The land flatworm *Amaga expatria* (Geoplanidae) in Guadeloupe and Martinique: new reports and molecular characterization including complete mitogenome (#49873)

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The land flatworm *Amaga expatria* (Geoplanidae) in Guadeloupe and Martinique: new reports and molecular characterization including complete mitogenome

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Background. The land flatworm *Amaga expatria* Jones & Sterrer, 2005 (Geoplanidae) was described from two specimens collected in Bermuda in 1963 and 1988 and not recorded since. **Methods.** On the basis of a citizen science project, we received observations in the field, photographs and specimens from non-professionals and local scientists in Martinique and Guadeloupe. We barcoded (COI) specimens from both islands and studied the histology of the reproductive organs of one specimen. Based on New Generation Sequencing, we obtained the complete mitogenome of *A. expatria* and some information on its prey from contaminating DNA. **Results.** We add records from 2006 to 2019 in two French islands of the Caribbean arc, Guadeloupe (six records) and Martinique (14 records), based on photographs obtained from citizen science and specimens examined. A specimen from Martinique was studied for histology of the copulatory organs and barcoded for the COI gene; its anatomy was similar to the holotype, therefore confirming species identification. The COI gene was identical for several specimens from Martinique and Guadeloupe and differed from the closest species by more than 10%; molecular characterisation of the species is thus possible by standard barcoding techniques. The mitogenome is 14962 bp in length and contains 12 protein coding genes, two rRNA genes and 22 tRNA genes; for two protein genes it was not possible to determine the start codon. The mitogenome was compared with the few available mitogenomes from geoplanids and the most similar was *Obama nungara*, a species from South America. An analysis of contaminating DNA in the digestive system suggests that *A. expatria* preys on terrestrial molluscs, and citizen science observations in the field suggest that prey include molluscs and earthworms; the species thus could be a threat to biodiversity of soil animals in the

Caribbean.

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Abstract

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Methods. On the basis of a citizen science project, we received observations in the field, photographs and specimens from non-professionals and local scientists in Martinique and Guadeloupe. We barcoded (COI) specimens from both islands and studied the histology of the reproductive organs of one specimen. Based on New Generation Sequencing, we obtained the complete mitogenome of *A. expatria* and some information on its prey from contaminating DNA.

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Introduction

The land flatworm *Amaga expatria* Jones & Sterrer, 2005 (Geoplanidae) was described from two specimens collected in Bermuda in 1963 and 1988 (Jones & Sterrer 2005). It has not been recorded since. Jones & Sterrer (2005) concluded that the species was an alien species in Bermuda, probably introduced from South America since other members of the genus have been collected in this region, including Colombia, Peru, Chile, Brazil, Paraguay and Argentina (Ogren & Kawakatsu 1990); the genus *Amaga* Ogren & Kawakatsu, 1990 currently includes 10 species (Grau et al. 2012).

In 2003, one of us (JLJ) undertook a citizen science program in France about alien land flatworms. Records were unexpectedly received from other locations including French overseas territories (Justine et al. 2014, 2015, 2018a, 2018b, 2019; Justine & Winsor 2020). Among these were several records of large land flatworms from the two Caribbean islands of Martinique and Guadeloupe, similar, on photographic evidence, to *A. expatria*. One specimen from Martinique has been subjected to both molecular characterization (COI barcoding) and histological examination. Another specimen from Martinique was included in a comparative study of the mitogenome of several land flatworms (Gastineau & Justine 2019, 2020; Gastineau et al. 2019).

We present here new records of *A. expatria* in two islands of the Caribbean, and provide the first barcoding characterisation of the species and its complete mitogenome.

Material and Methods

Citizen science and collection of information

Records were collected from 2013 to 2019, a period of 7 years (single records from 2006 and 2012 are also included). We used the same methods as for our previous research on land flatworms (Justine et al. 2014, 2015, 2018a, 2018b, 2019). A blog (Justine 2019) and a twitter account (@Plathelminthe4) were the main tools for collecting and transmitting information. The collaboration of local natural history associations and of the FREDON (Regional federations for the control of pests) in the departments of Martinique and Guadeloupe was also instrumental. Reports of sightings were received from the general public and from professionals, generally by email. We solicited and obtained specimens. Specimens were obtained alive, fixed in near boiling water and preserved in 95% ethanol, or sometimes fixed directly in cold ethanol. They were sent to the Muséum National d'Histoire Naturelle (MNHN) in Paris, registered, and processed for molecular studies.

Histology

A specimen from Martinique, MNHN JL146, was used for histology. It was killed alive in boiling water, then kept in 80% ethanol. A portion about 1.7 cm long containing the copulatory apparatus was removed for sectioning. Horizontal longitudinal sections (HLS) were cut at 12 µm thickness, mounted on 41 slides, eight or nine sections per slide, stained in haematoxylin and eosin and mounted in Canada balsam (slides 1-5 and 38-41 remain unstained in wax). HLS sections were cut in order to reduce the number of sections and slides. The worm was about 9 mm wide, so there would have been about 750 x 12 µm transverse longitudinal sections, circa 75 slides. Slides are deposited in the MNHN, Paris, registration number MNHN JL146.

