

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 seen 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 (Megléczy *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 MgCl₂ 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 GenAEx 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 were 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-Rodríguez *et al.* 2013, Sanvicente *et al.* 2016; Table 2). Several attempts of amplification using two hummingbird microsatellites developed in other studies, Aca3-3 and Hxan07 (Gutiérrez-Rodríguez *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	<i>Cypseloides fumigatus</i>						<i>Streptoprocne zonaris</i>		
				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 R: GCCACAATAGCAGCACAGAA	MF568530	(AG) ₅	58	2	282–286	0.032	0.032	-	NSA	-	-
Cyps8	F: GGCTTGACCATGAGAACCAT R: CAACATTGTCCCTGTGATCG	MF568531	(CT) ₅	60	3	107–129	0.029	0.189	0.0001*	60	1	121
Cyps9	F: GGTGATGTCAITTTCCCTCT R: TTAGAAAGTGCCAGAGAAGTATCA	MF579401	(AGC) ₅	NSA	-	-	-	-	-	52	2	76–97
Cyps12	F: GAGGCTGCAGAAAAGCTGTC R: ACCCTGCTGTTCAAGGTGTT	MF579402	(AG) ₅	58	3	179–193	0.118	0.112	1	58	2	179–181
Cyps14	F: AGGGGTGGAGATCAGACTCA R: AGTCCCTTTCTTCCCCTCTG	MF579403	(AC) ₅	58	3	128–142	0.125	0.420	0.0000*	NSA	-	-
Cyps20	F: CATGGCTTCCTCTTTCTGT R: TGGGATGACTTGTTTCTCTG	MF579404	(TG) ₅	58	3	102–130	0.212	0.195	1	58	2	109–131
Cyps22	F: CCCTCGTGACCATTTTCTGT R: GGTCACACAGAGGGGAAAAA	MF579405	(CT) ₅	58	2	203–207	0.065	0.062	1	58	1	203
Cyps23	F: CGGCTAAACTGCAAGGAAAA R: CCTATGGGCTGCTCTGCTAC	MF579406	(GA) ₉	62	-	-	-	-	-	62	-	-
Cyps24	F: GACAGAAGCCTTTCCAGTGC R: TGAGACCGGAGCTGTCTTTT	MF579407	(CA) ₅	64	4	200–208	0.138	0.219	0.0239	64	-	-
Cyps25	F: CATCTCCCAGGTGTTTTCGT R: AGTTGGGAAAAGAGCACAGC	MF579408	(AG) ₅	56	3	232–240	0.067	0.065	1	56	2	228–232
Cyps26	F: AGGAAAGAGCCCTCTGCAAT R: TGGGGAGCAGAAGTAGCTGT	MF579409	(TC) ₅	56	8	129–173	0.200	0.610	0.0000*	56	1	149
Cyps27	F: AAATGCTGGCAAAGGTCTTG R: CCGTGTCCCTCACTCAGACT	MF579410	(TG) ₅	NSA	-	-	-	-	-	NSA	-	-
Cyps28	F: CAAACATCTGCACCCCTTTT R: CTGACACTCGGCACAGACAT	MF579411	(GT) ₅	56	1	153	-	-	-	60	1	151
Cyps30	F: GATTCAATGGAGTAAATGGGTAG R: TGAAGGTCTAAAGCCTCCTCAG	MF579412	(AAT) ₅	56	4	229–241	0.071	0.103	0.0506*	NSA	-	-
Cyps31	F: GCGATAATGGGTGGACACTT R: GATCGCTCCTCCAAAATGTG	MF579413	(TA) ₅	NSA	-	-	-	-	-	NSA	-	-
Cyps32	F: GGAGTAGGAGCAGCACAAAGC R: ATCAGACACTGAGGCCATCC	MF579414	(GAG) ₅	NSA	-	-	-	-	-	NSA	-	-
Cyps33	F: TATTTCTTTTGGGGGTGCTG R: CACACTGTCAACCCACCTTG	MF579415	(TG) ₅	62	3	148–156	0.033	0.235	0.0000*	NSA	-	-
Cyps34	F: GTCTGGGAAGTGTCCCTTTT R: AGACTGGGACCCAAGGATG	MF579416	(TG) ₆	60	-	-	-	-	-	60	1	161
Cyps35	F: GGCCAGTATTAATGAAGCAGATG R: GCCTGTGGGGCATTAAAGAT	MF579417	(CT) ₉	58	4	149–155	0.560	0.671	0.0059*	58	1	149
Cyps36	F: GGGATGCTACAGTGAAAGG R: TTCTTGCCAGCAACTTTGAA	MF579418	(GA) ₅	58	3	152–156	0.533	0.638	0.3839	58	1	152
Cyps37	F: TGTATTAAGCAACCTTTCAGTGC R: CCAGCCACACCTTTTACTGC	MF579419	(AAAAC) ₈	NSA	-	-	-	-	-	NSA	-	-

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	<i>Cypseloides fumigatus</i>	13	2–8	0.156	0.254	This study
Trochilidae	<i>Hylocharis xantusii</i>	16	3–10	0.68	0.7	Sanvicente <i>et al.</i> 2016
Trochilidae	<i>Hylocharis leucotis</i>	14	2–8	0.35	0.41	Sanvicente <i>et al.</i> 2016
Trochilidae	<i>Calypte costae</i>	14	2–6	0.49	0.53	Sanvicente <i>et al.</i> 2016
Trochilidae	<i>Campylopterus curvipennis</i>	10	2–13	0.529	0.610	González <i>et al.</i> 2010
Trochilidae	<i>Amazilia cyanocephala</i>	10	2–13	-	-	Gutiérrez-Rodríguez <i>et al.</i> 2013
Trochilidae	<i>Selasphorus platycercus</i>	10	2–16	-	-	Oyler-McCance <i>et al.</i> 2011
Trochilidae	<i>Trochilus</i> spp.	15	2–10	-	-	Lance <i>et al.</i> 2009

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