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1

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The complete mitochondrial genome and description of a new cryptic Brazilian species of *Metopiellus* Raffray (Coleoptera: Staphylinidae)

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Metopiellus Raffray is a genus of South American rove beetles typically found in tropical humid forests. Here we describe a new cryptic species from Eastern Amazon, in northern Brazil, *Metopiellus crypticus* **sp. nov.**, and its major morphologic diagnostic features, which were photographed and illustrated. In addition, we bring the complete mitochondrial genome sequence of *M. crypticus*, and its position within the phylogenetic context of the family, including previously available mitogenomes of Staphylinidae species.

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Abstract

Metopiellus Raffray is a genus of South American rove beetles typically found in tropical humid forests. Here we describe a new cryptic species from Eastern Amazon, in northern Brazil, *Metopiellus crypticus* **sp. nov.**, and its major morphologic diagnostic features, which were photographed and illustrated. In addition, we bring the complete mitochondrial genome sequence of *M. crypticus*, and its position within the phylogenetic context of the family, including previously available mitogenomes of Staphylinidae species.

Keywords: Amazon basin, beetle, Brazil, Pselaphinae, taxonomy

Introduction

All species of the genus *Metopiellus* are distributed from Colombia to the North of Argentina (Asenjo *et al.* 2019, Fiorentino *et al.* 2022). To date, species of the genus were recorded in the Colombian Amazon (*M. guanano* Fiorentino, Tocora & Ramirez 2022), three species in the Brazilian Atlantic Forest (*M. aglenus* Reitter 1885, *M. hirtus* Reitter 1885, and *M. painensis* Asenjo *et al.* 2017), and one in Argentina (*M. sylvaticus* Bruch 1933) (Asenjo *et al.* 2013, Asenjo *et al.* 2017, Fiorentino *et al.* 2022). Members of *Metopiellus* are usually found inhabiting humid microenvironments on the forest floor consisting in decaying plant parts and their possible association with social insects as ants continue to be uncertain (Reitter 1885, Park 1942, Wasmann 1894, Bruch 1993). Asenjo *et al.* (2017) found *M. painensis* inside the Loca dos Negros II and the Cerâmica caves in southeastern Brazil. This latter species was the only troglotic Pselaphinae recorded from Brazil, up to date (Asenjo *et al.* 2017).

The aim of this study was to describe a new species from Brazil that was collected in the state of Pará, northern Brazil. The new species was found inhabiting forest areas, similar to other species in the genus, but it was also found in savanna-like environments. We described, for the first time, the complete mitochondrial genome of *Metopiellus crypticus* **sp. nov.** positioning the new species in the phylogenetic context of Staphylinidae.

Materials & Methods

Field collection and sequencing

A total of nine specimens of *Metopiellus crypticus* **sp. nov.** were collected in forest areas where individuals were more abundant, and only two specimens from the *canga*, a savanna-like environment from the Serra dos Carajás, in Pará (Fig. 1B), according to the sampling permit 49.994, granted by ICMBio/MMA. Various collection methods were used in all places (hand collection, litter sampling, hay-bait traps and soil sampling), but specimens were found only in litter-associated traps (hay-bait traps and soil sampling). Therefore, it is likely that the edaphic environment is characterized as a preferred habitat for populations of *Metopiellus crypticus* **sp. nov.** The specimens were immediately fixed in 99% ethanol within a 2 mL centrifuge tube and transported to the laboratory.

Total genomic DNA was extracted from three specimens from the type population with the DNeasy Blood & Tissue kit (Qiagen), following the manufacturer's protocol for insect samples, being deposited at the DNA bank of the Instituto Tecnológico Vale (ITV) under the accession numbers ITV10661, ITV21026 and ITV21027. Paired-end libraries were constructed from ~50

ng of genomic DNA using the QXT SureSelect kit (Agilent Technologies), which were
sequenced in an Illumina NextSeq 500 with the high-output v2 kit (300 cycles).

Afterwards, raw sequencing reads with base quality < Phred 20 and length < 70 bp were
trimmed with AdapterRemoval v.2 (Schubert *et al.* 2016), resulting in, ~~at least~~, 21,140,835 high
quality pairs of reads. The mitochondrial genomes were assembled with ~~at least~~ 21,141 pairs of
mapped reads using NovoPlasty 3.6 (Dierckxsens *et al.* 2017) and annotated with MITOS2
(Bernt *et al.* 2013), with minor corrections using Geneious Prime 2021 (Biomatters).

Morphological study

Specimens. The apical segments were cleared in a double boiler using 10% KOH during
three minutes. Dissections were made under a Leica S8APO (16×-128×) stereo-microscope.
Pictures were obtained using the AxioCam 506 color camera connected to an Axio ZoomV16
(ZEISS) stereo microscope and Photoshop CC 2021 was used for image processing, with final
plates being assembled in Adobe Illustrator CC 2021. Morphological character terminology,
including foveation and ~~initials of its, followed~~ Chandler (2001). All measurements are in
millimeters and were based on the holotype. Measurements were done using the Leica S8APO
(16×-128×) stereo-microscope and width/length measurements were made on the widest and
longest parts of the respective structure.

Measurements symbols:

- BL body length (from margin of prolongation of head to tergite IX posterior margin)
- BW body width (maximum width of elytra)

- 88 EL elytral length (maximum)
- 89 EW elytral width (maximum)
- 90 HL head length (from anterior margin of prolongation of head to head disc posterior margin)
- 91 HW head width (maximum)
- 92 NW neck width (minimum)
- 93 PL pronotum length (maximum)
- 94 PW pronotum width (maximum)

95

96 In the type label data, quotation marks (“ ”) separate different labels and a slash (/)
 97 separates different lines within a label. Text within square brackets [] is explanatory and is not
 98 included in the original labels.

