and genotype distributions (microsatellites) and haplotype distribution (mtDNA) between pairs of samples by using the same Markov-chain approach as above. The degree of divergence in microsatellites and mtDNA between sample pairs was estimated as F_{ST} and Φ_{ST}, respectively, in Arlequin. The F_{ST} and Φ_{ST} are measures of the extent of genetic divergence among subpopulations and range from 0.0 (no divergence) to 1.0 (complete divergence).

RESULTS AND DISCUSSION

Summary statistics for microsatellites at all four sample localities are given in Table A.1. Significant departures from Hardy–Weinberg equilibrium expectations were detected at 11 microsatellites before Bonferroni correction; only two (*Rca1BD10*, Virginia; and *Rca1H01*, Taiwan) remained significant after Bonferroni correction. Estimates of F_{IS} at both of these microsatellites were positive, indicating a deficit of heterozygotes. Subsequent tests of homogeneity in allelic richness, gene diversity, and allele and genotype distributions across sample localities were performed both with and without these two microsatellites. The results in all cases remained essentially unchanged; hence, the results reported hereafter for these tests included *Rca1BD10* and *Rca1H01*. Analysis with Micro-Checker indicated possible occurrences of null alleles at *Rca1BF06* (Louisiana and Taiwan), *Rca1BD10* (Virginia), *Rca1E05* (Taiwan), and *Rca1H01* (Taiwan). In total, 42 of the 1,512 pairwise tests of genotypic equilibrium were significant before sequential Bonferroni correction. Excluding comparisons of *Rca1BE08A* and *Rca1BE08B*, which were expected to be significant because these microsatellites were isolated from the same clone (Renshaw et al. 2005), none of the pairwise tests remained significant after Bonferroni correction. Because of the expected (and confirmed) tight linkage between *Rca1BE08A* and *Rca1BE08B*, we omitted *Rca1BE08B* from all subsequent analyses.

Average ($\pm$ SE) allelic richness and (unbiased) gene diversity across all microsatellites were 4.69 $\pm$ 0.68 and 0.445 $\pm$ 0.066, respectively, for the sample from Virginia; 4.66 $\pm$ 0.70 and 0.436 $\pm$ 0.067 for the sample from Mississippi; 4.65 $\pm$ 0.68 and 0.462 $\pm$ 0.066 for the sample from Louisiana; and 7.01 $\pm$ 0.69 and 0.691 $\pm$ 0.047 for the sample from Taiwan. Allelic richness and gene diversity differed significantly (Friedman rank test) among the four sample localities (allelic

TABLE 1. Pairwise F_{ST} values (above diagonal) and exact probabilities that F_{ST} was equal to 0 (below diagonal) for pairwise comparisons of three Cobia samples (U.S. Atlantic = Virginia; Gulf of Mexico = Mississippi and Louisiana, pooled; and Taiwan). Values in bold italics indicate significance before and after sequential Bonferroni correction.

Sample	U.S. Atlantic	Gulf of Mexico	Taiwan
U.S. Atlantic		0.003	0.373
Gulf of Mexico	0.096		0.387
Taiwan	***<0.001***	***<0.001***	

richness: $Q_3 = 21.189$, $P = 0.000$; gene diversity: $Q_3 = 21.320$, $P = 0.000$). Wilcoxon's signed rank tests revealed significant differences ($P = 0.000$) in both parameters only in pairwise comparisons involving the sample from Taiwan.

Due to the small size of the Louisiana sample ($n = 14$), exact tests of allele and genotype distributions (microsatellites) were carried out to determine whether the two samples from the Gulf (Louisiana and Mississippi) could be pooled into a single Gulf locality. Exact tests for alleles and genotypes were nonsignificant ($P = 0.530$ and $P = 0.636$, respectively); consequently, all remaining tests of microsatellites utilized three localities: the U.S. Atlantic (Virginia), the Gulf (Mississippi and Louisiana combined), and Taiwan. Global exact tests of the homogeneity of allele and genotype distributions among the three localities were significant ($P = 0.000$ for both allele and genotype distributions). Results from AMOVA also indicated significant genetic heterogeneity ($F_{ST} = 0.292$, $P = 0.000$). Exact tests of pairwise comparisons (Table 1) indicated that only comparisons between Cobias from Taiwan and those from U.S. localities differed significantly both before and after sequential Bonferroni correction. The pairwise comparison between Cobias from the Gulf and those from Virginia was not significant.

