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SHORT-COMMUNICATION

Novel microsatellites for *Cypseloides fumigatus*, cross-amplifiable in *Streptoprocne zonaris*

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ABSTRACT: Based on microsatellite prospection, we isolated and characterized 21 microsatellite markers for the Sooty Swift (*Cypseloides fumigatus*) and tested the cross-amplification in the White-collared Swift (*Streptoprocne zonaris*). Both species are New World species included in the Apodidae family. From these 21, only 13 loci were polymorphic in the Sooty Swift, and their levels of polymorphism were surprisingly low compared to related species. Cross-amplification in the White-collared Swift was successful for 11 loci of the 13 polymorphic found for the Sooty Swift, but seven were monomorphic and four were biallelic. The microsatellites described here could be useful in future genetic population studies for Sooty Swifts and related species.

KEY-WORDS: Cypseloidinae, Sooty Swift, Ultraconserved elements (UCEs), White-collared Swifts.

Sooty Swifts, *Cypseloides fumigatus*, and White-collared Swifts, *Streptoprocne zonaris*, are New World apodid species. Whereas the White-collared Swift has a wide distribution, ranging from southern USA to southwestern Argentina, the Sooty Swift ranges from Argentina to Bolivia, Brazil and Paraguay (Chantler 1999). Both species present highly aerial behavior and are frequently seeing foraging and nesting together (Marín & Stiles 1992, Pearman *et al.* 2010, Biancalana *et al.* 2012, Biancalana 2014 & 2015). They nest in colonies, usually next to waterfalls and wet caves. Like other swift species they are philopatric, returning to use the same nest site over several years (Marín & Stiles 1992, Collins & Foerster 1995, Biancalana, pers. obs.). Here, we describe and characterize 13 novel polymorphic microsatellites for the Sooty Swift. In addition, we cross-amplified 11 of the 13 loci in the White-collared Swift from which seven were monomorphic and four were biallelic.

Microsatellite prospection was based on off-target sequences obtained through sequence capture and next-generation sequencing of Ultraconserved Elements (UCEs), as described in Amaral *et al.* (2015). Genomic DNA was obtained from a muscle sample from a Sooty Swift individual deposited at the *Laboratório de Genética e Evolução Molecular de Aves* (Universidade de São Paulo USP, Brazil – LGEMA #11411), collected at Ortigueira, Paraná state (24°12'S; 50°55'W) using the Qiagen DNeasy kit (Valencia, CA) with an RNAse treatment. Sequencing was performed at Rapid Genomics (Gainesville, FL, USA). The contigs obtained were screened for perfect di-, tri-, tetra-, penta-, and hexa-nucleotide with at least five repeats using QDD (Meglécz *et al.* 2010). QDD and Primer3 (Koressaar & Remm 2007, Untergasser *et al.* 2012) were used to design primers with default parameters and minimum fragment length of 100 bp. Each forward primer was designed with a M13 sequence (CACGACGTTGTAAAACGAC) added to its 5' end in order to pair with a third fluorescently labeled *primer*, according to the universal labeling method described by Boutin-Ganache *et al.* (2001).

For the characterization of the prospected microsatellites, 34 samples of Sooty Swifts were collected: 19 at Intervales State Park (ISP, Ribeirão Grande, São Paulo state, Brazil) and 15 at Sussuapara Canyon (SC, Ponte Alta do Tocantins, Tocantins state, Brazil). Both sites are known to have nests of the species and are monitored since 2010 (SC) and 2012 (ISP). To check the cross-amplification for White-collared Swifts, 10 adult samples were collected at ISP. Adults were mist netted and nestlings were captured in their nests and returned after sampling. Blood samples were collected from the brachial vein using microcapillary tubes and stored in absolute ethanol under room temperature. Genomic DNA was extracted from whole blood with a salt protocol adapted

from Aljanabi & Martinez (1997).

