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# New taxonomic insights for Brazilian *Syrbatus* Reitter (Coleoptera: Staphylinidae: Pselaphinae), including three new species and their mitochondrial genomes

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Here we present a taxonomic treatment for the Brazilian species of *Syrbatus* (Reitter, 1882), including the description of three new species (*Syrbatus moustache* Asenjo & Valois **sp. nov.**, *Syrbatus obsidian* Asenjo & Valois **sp. nov.** and *Syrbatus superciliata* Asenjo & Valois **sp. nov.**) from the Quadrilátero Ferrífero (Minas Gerais, Brazil). In addition, we designated new lectotypes for the Brazilian species of species-group 2, *Syrbatus centralis* (Raffray, 1898), *Syrbatus hetschkoi* (Reitter, 1888), *Syrbatus hiatusus* (Reitter, 1888), *Syrbatus transversalis* (Raffray, 1898), and *Syrbatus trinodulus* (L.W.Schaufuss, 1887), besides recognizing the holotype for *Syrbatus brevispinus* (Reitter, 1882), *Syrbatus bubalus* (Raffray, 1898), and *Syrbatus grouvellei* (Raffray, 1898). The mitochondrial genomes (mitogenomes) of the three new species are presented, for which we present the phylogenetic placement among Staphylinidae species with previously published data.

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2	Pselaphinae), including three new species and their mitochondrial genomes
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## **Abstract** 19 Here we present a taxonomic treatment for the Brazilian species of *Syrbatus* (Reitter, 1882), 20 including the description of three new species (Syrbatus moustache Asenjo & Valois sp. nov., 21 Syrbatus obsidian Asenjo & Valois sp. nov. and Syrbatus superciliata Asenjo & Valois sp. nov.) 22 from the Quadrilátero Ferrífero (Minas Gerais, Brazil). In addition, we designated new 23 24 lectotypes for the Brazilian species of species-group 2, Syrbatus centralis (Raffray, 1898), Syrbatus hetschkoi (Reitter, 1888), Syrbatus hiatusus (Reitter, 1888), Syrbatus transversalis 25 (Raffray, 1898), and Syrbatus trinodulus (L.W.Schaufuss, 1887), besides recognizing the 26 holotype for Syrbatus brevispinus (Reitter, 1882), Syrbatus bubalus (Raffray, 1898), and 27 Syrbatus grouvellei (Raffray, 1898). The mitochondrial genomes (mitogenomes) of the three 28 new species are presented, for which we present the phylogenetic placement among 29 Staphylinidae species with previously published data. 30 31 32 **Keywords:** Quadrilátero Ferrífero, mtDNA, Pselaphinae, rove beetle, taxonomy 33 **INTRODUCTION** 34 35 The species of *Syrbatus* (Reitter, 1882) are distributed in Africa (89 spp.) and South America (30 spp.) (Newton & Chandler, 1989; Asenjo et al., 2019), where, to date, 25 species 36 37 were recorded for Brazil, three for Paraguay and two for Argentina (Asenjo et al., 2019; Newton, 38 2022). In Brazil, the genus is known to occur in the states of Santa Catarina (13 spp.), São Paulo (8 spp.), Bahia (3 spp.), Rio de Janeiro (2 spp.) and Minas Gerais (1 sp.) (Asenjo et al., 2013). 39

Since the description of *Syrbatus* as a subgenus of *Batrisus* (Aubé, 1833) by Reitter (1882),

based on the characters of the pronotum, Raffray (1897) raised it to the rank of genus, discussing



42	mainly the characters of head and pronotum among the species of these two taxa and Arthmius
43	(LeConte, 1849). Afterwards, Raffray (1904) used characters of the antenna and head to organize
44	the South American species of the genus into six species-group, with posterior reorganization of
45	these subdivisions made by Park (1942) and designation of <i>Batrisus clypeatus</i> (Reitter, 1882) as
46	the type species of Syrbatus by Jeannel (1949).
47	The biology of the genus is poorly known, with its species, in general, being part of the
48	soil fauna, as Syrbatus demoniacus (Raffray, 1898) and Syrbatus leleupi (Jeannel, 1950), for
49	instance, that were originally found in tobacco plantations in Brazil (Raffray, 1898) and in the
50	entrance a of cave in the Democratic Republic of the Congo (Jeannel, 1950), respectively.
51	Interestingly, several authors have related cave specimens of undescribed species of Syrbatus
52	from Brazil in the last three decades (Gnaspini & Trajano, 1994; Pinto da Rocha, 1995; Trajano
53	& Bichuette, 2010; Gallão & Bichuette, 2018). Therefore, the aim of this study was to describe
54	three new species of the group from Brazil, Syrbatus moustache Asenjo & Valois sp. nov.,
55	Syrbatus obsidian Asenjo & Valois sp. nov. and Syrbatus superciliata Asenjo & Valois sp. nov.,
56	all of which collected in caves of the Quadrilátero Ferrífero, in the state of Minas Gerais. In
57	addition, we sequenced the mitochondrial genomes (mitogenomes) of the three, exploring the
58	phylogenetic relationships within Staphylinidae and Pselaphinae.
59	
60	MATERIAL & METHODS
61	Field collection
62	The three species described in this work are associated with siliciclastic caves in the
63	Quadrilátero Ferrífero, one of the most important regions for the conservation of subterranean
64	biodiversity in Minas Gerais. This region comprises a large mosaic of phytophysiognomies



65	shaped by the conjunction of topography, lithology, climate, and altitude (Jacobi & Carmo,
66	2008), being inserted in a transition zone between two Brazilian biodiversity hotspots, the
67	Atlantic Forest and the Cerrado biomes (Mittermeier et al., 2004). In general, the climate is
68	characterized as Cwb (subtropical highland climate), with mild and humid summers, and cool
69	and dry winters (Köppen, 1948), although it can be strongly influenced by the relief since the
70	average altitude exceeds 1,000 m, with the tallest regions reaching 2× this height. The annual
71	precipitation ranges between 1,250-1,550 mm, with an average annual temperature between 18-
72	19 °C.
73	The Quadrilátero Ferrífero has an area of approximately 7,200 km², being considered one
74	of the most important mineral provinces in Brazil, mainly due to its gold and iron deposits. At
75	the same time, the region presents one of the most diverse floras in South America with high
76	rates of endemism (Giulietti, Pirani & Harley, 1997). The area is of special biological relevance
77	due to the presence of ferruginous fields, the occurrence of several endemic plant species,
78	besides constituting a unique environment in the country. Formed by ancient and geologically
79	complex terrains of the Minas Super Group, with varied lithologies (Alkmim & Marshak, 1998;
80	Klein & Ladeira, 2000), with more than two thousand caves currently known in the region
81	(CECAV, 2021), and dozens of cave species have been discovered and described in recent years
82	This large set of caves are distributed in different mountain ranges (geomorphological units),
83	with the species described in this work found in caves in the Serra da Moeda and the Escarpa
84	Oriental do Caraça (Figure 1). All studied specimens were collected in accordance with the
85	sampling permits 065/2013 NUFAS/MG and 424.033/2018, granted by IBAMA/MMA and
86	SEMAD/MG, respectively.



Morphological analyses

89	Specimens. The apical segments were cleared in a double boiler using 10% KOH for 3
90	min. Dissections were made under a Zeiss Discovery V12 (4×-125×) stereomicroscope. Pictures
91	were obtained using an AxioCam 506 (ZEISS, Oberkochen, Germany) connected to an Axio
92	Zoom V16 (ZEISS) stereomicroscope, and Photoshop CC 2021 was used for image processing.
93	Morphological character terminology, including foveation and its abbreviation followed
94	Chandler (2001). All measurements were based using a ZEISS Discovery V12 (4×-125×)
95	stereomicroscope, and the width/length ratios were acquired using the widest and longest parts of
96	the respective structures, being presented in millimeters, based on the holotype. We also
97	performed additional measurements using the paratypes, comparing with the data obtained for
98	the holotypes (Data S1).
99	
100	Measurements symbols:
101	
102	BL: body length (from margin of clypeus to posterior margin of tergite VIII)
103	BW: body width (maximum width of elytra)
104	EL: elytral length (maximum)
105	EW: elytral width (maximum)
106	HL: head length (from anterior margin of clypeus to posterior margin of head disc)
107	HW: head width (maximum, including eyes)
108	NW: neck width (minimum)
109	PL: pronotum length (maximum)
110	PW: pronotum width (maximum)



111	
112	In the type label data, quotation marks ("") separate different labels, and a slash (/)
113	separates different lines within a label. Text within square brackets [] is explanatory and is not
114	included on the original labels.
115	
116	Depositories
117	The specimens examined in this revision are deposited in the following collections
118	(curators in parenthesis):
119	
120	CEMT – Setor de Entomologia da Coleção Zoológica da Universidade Federal de Mato
121	Grosso, Departamento de Biologia e Zoologia, Cuiabá, Mato Grosso, Brazil (Fernando Vaz-de
122	Mello).
123	ISLA – Coleção de Invertebrados Subterrâneos de Lavras, Setor de Zoologia,
124	Departamento de Biologia, Universidade Federal de Lavras, Lavras, Minas Gerais State, Brazil
125	(Rodrigo Lopes Ferreira).
126	ITV – Coleção de DNA do Instituto Tecnológico Vale, Belém, Pará, Brazil (Santelmo
127	Vasconcelos).
128	MPEG – Museu Paraense Emilio Goeldi, Terra Firme, Belém, Brazil (Orlando Silveira)
129	MNHN – Muséum National d'Histoire Naturelle, Paris, France (Antoine Mantilleri).
130	SDEI – Senckenberg Deutschen Entomologischen Institut, Eberswalder, Germany
131	(Mariana Simões).
132	
133	Nomenclatural acts



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0A9D9C839434.
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## DNA sequencing, mitogenome assemblies and phylogenetic analysis

A total of seven specimens of the three new species described here were separated and processed to obtain total genomic DNA, being one individual of *Syrbatus moustache* Asenjo & Valois **sp. nov.** (MPEG-01052552/ITV22155), three of *Syrbatus obsidian* Asenjo & Valois **sp. nov.** (ISLA-110327/ITV22166, ISLA-110326/ITV22168, and MPEG-01052558/ITV22169), and three of *Syrbatus superciliata* Asenjo & Valois **sp. nov.** (ITV10831, ITV10835 and MPEG-01052550/ITV39056). The DNA samples were obtained using the DNeasy Blood & Tissue kit (Qiagen), following the manufacturer's protocol for insect samples, subsequently being deposited at the DNA bank of the Instituto Tecnológico Vale (ITV).

