Giant worms *chez moi!* Hammerhead flatworms (Platyhelminthes, Geoplanidae, *Bipalium* spp., *Diversibipalium* spp.) in metropolitan France and overseas French territories

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Background Species of the genera *Bipalium* and *Diversibipalium*, or bipaliines, are giants among land planarians (family Geoplanidae), reaching length of 400 mm; they are also easily distinguished from other land flatworms by the characteristic hammer shape of their head. Bipaliines, which have their origin in warm parts of Asia, are invasive species, now widespread worldwide. However, the scientific literature is very scarce about the widespread repartition of these species, and their invasion in European countries has not been studied. **Methods** In this paper, on the basis of a 4-year survey based on citizen science, which yielded observations from 1999 to 2017, we provide information about the 5 species present in Metropolitan France and French overseas territories. We also investigated the molecular variability of cytochrome-oxidase 1 (COI) sequences of specimens. **Results** Three species are reported from Metropolitan France: *Bipalium kewense*, *Diversibipalium multilineatum*, and an unnamed *Diversibipalium* "black" species. We also report the presence of *B. kewense* from overseas territories, such as French Polynesia (Oceania), French Guiana (South America), the Caribbean French islands of Martinique, Guadeloupe, Saint Martin and Saint Barthélemy, and Montserrat (Central America), and La Réunion island (off South-East Africa). For *B. vagum*, observations include French Guiana, Guadeloupe, Martinique, Saint Barthélemy, Saint Martin, Montserrat, La Réunion, and Florida (USA). A probable new species, *Diversibipalium* sp. "blue", is reported from Mayotte Island (off South-East Africa). *Bipalium kewense*, *B. vagum* and *D. multilineatum* each showed 0% variability in their COI sequences, whatever their origin, suggesting that the specimens are clonal, and that sexual reproduction is probably absent. COI barcoding was efficient in identifying species, with differences over 10% between species; this suggests that barcoding can be used in the future for identifying these

invasive species. In Metropolitan south-west France, a small area located in the Department of Pyrénées-Atlantiques was found to be a hot-spot of bipaliine biodiversity and abundance for more than 20 years, probably because of the local mild weather.
Discussion Our numerous records in the open in Metropolitan France raise questions: as scientists, we were astonished that these long and brightly coloured worms could escape the attention of scientists and authorities in a European developed country for such a long time; improved awareness about land planarians is probably necessary.

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Abstract

Background. Species of the genera *Bipalium* and *Diversibipalium*, or bipaliines, are giants among land planarians (family Geoplanidae), reaching length of 400 mm; they are also easily distinguished from other land flatworms by the characteristic hammer shape of their head. Bipaliines, which have their origin in warm parts of Asia, are invasive species, now widespread worldwide. However, the scientific literature is very scarce about the widespread repartition of these species, and their invasion in European countries has not been studied.

Methods. In this paper, on the basis of a 4-year survey based on citizen science, which yielded observations from 1999 to 2017, we provide information about the 5 species present in Metropolitan France and French overseas territories. We also investigated the molecular variability of cytochrome-oxidase 1 (COI) sequences of specimens.

Results. Three species are reported from Metropolitan France: *Bipalium kewense*, *Diversibipalium multilineatum*, and an unnamed *Diversibipalium* "black" species. We also report the presence of *B. kewense* from overseas territories, such as French Polynesia (Oceania), French Guiana (South America), the Caribbean French islands of Martinique, Guadeloupe, Saint Martin and Saint Barthélemy, and Montserrat (Central America), and La Réunion island (off South-East Africa). For *B. vagum*, observations include French Guiana, Guadeloupe, Martinique, Saint Barthélemy, Saint Martin, Montserrat, La Réunion, and Florida (USA). A probable new species, *Diversibipalium* sp. "blue", is reported from Mayotte Island (off South-East Africa). *Bipalium kewense*, *B. vagum* and *D. multilineatum* each showed 0% variability in their COI sequences, whatever their origin, suggesting that the specimens are clonal, and that sexual reproduction is probably absent. COI barcoding was efficient in identifying species, with differences over 10% between species; this suggests that barcoding can be used in the future for identifying these invasive species. In Metropolitan south-west France, a small area located in the Department of Pyrénées-Atlantiques was found to be a hot-spot of bipaliine biodiversity and abundance for more than 20 years, probably because of the local mild weather.