99

100 Depositories. The specimens examined in this revision are deposited in the following
 101 collections (curators in parenthesis):

102

103 CEMT Setor de Entomologia da Coleção Zoológica da Universidade Federal de Mato Grosso,
 104 Departamento de Biologia e Zoologia, Cuiabá, Mato Grosso, Brazil (Fernando Vaz-
 105 de-Mello).

106 ISLA Coleção de Invertebrados Subterrâneos de Lavras, Setor de Zoologia, Departamento
 107 de Biologia, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil (Rodrigo
 108 Lopes Ferreira).

109 ITV Coleção de DNA do Instituto Tecnológico Vale, Belém, Pará, Brazil (Santelmo
 110 Vasconcelos).

111 MPEG Museu Paraense Emilio Goeldi, Belém, Pará, Brazil (Orlando Tobias Silveira).

112

113 Results

114

115 Description

116 Family Staphylinidae Latreille, 1802

117 Subfamily Pselaphinae Latreille, 1802

118 Tribe Metopiasini Raffray, 1904

119 Subtribe Metopiasina Raffray, 1904

120 Genus *Metopiellus* Raffray, 1908

121

122 *Metopiellus crypticus* Asenjo new species

123 *Type material* (7 males, 2 females). *Holotype*: BRAZIL, male, labeled “BRAZIL: Pará, /

124 Curionópolis, Serra / Leste, 22M, 650137mE, / 9339970mN, WGS84, / 25.iv.2017, BioEspeleo

125 *leg.*”; “Hay-bait trap, / Transect: T2, / Quadrant: E, Parcel: d”; “HOLOTYPE ♂ [red label] /

126 *Metopiellus / crypticus* sp. nov. / Desig. Asenjo *et al.*, 2022” (CEMT-00120424). *Paratype*: (6

127 males, 2 females), labeled: “BRAZIL: Pará, / Curionópolis, Serra / Leste, 22M, 652136mE, /

128 9339073mN, WGS84, / 26.iv.2017, BioEspeleo *leg.*”; “Soil sampling, / Transect: T3, / Quadrant:

129 C, Parcel: -” (1 male, ISLA-103823). “BRAZIL: Pará, / Curionópolis, Serra / Leste, 22M,

130 650360mE, / 9339477mN, WGS84, / 25.iv.2017, BioEspeleo *leg.*”; “Soil sampling, / Transect:

131 T1, / Quadrant: D, Parcel: -” (1 female, ISLA-103824). “BRAZIL: Pará, / Curionópolis, Serra /

132 Leste, 22M, 652013mE, / 9339211mN, WGS84, / 26.iv.2017, BioEspeleo *leg.*”; “Soil sampling, /

133 Transect: T4, / Quadrant: C, Parcel: -” (1 male, MPEG-01051329). “BRAZIL: Pará, /

Curionópolis, Serra / Leste, 22M, 650095mE, / 9339732mN, WGS84, / 25.iv.2017, BioEspeleo
leg.”; “Hay-bait trap, / Transect: T2, / Quadrant: A, Parcel: d” (1 male, MPEG-01051330).
“BRAZIL: Pará, / Curionópolis, Serra / Leste, 22M, 650095mE, / 9339732mN, WGS84, /
25.iv.2017, BioEspeleo leg.”; “Hay-bait trap, / Transect: T2, / Quadrant: A, Parcel: c” (2 male,
CEMT-00120425 and CEMT-00120426, and 1 female, CEMT-00120427). “BRAZIL: Pará, /
Curionópolis, Serra / Leste, 22M, 650070mE, / 9339845mN, WGS84, / 25.iv.2017, BioEspeleo
leg.”; “Hay-bait trap, / Transect: T2, / Quadrant: C, Parcel: b” (1 male, MPEG-01051331). All
paratypes with label “PARATYPE [yellow label] / *Metopiellus* / *crypticus* sp. nov. / Desig.
Asenjo *et al.*, 2022”.

Additional specimens. BRAZIL: Pará, Curionópolis, Serra Leste, 22M, 650137mE,
9339970mN, WGS84, 25.iv.2017, BioEspeleo leg., HBT-T2 E(B) (1 male, ITV10661).
BRAZIL: Pará, Curionópolis, Serra Leste, 22M, 650070mE, 9339845mN, WGS84, 25.iv.2017,
BioEspeleo leg., HBT-T2 C(A), (1 female, ITV21026). BRAZIL: Pará, Curionópolis, Serra
Leste, 22M, 650070mE, 9339845mN, WGS84, 25.iv.2017, BioEspeleo leg., HBT-T2 C(A), (1
female, ITV21027).

Diagnosis. *Metopiellus crypticus* **sp. nov.** is very similar to *M. painensis* because both
have a similar habitus (Figs. 2A-B; Fig. 1 in Asenjo *et al.* 2017) and eyes nearly absent (Figs.
2A-C; Fig. 3 in Asenjo *et al.* 2017), but the new species differs by having rounded the
antennomere 7 (Fig. 2E) (rectangular in *Metopiellus painensis* [Fig. 5 in Asenjo *et al.* 2017]).
Furthermore, *Metopiellus crypticus* **sp. nov.** further differs by the paramere asymmetric elongate
and apex bifurcated (Figs. 2H-J) (paramere asymmetric no bifurcated in *M. painensis* [Figs. 10-
12 in Asenjo *et al.* 2017]). Also, *Metopiellus crypticus* **sp. nov.** differs by the mediam lobe

curved and edge with long line of small teeth (Figs. 2H-2J) (median lobe almost right with the apex curved in *M. painensis* [Figs. 10-12 in Asenjo *et al.* 2017]).