Paired-end libraries were constructed from ~5 ng of the obtained genomic DNA using the Illumina DNA Prep kit (Illumina, Inc., San Diego, CA, USA), following the manufacturer's



157	instructions for low-input samples. After the final purification step, the resultant libraries were
158	quantified with a Qubit 3.0 (Invitrogen, Waltham, MA, USA) fluorimeter, using the Qubit
159	dsDNA High Sensitivity kit (Invitrogen), and analyzed for fragment sizes in a 4200 TapeStation
160	(Agilent Technologies, Santa Clara, CA, USA). Then, the libraries were sequenced in an
161	Illumina NextSeq 2000 platform, with a P1 kit (240 cycles, 2× 120 bp). Resulting raw
162	sequencing reads with base quality < Phred 20 and length <50 bp were trimmed with
163	AdapterRemoval v.2 (Schubert, Lindgreen & Orlando, 2016), and the resulting high-quality
164	reads were used to assemble the mitochondrial genomes using NovoPlasty v.4.2 (Dierckxsens,
165	Mardulyn & Smits, 2017). Finally, the mitogenomes were annotated with MITOS2 (Bernt et al.,
166	2013), with subsequent minor manual corrections using Geneious Prime v.2023 (Biomatters), by
167	comparing the annotated mitogenomes with previously available data for Pselaphinae species.
168	To position the three new described species in the context of the phylogenetic tree of the
169	family, we obtained all previously published mitogenomes of Staphylinidae species available in
170	the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) which presented, at least, the
171	complete sequences of the 13 protein coding genes (PCGs), totaling 84 species from 14
172	subfamilies, plus one species of Hydrophilidae (Cercyon borealis) and one of Histeridae
173	(Euspilotus scissus) to be used as outgroups. Amino acid sequences of the 13 PCGs were aligned
174	with MAFFT v7.45 (Katoh et al., 2002; Data S2) and maximum likelihood (ML) and Bayesian
175	inference (BI) phylogenetic trees were obtained using RAxML v8 (Stamatakis, 2014) and
176	MrBayes v3.2.7 (Ronquist et al., 2012), as implemented in the CIPRES portal
177	(http://www.phylo.org/). The ML analysis was performed using the model PROTGAMMA and
178	1,000 replicates of rapid bootstrapping, and the BI trees were obtained using four simultaneous
179	runs, each with four Markov chains ( $T = 0.2$ ) extended through 20,000,000 generations,



sampling every 2,000, and using a burn-in fraction of 25% of the trees. Additionally, to further
investigate the differentiation among the mitogenomes of the three new species, we employed
two different lineage delimitation approaches, using the sequences of three specimens of the
recently described <i>Metopiellus crypticus</i> (Asenjo et al., 2023), which was identified as the closest
Pselaphinae species among the sampled mitogenomes in the phylogenetic reconstruction. In the
first, Assemble Species by Automatic Partitioning (ASAP; Puillandre, Brouillet & Achaz, 2021),
which uses a genetic distance matrix to identify putative species using a genetic distance matrix,
we employed a concatenated matrix of nucleotide sequences of all 13 PCGs as a single locus
with the mitogenomes as a single locus, choosing the K80 model ( $ts/tv = 2.0$ ). The second
approach, Bayesian Phylogenetics and Phylogeography (BPP; Yang, 2015), is based on the
multispecies coalescent model under a Bayesian Markov chain Monte Carlo (MCMC) algorithm,
for which we provided the nucleotide alignments of the 13 PCGs as independent loci and a guide
tree as obtained in the ML phylogenetic reconstruction, using the default parameters.
RESULTS
Description
Family Staphylinidae (Latreille, 1802)

- Subfamily Pselaphinae (Latreille, 1802)
- Tribe Batrisini (Reitter, 1882)
- Subtribe Batrisina (Reitter, 1882)
- Genus Syrbatus (Reitter, 1882)

