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RESEARCH ARTICLE

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Genetic variability of the *sws1* cone opsin gene among New World monkeys

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Abstract

Vision is a major sense for Primates and the ability to perceive colors has great importance for the species ecology and behavior. Visual processing begins with the activation of the visual opsins in the retina, and the spectral absorption peaks are highly variable among species. In most Primates, LWS/MWS opsins are responsible for sensitivity to long/middle wavelengths within the visible light spectrum, and SWS1 opsins provide sensitivity to short wavelengths, in the violet region of the spectrum. In this study, we aimed to investigate the genetic variation on the *sws1* opsin gene of New World monkeys (NWM) and search for amino acid substitutions that might be associated with the different color vision phenotypes described for a few species. We sequenced the exon 1 of the *sws1* opsin gene of seven species from the families Callitrichidae, Cebidae, and Atelidae, and searched for variation at the spectral tuning sites 46, 49, 52, 86, 90, 93, 114, 116, and 118. Among the known spectral tuning sites, only residue 114 was variable. To investigate whether other residues have a functional role in the SWS1 absorption peak, we performed computational modeling of wild-type SWS1 and mutants A50I and A50V, found naturally among the species investigated. Although in silico analysis did not show any visible effect caused by these substitutions, it is possible that interactions of residue 50 with other sites might have some effect in the spectral shifts in the order of ~14 nm, found among the NWM. We also performed phylogenetic reconstruction of the *sws1* gene, which partially recovered the species phylogeny. Further studies will be important to uncover the mutations responsible for the phenotypic variability of the SWS1 of NWM, and how spectral tuning may be associated with specific ecological features such as preferred food items and habitat use.

KEYWORDS

Atelidae, Callitrichidae, Cebidae, genetics of color vision, primate visual ecology

[Correction added on 9th October 2020, after first online publication: author name has been changed from "Dora Ventura Fix" to "Dora Fix Ventura"]

1 | BACKGROUND

Color discrimination is a major visual ability that enables animals to perceive hue variations and discriminate objects of the visual scene against the background, improving the capacity to find food sources, potential mates, and see the approach of predators (Caine & Mundy, 2000; Dominy et al., 2001; Pessoa et al., 2014; Regan et al., 2001). In order Primates, vision is a paramount sense, and the ability to discriminate colors is associated with the species ecological niche and behavior and is enabled by the presence of different visual pigments in the retinas (Jacobs, 1998). The visual pigments are located in the outer segments of rod and cone photoreceptors. They consist of a G protein-coupled receptor, opsin or rhodopsin, covalently bound to a retinal chromophore, through a Schiff base linkage, in a highly conserved lysine residue (K296). The absorption of a photon by the chromophore causes its photo-isomerization, which activates the visual pigment and triggers a phototransduction cascade within the photoreceptor, leading to the transduction of light information into neural signals (Bowmaker, 1991; Nathans et al., 1986). The signals generated by each photoreceptor type are processed by post-receptoral neural pathways, to generate color vision sensation. Different visual pigment classes have specific wavelength ranges of maximal absorption, and variations in the spectral absorption peak (λ_{max}) of the opsins are determined by specific amino acids located at spectral tuning sites (Bowmaker et al., 1987; Hunt et al., 2007; Neitz & Jacobs, 1984; Yokoyama & Shi, 2000).

In terms of color discrimination capacity, Primates are unique among mammals. Most mammals have a dichromatic color vision, based on the presence of a short-wavelength-sensitive opsin, SWS1, with absorption peaks that can range from ultraviolet to violet light, and a middle-/long-wavelength-sensitive opsin (MWS/LWS; Bowmaker, 1991; Hunt & Peichl, 2013; Hunt et al., 2009). In Old World monkeys (OWM) an ancestral duplication of the X-linked *lws* opsin gene, followed by mutations at important functional residues, generated two distinct opsins, one sensitive to middle wavelengths, MWS, with λ_{max} values around 530 nm, and the other sensitive to long wavelengths, LWS, with λ_{max} around 560 nm. The trichromatic phenotype of OWM results from the combination of post-receptoral signals derived from three opsin types, these two—MWS and LWS—plus the violet-sensitive SWS1 opsin (Jacobs, 1998). In contrast, in New World monkeys (NWM), polymorphisms in a single X-linked *lws* opsin gene can generate two or more distinct opsins, variably sensitive to middle and long wavelengths, providing a trichromatic color vision condition only in heterozygous females, while males and homozygous females are dichromatic as most mammals (Jacobs, 2007; Mollon et al., 1984). Several studies have investigated the variability and polymorphisms of LWS/MWS opsins in NWM (Bonci et al., 2013; Hiramatsu et al., 2005; Kawamura et al., 2001; Shyue et al., 1998; Soares et al., 2010; Travis et al., 1988; Yokoyama & Radlwimmer, 2001), and how the diversity of sensitivity to middle and long wavelengths might be associated with the species visual ecology, including the ability to find food sources (Abreu et al., 2019; Melin et al., 2007; Tovée et al., 1992; Vorobyev, 2004). However, less attention has been given to the variability of spectral sensitivity to short wavelengths within this group, and how possible variations may be associated with the species ecology and behavior (Hunt et al., 1995; Shimmin et al., 1997, 1998).