Discussion. Our numerous records in the open in Metropolitan France raise questions: as scientists, we were astonished that these long and brightly coloured worms could escape the attention of scientists and authorities in a European developed country for such a long time; improved awareness about land planarians is probably necessary.

Introduction

Land planarians (Platyhelminthes, Geoplanidae) are predatory soil-associated animals. Although small species (generally less than 1 cm in length) such as *Microplana* spp. or *Rhynchodemus* spp. are autochthonous in Europe (Álvarez-Presas et al. 2012), large species are not. Reports of invasive alien flatworms in Europe in recent years (Sluys 2016) include *Arthurdendyus triangulatus* from New Zealand, *Platydemus manokwari* originally from Papua New Guinea, *Obama nungara* from Brazil, and *Parakontikia ventrolineata*, *Caenoplana coerulea* and *Caenoplana bicolor* from Australia (see **Table 1** for authors of taxa and key references). All these species are conspicuous animals, several centimetres in length. Even larger are the species of *Bipalium* (and close genera), or "hammerhead flatworms": these can be longer than 20 centimetres (von Graff 1899). In this paper, we focus on these giant species, and we report new findings obtained mainly by citizen science in metropolitan France and overseas French territories in the Caribbean (Guadeloupe, Martinique, and Saint Barthélemy), South America (French Guiana) and Indian Ocean (La Réunion, Mayotte). Five species were found, among which three can be attributed to known binomial taxa (*Bipalium kewense*, *Bipalium vagum* and *Diversibipalium multilineatum*) and two are unnamed.

Winsor summarized knowledge about the world distribution of *Bipalium kewense,* listing the occurrence of the species in 39 territories (Winsor 1983a); by 2004 the species was recorded in 45 territories (Winsor et al. 2004), and subsequently reported in Northern and Peninsula Italy, Sardinia, and Sicily (Gremigni 2003); Czech Republic and Slovakia (Košel 2002); Cuba (Morffe et al. 2016); Ecuador (Wizen 2015); and Pakistan (M. Darley, personal communication to LW). As Sluys (2016) commented: "Almost every year *B. kewense* is found in new places: for example, this year (2016) it was found on São Miguel Island in the Azores and on São Tomé Island in the Gulf of Guinea". Although such reports from small remote islands are important for our knowledge of these invasive species (and we indeed add many new records of this type in this paper), we consider that the major finding of this paper is that several species of hammerhead flatworms are established in a European country, France, probably for more than 20 years. This highlights an unexpected blind spot of scientists and authorities facing an invasion by conspicuous large invasive animals.

The identification of land planarians from specimens or photographs is sometimes a futile exercise, in the absence of detailed anatomical study. In this paper, we tested identification with sequences of the cytochrome-oxidase 1 (COI). We confirm that barcoding with COI is efficient for the species studied here; in addition, our barcoding study revealed that all specimens in each species showed no genetic variability, suggesting that they are clonal, without sexual reproduction.

Material and methods

Citizen science and collection of information

In 2013, one of us (JLJ) organized a citizen science network in France for collecting information about land planarians. This included a blog (http://bit.ly/Plathelminthe) and a twitter account (https://twitter.com/Plathelminthe4). These efforts were advertised through the media (radio, television, and newspapers).

Reports of sighting of land planarians were received from citizens, mainly by email, sometimes by telephone. Photographs and details about locality were solicited, and only reports including this information were considered. Wrong records (slugs, myriapods, earthworms, leeches, caterpillars, nematomorphs, and nemerteans) were eliminated. Information collected from citizen science allowed monitoring of several land planarians (Justine et al. 2014a). Photographs were studied, and species were identified whenever possible. Only information relative to bipaliines is reported in this paper). Sometimes citizens provided records dating from before the survey, such as an amateur movie taken in 1999. Most citizens provided an authorisation to use the photographs at the time of the initial contact by email. When we prepared this paper for publication, we sought authorization to use the photographs and to publish them under a Creative Commons Licence; none of the citizens refused to provide the authorization, but some of them did not respond, probably simply because they changed their emails or did not check them. In this case, we provide the scientific information about the presence of species, but we do not include the photograph or the names of the citizen in the paper.

Although these efforts were originally aimed at collecting information from Metropolitan France, they unexpectedly reached French territories in other continents and provided additional information and specimens.

Collection of specimens

In some cases, after examination of photographs, specimens were solicited from citizens who reported sightings; they were sent either alive or in ethanol by the citizens, registered in the collections of the Muséum National d'Histoire Naturelle, Paris (MNHN), and processed for molecular analysis.