Molecular barcoding

For molecular analysis, a small piece of the body (1–3 mm^3) was taken from the lateral edge of ethanol-fixed individuals. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen). Two sets of primers were used to amplify the COI gene. A fragment of 424 bp was amplified with the primers JB3 (=COI-ASmit1) (forward 5′-TTTTTGGGCATCCTGAGGTTTAT-3′) and JB4.5 (=COI-ASmit2) (reverse 5′-TAAAGAAAGAACATAATGAAAATG-3′) (Bowles et al. 1995; Littlewood et al. 1997). PCR reactions were performed in 20 µl, containing 1 ng of DNA, 1× CoralLoad PCR buffer, 3 mM $MgCl_2$, 66 µM of each dNTP, 0.15 µM of each primer, and 0.5 units of Taq DNA polymerase (Qiagen). The amplification protocol was: 4’ at 94 °C, followed by 40 cycles of 94 °C for 30’’, 48 °C for 40’’, 72 °C for 50’’, with a final extension at 72 °C for 7’. A fragment of 825 bp was amplified with the primers BarS (forward 5′-GTTATGCCTGTAATGATTG-3′) (Álvarez-Presas et al. 2011) and COIR (reverse 5′-CCWGTYARMCCHCCWAYAGTAAA-3′) (Lázaro et al. 2009; Mateos et al. 2013). PCR products were purified and sequenced in both directions on a 96-capillary 3730xl DNA Analyzer sequencer (Applied Biosystems). Results of both analyses were concatenated to obtain a COI sequence of 903 bp in length. Sequences were edited using CodonCode Aligner software (CodonCode Corporation, Dedham, MA, USA), compared to the

GenBank database content using BLAST, and deposited in GenBank under accession number xxx-xxx.

Next Generation Sequencing, phylogeny and identification of contaminant DNA

A slice of flesh from specimen MNHN JL305 was sent to the Beijing Genomics Institute (BGI) in Shenzhen, which provided DNA extraction and sequencing. Sequencing was performed on a DNBSEQ platform. A total of ca. 60 million clean paired-end reads were obtained. Reads were assembled using SPAdes 3.14.0 (Bankevich et al. 2012) with a k-mer of 85. Contigs corresponding to the mitogenome and the nuclear ribosomal RNA genes were retrieved by customized blastn command line analyses, using already available sequences downloaded from GenBank as a custom database. In addition to the sequences related to *A. expatria*, other positive matches belonging to contaminant DNA were obtained as explained in the results. The mitogenome was verified using the Consed package (Gordon et al. 1998), and gene identification was performed using MITOS (Bernt et al. 2013). The genomic map was drawn using OGDRAW (Lohse et al. 2013).

Amino acid sequences of the protein coding genes were concatenated following a protocol already described (Gastineau & Justine 2020; Gastineau et al. 2019; Gastineau et al. 2020), and aligned with corresponding sequences from other species using MAFFT (Katoh & Standley 2013). A maximum likelihood phylogeny was inferred from this alignment using RaxML version 8.0 (Stamatakis 2014) using the MtArt substitution model (Abascal et al. 2007). The best tree out of 100 was computed for 100 bootstrap replicates.

COI trees and distances

MEGA7 (Kumar et al. 2016) was used to evaluate distances, and construct trees. For the outgroup, we chose a sequence in GenBank from the South American species *O. nungara* (MN529572) which had a 100% query cover with our sequences.

Results

Information obtained from citizen science and other scientists

We obtained 14 verified records from Martinique (map in Figure 1), from 2006 to 2018, and six verified records from Guadeloupe, from 2012 to 2018 (map in Figure 2). Records were generally obtained as photographs, but we also received five specimens from Martinique and three specimens from Guadeloupe (Table 1). In addition, François Meurgey (email, 29.01.2016) added information about Guadeloupe: "*Amaga expatria* is quite common in the high series of the mesophilic forest and between 400 and 700 m altitude in the moist forest. I met it in the municipalities of Baillif (St Louis river), Matouba, Trois-Rivières, and Gourbeyre. It seems more frequent in the South of Basse-Terre. I have observed it attacking snails of the genera *Helicina*, *Pleurodonte* and earthworms." Laurent Charles (email, 15.05.2020) sent a photograph showing predation on a snail identified as *Helicina platychila*.

Morphology and histology

Based on photographs, Jones & Sterrer (2005) describe the holotype specimen from Bermuda as follows: "the extended living worm was about 90 mm long, 8 mm wide and flattened. The dorsal ground colour was mid-brown with a dark brown anterior tip. Two narrow dark blue lines are present dorsally in the anterior third. These fade towards the posterior extremity, though some photographs show these to be just visible almost to the posterior end. An irregular series of blue/black marks occurs on either side a little medial to the two lines. These marks are not continuations of the lines and continue to about the level of the pharynx. They may indicate the position of the testes." The creeping sole covers the entire ventral surface. In the holotype, eyes are absent from the anterior tip but numerous multiserial eyes are present laterally and dorsally. The pharynx is of the plicate type.

Live specimens mentioned in this study, measured on photographs (Figures 3-5), were 128-132 mm in length and 5.5-9 mm in width in extended state, and 35 × 12 mm in contracted state.

Description of Specimen MNHN JL146.

Living dimensions (Figure 3): length 128 mm, width 5.5 mm. Preserved dimensions: length 108 mm, width 9 mm, height 2 mm; mouth 53%; genital pore 75%.

The copulatory apparatus (Figure 6) is about 8 mm long from the anterior of the male system to the posterior of the female system. The male system is about 5 mm long and the female system about 3 mm long.

Two sperm ducts, about 1.2 mm apart, each with copious stored sperm (cyanophylic) approach the copulatory apparatus, briefly turn anteriorly before opening separately into the ventral end of a single duct (Figure 6d). This duct has a thick muscular wall and runs vertically from ventral to dorsal for about 1080 µm (runs through 90 sections x 12 µm). At its dorsal end this duct continues posteriorly as a narrow sinuous duct before broadening into the ejaculatory duct. The ejaculatory duct terminates in a short penis about 800 µm long and 600 µm wide (Figure 6a,b). There are atrial folds outside the penis in the common antrum.

The two ovovitelline ducts (Figure 6a,b) are about 2 mm apart anterior to the penis, they run posteriorly and at about the level of the penis they turn dorsally to join and open into the combined female duct. Copious shell glands (eosinophilic) are present and open into both ovovitelline ducts before they join (Figure 6c) to form the combined female duct. The combined female duct broadens, and has one or two longitudinal folds, before opening into the common antrum.

The gonopore opens from the common antrum via a short, narrow duct.