The molecular mechanisms underlying the spectral tuning of SWS1 opsins of vertebrates are complex (Shi et al., 2001) and not as clear as those of the MWS/LWS photopigments (Yokoyama & Radlwimmer, 1998). The SWS1 opsin class may be sensitive to light in the ultraviolet (UV) range, with λ_{max} ranging from 355 to 390 nm, or in the violet range (VS), with λ_{max} from 390 to 455 nm (Bowmaker et al., 1987; Fasick et al., 1999; Jacobs & Deegan, 2001; Jacobs et al., 2002; Schnapf et al., 1988; Travis et al., 1988). Epistatic interactions among amino acid residues close to the chromophore Schiff base linkage (46, 49, 52, 86, 90, 93, 114, 116, and 118) result in different spectral shifts of the SWS1 opsins, depending on the protein background (Hunt et al., 2004; Shi et al., 2001; Shi & Yokoyama, 2003; Yokoyama & Shi, 2000). In most vertebrates, except birds, site 86 is responsible for major shifts between UV and violet opsins. Residue Phe86 is found in UV opsins, while different substitutions at this site cause wide shifts toward the violet spectrum (Cowing et al., 2002; Fasick et al., 2002; Nathans et al., 1986). However, in the nocturnal prosimian aye-aye (*Daubentonia madagascariensis*), a Phe86 residue is found in a violet opsin, with an absorption peak at ~406 nm (Carvalho et al., 2011; Melin et al., 2012). This indicates that in this group of mammals other mechanisms might be involved in the shift from UV to violet sensitivity, such as the conserved residue Pro93, which is consistently found in Primate violet opsins (Carvalho et al., 2011, 2017).

In all Primates studied heretofore, the SWS1 opsins are sensitive to violet light. However, despite its importance for color vision, the variation of the spectral sensitivity of the violet photopigments in NWM was only described in a few studies and the molecular mechanisms underlying the spectral shifts within the violet range in this group are poorly understood. Only a few studies in the literature described the *sws1* opsin genes of NWM, including the species *Callithrix jacchus* (Hunt et al., 1995), *Saimiri boliviensis* (Shimmin et al., 1997), *Cebus olivaceus*,and *Alouatta palliata* (Shimmin et al., 1998). Previous studies using microspectrophotometry (MSP) and electroretinography (ERG) described the spectral tuning of SWS1, and found variations that range from 423 to 437 nm, in a few species of NWM (Baylor et al., 1987; Bowmaker et al., 1987; Jacobs & Deegan, 2001, 2003; Mollon et al., 1984; Travis et al., 1988). Although contained within the violet range, the λ_{max} variation may have ecological and evolutionary implications as it may be associated with different features of the species lifestyle, such as daily activity patterns, feeding strategies, and habitat selection, given that spectral variation in different habitats influences color and brightness contrast, which can optimize the detection of prey, fruit, and flower against the background (Emerling et al., 2015; Endler, 1993, 1997; Veilleux & Cummings, 2012).

Based on those premises, in this study, we aimed to compare the *sws1* opsin gene from different species of NWM and map the variations in the amino acids located at known spectral tuning sites, as well as to explore the possibility of polymorphisms within this gene in NWM species. With this objective, we sequenced the exon 1 of the *sws1* opsin gene of individuals from seven species that inhabit the Brazilian forests and/or savannahs: one species from the Callitrichidae family (*C. jacchus*), one species from the Cebidae family

(*Sapajus apella*), and five species from the Atelidae family (*Brachyteles arachnoides*, *Alouatta clamitans*, *Alouattacaraya*, *Ateles belzebuth*, and *Lagothrix lagotricha lagotricha*). Our findings were discussed based on a context of the phenotypic variations of the spectral sensitivity of the SWS1 of NWM described in previous studies and presumed ecological implications. In addition, the *sws1* opsin gene was described to be highly conserved among different vertebrate groups, and thus, a good phylogenetic marker (van Hazel et al., 2006). Based on that, we performed molecular phylogenetic reconstructions to investigate the phylogenetic history of this gene within the group of NWM investigated. Our study brings new relevant data on the genetics of color vision of the New World Primates.

2 | METHODS

2.1 | Sample information

To perform the genetic analysis of the *sws1* opsin gene, blood, feces, or hair were collected from individuals of seven species of NWM from the families Cebidae: *S. apella* ($n = 29$), Callitrichidae: *C. jacchus* ($n = 34$), and Atelidae: *B. arachnoides* ($n = 6$), *L. lagotricha lagotricha* ($n = 2$), *A. caraya* ($n = 3$), *Alouatta guariba clamitans* ($n = 5$), and *A. belzebuth* ($n = 4$; Table 1). The project was approved by the Ethics Committee for the Use of Animals of the Federal University of Rio de Janeiro (UFRJ; CEUA license no. 01200.001568/2013-87), the Federal University of Rio Grande do Norte (UFRN; CEUA license no. 135/2014), the Federal University of Pará (UFPA; CEUA license no. PS040/2015), and the Federal University of the State of São Paulo (UNIFESP; CEUA license no. 3367270317). This study complies with the American Society of Primatologists Ethical Principles for the Treatment of Non-Human Primates.