When specimens were obtained alive, they were fixed in hot water then preserved in 95% ethanol. In some cases, some specimens were also fixed in hot water and preserved in 4% formaldehyde solution.

Molecular sequences

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 (designated in this text as "short sequence") was amplified with the primers JB3 (=COI-ASmit1) (forward 5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and JB4.5 (=COI-ASmit2) (reverse 5′-TAAAGAAAGAACATAATGAAAATG-3′) (Bowles et al. 1995; Littlewood et al. 1997). The PCR reaction was performed in 20 µl, containing 1 ng of DNA, 1× CoralLoad PCR buffer, 3Mm MgCl2, 66 µM of each dNTP, 0.15µM 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), following (Mateos et al. 2013). PCR products were purified and sequenced in both directions on a 3730xl DNA Analyzer 96-capillary sequencer (Applied Biosystems). Results of both analyses were concatenated to obtain a COI sequence of 909 bp in length (designated in this text as "long sequence"). 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 xxxx-xxxx. For several specimens only "short" sequences were obtained (**Table 2**).

Trees and distances

MEGA7 (Kumar et al. 2016) was used to estimate genetic distances (kimura-2 parameter distance) and the evolutionary history was inferred from the kimura-2 parameter distance using

the Neighbour-Joining method (Saitou & Nei 1987); all codon positions were used, with 1000 bootstrap replications. The evolutionary history was also inferred using Maximum Likelihood (ML) method. The best evolutionary model for the data set was estimated in MEGA7 (Kumar et al. 2016) under the Bayesian Information Criterion (BIC) to be Hasegawa–Kishino–Yano model (Hasegawa, Kishino & Yano, 1985) with a discrete Gamma distribution and some sites invariables (HKY + G +I). The ML tree was computed in MEGA7, with 100 bootstrap replications. Both NJ and ML trees showed comparable topologies, but the bootstrap values of branches, in both trees, were contrasted: 100% for all branches representing species, and very low for upper nodes. We thus considered that the trees were informative for showing the genetic identity of all specimens within a species, but not for inferring relationships between taxa; thus, no further comment about interspecies relationships are given in the rest of this text.

A note about taxonomy of *Diversibipalium*

Morphology-based taxonomy of land planarians is based on a suite of characters, especially those afforded by internal anatomy, and in particular those of the reproductive system (Winsor et al. 1998). Reproductive organs are only available in sexually mature specimens and require extensive histological preparations for their description. Unfortunately, many species of land planarians have been described from external morphology only. Some species can use only asexual reproduction and thus do no show mature organs; this is especially the case of some invasive species when they are not in their region of origin. However, the bipaliines represent a special case because the external morphology, i.e. the presence of a “hammer” head is distinctive of the subfamily, which thus can be easily differentiated if a photograph of the head is available. The genus *Diversibipalium* Kawakatsu et al., 2002 is a collective group created to temporarily accommodate species of the subfamily Bipaliinae whose anatomy of the copulatory apparatus is still unknown (Kawakatsu et al. 2002). For this reason, we attribute our two undescribed species, “black” and “blue” to this genus. We insist that attribution of species to the genus *Diversibipalium* does not mean that these species have characters in common – the only feature they share is our ignorance of their internal anatomy.

Results

Collection of information from citizen science

After the initial finding in June 2013 of two species in his garden by Pierre Gros, an amateur entomologist and photographer, more than 600 reports were received over 4 years (June 2013-September 2017). Most records were from citizens, some from scientists or other professionals. Unexpectedly, these reports included mentions of more than 8 species of land planarians (Justine et al. 2014a), the most recent being *Marionfyfea adventor*. Among these, 106 reports concerned bipaliines. **Figure 1** is a map of these records in Metropolitan France.

Results are presented here as follows: after an assessment of the identification of specimens from both morphology and molecules, separate paragraphs provide, for each species, a brief description and its range in Metropolitan France and overseas French territories, from both sampled specimens and photographs obtained through citizen science.

Molecular identification of sampled specimens

Sequences were obtained from specimens belong to five species (**Table 2**), including three named species, *Bipalium kewense* (specimens from 13 localities, 17 sequences including replicates), *Diversibipalium multilineatum* (specimens from 4 localities, 8 sequences including replicates), *Bipalium vagum* (specimens from 3 localities, 5 sequences including replicates) and two unnamed species, *Diversibipalium* “black” (1 specimen from 1 locality, 1 sequence) and *Diversibipalium* “blue” (specimens from 2 localities, 6 sequences including replicates).

