# Biodiversity genomics for species at risk: patterns of DNA sequence variation within and among complete mitochondrial genomes of three species of wolffish (*Anarhichas* spp.)

# K.A. Johnstone, H.D. Marshall, and S.M. Carr

**Abstract:** The first marine fish species to be listed under the Canadian Species At Risk Act as Threatened with extinction are the spotted wolffish (*Anarhichas minor* Olafsen, 1774) and the broadhead wolffish (*Anarhichas denticulatus* Krøyer, 1844); a third species, the striped wolffish (*Anarhichas lupus* L., 1758), is listed as a species of special concern. As part of the recovery plan for wolffish, we determined the complete mitochondrial DNA (mtDNA) genome sequences of all three species to identify the most variable gene regions for population analysis. *Anarhichas* genomes comprise either 16519 or 16520 base pairs (bp), among which there are 449 single nucleotide polymorphisms (SNPs). The most variable protein-coding loci are ND4, CYTB, and ND2, with 4.40, 4.22, and 4.19 SNPs/100 bp, respectively. Comparisons of rates of synonymous and nonsynonymous nucleotide substitutions indicate no evidence of selection. The control region, characterized in many species as hypervariable, was less variable than 9 of 13 protein-coding loci (2.45 SNPs/100 bp). Phylogenetic analysis shows that *A. lupus* and *A. minor* are more closely related to each other than either is to *A. denticulatus*. Amplification and sequence analysis of a contiguous block of 6392 bp that spans the ND4, ND5, ND6, and CYTB loci is an efficient strategy for evaluating patterns of intraspecific mtDNA variability.

**Résumé :** Les premières espèces de poissons marins à être placées sur la liste des espèces « menacées » d'extinction selon la Loi canadienne sur les espèces en péril sont le loup tacheté (*Anarhichas minor* Olafsen, 1774) et le loup à tête large (*Anarhichas denticulatus* Krøyer, 1844); une troisième espèce, le loup atlantique (*Anarhichas lupus* L., 1758), est incluse comme espèce « préoccupante ». Comme contribution au plan de récupération des loups, nous avons déterminé les séquences du génome complet de l'ADN mitochondrial (ADNmt) chez les trois espèces afin d'identifier les régions les plus variables en vue d'une analyse de population. Les génomes d'*Anarhichas* comprennent ou 16519 ou 16520 paires de bases(pb), parmi lesquelles il y a 449 polymorphismes simple nucléotide (SNPs). Les locus codant pour les protéines les plus variables sont ND4, CYTB et ND2 qui possèdent respectivement 4,40, 4,22 et 4,19 SNPs/100 pb. La comparaison des taux de substitutions synonymes et non synonymes de nucléotides ne révèle aucun signe de sélection. La région de contrôle, caractérisée d'hypervariable chez de nombreuses espèces, est moins variable que 9 des 13 locus codant pour les protéines (2,45 SNPs/100 pb). L'analyse phylogénétique montre que les *A. lupus* et *A. minor* sont plus apparentés l'un à l'autre que chacun ne l'est avec l'*A. denticulatus*. L'amplification et l'analyse des séquences d'un bloc contigu de 6392 pb qui comprend les locus ND4, ND5, ND6 et CYTB constituent une stratégie efficace pour l'évaluation des patrons de variabilité interspécifique de l'ADNmt.

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### Introduction

The wolffish family Anarhichadidae is a small family of blenny-like marine fishes found on rocky and hard bottom areas of the northern oceans (O'Dea and Haedrich 2002). Wolffish are sedentary, slow-growing fish with stout bodies and large, blunt heads (Templeman 1986*a*, 1986*b*). Their dentition comprises large, conical anterior teeth and molariform lateral teeth, which gives them a wolf-like appearance

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and enables them to feed on crustaceans and invertebrates (Scott and Scott 1988). There are two genera, the monotypic Pacific wolfeel (*Anarrhichthys ocellatus* Ayres, 1855) and *Anarhichas* L., 1758, which comprises four species — the Bering wolffish (*Anarhichas orientalis* Pallas, 1814) and three species in the North Atlantic Ocean, the striped or Atlantic wolffish (*Anarhichas lupus* L., 1758), the spotted wolffish (*Anarhichas minor* Olafsen, 1774), and the broadhead or northern wolffish (*Anarhichas denticulatus* Krøyer, 1844). *Anarhichas lupus* are recognized by a series of dark transverse bars, whereas *A. minor* and *A. denticulatus* are covered in irregular blackish brown spots, which are somewhat larger in the former than in the latter. All three species occur on both sides of the Atlantic Ocean and into Arctic waters (Fig. 1).

Although not recently the targets of directed fisheries, wolffish populations and habitats have been heavily impacted by bottom-trawling associated with the fishery for

**Fig. 1.** Distribution of three species of wolffish from the North Atlantic Ocean: from top to bottom, striped or Atlantic wolffish (*Anarhichas lupus*), spotted wolffish (*Anarhichas minor*), and broadhead or northern wolffish (*Anarhichas denticulatus*) (maps drawn from descriptions in Scott and Scott 1988).



Atlantic cod (*Gadus morhua* L., 1758). Although this decreased after the Atlantic cod moratorium in 1992 (O'Dea and Haedrich 2002), *A. minor* and *A. denticulatus* are still caught in directed fisheries for halibut and crab, and almost 1500 t were taken annually as bycatch between 2000 and 2002 (Department of Fisheries and Oceans 2004). Wolffish not caught may be injured by groundfish trawls, which dislocate the rocks and boulders where wolffish live, nest, and spawn. Dredging for scallops and clams also promotes resuspension of bottom sediments, and can destroy spawning habitats and damage fish gills (O'Dea and Haedrich 2002).

Indices of wolffish abundance declined by more than 90% on the Grand Banks and the Northeast Newfoundland and Labrador Shelf from the late 1970s to the early 1990s, about two wolffish generations (O'Dea and Haedrich 2002; Department of Fisheries and Oceans 2004). Consequently, *A. minor* and *A. denticulatus* became in 2001 the first marine species to be assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as Threatened with extinction, and were accordingly listed on schedule 1 of the Species At Risk Act (SARA) by the Minister in 2005. *Anarhichas lupus* were listed in 2000 as a species of Special Concern and *A. orientalis* were listed in 2002 as being Data Deficient (i.e., the available data are insufficient to assign a status).

Wolffish in the North Atlantic Ocean are currently the

subjects of a Department of Fisheries and Oceans (now Fisheries and Oceans Canada) species recovery plan that is intended to identify and correct the factors responsible for the observed decline. This plan includes a genetic analysis of their population structure to determine whether any of the species comprises distinctive subcomponents consistent with a stock structure (cf. Pepin and Carr 1993) or designatable units under SARA. Preliminary genetic studies of mitochondrial DNA (mtDNA) sequence variation in more than 400 wolffish from all three species showed very little variation within the species in the control region (K.A. Johnstone, H.D. Marshall, and S.M. Carr, unpublished observation), a gene region considered "hypervariable" in many fish species (Faber and Stepien 1997). These data gave no indication of population structure within species, and pairwise genetic differentiation among species was small, which is indicative of recent speciation that might also explain the low intraspecific variation.

To clarify the evolutionary, population, and conservation genetic status of wolffish, we obtained the complete mtDNA genome sequences of all three species to identify loci most suitable for population genetic analysis. Interspecific divergence should predict intraspecific variability at selectively neutral loci, as both are functions of the neutral mutation rate (Kimura 1983). Along with comparative genomic data on codfish, including *G. morhua*, which has also been assessed by COSEWIC as Endangered, this study represents the first genomic study of Canadian species at risk under SARA.

#### Materials and methods

Frozen hearts were collected at sea by Department of Fisheries and Oceans' personnel from bycatch fish of all three species. Preliminary experiments indicated that DNA obtained from most of these samples consisted of fragments of low molecular weight. To obtain high molecular weight DNA for long-range polymerase chain reactions (PCR; see below), we obtained a live A. lupus under a Department of Fisheries and Oceans' incidental harm permit at the Bonne Bay Marine Station on the west coast of the island of Newfoundland (Northwest Atlantic Fisheries Organization (NAFO) division 4R). Fresh heart, muscle, and liver tissues were collected from the fish immediately after being euthanized. DNA from frozen specimens was obtained from individual A. minor and A. denticulatus from NAFO divisions 30 and 3L, respectively. mtDNA was extracted with the QIAamp DNA Mini Kit (QIAGEN, Valencia, California), and the tissue protocol provided by the manufacturer was followed.

Initial studies of *Anarhichas* mtDNA employed the "universal" primers of Kocher et al. (1989) for the CYTB and control region (CR) loci. Of the 18 primer pairs designed by Coulson et al. (2006) for amplification and sequencing of *Gadus* and related gadiforms, three pairs successfully amplified *Anarhichas* spp.: a fish primer wCR2F and a mammalian primer *Cytb811R* for a region spanning the 3' end of the CYTB locus and the 5' end of the CR locus, g14F and g14R for part of the ND4 locus, and g08F and g08R for part of the COX1 locus. By pairing *Cytb811R* with g14F, g14R with g08F, and g08R with wCR2F, and modifying

**Fig. 2.** Schematic diagram of the wolffish mitochondrial genome, with three regions >4 kbp amplified by long-range PCR. Forward primers are in roman type and reverse primers are in italic type. The 466 bp fragment of the genome between the 5' ends of the *cytb811R* and wCR2F primers spans the 3' end of the CYTB locus and the 5' end of the control region (CR) locus, for which the sequence was already known.



standard PCR protocols to amplify fragments >5 kbp, we were able to amplify the entire genome (except for a 1 kbp portion that included the CR locus, the sequence of which was already known) of *A. lupus* as three overlapping fragments of >4 kbp each (Fig. 2). In the process of DNA sequencing, 42 *Anarhichas* specific primers were designed and were used along with 19 *Gadus* specific primers and 1 mammalian primer (Table 1).

For long-range PCR amplification of mtDNA from A. lupus, the TripleMaster system and High-Fidelity PCR protocol were used (Eppendorf, New York, New York). The reaction mixture was prepared in two parts. The first mixture comprised 8.4 µL of distilled deionized water (ddH<sub>2</sub>O), 0.3  $\mu L$  each of the 10  $\mu mol/L$  forward and reverse primers, and 1 µL of the template DNA. The second mixture comprised 7.4 µL of ddH<sub>2</sub>O, 2 µL of 10× High-Fidelity buffer with Mg<sup>2+</sup>, 0.4 µL of 40 nmol/L dNTP, and 0.2 µL of TripleMaster polymerase mix. The two mixtures were combined and immediately preheated to 94 °C for 2 min to denature the double-stranded DNA template. The PCR cycle was 94 °C for 20 s, 54 °C for 15 s, and 68 °C for 4 min, which was repeated for 35 cycles. The reactions were thereafter cooled to 5 °C. Size of amplicons were estimated by electrophoresis of 4 µL of the PCR product through 1% or 2% agarose gel with 8 µL of ethidium bromide in 1× TBE buffer at 60 V, for up to 6 h according to the expected sizes. PCR products were purified with the QIAGEN QIAQuick PCR purification kit according to the manufacturer's directions.

For low molecular weight DNA from A. minor and A. denticulatus, standard PCR reactions for amplicons with ex-