2.2 | Genotyping and spectral tuning prediction

DNA was extracted from blood using the PUREGENE® DNA Purification Kit (Gentra Systems, Qiagen), from feces with QIAamp DNA Stool Mini Kit (Gentra Systems, Qiagen), and from hair using the QIAamp DNA Investigator Kit (Gentra Systems, Qiagen), according to the manufacturer's protocols. Polymerase chain reactions (PCRs) were performed to amplify the exon 1 of the *sws1* opsin gene using the primer pair SWS1exon1_Fw (5′-AAGAGGACTCAGAGGAGGGTGTG′) and SWS1exon1_Rv (5′-CTAACCCCTTTTTCCCCTGC′). The PCRs were carried out using High-Fidelity Platinum Taq Polymerase, 10× High-Fidelity Buffer and $MgCl_2$, 10-mM GeneAmp dNTPs (Life Technologies), and 10-μM primers in 50-μl reactions. The PCR conditions were (1) an initial denaturation at 94°C for 1 min; (2) 37 cycles of 15 s at 94°C, 30 s at 60°C (annealing temperature), and 30 s at 72°C; (3) a final extension at 72°C, for 10 min. The PCR products were visualized by electrophoresis in 1% agarose gel, and purified with Illustra GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare). Sanger sequencings were carried out with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies) and the 3500 Applied Biosystems Sequencer (Life Technologies). Chromatograms were analyzed and sequences were aligned in the MEGA7 software (Kumar et al., 2016) with ClustalW algorithm (Thompson et al., 1994). The amino acids located at spectral tuning sites 46, 49, 52, 86, 90, 93, 114, 116, and 118 (Shi et al., 2001; Shi & Yokoyama, 2003), all encoded by exon 1 of the *sws1* gene, were analyzed and compared with the amino acid sequences from other primate species that had their SWS1 opsin spectral absorption peak measured in previous studies by site-directed mutagenesis and in vitro expression (Fasick et al., 1999), MSP (Baylor et al., 1987; Bowmaker et al., 1987; Mollon et al., 1984; Travis et al., 1988), or ERG (Jacobs & Deegan, 2001, 2003).

2.3 | Homology modeling of wild-type and mutant SWS1 opsins

We performed computational modeling of the SWS1 opsin using the SwissModel server (https://swissmodel.expasy.org/; Waterhouse et al., 2018). Wild-type SWS1 opsin and mutants A50I and A50V were modeled to investigate possible effects caused by residue 50. Although this residue was not previously described as a spectral tuning site, substitutions found among the NWM species analyzed might indicate that this site could play a role in the opsin function. As template, we used the full amino acid sequence of the SWS1 opsins

TABLE 1 Sample information of New World monkey species analyzed in this study

Family	Species	Number of individuals	Type of sample	Maintained	Location (city and state)
Cebidae	*Sapajus apella*	13	Blood	Captivity	Rio de Janeiro, Rio de Janeiro
		16	Blood	Captivity	Belém, Pará
Callitrichidae	*Callithrix jacchus*	15	Feces	Wild	Cabeceiras, Paraíba
		19	Hair	Captivity	Natal, Rio Grande do Norte
Atelidae	*Brachyteles arachnoides*	6	Feces	Wild	Capão Bonito, São Paulo
	Alouatta guariba clamitans	5	Feces	Wild	
	Alouatta caraya	3	Feces	Captivity	Sorocaba, São Paulo
	Ateles belzebuth	4	Feces	Captivity	
	Lagothrix lagotricha lagotricha	2	Feces	Captivity	

of *S. boliviensis* available at GenBank database (U53875.1). The resulting sequence was built using the crystalline structure of bovine rhodopsin as a template (PDB accession number: 5DYS; Singhal et al., 2016). Evaluation of the model structural quality was performed according to the Ramachandran diagram, with the PROCHECK server (https://servicesn.mbi.ucla.edu/PROCHECK/). The inferred model had good structural quality, with >90% of the residues disposed in highly favorable regions. The A50V and A50I mutations were inserted into the wild-type model using UCSF CHIMERA software v1.11.2 (Pettersen et al., 2004).

2.4 | Phylogenetic analyses

The seven sequences of NWM obtained in this study were aligned with the *sws1* opsin gene coding sequences of 38 other species of mammals, including 15 additional species of Primates, obtained from GenBank (accession numbers in Table S1) using ClustalW algorithm (Thompson et al., 1994). The sequence of the *sws1* opsin gene of *Gallus gallus* was used as an outgroup. A 307-bp alignment with the 46 nucleotides sequences was conducted in MEGA7 (Kumar et al., 2016).

Phylogenetic reconstruction was obtained using maximum likelihood based on Kimura's two-parameter model (Kimura, 1980). All positions of the alignment with <95% site coverage were eliminated. Initial tree(s) for the heuristic search were obtained automatically by applying neighbor-join and BioNJ algorithms to a matrix of pairwise distances estimated using maximum composite likelihood approach, and then selecting the topology with superior log likelihood value (Gascuel, 1997; Saitou & Nei, 1987). A discrete gamma distribution was used to model evolutionary rate differences among sites. The bootstrap consensus tree was visualized and edited in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). Branches corresponding to partitions reproduced in <60% bootstrap replicates were collapsed.