## Syrbatus moustache Asenjo & Valois, new species



```
(Figs. 1, 2 and 5)
203
             http://zoobank.org/urn:lsid:zoobank.org:act:469E3B88-F2C7-4724-9F4C-
204
      7A51D1F5FABC
205
             Type material (one male, two females). Holotype: BRAZIL: male, labeled "BRAZIL:
206
      Minas Gerais, / Nova Lima, SM 0046 / cave, 29.vii[July]-02.viii[August].2019, / -20.188606, -
207
      43.838583 [20°11'18.98"S, 43°50'18.89"W], / Spelayon et al.", "[image data matrix] / Instituto /
208
      Tecnológico / Vale / ITV22154", "HOLOTYPE of [red label] / Syrbatus / moustache sp. nov. /
209
      Desig. Asenjo et al., 2023" (one male, ISLA-110319). Paratype: (two females), labeled:
210
      "BRAZIL: Minas Gerais, / Nova Lima, SM 0046 / cave, 29.vii[July]- / 02.viii[August].2019, / -
211
      20.188606, -43.838583 [20°11'18.98"S, 43°50'18.89"W], / Spelayon et al.", "[image data
212
      matrix] / Instituto / Tecnológico / Vale / ITV22156" (one female, ISLA-110320). "BRAZIL:
213
      Minas Gerais, / Nova Lima, SM 0042 / cave, 29.vii[July]-02.viii[August].2019, / -20.192874, -
214
      43.836350 [20°11'34.34"S, 43°50'10.86"W], / Spelayon et al.", "[image data matrix] / Instituto /
215
      Tecnológico / Vale / ITV22158" (one female, MPEG-01052551). All paratypes with label
216
      "PARATYPE [yellow label] / Syrbatus / moustache sp. nov. / Desig. Asenjo et al., 2023".
217
             Molecular voucher. "BRAZIL: Minas Gerais, / Nova Lima, SM 0042 / cave, -20.192874,
218
219
      /-43.836350 [20°11'34.34"S, 43°50'10.86"W], 26-31.iii[March].2019, / Spelayon et al.",
      "[image data matrix] / Instituto / Tecnológico / Vale / ITV22155" (one female, MPEG-
220
221
      01052552).
222
             Diagnosis. Syrbatus mustache Asenjo & Valois sp. nov. belong to species-group 2 (see
223
      the Discussion section below), being similar to Syrbatus superciliata Asenjo & Valois sp. nov.,
224
225
      considering both the habitus and head shape. The main differences between the two are the
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darker color of the body of *Syrbatus mustache* **sp. nov.**, which is reddish brown, while *Syrbatus superciliata* Asenjo & Valois **sp. nov.** is a yellowish brown; head with oval transversal area in the anterior region (sling transversal area in *Syrbatus superciliata* Asenjo & Valois **sp. nov.**); posterior margin of sternum VIII with a stout conical projection and its apex rounded in *Syrbatus mustache* **sp. nov.**, posterior margin of sternum VIII with thin pointed projection at middle (thin pointed projection at the middle in with its apex rounded in *Syrbatus superciliata* Asenjo & Valois **sp. nov.**); and aedeagus with thin paramere in *Syrbatus mustache* **sp. nov.** Asenjo & Valois (paramere thick in *Syrbatus superciliata* Asenjo & Valois **sp. nov.**).

Holotype male (Fig. 2A). BL: 2.11. Head, pronotum, elytra and abdomen reddish brown; antennae, mouth parts, tibial apex brown.

Head. Subrectangular (Figs. 2A and 2C-D), longer (HL: 0.58) than wide (HW: 0.42). Antennal insertions on head not visible in dorsal view. Anterior margin rounded, genal edge rounded and not carinated. Eye composed of 13 ommatidia and situated more posteriorly at head length in lateral view. Neck almost one-half width of head (NW: 0.20), with margins rounded. Anterior region with wide oval transversal area, 2.0 wider than long; and its surface covered with long setae (Fig. 2C). Eyes connected by long curved transversal slim carina covered of long setae. Both regions cited anteriorly connected by short medial longitudinal keel covered by long setae. The vertex area between eyes with one deep sulcus in format of W-shaped (Fig. 2D) and with vertexal foveae [vf]. Ventral surface of head with one gular fovea, without visible gular sulcus. Antennae (Fig. 2A) 2/3 body length. Scape rectangular, deeply impressed medially with margins rounded. Pedicel subclaviform, only slightly shorter than scape. Antennomeres 3-7 cylindrical, gradually broadening to apex, about 2× longer than wide. Antennomere 8 oval, only



slightly longer than wide; smaller than the other antennomeres. Antennomeres 9-10 rounded, approximately  $2\times$  longer than wide. Antennomere 11 sub-fusiform,  $2.5\times$  longer than wide. All antennomeres covered with short and long microsetae.

Thorax: Pronotum hexagonal (Figs. 2A and 2E), slightly longer than wide (PL: 0.45; PW: 0.44) broader at two-third anterior portion and narrower at posterior one-third (Fig. 2F). Surface of pronotum shinning, with long setae direct to center. Pronotal disc distinctly convex in lateral view. Antebasal sulcus [as] present and complete, curved and slender, reaching the posterior ending of the longitudinal sulcus; and one strong lateral longitudinal sulcus on each side of pronotum. Pronotum with anterior margin almost right and posterior margin convex; with a lateral antebasal fovea [laf], an outer basolateral fovea [oblf], and an inner basolateral fovea [iblf]. Posterior angles of pronotum slightly obtuse (Fig. 2E). Prosternum with one lateral procoxal fovea [lpcf]. Mesoventrite with one lateral mesosternal foveae [lmsf], one lateral mesocoxal fovea [lmcf], and two median metasternal fovea [mmtf].

Elytra: Trapezoidal (EL: 0.70; EW: 0.80), posterior margin 2× larger than anterior margin (Figs. 2A and 2F). Surface of elytra shining, with long setae sparse and direct posteriorly.

Posterior margin curved, with sutural stria [ss] present. Elytron with three basal elytral fovea [bef] at anterior margin. Flight wings extremely reduced (brachypterous species).

Legs: Elongated and slender (Fig. 2A). Femora thickened medially; tibiae slightly widened toward apex, similar in length to femora. Protibiae with microsetae and one apical spur on its internal face. Tarsi 3-segmented, with basal tarsomere minute and the two other segments longer. Second segment longer than third. All tarsi with two apical claws; one thicker and other setiform and slender. Pro- and mesocoxae conical, prominent and contiguous. Metacoxae transversely and weakly separated. Procoxae with small prosternal processed, rounded at apex.



272	Abdomen: With five visible tergites (morphological tergites IV-VIII) bordered by fine
273	carina. Tergite IV with a short discal carina [dc] and one basolateral fovea [blf]. Sternite IV with
274	two basolateral fovea [blf] and a mediobasal fovea [mbf]. Tergite VIII with apex convex.
275	Tergum IX small, subtriangular approximately 8× wider than long (Fig. 2M). Sternum VIII
276	deeply impressed medially, posterior margin sinuous having a conical projection at middle, apex
277	of projection rounded (Figs. 2J-K). Tergum VIII subrectangular with anterior and posterior
278	margins rounded (Fig. 2L).
279	Aedeagus: Asymmetric (Figs. 2G-I) strongly asymmetric, length 0.53 mm; median lobe
280	with large basal capsule and triangular basal foramen; the median lobe strongly bifurcated, with
281	both branches twist. Paramere fused to median lobe to with apex divided into two long curved
282	prolongation. The genital plate divided in two small elongated plates, both attached to aedeagus
283	by a membrane almost transparent.
284	Female. It differs from males by frons of head with not distinctly expanded over antennal
285	insertions and the absence of excavation, oblique impressions, or cephalic keel (Fig. 5A). The
286	sternum VIII lacking modification such as projections or impressions. Genital complex (Fig. 5D)
287	transversal with lateral corns elongated; anterior border with two central prolongations and one
288	small prolongation on each side.
289	Etymology. The specific epithet name "moustache" refers to long setae on the anterior
290	region of front. This is a noun in apposition.
291	Distribution. Known only from Nova Lima, Minas Gerais, Brazil (Fig. 1).
292	
293	Syrbatus obsidian Asenjo & Valois, new species
294	(Figs. 1, 3 and 5)



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http://zoobank.org/urn:lsid:zoobank.org:act:C42D432B-4893-4324-A859-
295
      1BD40E47E6D7
296
297
             Type material (seven males, four females). Holotype: BRAZIL: male, labeled: "BRAZIL:
298
      Minas Gerais, / Catas Altas, FZ 0054 / cave, 04.ix[September].2019, / -20.116420, -43.417677
299
      [20°6'59.11"S, 43°25'3.63"W], / Spelayon et al.", "[image data matrix] / Instituto / Tecnológico
300
      / Vale / ITV22170", "HOLOTYPE of [red label] / Syrbatus / obsidian sp. nov. / Desig. Asenjo et
301
      al., 2023" (MPEG-01052553). Paratype: (six males, four females), the first label as the holotype;
302
      "[image data matrix] / Instituto / Tecnológico / Vale / ITV22172" (one male, ISLA-110321);
303
      "[image data matrix] / Instituto / Tecnológico / Vale / ITV22171" (one female, MPEG-
304
      01052554); "[image data matrix] / Instituto / Tecnológico / Vale / ITV22173" (one female,
305
      ISLA-110322). "BRAZIL: Minas Gerais, / Catas Altas, FZ 0050 / cave, 04.ix[September].2019,
306
      /-20.117065, -43.416092 [20°7'1.43"S, 43°24'57.93"W], / Spelayon et al.", "[image data
307
      matrix] / Instituto / Tecnológico / Vale / ITV22161" (one male, CEMT-00138995). "BRAZIL:
308
      Minas Gerais, / Catas Altas, FZ 0050 / cave, 04.ix[September].2019, / -20.117065, -43.416092
309
      [20°7'1.43"S, 43°24'57.93"W], / Spelayon et al.", "[image data matrix] / Instituto / Tecnológico
310
311
      / Vale / ITV22162" (one male, MPEG-01052555). "BRAZIL: Minas Gerais, / Catas Altas,
      FZ 0050 / cave, 04.ix[September].2019, /-20.117065, -43.416092 [20°7'1.43"S,
312
      43°24'57.93"W], / Spelayon et al.", "[image data matrix] / Instituto / Tecnológico / Vale /
313
314
      ITV22163" (one male, ISLA-110323). "BRAZIL: Minas Gerais, / Catas Altas, FZ 0050 / cave,
      04.ix[September].2019, /-20.117065, -43.416092 [20°7'1.43"S, 43°24'57.93"W], / Spelayon et
315
316
      al.", "[image data matrix] / Instituto / Tecnológico / Vale / ITV22164" (one male, ISLA-
317
      110324). "BRAZIL: Minas Gerais, / Catas Altas, FZ 0050 / cave, 04.ix[September].2019, / -
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20.117065, -43.416092 [20°7'1.43"S, 43°24'57.93"W], / Spelayon et al.", "[image data matrix] /
318
      Instituto / Tecnológico / Vale / ITV22165" (one male, ISLA-110325). "BRAZIL: Minas Gerais, /
319
      Catas Altas, FZ 0053 / cave, 04.ix[September].2019, / -20.116519, -43.417599 [20°6'59.46"S,
320
      43°25'3.35"W], / Spelayon et al.", "[image data matrix] / Instituto / Tecnológico / Vale /
321
      ITV22167" (one female, MPEG-01052556). "BRAZIL: Minas Gerais, / Catas Altas, FZ 0049 /
322
323
      cave, 04.ix[September].2019, /-20.117099, -43.415814 [20°7'1.55"S, 43°24'56.93"W], /
      Spelayon et al.", "[image data matrix] / Instituto / Tecnológico / Vale / ITV22159" (one female,
324