Molecular characterization – COI

For 4 specimens, the COI sequences obtained were identical along their whole length (903 bp). These specimens were JL289, JL305 (obtained both from Sanger sequencing and from the mitogenome) and JL310 from Martinique, and JL319 from Guadeloupe. This demonstrates that the same species was found in both islands. Specimen JL146 from Martinique, which was processed for histology, and three other specimens, JL216 and JL217 from Guadeloupe and JL262 from Martinique, provided shorter sequences but these were also identical between them and with the 4 sequences above for their portion in common. This demonstrates that the specimen studied for histology is from the same species, therefore confirming that the species in both islands is *A. expatria*. Differences with *O. nungara*, calculated on the 903 bp in common, were 12%.

Mitogenome

The mitogenome (Figure 7) is 14962 bp long (GenBank accession number: MT527191). It contains 12 protein coding genes, 2 ribosomal RNA genes and 22 transfer RNA genes. The mitogenome is completely colinear with that of *O. nungara* (KP208777) (Solà et al. 2015) and similar in size (14909 bp for *O. nungara*). A megablast query using the whole sequence of the mitogenome shows a global 83.77 % identity between these two species. The mitogenome is also colinear with those of *Bipalium kewense*, but not with those of *Platydemus manokwari* and *Parakontikia ventrolineata*. For 3 genes, atp6, cox2 and ND3, it was not possible to determine the start codon. The first methionine of the predicted proteins occurs at position 72/224 for *atp6*, 112/260 for *cox2* and 44/112 for *ND3*. It is worth mentioning that the impossibility to evidence a start codon for these genes was observed with *O. nungara*, but not for example with *B. kewense*, *Pl. manokwari* or *Pa. ventrolineata*. Unlike *O. nungara*, however, no overlap between the *ND4L* and *ND4* genes was evidenced.

The tree obtained from a maximum likelihood phylogeny of amino acid sequences of protein coding genes closely associates *A. expatria* with *O. nungara* (Figure 8).

Detection of an alien DNA

After assembly, sequences linked with contaminating DNA were identified. Three contigs of 3080 bp, 7274 bp and 15202 bp were retrieved. Megablast analyses were performed on the NCBI portal. The best result are listed thereafter. The 3080 bp fragment displayed a 99.66% of identity with the 18S ribosomal genes of sequences identified as the molluscs *Subulina striatella* (MN022690), *Lissachatina fulica* (MN022692) and *Achatina fulica* (KU365375). The 7274 bp fragment showed a 99.97% of identity with the internal transcribed spacer 2 of a sequence identified as *Subulina octona* (MF444887). The longest fragment appeared to be a nearly complete mitochondrial genome. The *cox1* gene was extracted from it, and it showed a 97.86% of identity with *S. octona* (JX988065).

Discussion

New records

Amaga expatria was described on the basis of two specimens from Bermuda, the holotype, collected in 1988, and a paratype, collected in 1963 (Jones & Sterrer 2005). The species has not been recorded since, but was mentioned in a book on molluscs of Martinique (Delannoye et al. 2015). These were originally identified by one of us (JLJ) and are also included in the present study. Our study has ten times more records than the original description, with 6 records from

Guadeloupe and 14 records from Martinique (Table 1). This exemplifies again the power of citizen science for recording land flatworms (Justine et al. 2018b, 2019).

Maps (Figures 1-2) show that the species is widely spread in both Martinique and Guadeloupe. In Guadeloupe, most records were from Basse Terre and a single record (Sainte-Anne) was from Grande Terre, and in Martinique, most records were from the North of the island, with only one in the South, in Sainte-Luce. This suggests that the species is more abundant in, but not exclusive to, the parts of the islands with higher rainfalls (Basse Terre in Guadeloupe and the North in Martinique).

Anatomy

Though there are slight differences, the copulatory apparatus of the Martinique specimen (Figure 6) is essentially the same as the type specimen of *A. expatria* from Bermuda (NHMUK.2002.10.16.1). The afferent male ducts have a similar structure, with two sperm ducts discharging into a ventro-dorsal duct which in turn opens into the ejaculatory duct and blunt penis via a sinuous duct. The duct wall is thickened in the same position about half way between the vertical duct and penis. We are confident of the identification of the Martinique specimen (JL146) as *A. expatria*.

Diet

Analysis of prey DNA is an efficient method to determine the diet of land planarians (Cuevas-Caballé et al. 2019). All blast analyses of the contaminant DNA in a specimen of *A. expatria* identified it as belonging to the Gastropoda, and it is likely that the prey was a specimen of *Subulina octona*, or a closely related species. *Subulina octona* is a tropical terrestrial snail, with a cosmopolitan distribution; this mollusc is indeed present in Martinique where it is considered recently introduced (Anonymous 2020).

The original description of *A. expatria* included no direct observation about the diet, but Jones & Sterrer (2005), on the basis of the presence of a plicate pharynx, wrote “it is likely that earthworms are the sole or principal prey of *A. expatria*”. The observations by François Meurgey (predation on snails of the genera *Helicina*, *Pleurodonte* and earthworms) and Laurent Charles (predation on *Helicina platychila*) reported here, and the finding of the sequence of a terrestrial mollusc in the gut, indicate a generalist diet, including both molluscs and earthworms. This diet might be one of the reasons of the success of the species to invade various islands in the Caribbean.

Molecular barcoding

One specimen from Martinique was characterised for morphology, histology, and barcoding and thus represents the first attempt at a molecular characterization of the species. Specimens from Martinique and Guadeloupe provided identical sequences, therefore unequivocally demonstrating that the same species is present on both islands, and, from morphology and anatomy, is *A. expatria*. The difference of 12% with *O. nungara* suggests that the COI sequences can valuably be used for barcoding *A. expatria*. The absence of genetic divergence between our sequenced specimens suggests that the species was recently introduced into the two islands from a single population.