The spatial distribution and the GenBank numbers of recovered mtDNA haplotypes are given in Table A.2. Haplotype 1 was the most common in U.S. waters; all five assayed Cobias from Taiwan possessed haplotype 4, which was not found in Cobias from U.S. waters. Significant heterogeneity in haplotype distribution was indicated by a global exact test ($P = 0.000$) and AMOVA ($\Phi_{ST} = 0.623$, $P = 0.000$). Exact tests of pairwise comparisons of haplotype distributions indicated that only the comparisons between Cobias from Taiwan and Cobias from U.S. localities differed significantly both before and after sequential Bonferroni correction (data not shown).

Our results indicate that in terms of both nuclear-encoded microsatellite genotypes and mtDNA sequences, Cobias in U.S. waters differ markedly from Cobias inhabiting the waters off Taiwan. The degree of genetic divergence indicates virtually no gene exchange between Cobias in the western Pacific and those in the western Atlantic, despite the species' pelagic lifestyle and broad distribution. This result is not surprising, given that genetic differences between populations in different ocean basins have been reported for several pelagic fish species (Díaz-Jaimes et al. 2010). We found that Cobias sampled from Taiwan also were more genetically variable than Cobias from U.S. waters, exhibiting significantly greater allelic richness and gene diversity. Reasons for this difference are not known. Nevertheless, to the extent that (presumed) selectively neutral microsatellite alleles and variable mtDNA sequences serve as surrogates for alleles at genes that impact adaptively important life history and production traits, the use of broodfish from Southeast Asia in U.S. aquaculture facilities would appear to be precluded.

Cobias that were sampled from the coastal waters of Virginia, Mississippi, and Louisiana were genetically homogeneous based on assays of microsatellite genotypes and mtDNA haplotypes. This finding is consistent with observed migration patterns and tag-and-release studies of Cobias. Adult Cobias appear to overwinter primarily off the Florida Keys and then undergo seasonal migrations during the spring, moving northward along the U.S. Atlantic as well as to the north and west into the Gulf (Shaffer and Nakamura 1989; Franks et al. 1991). Limited tagging studies (Franks et al. 1991; Hammond 2001) indicate fairly regular mixing of Cobias between the Gulf and U.S. Atlantic. Interestingly, Cobias have been reported to overwinter in deep waters of the Gulf (Franks et al. 1991), and fish tagged in both the Gulf and the U.S. Atlantic have been recaptured near the release locality over 1 year later (Franks et al. 1991; Hammond 2001). The range of sample localities in our study approximates the range where Cobia aquaculture facilities occur in U.S. waters (Benetti and Orhun 2002), suggesting that broodstock from the Gulf or the U.S. Atlantic could be used for Cobia aquaculture in either region.

There are two caveats to the suggestion that Cobias in the Gulf and those in the U.S. Atlantic can be used interchangeably for aquaculture. First, microsatellites generally are presumed to be selectively neutral and are not necessarily indicative of geographic patterns at selectively adaptive genes affecting the quantitative traits that are important to life history or aquaculture production (McKay and Latta 2002). This means simply that there could be adaptively useful alleles at coding genes in Cobias that differ between the Gulf and U.S. Atlantic; in addition, Cobias from the Gulf and U.S. Atlantic reportedly differ in growth rate, adult size, and longevity (Burns et al. 1998). Second, even though a data set of 27 microsatellites is rather large compared to the data sets used in most genetic studies of stock structure in marine fishes (e.g., Carson et al. 2009; Griffiths et al. 2010; Saillant et al. 2012), Cobias possess 24 haploid chromosomes (Jacobina et al. 2011), meaning that there is relatively little genome coverage even when 27 markers are examined. Future studies prompted by interest in this issue will need to utilize next-generation sequencing technology (Mardis 2008; Stapley et al. 2010) to achieve wider genome coverage. A final note is that Cobias are being raised to market elsewhere in the western Atlantic, including Martinique, Mexico, Belize, Panama, and Brazil (Benetti et al. 2008). Generating more complete genetic profiles of Cobias from additional localities in the western Atlantic could be useful relative to permitting decisions about the selection of broodstock for use in U.S. facilities.