PCRs were carried out in volumes of 12 µl containing 1.5 µl of extracted DNA (30–50 ng/µl), 0.2 mM of dNTPs, 1 × PCR buffer, 3 pmol of the reverse primer, 1 pmol of forward primer, 2 pmol of FAM/HEX M13 primer, 2.5 mM of $MgCl_2$ and 0.5 U of Taq Polymerase (Sinapse, Inc.). Thermocycling conditions consisted of 95° (5 min), 35 cycles at 94°C (30 s), T_A°C (30 s), 72°C (30 s) with a final extension at 72°C (10 min). The optimal annealing temperature for each primer pair was determined using a temperature gradient cycle from 56 to 64 °C with a 2 °C difference between steps. PCR products were visualized on a 1.5% agarose gel using a 100 bp ladder. Successful PCR products were genotyped on a ABI 3730 (Applied Biosystems) automated sequencer and analyzed with GeneMarker 2.7.0 (Softgenetics).

We used GenAlEx 6.5 (Peakall & Smouse 2012) to estimate the number of alleles, and expected and observed heterozygosities. GENEPOP 4.2 (Raymond & Rousset 1995, Rousset 2008) was used to search for deviations from Hardy-Weinberg and linkage equilibrium. Benjamini & Yekutieli (2001) correction was applied to adjust the critical values for multiple comparisons. The search for null alleles and the estimation of their frequencies was done using MICRO-CHECKER 2.2.3 (van Oosterhout *et al.* 2004).

A total of 423 microsatellites were prospected for the Sooty Swift. From these, 138 (32.6%) were linked to UCEs regions and were discarded from primer design as they may be under purifying selection (Harvey *et al.* 2016) and are possibly monomorphic. From the remaining 285 microsatellites, we designed primers for 21 perfect loci (Table 1). Most were dinucleotides (81.0%), followed by trinucleotides (14.3%) and one pentanucleotide (4.8%). From the 21 loci tested in the 34 samples of Sooty Swifts, 16 were successfully amplified in Sooty Swifts (Table 1). Although the loci Cyps23 and Cyps34 successfully amplified, they did not produce consistent genotypes due to an excess of stutter bands. Thirteen loci were polymorphic with the number of alleles ranging from 2 to 8 (mean ± SD: 3.3 ± 0.43, Table 1). Observed heterozygosities ranged from 0.03 to 0.56 (mean *Ho* ± SD: 0.16 ± 0.05) and expected heterozygosities from 0.03 to 0.67 (mean *He* ± SD: 0.25 ± 0.06, Table 1). These heterozygosities were low when compared to that of phylogenetically close species, though the microsatellites used in the reference literature where not the same as those designed and tested in this study (Lance *et al.* 2009, González *et al.* 2010, Oyler-McCance *et al.* 2011, Gutiérrez-Rodriguez *et al.* 2013, Sanvicente *et al.* 2016; Table 2). Several attempts of amplification using two hummingbird microsatellites developed in other studies, Acya3-3 and Hxan07 (Gutiérrez-Rodriguez *et al.* 2013, Sanvicente *et al.* 2016), were done by RNB, with different settings of temperature and thermocycling conditions, but resulted in no amplifications for Sooty and White-collared Swifts.

The test on Hardy-Weinberg equilibrium (HWE) for each locus revealed deviations in 5 loci after Benjamini & Yekutieli (2001) correction (Cyps8, Cyps14, Cyps26, Cyps33, and Cyps35 – Table 1). The following loci had evidence of null alleles (with their respective frequencies): Cyps8 (0.237), Cyps14 (0.281), Cyps24 (0.156), Cyps26 (0.310), and Cyps33 (0.279). Among 91 paired loci, four cases of linkage disequilibrium were detected for the loci Cyps8 and Cyps12, Cyps12 and Cyps25, Cyps14 and Cyps26, and Cyps24 and Cyps35 [$P < 0.01$ after Benjamini & Yekutieli (2001) correction]. The deviations to HWE might be caused by the presence of null alleles in some loci (Cyps8, Cyps14, Cyps26, Cyps33 and Cyps36), or due to population substructure, since samples were collected in different localities. The cross-amplification of the prospected loci in White-collared Swifts was successful for 11 of them. However, the loci were either monomorphic or biallelic (Table 1). Locus Cyps9, that did not amplify in Sooty Swifts, was successfully amplified for White-collared Swifts.