Mitochondrial genome and multigene phylogeny

The maximum likelihood multigene phylogeny associates *A. expatria* with *O. nungara* with strong node support of 100, which is congruent with their position in the same Geoplaninae sub-family (Figure 8). It clearly discriminates them from the Bipaliinae *B. kewense*, the Caenoplaninae *Pa. ventrolineata* and the Rhynchodeminae *Pl. manokwari*.

Among the features conserved between the mitogenomes of *A. expatria* and *O. nungara*, we would like to emphasize the conserved absence of canonical start codons for the three genes *atp6*, *cox2* and *ND3*. Instead, the first amino-acids evidenced from the putative proteins are always a leucine. This leucine is always coded by a TTG codon, except for *A. expatria* where it is replaced by TTA. While no such thing has been evidenced among the recently sequenced mitogenomes of *B. kewense*, *Pl. manokwari* and *Pa. ventrolineata* (Gastineau & Justine 2020; Gastineau et al. 2019, 2020), similar features have also been observed among several Dugesiidae such as *Dugesia japonica* AB618487, *Dugesia ryukyuensis* AB618488 (both in Sakai & Sakaizumi 2012)), *Girardia* sp. KP090061 and *Schmidtea mediterranea* NC022448 and KM821047 (both in Ross et al. 2016)). The possibility that TTG could act as an alternative start codon was already suggested by Ross et al. (2016). Based on our data, we may suggest that TTA could also be considered. Addressing properly this question might require N-terminal sequencing of these proteins.

We note that *Amaga* and *Obama* belong to the subfamily Geoplaninae, whereas *Platydemus* and *Parakontikia* are members of the Rhynchodeminae and *Bipalium* is a member of the Bipaliinae. This possible variation of the genetic code could thus be limited to a single subfamily within the Geoplanidae, the Geoplaninae.

Conclusion

Our study shows that a relatively large land flatworm species is common in two islands of the Caribbean, and, with 20 new records, adds ten times the previous number of records of the species, which were from a single location, Bermuda, an island located in the Northeast Atlantic Ocean. Jones & Sterrer (2005) hypothesized that the species originated from continental South America and was recently introduced in the Bermuda. Our genetic results show that COI sequences from Martinique and Guadeloupe were identical and thus suggest that the introduction is recent in these islands. It remains that the locality of origin of the species in South America is still unknown. The species preys on molluscs and earthworms and might be a threat to the biodiversity of soil animals, especially molluscs which include endemic and rare species in the Caribbean islands (Delannoye et al. 2015). However, no proliferation was recorded and the threat may be minor, but it might also be that *A. expatria* is only in the first stages of invasion and that it will become an invading species in the future; similarly, recent observations have shown that the highly invasive species *Platydemus manokwari* is now invading Guadeloupe (Justine & Winsor 2020). The presence of *A. expatria* in two islands of the Caribbean suggests that it might be present in other islands, and perhaps in continental North America.

Acknowledgments

We are grateful to all non-professionals and professionals who provided records and specimens; they are listed in Table 1. All individuals listed in Table 1 kindly agreed to have their photographs published in this paper. François Meurgey and Laurent Charles kindly communicated some field observations; Laurent Charles provided information about the status of *Subulina octona*; they both agreed to have their information communicated here. Leigh Winsor (James Cook University, Australia) kindly helped in identifying specimens on photograph at early stages of this work.

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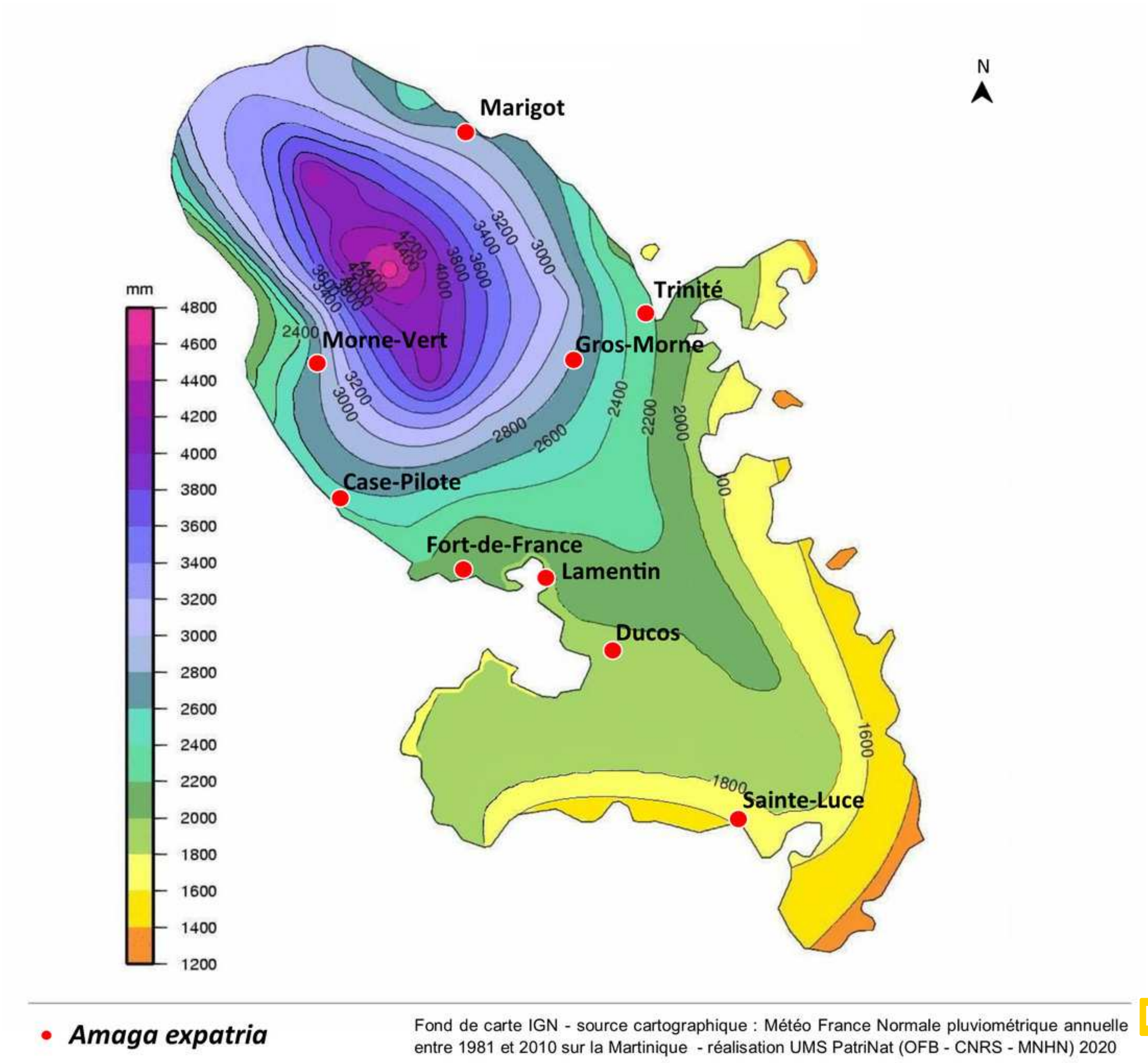
Figure 1

