

FIGURE S1 | Photograph of the sampling site in Córrego do Jaque, tributary of the left bank of rio das Velhas, rio São Francisco basin, Lagoa Santa (19°34'28.58"S 43°55'28.64"W), where *Phalloceros uai* was collected. Photo by EBH.

TABLE S2 | Molecular samples indicating the specimen phenotype (left side x right side), sex, sampling location, concentration (ng/µL), absorbance 260/290 nm (A260/A280) and GenBank accession number.

Phenotype	Sex	Location	Concentration	A260/A280	Museum voucher	GenBank accession number
1x1	Male	Lagoa Santa, MG, Brazil	213	1.94	DZUFMG 161	PX673585
1x1	Male	Lagoa Santa, MG, Brazil	271	1.94	DZUFMG 161	PX673590
1x1	Male	Lagoa Santa, MG, Brazil	339	1.95	DZUFMG 161	PX673581
1x1	Male	Santa Rita Durão, Mariana, MG, Brazil	638	1.95	DZUFMG 171	PX673585
1x1	Male	Santa Rita Durão, Mariana, MG, Brazil	563	1.95	DZUFMG 171	PX673584
1x2	Male	Santa Rita Durão, Mariana, MG, Brazil	410	1.96	DZUFMG 171	PX673595
2x2	Female	Santa Rita Durão, Mariana, MG, Brazil	306	1.95	DZUFMG 171	PX673588
2x3	Male	Santa Rita Durão, Mariana, MG, Brazil	93	1.95	DZUFMG 171	PX673591
1x2	Male	Santa Rita Durão, Mariana, MG, Brazil	207	1.95	DZUFMG 171	PX673580
1x1	Male	Santa Rita Durão, Mariana, MG, Brazil	328	1.93	DZUFMG 171	PX673592
2x2	Male	Santa Rita Durão, Mariana, MG, Brazil	187	1.95	DZUFMG 171	PX673593
1x2	Male	Santa Rita Durão, Mariana, MG, Brazil	498	1.97	DZUFMG 171	PX673582
1x1	Female	Brumadinho, MG, Brazil	843	2.00	DZUFMG 159	PX673583
2x2	Female	Brumadinho, MG, Brazil	579	1.98	DZUFMG 159	PX673589
1x1	Male	Brumal, Santa Bárbara, MG, Brazil	544	1.93	DZUFMG 160	PX673594
1x1	Female	Brumal, Santa Bárbara, MG, Brazil	453	1.95	DZUFMG 160	PX673587

TABLE S3 | Specimens of *Phalloceros uai* dissected for stomach content analysis, divided by size categories, sex and sampling area.

Females				Males			
Sampling area	Size category (mm)		Number of specimens	Sampling area	Size category (mm)		Number of specimens
Site 1	1	24.41–29.16	6	Site 1	1	24.41–29.16	2
	2	29.16–33.91	5		2	29.16–33.91	3
	3	33.91–38.66	5		3	33.91–38.66	4
	4	38.66–43.41	5		4	38.66–43.41	4
	5	43.41–48.12	4		5	43.41–48.12	2
			Total = 25				Total = 20
Site 2	1	24.41–29.16	3	Site 2	1	24.41–29.16	-
	2	29.16–33.91	1		2	29.16–33.91	2
	3	33.91–38.66	1		3	33.91–38.66	1
	4	38.66–43.41	-		4	38.66–43.41	2
	5	43.41–48.12	-		5	43.41–48.12	-
			Total = 5				Total = 5
Site 3	1	24.41–29.16	4	Site 3	1	24.41–29.16	2
	2	29.16–33.91	4		2	29.16–33.91	4
	3	33.91–38.66	5		3	33.91–38.66	2
	4	38.66–43.41	5		4	38.66–43.41	2
	5	43.41–48.12	2		5	43.41–48.12	-
			Total = 20				Total = 10
Site 4	1	24.41–29.16	3	Site 4	1	24.41–29.16	-
	2	29.16–33.91	1		2	29.16–33.91	-
	3	33.91–38.66	1		3	33.91–38.66	-
	4	38.66–43.41	-		4	38.66–43.41	-
	5	43.41–48.12	-		5	43.41–48.12	-
			Total = 5				Total = 0

