

Unveiling the Diversity of Avastroviruses in Tropical Wild Birds from the Atlantic Forest, Brazil

Amanda Haisi¹, Fabio Schunck², Laís Sampaio de Azevedo³, Mariana Sequetin Cunha³, Raquel Guiducci³, Vanessa Cristina Martins Silva³, Adriana Luchs^{3,x}, João Pessoa Araújo Junior^{1,x,*}

1. Biotechnology Institute, Sao Paulo State University (UNESP), Botucatu, SP, Brazil, 18607-440.

2. Independent researcher, Sao Paulo, Brazil, 04785-040.

3. Virology Center, Adolfo Lutz Institute, Sao Paulo, Brazil, 01246-902.

*Jointly supervised this research.

*Corresponding author: João Pessoa Araujo Junior

Phone: +55 (14) 3880-0869 Email: joao.pessoa@unesp.br

Supplementary Table 1. Conserved domains of PasAstV M1580 strain genome, containing the ORFs and their respective domains, number of domains and amino acid position. NLS: nuclear localization signal, VPg: genome-linked protein; RdRp: RNA-dependent RNA polymerase.

	Domain	Number of domains: aa position	Number of motifs: aa position
ORF1a (917aa)	Coiled coil	2: 154; 708	NI
	Transmembrane helix	6: 191-200; 316-330;340-350;380-395;406-417;431-440	NI
	Protease	1:512-637	NI
	NLS	1:859	NI
	Vpg	1:735-917	2:777;859
ORF1b (402aa)	RdRp	1:56-303	3:136-150;204-277;248-262.
ORF2 (762aa)	Conserved domain	61-408	NI
	Hypervariable domain	409-762	NI

NI: No conserved motif identified

Supplementary Table 2. Estimates of percentage nucleotide and amino acid identity and genetic distances between PasAstV M1580 strain and 29 prototype AAstV sequences.

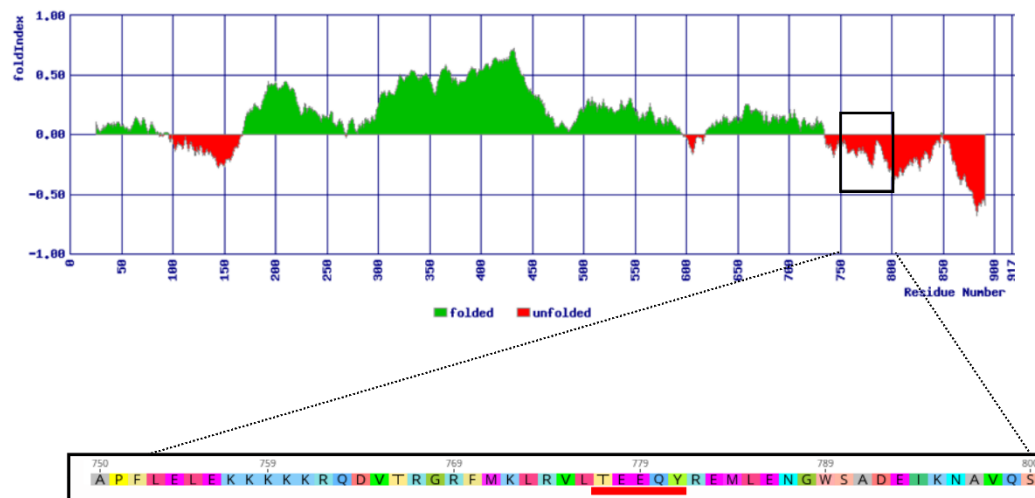
Clade	Viruses	Acession n°	PasAstV - M1580		p- distance
			Percentage identity %		
			Genome (nt)	ORF2 (aa)	ORF2 (aa)
Avastrovirus 1	Turkey astrovirus	Y15936	32.46	31.42	0.677
Avastrovirus 2	Avian nephritis virus	MN732559	31.62	27.47	0.730
	Avian nephritis virus 2	MH028405	31.66	26.19	0.739
	Avian nephritis virus 1	AB033998	31.71	29.26	0.712
	Avian nephitis virus	KM985694	NA	28.52	0.709
	Chicken Astrovirus	MG846421	NA	26.76	0.720
	Wood pigeon astrovirus	FR727149	NA	28.72	0.693
	Feral pigeon astrovirus	FR727147	NA	26.75	0.704
	Avastrovirus 3	Goose astrovirus	NC_034567	33.61	29.24
Duck astrovirus		KF753807	33.16	29.17	0.687
Chicken astrovirus		JF414802	33.59	28.05	0.711
Astrovirus CDB-2012 277		JQ307833	NA	28.29	0.701
Duck astrovirus		NC_012437	34.74	27.50	0.687
Duck astrovirus		JX439643	34.64	27.66	0.687
Duck astrovirus		FJ919228	34.82	27.82	0.687
Duck astrovirus		FJ919225	34.59	27.66	0.694
Duck astrovirus		KY646154	34.90	28.15	0.689
Avastrovirus 3		AF20663	33.18	28.24	0.691
Turkey astrovirus		EU143844	33.32	28.20	0.691
Turkey astrovirus		EU143845	33.33	28.31	0.692
Avastrovirus marecae	Avian astrovirus 4 MP22-196	PP623814	33.56	25.18	0.734
Unclassified astroviruses	Ruddy turnstone astrovirus	MK189093	30.64	25.09	0.738
Unclassified (Avastrovirus 5)	Passerine astrovirus 3	MK096775	46.73	27.84	0.717
	Passerine astrovirus 4	MK096776	47.10	30.54	0.694
	Passerine astrovirus 2	MK096774	47.10	30.21	0.682
	Passerine astrovirus 1	MK096773	37.27	<u>33.23</u>	0.671
Unclassified (Avastrovirus 4)	Avastrovirus 3 MPJ1332	JX985650	NA	25.18	0.734
	Avastrovirus 4 MPJ1442	JX985652	NA	26.94	0.735
	Avastrovirus 5 MPJ1433	JX985651	NA	27.12	0.734