Amaga expatria, map of records in Martinique.

The background colours indicate annual raindrop falls. Most records are from the Northern part of the island where raindrops are high, but the record in Sainte-Luce in the South is a from a relatively drier part.



Fond de carte IGN - source cartographique : Météo France Normale pluviométrique annuelle entre 1981 et 2010 sur la Martinique - réalisation UMS PatriNat (OFB - CNRS - MNHN) 2020

Figure 2

Amaga expatria, map of records in Guadeloupe.

The background colours indicate annual raindrop falls. Most records are from Basse Terre, where raindrops are high, but the record in Ste-Anne in Grande Terre is from a relatively drier part.

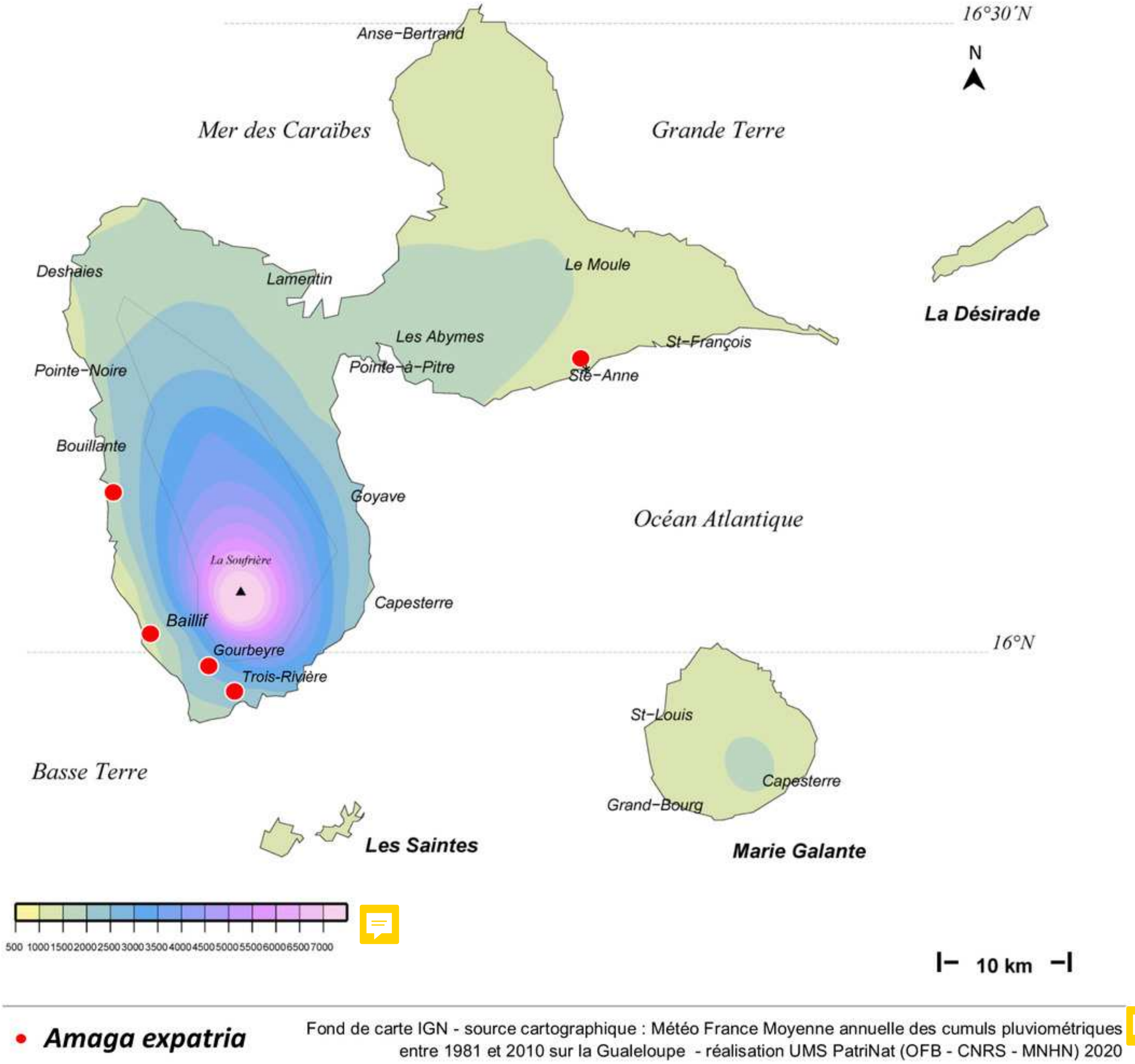


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Figure 3

Amaga expatria, specimen MNHN JL146 from Martinique

A, living specimen, photograph by Clément Dromer; scale: the diameter of the coin is 24 mm; B, preserved specimen, photograph by Jean-Lou Justine. This specimen was used for anatomy.

