Fifteen novel microsatellites for the Louisiana Waterthrush (Aves: Parulidae: *Parkesia motacilla*) using MiSeq sequencing

R. Lynn Von Hagen [1,2]

Rafia A. Khan [1,3]

Stefan Woltmann [1,4]

[1] Dept. Biology, and Center of Excellence for Field Biology, Austin Peay State University, Clarksville, TN 37040 USA

[2] current address: Dept. Biology, Western Kentucky University, Bowling Green, Kentucky 42101 USA

[3] current address: Dept. Entomology and Nematology, University of Florida, Gainesville, Florida 32611 USA

[4] corresponding author: woltmanns@apsu.edu

Abstract

The Louisiana Waterthrush (*Parkesia motacilla*) breeds along wooded streams throughout much of eastern North America, and winters in the Caribbean and Central America. Because of its dependence on stream macroinvertebrates—which are themselves dependent on high water quality—the Louisiana Waterthrush may serve as a useful bioindicator of both stream and landscape integrity. Perhaps unique among eastern North American songbirds, the Louisiana Waterthrush often maintains essentially linear territories along streams, and this provides a unique context in which to ask questions about the genetic (as opposed to social) mating system of this species. We developed 15 microsatellite loci for Louisiana Waterthrush using MiSeq sequencing. All loci presented here are polymorphic, with 3-15 alleles detected in a reference sample of 35-43 individuals. For parentage analyses, these loci have a combined non-exclusion probability of 0.0011 if neither parent is known *a priori*, and a non-exclusion probability of < 0.0001 if one parent is known. These 15 loci thus provide high discriminatory power to assign parentage to nestlings, and can also be used to examine population genetic structure within the species.

Introduction

The Louisiana Waterthrush (*Parkesia motacilla*) is a migratory warbler (Aves: Parulidae) that breeds throughout much of eastern North America and over-winters in Central America and the Caribbean (Mattsson et al. 2009). Louisiana Waterthrushes are highly dependent on forested streams with good-to-excellent water quality (Mattsson and Cooper 2006, Mulvihill et al. 2008), where they maintain essentially linear territories (Mattsson et al. 2009). During the breeding season, North American parulids are typically socially monogamous (as is Louisiana Waterthrush), but are often genetically polygamous (e.g., Thusius et al. 2001, Webster et al. 2001, Reudink et al. 2009). In the Louisiana Waterthrush, whose genetic mating system has not been examined, it is the often linear arrangement of territories, combined with observations of males at least occasionally attending multiple nests simultaneously (Mulvihill et al. 2002) that suggest a need to better understand the genetic mating patterns within the species. We describe here the development of 15 independent microsatellite loci that will be useful in studies of the genetic mating patterns of Louisiana Waterthrush.

We collected blood samples from Louisiana Waterthrush during April-June in 2014 and 2015 from Cheatham, Davidson, and Montgomery counties in Tennessee, USA. Blood was stored in Queen's lysis buffer (Seutin et al. 1991). Genomic DNA was isolated using Qiagen DNEasy Blood and Tissue kits following the manufacturer's protocols (Qiagen Inc., Valencia, California, USA). Methods for initial marker discovery using the Illumina MiSeq platform are presented in detail by Nali et al. (2014).

MSATCOMMANDER (v 1.0.3; Faircloth 2008) was used to scan the assembled .fasta file for trimeric and tetrameric microsatellites (minimum repeat number = 6), and to design primers. We optimized 15 microsatellites that contained at least 3 alleles, amplified reliably, and produced repeatable genotypes. We modified forward primers with a 19 bp M13 tag (CACGACGTTGTAAAACGAC) on the 5' end to allow the use of a third, dye labeled M13 primer in the PCR for subsequent genotyping (Boutin-Ganache et al. 2001). Reverse primers were modified with a "pig tail" (GTTTCTT) on the 5' end to help reduce stutter. PCRs were done in 10 µL reactions containing 1X buffer (New England Biolabs Inc., Ipswich, Massachusetts, USA), locus-specific $MgCl_2$ (see Table 1), 0.15 µM forward primer, 0.30 µM reverse primer, 0.10 µM dye-labled primer, and 0.5 units *taq* polymerase (New England Biolabs). PCR conditions were as follows: initial denaturation for 1 min at 94°C, followed by 33 cycles of: 30 s denaturation at 94°C, annealing for 30 s at 60°C, extension for 30 s at 72°C, and a final extension for 5 min at 72°C. PCR products were pooled post-PCR (using up to four different dyes) and run on an ABI 3730 capillary sequencer.