      CEMT-00138996). All paratypes with label "PARATYPE [yellow label] / Syrbatus / obsidian
325
      sp. nov. / Desig. Asenjo et al., 2023".
326
             Molecular voucher. "BRAZIL: Minas Gerais, / Catas Altas, FZ 0053 / cave,
327
      24.ix[September].2019, /-20.116519, -43.417599 [20°6'59.46"S, 43°25"3.35"W], / Spelayon et
328
      al.", "[image data matrix] / Instituto / Tecnológico / Vale / ITV22166" (one female, ISLA-
329
      110327). "BRAZIL: Minas Gerais, / Catas Altas, FZ 0054 / cave, 04.ix[September].2019, / -
330
      20.116420, -43.417677 [20°6'59.11"S, 43°25'3.63"W], / Spelayon et al.", "[image data matrix] /
331
      Instituto / Tecnológico / Vale / ITV22168" (one male. ISLA-110326). "BRAZIL: Minas Gerais, /
332
      Catas Altas, FZ 0054 / cave, 04.ix[September].2019, /-20.116420, -43.417677 [20°6'59.11"S,
333
      43°25'3.63"W], / Spelayon et al.", "[image data matrix] / Instituto / Tecnológico / Vale /
334
      ITV22169" (one female, MPEG-01052558).
335
336
337
             Diagnosis. Syrbatus obsidian Asenjo & Valois sp. nov. belong to species-group 5 (see
      the Discussion section). Among the species of species-group 5, Syrbatus obsidian Asenjo &
338
339
      Valois sp. nov. is similar to Syrbatus nasutus (Reitter, 1888) in the shape of head and pronotum;
340
      Syrbatus obsidian Asenjo & Valois sp. nov. presents scape moderately dilated (Syrbatus nasutus
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341	with scape strongly dilated), anterior margin of head rounded (right in Syrbatus nasutus), and
342	genal edge rounded (right angulated in Syrbatus nasutus).
343	
344	Holotype male (Fig. 3A). BL: 1.98 mm. Head, pronotum and abdomen dark brown;
345	antennae, mouth parts legs and elytra reddish brown.
346	Head: Subtriangular (Figs. 3A and 3C-D), slightly wider (HW: 0.59) than long (HL:
347	0.44). Antennal insertions on head not visible in dorsal view. Anterior margin rounded and
348	emarginated, genal edge rounded and carinated. Eyes composed of right side 19 and left 20
349	ommatidia situated at posterior third of head length. Neck almost one-half width of head (NW:
350	0.23), with margins rounded. Dorsal region with longitudinal oval deep excavation (Fig. 3A).
351	The basal margin of excavation with U-shaped carinated and connected to neck by deep
352	longitudinal sulcus. Head with two vertexal foveae [vf]. Ventral surface of head with one gular
353	fovea [gf], with indistinct gular sulcus. Antennae (Fig. 3A) 2/3 body length. Scape thick and
354	claviform, apex one-third wider than base, deeply impressed medially and lateral margins
355	strongly margined (Fig. 3B). Pedicel subrectangular, only slightly shorter than scape.
356	Antennomeres 3-7 cylindrical, gradually broadening to apex, about 2× longer than wide.
357	Antennomere 8 oval, only slightly longer than wide; smaller than the other antennomeres.
358	Antennomeres 9-10 rounded, approximately 2× longer than wide. Antennomere 11 sub-fusiform
359	2.5× longer than wide. All antennomeres covered by long and short microsetae.
360	Thorax: Pronotum hexagonal (Figs. 3A and 3E), as long as wide (PL: 0.48; PW: 0.48)
361	broader at two-third anterior portion and narrower at posterior one-third (Fig. 3F). Surface of
362	pronotum shinning, with sparse long setae direct to center. Pronotal disc distinctly convex in
363	lateral view. Antebasal sulcus [as] sinuous and widened medially, reaching the posterior ending



364	of the longitudinal sulcus; and one strong lateral longitudinal sulcus on each side of pronotum.
365	Pronotum with anterior margin convex and posterior margin straight; with a lateral antebasal
366	fovea [laf], an outer basolateral fovea [oblf], and an inner basolateral fovea [iblf]. Posterior
367	angles of pronotum straight (Fig. 3E). Prosternum with lateral procoxal fovea [lpcf].
368	Mesoventrite with lateral mesosternal foveae [lmsf], lateral mesocoxal fovea [lmcf], and two
369	median metasternal fovea [mmtf].
370	Elytra: (Figs. 3A and 3F). Subrectangular (EL: 0.79; EW: 0.87), apex 2× larger than base
371	Surface of elytra shinning, with long setae and direct posteriorly; setae spars and lacking on the
372	center of elytral disc. Posterior margins curved, with sutural stria [ss] present. Elytron with three
373	basal elytral fovea [bef] at anterior margin. Flight wings extremely reduced (brachypterous
374	species).
375	Legs: Elongated and slender (Fig. 3A). Femora thickened medially; tibiae slightly
376	widened toward apex, similar in length to femora. Protibiae with microsetae and one apical spur
377	on its internal face. Tarsi 3-segmented, with basal tarsomere minute and the two other segments
378	longer. Second segment longer than third. All tarsi with two apical claws; one thicker and other
379	setiform and slender. Pro- and mesocoxae conical, prominent and contiguous. Metacoxae
380	transversely and weakly separated. Procoxae with small prosternal processed, rounded at apex.
381	Abdomen: With five visible tergites (morphological tergites IV-VIII) bordered by fine
382	carina. Tergite IV with a short discal carina [dc] and one basolateral fovea [blf]. Sternite IV with
383	one basolateral fovea [blf] and a mediobasal fovea [mbf]. Tergite VIII with apex convex.
384	Tergum IX triangular approximately 5× wider than long (Fig. 3M). Sternum VIII with a small
385	conical projection at posterior margin and a round impression medially (Figs. 3J-K). Tergum
386	VIII subrectangular with anterior and posterior margins rounded (Fig. 3L).



387	Aedeagus (Figs. 3G-I): strongly asymmetric, length 0.35 mm; median lobe with stout
388	large basoventral projection in the basal capsule and roundly basal foramen; the median lobe
389	strongly bifurcated, in one large and one small branches. Paramere fused to median lobe and
390	broaden to apex. The genital plate large and curved not divided attached to aedeagus by a
391	membrane almost transparent.
392	Female. It differs from male by frons on head not distinctly expanded over antennal
393	insertions and the absence of excavation, oblique impressions, or cephalic keel (Fig. 5B). The
394	sternum VIII without modifications. Genital complex (Fig. 5E) with semicircular shape; and
395	anterior border with one wide emargination.
396	Etymology. The specific epithet name "obsidian" refers to black light color of body. This
397	is a noun in apposition.
398	Distribution. Known only from Catas Altas, Minas Gerais, Brazil (Fig. 1).
399	
400	Syrbatus superciliata Asenjo & Valois, new species
401	(Figs. 1, 4 and 5)
402	http://zoobank.org/urn:lsid:zoobank.org:act:489AB8BC-FF86-4214-AC28-
403	850C99EA37B1
404	Type material (one male, two females). Holotype: BRAZIL: male, labeled "BRAZIL:
405	Minas Gerais, / Nova Lima, ABOB_0028 / cave, -20.163733, / -43.863682 [20°9'49.44"S,
406	43°51'49.25"W], 29.iv[April].2019, / R.Zampaulo & M.Simões", "[image data matrix] /
407	Instituto / Tecnológico / Vale / ITV39053", "HOLOTYPE 🖒 [red label]/ Syrbatus / superciliata
408	sp. nov. / Desig. Asenjo et al., 2023" (one male, ISLA-110317). Paratype: (two females),
409	labeled: "BRAZIL: Minas Gerais, / Nova Lima, ABOB_0028 / cave, -20.163733, -43.863682



410	[20°9'49.44"S, 43°51'49.25"W], / 29.iv[April].2019, R.Zampaulo & / M.Simões", "[image data
411	matrix] / Instituto / Tecnológico / Vale / ITV39054" (one female, ISLA-110318). "BRAZIL:
412	Minas Gerais, / Nova Lima, ABOB_0015 / cave, 29.iv[April].2019, / -20.165292, -43.861671
413	[20°9'55.05"S, 43°51'42.01"W], / R.Zampaulo & M.Simões", "[image data matrix] / Instituto /
414	Tecnológico / Vale / ITV39055" (one female, MPEG-01052549). All paratypes with label
415	"PARATYPE [yellow label] / Syrbatus / superciliata sp. nov. / Desig. Asenjo et al., 2023".
416	Molecular vouchers. "BRAZIL: Minas Gerais, Nova Lima, ABOB_0015 / cave,
417	01.viii[August].2013, / -20.165292, -43.861671 [20°9'55.05"S, 43°51'42.01"W], / M.Simões",
418	"[image data matrix] / Instituto / Tecnológico / Vale / ITV10831" (one female, ITV10831).
419	"BRAZIL: Minas Gerais, Nova Lima, ABOB_0015 / cave, 01.viii[August].2013, / -20.165292, -
420	43.861671 [20°9'55.05"S, 43°51'42.01"W], / M.Simões, "[image data matrix] / Instituto /
421	Tecnológico / Vale / ITV10835" (one female, ITV10835). "BRAZIL: Minas Gerais, / Nova
422	Lima, ABOB_0015 / cave, 29.iv[April].2019, / -20.165292, -43.861671 [20°9'55.05"S,
423	43°51'42.01"W], / R.Zampaulo & M.Simões", "[image data matrix] / Instituto / Tecnológico /
424	Vale / ITV39056" (one female, ITV39056).
425	
426	Diagnosis. As mentioned above, Syrbatus superciliata Asenjo & Valois sp. nov. is, in
427	general, similar to Syrbatus mustache Asenjo & Valois sp. nov. within the species-group 2. For
428	diagnostical characters, see the diagnosis of Syrbatus mustache Asenjo & Valois sp. nov. above.
429	
430	Holotype male (Fig. 4A). BL: 1.52 mm. Head, pronotum and abdomen brown; antennae,
431	mouth parts legs and elytra light brown.





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Head: slightly oval (Figs. 4A and 4C-D), longer (HL: 0.47) than wide (HW: 0.37). Antennal insertions on head not visible in dorsal view. Anterior margin rounded, genal edge rounded and not carinated. Eyes composed of 10 (right side) and 9 (left side) ommatidia situated at posterior third of head length. Neck almost one-half width of head (NW: 0.21), with margins rounded. Anterior region with slim rounded transversal area, 3.5× wider than long; surface with very long setae concentrated near margin (Fig. 4C). Eyes connected by long curved transversal slim carina covered of long setae. Both regions cited anteriorly connected by short medial longitudinal keel covered by small setae. The vertex area between eyes with one deep sulcus in format of W-shaped (Fig. 4D) and with vertexal foveae [vf]. Ventral surface of head with one gular fovea [gf], without visible gular sulcus. Antennae (Fig. 4B) 2/3 body length. Scape rectangular and slightly stout, deeply impressed medially with margins rounded. Pedicel subclaviform, only slightly shorter than scape. Antennomeres 3-7 cylindrical, gradually broadening to apex, about 2× longer than wide. Antennomere 8 oval, only slightly longer than wide; smaller than the other antennomeres. Antennomeres 9-10 rounded, approximately 2× longer than wide. Antennomere 11 sub-fusiform, 2.5× longer than wide. All antennomeres covered by long microsetae. Thorax: Pronotum hexagonal (Figs. 4A and 4E), slightly longer than wide (PL: 0.46; PW: 0.42) broader at medio-anterior portion and narrower at posterior one-third (Fig. 4F). Surface of pronotum shinning, with long setae direct to center. Pronotal disc distinctly convex in lateral view. Antebasal sulcus [as] present and complete, regularly curved and slender, reaching the posterior ending of the longitudinal sulcus on the lateral portions of the pronotal disc. Pronotum with basal and anterior margins weakly convex; with a lateral antebasal fovea [laf], an outer basolateral fovea [oblf], and an inner basolateral fovea [iblf]. Posterior angles of pronotum