Figure 4

Amaga expatria from Martinique, living specimens.

A, photograph by Cédric Rareg; B,C photograph by Régis Delannoye; C, scale in mm; D, photograph by Mathieu Coulis, specimen MNHN JL305

Figure 5

Amaga expatria from Guadeloupe, living specimens in the field.

A, photograph by Pierre and Claude Guezennec; B, C, photographs by Laurent Charles, B, specimen MNHN JL216, C, MNHN JL217; the prey snail is *Helicina platychila*; D, photograph by Mathieu Coulis, specimen MNHN JL310; E-G, photographs by Guy van Laere, E,F, specimen MNHN JL319, E, dorsal side, F, showing ventral side.


A
B
C
D
E
F
G

Figure 6

Amaga expatria, specimen MNHN JL146 from Martinique, anatomy.

Anatomy of the copulatory apparatus, anterior to the right: a & b respectively an HLS section and a diagrammatic reconstruction through the copulatory apparatus to the same scale; c, posterior of the female ducts showing the junction of the ovovitelline ducts; d, anterior of the male ducts showing the junction of the sperm ducts, both with copious stored sperm (cyanophylic) with the ventral end of the vertical sperm duct. Scale lines 2 mm in (a), 0.5 mm in (c) and (d). Abbreviations: ed, ejaculatory duct; jod, junction of the ovovitelline ducts; jsd, junction of the sperm ducts and the vertical sperm duct; od, ovovitelline duct; p, penis; sd, sperm duct; shg, shell gland; vsd, vertical sperm duct.

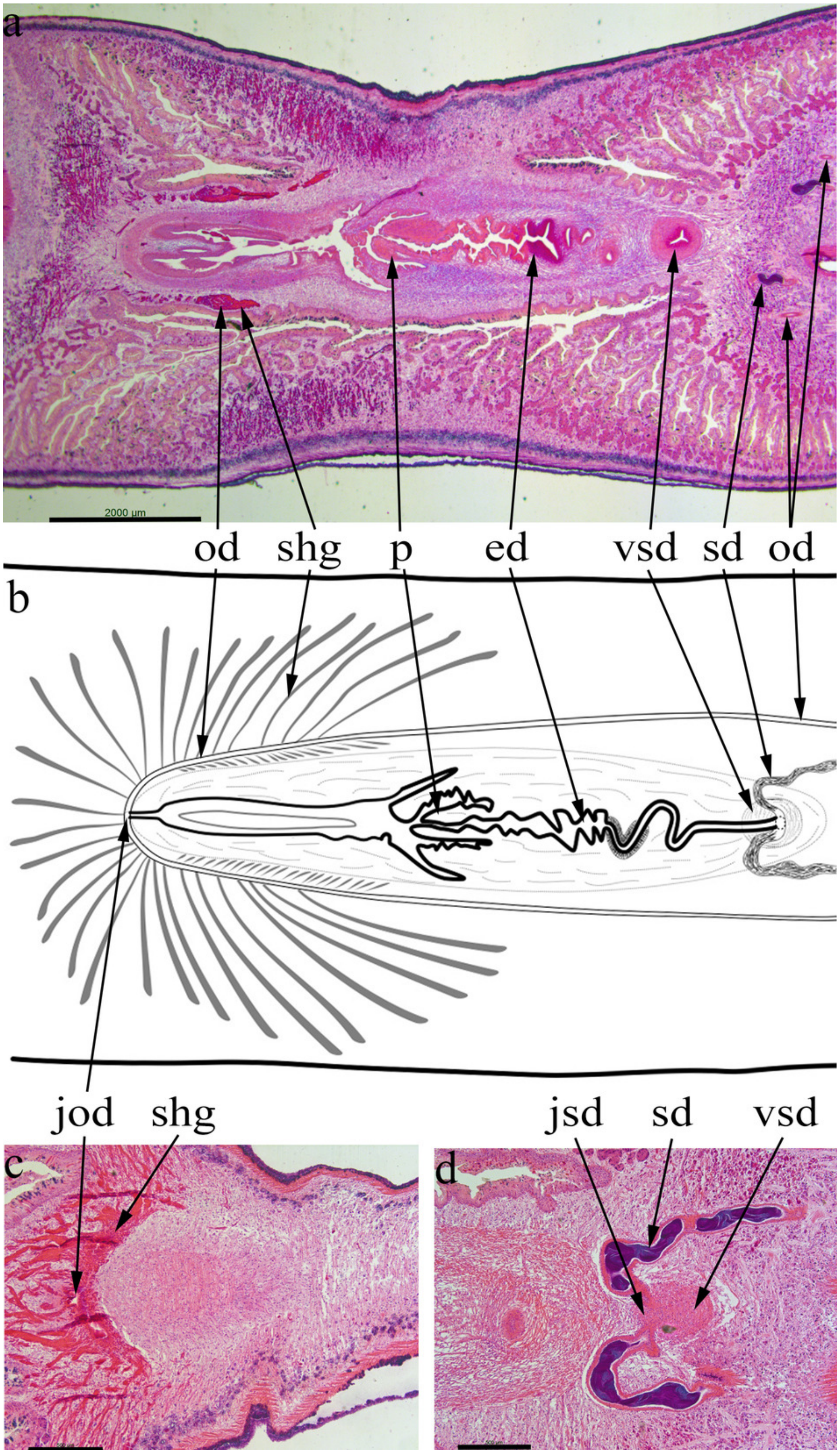
a
2000 µm
od
shg
p
ed
vsd
sd
od
b
jod
shg
jsd
sd
vsd
c
d

Figure 7

Amaga expatria, genomic map of the mitochondrial genome.

The mitogenome is 14,962 bp long and contains 12 protein coding genes, 2 ribosomal RNA genes and 22 transfer RNA genes. For 3 genes, atp6, cox2 and ND3, it was not possible to determine the start codon.