**Table 1.** Primers used to amplify and (or) sequence the mitochondrial genomes of *Anarhichas* species.

g20RGGCAGGACATTAAGGGCATTCTCAC29wCR1FACCTCCCACCCCTAACTCCCAAAGC53wCR1FATCCTGCATCTGGTAACTCCCAAAGC53wCR2FATTCCTGACATTTGGCTCTACTTC176wCR2FCTAGGGCCATCTTAACATCTTCAG739g01FCTGAAGATATTAGGATGGACCTAG1 358g02FCCAAAAACGTCAGGCCAGGGTGTAG1 499w02FTGTTCCGCTGAAATTGGCCTGAAGC2 019w02FTGTTCCGCTGAAATTGGCCTCAGC2 618g03FACCCCGAAACTGAGCGAGCTATCC2 618g03FTTACCCAAAACTGGCCTCTTG3 276g04FTTTACCAAAACTGGCCTCTTG3 244g04FTTCACGCGGGGTATGGCCCAGGGCCAGGG5 026w04FTTCAGACCGGAGTAATCCAGGTCAG4 020g05RATGTTCGGGGTATGGCCCAGGAGCC5 026w05FAGTATGCTCTTATCGGAGCCTTGG5 107w06FGTGCTCCCACTACACCACTTCCTAG5 233g06RAGCTTAATTAAGGGCCTTGTTACC6 296g07FAAACTGAGCCAAGGAGAGTATAGTTGC5 931w07RCTGGTTAGACGGCTTAGCTGTTAAC6 407w08FCAAAGGTAAAGTAAGCCGCTGTGTC6 407w08FACAACGAATGTGGGGCATACAACG7 498w08FACAACGAATGTGGGGTGACTACACG8 555w09FGCCATATTAGCTTCTTGGTGGGGGAGTAGTAG10 44w10F1ATGCGGAAAGTAGTGGGGTGACTACACG10 274w11FCTGACTCACGTGGTGGGGGGGGGGGGGGGGGGGGGGGGG	Primer	DNA sequence (5'→3')	5' base
WCR1FACCTCCCACCCTAACTCCCAAAGC53WCR1FGGCATCCTGGGTTATCCTGCTTATG161WCR2FATTCCTGGACATTTGGCTCTACGCTTATG176WCR2FCTAGGGCCATCTTAACATCTTCAG739WCR2F1TGGTATCAGGCCATCTTAACATCTTCAG739WCR2F1TGGTATCAGGCCACTCTTAACATCTTCAG1358g02FCCAAAAACGTCAGGTCGAGGTGAG1499g02RCTATTCATTCATTACAGGCAACCACCT2019y02FTGTCCGCGGAAATTGGCCCTAGAG2176g03FACCCCGAAACTGAGCGAGCTACCC2618g03RTAAGCCCTCGTAGAAAGGGCTAGG3954w04FTTCACACCGGAGTATCCCCCTTG3276g04RTGAACCTCTGTAGAAAGGGCTAGG5026w05FAGTATGCTCTTATCGGAGATAGGCCCAGGC5026w05FAGTATGCCACTAACACGACCCTTCG5931w07RCTGGTTGAGCACAGAAGAGTAATTCC5931w07RCTGGTTAGACGAGGCCTTAGCTGTTAAC6950w08F1CCACAGGTAAAGTAAGCAGCGTGTGAA6407w08RCAAGGGAAAGGAGTAATCAAAGG7908w08FACACCACATTCTGAGCAATGGTGTTACC7165g08RTAACCCCACATTCTGAGCTATGAGG7908w08FACACCAAATGTGGGGGCATCAAAGCAATG9094w10F1ATGGGAATGGAATGAATAACTAATTG9169g10RAGAGGGGAATGAATAAACTAATTG9169g10RAGAGGGGAATGGAATCAACCACCCATGGT1044w13RTAACCCCCAATTGTAGGGGTACCAAGG1258w14FCTGTCTGCGTGTGAAGGAAGCAAGGGGGGGGGGGGGGGG	q20R	GGCAGGACATTAAGGGCATTCTCAC	29
wCR1R         GGCATGCTGGGTTATCTCGCTATG         161           wCR2F         ATTCCTGACATTGGCTCCTACTTC         176           wCR2F1         TGGTATCAGGCCATCTTAACATCTTCAG         739           wCR2F1         TGGTATCAGGCCATCTTAACATCTCAGGAG         870           g01F         CTAAGGATATTAGGATGGAGGTGAGG         1499           g02F         CCAAAACGTCAGGTCGAGGTGAGG         2019           w02F         TGTTCCGCGTGAAATGGCCATCATCC         2618           g03F         ACCCCGGAACTGAAACGGCCTTCATAC         2841           g04F         TTACCAAAAACTCGCCTTGG         3276           g04R         TGTACCGGAACTGAAACGGCCTTAGG         4020           g05F         AGTATCGGGGTATGGGACCATGGG         5107           wO4F         TTCCAGCTGAAGGAAGATAAGTTGC         523           g06R         AGCTGATAATAAAGGCCTTGTGAG         525           w06F         GATGGCCAACGAAGAAGATAAGTTGC         531           w07F         CTGGTTTGACGCTTAGCTGTTAACT         650           g07F         AAACTAGCCAAGGAATAGCTGCTGAGG         748           g08R         TAACCAAATTGTGCGCTTGACGGG         748           g08F         ACGAAGTGGGTATGATGAGG         904           w08F         ACGAAGTGGGGTATGAGGAGGAGTAGG         1649           w11R	wCR1F	ACCTCCCACCCCTAACTCCCAAAGC	53
wCR2FATTCCTGACATTTGGCTCCTACTTC176wCR2FCTAGGGCCCATCTTAACATCTTCAGG739wCR2F1TGGTACAGGCACATCTTAAGATCTGAGA870g01FCTGAAGATATTAGGATGGACCCTAG1358g02FCCATACAGTCAGGCCATCCAGGCTGAGG2176g03FACCCCGAAACTGAGCGATCCCAGGC2618g03FTAAGCCTCGTGAGTGCACTTCATAC2841g04FTTTACCAAAACAGCGCCTTCATAC2641g04FTTCACACGGGGTATGGCCATCATCC2619g04FTCAACCGCGGGTATGGCCCATCAGC5026w04FTCCAACAGCGGGATAGGCCCATCGC5026w05FAGTATGCTCTTATCGGAGCCATCCTAG5225w06RGATGGCGACAGGAAGGTAAGCAGGCC5031w07RCTGGGTATGACCATACCAGTCCC6950w08FAGGTATTAAAGTATTGCTGAGCGTTGC6950w08FAAGGTAAAAAAGACGGGGTGTGC6407w08RCAGAGGGTAAAGTAAGTAGGGGGTATGG798g08RTAACCCACAATCCGAGGATTCCTAAAGC6407w08FACGGAAGGAAAAATAAGCGCGTGTGTC6950w08FACGGAATAGTAAGTAGCGGGGGTGTGC798g08RTAACCCACAATCCGAGGATATCACG8473g09RACCCATATTAGCTCTTAGTGAGGG798y08FAGGGCAATCGTGGGTCACAGGCTAGTGG1044y13RTAACCCACAATCTGGGTTGGGTCC10274y11FCTGCGCATAGTGGTATGGAGGATAGTG11044y13RTACCGCGTTAAGTGTGGGTCACAGG1583g14FCCGCTCAATGGGTTAGTGGGGGAGGAGGG1670g14FCTGTGGGGTGATGTGGGGTGACGAGGGGGGGGGGGGGGG	wCR1R	GGCATGCTGGGTTATCTCGCTTATG	161
wCR2RCTAGGGCCATCTTAACATCTTCAG739wCR2F1TGGTATCAGGCCATCTTAACATCTCAG870g01FCTGAACAGCAGCACCCTAGGCTGAGGTGTAG1358g02FCCAAAAACGTCAGGTCGAGGTGTAG2176g03FACCCCGAAATGGCCGACCATCTCC2618g03RTAAGCCTCGTGAAGCCATCATCC2841g04FTTTACCATAAACATCCAGGTCAG3276g04RGOACCTCTGTAGAAAGGCCTATGG3276g04FTTCACACCGGGAGTAATCCAGGTCAG5026w04FTTCACACCGGGGTAAGGCCCATCGG5026w05FAGTATGCTCTTATCGGAGCCCATCGG5026w05FGTGCTCCACCTACACCACTTCCTAG5225w06RGATGGCGCACAGGAAGGTAAGTAGC5231w07RCTGGTTTGAGGGCTTAGCTGTTACC6296g07FAAACTAGACCACGGCTTAGCTGTTACC6407w08FCAGAGGTAAAGTAAGCGCGTGTGTC6950w08FACACGAATGTGGAGGAAGGTAACTGC7488g08RACACGAATGTGGAGGACTACACGG8473g09RACCCCATATTGACCTATGCTGTGACCAACG1659w01F1ATGGGAATGGAGGAACTACACG1659w11FCTGACCTCAGGGTAACGACCATGGC10w11RTTGACTCCCCACGGTACGCATGAGG10w11RTTGACTCCCCACGGTACGCTAGGCGAGGAGGG1670g14FCCCCTTATATAGCTTCTGGGGGGAGGAGGG11w14F2GAACACCATTCTGGGGTCACAGG14w13RTACCGCGTTAGGCGTAGGAGCAGGGGGGGGGGGGGGGGG	wCR2F	ATTCCTGACATTTGGCTCCTACTTC	176
wCR2F1TGGTATCAGGCACATCTCTAGTGAG870g01FCTGAAGTATTAGATGGACCCTAG1388g02FCCAAAAGGTCAGGTGAGGGTAG2019w02FTGTTCCGCTGAAATTGACCAGCT2019w02FTGTTCCGCTGAAATGACCAGCTAGAG2176g03FACCCCGAAACTGACGACGACCAGCT2618g04RTTACCAAAACGTCGCCCTTGG3276g04RTGAACCTCTGTAGAAAGGCCTAGG3954w04FTTCCACACCGGAGTAATCCAGGCCCAAGAGC5026w05FAGTATGCTCTTATCGAGACCCTTCG5107w06FGTGCTCCCATCACACCTTCCTAG5225w06RGATGGCGACAGGAAGAGTAAGTTAGC5293g06RAGCTTAATTAAGTATTGTTTGC5981w07RCTGGTTGAGCGCTAGGCCTTGACC6960w08F1CCCTCACCTAGCAGGCCTTCCAACC6950w08F1CCCCTCACCAAGGGCATTCTTACAGG7908w08FACACGAATTCTGCGGTTATGATGG9169g10RAGAGGGCAATGAATTCTTGAAGG9169g10RAGAGGGCAATGAAATTGGGGTAAGTGAGG1699w11FCTGACTCAGCAGGTATGTTGAGGG1694w11FTGACACCAACCAACCCATGCTGGTTG11044w13RTACCGAGTTAGGTGTACGGGGAATGATAGTAG1594w14F2GAACCTTCTTAGGCGTTAGCGGGGGTAAGG1594w14F2GAACCTTCTTAGTGGGTCCAACG1828y14FCTGTTGCAGGTTAGGGGGCAAGGAAGGAGGGGGGGGGGG	wCR2R	CTAGGGCCCATCTTAACATCTTCAG	739
901FCTGAAGATATTAGGATGGACCCTAG1 358902FCCAAAACGTCAGTCGAGGTGTAG1 499902RCTATTCATTTCATTCCAGGCACCAGCT2 019903FACCCCGAAACTGAGCGAGCTACTCC2 618903RTAAGCCTCGTGATGCATCATAC2 841904FTTTACCAAAAACATCGCCTCTG3 276904FTGAACCTCGTGTAGAACAGGCCTAGG4 020905RAGTGTCGGGGATAATCCAGGTCAG5 026w04FTCCAGACGGAGATAATCCAGGTCCAG5 026w05FAGTGTCCGGGATATGGCCCAAGAGC5 223w06RGATGGCGCACAGGACGATAAGTTGC5 931w07RCTGGTTTCAGCGCTTAGCTGTTACC6 296907FAACTTAATTAAAGTATTGTTTGC5 931w07RCTGGTTGAGCGCTTAGGCGTGTGC6 950w08RCAGAGGTAAAGTAAGCGGCGTGAGTC6 950w08FACGAGGGTAAATCTCGCGTGAGCTC7 185908FACACGAATGTGGAGCATCAAGGG7 908909RACCCATATTAGCTCTGAGAGCATCAAGG8 585w09FGGCCATCAGTGGAGCATCAAGGG8 585w09FGGCCATCAGTGGAGCAATCAAGTG9 904w11FCTACACTAGGCAATGAAGTAGGGCTC10 274w11RTGGAGAAGTTAGGGCGTACGCAAGGATTAG1 996w11FCTACACTTGGCCTTGGAGGAGTAGG11 594w14FCCCATATATGGCTTAGGAGGAGTAGG1 593w14FCCCATATATGGCTTAGGAGGAGTAGG1 593w14FCCCATATTGGGCTGGAGGAGGAGGGAGG1 593w14FCCCATATTGGGCTTAGGAGGAGCACCAAGC1 833g14RTCCAGAGTGGAAGCATTCAACC2 358w14FCCCATATTGGATGGAGGAGCTCAAGC1 833<	wCR2F1	TGGTATCAGGCACATCTCTAGTGAG	870
902F         CCAAAAACGTCAGGTCAGGTGTAG         1 499           902R         CTATTCATTTCACAGGCAACCAGCT         2 019           w02F         TGTTCCGCTGAAATTGGCCCTGAAG         2 176           903R         TAAGCCTCGTGATGCCATCATCC         2 618           903R         TAAGCCTCGTGATGCCATTCATAC         2 841           904F         TTTACCAAAAACATCGCCCTTG         3 254           w04F         TCAGACCGGAGTAATCCAGGTCAG         4 020           905R         ATGTTGCGCTTATGGAGCCCAAGAGC         5 026           w05F         AGTGTGCACTATGGGGCCCAAGAGC         5 026           w05F         GGGCTTCCACTACCACCTTCCTAG         5 233           g06R         AGCTTAATTAAGGACCGTGTGTAAC         6 296           907F         AAACTAGACCAAGGCCTTCAAAGC         6 407           w08R         CAGAGGTAAAGTAAGCGCGTGTGTC         6 950           w078F         ACGACGAAAGTAAGCGCGTGTGTC         6 950           w08F         ACGCAATATGCGAGAATTCTCTCAATCC         7 165           g08R         TAACCCACAATTGCGGAGGACTACACG         8 473           g09R         ACCCCATATTGGCGAGGAGTACACG         8 473           g09R         ACCCCATATTGGCACGAGGAGTAGTAG         9 169           g10R         AGAGGGCAATGAAAAGTAGCGGGGGAGTAGTAG         1 044      <	g01F	CTGAAGATATTAGGATGGACCCTAG	1 358
g02R         CTATTCATTCACAGGCAACCAGCT         2019           w02F         TGTTCCGCTGAAATTGGCCTGAAG         2176           g03F         ACCCCGAAACTGAGCGAGCTACTCC         2618           g03R         TAAGCCCTCGTGAGTGCCATTCATAC         2841           g04F         TTTACCAAAAACATCGCCTCTG         3276           g04R         TGAACCTCCTGTAGAAAGGGCTATGG         4020           g05R         ATGTTCGGGGTATGGGCCCAGGACA         4020           g05R         ATGTTCGGGGTATGGGCCCAAGAC         5026           w05F         AGTATGCTCTATTGGGGCCCAAGACC         5026           w05F         AGTGTACCACAGGACACCACTCCTAG         5233           g06R         AGCTTAATTAAGGCCGCTTAACTGTTAAC         6296           g07F         AAACTAGCCAAGGACTTCAAAGC         6407           w08R         CAGAGGTAAAGTAAGTTGCAAGGC         6407           w08F         ACAGGTATAGCTGAGGCATTGAAGC         6490           w08F         ACAGCAATGGCAGGAGTACCAAGG         7908           g08R         TAACCACACTGCATGGAGGACTACACG         8473           g09R         ACCCATATGGGAGGAGCTACACG         8473           g09R         ACCCATATGGGACGAAGGACTACACG         10274           w10F1         ATGCGAAACCAACCAACCAACCAAGCAAGGATAAGTGTAG         904	g02F	CCAAAAACGTCAGGTCGAGGTGTAG	1 499