ACKNOWLEDGMENTS

We thank B. Falterman, J. Franks, and J. Graves for assistance in procuring specimens from U.S. waters, and K. Burns and J. Franks for comments on a draft of the manuscript. Work was supported by TexasAgriLife (Project H-6703) and by the National Science Council of Taiwan. This paper is Number 89 in the series "Genetics Studies in Marine Fishes" and is Contribution Number 212 of the Center for Biosystematics and Biodiversity at Texas A&M University.

REFERENCES

Benetti, D. D., and M. R. Orhun. 2002. Aquaculture of pelagic fish: IV. Cobia (*Rachycentron canadum*). Global Aquaculture Alliance Advocate 5: 61–62.

Benetti, D. D., M. R. Orhun, B. Sardenberg, B. O'Hanlon, A. Welch, R. Hoenig, I. Zink, J. A. Rivera, B. Denlinger, D. Bacoat, K. Palmer, and F. Cavalin. 2008. Advances in hatchery and grow-out technology of Cobia *Rachycentron canadum* (Linnaeus). Aquaculture Research 39:701–711.

Biesiot, P. M., A. W. Hrincevich, and J. S. Franks. 1993. Mitochondrial DNA analysis of Cobia, *Rachycentron canadum*, from the northern Gulf of Mexico. Mississippi/Alabama Sea Grant Consortium, Final Report for Sea Grant Contract R/LR-26, Ocean Springs.

Briggs, J. C. 1958. A list of Florida fishes and their distribution. Bulletin of the Florida State Museum Biological Science 2:221–318.

Burns, K. M., C. Neidig, J. Lotz, and R. Overstreet. 1998. Cobia (*Rachycentron canadum*) stock assessment study in the Gulf of Mexico and in the South Atlantic. Mote Marine Laboratory, Technical Report 571, Sarasota, Florida.

Carson, E. W., S. Karlsson, E. Saillant, and J. R. Gold. 2009. Genetic studies of hatchery-supplemented populations of Red Drum, *Sciaenops ocellatus*, in four Texas bays. North American Journal of Fisheries Management 29:1502–1510.

Díaz-Jaimes, P., M. Uribe-Alcocer, A. Rocha-Olivares, F. J. García-de-León, P. Nortmoon, and J. D. Durand. 2010. Global phylogeography of the Dolphinfish (*Coryphaena hippurus*): The influence of large effective population size and recent dispersal on the divergence of a marine pelagic cosmopolitan species. Molecular Phylogenetics and Evolution 57:1209–1218.

Estoup, E., C. R. Lagiader, E. Perrot, and D. Chourrout. 1996. Rapid one-tube extraction for reliable PCR detection of fish polymorphic markers and transgenes. Molecular Marine Biology and Biotechnology 5: 295–29.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564–567.

FAO (Food and Agricultire Organization of the United Nations). 2007. *Rachycentron canadum*: FAO cultured aquatic species information. FAO, Rome. Available: http://www.fao.org/fishery/culturedspecies/Rachycentron_canadum/en. (September 2012).

Finnerty, J. R., and B. A. Block. 1995. Evolution of cytochrome *b* in the Scombroidei (Teleostei): molecular insights into Billfish (Istiophoridae and Xiphiidae) relationships. U.S. National Marine Fisheries Service Fishery Bulletin 93:78–96.

Franks, J. S., J. R. Warren, and M. V. Buchanan. 1999. Age and growth of Cobia, *Rachycentron canadum*, from the northeastern Gulf of Mexico. U.S. National Marine Fisheries Service Fishery Bulletin 97:459–471.

Franks, J. S., M. H. Zuber, and T. D. McIlwain. 1991. Trends in seasonal movements of Cobia, *Rachycentron canadum*, tagged and released in the northern Gulf of Mexico. Journal of the Mississippi Academy of Science 36:55.

Garber, A. F., W. D. Grater, K. C. Stuck, and J. S. Franks. 2002. Characterization of the mitochondrial DNA control region of Cobia, *Rachycentron canadum*, from Mississippi coastal waters. Gulf and Caribbean Fisheries Institute 53:570–580.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available: http://www2.unil.ch/popgen/softwares/fstat.htm. (November 2012).

Griffiths, A. M., G. Machado-Schiaffino, E. Dillane, J. Coughlan, J. L. Horreo, A. E. Bowkett, P. Minting, S. Toms, W. Roche, P. Gargan, P. McGinnity, T. Cross, D. Bright, E. Garcia-Vazquez, and J. R. Stevens. 2010. Genetic stock identification of Atlantic Salmon (*Salmo salar*) populations in the southern part of the European range. BMC Genetics [online serial] 11:31.