455	obtuse (Fig. 4F). Prosternum with lateral procoxal fovea [lpcf]. Mesoventrite with one lateral
456	mesosternal foveae [lmsf], one lateral mesocoxal fovea [lmcf], and two median metasternal
457	fovea [mmtf].
458	Elytra: (Figs. 4A and 4F). Subrectangular (EL: 0.75; EW: 0.77), apex 2× larger than base.
459	Surface of elytra shinning, with long setae directed posteriorly, setae sparsely distributed.
460	Posterior margins curved, sutural stria [ss] present. Elytron with three basal elytral fovea [bef] at
461	anterior margin. Flight wings extremely reduced (brachypterous species).
462	Legs: Elongated and slender (Fig. 4A). Femora thickened medially; tibiae slightly
463	widened toward apex, similar in length to femora. Protibiae with microsetae and one apical spur
464	on its internal face. Tarsi 3-segmented, with basal tarsomere minute and the other two tarsomeres
465	longer. Second tarsomere longer than third. All tarsi with two apical claws; one thicker and other
466	setiform and slender. Pro- and mesocoxae conical, prominent and contiguous. Metacoxae
467	transversely and weakly separated. Procoxae with small prosternal process, rounded at apex.
468	Abdomen: With five visible tergites (morphological tergites IV-VIII) bordered by fine
469	carina. Tergite IV with a short discal carina [dc] and one basolateral fovea [blf]. Sternite IV with
470	two basolateral fovea [blf] and a mediobasal fovea [mbf]. Tergite VIII with apex convex.
471	Tergum IX triangular approximately 5× wider than long (Fig. 4M). Sternum VIII deeply
472	impressed medially, posterior margin sinuous having a pointed projection at middle (Figs. 4J-K).
473	Tergite VIII subrectangular with anterior and posterior margins rounded (Fig. 4L).
474	Aedeagus (Figs. 4G-I): strongly asymmetric, length 0.47 mm; median lobe with large
475	basal capsule and roundly basal foramen; the median lobe strongly bifurcated, with both
476	branches twist. Paramere fused to median lobe with apex divided into two long and slim



477	prolongation curved. The genital plate divided in two small elongated plates, both attached to
478	aedeagus by a membrane almost transparent.
479	Female. It differs from males by frons not distinctly expanded over antennal insertions
480	and the absence of excavation, oblique impressions, or cephalic keel (Fig. 5C). The sternum VIII
481	lacks modifications such as projections or impressions. Genital complex (Fig. 5F) oblong with
482	anterior border with broad two central prolongations and one broad prolongation on each side.
483	Etymology. The specific epithet name "superciliata" refers to similar eyebrow above
484	compound eyes. This is a noun in apposition.
485	Distribution. Known only from Nova Lima, Minas Gerais, Brazil (Fig. 1).
486	
487	Syrbatus brevispinus (Reitter, 1882)
488	(Fig. S1)
489	Batrisus (Syrbatus) brevispinus: Reitter, 1882: 137 (original description, drawings pl. 5,
490	fig. 8. Type locality: "Brasilia: Sao Paolo, 3000 Fufs über dem Meere"), Reitter, 1888: 245
491	(revision).
492	Batrisus brevispinus: C.Schaufuss, 1888: 11 (distribution).
493	Arthmius (Syrbatus) brevispina: Raffray, 1898: 446, 454 (revision), Raffray, 1904: 70
494	(catalogue, species-group 2, and distribution), Raffray, 1908: 150 (checklist, distribution),
495	Raffray, 1911: 55 (catalogue, distribution).
496	Arthmius brevispina: Blackwelder, 1944: 91 (checklist Latin American species,
497	distribution).
498	Syrbatus brevispinus: Park, 1942: 234, 237 (revision, species-group 2, and distribution),
499	Asenjo et al., 2013: 14 (checklist Brazilian species, distribution).



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500
             Type material (Fig. S1). "Brésil / Sao. Paulo", "80 Juli / Brasilien / Sao Paolo / 3000 FB /
      leg. [unreadable]", "Syrbatus / brevispina / Type Reitter / Bresil", "HOLOTYPE / Syrbatus /
501
      brevispina (Reitter, 1882)", "MUSÉUM PARIS / 1917 / COLL. A. RAFFRAY", "S. Brevispina
502
      / A. Raffray det.", "HOLOTYPE", "TYPE", "MNHN, Paris / EC21615 / [image data matrix]".
503
             Comments. Reitter (1882), in the original description, specified that he studied one
504
505
      incomplete female. We studied, by photography, one specimen of the Raffray collection with the
      abdomen absent, probably female, deposited in the MNHN Entomological Collection labeled as
506
      "Type", and we recognize it as the Holotype.
507
             Distribution. Full record and distribution are described in Asenjo et al. (2013).
508
509
      Syrbatus bubalus (Raffray, 1898)
510
      (Fig. S2)
511
             Arthmius (Syrbatus) bubalus: Raffray, 1898: 444, 454 (original description, drawings pl.
512
      17, fig. 1. Type locality: "Brésil: Bahia"), Raffray, 1904: 70 (catalogue, species-group 2, and
513
      distribution), Raffray, 1908: 150 (checklist, distribution), Raffray, 1911: 55 (catalogue,
514
      distribution).
515
516
             Arthmius bubalus: Blackwelder, 1944: 91 (checklist Latin American species,
      distribution).
517
518
             Syrbatus bubalus: Park, 1942: 234, 237 (revision, species-group 2, and distribution),
519
      Asenjo et al., 2013: 14 (checklist Brazilian species, distribution).
             Type material (Fig. S2). "Bahia", "S. Bubalus / A. Raffray det.", "MUSÉUM PARIS /
520
      1917 / COLL. A. RAFFRAY", "Syrbatus Reitt. / bubalus Raffr. / male symbol type / Bahia",
521
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"HOLOTYPE / Syrbatus / bubalus (Raffray, 1898)", "HOLOTYPE", "TYPE", "MNHN, Paris / 522 EC21624 / [image data matrix]". 523 Comments. Raffray (1898), in the original description, specified that he studied one male 524 collected by A. Grouvelle [Antoine Henri Grouvelle] from Brazil (Bahia). We studied, by 525 photography, one specimen of Raffray collection deposited in the MNHN Entomological 526 527 Collection labeled as "Type", and we recognize it as the Holotype. Distribution. Full record and distribution are described in Asenjo et al. (2013). 528 529 Syrbatus centralis (Raffray, 1898) 530 (Fig. S3) 531 Arthmius (Syrbatus) centralis: Raffray, 1898: 445, 454 (original description. Type 532 locality: "Brésil: Blumenau"), Raffray, 1904: 70 (catalogue, species-group 2, and distribution), 533 Raffray 1908: 150 (checklist, distribution), Raffray, 1911: 55 (catalogue, distribution). 534 535 Arthmius centralis: Blackwelder, 1944: 91 (checklist Latin American species, distribution). 536 Syrbatus centralis: Park, 1942: 234, 237 (revision, species-group 2, and distribution), 537 538 Asenjo et al., 2013: 14 (checklist Brazilian species, distribution). Arthmius centralis Reitter: Raffray, 1898: 454 (in litteris name). 539 Type material (Fig. S3). "Brésil / Blumenau", "S. centralis / A. Raffray det.", "B. 540 centralis / m. Blūmenaū", "MUSEUM PARIS / 1917 / COLL. A. RAFFRAY", "Syrbatus / 541 centralis 3 / Type Reitter / Brésil", "HOLOTYPE / Syrbatus / centralis (Raffray, 1898)", 542 "HOLOTYPE", "TYPE", "MNHN, Paris / EC21623 / [image data matrix]". 543



544	Comments. Rathay (1898), in the original description, does not specify the number of
545	specimens that he studied, but he, at least, studied one male specimen collected from Brazil
546	(Blumenau). We studied, by photography, one specimen of the Raffray collection deposited in
547	the MNHN Entomological Collection labeled as "Type", and we are designating it as Lectotype.
548	Distribution. Full record and distribution are described in Asenjo et al. (2013).
549	
550	Syrbatus grouvellei (Raffray, 1898)
551	(Fig. S4)
552	Arthmius (Syrbatus) grouvellei: Raffray, 1898: 445, 454 (original description, drawings
553	pl. 17, fig. 3. Type locality: "Brésil: Bahia"), Raffray, 1904: 70 (catalogue, species-group 2, and
554	distribution), Raffray 1908: 150 (checklist, distribution), Raffray, 1911: 55 (catalogue,
555	distribution).
556	Arthmius grouvellei: Blackwelder, 1944: 91 (checklist Latin American species,
557	distribution).
558	Syrbatus grouvellei: Park, 1942: 234, 237 (revision, species-group 2, and distribution),
559	Asenjo et al. 2013: 15 (checklist Brazilian species, distribution).
560	Type material (Fig. S4). "Bahia", "S. Grouvellei / A. Raffray det.", "MUSÉUM PARIS /
561	1917 / COLL. A. RAFFRAY", "'Syrbatus Reitt. / grouvellei / Type. Raffr. / Bahia.',
562	HOLOTYPE / Syrbatus / grouvellei (Raffray, 1898)", "TYPE", "HOLOTYPE", "MNHN, Paris /
563	EC21622 / [image data matrix]"
564	Comments. Raffray (1898), in the original description, specified that he studied one male
565	collected by A. Grouvelle [Antoine Henri Grouvelle] from Brazil (Bahia). We studied, by



photography, one specimen of the Raffray collection deposited in the MNHN Entomological 566 Collection labeled as "Type", and we recognize it as the Holotype. 567 Distribution. Full record and distribution are described in Asenjo et al. (2013). 568 569 Syrbatus hetschkoi (Reitter, 1888) 570 (Figs. S5 and S6) 571 Batrisus (Syrbatus) hetschkoi: Reitter, 1888: 245, 250 (original description. Type locality: 572 "Brésil: Bahia"). 573 Arthmius (Syrbatus) hetschkoi: Raffray, 1898; 446, 455 (revision, drawings pl. 17, fig. 8), 574 Raffray, 1904: 70 (catalogue, species-group 2, and distribution), Raffray, 1908: 150 (checklist, 575 distribution), Raffray, 1911: 55 (catalogue, distribution). 576 Arthmius hetschkoi: Blackwelder, 1944: 91 (checklist Latin American species, 577 distribution). 578 Syrbatus hetschkoi: Park, 1942: 234, 237 (revision, species-group 2, and distribution), 579 Asenjo et al., 2013: 15 (checklist Brazilian species, distribution). 580 Type material. First specimen (Fig. S5): "Brésil / Blumenau", "S. Hetschkoi / A. Raffray 581 det.", "MUSÉUM PARIS / 1917 / COLL. A. RAFFRAY", "Syrbatus Reitt. / Hetschkoi Reitt. / & 582 Type / Brésil", "SYNTYPE / Syrbatus / hetschkoi (Reitter, 1888)", "SYNTYPE", "TYPE", 583 "MNHN, Paris / EC21619 / [image data matrix]"; second specimen (Fig. S6): "Brésil / 584 Blumenau", "S. Hetschkoi / A. Raffray det.", "MUSÉUM PARIS / 1917 / COLL. A. 585 RAFFRAY", "SYNTYPE / Syrbatus / hetschkoi (Reitter, 1888)", "SYNTYPE", "MNHN, Paris / 586 EC21620 / [image data matrix]". 587



588	Comments. Reitter (1888), in the original description, does not specify the number of
589	specimens that he studied; the specimen(s) was(were) collected by Lothar Hetschko from Brazil
590	(Blumenau). We studied by photography two specimens of Raffray collection deposited in the
591	MNHN Entomological Collection labeled as "Type" [first specimen] and "syntype" [second
592	specimen], and we are designating Lectotype and Paralectotype respectively.
593	Distribution. Full record and distribution are described in Asenjo et al. (2013).
594	
595	Syrbatus hiatusus (Reitter, 1888)
596	(Figs. S7 and S8)