W02FTGTTCCGCTGAAATTGGCCCTGAAG2 176g03FACCCCCGAAACTGACCCTGGCATCCATAC2 618g03RTAAGCCCCGGTAGTGCCATCCATAC2 841g04FTTTACCAAAACATCGCCTCTTG3 276g04FTGAACCCCCGGAGTAATCCAGGCAG4 020g05RATGTTCGGGTATGGCCCAAGAGC5 026w05FAGTATGCTCTTATCGGAGCCCTTCG5 107w06FGTGCTTCCACTACACCACTTCCTAG5 225w06RGATGGCGACAGGAAGAGTAAGTTGC5 931w07RCTGGTTTGGACGCCTAACTGGTTAAC6 296g07FAAACTAGACCAAGGAATAAGTTGC6 950w08F1CCCTTCACCAGGGCCTTCAAAGC6 407w08RCAAGGGTAAAGTAAGCGCGTGGTCC6 950w08F1CCCTTCACCAGGGGCTTACAGGG7 498g08RTAACCACAATTCTGCCTTGACAGG7 908w08FACACCAATATGGGAGTACTACACG8 473g09RACCCATATTAGCTCTTAGTGAGG9 904y10F1ATGCGAAACCAACCAACCAATGTG9 199y10RAGAGGGAATGGATACTACAGG1 0274w11RTTGATCCCCAGTGGTAGCGGGAGTAGTAGG11 044w13RTACCGCGTTAGGCGTTAGGGGGATAGTAG11 654w14FCCTTTAGACCCATTGGGGTCTACGGG12 258y14FCTGTCCGGTTGGGTTGGGTTGGTTG11 258y14FCGCTATATATATTCTTATGGGCCTTCCC13 025w14FCCCCTATATATATTCTTATGGGAGGATAGG11 670y14FCTGCCATAGGCTTAGGGGGGATAGTAG14 1693y14FCCCCTATATATATTCTTATGGGAGGATAGG11 670y14FCGCCATAGGGCTTAGGGGAGGAGGGGAGGGGGGGGGGAGGGG13 615w15RCTAGCGGGAGCTGGG	g02R	CTATTCATTTCACAGGCAACCAGCT	2 019
g03F         ACCCCGAAACTGAGCGAGCTACTCC         2 618           g03R         TAAGCCCTCGTGATGCCATTCATAC         2 841           g04F         TTTACCAAAAACATCGCCTTTG         3 254           g04R         TGAACCTCTGTAGAAAGGCTTAGG         3 954           w04F         TTCAGACCGGAGTAATCCAGGTCAG         4 020           g05R         ATGTTCGGGGTATGGGCCCAAGAGC         5 107           w06F         GTGCTTCCACTACCACCTTCCTAG         5 225           w06R         GATGGCGACAGGAAGGTAAAGTGTGC         5 293           g06R         AGCTTAATAAAGTATTTGTTTGC         5 931           w07R         CTGGTTGAACGCGTTGTGTAAC         6 296           g07F         AAACTAGACCAAGGGCCTTCAAAGC         6 407           w08R         CAGAGGTAAAGTAAGCGCGTGTGTC         6 950           w08F1         CCCTTCACCTAGCAGAGATTCTCAATGC         7 498           g08R         TAACCCACAATTGGGAGGAGCTACTGAAGG         7 908           w08F         ACACGAATGTAGGAGGAGCTAACGG         8 555           w09F         GGCCATCAGTGGTACTGAAGCTAGTGGT         9 169           g10R         AGAGGCGAATGAATGAACTAACCC         10 459           w11R         TGAGTGAGAGGTGTGTGGGTCACAGT         1 044           w13R         TACCCCACATTGGGTCACATGTGTGGTAGTAG         1 599	w02F	TGTTCCGCTGAAATTGGCCCTGAAG	2 176
903R         TAAGCCCTCGTGATGCCATTCATAC         2 841           904F         TTTACCAAAAACATCGCCTCTTG         3 276           904R         TGAACTCTGTAGAAAAGGCTTAGG         3 954           WOAF         TTCAGACCGGAGTAATCCGGCTAGG         4 020           905R         ATGTTCGGGGTATGGGCCCAAGAGC         5 026           WO5F         AGTATGCTCTTATCGGAGCCCTTCG         5 027           WO6F         GTGCTCCACTACACCACTTCTAG         5 225           W06R         GATGGCGACAGGAAGAGTAAGTTGC         5 293           g06R         AGCTTAATTAAAGTATTGTTTGC         5 931           W07R         CTGGTTTGACGCGTTAGCTGTTACC         6 950           W08F         CCAGAGGTATAGTCGAGGCGTGTGTC         6 950           W08F         ACGCACAAATGGGAGCATACACG         7 498           g08R         TAAGCCACAATGGGAGCATACACG         8 473           g09R         ACCCATATTAGGTGCTAGAGGAG         7 908           w08F         ACGCATAGTGGTAGAGCATATCACG         8 473           g09R         ACCCATATTAGCTCCTAGGGGAGGAGGG         8 585           w09F         GGCCATCAGTGGTAGACAACCACCATGCT         10 274           w11R         TTGACTCTCCAGGGGAGGAGGAGGAGGAGGGGGGGGGGG	g03F	ACCCCGAAACTGAGCGAGCTACTCC	2 618
g04F         TTTACCAAAACATCGCCTCTTG         3 276           g04R         TGAACCTCTGTAGAAAAGGCTTAGG         3 954           w04F         TTCAGACCGGAGTAATCCAGGTCAG         4 020           g05R         ATGTTCGGGGTATGGCCCAAGAGC         5 026           w05F         AGTATGCTCTTATCGGAGCCTTCG         5 107           w06F         GTGCTTCCACTACACCACTTCCTAG         5 225           w06R         GATGGCGACAGGAAGAGTAAGTTGC         5 931           w07R         CTGGTTTGAGCGCTTAGCTGTTAAC         6 296           g07F         AAACTAGCCAAGGGCTTCAAAGC         6 407           w08R         CAGAGGTAAAGTAAGCGGGTGTGTC         6 950           w08F         ACGCATAATTAGCTCTGACAAGG         7 498           g08R         TAACCCACAATTCTGCCTTGACAAG         7 908           w08F         ACACGAATGTGGAGTATCACAG         8 473           g09R         ACCCATATTAGCTCTTAGTGAAGG         8 685           w09F         GGCCATCAGTGGTACTGGAGCTATGG         9 904           w10F1         ATGAGGAGACCAATCAACCCATGCTC         10 274           w11R         TTGAGTGAGGTTAGCGGGAGTAGTAG         10 459           w11F         CTACACTTGGCTTAGGGGAGATAGAG         11 594           w14R         ACCCCATATGGCTTAGGGGGAGAGGGGAGAGGG         11 670 <tr< td=""><td>g03R</td><td>TAAGCCCTCGTGATGCCATTCATAC</td><td>2 841</td></tr<>	g03R	TAAGCCCTCGTGATGCCATTCATAC	2 841
g04R         TGAACCTCTGTAGAAAGGGCTTAGG         3 954           w04F         TTCAGACCGAGGTAATCCAGGTCAG         4 020           g05R         ATGTTCGGGGTATGGGCCCAAGAGC         5 026           w05F         AGTATGCTCTATATGGGACCCTTCG         5 107           w06F         GTGCTCCACTACACACCACTTCCTAG         5 225           w06R         GATGCGGACAGGAAGAGTAAGTTGC         5 293           g06R         AGCTTAATTAAAGTATTTGTTTTGC         5 931           w07R         CTGGTTGAGCGCTTAGCTGGTAAC         6 296           g07F         AAATTGACCCAAGGGCTTCAAGCG         6 407           w08R         CAGAGGTAAAGTAGCGGTGTGTC         6 950           w08F1         CCCTTCACCTAGCAGGAGTTTCTTCAAATCC         7 165           g08R         TAACCCACAATTGGCTGTGACAGG         7 498           g09R         ACCCATATTAGCTGCTTGACAGG         8 473           g09R         ACCCATATTAGCTGCTTAGAGG         8 685           w09F         GGCCATCAGTGGTACTAAGCTATG         9 044           w10F1         ATGGTGAGAGTAAACCAACCAACCAATGTATG         9 044           w10F1         ATGGTGAGAGTAGATAGTGGC         10 274           w11R         TTGACACTGAGCGTATGAAGGGCTAGTAG         10 44           w13R         TACCTGCGTTTGGGTCCACAGT         11 044 <t< td=""><td>g04F</td><td>TTTACCAAAAACATCGCCTCTTG</td><td>3 276</td></t<>	g04F	TTTACCAAAAACATCGCCTCTTG	3 276
wO4F         TTCAGACCGGAGTAATCCAGGTCAG         4 020           g05R         ATGTTCGGGGTATGGGCCCAAGAGC         5 026           w05F         AGTATGCTCTTATCGGAGCCTTCG         5 107           wO6R         GATGGCGACAGGAAGAGTAAGTTGC         5 293           g06R         AGCTTAATTAAAGTATTTGTTTGC         5 931           w07R         CTGGTTTGAGCGCTTCAAAGC         6 296           g07F         AAACTAGACCAAGGGCCTTCAAAGC         6 407           w08R         CAGAGGTAAAGTAAGCGCGTGTGTC         6 950           w08F         ACGCTACAATGCGAGGAATTTCTTCAATCC         7 165           g08F         TAACCACCAATTCGCCTTGGACTAGCG         8 473           g09R         ACCCATATTAGCTTCTAGTGAGG         7 998           w08F         ACACGAATGTGGAGGACTACCAG         8 473           g09R         ACCCATATTAGCTCTTAGTGAGG         9 064           w10F1         ATGGCGAACGACCAACCCATCCATGG         9 044           w10F1         ATGGCGAACGACCAACCCCATGCTC         10 274           w11R         TTGACCCCCAGGTGTGGCGCCAAGGT         11 044           w12R1         TGAGGAGCTTGGGGTCAAGGT         11 044           w13R         TACCTGCGCTTGAGAGGAGATAGG         12 58           g13F         CTTTCCCGCTTGTGAGGAGCAAGG         12 58	g04R	TGAACCTCTGTAGAAAGGGCTTAGG	3 954
g05R         ATGTTCGGGGTATGGGCCCAAGAGC         5 026           w05F         AGTATGCCTTTATCGGAGCCCTTCG         5 107           w06F         GTGCTTCCACTACACCACTCCTAG         5 225           w06R         GATGGCGACAGGAGAGAGTAAGTTGC         5 931           w07R         CTGGTTTGAGCGCTTAGCTGTTAAC         6 296           g07F         AAACTAGACCAAGGGCTTCAAAGC         6 407           w08R         CAGAGGTAAGGTAAGGCGTGTC         6 950           w08F         ATGGGTATAGTCTGAGCATGATGAG         7 498           g08R         TAACCCACAATTGGAGCTATGATGG         7 498           g08R         TAACCCACATTCTGACCTGACAAG         7 908           w08F         ACACCATATTAGCTCTAGAGGAGGACTACACG         8 473           g09R         ACCCATATTAGCTCTAGAGGTATGG         9 169           g10R         AGAGGCGAATGAATAAACTAATTG         9 904           w10F1         ATGCGAAACCAACCAACCCATGCTC         10 274           w11R         TTGGACCACCACCATGCGTCGGAGGAGTAGG         11 044           w13R         TACCTGCGTTTAGTGGGCCACAGT         11 044           w13R         TACCTGCGTTGAGGGCTCAAGG         12 58           g13F         CTTTCCCGCTTGGGGTCAAGG         11 58           g13F         CTTCTCCGCTTGGGGGTCAAGG         11 593	wO4F	TTCAGACCGGAGTAATCCAGGTCAG	4 020
W05F         AGTATGCTCTTATCGGAGCCCTTCG         5 107           W06F         GTGCTTCCACTACACCACTTCCTAG         5 225           W06R         GATGGCGACAGGAAGATAAGTTGC         5 293           g06R         AGCTTAATTAAAGTATTGTTTTGC         5 931           W07R         CTGGTTTGAGCGCTTAGCTGTTAAC         6 296           g07F         AAACTAGACCAAGGGCCTTCAAAGC         6 407           W08R         CAGAGGTAAAGTAAGCGCGTGTGTC         6 950           W08F1         CCCTTCACCTAGCAGGAATTTCTTCAATCC         7 165           g08F         ATGGGTATAGTCTGAGCTATGATGG         7 498           g08R         TAACCCACATTTGGCCTTGACAAG         7 908           w08F         ACACGAATGTGGAGTGACTACACG         8 473           g09R         ACCCATATTAGCTCTTAGTGGAGG         8 585           w09F         GGCCATCAGTGGTACTGAAGCTATG         9 169           g10R         AGGGGGAATGAATAAACTAATTG         9 904           w10F1         ATGGGAAGGTTGAGCAACCCAACCCAAGCT         10 274           w11R         TTGATGGAAGTTAGGGTACGAAGG         10 274           w11R         TTGATGGAGGTTACGAGGGAGTAGTGG         10 44           w13R         TACCTGCGTTTAGTGCGTTGGGTTGG         11 258           g13F         CTTTCTCCGCGTTGGAGGAGGAGTAGGG         11 594 <td>q05R</td> <td>ATGTTCGGGGTATGGGCCCAAGAGC</td> <td>5 026</td>	q05R	ATGTTCGGGGTATGGGCCCAAGAGC	5 026