3 | RESULTS

In this study, we amplified and sequenced approximately 500 bp, corresponding to exon 1 of the *sws1* opsin gene of seven species of NWM from three different families: Callitrichidae, *C. jacchus*; Cebidae, *S. apella*; and Atelidae, *B. arachnoides*, *L. lagotricha lagotricha*, *A. caraya*, *A. guariba clamitans*, and *A. belzebuth*. Comparisons of the sequences obtained from individuals of each species did not show any species polymorphisms. The sequences were deposited at the NCBI GenBank under the accession numbers MW009256–MW009262. The comparative analysis of the nine spectral tuning sites, 46, 49, 52, 86, 90, 93, 114, 116, and 118, showed that only residue 114 varied among the species (Figure 1). In *C. jacchus*, *A. caraya*, and *A. guariba clamitans* we found the amino acids I46, L49, L52, L86, S90, P93, G114, L116, and T118. The other four species, *S. apella*, *B. arachnoides*, *L. lagotricha lagotricha*, and *A. belzebuth*, had the substitution G114A. No amino acid variation at residues 86 and 93 was observed, and all species have L86 and P93, indicating a violet visual pigment.

Within exon 1, we also found variations at residue 50 among the species analyzed, and we performed computational modeling to investigate possible effects of the amino acid substitutions A50I and A50V on the protein structure. *Lagothrix*, *Ateles*, and *Brachyteles* had the amino acid A50, *Sapajus* had the substitution A50V, and *Callithrix* had A50I. However, our computational modeling did not show any change in the hydrogen-bond network profile formed between

Family	Species	46	49	50	52	86	90	93	114	116	118
Hominidae	*Homo sapiens*	T	L	I	F	L	S	P	G	L	T
	Pan paniscus	.	.	.	.	.	.	.	.	.	.
	Gorilla gorilla	.	.	.	.	.	.	.	.	.	.
	Pongo abelii	.	.	V	L	.	.	.	.	.	.
Cercopithecidae	*Macaca fascicularis*	.	.	A	.	.	.	.	A	.	.
	Miopithecus talapoin	.	.	A	.	.	.	.	A	.	.
Atelidae	***Alouatta caraya***	**I**	**.**	**A**	**L**	**.**	**.**	**.**	**.**	**.**	**.**
	Alouatta guariba clamitans	**I**	**.**	**A**	**L**	**.**	**.**	**.**	**.**	**.**	**.**
	Alouatta palliata	I	.	A	L	.	.	.	.	.	.
	Lagothrix lagotricha	**I**	**.**	**A**	**L**	**.**	**.**	**.**	**A**	**.**	**.**
	Ateles belzebuth	**I**	**.**	**A**	**L**	**.**	**.**	**.**	**A**	**.**	**.**
	Brachyteles arachnoides	**I**	**.**	**A**	**L**	**.**	**.**	**.**	**A**	**.**	**.**
Cebidae	***Sapajus apella***	**I**	**.**	**V**	**L**	**.**	**.**	**.**	**A**	**.**	**.**
	Cebus olivaceus	I	.	V	L	.	.	.	A	.	.
	Saimiri boliviensis	I	.	A	L	.	.	.	.	.	.
Callitrichidae	***Callithrix jacchus***	**I**	**.**	**.**	**L**	**.**	**.**	**.**	**.**	**.**	**.**
Aotidae	*Aotus nancymaae*	I	.	A	L	.	.	.	A	.	.
Tarsiidae	*Tarsius bancanus*	V	S	V	I	.	.	.	.	.	S
Lemuridae	*Eulemur fulvus*	L	F	A	A	C	.	.	.	.	S
	Lemur catta	L	F	A	A	C	.	.	.	.	S
	Daubentonia madagascariensis	F	F	A	T	F	.	.	.	.	S

FIGURE 1 Spectral tuning sites of the SWS1 visual pigment of species of Primates. Species sequenced in this study are represented in bold

residues A50, V50, and I50, and other amino acids, including known spectral tuning sites close to this residue (Figure 2).

The phylogenetic reconstruction of the *sws1* gene included 22 species of Primates, seven of which were sequenced in this study. The maximum likelihood tree partially recovered the Primate tree topology, according to the phylogenies proposed by Goodman et al. (1998) and Perelman et al. (2011; Figure 3). The phylogeny recovered the suborders Lemuriformes and Tarsiiformes as outgroups, and the clade Anthropoidea, with the Hominidae and Cercopithecidae families, as sister to the NWM clade. However, within the parvorder Platyrrhini, we found some inconsistencies and the phylogeny of the group was partially recovered. For instance, the Cebidae family was not reconstructed as a monophyletic clade, as expected for the group. In addition, *Aotus* species, which has an *sws1* pseudogene, grouped with the Atelidae family, although with low bootstrap support.

4 | DISCUSSION

In this study, we sequenced the exon 1 of the *sws1* opsin gene of seven species of NWM, from three different families, Cebidae, Atelidae, and Calitrichidae. In our analysis, we searched for divergences in the spectral tuning sites of the short-wavelength sensitive opsin that might be responsible for the different spectral absorption peaks within the violet range, in the NWM species, described previously based on in vivo and in vitro approaches. Based on previous studies using MSP (Baylor et al., 1987; Bowmaker et al., 1980; Bowmaker et al., 1987; Mollon et al., 1984; Travis et al., 1988) and ERG (Jacobs & Deegan, 2001, 2003), the spectral range of the violet opsins in this group of Primates varies from 423 to 437 nm.