The low polymorphism found in Sooty Swift microsatellites may result from many factors, ranging from loci linked to conserved regions to ecological and historical factors. Milot *et al.* (2007) suggested, for example, that some life history traits associated with demographic patterns may result in a small effective population size, that in long periods of time can result in loss of genetic diversity. This means that not only bottlenecks might be the main cause for low genetic variability in birds (Amos & Harwood 1998). Because Sooty Swifts usually establishes small populations (ranging from two to ten individuals in general), exhibits philopatry, presents a long breeding period and raises just one chick per year (Biancalana *et al.* 2012, Biancalana 2015), the population might be suffering from inbreeding and/or might naturally have low genetic variability. Alternatively, population size variation due to historical factors - as climate change in the past - could also explain low genetic variation. Additional studies will be important to test alternative scenarios related to the low nuclear variation found here. The microsatellites described here will be useful to explore both ecology and evolution of Sooty Swift and closely related species.

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Table 1. Characterization of 21 microsatellite loci isolated from *Cypseloides fumigatus* and their cross-amplification in *Streptoprocne zonaris*

Locus	Primer sequence (5'-3')	GenBank accession No.	Repeat motif	*Cypseloides fumigatus*						*Streptoprocne zonaris*		
				T_a (°C)	n_a	Size range (bp)	H_o	H_e	*P*-value	T_a (°C)	n_a	Size range (bp)
Cyps3	F: TGCCCAGGGCTCTAAAAGTA	MF568530	$(AG)_5$	58	2	282–286	0.032	0.032	-	NSA	-	-
	R: GCCACAATAGCAGCACAGAA											
Cyps8	F: GGCTTGACCATGAGAACCAT	MF568531	$(CT)_5$	60	3	107–129	0.029	0.189	0.0001*	60	1	121
	R: CAACATTGTCCCTGTGATCG											
Cyps9	F: GGTGATGTCATTTCCCCTCT	MF579401	$(AGC)_5$	NSA	-	-	-	-	-	52	2	76–97
	R: TTAGAAAGTGCCAGAGAAGTATCA											
Cyps12	F: GAGGCTGCAGAAAAGCTGTC	MF579402	$(AG)_5$	58	3	179–193	0.118	0.112	1	58	2	179–181
	R: ACCCTGCTGTTCAAGGTGTT											
Cyps14	F: AGGGGTGGAGATCAGACTCA	MF579403	$(AC)_5$	58	3	128–142	0.125	0.420	0.0000*	NSA	-	-
	R: AGTCCCTTTCTTCCCCTCTG											
Cyps20	F: CATGGCTTCCTCCTTTCTGT	MF579404	$(TG)_5$	58	3	102–130	0.212	0.195	1	58	2	109–131
	R: TGGGATGACTTGTTTCTCCTG											
Cyps22	F: CCCTCGTGACCATTTTCTGT	MF579405	$(CT)_5$	58	2	203–207	0.065	0.062	1	58	1	203
	R: GGTCACACAGAGGGGAAAAA											
Cyps23	F: CGGCTAAACTGCAAGGAAAA	MF579406	$(GA)_9$	62	-	-	-	-	-	62	-	-
	R: CCTATGGGCTGCTCTGCTAC											
Cyps24	F: GACAGAAGCCTTTCCAGTGC	MF579407	$(CA)_5$	64	4	200–208	0.138	0.219	0.0239	64	-	-
	R: TGAGACCGGAGCTGTCTTTT											
Cyps25	F: CATCTCCCAGGTGTTTTCGT	MF579408	$(AG)_5$	56	3	232–240	0.067	0.065	1	56	2	228–232
	R: AGTTGGGAAAAGAGCACAGC											
Cyps26	F: AGGAAAGAGCCCTCTGCAAT	MF579409	$(TC)_5$	56	8	129–173	0.200	0.610	0.0000*	56	1	149
	R: TGGGGAGCAGAAGTAGCTGT											
Cyps27	F: AAATGCTGGCAAAGGTCTTG	MF579410	$(TG)_5$	NSA	-	-	-	-	-	NSA	-	-
	R: CCGTGTCCCTCACTCAGACT											
Cyps28	F: CAAACATCTGCACCCCTTTT	MF579411	$(GT)_5$	56	1	153	-	-	-	60	1	151
	R: CTGACACTCGGCACAGACAT											
Cyps30	F: GATTCAATGGAGTAAATGGGTAG	MF579412	$(AAT)_5$	56	4	229–241	0.071	0.103	0.0506*	NSA	-	-
	R: TGAAGGTCTAAAGCCTCCTCAG											
Cyps31	F: GCGATAATGGGTGGACACTT	MF579413	$(TA)_5$	NSA	-	-	-	-	-	NSA	-	-
	R: GATCGCTCCTCCAAAATGTG											
Cyps32	F: GGAGTAGGAGCAGCACAAGC	MF579414	$(GAG)_5$	NSA	-	-	-	-	-	NSA	-	-
	R: ATCAGACACTGAGGCCATCC											
Cyps33	F: TATTTCTTTTGGGGGTGCTG	MF579415	$(TG)_5$	62	3	148–156	0.033	0.235	0.0000*	NSA	-	-
	R: CACACTGTCAACCCACCTTG											
Cyps34	F: GTCTGGGAACTGTCCCCTTT	MF579416	$(TG)_6$	60	-	-	-	-	-	60	1	161
	R: AGACTGGGACCCAAGGATG											
Cyps35	F: GGCCAGTATTAATGAAGCAGATG	MF579417	$(CT)_9$	58	4	149–155	0.560	0.671	0.0059*	58	1	149
	R: GCCTGTGGGGCATTAAAGAT											
Cyps36	F: GGGATGCCTACAGTGAAAGG	MF579418	$(GA)_5$	58	3	152–156	0.533	0.638	0.3839	58	1	152
	R: TTCTTGCCAGCAACTTTGAA											
Cyps37	F: TGTATTAAAGCAACCTTTCAGTGC	MF579419	$(AAAAC)_8$	NSA	-	-	-	-	-	NSA	-	-
	R: CCAGCCACACCTTTTACTGC											