wO6FGTGCTTCCACTACACCACTTCCTAG5 225w06RGATGGCGACAGGAAGAGTAAGTTGC5 233g06RAGCTTAATTAAAGTATTTGTTTTGC5 931w07RCTGGTTTGAGCGCTTAGCTGTTACC6 296g07FAAACTAGACCAAGGCCTTCTAAAGC6 407w08RCAGAGGTAAAGTAAGCGCGTGTGTC6 950w08F1CCCTTCACCTAGCAGGAATTTCTTCAATCC7 165g08FATGGTATAGTCTGAGCTAGATGG7 908w08FACACCAAATTCTGCACTGACTAGAGG7 908w08FACACGAATGTGGAGTGACTACAGG8 473g09RACCCATATTAGCTTCTAGTGAGG8 585w09FGGCCATCAGTGTACTGAAGCTATG9 044w10F1ATGCGAAACCAACCAACCCATGCTC10 274w11RTTGGCAAACCAACCAACCCATGCTC10 459w11FCTACACTTGGCTTAGGGTGACTAGAG11 644w13RTACCTGCGTTTAGTGGGTCACAGT11 044w13RTACCTGCGTTGGAAGCAAGG11 594w14FCCGTATAGTGCTACGGAGGAGTAGGG11 594w14FCTGTTGCAGGCTCAATGTCTTGC13 833g14RTTCGAGGAGCCTTGGGGTCTAACC12 358w14FCCTGTATATATTCTTATAGCCCACCGGAGG12 915w14F2GAACACTTCTTCTTGGGGGCTAAAGC13 515w15FTCGCCATAGTCATTCTAGTGGAAGAC14 216w17FTTACCACTCACCACCTTCTCAGC13 632w16FCCACAGCTTGATGTGGAGGAACAC14 216w17FTTACCACTCACCACCTTCTCCAAC14 893w18RAGCAAAGGCCGAGTAGGGAACCAAAGCAACCAACGAACCAACC	w05F	AGTATGCTCTTATCGGAGCCCTTCG	5 107
w06RGATGGCGACAGGAAGAGTAAGTTGC5 293g06RAGCTTAATTAAAGTATTTGTTTGC5 931w07RCTGGTTTGAGCGCTTAGCTGTTAAC6 296g07FAAACTAGCGAAGGGCCTTCAAAGC6 407w08RCAGAGGTAAAGTAAGCGCGTGTGTC6 950w08F1CCCTTCACCTAGCAGGAATTTCTTCAATCC7 165g08FATGGGTATAGTTGGAGCTATGACAG7 908w08FACAACGAATGTGGAGGTGACTACAGG8 473g09RACCCATATTAGCTGGAGTGACTACAGG8 585w09FGGCCATCAGTGGTACTGAAGGTAACTAG9 169g10RAGAGGGCGAATGAATAAACTAATTG9 904w10F1ATGCGAAACCAACCCATGCTC10 274w11RTTGACTCTCCCACGGTACGGGTAGTAGG11 549w11FCTACACTTGACCCATTGAAGGTACGGG11 594w12R1TGAGTGAGAGTGTGGGGTCACAGT11 044w13RTACCTGCGTTTGGAGCAGCAGGGAGTAGGG11 594w14FCCGATTAGTGTTGGGGTCACAGT11 893g14RTTCGAGGGAGCCTTGGGGTCTAACC12 358w14FCCTGTTGCAGGCTTAGGGGTCTAACC13 025w14FCTGTTGCAGGCTTAGTGGAGCAGGG13 515w15FTGCCCATAGTCATTCTAGTGACAGC13 535w16RGCTGTTTGTTAGGAGGACTAGTC13 842w16FCCACAGCTTGAGGATAAGTCGAGGAAC14 216w17FTTACCACTCCACCACTTCTCCCAAC14 893w18RAGCAAAGGCCGAGTAGGGAACCAAAGTTG14 915w18RAGCAAAGGCCGAGTAGGGAACCAAAGCTACACC14 639w18FCCGCTACAACCAACCACCCTAAAGC15 631w18FCCGCCATATTTTGGTTATCGAGGAACCAACCAACCAACCA	wO6F	GTGCTTCCACTACACCACTTCCTAG	5 225
g06RAGCTTAATTAAAGTATTTGTTTTGC $5931$ $w07R$ CTGGTTGAGCGCTTAGCTGTAAC $6296$ $g07F$ AAACTAGACCAAGGGCCTTCAAAGC $6407$ $w08R$ CAGAGGTAAAGTAAGCGGTGTC $6950$ $w08F$ ACGGTATAGTCTGAGCATGATGG $7498$ $g08F$ ATGGGTATAGTCTGAGCTATGATGG $7498$ $g08R$ TAACCCACAATTCGCCTTGACAAG $7908$ $w08F$ ACAACGAATGTGGGAGTGACTACACG $8473$ $g09R$ ACCCATATTAGCTGTAGGAGCAACACG $8473$ $g09R$ ACCCATATTAGCTCAGAGGTAATGAGG $8585$ $w09F$ GGCCATCAGTGGTACTGAAGCTATG $9169$ $g10R$ AGAGGCGAATGAATAAACTAATTG $9904$ $w10F1$ ATGCGAAACCAACCAACCCATGCTC $10274$ $w11R$ TTGACTCCCCCAGGGTAGGAGGAGTAGGG $10459$ $w11F$ CTACACTTGACCCAATGTGTGGGTCACAGT $11044$ $w12R1$ TGAGTGAGAGTTGTGGGTCACAGT $11044$ $w13R$ TACCCGCTTTGAGCGCGGTTGG $11258$ $g13F$ CTTTCTCCGCTTGGAGGCCAAGG $11594$ $w14R$ ACCCATATGGCTTAGGGGTCAAAG $11594$ $w14F$ CCCCATATGGCTTGAGGGCCCATAGC $12358$ $w14F$ CCTCTATATATTTCTTATAGCCCGCGGG $12558$ $w14F$ CTGCCATAGTCATAGGCAGGCAAGAC $14219$ $w17R$ TAACGCGAGGATAAGGCGAGCAAGAC $14219$ $w17R$ TAACGCGAGGATAGGGAGCAAGAC $14219$ $w17R$ TAACGCGAGGATAGGGAGCAAGAC $14219$ $w17R$ TAACGCGAGGATAGGGAGCAAGAC $14219$ $w17R$ TAACGCGAGATAGGCGAGGAAGACCAAAGCACAAGCA $14219$	w06R	GATGGCGACAGGAAGAGTAAGTTGC	5 293
W07R         CTGGTTTGAGCGCTTAGCTGTTAAC         6 296           g07F         AAACTAGACCAAGGGCCTTCAAAGC         6 407           W08R         CAGAGGTAAAGTAAGGGCGTGTGTC         6 950           w08F1         CCCTTCACCTAGCAGGAATTTCTTCAAATCC         7 165           g08F         ATGGGTATAGTTCGAGCTATGATGG         7 498           g08R         TAACCCACAATTCTGCCTTGACAAG         7 908           w08F         ACACCACAATTCTGCCTTGACAAG         7 908           w08F         ACACCACAATTCTGGCTATCAAGG         8 473           g09R         ACCCCATATTAGCTCTAAGTGAGG         8 585           w09F         GGCCATCAGTGGTACTGAAGCTATG         9 169           g10R         AGAGGGCGAATGAATAAACTAATTG         9 904           w10F1         ATGCGAAACCAACCAACCCATGCTC         10 274           w11R         TTGATCTCCAGGGTAGGGAGGATAGTAG         10 44           w13R         TACCTGCGTTTAGTCGTTCGGTTGG         11 258           g13F         CTTTCTCCGCTTTGGAGCACAGG         11 670           g14F         CTGTTGCAGGCTCAATAGTTCTCTGC         12 358           w14F         CCCATATGCTTAGTGTGTGGGGCCAAGGAC         14 593           g14R         TTCGAGGGAGCCTTGGGGTCAACGC         13 535           w14F         CCTGTTGTTGTATGTTAGTGTGAGGAACC         14 519 <td>q06R</td> <td>AGCTTAATTAAAGTATTTGTTTTGC</td> <td>5 931</td>	q06R	AGCTTAATTAAAGTATTTGTTTTGC	5 931
g07F         AAACTAGACCAAGGGCCTTCAAAGC         6 407           w08R         CAGAGGTAAAGTAAGCGCGTGTGTC         6 950           w08F1         CCCTTCACCTAGCAGGAATTTCTTCAATCC         7 165           g08F         ATGGGTATAGTCTGAGCTATGATGG         7 498           g08R         TAACCCACAATTCTGCCTTGACAAG         7 908           w08F         ACACGAATGTGGAGTGACTACACG         8 473           g09R         ACCCATATTAGCTCTTAGTGAGG         8 585           w09F         GGCCATCAGTGGTACTGAAGCTATG         9 169           g10R         AGGGGGGAATGAATAAACTAATTG         9 904           w10F1         ATGCGAAACCAACCAACCCAACCCATGTGA         10 459           w10F1         ATGCGAAGCAACCAACCAACCAACGACC         10 274           w11R         TTGATGGAGAGTTGTGGGTACGGGGAGTAGTAG         10 459           w11F         CTACACTTGACCCATTGAAGTGCTC         10 274           w12R1         TGAGTGACGAGTTAGTGGTCCACAGT         11 444           w13R         TACCTGCGTTTAGGGTACGAGGT         11 258           g13F         CTTTCTCCGCTTGGAGGTAGG         11 594           w14R         ACCCATATGGCTTACGGAGGAGTAGG         12 515           w14F         CGTGTTGCTAGGGTTAACC         12 358           g14F         CTGTGTGCAGGCTTAGGGCTTAACC         12 355	w07R	CTGGTTTGAGCGCTTAGCTGTTAAC	6 296
W08R         CAGAGGTAAAGTAAGCACGCGTGTGTC         6 950           W08F1         CCCTTCACCTAGCAGGAATTTCTTCAATCC         7 165           g08F         ATGGGTATAGTCTGAGCTAGATGG         7 498           g08R         TAACCCACAATTCTGCACTTGACAAG         7 908           w08F         ACACGAATGTGGAGTGACTACACG         8 473           g09R         ACCCATATTAGCTTGTGAGTGACTACACG         8 473           g09R         ACCCATATTAGCTTCTAGTGAGG         8 585           w09F         GGCCATCAGTGGTACTGAAGCTATG         9 904           w10F1         ATGGGAAACCAACCAACCAACCATATTG         9 904           w10F1         ATGGGAAACCAACCAACCAACCAACGAGTAGTAG         10 459           w11F         CTACACTTGACCATTGAAGTGCC         10 812           w12R1         TGAGTGAGAGTTATGGGTCACAGT         11 044           w13R         TACCTGCGTTTGGAGTCACAGT         11 044           w13R         TACCTGCGTTGGAGGTCACAGT         11 670           g14F         CTGTTGCAGGCTCAATAGTCTTGC         11 893           g14R         TTCGAGGAGCCTTGGGGGTCAACC         12 258           w14F         CCCCTATATTCTTATGGCCTTCACC         13 025           w14F2         GAACACTCTCTTATGTGGAGAGAC         14 519           w14F2         GAACACTCTTCTTAGGGCTTACACC         13 515 <td>q07F</td> <td>AAACTAGACCAAGGGCCTTCAAAGC</td> <td>6 407</td>	q07F	AAACTAGACCAAGGGCCTTCAAAGC	6 407
w08F1         CCCTTCACCTAGCAGGAATTTCTTCAATCC         7 165           g08F         ATGGGTATAGTCTGAGCTATGATGG         7 498           g08R         TAACCCACATTCTGCCTTGACAAG         7 908           w08F         ACAACGAATGTGGAGGAGTGACTACACG         8 473           g09R         ACCCATATTAGCTCTGAGCAGG         8 585           w09F         GGCCATCAGTGGTACTGAAGGAGG         9 904           w10F1         ATGCGAAACCAACCAACCATGCTC         10 274           w11R         TTGACTCCCACGGGTAGCGGGAGTAGTAG         10 459           w11F         CTACACTTGACCCATGCTC         10 812           w12R1         TGAGTGAGAGTTGTGGGTCACAGT         11 044           w13R         TACCCGCGTTTAGTGGGTCACAGT         11 644           w13R         TACCCGCTTGGAGGTCACAGT         11 044           w13R         TACCCGCGTTGGAGGTCACAGT         11 893           g14F         CTGTTGCAGGCTCAATAGTCTTGC         12 88           w14F         CCCCATATGCCTTGCGGGGTCTAACC         12 88           w14F         CCTCTATATATTCTTATAGCCCACGGGGG         12 515           w14F2         GAACATCTTCTTATGGTCTGACG         13 032           g14R         TTCGAGGAGCTATGTCAGGCAAGAC         14 893           w14F2         GAACATCTTCTTATGTCTACC         13 035 <t< td=""><td>w08R</td><td>CAGAGGTAAAGTAAGCGCGTGTGTC</td><td>6 950</td></t<>	w08R	CAGAGGTAAAGTAAGCGCGTGTGTC	6 950
g08FATGGGTATAGTCTGAGCTATGATGG7 498g08RTAACCCACAATTCTGCCTTGACAAG7 908w08FACACCGAATTGGCAGTGACTACACG8 473g09RACCCATATTAGCTCTTAGTGAGG8 585w09FGGCCATCAGTGGTACTGAGAGCATATG9 169g10RAGAGGGCGAATGAATAAACTAATTG9 904w10F1ATGCGAAACCAACCAACCCATGCTC10 274w11RTTGATCTCCTCAGGGTAGCGGGAGTAGGAGTAGG10 459w11FCTACACTTGACCCATTGAAGTGCC10 812w12R1TGAGTGAGAGTTGTGGGTCACAGT11 044w13RTACCGCGTTTAGTCGTTCGGTTGG11 258g13FCTTTCTCCGCTTGGAAGCAAG11 594w14RACCCATATGGCTCAGGGTAGG11 670g14FCTGTTGCAGGCTCAATAGTCTTGC12 358w14FCCTCTTATATATTCTTATAACCCAGCGAGG12 515w14F2GAACATCTTCTTATGGCCCTCTCACC13 255w15FTCGCCATAGTCATTCTAGTGACAGC13 515w15FTCGCCATAGTCATTCTAGTGACAGC13 842w16FCCACAGCTTGATGATGAGGAACC14 216w17RTAACGCGAGGATAGGCAAGAC14 216w17RTAACGCGAGGATAGGCAAGACC14 216w17RTAACGCGAGGATAGGGAACCAAAGACC14 915w18RAGCAAAGGCCGAGTAGGGAACCAAAGCTTCG14 933w18FCCGCTACAACGAACCAACCAAACCAACCAACGCACGAG15 631w18FCCGCATACGAAACAACCAACCAAACCAACCACCCTAAGC15 631w18FCCGCATACGAAACCAACCAACCAACCACCCTAAGC15 631w18FCCGCATCAACGAAACCAACCAACCAACCAACCACC16 060g19FGAGGAGGTTTCTCAGTGAGAAATACCACC16 174	w08F1	CCCTTCACCTAGCAGGAATTTCTTCAATCC	7 165