Holotype male, BL: 2.68. Body, mouthparts, antennae and tarsi light brown (Figs. 2A-B).

Head: pyriform (Figs. 2A and 2C), length (HL: 0.44) similar to ~~wide~~ (HW: 0.44), anterior region distinctly narrower, apex ending and slightly raised at the antennal tubercle. Posterior margin of head narrowing, with posterolateral angles rounded. Neck almost half of width (NW: 0.19) of head. Head with two vertexal foveae [VF] (Figs. 2A and 2C), foveae connected by a transverse sulcus near posterior margin. Vertex longitudinally impressed, with weak sulcus running from anterior margin of antennal tubercle to neck. Ventral surface of head without gular sulcus and posterior region with two gular foveae [GF] that are connected by curved sulcus. Eyes (Figs. 2A-C) composed of ~~some~~ ommatidium situated at middle of head length in lateral view.

Antennae (Fig. 2E) almost 3/4 body length, scape almost half antenna length, last three antennomeres gradually broadening. Scape length (~~all length of antennomeres without peduncle and wide maximum~~) 0.92 mm, width (~~all width of antennomeres maximum~~) 0.09 mm, pedicel shorter than scape (0.39 : 0.08), antennomere 3 (0.06 : 0.06), antennomere 4 (0.05 : 0.06), antennomere 5 (0.07 : 0.06), antennomere 6 (0.06 : 0.06), antennomere 7 (0.06 : 0.08), antennomere 8 (0.03 : 0.06), antennomere 9 (0.06 : 0.1), antennomere 10 (0.06 : 0.12), antennomere 11 (0.15 : 0.14); all antennomeres covered by long microsetae.

Thorax: pronotum (Figs. 2A, 2C) slightly wider than long (PL: 0.45; PW: 0.52) widest at anterior half. Pronotum convex with weak median longitudinal sulcus, each side with lateral sulcus, with transversal antebasal sulcus. Pronotum with basal and anterior margins weakly emarginated; with median antebasal fovea [MAF] and lateral antebasal fovea [LAF]. Prosternum

with lateral procoxal fovea [LPCF]. Mesoventrite with median mesocoxal fovea [MMNF], lateral mesosternal foveae [LMNF] lateral mesocoxal foveae [LMCF], and with lateral metasternal foveae [LMTF]. Metaventre with median metasternal fovea [MMTF] and one flat median triangle area before of metacoxal cavities.

Elytra: subquadrate (EL: 0.74; EW: 0.80), sides gradually broadening apically (Fig. 2A). Posterior margins slightly concave, discal stria [DS] and sutural stria [SS] present. Elytron with two basal elytral foveae [BEF] at anterior margin, one at side of base the elytral sutural stria, second on the base of discal stria. Apico-lateral margin of elytra with small notched. Flight wings absent.

Legs: Legs long and slender (Figs. 2A-B). Femora thickened in apical half. Tibiae curved and similar in length to femora, all tibiae thickened at apex. Protibiae lacking microsetae on concave, mesial face, which is carinate and open at base (Fig. 2D). Tarsi 3-segmented (Fig. 2D), first tarsomeres very short, last 2 tarsomeres longer, tarsomere 2 longer ~~that~~ segment 3; all tarsi with single claw and minute accessory seta. Procoxae conical and prominent, mesocoxae rounded and prominent, metacoxae transverse, region that articulates with trochanter conical in shape. Procoxae with small, apically pointed prosternal process, mesocoxae weakly separated, metacoxae contiguous.

Abdomen: strongly margined (Fig. 2A), with five visible tergites (morphological tergites IV-VIII), tergite III reduced to translucent plate beneath elytra, tergite IV with basolateral fovea [BLF], tergite VIII with apex straight. Tergites IV-VII bordered by distinct paratergites, paratergite in abdominal segment IV with one small tooth in the middle. Sternite III with transverse depressed plate completely bare and beneath metacoxae, transverse plate with longitudinally projecting carina at middle. Sternite IV with baselateral fovea [BLF]. Tergum IX

divided into two plates; right plate (Fig. 2K) larger and more sclerotized than left (Fig. 2J).

Sternite VIII (Fig. 2F) with apex deeply emarginate.

Aedeagus (Figs. 2H-J): asymmetric with parameres fused to form elongate plate with the apex forked, the median lobe slightly bulbous at base, elongate and narrow, stronger curved laterally at apex, on edge with a line of small teeth. The apex with forked.

Female. Similar to male, except apex of tergite VIII convex (Fig. 2G).

Distribution. Only known from the type locality (Figs. 1A-B).

Etymology. The specific epithet “crypticus” is a noun in apposition.

Mitogenome sequence and phylogenetic placement

All three assembled mitogenomes [GenBank accession numbers MZ576843 (ITV10661), MZ576844 (ITV21026) and MZ576845 (ITV21027)] presented the standard structure sequence and gene content for Metazoa, consisting of 13 PCG, 22 tRNA genes and two rRNA genes (Fig. 3). The three mitogenome assemblies ranged in size from 14353 to 14984 bp, with similar GC contents between 16.2% and 16.5%, and 98.3% identical sites (1.7% differences). We observed differences in nucleotide composition among the three mitogenomes of *Metopiellus crypticus* **sp. nov.**, with indel events mostly occurring in the rRNA genes (three in each locus). Also, rrnL presented 33 site substitutions as indicated by the mismatches in the alignment, one of the highest proportions of polymorphic sites within the analyzed mitogenomes (2.68% of the 1232 bp), behind only of NAD6 (3.73% of the 456 bp), excluding the tRNAs, which are considerably shorter with 63 bp on average) (Table 1).