The variability of the SWS1 absorption peaks results from substitutions at specific amino acid residues that influence the strength of the linkage between the retinal chromophore and the opsin protein, which alters the energy necessary to isomerize the chromophore molecule (Blatz et al., 1972). Estimates of the spectral tuning of SWS1 opsins are particularly complex, given that epistatic interactions among the amino acids result in different tuning effects depending on the protein background (Shi et al., 2001; Shi & Yokoyama, 2003; Yokoyama & Shi, 2000). Among the known spectral tuning sites, only residue P93 is consistently conserved in the violet SWS1 opsins of the species of Primates studied so far, and therefore may represent the main residue responsible for the major shift from UV to violet sensitivity in this lineage (Carvalho et al., 2011). Our findings corroborate this proposal, as all species with sensitivity in the violet range have P93. However, the molecular mechanisms responsible for shifts within the violet range, including the effects caused by single amino acid substitutions, are not clear.

Comparisons from genetic data and MSP analysis from the groups of OWM (Baylor et al., 1987; Fasick et al., 1999) and NWM (see Table 2), indicate that the substitution T46I is responsible for major shifts to longer wavelengths, within the violet range, in NWM.

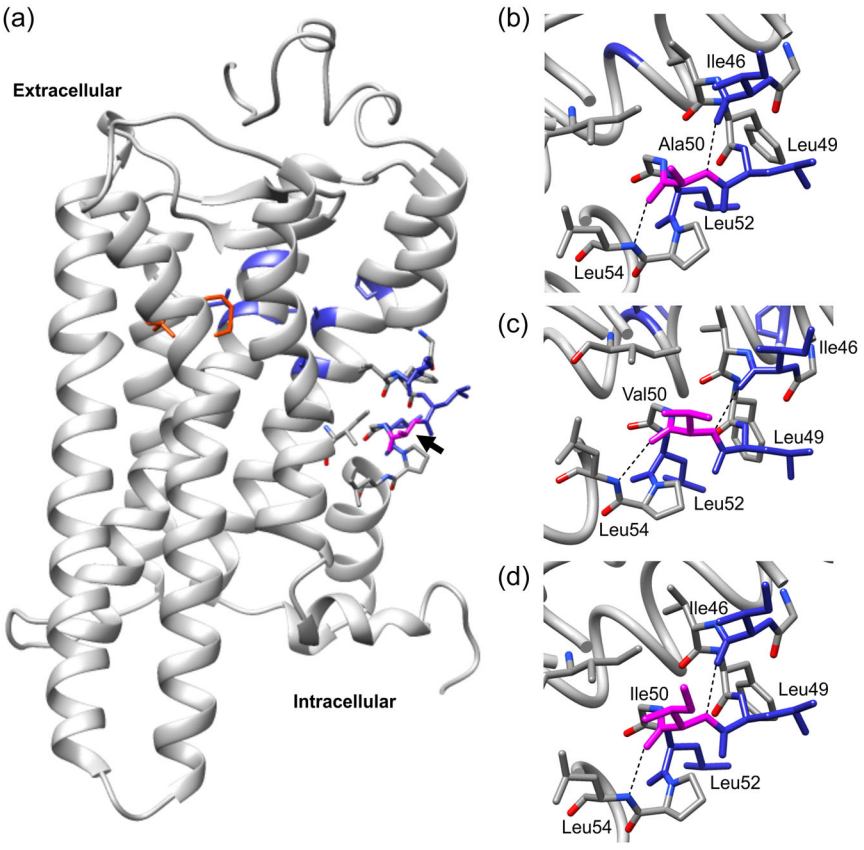

FIGURE 2 Homology model of the SWS1 opsin of *Saimiri boliviensis*. (a) Overall structure of the wild-type SWS1 opsin. The known spectral tuning sites are indicated in blue, and residue 50 is shown in magenta (arrow). Retinal chromophore is depicted in orange. (b–d) Magnifications showing the hydrogen-bond network formed between residue 50 and the amino acids located at about 5 Å distance: (b) wild-type SWS1; (c) mutant Ala50Val; (d) mutant Ala50Ile. In the wild-type and in both mutants, hydrogen bonds (dashed black line) are formed with two amino acids, Leu54, and the spectral tuning residue Ile46. The amino acid substitutions do not appear to cause an impact on the hydrogen bonds network configuration