A tree was constructed from an analysis of our new COI sequences and sequences from GenBank. Each of the three-named species belonged to a clade with high (100%) bootstrap support (**Figure 2**). As explained in Materials and Methods, we do not comment upon the relationships between nodes because of very low bootstrap values.

For *Bipalium kewense*, the clade includes GenBank sequences from Spain, Acores Islands, and Cuba; our 13 new sequences (excluding replicates) are from 7 localities in metropolitan France, 3 overseas French territories (Guadeloupe, Martinique, French Guiana) and 2 other countries, Monaco and Portugal. All COI sequences were strictly identical.

For *Diversibipalium multilineatum*, the clade includes GenBank sequences from Italy and France (sequence from specimen MNHN JL177, already published (Mazza et al. 2016)), and our 6 new

sequences (excluding replicates) are from 3 localities in metropolitan France. All COI sequences were strictly identical.

For *Bipalium vagum*, no sequence was found in GenBank. Our 5 new sequences are from 1 overseas French territory (Guadeloupe) and 2 other countries, Montserrat (West Indies) and Florida, USA. All COI sequences were strictly identical.

For *Diversibipalium* "black" from Metropolitan France and *Diversibipalium* "blue" from Mayotte, each sequence was found to have no close match in GenBank sequences or our new sequences, suggesting that they each belong to a species which has never been sequenced for COI gene.

Distances between taxa

"Short" sequences were obtained from all specimens and "long" sequences" were obtained from only some of them. Distances between species of bipaliines were computed from two sets of sequences, "short" sequences and "long" sequences.

The first set included "short" sequences and 7 bipaliin taxa were available. Distances varied from 10.9% to 21.2% **(Table 3)**. The closest taxa were *B. kewense – D. multilineatum* with an interspecific distance of 10.9%, and the most distant were *Diversibipalium* "blue" and *B. adventitium* with 21.2%.

The second set included only "long" sequences and 4 bipaliin taxa were available. Distances were higher than with short sequences and varied from 15.9% to 25.9% **(Table 4)**. The closest taxa were, again, *B. kewense – D. multilineatum* with an interspecific distance of 15.9%, and the most distant were *Diversibipalium* "blue" and *D. multilineatum* with 25.9%.

Information for each species

Bipalium kewense **Moseley, 1878**

Morphology

A long, thin bipaliine, living specimens of B. kewense may attain a length of up to 350 mm. The dorsal ground colour is usually a light ochre **(Figure 3)**, with five black to grey-coloured longitudinal stripes: a median, paired lateral, and paired marginal stripes which begin at or near

the base of the headplate where it joins the body the "neck". The headplate **(Figure 4)** is usually the same colour as the body, or slightly darker, with recurved posterior margins. The median stripe is black, narrow, with sharp margins, extending caudally from below the neck over the entire body length, and is broadest over the pharyngeal area. Paired dark to pale brown coloured lateral stripes with diffuse margins, constant over the entire body length, are separated from the median and marginal stripes by an equal width of ground colour. The paired black, fine, marginal stripes, with sharp margins, extend the entire body length. The paired lateral and marginal stripes unite just behind the neck to form an incomplete black transverse neck band, interrupted dorsally by a small median gap, and ventrally by the creeping sole. The headplate is a greyish colour with a light ochre margin. The ventral surface **(Figure 5)** is a light ochre colour, with a distinct off-white creeping sole, delineated by paired, narrow, longitudinal diffuse grey-violet stripes beginning at the ventral termination of the collar, and extending the entire body length. In a preserved sexual specimen, the mouth is situated ventrally at 31.2 mm (39.7 % of the body length) from the anterior end, and gonopore 6 mm (7.7% of the body length) posterior to the mouth (Winsor 1983a). *Bipalium kewense* is differentiated externally from similar striped species by the incomplete black transverse band at the neck (the "collar"), the thin dorsal median longitudinal stripe that begins at or below the transverse neck band, the pattern and form of the dorsal and ventral stripes, and the relative position of body apertures.