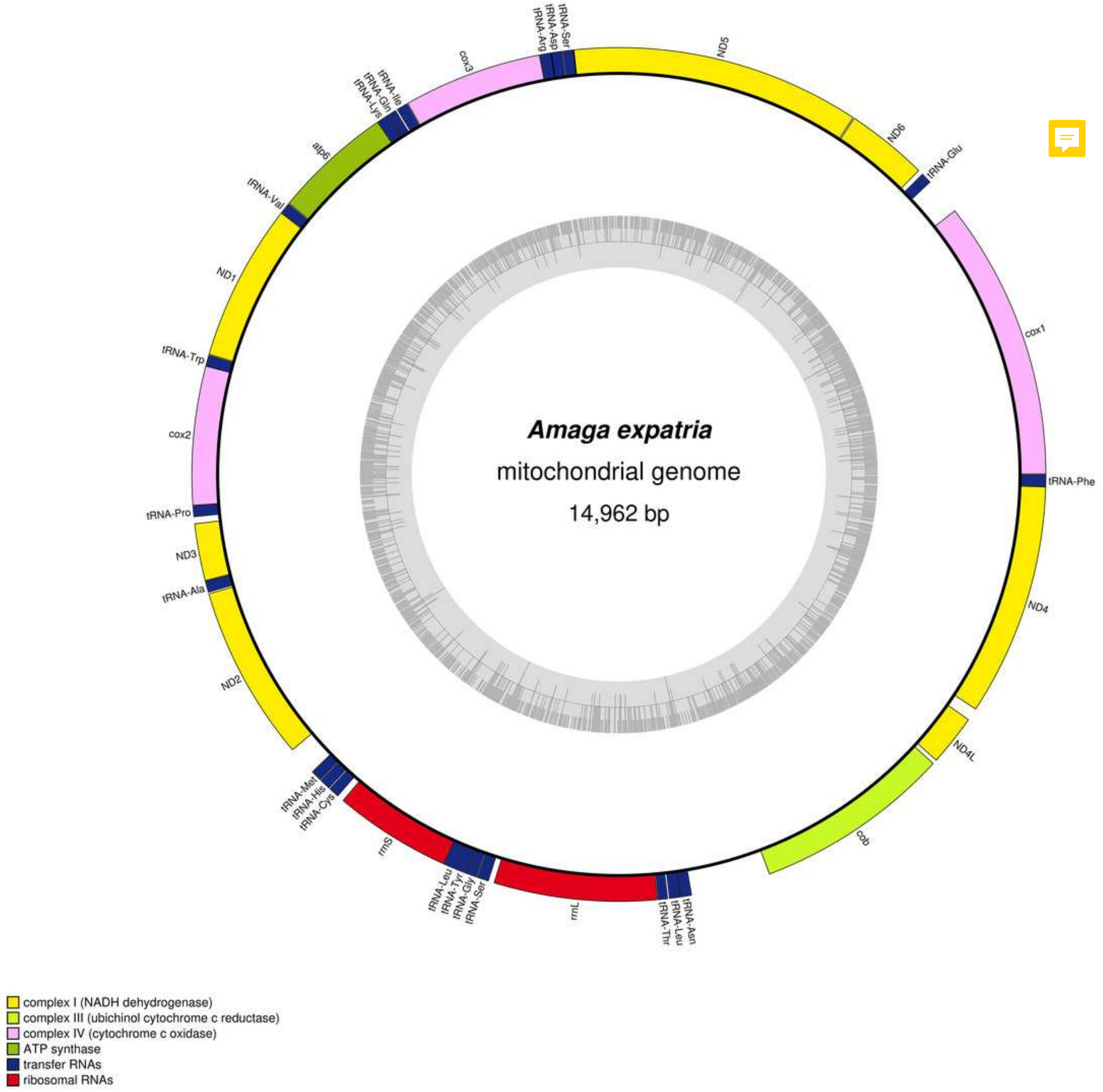
Amaga expatria
mitochondrial genome
14,962 bp
tRNA-Ser
tRNA-Asp
tRNA-Arg
ND5
ND6
tRNA-Glu
cox1
tRNA-Phe
ND4
ND4L
cob
tRNA-Asn
tRNA-Leu
tRNA-Thr
rrnL
tRNA-Ser
tRNA-Gly
tRNA-Tyr
tRNA-Leu
rrnS
tRNA-Cys
tRNA-His
tRNA-Met
ND2
tRNA-Ala
ND3
tRNA-Pro
cox2
tRNA-Trp
ND1
tRNA-Val
atp6
tRNA-Lys
tRNA-Gln
tRNA-Ile
cox3
complex I (NADH dehydrogenase)
complex III (ubichinol cytochrome c reductase)
complex IV (cytochrome c oxidase)
ATP synthase
transfer RNAs
ribosomal RNAs

Figure 8

Maximum likelihood tree of mitogenome proteins.

Mitogenome proteins were obtained from concatenated amino-acid sequences of all mitochondrial protein coding genes of *Amaga expatria* and other Platyhelminthes obtained using the MtArt model of evolution after 100 bootstrap replications. The tree with the best likelihood is shown (-62178.796969).