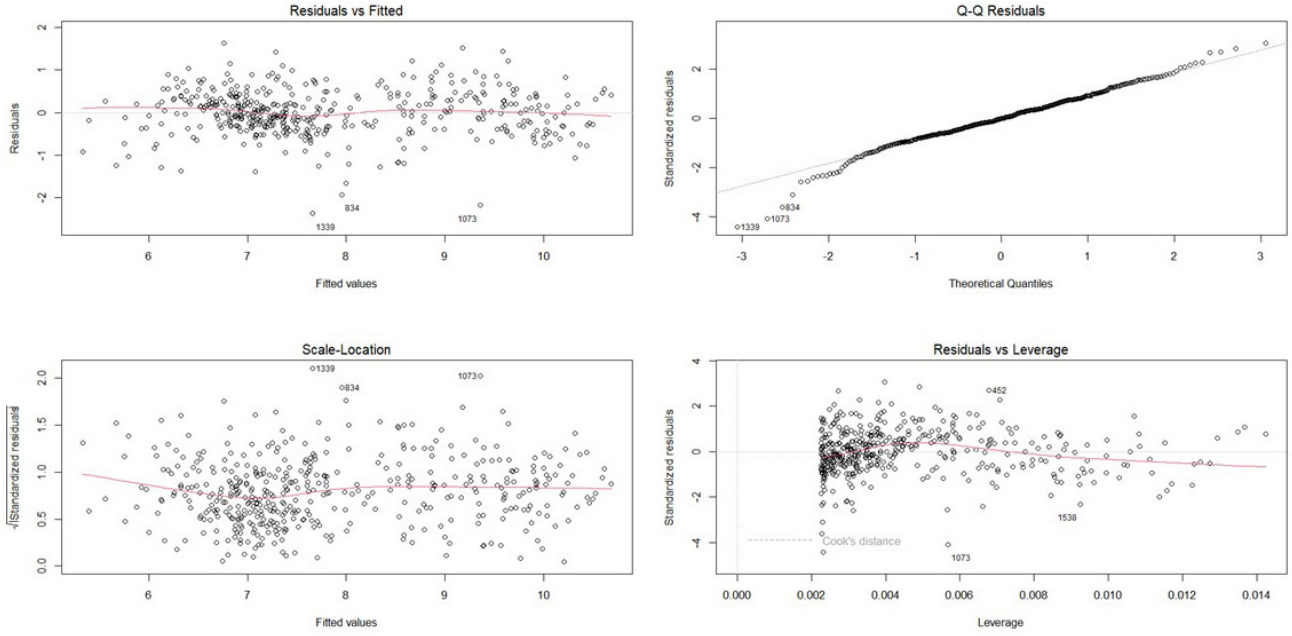

FIGURE S4 | Graphical representation of residuals from the linear regression analysis between male gonopodium length and total length.



FIGURE S5 | Gonopodium of males of *Phalloceros uai*. **A.** DZUFMG 160 - 1x1 Phenotype. **B.** DZUFMG 159 - 2x3 Phenotype. All specimens possessed a left hook on the gonopodium, an asymmetrical gonopodial appendage (right wider than the left), and a serrated structure on ray 4p, consistent with original description.

TABLE S6 | Genetic distance of mitochondrial COI gene sequences from *Phalloceros* by K2P model.