Most genes were encoded in the L-strand, including nine PCGs (ATP6, APT8, COB, COX1, COX2, COX3, NAD2, NAD3 and NAD6) and 14 tRNA genes (trnA, trnD, trnE, trnG,

trnI, trnK, trnL2, trnM, trnN, trnR, trnS1, trnS2, trnT and trnW). Also, ATT was the most frequent start codon, being observed in seven genes, followed by ATA in four genes, and ATG in three (Table 1). On the other hand, almost all genes presented the TAA stop codon, except for NAD3 with TAG, and the three COX genes with an incomplete stop codon (Table 1).

Previously published mitogenome sequences of Staphylinidae species from 11 subfamilies, plus one species of Hydrophilidae (*Cercyon borealis*) and one of Histeridae (*Euspilotus scissus*) to be used as outgroups, were obtained from GenBank, totaling 61 accessions. Sequences of the 13 protein coding genes (PCG) were aligned with MAFFT v7.45 (Kato et al. 2002) and maximum likelihood (ML) phylogenetic trees were obtained using RAxML v8 (Stamatakis 2014), implemented in raxmlGUI v2 (Edler et al. 2021) using the model GTR+PROTGAMMA and the rapid bootstrapping option with 1,000 replicates.

In the phylogenetic analysis, most of the subfamilies were recovered as monophyletic and well supported, except for Tachyporinae and Paedrinae, which were polyphyletic and paraphyletic, respectively, and Staphylininae, presenting a low bootstrap support (BS = 55) (Fig. 4). Within Pselaphinae, the relationships among the sampled species were mostly unsupported (BS < 70). The three specimens of *Metopiellus crypticus* sp. nov. grouped with maximum statistical support (BS = 100) in the longer branch within the subfamily, being recovered as sister to *Batrisodes* sp., although with low statistical support (BS = 42) (Fig. 4).

Discussion

The new species belongs to the genus *Metopiellus* based on the third antennal segment being much shorter than the second (Fig. 2E); the posterior coxae contiguous or nearly so; and

the mesial face of protibia being carinate and open at its base and apex (Fig. 2B) (Raffray 1908, Park 1942, Asenjo *et al.* 2017). One of the characters “pronotum not being spinose” for *Metopiellus*, should not be considered a good character as considered by previously authors to define the genus since *M. guanano* has pronotum with four small spines (Fiorentino *et al.* 2022).

Specimens of the know species on the genus *Metopiellus* were collected in litter of ants or in caves (Asenjo *et al.* 2017). Unlike the other species of the genus, which have been recorded in forested areas or inside caves, the new species has been found in forested areas of the Serra dos Carajás, as well as in a savanna-like environment, although being less abundant in the latter.

For the first time, the mitochondrial genome of a *Metopiellus* species is described focusing on the phylogenetic position of *Metopiellus crypticus* **sp. nov.** within the subfamily Pselaphinae. In the obtained topology, the new species was recovered as sister to *Batrisodes* sp., although with low statistical support (BS = 42). However, this grouping was probably an artefact influenced by the absence of published mitogenomes of the others representatives of the Metopiasini.

Despite of all three assembled mitogenomes presenting the standard structure sequence and gene content for Metazoa, we could not obtain a circularized assembly for any of them, probably due to a high repetitive DNA content in the D-loop control region (Sayadi *et al.* 2017), as indicated by the several mononucleotide repeats in both ends of the assembled sequences. Such a pattern has been frequently reported for beetle species, with several Coleoptera mitogenomes available in the GeneBank database containing all expected genes, but missing part of the control region, and thus being reported as partial sequences.

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272

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277 project (RCUK/BB/P027849/1).

278

279 **Competing Interests**

280 The authors declare no competing interests.

281

282 **Author Contributions**

- 283 • Angélico Asenjo conceived and designed the experiments, analyzed the data, performed
284 species identification and description, prepared figures and/or tables, authored or
285 reviewed drafts of the paper, approved the final draft.
- 286 • Marcus Paulo Alves de Oliveira designed the experiments, collected specimens, approved
287 the final draft.
- 288 • Renato R. M. Oliveira contributed reagents/materials/analysis tools, analyzed the data,
289 prepared figures and/or tables, approved the final draft.
- 290 • Eder S. Pires contributed reagents/materials/analysis tools, approved the final draft.
- 291 • Marcely C. Valois authored or reviewed drafts of the paper, approved the final draft.
- 292 • Guilherme Oliveira contributed reagents/materials/analysis tools, approved the final draft.

- Santelmo Vasconcelos conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

Data Availability

The assembled mitogenomes were deposited in GenBank under the accession numbers MZ576843, MZ576844 and MZ576845, and raw data were deposited in the BioProject PRJNA862473.

References

- Asenjo A, Irmeler U, Klimaszewski J, Chandler DS, Fierros-Lopéz HE, Vieira JS. 2019.** Staphylinidae (Insecta: Coleoptera) in Latin America: synopsis, annotated catalog, diversity and distribution. *Zootaxa* **4621(1)**:1–406 DOI [10.11646/zootaxa.4621.1.1](https://doi.org/10.11646/zootaxa.4621.1.1).
- Asenjo A, Irmeler U, Klimaszewski J, Herman LH, Chandler DS. 2013.** A complete checklist with new records and geographical distribution of the rove beetles (Coleoptera, Staphylinidae) of Brazil. *Insecta Mundi* **0277**:1–419.
- Asenjo A, Ferreira RL, Zampaulo RdeA. 2017.** Description of *Metopiellus painensis* sp. nov. (Coleoptera, Staphylinidae), first troglobitic Pselaphinae from Brazil. *Zootaxa* **4269(1)**: 115–123 DOI [10.11646/zootaxa.4269.1.5](https://doi.org/10.11646/zootaxa.4269.1.5).
- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsche G, Pütz J, Middendorf M, Stadler PF. 2013.** MITOS: improved *de novo* metazoan mitochondrial genome

annotation. *Molecular Phylogenetics and Evolution* **69(2)**:313–319 [DOI](#)

[10.1016/j.ympev.2012.08.023](https://doi.org/10.1016/j.ympev.2012.08.023).