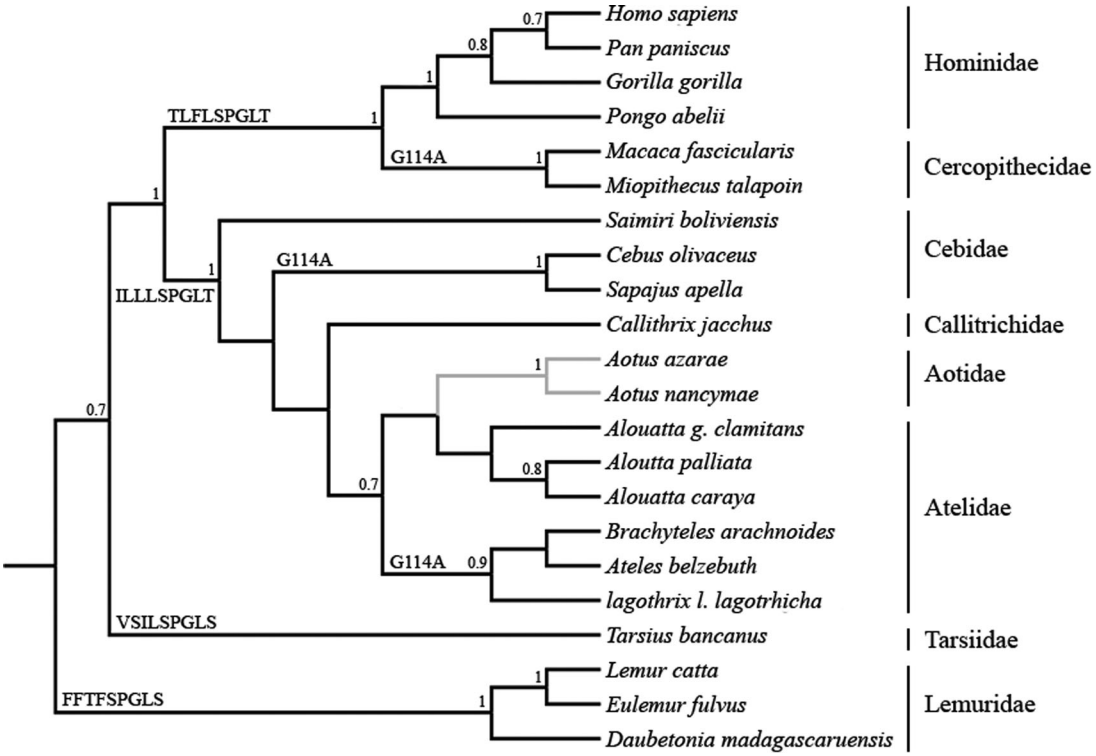

FIGURE 3 Maximum likelihood gene tree reconstruction of the *sws1* opsin gene of Primates. The amino acids at spectral tuning sites are indicated: 46, 49, 52, 86, 90, 93, 114, 116, and 118. The gray branches represent species with *sws1* pseudogene. Bootstraps supports are indicated for each resolved node. The sequence of *Gallus gallus* was used as outgroup (data not shown)

In our survey, within the NWM species analyzed, we found variations only in a single amino acid residue, 114, among the nine known spectral tuning sites investigated. All species had the amino acids I46, L49, L52, L86, S90, P93, L116, and T118, and the substitution G114A was observed in different lineages. Considering that residue 114 is known to cause minor shifts, around 1 nm, in the spectral peak of SWS1 opsins (Yokoyama et al., 2014), we suggest that this substitution by itself can hardly explain the shifts in spectral absorbance found among the NWM species (Table 2). Thus, the differences found may result from other amino acid interactions at unknown residues.

In four species, *C. jacchus*, *S. boliviensis*, *A. guariba clamitans*, and *A. caraya*, residue 114 has the nonpolar amino acid glycine, G114. The spectral absorption peak of the opsins was measured by MSP for *C. jacchus*, with λ_{max} at 423 nm (Travis et al., 1988). Interestingly, in *Saimiri sciureus*, the spectral absorption peak of the SWS1 opsin, measured by MSP, was 430/433 nm (Bowmaker et al., 1987; Mollon et al., 1984). Although the amino acid sequence of *S. sciureus* is not available, considering the genetic data from the closely related species, *S. boliviensis*, no difference in the known spectral tuning sites was found among the Cebidae and the Callitrichidae species. Therefore, it would be highly valuable to investigate the specific sequence of *S. sciureus*, as well as the presence of other unknown residues that might cause the 10-nm spectral shift observed between *S. sciureus* and *C. jacchus*. Our computational modeling of residue 50, that varied among those species, did not indicate any visible effect on the structure and the hydrogen-bound network profile, by inferring the substitutions A50I and A50V, naturally found among the NWM species analyzed. However, considering the complexity of the epistatic interactions and spectral tuning described for the SWS1 opsin (Shi et al., 2001), it is plausible to suggest that interactions among residue 50 and other sites may also play a role in the spectral tuning of the SWS1 opsins of NWM, and deserve further investigations.

In the other four species analyzed in this study, *S. apella*, *L. lagotricha lagotricha*, *A. belzebuth*, and *B. arachnoides*, we found the substitution G114A. For one of those species, *S. apella*, a study using ERG described a sensitivity at 425–427 nm (Jacobs & Deegan, 2003). As mentioned previously, the G114A substitution was described to cause a ~1-nm redshift in the opsin λ_{max} (Yokoyama et al., 2014), and thus, the comparison from *S. apella* (λ_{max} 425 nm) and *C. jacchus* (λ_{max} 423 nm), would be consistent with the genetic data. Based on that, we presumed a similar range of absorption for the other three species sequenced in our study' *L. lagotricha lagotricha*, *A. belzebuth*, and *B. arachnoides*. However, in other species from those same three genera, previous ERG analyzes showed spectral peaks shifted toward longer wavelengths, at 432 nm in two *Ateles* species (*A. geoffroyi* and *A. fusciceps*), and at 437 nm in two subspecies of *L. lagotricha* (*L. lagotricha lugens* and *L. lagotricha poeppigii*; Jacobs & Deegan, 2001). The redshifts found in these two genera of NWM, compared with *Sapajus*, might indicate that other unknown sites may play a role in the opsin absorption peak, or they might be due to artefacts from the different techniques applied to measure the spectral sensitivity.