	P. elachistos RDOCE211	P.enneaktinos MNLM7084	P. reisi LBPV43526	P. tupinamba MNLM7028l	P. uai MNLM7019	P. uai DCC01181	P. uai DCC31592	P. uai DZUFMG161 1x1	P. uai DZUFMG161 1x1	P. uai DZUFMG161 1x1	P. uai DZUFMG171 1x1	P. uai DZUFMG171 1x1	P. uai DZUFMG171 2x1	P. uai DZUFMG171 2x2	P. uai DZUFMG171 3x2	P. uai DZUFMG171 1x2	P. uai DZUFMG171 1x1	P. uai DZUFMG171 2x2	P. uai DZUFMG171 1x2	P. uai DZUFMG159 1x1	P. uai DZUFMG159 2x2	P. uai DZUFMG160 1x1	P. uai DZUFMG160 1x1
P. elachistos RDOCE211																								
P.enneaktinos MNLM7084	0.109																							
P. reisi LBPV43526	0.098	0.064																						
P. tupinamba MNLM7028l	0.082	0.098	0.087																					
P. uai MNLM7019	0.099	0.063	0.026	0.085																				
P. uai DCC01181	0.100	0.063	0.026	0.086	0.000																			
P. uai DCC31592	0.099	0.063	0.026	0.085	0.000	0.000																		
P. uai DZUFMG161 1x1	0.105	0.075	0.058	0.098	0.011	0.009	0.009																	
P. uai DZUFMG161 1x1	0.102	0.070	0.056	0.092	0.006	0.006	0.006	0.025																
P. uai DZUFMG161 1x1	0.109	0.077	0.065	0.099	0.012	0.011	0.011	0.030	0.027															
P. uai DZUFMG171 1x1	0.107	0.073	0.055	0.096	0.009	0.008	0.008	0.032	0.030	0.032														
P. uai DZUFMG171 1x1	0.113	0.079	0.063	0.101	0.014	0.012	0.012	0.032	0.029	0.024	0.033													
P. uai DZUFMG171 2x1	0.114	0.080	0.066	0.103	0.015	0.014	0.014	0.035	0.036	0.039	0.035	0.041												
P. uai DZUFMG171 2x2	0.116	0.082	0.060	0.104	0.017	0.015	0.015	0.024	0.038	0.032	0.025	0.030	0.039											
P. uai DZUFMG171 3x2	0.102	0.068	0.058	0.091	0.005	0.005	0.005	0.025	0.016	0.033	0.029	0.033	0.036	0.036										
P. uai DZUFMG171 1x2	0.110	0.073	0.061	0.096	0.009	0.008	0.008	0.039	0.041	0.038	0.029	0.042	0.038	0.038	0.042									
P. uai DZUFMG171 1x1	0.112	0.078	0.066	0.101	0.014	0.012	0.012	0.036	0.038	0.041	0.026	0.041	0.039	0.024	0.038	0.038								
P. uai DZUFMG171 2x2	0.105	0.075	0.058	0.098	0.011	0.009	0.009	0.028	0.033	0.036	0.024	0.035	0.038	0.032	0.033	0.038	0.038							
P. uai DZUFMG171 1x2	0.107	0.075	0.057	0.098	0.011	0.011	0.011	0.032	0.030	0.039	0.033	0.032	0.036	0.032	0.032	0.036	0.039	0.028						
P. uai DZUFMG159 1x1	0.105	0.075	0.066	0.097	0.011	0.009	0.009	0.028	0.030	0.024	0.030	0.039	0.033	0.036	0.031	0.030	0.034	0.027	0.031					
P. uai DZUFMG159 2x2	0.106	0.072	0.060	0.094	0.009	0.008	0.008	0.031	0.031	0.036	0.036	0.032	0.030	0.033	0.038	0.039	0.036	0.031	0.036	0.028				
P. uai DZUFMG160 1x1	0.103	0.071	0.038	0.094	0.008	0.008	0.008	0.012	0.009	0.014	0.012	0.014	0.020	0.020	0.009	0.018	0.017	0.011	0.012	0.009	0.014			
P. uai DZUFMG160 1x1	0.107	0.073	0.055	0.096	0.009	0.008	0.008	0.025	0.027	0.030	0.019	0.029	0.039	0.024	0.035	0.030	0.028	0.033	0.035	0.033	0.033	0.012		