597	Batrisus (Arthmius) hiatusus: Reitter, 1888: 247, 257 (original description. Type locality:
598	"Brésil: Blumenau").
599	Arthmius (Syrbatus) hiatusus: Raffray, 1898: 445, 454 (revision), Raffray, 1904: 70
600	(catalogue, species-group 2, and distribution), Raffray, 1908: 150 (checklist, distribution),
601	Raffray, 1911: 55 (catalogue, distribution), Bruch, 1914: 304 (catalog Argentinean species).
602	Arthmius hiatusus: Blackwelder, 1944: 91 (checklist Latin American species,
603	distribution).
604	Syrbatus hiatusus: Jeannel, 1949: 128 (drawings fig. 57), Park, 1942: 234, 237 (revision,
605	species-group 2, and distribution), Asenjo et al., 2013: 15 (checklist Brazilian species,
606	distribution).
607	Type material. First specimen (Fig. S7): "Brésil / Blumenau", "B. hiatusus / m.
608	Blūmenaū", "[red square label]", "MUSÉUM PARIS / 1917 / COLL. A. RAFFRAY", "S.
609	Hiatúsus / A. Raffray det.", "Syrbatus Reitt. / hiatusus Reitt. / 💍 Type / Brésil", "HOLOTYEPE /
610	Syrbatus / hiatusus (Reitter, 1888)", "HOLOTYPE", "MNHN, Paris / EC21616 / [image data



611	matrix]"; second specimen (Fig. S8): "Brésil / Blumenau", "S. Hiatusus / A. Raffray det.",
612	"MUSÉUM PARIS / 1917 / COLL. A. RAFFRAY", "MNHN, Paris / EC21617 / [image data
613	matrix]".
614	Comments. Reitter (1888), in the original description, mentioned that he studied some
615	specimens, but without specifying the number, collected by Lothar Hetschko from Brazil
616	(Blumenau). We studied, by photography, two specimens of the Raffray collection deposited in
617	the MNHN Entomological Collection first specimen and second specimen, and we are
618	designating the specimen labeled as Lectotype and Paralectotype, respectively.
619	Distribution. Full record and distribution are described in Asenjo et al. (2013), and for
620	Argentina (Buenos Aires) in Bruch (1914).
621	
622	Syrbatus transversalis (Raffray, 1898)
623	(Fig. S9)
624	Arthmius (Syrbatus) transversalis: Raffray, 1898: 447, 455 (original description. Type
625	locality: "Brésil: S. Antonio"), Raffray, 1904: 70 (catalogue, species-group 2, and distribution),
626	Raffray 1908: 150 (checklist, distribution), Raffray, 1911: 55 (catalogue, distribution).
627	Arthmius transversalis: Blackwelder, 1944: 92 (checklist Latin American species,
628	distribution).
629	Syrbatus transversalis: Park, 1942: 234, 237 (revision, species-group 2, and distribution)
630	Asenjo et al., 2013: 17 (checklist Brazilian species, distribution).
631	
632	Type material (Fig. S9). "Brésil / St.Antonio", "S.Transversalis / A. Raffray det.",
633	"MUSÉUM PARIS / 1917 / COLL. A. RAFFRAY", "Syrbatus Reitt. / transversalis / Type.



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Raffr. / Brésil", "HOLOTYEPE / Syrbatus / transversalis (Raffray, 1898)", "TYPE",
634
      "HOLOTYPE", "MNHN, Paris / EC21621 / [image data matrix]".
635
             Comments. Raffray (1898), in the original description, does not specify the number of
636
      specimens that he studied, but he, at least, studied one male specimen collected by S. Antonio
637
      from Brazil. We studied, by photography, one specimen of the Raffray collection deposited in
638
      the MNHN Entomological Collection labeled as "Type", and we are designating it as Lectotype.
639
             Distribution. Full record and distribution are described in Asenjo et al. (2013).
640
641
      Syrbatus trinodulus (L.W.Schaufuss, 1887)
642
      (Fig. S10)
643
             Batrisus (Syrbatus) trinodulus: L.W.Schaufuss, 1887: 145 (original description. Type
644
      locality: "Minas Geraes, Brasilia").
645
             Batrisus trinodulus: Gaedike, 1984: 456 (catalogue of type specimens).
646
             Arthmius trinodulus: C.Schaufuss, 1888: 17 (distribution).
647
             Arthmius (Syrbatus) trinodulus: Raffray, 1904: 71 (catalogue, distribution and unplaced
648
      any group), Raffray, 1908: 151 (checklist, distribution), Raffray, 1911: 55 (catalogue,
649
      distribution), Park, 1942: 237 (revision, unplaced any group, and distribution), Blackwelder,
650
      1944: 92 (checklist Latin American species, distribution).
651
             Syrbatus trinodulus: Asenjo et al., 2013: 17 (checklist Brazilian species, distribution).
652
653
             Type material (Fig. S10). "min geraes [Minas Gerais state in Brazil]", "Minas / geraes",
      "Syntypus", "Batrisus / (Syrbatus) / 3-nodulus.", "SDEI Coleoptera / # 302700".
654
             Comments. L.W.Schaufuss (1887), in the original description, does not specify the
655
656
      number or sex of the specimens studied. We studied, by photography, one specimen deposited in
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the SDEI Entomological Collection labeled as "syntypus", and we are designating it as Lectotype. The species is considered unplaced in any species-group, as proposed by Raffray (1904).

Distribution. Full record and distribution are described in Asenjo et al. (2013).

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## Mitogenomes and phylogenetic placement

All seven assembled mitochondrial genomes [Syrbatus moustache Asenjo & Valois sp. nov.: OR625193 (ITV22155); Syrbatus obsidian Asenjo & Valois sp. nov.: OR625194 (ITV22166), OR625195 (ITV22168) and OR625196 (ITV22169); and Syrbatus superciliata Asenjo & Valois sp. nov.: OR625197 (ITV10831), OR625198 (ITV10835) and OR625199 (ITV39056)] presented the standard structure and gene content of Metazoans, although the genes were arranged in an unusual order, considering the pattern described for all other Staphylinidae species with available mitogenomes (Fig. 6A; Table 1). Most genes were encoded in the light (L) strand in the mitogenomes of the three new species, except for four PCGs (ND1, ND4, ND4L and ND5), the two rRNA genes (rrnL and rrnS), and eight tRNA genes (trnC, trnF, trnH, trnL1, trnP, trnP, trnQ, trnV and trnY), which were observed in the heavy (H) strand (Table 1). Thus, using the L strand as the standard, we observed the following gene order for the three new species: trnM, COX1, trnK, ND3, trnR, trnC, COX3, ND4, trnT, trnP, ND6, CYTB, trnI, trnQ, ND2, trnW, trnY, trnL2, COX2, trnD, ATP8, ATP6, trnG, trnA, trnN, trnS1, trnE, trnF, ND5, trnH, ND4L, trnS2, ND1, trnL1, rrnL, rrnS and trnV. Moreover, since some of the mitogenome assemblies presented different levels of completeness, we could not recover genes in the extremities for some specimens, as in the case of all three accessions of Syrbatus obsidian Asenjo & Valois sp. nov. (OR625194, OR625195 and OR625196), missing part of the rrnL,



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besides the whole rrnS and trnV genes, and one accession of *Syrbatus superciliata* Asenjo & Valois **sp. nov.** (OR625199), missing part of the rrnL, and the complete sequences of rrnS, trnM and trnV (Table 1).

The mitogenomes of the three new species were largely syntenic, presenting intergenic regions and gene superpositions with similar lengths along the fully aligned extension (from COX1 to mid rrnL, due to the missing parts of some accessions), except for a non-coding intergenic region with 473 bp between trnS2 and NAD1, which was only present in the mitogenomes of Syrbatus obsidian Asenjo & Valois sp. nov. (Figure 6B). We also observed similar nucleotide contents between Syrbatus moustache Asenjo & Valois sp. nov. and Syrbatus superciliata Asenjo & Valois sp. nov., with %GC = 17.3 and 17.0, respectively, and Syrbatus obsidian Asenjo & Valois sp. nov. as the most diverging species, with %GC = 18.9. Overall, the three species shared most of the start codons, which presented the usual ATN configuration, except in the case of COX1 of Syrbatus obsidian Asenjo & Valois sp. nov., which presented TTA, which is an alternative start codon (encoding the amino acid leucine) for invertebrate mitogenomes (Table 1). In addition, only two genes were recorded with diverging stop codons among the three species: COX3, for which Syrbatus obsidian Asenjo & Valois sp. nov. presented TAG instead of TAA; and CYTB, with an incomplete stop codon T in Syrbatus superciliata Asenjo & Valois sp. nov., also TAA in the other two species (Table 1).

The phylogenetic reconstruction within Staphylinidae based on ML and BI analyses using a concatenated matrix with amino acid sequences of all 13 PCGs resulted in mostly well-resolved trees, with most of the 14 sampled subfamilies being recovered as well-supported clades (Figure 7). However, the only two species of Tachiporinae included in the phylogenetic analyses appeared within two distinct lineages, in which *Sepedophilus bipunctatus* (Gravenhorst, 1802)



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subterraneus (Linnaeus, 1758) was more closely related to Apateticinae and Omaliinae. Also, 704 Paederinae was recovered as paraphyletic, with *Habrocerus capillaricornis* (Gravenhorst, 1806), 705 from Habrocerinae, appearing deeply nested within the former subfamily. On the other hand, 706 while Staphylininae was poorly supported in the ML analysis (BS = 68), the group presented the 707 708 maximum value of posterior probability in the BI trees (PP = 1). Regarding Pselaphinae, the subfamily was recovered as monophyletic with strong support 709 (BS = 100; PP = 1), forming, with Neophoninae, the sister clade to the remaining Staphylinidae 710 subfamilies (Figure 7). The three new species of *Syrbatus* formed a fully supported monophyletic 711 group (BS = 100; PP = 1) with well-resolved interspecific relationships, forming, alongside 712 Metopiellus crypticus, another clade with maximum statistical support (BS = 100; PP = 1). As 713 also indicated by the morphological similarity, Syrbatus moustache Asenjo & Valois sp. nov. 714 and Syrbatus superciliata Asenjo & Valois sp. nov. were more closely related to each other, with 715 a considerably lower interspecific divergence in comparison to the phylogenetic distance of both 716 to *Syrbatus obsidian* Asenjo & Valois **sp. nov.** (Figure 7). 717 In addition, we also recovered the three new species as clearly separated clusters in both 718 719 lineage delimitation approaches. In the ASAP analysis, the best score was obtained for four subsets and a threshold distance of 0.0467 (asap-score = 1.00; P-val = 0.0019; W = 0.0387; 720 Table S1), considering the three species of *Syrbatus* and *Metopiellus crypticus*, grouping in the 721 722 same pattern as observed in the phylogenetic reconstruction. Similarly, the four species were recovered as independent lineages with strong support (PP = 0.9828), all of which presenting 723 individual high posterior probabilities in the BPP analysis, being 0.9829 for *Syrbatus moustache* 724

appeared as sister to the clade composed of Steninae and Euaesthetinae species, and Tachinus