Predation of earthworms

The main stages in *B. kewense* hunting, attacking, capturing, and consuming an earthworm are illustrated in **Figures 6-16.**

Morphological evidences of reproduction by scissiparity

Some 1-2 days following feeding, the fission process in *B. kewense* is first manifested by a slight pinching of the body, some 1-2 cm. from the tail tip **(Figures 17-18)**. Severance occurs when the tail tip adheres to the substratum and the rest of the planarian pulls away. The free tail fragment is immediately motile.

Remarks

We obtained 44 records of *B. kewense*, including 14 confirmed by molecules and 30 from photographs only (**Tables 2, 5**). Localities where bipaliines were found in the open, generally in gardens, include Portugal (1 record), Martinique (3), Guadeloupe (7), French Guiana (1), La

Réunion (1), Monaco (1), i.e. from 6 territories in 4 continents, and 30 from Metropolitan France (**Figure 1**), from 8 departments: Corse-Sud (Corsica) (2), Var (2), Gironde (1), Loire-Atlantique (1), Landes (3), Alpes-Maritimes (2), Yonne (1), and Pyrénées-Atlantiques (15). In addition, we received two reports in hothouses in the Department of Yonne. Among the 28 records in the open in Metropolitan France, 15, i.e. more than half, were from the department of Pyrénées-Atlantiques. The distribution of our records is shown in Figure 5 for Metropolitan France (including Corsica). Dates of records ranged 1999-2017; the oldest record (1999) was in the Pyrénées-Atlantiques.

The COI sequences were strictly identical for specimens from all localities where specimens were sequenced.

Earthworms appear to be the sole natural prey of *B. kewense*. The hunting, capture and consumption of this prey have been vividly described (Barnwell et al. 1965 ; Johri 1952; Lehnert 1891).

Diversibipalium multilineatum **(Makino and Shirasawa, 1982)**

Morphology

A long thin bipaliine, living specimens of *D. multilineatum* may attain lengths of more than 200 mm **(Figure 19)**. The dorsal ground colour including the headplate is usually a light brown-ochre with five evenly spaced, black to dark brown longitudinal stripes: a median, paired lateral, and paired marginal longitudinal stripes. The median stripe is black, and narrow with sharp margins. It has a pronounced characteristic oblanceolate shape beginning at the anterior third of the headplate **(Figure 20)**, then tapering to a thin dark stripe extending caudally along the entire body length, broadest over the pharyngeal area. Either side of the median stripe, each separated by an equal width of ground colour is a lateral stripe and submarginal stripe both of which join at the neck in the inner curvature of the headplate at the “neck”, and extend the entire body length. The lateral stripes are a black to dark brown colour with diffuse margins, approximately 2-3 times the width of the median stripe; the narrow, brown paired marginal stripes are approximately the same thickness as the median stripe. The ventral surface **(Figure 21)** is a light brown ochre colour, generally slightly paler than that dorsally, with a distinct white creeping sole, delineated by paired, narrow, longitudinal brown stripes beginning faintly on the anterior third of the headplate **(Figure 22)**, and extending the entire body length. A finer, generally discontinuous mid ventral

dark stripe extends from the anterior third of the ventral headplate to the posterior end **(Figure 23)**. The posterior margins of the headplate are not recurved, giving the head a club-shape. Preserved specimens are 24-138 mm long. In a preserved specimen (MNHN JL161A) 71 mm long and 2 mm wide, the mouth is situated ventrally at 71 mm (36.6% of the body length) from the anterior end. All specimens found to date are non-sexual and have no gonopore. They reproduce by scissiparity, evidenced by the puckered, or bluntly rounded posterior tip. *Diversibipalium multilineatum* is differentiated externally from similar elongate striped species by the oblanceolate-shape of the beginning of the median stripe on the headplate, presence of distinct dark paired ventral median stripes, the thin, dark, generally incomplete midventral longitudinal stripe, and the relative position of the mouth.

Remarks

We obtained 1 record of *D. multilineatum* from Switzerland and 16 records in the open in Metropolitan France, in the departments of Ariège (1), Haute-Garonne (3), Isère (2), Landes (2), Val d'Oise (2), and Pyrénées-Atlantiques (6). One record was confirmed two years in a row (2014-2015) in the same garden in Bellocq (Pyrénées-Atlantiques). In addition, two records were from hot-houses, in the Department of Lot (1) and Val d'Oise (1). Among the 16 records in the open in Metropolitan France, more than one third (6) are from the department of Pyrénées-Atlantiques. The distribution of our records is shown in Figure 5 for Metropolitan France (including Corsica). Dates of records ranged 2010-2017; the oldest record (2010) was in the Pyrénées-Atlantiques (**Tables 2, 6**).