Bruch C. 1933. Coleópteros mirmecófilos de Misiones (Staph. Pselaph. Hister.). *Revista de Entomologia* **3(1)**:12–37.

Dierckxsens N, Mardulyn P, Smits G. 2017. NOVOPlasty: *de novo* assembly of organelle genomes from whole genome data. *Nucleic Acids Research* **45(4)**:e18 [DOI](#)

[10.1093/nar/gkw955](https://doi.org/10.1093/nar/gkw955).

Edler D, Klein J, Antonelli A, Silvestro D. 2021. raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAXML. *Methods in Ecology and Evolution* **12(2)**:373–377 [DOI 10.1111/2041-210X.13512](https://doi.org/10.1111/2041-210X.13512).

Fiorentino G, Tocora M, Ramirez S. 2022. A new ant-mimetic species of *Metopiellus* (Staphylinidae: Pselaphinae) from the Northern Colombian Amazon. *ZooKeys* **1108**:1–9. [DOI 10.3897/zookeys.1108.76077](https://doi.org/10.3897/zookeys.1108.76077).

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30(14)**:3059–3066 [DOI 10.1093/nar/gkf436](https://doi.org/10.1093/nar/gkf436).

Park O. 1942. A study in neotropical Pselaphidae. *Northwestern University Studies in the Biological Sciences and Medicine, Number 1*, 1, Northwestern University, Evanston and Chicago. x + 403 pp, 21 pls.

Raffray A. 1908. Coleoptera. Fam. Pselaphidae, pp. 1–487, 9 pls. *In*: Wytsman, P. (Ed.), *Genera Insectorum*. Fasc. 64. V. Verteneuil & L. Desmet, Bruxelles.

- 336 **Reitter E. 1885.** Abbildungen und Bemerkungen zu wenig gekannten Pselaphiden-Gattungen
337 mit Beschreibungen neuer Arten. *Deutsche Entomologische Zeitschrift* **29**:333–339, pls. 2–3
338 [DOI 10.1002/mmnd.48018850226](https://doi.org/10.1002/mmnd.48018850226).
- 339 **Sayadi A, Immonen E, Tellgren-Roth C, Arnqvist G. 2017.** The evolution of dark matter in
340 the mitogenome of seed beetles. *Genome Biology and Evolution* **9(10)**:2697–2706 [DOI](https://doi.org/10.1093/gbe/evx205)
341 [10.1093/gbe/evx205](https://doi.org/10.1093/gbe/evx205).
- 342 **Schubert M, Lindgreen S, Orlando L. 2016.** AdapterRemoval v2: rapid adapter trimming,
343 identification, and read merging. *BMC Research Notes* **9(1)**:88 [DOI 10.1186/s13104-016-](https://doi.org/10.1186/s13104-016-1900-2)
344 [1900-2](https://doi.org/10.1186/s13104-016-1900-2).
- 345 **Stamatakis A. 2014.** RAxML version 8: a tool for phylogenetic analysis and post-analysis of
346 large phylogenies. *Bioinformatics* **30(9)**:1312–1313 [DOI 10.1093/bioinformatics/btu033](https://doi.org/10.1093/bioinformatics/btu033)
- 347 **Wasmann E. 1894.** *Kritisches Verzeichniss der Myrmekophilen und Termitophilen Arthropoden.*
348 *Mit Angabe der Lebensweise und mit Beschreibung neuer Arten.* F. L. Dames, Berlin, xiii +
349 231 pp. [DOI 10.5962/bhl.title.122977](https://doi.org/10.5962/bhl.title.122977).
- 350 **Yin ZW, Shen JW, Li LZ. 2015.** New species and new combinations of Asian Batrisodes
351 Reitter (Coleoptera, Staphylinidae, Pselaphinae), and synonymy of *Batrisodellus* Jeannel with
352 *Batrisodes*. *Deutsche Entomologische Zeitschrift* **62**:45.

Figure captions

Figure 1: Geographic location of collected specimens of *Metopiellus crypticus* sp. nov.

Brazil and Pará state are in orange, Carajás mineral Province in red and the specific type locality indicated by a white star (A); exact locations where the specimens were collected in the forest ground (yellow dots) and savanna regions (red dots) of Serra Leste (B); panoramic view of the vegetation in Serra Leste, the savanna vegetations are in the flat area on mountain tops, and forest vegetation on the slopes and valley (picture by Alan Calux) (C).

Figure 2: *Metopiellus crypticus* sp. nov.

Habitus, dorsal view (A); habitus, left lateral view (B); head and pronotum, dorsal view (C); proleg (D); left antenna, lateral view (E); abdomen of male, ventral view (F); abdomen of female, ventral view (G); aedeagus, ventral view (H); aedeagus, lateral view (I); aedeagus, dorsal view (J); left tergum IX (K); right tergum IX (L). Scale bars: 1 mm (A-B); 0.5 mm (C, E); 0.2 mm (D, F-L). Holotype male (A-F, H-L). Paratype female (G).