Guo, S., and E. Thompson. 1992. Performing the exact test of Hardy–Weinberg proportion from multiple alleles. Biometrics 48:361–372.

Hammond, D. L. 2001. Status of the South Carolina fisheries for Cobia. South Carolina Marine Resources Center Technical Report 83.

Jacobina, U. P., M. de B. Cioffi, L. G. R. Souza, L. L. Calado, M. Tavares, J. Manzella Jr., L. A. C. Bertollo, and W. F. Molina. 2011. Chromosome mapping of repetitive sequences in *Rachycentron canadum* (Perciformes: Rachycentridae): implications for karyotypic evolution and perspectives for biotechnological uses. Journal of Biomedicine and Biotechnology [online serial] 2011:218231. DOI: 10.1155/2011/218231.

Mardis, E. R. 2008. Next-generation DNA sequencing methods. Annual Review of Genomics and Human Genetics 9:387–402.

McKay, J. K., and R. G. Latta. 2002. Adaptive population divergence: markers, QTL and traits. Trends in Ecology and Evolution 17:285–291.

NOAA (National Oceanic and Atmospheric Administration). 2011. Draft aquaculture policy. NOAA. Available: http://www.nmfs.noaa.gov/aquaculture/docs/noaadraftaqpolicy.pdf. (September 2012).

Renshaw, M. A., C. L. Pruett, E. Saillant, J. C. Patton, C. E. Rexroad III, and J. R. Gold. 2005. Microsatellite markers for Cobia, *Rachycentron canadum*. Gulf of Mexico Science 23:248–251.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Raymond, M., and F. Rousset. 1995. Genepop (version 2.1): population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248–249.

Rousset, F. 2008. Genepop007: a complete re-implementation of the Genepop software for Windows and Linux. Molecular Ecology Resources 8:103–106.

Saillant, E. A., M. A. Renshaw, N. J. Cummings, and J. R. Gold. 2012. Conservation genetics and management of Yellowtail Snapper, *Ocyurus chrysurus*, in the U.S. Caribbean and south Florida. Fisheries Management and Ecology 19:301–312.

Seutin, G, B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69:82–90.

Shaffer, R. V., and E. L. Nakamura. 1989. Synopsis of biological data on Cobia, *Rachycentron canadum* (Pisces: Rachycentridae). NOAA Technical Report NMFS 82.

Stapley, J., J. Reger, P. G. D. Feulner, C. Smadja, J. Galindo, R. Ekblom, C. Bennison, A. D. Ball, A. P. Beckerman, and J. Slate. 2010. Adaptation genomics: the next generation. Trends in Ecology and Evolution 25: 705–712.

Triantafyllidis, A., N. Karaiskou, F. Bonhomme, L. Colombo, D. Crosetti, D. Danancher, E. García-Vázquez, J. Gilbey, T. Svåsand, E. Verspoor, and C. Triantaphyllidis. 2007. Management options to reduce genetic impacts of aquaculture activities. Pages 162–167 *in* T. Svåsand, D. Crosetti, E. Garcia-Vazquez, and E. Verspoor, editors. Genetic impact of aquaculture activities on native populations. Genimpact, Final Scientific Report, EU Contract n. RICA-CT-2005-022802. Available: http://genimpact.imr.no/. (September 2012).

van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538.

APPENDIX: MICROSATELLITE SUMMARY STATISTICS AND MITOCHONDRIAL DNA HAPLOTYPE DISTRIBUTION

TABLE A.1. Summary statistics for 28 nuclear-encoded microsatellites in Cobias sampled from Virginia (VA), Mississippi (MS), Louisiana (LA), and Taiwan (TW); n is sample size, A is number of alleles, A_R is allelic richness, H_E is gene diversity (expected heterozygosity), P_{HW} is the probability of conformity to expected Hardy–Weinberg (HW) genotypic proportions, and F_{IS} is the inbreeding coefficient (measured as f of Weir and Cockerham 1984). The P_{HW} values in bold italics indicate significant departures from HW equilibrium after (sequential) Bonferroni correction.