Forward (F) and reverse (R) primer sequence, T_a annealing temperature, NSA no successful amplification, n_a number of alleles, observed (H_o) and expected (H_e) heterozygosity, *P*-value of the Hardy-Weinberg equilibrium test. * Significant values, considering Benjamini & Yekutieli (2001) correction ($P < 0.015$).

Table 2. Comparison of the number of alleles ranges, mean observed (H_o) and expected (H_e) heterozygosities between microsatellite loci developed for *Cypseloides fumigatus* and that developed for some hummingbird species.

Family	Species	Number of loci	n_a	H_o	H_e	Reference
Apodidae	*Cypseloides fumigatus*	13	2–8	0.156	0.254	This study
Trochilidae	*Hylocharis xantusii*	16	3–10	0.68	0.7	Sanvicente *et al.* 2016
Trochilidae	*Hylocharis leucotis*	14	2–8	0.35	0.41	Sanvicente *et al.* 2016
Trochilidae	*Calypte costae*	14	2–6	0.49	0.53	Sanvicente *et al.* 2016
Trochilidae	*Campylopterus curvipennis*	10	2–13	0.529	0.610	González *et al.* 2010
Trochilidae	*Amazilia cyanocephala*	10	2–13	-	-	Gutiérrez-Rodriguez *et al.* 2013
Trochilidae	*Selasphorus platycercus*	10	2–16	-	-	Oyler-McCance *et al.* 2011
Trochilidae	*Trochilus* spp.	15	2–10	-	-	Lance *et al.* 2009

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REFERENCES

Aljanabi S.M. & Martinez I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research* 25: 4692–4693.

Amaral F.R., Neves L.G., Resende-Jr. M.F.R., Mobili F., Miyaki C.Y., Pellegrino K.C.M. & Biondo C. 2015. Ultraconserved elements sequencing as a low-cost source of complete mitochondrial genomes and microsatellite markers in non-model amniotes. *PLoS One* 10: e0138446.

Amos W. & Harwood J. 1998. Factors affecting levels of genetic diversity in natural populations. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 353: 177–186.

Benjamini Y. & Yekutieli D. 2001. The control of the false discovery rate in multiple testing under dependency. *Annals of Statistics* 29: 1165–1188.

Biancalana R.N. 2014. Breeding biology of the White-collared Swift *Streptoprocne zonaris* in southeastern Brazil. *Revista Brasileira de Ornitologia* 22: 341–346.

Biancalana R.N. 2015. Breeding biology of the Sooty Swift (*Cypseloides fumigatus*) in São Paulo, Brazil. *Wilson Journal of Ornithology* 127: 402–410.