Figure 3: Representative genetic map of the mitogenome of *Metopiellus crypticus* sp. nov.

Disposition of all 37 mitochondrial genes. 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. The green and brownish bars above the arrows indicate monomorphic and polymorphic nucleotide sites among the three analyzed genomes, respectively.

377

378 **Figure 4: Mitogenome phylogenetic relationships among Staphylinidae species.**

379 Majority-rule consensus phylogram of the maximum likelihood analysis evidencing the
 380 phylogenetic relationships among Staphylinidae species with available mitogenomes in the
 381 GenBank database and the three specimens of *Metopiellus crypticus* **sp. nov.** sequenced here,
 382 indicating their respective subfamily affiliations. Well-supported groups ($BS \geq 70$) are indicated
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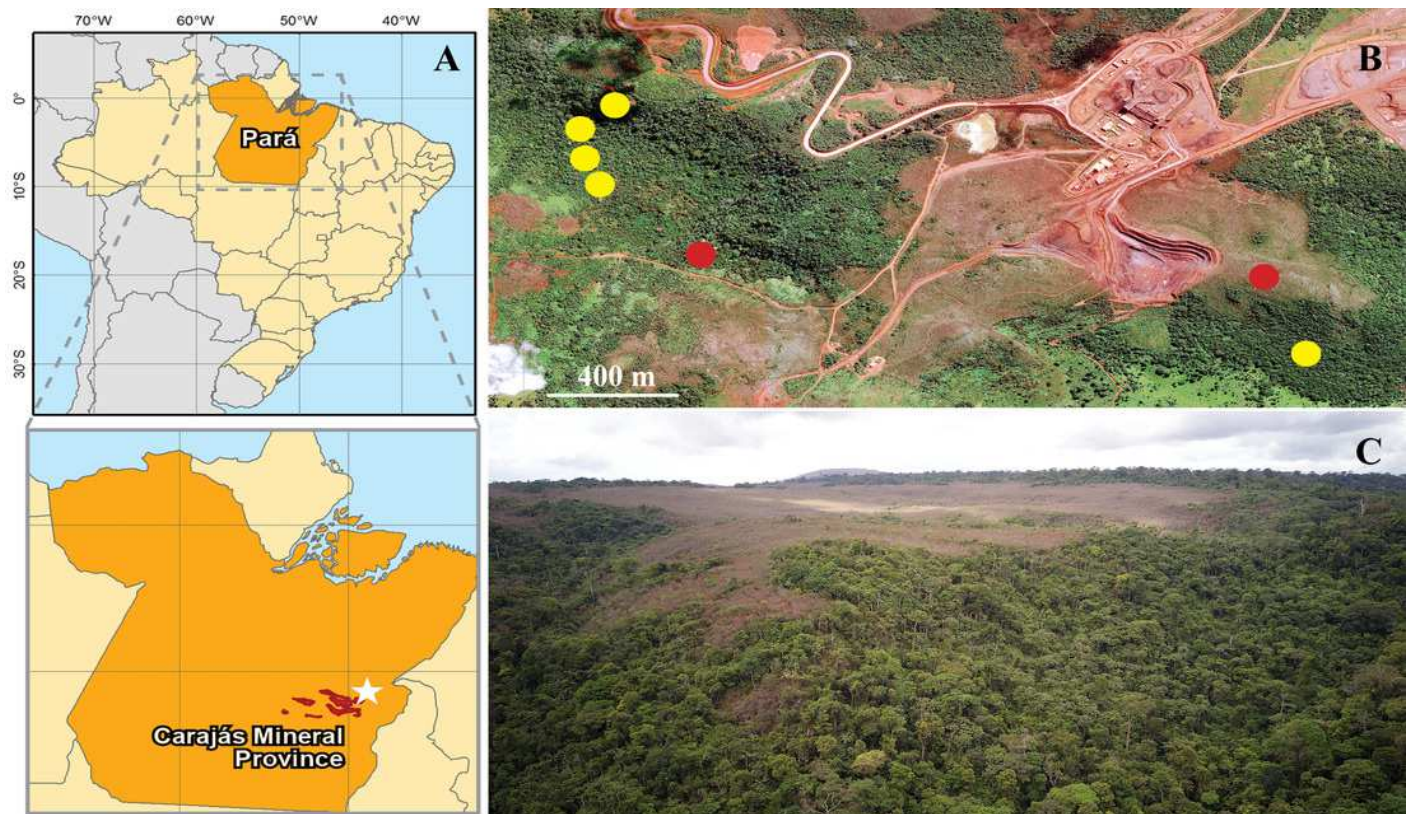


Figure 2

Metopiellus crypticus sp. nov.