Statistic	VA	MS	LA	TW	Statistic	VA	MS	LA	TW
		Rca1A08					***Rca1A11***		
n	35	52	14	36	n	35	52	14	36
A	2	4	1	4	A	13	16	9	14
A_R	1.40	2.35	1.00	2.56	A_R	9.93	10.30	9.00	9.53
H_E	0.028	0.112	0.000	0.134	H_E	0.858	0.868	0.810	0.826
P_{HW}	1.000	0.130	1.000	1.000	P_{HW}	0.151	0.072	0.467	0.741
F_{IS}	0.000	0.141	—	−0.039	F_{IS}	0.137	0.048	0.031	−0.112
		Rca1B12					***Rca1C04***		
n	35	52	14	36	n	35	52	14	36
A	3	3	2	3	A	12	12	8	15
A_R	2.39	2.61	2.00	2.99	A_R	8.78	8.42	8.00	10.75
H_E	0.252	0.359	0.423	0.505	H_E	0.763	0.810	0.839	0.873
P_{HW}	0.047	0.344	1.000	0.341	P_{HW}	0.268	0.554	0.343	0.554
F_{IS}	0.209	0.144	−0.013	0.178	F_{IS}	0.141	−0.020	−0.112	−0.019
		Rca1D04					***Rca1D07***		
n	35	52	14	36	n	35	52	14	36
A	3	3	3	3	A	3	3	2	8
A_R	2.52	1.88	3.00	2.39	A_R	1.80	1.74	2.000	6.20
H_E	0.162	0.075	0.140	0.372	H_E	0.056	0.057	0.071	0.718
P_{HW}	1.000	1.000	1.000	0.262	P_{HW}	1.000	1.000	1.000	0.130
F_{IS}	−0.057	−0.023	−0.020	−0.274	F_{IS}	−0.007	−0.013	0.000	0.072
		Rca1D08					***Rca1D11***		
n	35	52	14	36	n	35	52	14	36
A	2	1	1	12	A	2	2	1	4
A_R	1.40	1.00	1.00	7.90	A_R	1.40	1.27	1.00	2.78
H_E	0.028	0.000	0.000	0.778	H_E	0.028	0.019	0.000	0.520
P_{HW}	1.000	1.000	1.000	0.228	P_{HW}	1.000	1.000	1.000	0.023
F_{IS}	0.000	N/A	N/A	0.072	F_{IS}	0.000	0.000	N/A	−0.341
		Rca1E04					***Rca1E05***		
n	35	52	14	36	n	35	52	14	36
A	2	2	2	3	A	13	11	6	18
A_R	1.88	1.72	2.00	2.02	A_R	7.12	6.24	6.00	12.80
H_E	0.109	0.075	0.198	0.082	H_E	0.657	0.519	0.675	0.895
P_{HW}	1.000	1.000	1.000	1.000	P_{HW}	0.559	0.530	0.340	0.002
F_{IS}	−0.046	−0.030	−0.083	−0.019	F_{IS}	0.001	0.010	0.045	0.258
		Rca1E11					***Rca1F01***		
n	35	52	14	36	n	35	52	14	36
A	5	5	6	10	A	2	2	2	5
A_R	4.50	4.06	6.00	7.45	A_R	1.98	1.96	2.00	3.89
H_E	0.613	0.563	0.746	0.830	H_E	0.182	0.176	0.349	0.575
P_{HW}	0.666	0.723	0.468	0.104	P_{HW}	1.000	1.000	0.489	1.000
F_{IS}	−0.025	0.010	−0.156	0.131	F_{IS}	−0.097	−0.097	0.186	−0.015

(*Continued on next page*)

TABLE A.1. Continued.