TABLE 2 Amino acid residues responsible for the differentiation of ultraviolet (UV) and violet (VS) pigments of Primates along with the residue 50

Family (Groves, 2005; Rylands & Mittermeier, 2009)	Species	Primary diet and secondary diet	Habitat	Spectral tuning residues[b]	Residue 50	λ_{max} measured
Hominidae	*Homo sapiens*	Omnivore	Terrestrial	TLFLSP**G**LT	I	414[c]
Cercopithecidae	*Macaca fascicularis*	Omnivore	Terrestrial	TLFLSP**A**LT	A	415/430[d]
Callitrichidae	*Callithrix jacchus*[a]	Gummivore and frugivore/ faunivore/nectarivore	Understory/lower canopy of forest edges and savannas	ILLLSP**G**LT	I	423[e]
Cebidae	*Sapajus apella*[a]	Omnivore	Understory/lower canopy of forests and open forests	ILLLSP**A**LT	V	425–427[g]
	Saimiri sciureus	Frugivore and faunivore/folivore	Understory/lower/main canopy of forests			430/433[f]
	Saimiri boliviensis	Frugivore/faunivore and nectarivore	Understory/lower/main canopy of forests	ILLLSP**G**LT	A	
Atelidae	*Ateles belzebuth*[a]	Frugivore and folivore	Upper canopy of rainforest	ILLLSP**A**LT	A	
	Ateles fusciceps robustus	Frugivore/folivore and faunivore	Upper canopy of rainforest			432[h]
	Ateles geoffroyi	Frugivore and folivore/and nectarivore	Upper canopy of rainforest			432[h]
	Lagothrix lagotricha lagotricha[a]	Frugivore and folivore/faunivore	Main canopy of forests	ILLLSP**A**LT	A	
	Lagothrix lagotricha lugens	Frugivore and folivore/faunivore	Main canopy of forests			437[h]
	Lagothrix lagotricha poeppigii	Frugivore and folivore/faunivore	Main canopy of forests			437[h]
	Brachyteles arachnoides[a]	Frugivore and folivore/nectarivore	Main canopy of rainforests	ILLLSP**A**LT	A	
	Alouatta guariba clamitans[a]	Frugivore and folivore/nectarivore	Main canopy of forests	ILLLSP**G**LT	A	
	Alouatta caraya[a]	Folivore and frugivore/nectarivore	Main canopy of forests	ILLLSP**G**LT	A	

Note: Ecological information taken from Fleagle (2013) and https://animaldiversity.org/.

Abbreviations: SM, site-directed mutagenesis; ERG, electroretinography; MSP, microspectrophotometry.

[a]Species sequenced in this study.

[b]46, 49, 52, 86, 90, 93, 114, 116, and 118. The amino acid variation at residue 114 is highlighted in bold.

[c]SM (Fasick et al., 1999).

[d]MSP (Baylor et al., 1987; Bowmaker et al., 1980).

[e]MSP (Travis et al., 1988).

[f]MSP (Bowmaker et al., 1987; Mollon et al., 1984).

[g]ERG (Jacobs & Deegan, 2003).

[h]ERG (Jacobs & Deegan, 2001).

The ERG, used to measure the sensitivity of those species with the longer absorption peaks, at 432 and 437 nm, is an electrophysiological approach that registers the electrical responses to a light stimulus generated by the whole retina, and captured by electrodes positioned at the cornea. Based on that, it is important to highlight that other features of the ocular structures influence the resulting spectral absorption data, compared with the MSP technique that measures directly the spectral absorption of the outer segment of single retinal photoreceptors. Therefore, the ERG results are influenced by the spectral absorption characteristics of the ocular media. In terrestrial vertebrates, it has been documented for many species that short wavelengths are filtered by the lens. This is associated with a better image quality due to the reduction of Rayleigh scattering and chromatic aberration, caused more intensely by shorter wavelengths (Emerling et al., 2015; Thibos et al., 1990; Walls, 1931), and the prevention of retinal tissue damage caused by UV light (Hunt et al., 2009; Van Norren & Gorgels, 2011; Yokoyama, 2008). Analysis of lens transmittance showed that different species of Primates have highly UV-blocking lenses, with considerable attenuation of the amount of short-wavelength light that passes through the optical media (Douglas & Jeffery, 2014). In the NWM species, *S. sciureus*, *Cebus apella*, *C. jacchus*, and *Ateles paniscus*, for instance, the degree of UV radiation transmitted by the lens, which is expressed as the wavelength of 50% transmission (λ_{T50}), was respectively, 420, 426, 427, and 438 nm (Douglas & Jeffery, 2014). It is interesting to mention that the spectral absorption measured by ERG for some species, coincided with lens transmittance data, in which species of the genus *Ateles* have a highly short-wavelength blocking lens (λ_{T50} = 438 nm; Douglas & Jeffery, 2014), and opsins λ_{max} at 432 nm (see Table 2; Jacobs & Deegan, 2001). Similarly, in *S. apella*, the ERG recorded a spectral peak at 425–427 nm (Jacobs & Deegan, 2001), while the lens had λ_{T50} = 426 nm (Douglas & Jeffery, 2014). Thus, the values obtained from ERG studies of the Atelidae species (Jacobs & Deegan, 2001), may result from influences of the optical media transmittance, instead of actual changes in the spectral tuning of the violet opsins, and in this way, the overall absorption of the eye at short wavelengths would be red-shifted. Therefore, we can consider that data from ERG analysis is a good starting point for comparisons of the retinal sensitivity, but should be cautiously evaluated when searching for the specific spectral peaks of a given opsin, especially those sensitive to short wavelengths.