Bolded values are the lowest and highest p-distance; The underlined value is the highest percentage identity (%) of each ORF region; NA – full length genome not available.

Supplementary Table 3. Distribution of AAsV-positive samples according to family, species, and sampling date among birds collected in the Curucutu Unit, Serra do Mar State Park, Southeast Brazil, between 2022 and 2023. Resampled positive birds are highlighted in bold. The symbols next to the scientific name indicate: * migratory species according to Sick (1997), Capelloch & Wagner (2009), and Schunck et al. (2019); ** species exhibiting little-known regional movements according to Sick (1997); + species that participate in mixed flocks in the Atlantic Forest according to Sick (1997) and Machado (1999); and # species with small territories that are less mobile than other. Current population trend according to the International Union for Conservation of Nature (IUCN, 2026) is indicated by symbols, where ↓ represents decreasing populations, ↔ represents stable, ↑ represents increasing. The IUCN Red List Category and Criteria – Global Assessment were also included, where LC indicates Least Concern.

Nº	ID	Banding numbers	Family	Common name	Species	Sampling date	IUCN last assessed	IUCN Red List Category and Criteria
1	M1382	G67183	<i>Cardinalidae</i>	Red-crowned Ant-Tanager	<i>Habia rubica</i> +↓	28/06/2022	January 2020	LC
2	M1383	G67184	<i>Thamnophilidae</i>	White-shouldered Fire-eye	<i>Pyriglena leucoptera</i> +↓	28/06/2022	June 2024	LC
3	M1391	E186470	<i>Thamnophilidae</i>	Squamate Antbird	<i>Myrmoderus squamosus</i> +↓	29/06/2022	August 2018	LC
4	M1392	E186471	<i>Vireonidae</i>	Rufous-crowned Greenlet	<i>Hylophilus poicilotis</i>↔	29/06/2022	August 2018	LC
5	M1402	D144970	<i>Parulidae</i>	Golden-crowned Warbler	<i>Basileuterus culicivorus</i> +↓	30/06/2022	December 2019	LC
6	M1414	D175152	<i>Parulidae</i>	Golden-crowned Warbler	<i>Basileuterus culicivorus</i> +↓	09/08/2022	December 2019	LC
7	M1474	E75850	<i>Troglodytidae</i>	Southern House Wren	<i>Troglodytes musculus</i>#↔	11/10/2022	June 2025	LC
8	M1476	D144983	<i>Vireonidae</i>	Rufous-crowned Greenlet	<i>Hylophilus poicilotis</i> ↔	11/10/2022	August 2018	LC
9	M1485	E75855	<i>Thraupidae</i>	Flame-crested Tanager	<i>Loriotus cristatus</i> ↔	11/10/2022	August 2018	LC
10	M1490	E75857	<i>Thamnophilidae</i>	Plain Antvireo	<i>Dysithamnus mentalis</i> +↓	11/10/2022	October 2020	LC
11	M1515	D144993	<i>Parulidae</i>	Golden-crowned Warbler	<i>Basileuterus culicivorus</i> +↓	13/10/2022	December 2019	LC
12	M1526	C101332	<i>Dendrocolaptidae</i>	Olivaceous Woodcreeper	<i>Sittasomus griseicapillus</i> +↓	14/10/2022	February 2020	LC
13	M1527	D14966	<i>Dendrocolaptidae</i>	Olivaceous Woodcreeper	<i>Sittasomus griseicapillus</i> +↓	14/10/2022	February 2020	LC