008R         TAACCCACAATTCTGCCTTGACAAG         7 908           w08F         ACAACGAATGTGGAGTGACTACACG         8 473           g09R         ACCCATATTAGCTTCTTGGAGG         8 473           g09R         ACCCATATTAGCTTCTTGGAGG         8 585           w09F         GGCATCAGTGGTACTGAAGCTATG         9 169           g10R         AGAGGCGAATGAATAAACTAATTG         9 904           w10F1         ATGCGAAACCAACCAACCCATGCTC         10 274           w11R         TTGATCTCCTCAGGGTAGCGGGAGTAGTAG         10 459           w11F         CTACACTTGACCATTTCAAGTGCC         10 812           w12R1         TGAGTGAGAGTTGTGGGTCACAGT         11 044           w13R         TACCGCGTTAGTGGAACCAAG         11 594           w14R         ACCCCATATGGCTTACGGAGGAGTAGG         11 670           g14F         CTGTTGCAGGCTCAATAGTCTTGC         12 858           g14F         CTGTTGGAGGCTCTAGGGCTCAACC         12 358           w14F         CCTCTATATATTTCTTATAACCCAGCGGAGG         12 915           w14F2         GAACATCTTCTTATGGCCTTCACC         13 025           w15F         TCGCCATAGTGATGATGAGAAAGC         13 515           w15F         TCGCCATAGTGATGACAGCAC         14 216           w16F         CCACAGCTTGAATGTTCTAGGAAGACC         14 216	q08F	ATGGGTATAGTCTGAGCTATGATGG	7 498
W08F         ACAACGAATGTGGAGTGACTACACG         8 473           g09R         ACCCATATTAGCTTCTTAGTGAGG         8 585           W09F         GGCCATCAGTGGTACTGAAGCTATG         9 169           g10R         AGAGGCGAATGAATAAACTAATTG         9 904           W10F1         ATGCGAAACCAACCAACCCAACGTC         10 274           w11R         TTGATCTCCTCAGGGTAGCGGGAGTAGTAG         10 459           w11F         CTACACTTGACCCATTGAAGTGC         10 812           w12R1         TGAGTGGAGAGTTGTGGGTCACAGT         11 044           w13R         TACCTGCGTTTAGGACTCCGGTTTG         11 258           g13F         CTTTCTCCGCTTGGAAGCAAG         11 594           w14R         ACCCATATGGCTTACGGAGGAGTAGG         11 670           g14F         CTGTTGCAGGCTCAATAGTCTTGC         12 358           w14R         ACCCATATGGCTTACGGAGGAGTAGG         12 915           w14F         CCTCTATATATTTCTTATAGCCCAGCGAGG         12 915           w14F         CTGTGTGGCTTGATGTGAGAAAGC         13 025           w14F         CTAGCTGGCTTGATGTGAGAAAGC         13 515           w15F         TCGCCATAGTCATTCTAGGAAAGC         13 632           w16F         CCAAGCTTGATGTGTGAGAAAGC         13 632           w16F         CCACAGCTTGAATGTAGCGAAGAAC         14 216 </td <td>a08R</td> <td>TAACCCACAATTCTGCCTTGACAAG</td> <td>7 908</td>	a08R	TAACCCACAATTCTGCCTTGACAAG	7 908
g09RACCCATATTAGCTTCTTAGTGAGG8 585w09FGGCCATCAGTGGTACTGAAGCTATG9 169 $g10R$ AGAGGCGAATGAATAAACTAATTG9 904w10F1ATGCGAAACCAACCAACCCATGCTC10 274w11RTTGACTCCTCAGGGTACCGGGAGTAGTAG10 459w11FCTACACTTGACCCATTGAAGTGCC10 812w12R1TGAGTGAGAGTTGTGGGTCACAGT11 044w13RTACCTGCGTTTGGATGCGTTGGGTTGG11 594w14RACCCATATGCTTGGGAGCAAG11 670g14FCTGTTGCAGGGCTCAATAGTCTTGC12 358w14F2GAACACCTTGTGGAGCCTTAGCGGAGGG12 915w14F2GAACATCTTCTTATGCCCTTCACC13 025w15FTCGCCATAGTCATGTGGAGCAAGC13 515w16FCCACAGCTTGATGTTGGAGCAAGC13 535w16FCCACAGCTTGATGTTGGAGCAAGC14 219w17FTTACCACTCCACCACTCTCCCAAC14 893w18RAGCAAAGGCCGAGTAGGGAACCAAAGTTG14 915w18FCCGCATAGTCATGGAGGAACCAAAGTTG14 915w18FCCGCATAGTAGGAAACCCAACGTTGG15 593w18FCCGCATAGTAGAGAAGCCCCTCAAGC15 631cytb811RCTGCCATTTTTGGTTTGTTAGGGAACACAACCAACCA16 060g19FGAGGAGGTTTCTCAGTAGAGAAACCAACCAACCCAACCC	w08F	ACAACGAATGTGGAGTGACTACACG	8 473
W09F         GGCCATCAGTGGTACTGAAGCTATG         9 169           g10R         AGAGGCGAATGAATAAACTAATTG         9 904           w10F1         ATGCGAAACCAACCAACCAATGATG         10 274           w11R         TTGACTCCCCAGGGTAGCAGGAGTAGTAG         10 274           w11R         TTGATCTCCCCAGGGTAGCAGGAGTAGTAG         10 459           w11F         CTACACTTGACCCATTGAAGTGCC         10 812           w12R1         TGAGTGAGAGTTGTGGGTCACAGT         11 044           w13R         TACCTGCGTTTGAAGCAAG         11 594           w14R         ACCCATATGGCTTGGAGGACAAGG         11 594           w14R         ACCCATATGGCTTGGGGTCTAACG         12 358           g14F         CTGTTGCAGGGCTCAATAGTTCTTGC         11 893           g14R         TTCGAGGGAGCCTTGGGGGTCTAACC         12 358           w14F         CCTCTATATATTCTTATAGCCCATCCACC         13 025           w14F2         GAACATCTTCTTATGGTCAGTCAGCGAGG         13 515           w15F         TCGCCATAGTCATTCTAGGACGCCAAGAC         14 216           w16R         GCTTGTTGATGATGAGGAAGACC         14 216           w17F         TTACGCGAGGATAAGCGAGGAAGAC         14 519           w17F         TTACCACTCACCACTCTCCCAAC         14 893           w18R         AGCAAAGGCCGAGTAGGGAACAAACCAAACCAAACCAACC	a09R	ACCCATATTAGCTTCTTAGTGAGG	8 585
g10R         AGAGGGCGAATGAATAAACTAATTG         9 904           w10F1         ATGCGAAACCAACCAACCCATGCTC         10 274           w11R         TTGATCTCCTCAGGGTACCGGGAGTAGTAG         10 459           w11F         CTACACTTGAAGCCATTGAAGTGCC         10 812           w12R1         TGAGGAGAGTTGTGGGTCACAGT         11 044           w13R         TACCTGCGCTTTAGTCGGTTGG         11 258           g13F         CTTTCTCCGCTTGTGAAGCAAG         11 594           w14R         ACCCATATGGCTTACGGAGGAGTAGG         11 670           g14F         CTGTGGAGGCCTAATAGTCCTTGC         12 358           g14R         TTCGAGGGAGCCTTGGGGTCTAACC         12 358           w14F         CCTCTATATATTTCTTATAACCCAGCGAGGG         12 915           w14F2         GAACATCTTCTATGGGCCCTTCACC         13 025           w15F         TCGCCATAGTGATGTTGTGAGAAAGC         13 515           v15F         TCGCCATGATGTTGTAGGAGCAGC         13 842           w16F         CCACAGCTTGAATGACGAGCAACAC         14 216           w17R         TAACGCGAGGATTAAGTCGAGGAAC         14 519           w17F         TTACCACTCCACCACTTCTCCCAAC         14 893           w18R         AGCAAAGCCAACCAACCAACCAAAGCAAACCAAAGCA         14 915           wCytbR         GTTGTCAAATGAGAAGCCCCTCCAG	w09F	GGCCATCAGTGGTACTGAAGCTATG	9 169
w10F1         ATGCGAAACCAACCAACCCATGCTC         10 274           w11R         TTGATCTCCTCAGGGTAGCGGAGTAGTAG         10 459           w11F         CTACACTTGACCATTTGAAGTGCC         10 812           w12R1         TGAGTGAGAGTTGTGGGTCACAGT         11 044           w13R         TACCTGCGTTTAGTCGGTTCGGTTG         11 258           g13F         CTTTCTCCGCTTGTGAAGCAAG         11 594           w14R         ACCCATATGGCTTACGGAGGAGTAGG         11 670           g14F         CTGTTGGCAGGCTCAATAGTCTTGCC         12 358           w14F         CCTCTATATATTTCTTATAACCCAGCGAGG         12 915           w14F2         GAACATCTTCTTATGGCCTTCACC         13 025           w15R         CTAGCTGGCTTGATGTGAGAAAGC         13 515           w15F         TCGCCATAGTCATTCTAGTGACAGC         13 535           w16F         CCACAGCTTGAATGTTGAGGAACC         14 216           w17F         TTACCACTCCACCACTTCTCCAAC         14 893           w18R         AGCAAAGCAAACCAACCAACCAAACCAAACCAACCAACC	g10R	AGAGGGCGAATGAATAAACTAATTG	9 904
w11RTTGATCTCCTCAGGGTAGCGGAGTAGTAG10 459w11FCTACACTTGACCCATTTGAAGTGCC10 812w12R1TGAGTGAGAGTTGTGGGTCACAGT11 044w13RTACCTGCGTTAGTCGTCGGTTTG11 258g13FCTTTCTCCGCTTGGAAGCAAG11 594w14RACCCATATGGCTTACGGAGGAGTAGG11 670g14FCTGTTGCAGGCTCAATAGTCCTGC12 838g14FCTGTTGCAGGGCCTAATAGTCCTGC12 358w14FCCTCTATATATTCTTATAACCCAGCGAGG12 915w14F2GAACATCTTCTTATGGCCTTCACC13 025w15FTCGCCATAGTCATTCTAGTGACAAGC13 535w16FCCACAGCTTGAATGACGAGCAAGAC14 216w17FTTACCACTCCACCACTCTCCAAC14 893w17FTTACCACTCCACCACTTCTCCAAC14 893w18FAGCAAAGGCCGAGTAGGGAACCAAAGTTG14 915w18FCCGCTACAACCAACCCAACCTCAAGC15 593w18FCCGCACAACTGAAGAACCCCTCAAGC15 631cytb811RCTGCCATTTTTGGTTTACAACAC16 060g19FGAGGAGGTTTCCCAGTAGATAATGC16 174	w10F1	ATGCGAAACCAACCAACCCATGCTC	10 274
w11FCTACACTTGACCCATTTGAAGTGCC10 812w12R1TGAGTGAGAGTTGTGGGTCACAGT11 044w13RTACCTGCGTTTAGTGTGGGTCACAGT11 258g13FCTTTCTCCGCTTGGAAGCAAG11 594w14RACCCATATGGCTTAGGAGGAGTAGG11 670g14FCTGTTGCAGGGCCTAATAGTCTTGC11 893g14RTTCGAGGGACCTTGGGGTCTAACC12 358w14FCCTCTATATATTTCTTATAACCCAGCGAGG12 915w14F2GAACATCTTCTTATGGCCCTCCACC13 025w15FTCGCCATAGTCATTCTAGTGACAGC13 535w16FCCACAGCTTGAATGACGAGGAAC14 216w17FTTACCCAGCAGAGACTAAGTCGAGGAAC14 519w17FTTTACCACTCCACCACCTCTCCAAC14 893w18RAGCAAAGGCCGAGTAGGGAACCAAAGTTG14 915w07bRGTTGTCAACTGAGAAACCAAACCAAACCAACCTAAGC15 593w18FCCGCTACAACCAAACCAAACCAAACCAACC16 060g19FGAGGAGGTTTCTCAGTAGAGAAATATGC16 174	w11R	TTGATCTCCTCAGGGTAGCGGGAGTAGTAG	10 459
w12R1         TGAGTGAGAGTTGTGGGTCACAGT         11 044           w13R         TACCTGCGTTTAGTCGTTCGGTTG         11 258           g13F         CTTTCTCCGCTTGGAAGCAAG         11 594           w14R         ACCCATATGGCTTAGGGAGGAGTAGG         11 670           g14F         CTGTTGCAGGCTCAAGTCTTGC         11 893           g14R         TTCGAGGGAGCCTTGGGGTCTAACC         12 358           w14F         CCTCTATATATTTCTTATAACCCAGCGAGG         12 915           w14F2         GAACATCTTCTTATGGCCCTCTCACC         13 0325           w15F         TGCCCATAGTCATTCTAGGACAGC         13 515           w15F         TCGCCATAGTCATTCTAGGACAGC         13 535           w16R         GCTTGTTTGTTAGGGAGCTAGTC         14 893           w17R         TAACGCGAGGATAAGGCAGGAAGAC         14 216           w17R         TAACGCGAGGATAGGGAACCAAAGTTG         14 915           w17R         AGCAAAGGCCGAGTAGGGAACCAAAGTTG         14 915           w17R         AGCAAAGGCCGAGTAGGGAACCAAAGTTG         14 915           w17R         GTGTCAACTGAAGAAGCCCCTCAAG         15 593           w18F         CCGCTACAACCAAACCAAACCAAACCAAACCAAACCAACC	w11F	CTACACTTGACCCATTTGAAGTGCC	10 812
w13R         TACCTGCGTTTAGTCGTTCGGTTTG         11 258           g13F         CTTTCTCCGCTTGTGAAGCAAG         11 594           w14R         ACCCATATGGCTTACGGAGGAGTAGG         11 670           g14F         CTGTTGCAGGCCAATAGTCTTGC         11 893           g14R         TTCGAGGGAGCCTTGGGGTCTAACC         12 358           w14F         CCTCTATATATTTCTTATAACCCAGCGAGG         12 915           w14F2         GAACATCTTCTATGGCCTTCACC         13 025           w15F         TCGCCATAGTGTTAGTGACAGC         13 515           w15F         TCGCCATAGTGTTAGGGAGGCAGCAC         13 842           w16F         CCACAGCTTGAATGACGAGCAAGAC         14 519           w17F         TTACCACTCCACCACTTCTCCAAC         14 893           w17F         TTACCACTCCACCACTTCTCCAAC         14 893           w18R         AGCAAAGGCCGAATAGGGAACCAAAGAC         14 519           w17F         TTACCACTCGAGGATAAGGCACCAAAGTTTG         14 915           wCytbR         GTTGTCAAACGAAAGCACCAACCAAAGTTTG         14 519           w18F         CCGCTACAACCGAAGCACCAAGCAAAGCA         15 593           w18F         CCGCTACAAACCAAACCAAACCAAAGCA         15 631           cytb811R         CTGCCATTTTTGGTTTACAACCA         16 060           g19F         GAGGAGGTTTCCAAGTAGAAAAATACCA	w12R1	TGAGTGAGAGTTGTGGGTCACAGT	11 044