Statistic	VA	MS	LA	TW	Statistic	VA	MS	LA	TW
		Rca1F11					***Rca1G02***		
n	35	52	14	36	n	35	52	14	35
A	1	1	1	8	A	3	3	2	18
A_R	1.00	1.00	1.00	6.01	A_R	2.52	2.17	2.000	12.22
H_E	0.000	0.000	0.000	0.676	H_E	0.162	0.145	0.071	0.910
P_{HW}	1.000	1.000	1.000	0.393	P_{HW}	1.000	1.000	1.000	0.950
F_{IS}	—	—	—	0.055	F_{IS}	−0.057	−0.064	0.000	−0.068
		Rca1G05					***Rca1H01***		
n	35	52	14	36	n	35	52	14	36
A	4	6	3	17	A	15	16	10	18
A_R	3.79	3.81	3.00	12.27	A_R	10.63	10.71	10.00	12.33
H_E	0.697	0.668	0.677	0.894	H_E	0.869	0.870	0.897	0.897
P_{HW}	0.750	0.898	0.613	0.057	P_{HW}	0.684	0.421	0.909	***0.000***
F_{IS}	0.059	−0.036	0.053	0.101	F_{IS}	−0.086	−0.062	−0.037	0.508
		Rca1H10					***Rca1BA10***		
n	35	52	14	36	n	35	52	14	36
A	10	10	7	7	A	3	3	3	7
A_R	6.99	7.19	7.00	5.98	A_R	2.64	2.25	3.00	5.94
H_E	0.723	0.785	0.582	0.782	H_E	0.428	0.223	0.442	0.792
P_{HW}	0.673	0.036	0.828	0.438	P_{HW}	0.687	0.117	1.000	0.598
F_{IS}	0.053	0.218	−0.109	0.041	F_{IS}	0.001	−0.035	0.031	0.054
		Rca1BC06					***Rca1BD09***		
n	35	52	14	36	n	35	52	14	36
A	15	20	14	13	A	2	1	2	5
A_R	11.37	11.91	14.00	10.21	A_R	1.40	1.00	2.00	3.52
H_E	0.904	0.908	0.939	0.880	H_E	0.028	0.000	0.071	0.233
P_{HW}	0.106	0.146	0.261	0.403	P_{HW}	1.000	1.000	1.000	1.000
F_{IS}	0.085	−0.081	0.090	0.054	F_{IS}	0.000	N/A	0.000	−0.075
		Rca1BD10					***Rca1BE06***		
n	34	52	14	36	n	35	52	14	36
A	19	22	14	22	A	6	6	5	8
A_R	14.61	14.77	14.00	13.93	A_R	4.09	3.87	5.00	6.54
H_E	0.937	0.944	0.942	0.929	H_E	0.573	0.586	0.566	0.760
P_{HW}	***0.000***	0.358	0.239	0.956	P_{HW}	0.035	0.666	0.040	0.703
F_{IS}	0.250	0.002	0.093	−0.078	F_{IS}	0.205	−0.048	−0.275	0.051
		Rca1BE08A					***Rca1BE08B***		
n	35	52	14	36	n	35	52	14	36
A	4	8	4	12	A	2	2	2	2
A_R	3.43	4.83	4.00	8.66	A_R	2.000	2.000	2.000	2.000
H_E	0.519	0.609	0.602	0.854	H_E	0.437	0.473	0.495	0.497
P_{HW}	0.609	0.906	1.000	0.024	P_{HW}	0.706	0.240	0.567	0.734
F_{IS}	0.066	−0.0107	0.050	−0.009	F_{IS}	0.086	−0.180	0.286	−0.062
		Rca1BF06					***Rca1BF07***		
n	35	52	14	36	n	35	52	14	36
A	14	16	11	23	A	3	5	2	7
A_R	10.81	10.73	11.00	15.06	A_R	2.04	2.95	2.00	5.06
H_E	0.904	0.900	0.921	0.938	H_E	0.084	0.181	0.071	0.577
P_{HW}	0.582	0.626	0.015	0.012	P_{HW}	1.000	0.133	1.000	0.734
F_{IS}	0.053	−0.047	0.310	0.143	F_{IS}	−0.020	0.154	0.000	−0.059

TABLE A.1. Continued.

Statistic	VA	MS	LA	TW	Statistic	VA	MS	LA	TW
		Rca1H04A					***Rca1BH09***		
n	35	52	14	36	n	35	52	14	36
A	4	3	3	4	A	12	17	9	18
A_R	2.80	2.27	3.00	3.38	A_R	10.13	11.36	9.00	12.43
H_E	0.533	0.501	0.537	0.497	H_E	0.903	0.908	0.902	0.917
P_{HW}	0.500	0.193	1.000	0.104	P_{HW}	0.658	0.488	0.282	0.718
F_{IS}	−0.126	0.195	−0.067	−0.007	F_{IS}	0.020	0.047	−0.030	0.093

TABLE A.2. Spatial distribution of mitochondrial DNA haplotypes based on sequences of the mitochondrial cytochrome-*b* gene in Cobias (total $n = 5$ fish/sample locality) sampled from Virginia (VA), Louisiana (LA), Mississippi (MS), and Taiwan (TW). GenBank accession numbers for the haplotypes are also presented.

Haplotype	VA	MS	LA	TW	GenBank
1	4	5	3		JX149559
2			1		JX149560
3			1		JX149561
4				5	JX149562
5	1				JX149563