Variations in the spectral tuning at short wavelengths have functional importance for the species activities (Caine & Mundy, 2000; Dominy et al., 2001; Regan et al., 2001). In aquatic environments, the spectral variations and light composition are relatively easier to measure (Bowmaker, 2008; Loew & Lythgoe, 1985; Lythgoe & Partridge, 1989), and the spectral tunings of the opsins of many vertebrate species were proven to be adjusted to the specific photic environment (Bowmaker & Hunt, 2006; Hunt et al., 1996). In contrast, in terrestrial environments, where the photic variability is not so easily measurable, many aspects such as geographic characteristics of latitude and longitude, habitat and microhabitat, and the vegetal composition, alter the available photic quality in different ways, making it harder to determine correlations between the photic environment and specific tuning of the opsins (Endler, 1990; Loew & Lythgoe, 1985; Lythgoe & Partridge, 1989). In terrestrial environments, it is also possible that spectral adjustments of violet-sensitive opsins are associated with variations in the preferred food items of different species. In nocturnal mammals, it was suggested that species that primarily feed on fruits and flowers have shifts in the SWS1 opsin toward shorter wavelengths (Veilleux & Cummings, 2012), which makes it easier to discriminate UV-reflecting flowers under dim light (Fleming et al., 2009). It is also possible to speculate that shifts toward shorter wavelengths, even within the violet range, may benefit the view of contrasts of the floral organs, against the green background of the vegetation (Allman, 1977; Cartmill, 1992, 2012; Crompton, 1995; Sussman, 1991). In our sampling, it is interesting to mention that gummivore and omnivore species (see Table 2), as *C. jacchus* and *S. apella* had the shorter spectral peaks measured (Jacobs & Deegan, 2003; Travis et al., 1988). However, species that mainly feed on leaves and fruits, had the longer SWS1 absorption peaks (Jacobs & Deegan, 2001, 2003), indicating a possible ecological and behavioral role of the SWS1 shifts in this group of primates. Additionally, as spectral light varies among the different environments (Endler, 1993), the habitat used by the species may have an important role in the range of the violet spectrum seen among Primates. The variation in spectral tuning of the SWS1 opsin also indicates that terrestrial species and species that dwell in more open spaces, such as *C. jacchus*, might have shorter λ_{max} values among NWM (Table 2). In contrast, genera that display a longer λ_{max}, as *Ateles* and *Lagothrix*, occupy the main forest canopy, where light is filtered by the foliage, and middle wavelengths are predominant (greenish light spectrum; Endler, 1993, 1997). These considerations point to the need for further investigation into the molecular mechanisms of the spectral tuning of the SWS1 opsins of primates, in combination with functional aspects of the phenotypic diversity of color vision, and their importance in the visual ecology of different species.

The maintenance of the SWS1 opsin function in most NWM primates indicates the importance of sensitivity to short wavelengths in color vision. Besides its functional importance, the *sws1* opsin gene may be considered a good marker for molecular phylogeny reconstitutions as it is highly conserved in most vertebrate groups, and evolving under strong evolutionary constraints (van Hazel et al., 2006). This assumption was confirmed in our study, in which the reconstituted phylogeny of the *sws1* gene is in agreement with accepted Primate phylogenies based on molecular data (Goodman et al., 1998; Perelman et al., 2011). The monophyly of the Platyrrhine group was recovered. However, the relationship between *Saimiri*, *Callithrix*, and *Aotus* as sister group to the subfamily Cebinae, an unresolved trichotomy (Osterholz et al., 2009; Perez et al., 2012; Singer et al., 2003), was not recovered (Figure 3). Our results reiterate that the phylogenetic relationship of Platyrrhines is a complex issue, which is still under ongoing debate in the literature (Ford, 1986; Groves, 2001; Kay, 1990, 2015; Perelman et al., 2011; Rosenberger, 1981;

Rylands & Mittermeier, 2009; H. Schneider, 2000). Gene tree disagreements around Platyrrhine families might reflect that they are ancient lineages that have undergone rapid radiation (Perez et al., 2013; H. Schneider, 2000; I. Schneider et al., 2004; Singer et al., 2003). Additionally, inconsistencies found in our phylogenetic reconstruction may result from low taxon sampling, thus a more representative analysis combining the *sws1* opsin gene sequence from all Platyrrhine families, including species from the Pithecidae family, which were not sampled in this study, is necessary for a better view of the *sws1* opsin gene evolution in NWM.

In conclusion, our study provides a first broad overview of the variability of the *sws1* opsin gene of NWM, in terms of functional sites important for the SWS1 opsin spectral tuning, and indicates that other residues might also play a role in the spectral shifts of violet sensitive opsins in this group. We suggest that even small shifts within the violet range may indicate important visual adaptations that can be associated with feeding strategies and habitat selection of different species of NWM. Our study points to the importance of further investigations on the molecular mechanisms underlying spectral tuning of the SWS1 opsins, as well as behavioral and ecological surveys to investigate the ecological importance of sensitivity to short-wavelengths for visual processing and color vision in primates.

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DATA AVAILABILITY STATEMENT

Additional support information is available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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