Asenjo & Valois **sp. nov.**, 0.9901 for *Syrbatus obsidian* Asenjo & Valois **sp. nov.**, 0.9997 for *Syrbatus superciliata* Asenjo & Valois **sp. nov.**, and 0.9930 for *Metopiellus crypticus*.

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#### DISCUSSION

The three new species described here of the Brazilian state of Minas Gerais state belong to Syrbatus, based on a sub-basal transverse sulcus on the base of pronotum (Figs. 2E, 3E and 4E), and lateral longitudinal sulcus on each side of the pronotum (Figs. 2E, 3E and 4E) (Reitter, 1882; Park, 1942). Raffray (1904) grouped the South American species of the genus in six species-group based on morphological characters of head and antenna. Posteriorly, the concept groups were updated by Park (1942), which were used here for the analysis and description of the three new Brazilian species of *Syrbatus* in the present work. In addition, to describe the new species with greater confidence, we found it necessary to study the other species of the speciesgroup 2 (Syrbatus brevispinus, Syrbatus bubalus, Syrbatus centralis, Syrbatus grouvellei, Syrbatus hetschkoi, Syrbatus hiatusus and Syrbatus transversalis), besides Syrbatus trinodulus, which is also known from Minas Gerais. As mentioned above, Syrbatus moustache Asenjo & Valois sp. nov. and Syrbatus superciliata Asenjo & Valois sp. nov. were included in the species-group 2, characterized by the dorsally excavated head of the male (Figs. 2C and 4C), antennae simple (similar in both sexes; Figs. 2C and 4C), and genal region of the head not carinated (Figs. 2C and 4C). On the other hand, Syrbatus obsidian Asenjo & Valois sp. nov. was included in the species-group 5, considering the genal area of the head longitudinally carinated (Fig. 3C), and antennae abnormal (Fig. 3B). Furthermore, the genital complex for female specimens of *Syrbatus* was studied,



illustrated, and described for the first time (Fig. 5), showing its usefulness for the identification of the species with female sexual characters.

The three species described in the present study were found in caves inserted in siliciclastic rocks. These caves have between 10-500 m of horizontal projection, being inserted in all compartments of the landscape (low, medium, and high slopes) at altitudes that vary between 900-1100 m. In general, such caves are formed by talus deposits, with perennial underground drainage, and the presence of aphotic zones in the larger ones. However, the occurrence of *Syrbatus moustache* Asenjo & Valois **sp. nov.**, *Syrbatus obsidian* Asenjo & Valois **sp. nov.** and *Syrbatus superciliata* Asenjo & Valois **sp. nov.** in caves of small dimensions, and therefore without aphotic zones, besides the absence of evident troglomorphisms (Christiansen, 2012), suggests that the distribution of these new species may also be related to surface environments, characterizing the three of them as troglophiles.

The gene content and order tend to be highly conserved among the subdivisions of Insecta, with the structure of the ancestral insect mitogenomes being almost ubiquitous (Cameron, 2014). Nevertheless, some deviations from the standard configuration have been observed in the class, as in the case of some species of seed beetles (Chrysomelidae; Bruchinae), with long intergenic repeats and a minor reshuffling related to the gene trnQ (Sayadi et al., 2017). On the other hand, the three new species described here presented the mitochondrial genes in quite different positions, while all other currently known Staphylinidae mitogenomes share the standard insect gene order, as represented in the Figure 6B. Additionally, a somewhat long intergenic region between trnS2 and ND1 was observed only for *Syrbatus obsidian* Asenjo & Valois **sp. nov.**, as another feature that differentiates this species from *Syrbatus moustache* Asenjo & Valois **sp. nov.** and *Syrbatus superciliata* Asenjo & Valois **sp. nov.**, besides the



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morphologic characters discussed above. Yet, the phylogenetic reconstruction with ML and BI, plus the high statistical support obtained in the ASAP and BPP analyses evidenced a robust delimitation among the three new species of *Syrbatus* described here.

Most of the sampled Staphylinidae subfamilies were recovered here as monophyletic and well supported in our phylogenetic reconstruction, at least in one of the two employed approaches, as, for instance, Staphylininae (BS = 68; PP = 1), which have been recovered only weakly supported in other analyses based on whole mitogenome data (Song, Zhai & Zhang, 2021; Ji et al., 2023). Considering the huge diversity of one of the largest Metazoan families (Grebennikov & Newton, 2009; Newton, 2022), one should expect the retelling of phylogenetic history of Staphylinidae to be, at least, very challenging. With its approximately 67,000 species (Newton, 2022), there are numerous difficulties in achieving relevant coverage and proportionality to reconstruct a robust molecular phylogeny of such an immense group. Depending on which taxa is included or left out, the resulting topology may vary considerably regarding recovered clades and support values, with genomic coverage and differences in evolutionary rates among used markers/genome portions also being an immense influence on the outcome (e.g., McKenna et al., 2015; Kim et al., 2020; Motyka et al., 2021; Song, Zhai & Zhang, 2021; Ji et al., 2023). Therefore, the divergences among topologies presented by different studies on the phylogenetic relationships within and among Staphylinidae subdivisions, even those employing the same set of markers/regions, such as whole mitogenomes, are not surprising. Yet, as the availability of genetic data for beetle taxa are continuously increasing, the causes of such conflicting phylogenetic signals may be clarified, allowing us to better address the many standing systematic issues of the infrafamilial relationships.

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793	Funding
794	This work was funded by Vale S.A. (Projeto Diversidade Biológica de Cavernas,
795	R100603.CD.0X; Projeto Centro de Triagem de Invertebrados, R100603.CT.0X). Guilherme
796	Oliveira is a CNPq (Conselho Nacional de Desenvolvimento Científico) fellow (307479/2016-1).
797	also being funded by CNPq (444227/2018-0, 402756/2018-5, 307479/2016-1). The funders had

no role in study design, data collection and analysis, decision to publish, or preparation of the

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#### **Competing Interests**

The authors declare that they have no competing interests.

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#### **Author Contributions**

- Angélico Asenjo conceived and designed the experiments, analyzed the data, performed species identification and description, prepared figures, authored and reviewed preliminary drafts of the text, and approved the final draft of the manuscript.
  - Marcely Valois analyzed the data, performed species identification and description,
     prepared figures, authored and reviewed preliminary drafts of the text, and approved the final draft of the manuscript.
  - Robson de A. Zampaulo collected part of the evaluated material, analyzed the biological data, prepared figures, authored and reviewed preliminary drafts of the text, approved the final draft of the manuscript.
  - Renato R. M. Oliveira analyzed and curated the data, reviewed preliminary drafts of the text, and approved the final draft of manuscript.





316	Michele Molina performed the experiments, analyzed the data, reviewed preliminary					
317	drafts of the text, and approved the final manuscript.					
318	Guilherme Oliveira conceived and designed the experiments, reviewed preliminary drafts					
319	of the text, and approved the final manuscript.					
320	• Santelmo Vasconcelos conceived and designed the experiments, analyzed the data,					
321	prepared figures and tables, authored and reviewed drafts of the paper, approved the final					
322	draft.					
323						
324	Data Availability					
325	The assembled mitogenomes were deposited in GenBank under the accession numbers					
326	OR625193, OR625194, OR625195, OR625196, OR625197, OR625198 and OR625199, and raw					
327	data were deposited in the BioProject PRJNA862473.					
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985								
986	FIGURE CAPTIONS							
987	Figure 1: Geographic distribution of Syrbatus moustache Asenjo & Valois sp. nov., Syrbatus							
988	obsidian Asenjo & Valois sp. nov. and Syrbatus superciliata Asenjo & Valois sp. nov.							
989	Geographic delimitation of geomorphological units in the Quadrilátero Ferrífero, Minas Gerais,							
990	Brazil (Map data: Google, ©2023 CNES/Airbus, Maxar Technologies), showing the occurrence							
991	sites of Syrbatus moustache Asenjo & Valois sp. nov. (yellow triangles) and Syrbatus							
992	superciliata Asenjo & Valois sp. nov. (red crosses) in the mountain range of Serra da Moeda (A							



and D), and Syrbatus obsidian Asenjo & Valois sp. nov. (pink stars) in the mountain range of the 993 Escarpa Oriental do Caraça (B and E). Habitus of Syrbatus superciliata Asenjo & Valois sp. 994 **nov.** (C) and general appearance of the interior of the cave ABOB 0015 (F). 995 996 Figure 2: Habitus and diagnostic characters of holotype male Syrbatus moustache Asenjo & 997 998 Valois sp. nov. (ISLA-110319). Habitus, dorsal view (A); antenna (B); head, dorsal view (C); head, frontal-dorsal view (D); 999 pronotum (E); elytra (F); aedeagus (G-I); sternum VIII (J-K); tergum VIII (L); sternite IX (M). f: 1000 1001 foramen, p: paramere. 1002 Figure 3: Habitus and diagnostic characters of holotype male Syrbatus obsidian Asenjo & 1003 Valois sp. nov. (MPEG-01052553). 1004 Habitus, dorsal view (A); antenna (B); head, dorsal view (C); head, frontal-dorsal view (D); 1005 pronotum (E); elytra (F); aedeagus (G-I); sternum VIII (J-K); tergum VIII (L); sternite IX (M). f: 1006 foramen, p: paramere. 1007 1008 Figure 4: Habitus and diagnostic characters of holotype male Syrbatus superciliata Asenjo 1009 & Valois sp. nov. (ISLA-110317). 1010 Habitus, dorsal view (A); antenna (B); head dorsal view (C); head frontal-dorsal view (D); 1011 1012 pronotum (E); elytra (F); aedeagus (G-I); sternum VIII (J); sternum VIII (K-L); sternite IX (M). f: foramen, p: paramere. 1013 1014 1015 Figure 5: Morphology of the female of the three new species of *Syrbatus*.