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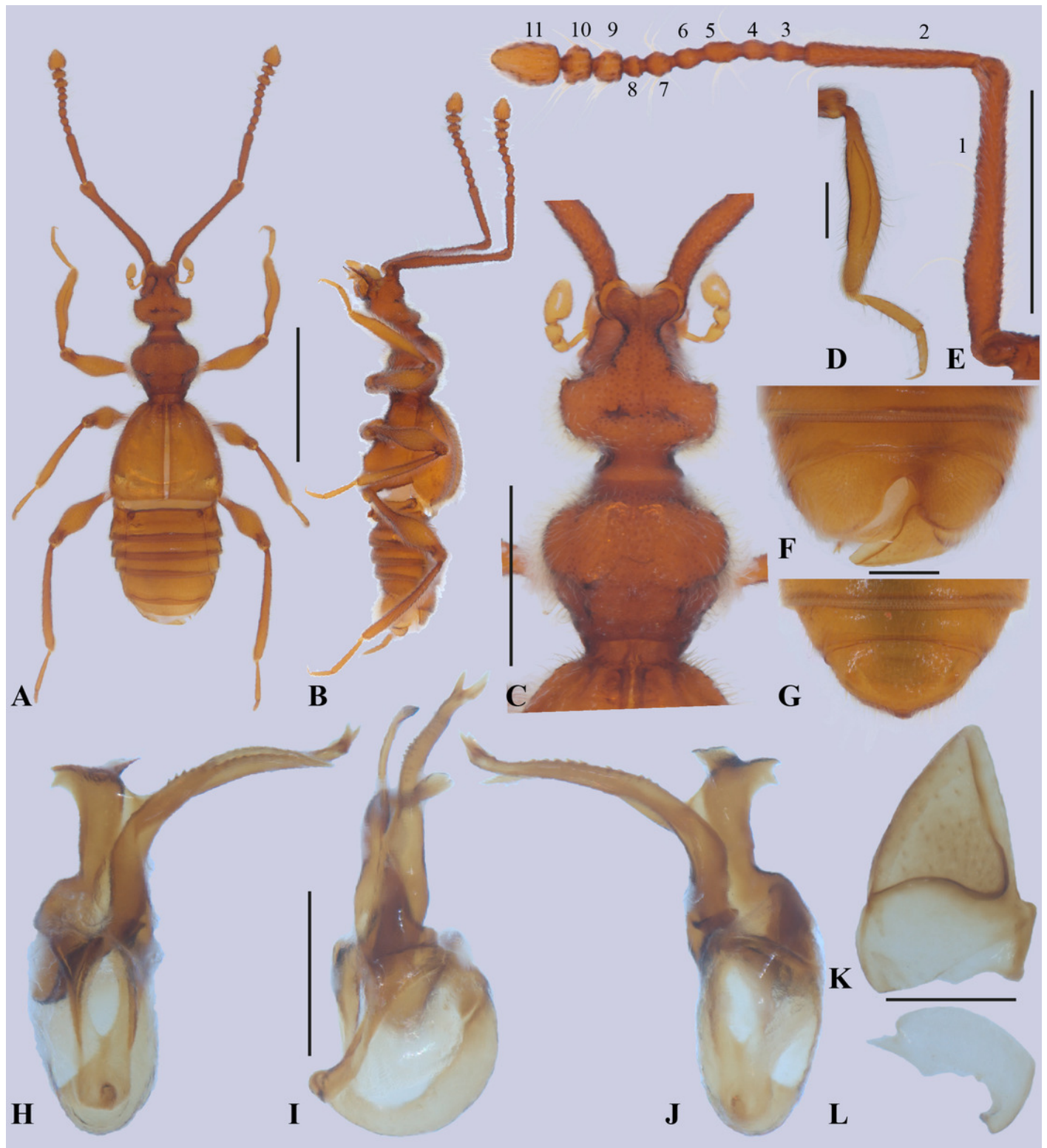


Figure 3

Representative genetic map of the mitogenome of *Metopiellus crypticus* sp. nov.

Disposition of all 37 mitochondrial genes. 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. The green and brownish bars above the arrows indicate monomorphic and polymorphic nucleotide sites among the three analyzed genomes, respectively.

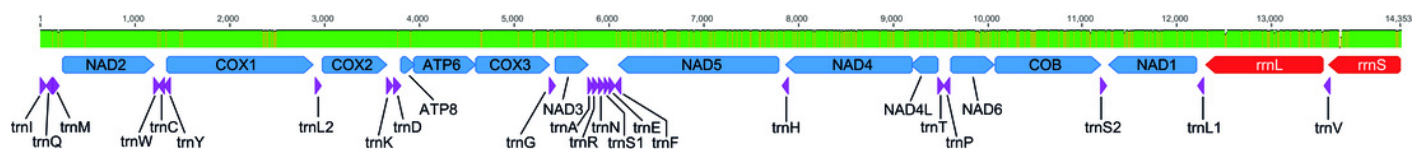
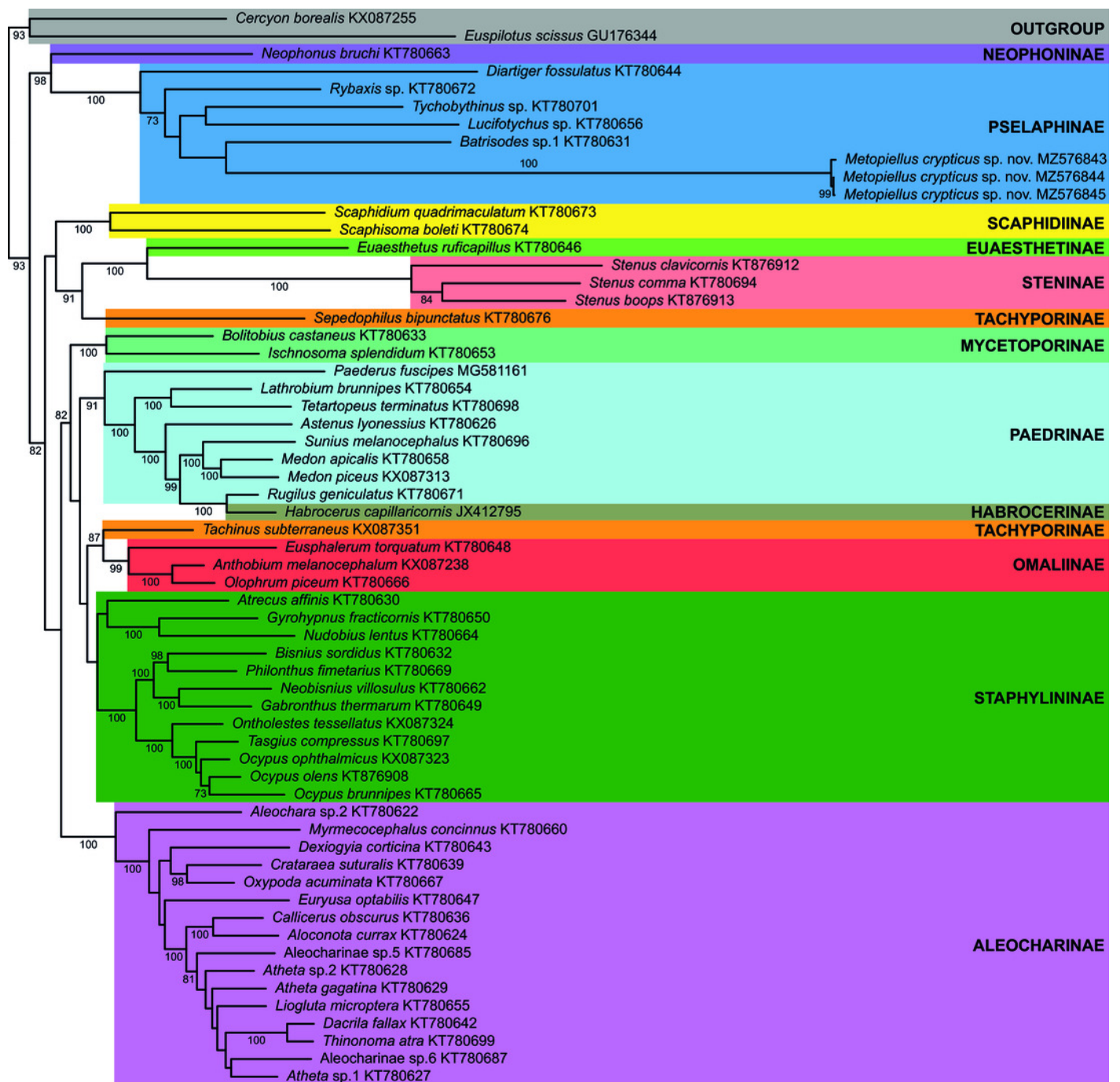