g13F         CTTTCTCCGCTTGTGAAGCAAG         11 594           w14R         ACCCATATGGCTTACGGAGGAGTAGG         11 670           g14F         CTGTTGCAGGCTCAATAGTTCTTGC         11 893           g14R         TTCGAGGGACCTAGGGCTCTAACC         12 358           w14F         CCTCTATATATTTCTTATAACCCAGCGAGGG         12 915           w14F2         GAACATCTTCTTATGGCCTTCACC         13 025           w15R         CTAGCTGGCTTGATGTTGAGAAAGC         13 515           w15F         TCGCCATAGTCATCTAGTGACAGCA         13 535           w16F         CCACAGCTTGAATGACGAGCAAGAC         14 216           w17R         TTAACGCGAGGATTAAGTCGAGGAAC         14 519           w17F         TTTACCACTCCACCACTTCTCCAAC         14 893           w18R         AGCAAAGGCCGAGTAGGGAACCAAAGTTG         14 915           wCytbR         GTTGTCAACTGAGAAGACCCCTCCAG         15 593           w18F         CCGCTACAACCAACCCACCTCTCAG         15 631           cytb811R         CTGCCATTTTTGGTTTACAACCA         16 060           g19F         GAGGAGGTTTCCAGGTAGATAATGC         16 174	w13R	TACCTGCGTTTAGTCGTTCGGTTTG	11 258
w14R         ACCCATATGGCTTACGGAGGAGTAGG         11 670           g14F         CTGTTGCAGGCTCAATAGTTCTGC         11 893           g14F         CTGTTGCAGGCTCAATAGTTCTGC         12 838           g14R         TTCGAGGGAGCCTTGGGGTCTAACC         12 358           w14F         CCTCTATATATTTCTTATAACCCAGCGAGG         12 915           w14F2         GAACATCTTCTTATGGCCTTCACC         13 025           w15R         CTAGCTGGCTTGATGTGAGAAAGC         13 515           w15F         TCGCCATAGTCATCTAGTGACAGCC         13 635           w16F         CCACAGCTTGAATGACGAGCAAGAC         14 216           w17R         TAACGCGAGGATTAAGTCGAGGAAAC         14 519           w17F         TTTACCACTCCACCACTTCTCCAAC         14 893           w18R         AGCAAAGGCCGAGTAGGGAACCAAAGTTG         14 915           w07bR         GTTGTCAACTGAGAAACCACCTTCAG         15 593           w18F         CCGCTACAACCAACCCAACCTAAAGC         15 631           cytb811R         CTGCCATTTTTGGTTTACAACCA         16 060           g19F         GAGGAGGTTTCTCAGTAGAGAAATAGC         16 174	g13F	CTTTCTCCGCTTGTGAAGCAAG	11 594
g14F         CTGTTGCAGGCTCAATAGTTCTTGC         11 893           g14R         TTCGAGGGAGCCTTGGGGTCTAACC         12 358           w14F         CCTCTATATATTTCTTATAACCCAGCGAGG         12 915           w14F2         GAACATCTTCTTATGGCCCTTCACC         13 025           w15R         CTAGCTGGCTTGATGTTCAGAAAGC         13 515           w15F         TCGCCATAGTCATCTAGTGACAGC         13 535           w16R         GCTTGTTGTTGTGAGGAGCAAGAC         14 216           w17F         TTACCACTCCACCACTCTCCCAAC         14 893           w18R         AGCAAAGGCCGAGTAGGGAACCAAAGTTG         14 915           w07bR         GTTGTCAACTGAGAAGACCCCTCAAG         15 593           w18F         CCGCTACAACCAACCCAACCTAAGC         15 693           w18F         CCGCTACAACAACAACCAACCAAACCAAACCA         16 060           g19F         GAGGAGGTTTCCCAGTGAGAAAATGC         16 174	w14R	ACCCATATGGCTTACGGAGGAGTAGG	11 670
g14R         TTCGAGGGAGCCTTGGGGTCTAACC         12 358           w14F         CCTCTATATATTTCTTATAACCCAGCGAGG         12 915           w14F2         GAACATCTTCTTATGGCCCTTCACC         13 025           w15R         CTAGCTGGCTTGATGTGAGAAAGC         13 515           w15F         TCGCCATAGTCATGTGAGAAGC         13 842           w16F         CCACAGCTTGAATGACGAGCAAGAC         14 216           w17R         TAACGCGAGGATAAGTCGAGGAAC         14 519           w17F         TTACCACTCCACCACTCTCCCAAC         14 893           w18R         AGCAAAGGCCGAAGGAAGCAAAGATC         15 53           w18F         CCGCTACAACAACCAACCAAAGTTG         15 93           w18F         CCGCTACAACAACCAACCCAAAGTTG         15 631           cytb811R         CTGCCATTTTTGTTACAACCAC         16 060           g19F         GAGGAGGTTTCCAGGTAGAGATAATGC         16 174	q14F	CTGTTGCAGGCTCAATAGTTCTTGC	11 893
w14F         CCTCTATATATTTCTTATAACCCAGCGAGG         12 915           w14F2         GAACATCTTCTTATGGCCCTTCACC         13 025           w15R         CTAGCTGGCTTGATGTTAGGAAGC         13 515           w15F         TCGCCATAGTCATCTAGTGACAGC         13 842           w16F         CCACAGCTTGAATGACGACGACAACAC         14 216           w17R         TAACGCGAGGATAAGTCGAGGAAC         14 519           w17F         TTACCACTCCACCACTTCTCCAAC         14 893           w18R         AGCAAAGGCCGAAGAACCCAACGTTG         15 533           w18F         CCGCTACAACTGAGAAGGCACCAAAGTTTG         14 915           wCytbR         GTTGTCAACTGAGAAGCCCCTCAG         15 631           cytb811R         CTGCCATTTTTGTTTACAACCAC         16 060           g19F         GAGGAGGTTTCCAAGTAGAGATAATGC         16 174	g14R	TTCGAGGGAGCCTTGGGGTCTAACC	12 358
w14F2         GAACATCTTCTTATGGCCCTTCACC         13 025           w15R         CTAGCTGGCTTGATGTTGAGAAAGC         13 515           w15F         TCGCCATAGTCATCTAGTGACAGC         13 535           w16R         GCTTGTTGTTAGGGAGCTAGTTC         13 842           w16F         CCACAGCTTGAATGACGAGCAAGAC         14 216           w17R         TAACGCAGGGATTAAGTCGAGGAAC         14 519           w17F         TTTACCACTCCACCACTCTCCCAAC         14 893           w18R         AGCAAAGCCGAGTAGGGAACCCAAGTTTG         14 515           wCytbR         GTTGTCAACTGAGAAGCCCCTCAGG         15 593           w18F         CCGCTACAACCAACCCAACCCTAAAGC         16 060           g19F         GAGGAGGTTTCTCAGTAGAGAAACACA         16 174	w14F	CCTCTATATATTTCTTATAACCCAGCGAGG	12 915
w15RCTAGCTGGCTTGATGTGAGAAAGC13 515w15FTCGCCATAGTCATCTAGTGACAGC13 635w16RGCTTGTTGTTAGGAGGACAGTC13 842w16FCCACAGCTTGAATGACGAGCAAGAC14 216w17RTAACGCGAGGATTAAGTCGAGGAAC14 519w17FTTTACCACTCCACCACTCTCCCAAC14 893w18RAGCAAAGGCCGAGTAGGGAACCAAAGTTG15 593w18FCCGCTACAACCAACCCTACGC15 631cytb811RCTGCCATTTTTGGTTACAACCA16 060g19FGAGGAGGTTTCCCAGTAGAGATAATGC16 174	w14F2	GAACATCTTCTTATGGCCCTTCACC	13 025
w15F         TCGCCATAGTCATTCTAGTGACAGC         13 535           w16R         GCTTGTTTGTTAGGGAGGCTAGTTC         13 842           w16F         CCACAGCTTGAATGACGAGGACAGAC         14 216           w17R         TAACGCGAGGATAAGTCGAGGAAC         14 519           w17F         TTTACCACTCCACCACTTCTCCCAAC         14 993           w18R         AGCAAAGGCCGAGTAGGGAACCAAAGTTG         14 915           w07tbR         GTTGTCAACTGAGAAGACCCTCTAG         15 593           w18F         CCGCTACAACCAACCTAAAGC         15 631           cytb811R         CTGCCATTTTTGGTTTACAACAC         16 060           g19F         GAGGAGGTTTCTCAGTAGATAATGC         16 174	w15R	CTAGCTGGCTTGATGTTGAGAAAGC	13 515
w16RGCTTGTTTGTTAGGGAGGCTAGTTC13 842w16FCCACAGCTTGAATGACGAGCAAGAC14 216w17RTAACGCGAGGATTAAGTCGAGGAAC14 519w17FTTTACCACTCCACCACTTCTCCAAC14 893w18RAGCAAAGGCCGAGTAGGGAACCAAAGTTG14 915wCytbRGTTGTCAACTGAGAAGCCTCCTCAG15 593w18FCCGCTACAACAACCACCCTAAAGC15 631cytb811RCTGCCATTTTTGGTTTACAACAC16 060g19FGAGGAGGTTTCTCAGTGAGAAATGC16 174	w15F	TCGCCATAGTCATTCTAGTGACAGC	13 535
w16F         CCACAGCTTGAATGACGAGCAAGAC         14 216           w17R         TAACGCGAGGATTAAGTCGAGGAAC         14 519           w17F         TTTACCACTCCACCACTTCTCCAAC         14 893           w18R         AGCAAAGGCCGAGTAGGGAACCAAAGTTTG         14 915           wCytbR         GTTGTCAACTGAGAAGAGCCTCCTAG         15 593           w18F         CCGCTACAACAACCCAACTCTAAGC         15 631           cytb811R         CTGCCATTTTTGGTTTACAACCA         16 060           g19F         GAGGAGGTTTCTCAGTAGAGAAAGCC         16 174	w16R	GCTTGTTTGTTAGGGAGGCTAGTTC	13 842
w17R         TAACGCGAGGATTAAGTCGAGGAAC         14 519           w17F         TTTACCACTCCACCACTTCTCCAAC         14 893           w18R         AGCAAAGCCGAGTAGGGAACCAAAGTTTG         14 915           wCytbR         GTTGTCAACTGAGAAGCCTCCAG         15 593           w18F         CCGCTACCAACCCACACCCTAAAGC         15 631           cytb811R         CTGCCATTTTTGGTTTACAACCA         16 060           g19F         GAGGAGGTTTCTCAGTAGATAATGC         16 174	w16F	CCACAGCTTGAATGACGAGCAAGAC	14 216
w17F         TTTACCACTCCACCACTTCTCCAAC         14 893           w18R         AGCAAAGCCGAGTAGGGAACCAAAGTTG         14 915           wCytbR         GTTGTCAACTGAGAAGCCTCCAG         15 593           w18F         CCGCTACAACCAAACCCAAAGC         15 631           cytb811R         CTGCCATTTTTGGTTTACAACCA         16 060           g19F         GAGGAGGTTTCTCAGTAGATAATGC         16 174	w17R	TAACGCGAGGATTAAGTCGAGGAAC	14 519
w18R         AGCAAAGGCCGAGTAGGGAACCAAAGTTTG         14 915           wCytbR         GTTGTCAACTGAGAAGCCTCCTCAG         15 593           w18F         CCGCTACAACAACCAACCCAAAGC         15 631           cytb811R         CTGCCATTTTTGGTTACAACAC         16 060           g19F         GAGGAGGTTTCTCAGTAGATAATGC         16 174	w17F	TTTACCACTCCACCACTTCTCCAAC	14 893
wCytbR         GTTGTCAACTGAGAAGCCTCCTCAG         15 593           w18F         CCGCTACAACAACCAACCCAAAGC         15 631           cytb811R         CTGCCATTTTTGGTTTACAACAC         16 060           g19F         GAGGAGGTTTCTCAGTAGATAATGC         16 174	w18R	AGCAAAGGCCGAGTAGGGAACCAAAGTTTG	14 915
w18F         CCGCTACAACAACCAACCCAAAGC         15 631           cytb811R         CTGCCATTTTTGGTTTACAACAC         16 060           g19F         GAGGAGGTTTCTCAGTAGATAATGC         16 174	wCytbR	GTTGTCAACTGAGAAGCCTCCTCAG	15 593
cytb811R         CTGCCATTTTTGGTTTACAACAC         16 060           g19F         GAGGAGGTTTCTCAGTAGATAATGC         16 174	w18F	CCGCTACAACAACCAACCCTAAAGC	15 631
g19F gaggaggtttctcagtagataatgc 16174	cytb811R	CTGCCATTTTTTGGTTTACAACAC	16 060
	g19F	GAGGAGGTTTCTCAGTAGATAATGC	16 174