Frontal-dorsal view of the head (A; ISLA-110320) and genital complex (MPEG-01052551) (D) 1016 of Syrbatus moustache Asenjo & Valois sp. nov.; frontal-dorsal view of the head (B; MPEG-1017 01052554) and genital complex (E; MPEG-01052554) (F) of Syrbatus obsidian Asenjo & Valois 1018 sp. nov.; and frontal-dorsal view of the head (C; MPEG-01052549) and genital complex (F; 1019 ISLA-110318) of Syrbatus superciliata Asenjo & Valois sp. nov. 1020 1021 Figure 6: Mitochondrial genome representation of the three new species of *Syrbatus*, 1022 comparing the gene order with Metopiellus crypticus. 1023 1024 Comparison of the order of all 37 mitochondrial genes between Syrbatus moustache Asenjo & Valois **sp. nov.** and *Metopiellus crypticus* (A); colored arrows pointing to the left and right 1025 represent the transcription regions of protein coding genes (blue), rRNA genes (red) and tRNA 1026 1027 genes (purple) on the L and H strands, respectively; green, orange, and pink and connections between genes indicate the reorganization of the mitogenome of Syrbatus moustache Asenjo & 1028 Valois **sp. nov.** in relation to the gene disposition of *Metopiellus crypticus*, a species presenting 1029 the ancestral pattern of insect species. Detail of the region between the genes trnS2 and ND1 in 1030 the mitogenomes of Syrbatus moustache Asenjo & Valois sp. nov., SM, Syrbatus obsidian 1031 1032 Asenjo & Valois sp. nov., SO, and Syrbatus superciliata Asenjo & Valois sp. nov., SS, evidencing the extended intergenic region in the three accessions of the former; the green and 1033 1034 brownish bars above the arrows indicate monomorphic and polymorphic nucleotide sites, 1035 respectively. 1036 1037 Figure 7: Phylogenetic placement of the three new species of *Syrbatus* within Staphylinidae, 1038 based on whole mitogenome data.

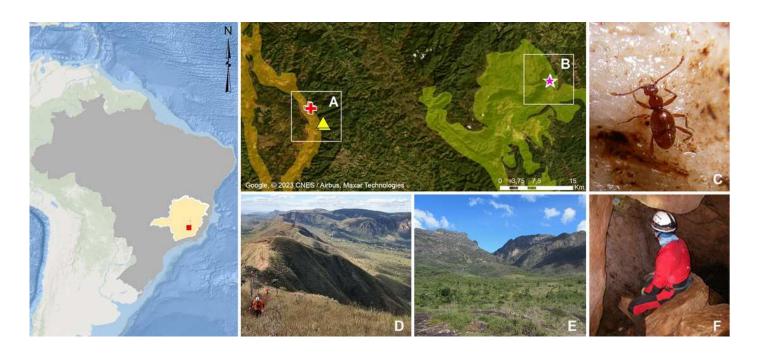




1039	Majority-rule consensus phylogram of the Bayesian inference analysis evidencing the
1040	phylogenetic relationships among Staphylinidae species with available mitogenomes in the
1041	GenBank database and the three specimens of Syrbatus moustache Asenjo & Valois sp. nov.,
1042	Syrbatus obsidian Asenjo & Valois sp. nov. and Syrbatus superciliata Asenjo & Valois sp. nov.,
1043	indicating their respective GenBank accessions and subfamily affiliations. Statistical support
1044	values (BS $\geq$ 70 and PP $\geq$ 0.90) are indicated near the branches.

Geographic distribution of *Syrbatus moustache* Asenjo & Valois sp. nov., *Syrbatus obsidian* Asenjo & Valois sp. nov. and *Syrbatus superciliata* Asenjo & Valois sp. nov.

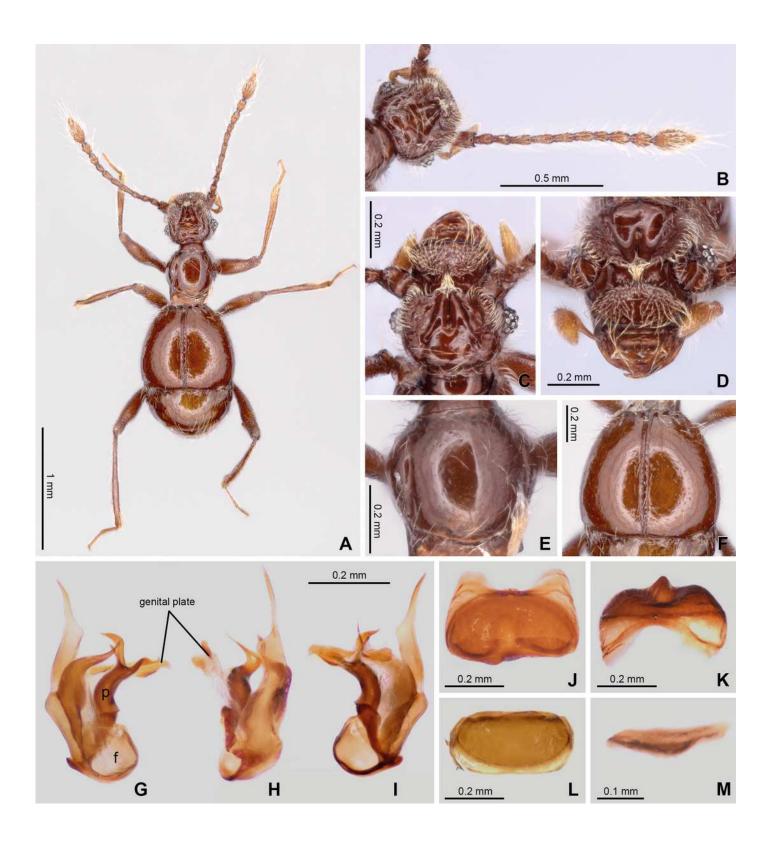
Geographic delimitation of geomorphological units in the Quadrilátero Ferrífero, Minas Gerais, Brazil (Map data: Google, ©2023 CNES/Airbus, Maxar Technologies), showing the occurrence sites of *Syrbatus moustache* Asenjo & Valois **sp. nov.** (yellow triangles) and *Syrbatus superciliata* Asenjo & Valois **sp. nov.** (red crosses) in the mountain range of Serra da Moeda (A and D), and *Syrbatus obsidian* Asenjo & Valois **sp. nov.** (pink stars) in the mountain range of the Escarpa Oriental do Caraça (B and E). Habitus of *Syrbatus superciliata* Asenjo & Valois **sp. nov.** (C) and general appearance of the interior of the cave ABOB\_0015 (F).





Habitus and diagnostic characters of holotype male *Syrbatus moustache* Asenjo & Valois sp. nov. (ISLA-110319).

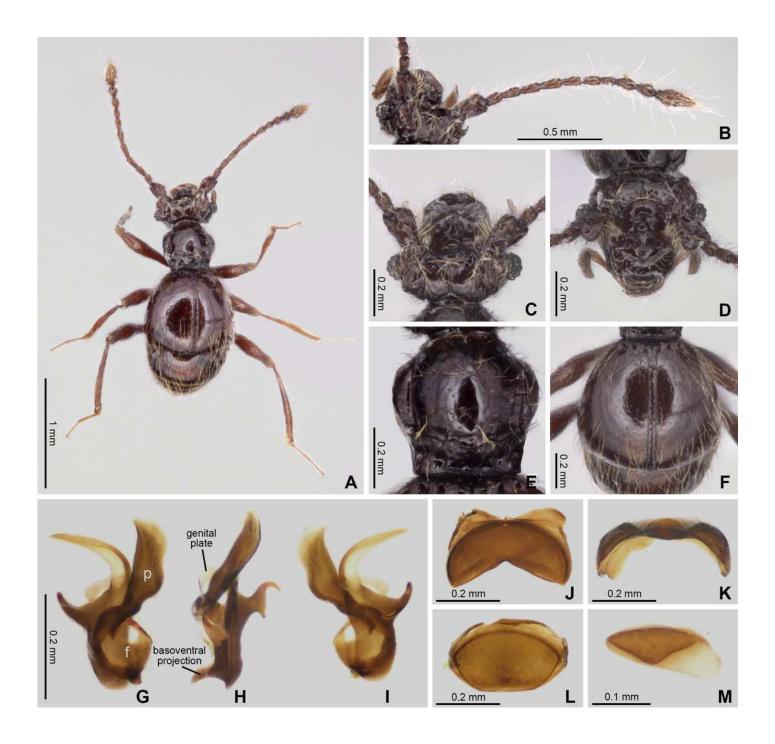
Habitus, dorsal view (A); antenna (B); head, dorsal view (C); head, frontal-dorsal view (D); pronotum (E); elytra (F); aedeagus (G-I); sternum VIII (J-K); tergum VIII (L); sternite IX (M). f: foramen, p: paramere.





Habitus and diagnostic characters of holotype male *Syrbatus obsidian* Asenjo & Valois sp. nov. (MPEG-01052553).

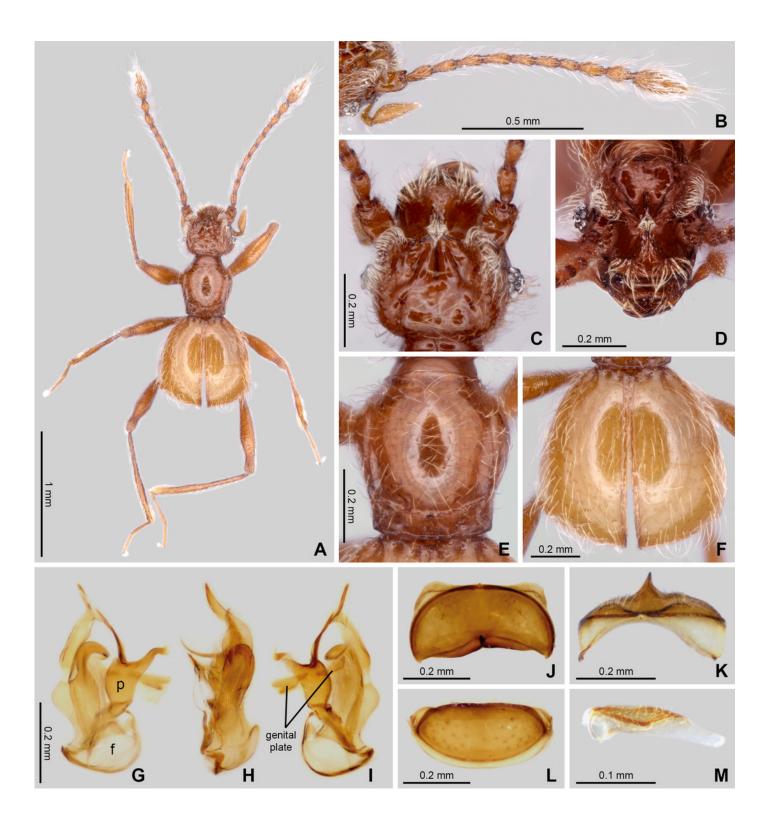
Habitus, dorsal view (A); antenna (B); head, dorsal view (C); head, frontal-dorsal view (D); pronotum (E); elytra (F); aedeagus (G-I); sternum VIII (J-K); tergum VIII (L); sternite IX (M). f: foramen, p: paramere.





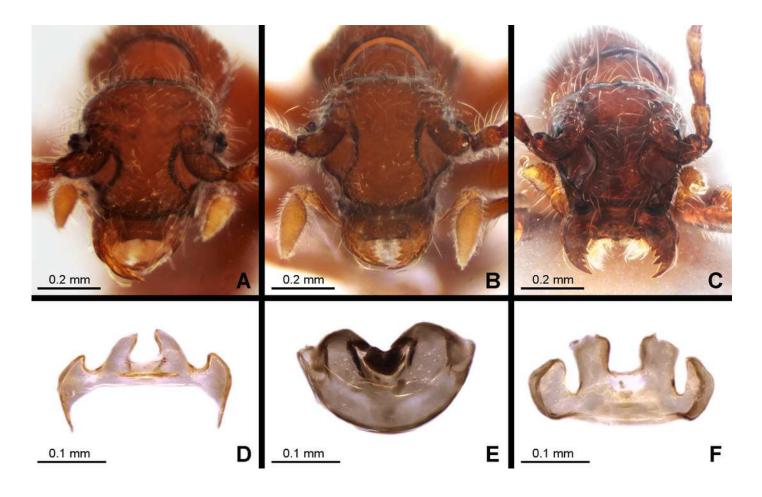
Habitus and diagnostic characters of holotype male *Syrbatus superciliata* Asenjo & Valois sp. nov. (ISLA-110317).

Habitus, dorsal view (A); antenna (B); head dorsal view (C); head frontal-dorsal view (D); pronotum (E); elytra (F); aedeagus (G-I); sternum VIII (J); sternum VIII (K-L); sternite IX (M). f: foramen, p: paramere.



Morphology of the female of the three new species of *Syrbatus*.

Frontal-dorsal view of the head (A; ISLA-110320) and genital complex (MPEG-01052551) (D) of *Syrbatus moustache* Asenjo & Valois **sp. nov.**; frontal-dorsal view of the head (B; MPEG-01052554) and genital complex (E; MPEG-01052554) (F) of *Syrbatus obsidian* Asenjo & Valois **sp. nov.**; and frontal-dorsal view of the head (C; MPEG-01052549) and genital complex (F; ISLA-110318) of *Syrbatus superciliata* Asenjo & Valois **sp. nov.** 



Mitochondrial genome representation of the three new species of *Syrbatus*, comparing the gene order with *Metopiellus crypticus*.

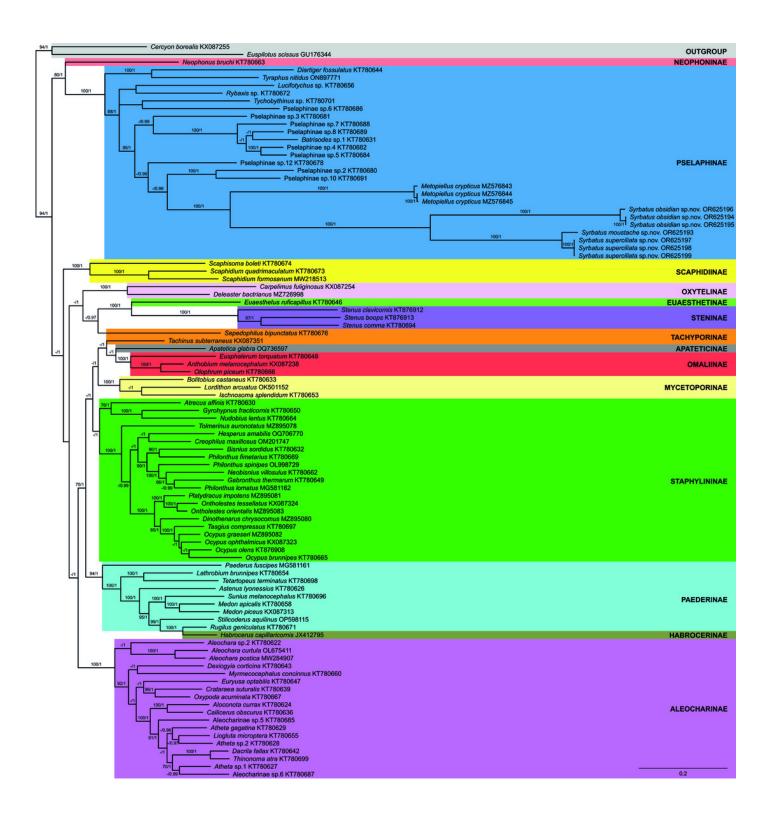
Comparison of the order of all 37 mitochondrial genes between *Syrbatus moustache* Asenjo & Valois **sp. nov.** and *Metopiellus crypticus* (A); colored arrows pointing to the left and right represent the transcription regions of protein coding genes (blue), rRNA genes (red) and tRNA genes (purple) on the L and H strands, respectively; green, orange, and pink and connections between genes indicate the reorganization of the mitogenome of *Syrbatus moustache* Asenjo & Valois **sp. nov.** in relation to the gene disposition of *Metopiellus crypticus*, a species presenting the ancestral pattern of insect species. Detail of the region between the genes trnS2 and ND1 in the mitogenomes of *Syrbatus moustache* Asenjo & Valois **sp. nov.**, SM, *Syrbatus obsidian* Asenjo & Valois **sp. nov.**, SO, and *Syrbatus superciliata* Asenjo & Valois **sp. nov.**, SS, evidencing the extended intergenic region in the three accessions of the former; the green and brownish bars above the arrows indicate monomorphic and polymorphic nucleotide sites, respectively.



Phylogenetic placement of the three new species of *Syrbatus* within Staphylinidae, based on whole mitogenome data.

Majority-rule consensus phylogram of the Bayesian inference analysis evidencing the phylogenetic relationships among Staphylinidae species with available mitogenomes in the GenBank database and the three specimens of *Syrbatus moustache* Asenjo & Valois **sp. nov.**, *Syrbatus obsidian* Asenjo & Valois **sp. nov.** and *Syrbatus superciliata* Asenjo & Valois **sp. nov.**, indicating their respective GenBank accessions and subfamily affiliations. Statistical support values (BS  $\geq$  70 and PP  $\geq$  0.90) are indicated near the branches.







### Table 1(on next page)

General features of the mitochondrial genes of *Syrbatus moustache* Asenjo & Valois sp. nov., *Syrbatus obsidian* Asenjo & Valois sp. nov. and *Syrbatus superciliata* Asenjo & Valois sp. nov.

Sequenced mitogenomes of *Syrbatus moustache* Asenjo & Valois **sp. nov.** (Sm - OR625193), *Syrbatus obsidian* Asenjo & Valois **sp. nov.** (So - OR625194, OR625195 and OR625196) and *Syrbatus superciliata* Asenjo & Valois **sp. nov.** (Ss- OR625197, OR625198 and OR625199), indicating the size of the transcription regions, presence of indel events, number of intraspecific mismatches after the alignment of the mitogenomes, coding strand, and sequences of both start and stop codons. <sup>a</sup>Whenever any of the accessions presented incomplete or missing genes in the mitogenome assembly, we showed the data for those with a complete sequence; also, in the cases with intraspecific variation in the gene sizes, we presented the data of the largest one; <sup>b</sup>the rrnL, rrnS and trnV genes were either incomplete or missing in the three mitogenomes assembled for *Syrbatus obsidian* Asenjo & Valois **sp. nov.** 

1 Table 1: General features of the mitochondrial genes of Syrbatus moustache Asenjo & Valois sp. nov., Syrbatus obsidian Asenjo

2 & Valois sp. nov. and Syrbatus superciliata Asenjo & Valois sp. nov.

Gene	Size (bp) <sup>a</sup> (Sm/So/Ss)	Indels (Sm/So/Ss)	Mismatches (Sm/So/Ss)	% Mismatches (Sm/So/Ss)	Coding strand	Start codon	Stop codon
ATP6	653/638/653	NA/0/0	NA/15/1	NA/2.35/0.15	L	ATA	TA
ATP8	138/150/135 <sup>b</sup>	NA/0/6	NA/3/0	NA/2.00/0.00	L	ATC	TAA
COX1	1566/1533/1566	NA/0/0	NA/26/0	NA/1.70/0.00	L	ATT/TTA/ATT	TAA
COX2	672/687/672	NA/0/0	NA/9/0	NA/1.31/0.00	L	ATA	TAA
COX3	792/783/792	NA/0/0	NA/24/3	NA/3.07/0.38	L	ATA/ATT/ATA	TAA/TAG/TAA
CYTB	1116/1077/1126	NA/0/0	NA/29/1	NA/2.69/0.09	L	ATG	TAA/TAA/T
ND1	927/927/927	NA/0/NA	NA/22/1	NA/2.37/0.11	Н	ATT/ATA/ATT	TAA
ND2	975/978/975	NA/0/0	NA/20/0	NA/2.04/0.00	L	ATT	TAA
ND3	351/351/348	NA/0/0	NA/4/0	NA/1.14/0.00	L	ATT/ATT/ATA	TAA
ND4	1353/1314/1347	NA/0/0	NA/25/0	NA/1.90/0.00	Н	ATG	TAA
ND4L	256/262/256	NA/0/0	NA/4/0	NA/1.45/0.00	Н	ATA	T
ND5	1695/1710/1695	NA/0/0	NA/36/2	NA/2.11/0.12	Н	ATT	TAA
ND6	465/468/465	NA/0/0	NA/9/0	NA/1.92/0.00	L	ATT	TAA
rrnL	1340/NAb/1336	NA/NA/2	NA/NA/1	NA/NA/0.00	Н	NA	NA
rrnS	747/NAb/764	NA/NA/0	NA/NA/0	NA/NA/0.00	Н	NA	NA
trnA (tgc)	63/70/63	NA/0/0	NA/3/0	NA/4.29/0.00	L	NA	NA
trnC (gca)	61/62/61	NA/0/0	NA/1/0	NA/1.61/0.00	Н	NA	NA
trnD (gtc)	64/63/62	NA/0/0	NA/0/0	NA/0.00/0.00	L	NA	NA
trnE (ttc)	63/63/61	NA/0/0	NA/0/0	NA/0.00/0.00	L	NA	NA
trnF (gaa)	61/60/61	NA/0/0	NA/0/0	NA/0.00/0.00	Н	NA	NA
trnG (tcc)	60/60/60	NA/0/0	NA/0/0	NA/0.00/0.00	L	NA	NA
trnH (gtg)	70/63/72	NA/0/0	NA/1/0	NA/1.59/0.00	Н	NA	NA
trnI (gat)	61/62/63	NA/0/0	NA/1/0	NA/1.61/0.00	L	NA	NA
trnK (ctt)	71/70/71	NA/0/0	NA/0/0	NA/0.00/0.00	L	NA	NA
trnL1 (tag)	62/67/62	NA/4/0	NA/1/0	NA/1.49/0.00	Н	NA	NA

trnL2 (taa)	64/63/64	NA/0/0	NA/0/0	NA/0.00/0.00	L	NA	NA
trnM (cat)	68/69/NA	NA/0/NA	NA/1/NA	NA/1.45/NA	L	NA	NA
trnN (gtt)	64/66/63	NA/0/0	NA/0/0	NA/0.00/0.00	L	NA	NA
trnP (tgg)	70/67/70	NA/0/0	NA/3/0	NA/4.48/0.00	Н	NA	NA
trnQ (ttg)	66/68/66	NA/0/0	NA/1/0	NA/1.47/0.00	Н	NA	NA
trnR (tcg)	53/57/54	NA/1/0	NA/2/0	NA/3.51/0.00	L	NA	NA
trnS1 (tct)	52/54/52	NA/0/0	NA/0/0	NA/0.00/0.00	L	NA	NA
trnS2 (tga)	61/61/62	NA/0/0	NA/0/0	NA/0.00/0.00	L	NA	NA
trnT (tgt)	63/61/63	NA/0/0	NA/1/0	NA/1.64/0.00	L	NA	NA
trnV (tac)	62/NAb/63	NA/NA/NA	NA/NA/NA	NA/NA/NA	Н	NA	NA
trnW (tca)	68/67/72	NA/0/2	NA/1/0	NA/1.49/0.00	L	NA	NA
trnY (gta)	61/64/61	NA/0/0	NA/1/0	NA/1.56/0.00	Н	NA	NA

Sequenced mitogenomes of *Syrbatus moustache* Asenjo & Valois **sp. nov.** (Sm – OR625193), *Syrbatus obsidian* Asenjo & Valois **sp. nov.** (So – OR625194, OR625195 and OR625196) and *Syrbatus superciliata* Asenjo & Valois **sp. nov.** (Ss– OR625197, OR625198 and OR625199), indicating the size of the transcription regions, presence of indel events, number of intraspecific mismatches after the alignment of the mitogenomes, coding strand, and sequences of both start and stop codons. <sup>a</sup>Whenever any of the accessions presented incomplete or missing genes in the mitogenome assembly, we showed the data for those with a complete sequence; also, in the cases with intraspecific variation in the gene sizes, we presented the data of the largest one; <sup>b</sup>the rrnL, rrnS and trnV genes were either incomplete or missing in the three mitogenomes assembled for *Syrbatus obsidian* Asenjo & Valois **sp. nov.**