Figure 4

Mitogenome phylogenetic relationships among Staphylinidae species.

Majority-rule consensus phylogram of the maximum likelihood analysis evidencing the phylogenetic relationships among Staphylinidae species with available mitogenomes in the GenBank database and the three specimens of *Metopiellus crypticus* **sp. nov.** sequenced here, indicating their respective subfamily affiliations. Well-supported groups ($BS \geq 70$) are indicated by their bootstrap values near the branches.



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Table 1(on next page)

General features of the mitochondrial genes of *Metopiellus crypticus* sp. nov.

Sequenced mitogenomes based on from three different specimens, indicating the size of the transcription regions, presence of indel events, number of mismatches after the alignment of the mitogenomes, coding strand, and sequences of both start and stop codons. ^aFor genes with indel events, we presented the length observed in two of the three specimens; ^bfor trnM(cat), the value presented correspond to the average size among the three mitogenomes.

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Gene	Size (bp)	Indels	Mismatches	% Mismatches	Coding strand	Start codon	Stop codon
ATP6	651	0	0	0.00	L	ATG	TAA
ATP8	153	0	1	0.65	L	ATT	TAA
COB	1110	0	26	2.34	L	ATA	TAA
COX1	1537	0	11	0.72	L	ATT	T
COX2	682	0	0	0.00	L	ATA	T
COX3	781	0	8	1.02	L	ATG	T
NAD1	924 ^a	1	18	1.95	H	ATA	TAA
NAD2	963	0	1	0.10	L	ATA	TAA
NAD3	348	0	2	0.57	L	ATT	TAG
NAD4	1332 ^a	0	29	2.18	H	ATG	TAA
NAD4L	273	0	4	1.47	H	ATT	TAA
NAD5	1692	0	35	2.07	H	ATT	TAA
NAD6	456 ^a	1	17	3.73	L	ATT	TAA
rrnL	1232	3	33	2.68	H	-	-
rrnS	728 ^a	3	4	0.55	H	-	-
trnA(tgc)	52	0	0	0.00	L	-	-
trnC(gca)	63 ^a	1	0	0.00	H	-	-
trnD(gtc)	64	0	1	1.56	L	-	-
trnE(ttc)	62	0	0	0.00	L	-	-
trnF(gaa)	63 ^a	1	1	1.59	H	-	-
trnG(tcc)	63	0	2	3.17	L	-	-
trnH(gtg)	63	0	1	1.59	H	-	-
trnI(gat)	63	0	0	0.00	L	-	-
trnK(ctt)	68	0	0	0.00	L	-	-
trnL1(tag)	61	0	0	0.00	H	-	-
trnL2(taa)	62	0	0	0.00	L	-	-
trnM(cat)	68 ^b	1	0	0.00	L	-	-
trnN(gtt)	63	0	0	0.00	L	-	-
trnP(tgg)	64 ^a	1	0	0.00	H	-	-
trnQ(ttg)	63	0	0	0.00	H	-	-
trnR(tcg)	60	0	0	0.00	L	-	-
trnS1(tct)	55	0	0	0.00	L	-	-
trnS2(tga)	64	0	2	3.13	L	-	-
trnT(tgt)	64	0	0	0.00	L	-	-
trnV(tac)	64	0	2	3.13	H	-	-
trnW(tca)	64 ^a	1	0	0.00	L	-	-
trnY(gta)	63	0	1	1.59	H	-	-