**Note:** Primer codes beginning with "g" were designed for *Gadus* spp. (Coulson 2004). Forward primers are in Roman type and reverse primers are in italic type.

pected sizes <1 kbp were done according to previously published methods (Coulson et al. 2006).

To sequence the PCR products, 5  $\mu$ L of the PCR product was dried by evaporation or under vacuum. The sequencing cocktail comprised 1.0 or 2.0  $\mu$ L of Big Dye (version 3.0 chemistry; Applied Biosystems Inc., Foster City, California), 1.6  $\mu$ L of 2  $\mu$ mol/L primer forward or reverse, and 2.2  $\mu$ L ddH<sub>2</sub>O. The sequencing cycle was an initial denaturation at 96 °C for 2 min, followed by 50 cycles of a 96 °C denaturation for 2 min, 50 °C annealing for 15 s, and 60 °C extension for 4 min. Excess reactants were removed by isopropanol precipitation. The DNA was resuspended in 5  $\mu$ L of a 5:1 mixture of deionized formamide and

(A) Sequence differences among the	ne genus Lycodes and Anarhic	chas species.		
	Lycodes	A. denticulatus	A. minor	A. lupus
Lycodes	0	1708	1706	1732
A. denticulatus	99.66	0	298	340
A. minor	98.79	17.44	0	283
A. lupus	100.85	20.22	16.64	0
(B) Number of SNP differences/k	bp (p distances) among Gadua	s species.		
	<i>G</i> . (= <i>T</i> .) <i>chalcogrammus</i> EPac	<i>G. morhua</i> EAtl	<i>G. macrocephalus</i> EPac	G. m. ogac WAtl
G. (= T.) chalcogrammus WPac	4.77	38.46	40.18	40.25
G. morhua WAtl	38.26	3.32	39.58	39.25
G. macrocephalus WPac	39.85	38.72	4.91	3.25
G. m. ogac WAtl	39.72	38.46	4.51	[1.33]

Table 2. Phenetic comparison of genome differentiation in Anarhichas species and Gadus codfish mtDNA genomes.

**Note:** In A, the sequence differences were calculated as the absolute number of differences in the aligned complete sequences (upper half of the matrix), or the number of SNPs/kbp for the 15082 bp aligned between zoarcoid and gadid sequences, less the CR locus, and missing or ambiguous sequences (lower half of the matrix). In B, the number of SNP differences/kbp (*p* distances) was calculated over the same 15082 bp (after Coulson et al. 2006). In B, interspecific and intraspecific differences are compared across oceans (italic type); the *Gadus macrocephalus ogac* Richardson, 1836 comparison (brackets) is for two individuals from the western Atlantic Ocean (cf. Carr et al. 1999; Coulson et al. 2006). EPac, eastern Pacific Ocean; EAtl, eastern Atlantic Ocean; WAtl, western Atlantic Ocean.

25 mmol/L Na<sub>2</sub>EDTA buffer, heated to 95 °C for 2 min, and cooled rapidly to 5 °C. The denatured samples were spotted onto a paper comb and electrophoresed in an ABI377 automated DNA sequencer. The ABI Data Collection (version 2.6) software was used to collect the trace data and the Sequence Analysis (version 3.2) software was used to extract the sequence chromatograms.

Sequencer version 4.1.2 (GeneCodes, Ann Arbor, Michigan) was used to assemble and edit the mtDNA fragments of the three *Anarhichas* species. These fragments were initially aligned against the *G. morhua* reference sequence (NCBI accession No. NC002081), in which the positions of the initial primer sets were known.

Phylogenetic and phenetic analyses were carried out using the phylogenetic analysis using parsimony (PAUP\*) program version 4.0b10 (Swofford 2002). The exhaustive search algorithm was used to evaluate the properties of each of the three bifurcating trees possible with the complete genomes of the three Anarhichas species and another zoarcoid perciform species, the eelpout Lycodes toyamensis (Katayama, 1944) (NCBI accession No. NC004409), as the outgroup. A bootstrap analysis with 10 000 replicates was done to estimate the statistical significance for the nodes of the tree. Neighbor-joining phenetic analysis of uncorrected average pairwise distances (p distances) was also done. To compare the extent of intra- and inter-specific mtDNA genome differentiation with other fish species, the four zoarcoid genome sequences were aligned with those of seven gadid codfish (Gadus sensu lato; Carr et al. 1999) described in Coulson et al. (2006) (NCBI accession Nos. DQ356937-DQ356941 and DQ356946), with the addition of a second sequence from Gadus (= Theragra) chalcogrammus Pallas, 1814 (NCBI accession No. NC004449) from the western Pacific Ocean. CR locus sequences, which were not available for all gadines, were removed, and PAUP\* was used to removed any missing and (or) ambiguous characters. For the consensus data set of 15082 bp, p distances were calculated and are shown as numbers of changes per kilo base pairs in Table 2.

Variability of individual Anarhichas genes within genomes was calculated three ways: first as the average of the three pairwise sequence divergences among species (p distance) and second as the density of interspecific single nucleotide polymorphisms (SNPs), calculated as the ratio of the number of SNPs observed among all three species to the total gene length, expressed as the number of SNPs/100 bp. The two indices are closely related: the former will underestimate the actual extent of nucleotide divergence by not correcting for multiple-hit nucleotide substitutions at the same site, whereas the latter will underestimate variability by not counting the homoplasic changes implied by the inferred phylogeny. The latter provides a more intuitive measure of observed variability. The third measure of variability is the difference between the rates of synonymous  $(d_S)$  and nonsynonymous  $(d_N)$  nucleotide substitutions in each gene, according to the algorithm of Nei and Gojobori (1986) as implemented in MEGA version 3.1 (Kumar et al. 2004). This measure detects unusual patterns of amino acid substitution, for example, as a result of selection (cf. Coulson et al. 2006). The expected Poisson distribution of SNPs over tRNA loci was calculated by the recursive algorithm described by Zar (1999: 181). Nonparametric rank-order correlations of SNP density among protein-coding loci between the three Anarhichas species and the three Gadus codfish species (G. morhua, G. (= T.) chalcogrammus, and Gadus macrocephalus Tilesius, 1810) (Coulson et al. 2006) (DQ356938, DQ356939, and DQ356946), and comparisons of variability indices among Anarhichas loci made independently of absolute magnitude, were calculated by Kendall's coefficient of rank correlation  $(\tau)$  as implemented in BIOMstat version 3.30m (Applied Biostatistics, Inc. 2002; Sokal and Rohlf 1995: 593).

# Results

The long-range PCR procedure amplified more than 97% of the complete mtDNA genome of *A. lupus* in three over-

**Fig. 3.** Phylogenetic relationships among the mitochondrial genomes of *Anarhichas* species and *Lycodes toyamensis*. The minimum length tree requires 2074 changes, with branch lengths as shown. The tree indicates that *A. lupus* and *A. minor* are each other's closest relatives; this arrangement is supported by 96.9% of bootstrap replications.



lapping fragments of 6835, 4555, and 4276 bp (Fig. 2). The sequence of the remaining 466 bp segment of the genome (including the 3' end of the CYTB locus and the 5' end of the CR locus) had been determined previously. The genomes of the other two wolffish species were amplified with various combination of the *Anarhichas* and *Gadus* specific primers in Table 1. The three genomes were sequenced with the same set of primers.

The mtDNA genome sequences comprised 16519 bp in *A. lupus* and 16520 bp in the other two species. The sequences have been submitted to GenBank and assigned the accession numbers EF427916, EF427917, and EF427918 for *A. lupus, A. minor,* and *A. denticulatus,* respectively. The gene order is identical to that of *L. toyamensis,* except that *L. toyamensis* has an extra 169 bp segment 3' to the tRNA–Val locus that is not present in *Anarhichas* species. This segment has been deleted from the alignment analyzed here.

A total of 449 SNP sites were observed among the individuals representing the three species. *Anarhichas lupus* and *A. minor* differed by 248 pairwise differences (226 transitions and 22 transversions), *A. minor* and *A. denticulatus* by 274 differences (248 transitions and 26 transversions), and *A. lupus* and *A. denticulatus* by 286 differences (254 transitions and 32 transversions) (Table 2A). Interspecific *p* distances among *Anarhichas* species range from 16.64 to 20.22 substitutions/kbp (subs/kbp), which is approximately one-half that among *Gadus* species (from 38.46 to 40.25 subs/kbp) (Table 2B) and about four to five times as large as the differences among transoceanic individuals within *Gadus* species (from 3.25 to 4.77 subs/kbp).