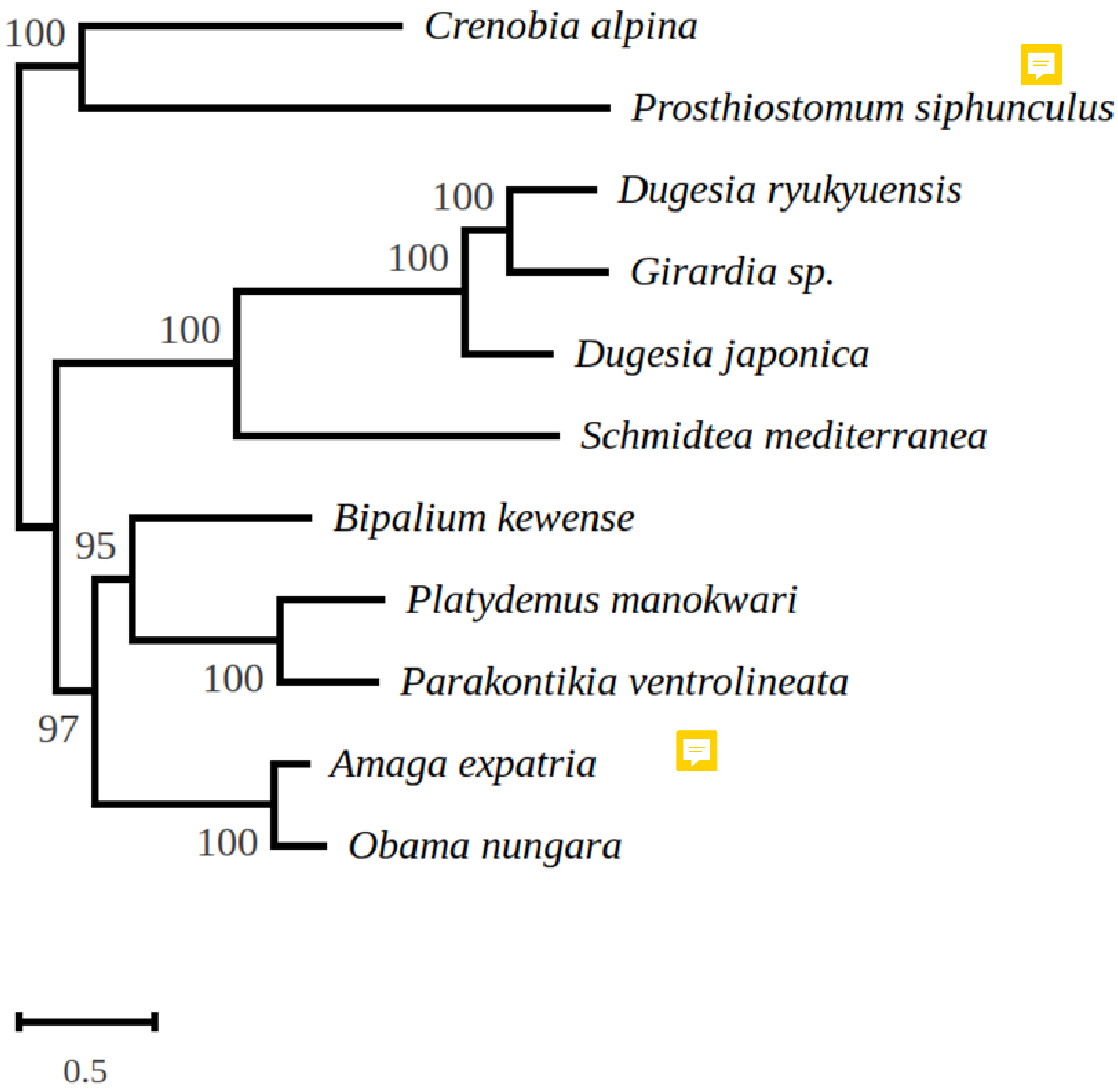
100
Crenobia alpina
Prosthiostomum siphunculus
100
Dugesia ryukyuensis
100
Girardia sp.
100
Dugesia japonica
Schmidtea mediterranea
Bipalium kewense
95
Platydemus manokwari
100
Parakontikia ventrolineata
97
Amaga expatria
100
Obama nungara
0.5

Table 1(on next page)

Records of *Amaga expatria* from Guadeloupe and Martinique.

The Table includes only observations based on photographs and specimens. François Meurgey provided additional findings in Guadeloupe: 10.10.2019, Goyave; 16.10.2019, Vieux-Fort; 22.06.2019, Petit Canal. * For MNHN JL305, we obtained both a COI sequence (GenBank xxxx) and the complete mitogenome (GenBank MT527191).

Date	Record (specimen number and/or photo)	Number of specimens	Commune	Department	COI Sequences	Collector/Observer
28/02/2006	photo	0	Case-Pilote	Martinique	no	Régis Delannoye
17/08/2013	photo	0	Case-Pilote	Martinique	no	Régis Delannoye
25/11/2013	movie	0	Fort de France	Martinique	no	Anonymous
20/12/2013	photo	0	Le Lamentin	Martinique	no	Pierre Damien Lucas
06/05/2014	JL146 + photo	1	Le Gros Morne	Martinique	xxxx	Clément Dromer
21/03/2015	photo	0	La Trinité	Martinique	no	Régis Delannoye
05/08/2015	JL262	1	La Trinité	Martinique	xxxx	Olivier Palcy
17/10/2015	photo	0	Le Gros Morne	Martinique	no	Pierre Damien Lucas
12/11/2015	JL305	1	Le Morne Vert	Martinique	xxxx *	Mathieu Coulis
13/11/2015	photo	0	Ducos	Martinique	no	Cedric Rareg
04/02/2016	JL289	1	Le Marigot	Martinique	xxxx	Régis Delannoye
18/06/2017	JL310 + photo	1	Le Lamentin	Martinique	xxxx	Mathieu Coulis
22/06/2017	photo	0	Fort de France	Martinique	no	Marcel Bourgade
27/01/2018	photo	0	Sainte-Luce	Martinique	no	Stéphane Bras
12/02/2019	photo	0	La Trinité	Martinique	no	Régis Delannoye
20/12/2012	photo	0	Trois Rivières	Guadeloupe	no	Guy van Laere
06/12/2014	JL216 + photo	1	Gourbeyre	Guadeloupe	xxxx	Laurent Charles
12/12/2014	JL217 + photo	1	Bouillante	Guadeloupe	xxxx	Laurent Charles
21/01/2016	photo	0	Baillif	Guadeloupe	no	Pierre et Claudine Guezennec
20/12/2017	JL319 + photo	1	Trois Rivières	Guadeloupe	xxxx	Guy van Laere
06/08/2018	photo	0	Sainte-Anne	Guadeloupe	no	Jean-Christian Rotger