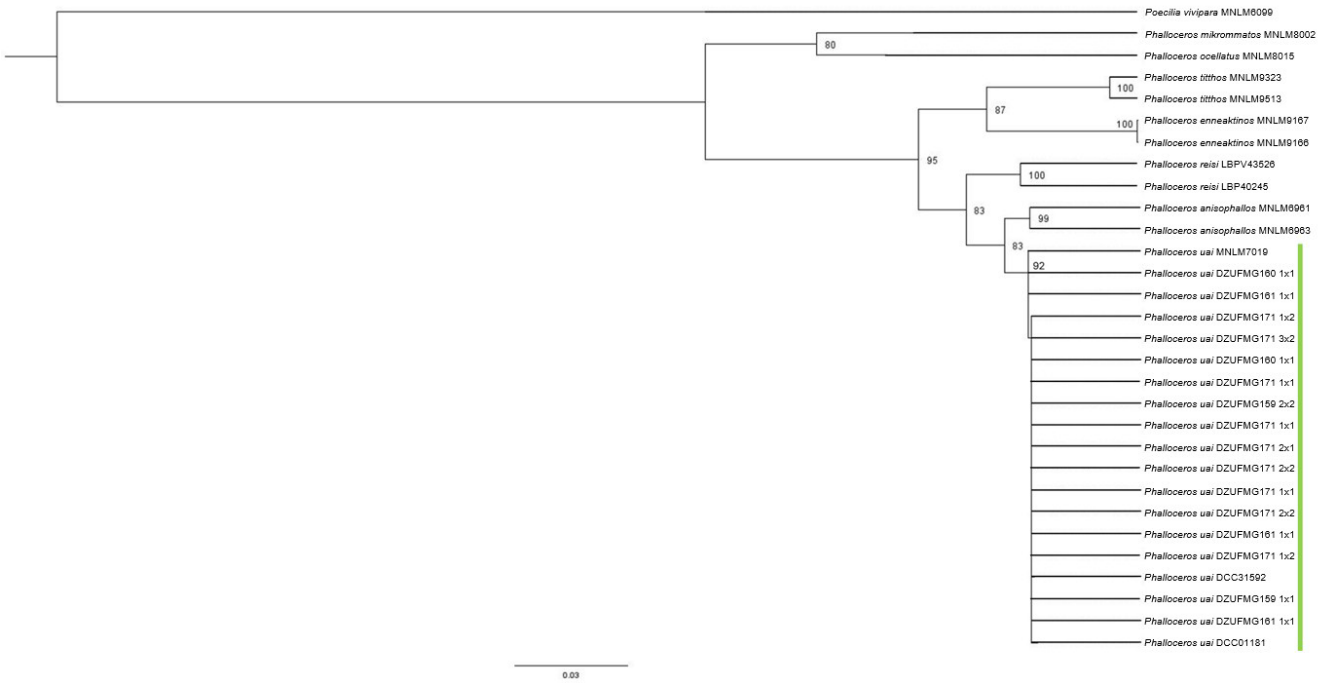

FIGURE S7 | Gene tree inferred from cytochrome c oxidase subunit I (CO_1) sequences of *Phalloceros* obtained in this study and from GenBank. Nodes with bootstrap support below 80% are omitted. *Phalloceros uai* forms a well-supported monophyletic clade (highlighted in green).

TABLE S8 | Total number and frequency of stomach contents separated by food item and population. N = Number of stomachs found with the food item. % = Frequency of the food item.

Food item		Population							
		Site 1 (n = 45)		Site 2 (n = 10)		Site 3 (n = 30)		Site 4 (n = 5)	
		N	%	N	%	N	%	N	%
Aquatic inverterbrates	Coleoptera (Adult)	-	-	-	-	-	-	1	25.00
	Coleoptera (Immature)	1	2.22	1	1.00	-	-	-	-
	Diptera (Adult)	1	2.22	-	-	1	3.33	1	25.00
	Diptera (Immature)	2	4.44	2	2.00	11	36.67	1	25.00
	Ephemeroptera (Immature)	-	-	1	1.00	-	-	-	-
	Odonata (Immature)	-	-	-	-	1	3.33	-	-
	Trichoptera (Immature)	-	-	-	-	1	3.33	-	-
	Unidentified adult insect	-	-	2	2.00	1	3.33	1	25.00
	Unidentified animal material	-	-	2	2.00	3	10	1	25.00
Plant material		3	6.67	-	-	-	-	-	-
Debris	Sand/Stone grains	16	35.56	1	1.00	5	16.67	2	50.00
Unidentified material		2	4.44	1	1.00	1	3.33	-	-
Empty		31	68.89	3	3.00	9	30.00	-	-

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