We used GENEMARKER (v 1.6; Soft Genetics Inc., State College, Pennsylvania, USA) to genotype 35-43 individuals at each locus. MICROCHECKER (van Oosterhout et al. 2004) found no evidence of null alleles, large allele drop-out, or problems arising from stutter. Observed (H_O) and expected heterozygosity (H_E) were calculated in GenAlEx (v 6.502; Peakall and Smouse 1996). Probability tests implemented in GENEPOP (Rousset 2008) found no deviations from Hardy-Weinberg expectations and no consistent evidence for linkage disequilibrium among the 15 loci (all P values > 0.05). For use in parentage analyses, the program CERVUS (Marshall et al. 1998) calculated combined non-exclusion probabilities assuming one, or both parents known of 0.0011 and <0.0001, respectively. These 15 loci thus provide high discriminatory power to assign parentage to nestlings, and can also be used to examine population genetic structure within the species.

77

78 **Additional Information and Declarations**

79 **Competing Interests**

80 The authors declare no competing interests.

81

82 **Author Contributions**

83 R. Lynn Vonn Hagen helped collect field data, performed the experiments, and reviewed drafts
84 of the paper.

85

86 Rafia A. Khan performed the experiments and reviewed drafts of the paper.

87

88 Stefan Woltmann conceived and designed the experiments, collected field data, analyzed the
89 data, and wrote the paper.

90

91 **Animal Ethics**

92 Animals were captured and released under the following permits to Stefan Woltmann: U.S.
93 Federal Bird Banding permit 23828, Tennessee Wildlife Resources Agency permit 3728, and
94 Austin Peay State University IACUC protocol 13-013.

95

96 **DNA Deposition**

97 GenBank Accession numbers: KX24524 – KX24538.

98

99 **Funding**

100 Support for this work came from the Summer Research Fellowship Program, the Biology
101 Department, and the Center of Excellence for Field Biology at Austin Peay State University.

102

103 **Acknowledgments**

104 S. M. Bogdanowicz and the Evolutionary Genetics Core Facility at Cornell University performed
105 the early stages of marker discovery (MiSeq). A. Lindley, M. Hart, and B. Bedal assisted with
106 sample collection and processing.

107

References

Boutin-Ganache, I., M. Raposo, M. Raymond, and C. F. Deschepper. 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. BioTechniques **31**:24-28.

Faircloth, B. C. 2008. msatcommander: detection of microsatellite repeat arrays and automated, locus-specific primer design. Molecular Ecology Resources **8**:92-94.

Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology **7**:639-655.

Mattsson, B. J., and R. J. Cooper. 2006. Louisiana waterthrushes (*Seiurus motacilla*) and habitat assessments as cost-effective indicators of instream biotic integrity. Freshwater Biology **51**:1941-1958.

Mattsson, B. J., T. L. Master, R. S. Mulvihill, and W. D. Robinson. 2009. Louisiana Waterthrush (*Parkesia motacilla*).*in* A. Poole, editor. The Birds of North America. Cornell Lab of Ornithology, Ithaca. Retrieved from the Birds of North America Online: http://dx.doi.org/10.2173/bna.151.

Mulvihill, R. S., A. M. Y. Cunkelman, L. Quattrini, T. J. O'Connell, and T. L. Master. 2002. Opportunistic polygyny in the Louisiana Waterthrush. The Wilson Bulletin **114**:106-113.

Mulvihill, R. S., F. L. Newell, and S. C. Latta. 2008. Effects of acidification on the breeding ecology of a stream-dependent songbird, the Louisiana waterthrush (*Seiurus motacilla*). Freshwater Biology **53**:2158-2169.

Nali, R. C., K. R. Zamudio, and C. P. Prado. 2014. Microsatellite markers for *Bokermannohyla* species (Anura, Hylidae) from the Brazilian Cerrado and Atlantic Forest domains. Amphibia-Reptilia **35**:355-360.

Peakall, R., and P. E. Smouse. 1996. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**:288-295.

Reudink, M. W., P. P. Marra, T. K. Kyser, P. T. Boag, K. M. Langin, and L. M. Ratcliffe. 2009. Non-breeding season events influence sexual selection in a long-distance migratory bird. Proceedings of the Royal Society B: Biological Sciences **276**:1619-1626.

Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources **8**:103-106.

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

Thusius, K. J., P. O. Dunn, K. A. Peterson, and L. A. Whittingham. 2001. Extrapair paternity is influenced by breeding synchrony and density in the common yellowthroat. Behavioral Ecology **12**:633-639.

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

Webster, M. S., H. C. Chuang-Dobbs, and R. T. Holmes. 2001. Microsatellite identification of extrapair sires in a socially monogamous warbler. Behavioral Ecology **12**:439-446.

Table 1. Description and summaries of 15 microsatellite loci for use in Louisiana Waterthrush (*Parkesia motacilla*). N_A = number of alleles. Size ranges reflect subtraction of the 19 bp of M13 tag from the forward primer and 7 bp of the "pig tail" from the reverse primer.

Locus GenBank#	**Primer Pair Sequence (5'-3')**	**Repeat Motif**	**$MgCl_2$ (mM)**	***N* (indivs)**	**Size Range (bp)**	**N_A**	***Ho***	***He***
Pm009 KX245424	F:ACCAACTGAAGACATCCATCTATC R:GGAGGATGAAGAGACAAGTGTTC	$(TCTA)_5$	3.0	38	150-224	15	0.895	0.872
Pm010 KX245425	F:GATCTTCCACGAAATGACCTCTG R:GTTGAGTACACCATCCACTGTTG	$(ACAG)_5$	3.0	43	322-338	4	0.558	0.602
Pm017 KX245426	F:ATAGTGTGATGGCCTAGAAATGC R:AATACTGCAGGTGTGATTTGAGC	$(AGAC)_6$	2.0	43	164-184	6	0.651	0.727
Pm030 KX245427	F:GCTCTCCAGTCTTCACAGTTTG R:TGATGGCTCACAAATCTCACTTC	$(ACAG)_6$	3.0	43	152-162	5	0.651	0.676
Pm042 KX245428	F:AGGGACCTAATAGCGGATCAATG R:GTCATTCTGAATCTTCCAGAGGC	$(TCTA)_5$	2.0	43	322-346	7	0.744	0.764
Pm047 KX245429	F:TGTCCTTTCTAGAGCACAGTAGG R:GGTTCTCCAAATGACAATGATGAC	$(TATC)_{15}$	2.0	43	192-248	15	0.884	0.890
Pm050 KX245430	F:ACATATGCATTCTGTAGGTGCTC R:AAGTGGCAGGGTAGGTAGGTATC	$(TATC)_{11}$	2.0	35	261-281	6	0.771	0.766
Pm052 KX245431	F:ATGCAAGCAAACAAGGTTCAAAG R:CCCATCAATATATACAGTGTGCAG	$(TCTA)_5$	2.0	41	282-298	4	0.415	0.416
Pm055 KX245432	F:GAAGCTTGGTGGAACTCTGAAG R:ACCAAATGTCAGTCTGCTCAAAG	$(TCTA)_{10}$	2.0	43	236-276	12	0.791	0.849
Pm056 KX245433	F:AGCACCTGTTGTTTGATAGATCTC R:TATCACTCATATTCTCTGCTGGC	$(TATC)_{11}$	2.0	43	137-177	9	0.884	0.850
Pm063 KX245434	F:TGCTGATTTGCTCTGTGTGTAAG R:AAAGGCAATGATGTAACACCCAC	$(ATC)_6$	3.0	43	165-192	8	0.698	0.771
Pm065 KX245435	F:ATAACACTTCTGAGCCTGTTTGC R:TCTCCTATTCATCTTTCTGGGAGG	$(ATC)_6$	3.0	43	145-154	4	0.674	0.578
Pm076 KX245436	F:AAACTGTGCACCATATCCACATC R:GTATATCAAGGTTGCAGTTTGGC	$(ATC)_6$	2.0	42	241-271	7	0.619	0.577
Pm077 KX245437	F:ATCACCAGGAATATGCTCTTGAG R:CCTATATCCACAGCACGGTTTC	$(ACT)_3…(ATC)_5$	2.0	43	285-299	5	0.721	0.733

Pm078 KX245438	F:TCGTTCCCTCAGTGGTATTCTAG R:GAGGCATCATCAGGTCCAATAAC	$(ACT)_7$	2.0	43	213-222	3	0.651	0.603