Biancalana R.N., Nogueira W., Bessa R., Pioli D., Albano C. & Lees A.C. 2012. Range extensions and breeding biology observations of the Sooty Swift (*Cypseloides fumigatus*) in the states of Bahia, Goiás, Minas Gerais and Tocantins. *Revista Brasileira de Ornitologia* 20: 87–92.

Boutin-Ganache I., Raposo M., Raymond M. & Deschepper C.F. 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques* 31: 24-27.

Chantler P. 1999. Family Apodidae (swifts). In: del Hoyo J., Elliott A. & Sargatal J. (eds.). *Handbook of the birds of the world, v. 5 (barn-owls to hummingbirds)*. Barcelona: Lynx Editions.

Collins C.T. & Foerster K.S. 1995. Nest site fidelity and adult longevity in the Black Swift (*Cypseloides niger*). *North American Bander* 20: 11–14.

González C., Gutiérrez-Rodríguez C. & Ornelas J.F. 2010. Isolation, characterization and cross species amplification of microsatellite loci in a lek-breeding hummingbird (*Campylopterus curvipennis*, Tochilidae). *Molecular Ecology Resources* 10: 232–236.

Gutiérrez-Rodríguez C., Covarrubias S., González C. & Ornelas J.F. 2013. Isolation, characterization and cross amplification of microsatellite loci in the azure-crowned hummingbird (*Amazilia cyanocephala*, Trochilidae). Permanent Genetic Resources added to Molecular Ecology Resources Database 01 February 2013–31 March 2013. Molecular Ecology Resources Primer Development Consortium. *Molecular Ecology Resources* 13: 760–762.

Harvey M.G., Smith B.T., Glenn T.C. Faircloth B.C. & Brumfield R.T. 2016. Sequence capture versus restriction site associated DNA sequencing for shallow systematics. *Systematic Biology* 65: 910–924.

Koressaar T. & Remm M. 2007. Enhancements and modifications of primer design program Primer3. *Bioinformatics* 23: 1289–1291.

Lance S.L., Hagen C., Glenn T.C., Brumfield R.T., Stryjewski K.F. & Graves G.R. 2009. Fifteen polymorphic microsatellite loci from Jamaican streamertail hummingbirds (*Trochilus*). *Conservation Genetics* 10: 1195–1198.

Marín M. & Stiles F.G. 1992. On the biology of five species of swifts (Apodidae, Cypseloidinae) in Costa Rica. *Proceedings of the Western Foundation of Vertebrate Zoology* 4: 287–351.

Meglécz E., Costedoat C., Dubut V., Gilles A., Malausa T., Pech N. & Martin J.F. 2010. QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. *Bioinformatics* 26: 403–404.

Milot E., Weimerskirch H., Duchesne P. & Bernatchez L. 2007. Surviving with low genetic diversity: the case of albatrosses. *Proceedings of the Royal Society of London B: Biological Sciences* 274: 779–787.

Oyler-McCance S.J., Fike J.A., Talley-Farnham T., Engelman T. & Engelman F. 2011. Characterization of ten microsatellite loci in the Broad-tailed Hummingbird (*Selasphorus platycercus*). *Conservation Genetics Resources* 3: 351–353.

Peakall R. & Smouse P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* 28: 2537–2539.

Pearman M., Areta J.I., Roesler I. & Bodrati A. 2010. Confirmation of the Sooty Swift (*Cypseloides fumigatus*) in Argentina with notes

on its nest placement, seasonality, and distribution. *Ornitología Neotropical* 21: 351–359.

Raymond M. & Rousset F. 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248–249.

Rousset F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.

Sanvicente C.G.-R., Rodríguez-Estrella R., Lozano-Garza O.A. & García-De-Léon F.J. 2016. Genetic diversity of the endemic Xantus' Hummingbird using 16 novel polymorphic microsatellite loci, and their cross amplification between six related species. *Open Journal of Genetics* 6: 19–27.

Untergasser A., Cutcutache I., Koressaar T., Ye J., Faircloth B.C., Remm M. & Rozen S.G. 2012. Primer3 – new capabilities and interfaces. *Nucleic Acids Research* 40: e115.

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

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