14	M1528	F32508	<i>Passerellidae</i>	Rufous-collared Sparrow	<i>Zonotrichia capensis</i> # ↑	14/10/2022	February 2020	LC
15	M1530	E75859	<i>Thraupidae</i>	Blue-black Grassquit	<i>Volatinia jacarina</i> ** ↑	12/10/2022	October 2020	LC
16	M1535	E186471	<i>Vireonidae</i>	Rufous-crowned Greenlet	<i>Hylophilus poicilotis</i>+↔	29/11/2022	August 2018	LC
17	M1544	C101333	<i>Furnariidae</i>	Streaked Xenops	<i>Xenops rutilans</i> +↔	29/11/2022	February 2020	LC
18	M1580	E75850	<i>Troglodytidae</i>	Southern House Wren	<i>Troglodytes musculus</i>#↔	30/11/2022	June 2025	LC
19	M1630	E75850	<i>Troglodytidae</i>	Southern House Wren	<i>Troglodytes musculus</i>#↔	23/12/2022	June 2025	LC
20	M1637	E189506	<i>Vireonidae</i>	Chivi Vireo	<i>Vireo chivi</i> *+↔	08/01/2023	August 2019	LC
21	M1675	E189529	<i>Vireonidae</i>	Chivi Vireo	<i>Vireo chivi</i> *+↔	12/01/2023	August 2019	LC
22	M1677	E75889	<i>Troglodytidae</i>	Southern House Wren	<i>Troglodytes musculus</i>#↔	12/01/2023	June 2025	LC
23	M1679	E189533	<i>Tyrannidae</i>	Chilean elaenia	<i>Elaenia chilensis</i> *↓	26/03/2023	June 2024	LC
24	M1692	D175171	<i>Parulidae</i>	Golden-crowned Warbler	<i>Basileuterus culicivorus</i> +↓	01/03/2023	June 2024	LC
25	M1701	D175172	<i>Tyrannidae</i>	Gray-hooded Flycatcher	<i>Mionectes rufiventris</i> +↓	01/03/2023	August 2018	LC
26	M1712	E189562	<i>Thamnophilidae</i>	Dusky-tailed Antbird	<i>Drymophila malura</i> #↓	02/03/2023	June 2024	LC
27	M1725	F32538	<i>Vireonidae</i>	Chivi Vireo	<i>Vireo chivi</i> *+↔	02/03/2023	August 2019	LC
28	M1726	E189581	<i>Vireonidae</i>	Chivi Vireo	<i>Vireo chivi</i> *+↔	02/03/2023	August 2019	LC
29	M1733	F32543	<i>Dendrocolaptidae</i>	Plain-winged Woodcreeper	<i>Dendrocincla turdina</i> +↓	03/03/2023	June 2024	LC
30	M1735	D175183	<i>Parulidae</i>	Golden-crowned Warbler	<i>Basileuterus culicivorus</i> +↓	03/03/2023	June 2024	LC
31	M1737	E189585	<i>Parulidae</i>	Golden-crowned Warbler	<i>Basileuterus culicivorus</i> +↓	03/03/2023	June 2024	LC
32	M1739	E189586	<i>Parulidae</i>	Masked Yellowthroat	<i>Geothlypis aequinoctialis</i> #↔	03/03/2023	June 2024	LC



Supplementary Figure 1. Prediction of putative viral genome-linked protein (VPg) region by FoldIndex and indicated TEEE-like motif (at 777 to 781).