With *L. toyamensis* included as an outgroup, there are 1944 variable sites, of which 146 are phylogenetically informative (sensu Nei 1987). The three possible bifurcating trees for four terminal taxa had lengths of 2074, 2094, and 2100 changes. The shortest tree indicates that *A. lupus* and *A. minor* are more closely related to each other than they are to *A. denticulatus*; this tree is supported by 96.9% of 10 000 bootstrap replicates (Fig. 3). Random resampling of 1, 2, 4, or 8 kbp of the complete genome supported the same tree in 57.2%, 66.2%, 77.4%, and 89.4% of bootstraps, respectively. This tree is also supported by neighbour-joining distance analysis (*p* distances) in 96.3% of bootstraps, as well

as by weighted parsimony and various distance models with >95% bootstrap support in all cases (results not shown).

The most variable protein-coding loci as measured by SNP density were ND4, CYTB, and ND2, with 4.40, 4.22, and 4.19 SNPs/100 bp, respectively. The least variable loci were ATP8 (also the shortest region), COX2, and ND3, with 1.19, 1.57, and 1.99 SNPs/100 bp, respectively (Table 3). The CR locus was less variable than 9 of 13 protein-coding regions (2.45 SNPs/100 bp) (Table 4). Most tRNA loci were either invariant or had only 1 SNP site, and the 20 tRNA loci collectively showed only 18 SNP sites over 1410 bp (1.28 SNPs/100 bp), which was lower than all except 1 protein-coding locus. Although 3 tRNA loci had three or more SNP sites and were thus more variable than any protein-coding locus (tRNA-Val, tRNA-Arg, and tRNA-Trp: >4 SNPs/100 bp), the observed distribution did not depart significantly from the expected random Poisson distribution of 18 SNPs over 20 tRNA loci ( $\chi^2_{0.05,[3]} = 4.21$ , 0.10 ). The 16S and 12S rRNA loci were the leastvariable gene regions (1.18 and 0.35 SNPs/100 bp, respectively). The relative ranks of locus variability as measured by SNP density and mean p distance are highly correlated (Kendall's  $\tau = 0.9731$ ,  $p \ll 0.01$ ), as are the rankings of SNP density and the rate of synonymous substitution  $(d_s)$ (Kendall's  $\tau = 0.7871$ ,  $p \ll 0.01$ ) and p distance vs.  $d_{\rm S}$ (Kendall's  $\tau = 0.8387$ ,  $p \ll 0.01$ ). The correlations of SNP density and p distance with  $d_{\rm N} - d_{\rm S}$  were slightly lower (Kendall's  $\tau = 0.7179$  and 0.7949, respectively), but still  $p \ll 0.01$  in both cases.

Similarly, there is a significant rank-order correlation of SNP densities between *Anarhichas* species and *Gadus* species (Kendall's  $\tau = 0.4615$ , p = 0.0140), but the association is far from exact. ND4 ranked first in *Anarhichas* and second in *Gadus*; however, the second- and third-ranked loci in *Anarhichas* (CYTB and ND2) ranked seventh and sixth in *Gadus*. The most highly ranked locus in *Gadus* (ND6) ranked fourth in *Anarhichas*, and the ATP6 and ND3 loci also had substantially lower ranks in *Anarhichas* (ninth and eleventh vs. fourth and fifth, respectively) (Fig. 4).

#### Discussion

The determination of the complete mtDNA genome se-

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Gene	Length	SNP occurrence	SNP density	р	$d_{\rm S}$	$d_{ m N}$	$d_{\rm N} - d_{\rm S}$
ND1	975	36	3.69	2.57	0.0858	0.0000	-0.0858
ND2	1048	44	4.19	2.54	0.0857	0.0054	-0.0803
COX1	1554	50	3.15	2.11	0.0728	0.0018	-0.0710
COX2	699	11	1.57	1.05	0.0424	0.0000	-0.0424
ATP8	168	2	1.19	0.80	0.0374	0.0000	-0.0374
ATP6	684	17	2.49	1.66	0.0568	0.0014	-0.0554
COX3	786	19	2.42	1.61	0.0538	0.0036	-0.0503
ND3	352	7	1.99	1.23	0.0416	0.0030	-0.0385
ND4L	297	8	2.69	1.80	0.0671	0.0000	-0.0671
ND4	1386	61	4.40	3.01	0.0837	0.0079	-0.0758
ND5	1839	59	3.21	1.90	0.0609	0.0060	-0.0550
ND6	523	21	4.02	2.55	0.0857	0.0035	-0.0822
CYTB	1162	49	4.22	2.64	0.0905	0.0047	-0.0851

**Table 3.** Length (bp), SNP occurrence, SNP density (no. of SNPs/100 bp), mean pairwise difference (p), rates of synonymous ( $d_S$ ) and nonsynonymous ( $d_N$ ) nucleotide substitutions, and the difference of the latter two measures for protein-coding loci of the mitochondrial genomes among *Anarhichas* species.

Note: The regions are listed in their linear order in the genome.

**Table 4.** Length (bp), SNP occurrence, and SNP density (no. of SNPs/100 bp) for the Control Region (CR),rRNA-coding, and tRNA-coding loci of the mitochondrial genomes of *Anarhichas* species.

Gene	Length	SNP occurrence	SNP density
CR	981	24	2.45
12S rRNA	948	3	0.32
16S rRNA	1697	20	1.18
tRNA-Phe	68	0	0
tRNA-Val	72	4	5.56
tRNA-Leu	74	0	0
tRNA-Ile	70	1	1.43
tRNA-Gln	68	0	0
tRNA-Met	69	0	0
tRNA-Trp	70	3	4.29
tRNA–Ala	72	0	0
tRNA-Asn	74	0	0
tRNA–Cys	66	1	1.52
tRNA–Tyr	67	1	1.49
tRNA-Ser	71	0	0
tRNA-Asp	69	1	1.45
tRNA-Lys	74	1	1.35
tRNA-Gly	69	1	1.45
tRNA-Arg	69	3	4.35
tRNA-His	64	0	0
tRNA-Ser	72	1	1.39
tRNA-Leu	73	0	0
tRNA-Glu	79	1	1.27
All tRNAs	1410	18	1.28

quences of *Anarhichas* species provides benchmark data for the investigation of their genetic biodiversity. The analysis presented here can better inform a recovery plan for these species by identifying the gene regions of highest variability, which would best be able to identify any distinctive fishery stocks and (or) designatable units under SARA.

The molecular analysis shows that *A. lupus* and *A. minor* are more closely related to each other than either is to *A. denticulatus*. The genomic difference between *A. lupus* 

**Fig. 4.** Total number of SNPs observed among three species of *Anarhichas* and three species of *Gadus* (*G. morhua*, *G. macrocephalus*, and *G.* (= *T.*) *chalcogramma*) (Coulson et al. 2006), expressed as number of SNPs/100 bp of gene length. Solid boxes indicate loci at which the relative rank of SNP density is higher in *Anarhichas* than in *Gadus* and shaded boxes indicate the reverse; ATP8 ranked last in both genera.



and A. minor (~20 subs/kbp) is about half that found between pairs of Gadus species (~40 subs/kbp), including another COSEWIC-assessed species, G. morhua. Because of the close relationships of the three species, the entire genome is required to resolve their branching order at >95% confidence. In contrast, Coulson et al. (2006) found that a random sample of about one-half of the genome sequence was sufficient to resolve almost all interspecific branches among gadines at >95% confidence. Given the estimated time of divergence of Atlantic G. morhua and Pacific G. macrocephalus at 3.5 million years before present, at the time of the last opening of the Bering strait (Carr et al. 1999; Coulson et al. 2006), extrapolation suggests that the three *Anarhichas* species separated from one another ca. 2 million years ago.

A closer relationship of A. lupus and A. minor is supported by an examination of several morphological and ecological characters. For example, both are typical benthophages with teeth adapted for feeding on bottomdwelling invertebrates (echinoderms, molluscs, and crustaceans), in contrast to A. denticulatus, which has teeth adapted for feeding on mobile invertebrates (ctenophores and jellyfish) (Albikovskaya 1982). All three species are pan-Atlantic (Fig. 1) but appear to have rather different niche requirements in this region. Anarhichas denticulatus have the widest distribution, found in the eastern Atlantic Ocean from Iceland to Novaya Zemlya and in the western Atlantic Ocean from Mould Bay to Sable Island Bank. They also have the greatest range of depths, from 151 to 600 m, and are known to feed close to the surface in open water — behaviours that are uncommon in the other two species. Anarhichas lupus are found in the eastern Atlantic Ocean from Iceland to the west coast of France, and from Greenland to the Gulf of Maine, and are thus the most southerly distributed species. They are the most abundant species in the area of Newfoundland and Labrador, where they are found from 101 to 350 m (Albikovskaya 1982). Anarhichas minor have the most restricted distribution, from Iceland to Bergen and from Greenland to the Grand Banks, where they are usually restricted to depths >450 m (Scott and Scott 1988). Given the phylogenetic relationships shown here, we can hypothesize that A. lupus and A. minor represent alternative, derived specializations at more restrictive, benthic strata, in contrast to a more widespread, generalist ancestral type represented by A. denticulatus.

The 449 SNPs observed in the mtDNA of the three individual representatives of the Anarhichas species occur at an average density of 26.8 SNPs/kbp, which varies from 3.2 to 44.0 SNPs/kbp among the major gene regions. SNP density among protein-coding loci in these individuals is exactly one-half of that among three individuals representative of three Gadus species (3.0 vs. 6.0 SNPs/100 bp), which is in agreement with the twofold difference in intrageneric genetic distances (18.8 vs. 39.58 subs/kbp) (Table 2). Such a correspondence is expected on the assumption of selective neutrality (Kimura 1983), as is the observation that all measures of locus variability are highly correlated. Rates of nonsynonymous substitution are very low at every locus, and the difference  $(d_N - d_S)$  is always negative, so that Fisher's exact test of the distribution of synonymous and nonsynonymous substitutions (Zhang et al. 1997) would not approach significance for any comparison. There are thus no indications of unusual patterns of selection at work on Anarhichas genomes. The significant rank-order correlation of SNP density among protein-coding loci between Anarhichas and Gadus indicates that patterns are broadly similar; however, there are many exceptions. The three most variable loci in Anarhichas ranked second, seventh, and sixth, respectively, in Gadus; this includes the CYTB locus (second vs. seventh) for which a large number of SNP variants have been identified in G. morhua (Carr and Marshall 1991; Pepin and Carr 1993; Arnason 2004). Whether such intergeneric differences are a function simply of variation in the neutral mutation rate among loci, or instead indicate differing patterns of natural selection, can be evaluated by further comparison of intraspecific patterns of nucleotide substitution in *Anarhichas* and *Gadus* with the interspecific patterns shown here (Kreitman 2000).

Although many studies of fish and other populations have used the CR locus on the expectation that it is routinely hypervariable (Brown et al. 1979; Kocher and Carleton 1997; Ingman et al. 2000), in wolffish this region is one of the least variable (less than 9 of 13 protein-coding loci) and is not a good candidate for intraspecific comparisons. The available population data confirm this (K.A. Johnstone, H.D. Marshall, and S.M. Carr, unpublished observation). As shown by this study and others (Faber and Stepien 1997), hypervariability of the CR locus is often not the rule in nonmammalian taxa. Other studies have advocated the use of loci in the NADH gene complex for single- and multi-locus population analyses (Russo et al. 1996). Recently, use of the 5' end of the COX1 locus as a "barcode" across species and taxa, including fish, has been advocated, with the expectation that the locus will be routinely variable among species and less so within (Ward et al. 2004). Again, the present data do not show a uniform pattern among taxa: COX1 is more variable interspecifically in Anarhichas species than in Gadus species (ranked sixth vs. tenth). The present data should emphasize that patterns of locus variability are not readily generalized across even relatively closely related taxa (zoarcoid perciform fishes) and that preliminary investigation of taxon-specific patterns is routinely warranted before carrying out a fullscale population genetic study.

The results of this study suggest several strategies for population genetic investigation of Anarhichas species. A single-locus analysis would best target the ND4 locus with primers g13F and g14R, which would amplify this gene within a fragment of 1476 bp. Where amplification of fragments of this size from samples with predominantly low molecular weight DNA is difficult, amplification of the ND4 region in two PCR reactions, with primer pairs of g13Fw14R (896 bp) and g14F-g14R (781 bp), should be feasible. A second strategy would be a multi-locus analysis of the contiguous ND4, ND5, CYTB, and COX1 genes, which includes two of the most variable loci. Use of long-range PCR amplification with the primers w13F and cytb811R would amplify a 6329 bp fragment that includes these four loci, and provide more than one-third of the genome for analysis. Alternatively, whole-genome approaches are also available that would examine variation in the complete 16.5 kbp genomes (Carr and Marshall 2005; Carr et al. 2007).

# Note added in proof

The original description of the walleye pollock is *Gadus* chalcogrammus Pallas, 1814 in Pallas' Zoographia rosso-asiatica. Volume 1 of this work was published in 1811, which is often cited as the year of publication of this combination (e.g., Cohen et al. 1990), but the description of the pollock occurs in volume 3, which was published in 1814, according to *International Commission of Zoological No-menclature* opinion 212. The correction should also be applied to the discussion in Carr et al. (1999) and Coulson et al. (2006).

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