As for *B. kewense*, the COI sequences of *D. multilineatum* were strictly identical for specimens from all localities.

***Bipalium vagum* Jones and Sterrer, 2005**

Morphology

A relatively small bipaliine, living specimens of this species attain a length of only some 35 mm. **Figures 24-27** show specimens from four localities, French Guiana, Guadeloupe, Martinique and La Réunion, with varied colour patterns. The dorsal ground colour is a pale brown, with three black to brown dorsal longitudinal stripes: a median sharply demarcated broad black stripe, and two lateral dark brown stripes, less sharply delineated, all beginning at the transverse neck band,

continuing the length of the full body, and often terminating in a well-defined black tip. The longitudinal stripes are separated from each other by an equal width of ground colour. Typically, the headplate ground colour is a dark brown marginally with two large grey-brown to black patches separated by an interval of lighter ground colour. The posterior margins of the headplate are slightly recurved. In a preserved sexual specimen 24 mm long and 1.5 mm wide, the mouth is situated ventrally at 12 mm (50% of the body length) from the anterior end, and the gonopore 5 mm (21% of the body length) posterior to the mouth (Jones & Sterrer 2005). *Bipalium vagum* is distinguished externally from species of similar morphology by the combination of characters, especially the transverse neck band that is continuous dorsally, from which the broad median black stripe originates.

Remarks

No record was obtained from Metropolitan France. We obtained 37 records, all in the open, from French Guiana (4 records) and from 5 islands in the West Indies, including Montserrat (1) and 4 French territories, namely Guadeloupe (8), Martinique (2), Saint Barthélemy (2), and Saint Martin (1), and, from the Indian Ocean island of La Réunion (15); specimens from Florida, USA, were also sequenced. Unfortunately, in spite of the many photographic records from La Réunion, no specimen was received for sequencing, but the morphology and colour pattern were similar to other localities (**Figures 24-27**). Dates of records ranged 2005-2017; the oldest record (2005) was from French Guiana (**Tables 2, 7**).

The COI sequences were strictly identical for specimens from all localities.

***Diversibipalium* sp. "black" from Metropolitan France**

Morphology

The dorsal ground colour of this small bipaliine is black, with no evidence of dorsal stripes **(Figures 28-30)**. The ventral surface is a light grey colour with paler creeping sole. The posterior margins of the headplate are not recurved, giving the headplate a shovel-shape. The living specimen attains a length of 20 – 25 mm. A preserved sexual specimen **(Figures 31-32)** is 20 mm long and 3.2 mm wide, with the mouth situated ventrally 6 mm (30 % of the body length) from the anterior end, and gonopore 1.8 mm (9% of the body length) posterior to the mouth.

This species was recorded in 2013 from a single garden in Saint-Pée-sur-Nivelle (Pyrénées Atlantiques) in which *B. kewense* was also present. According to the owner, the species was present for years in the garden and is still present in 2017.

Remarks

The COI barcode of this specimen is clearly different from all other known sequences. We can safely claim that this species has never been sequenced before. Whether the species is already described or not is not an easy question to answer, and would require examination of mature specimens; in the discussion we list a few species which have the same black colour pattern.

***Diversibipalium* sp. "blue" from Mayotte (Indian Ocean)**

Morphology

The headplate in this beautiful, small planarian is a rusty-brown colour that extends to some irregular patches on the "neck" **(Figures 33-37)**. The dorsal ground colour is an iridescent blue-green ("dark turquoise glitter"), and the ventral surface a very pale brown colour, with the creeping sole white to pale green. The iridescence and blue-green colour are lost on fixation, leaving a dark brown ground colour. The posterior margins of the headplate are not recurved, but rounded, giving the headplate a club-shape. The living specimens are about 45 mm in length. The preserved sexual specimen is 9 mm long and 1 mm wide, with the mouth situated ventrally approximately 3.5 mm (39% of the body length) from the anterior end, and gonopore 3 mm (33% of the body length) posterior to the mouth.

Remarks

We obtained records of this species only from Mayotte, from two independent observers, one who provided specimens and photographs and one who provided only photographs (**Tables 2, 8**).The COI barcode of this specimen is clearly different from all other known sequences. We can safely claim that this species has never been sequenced before. Whether the species is already described or not is not an easy question to answer; see the discussion.

Discussion

Validity of COI for barcoding of bipaliine flatworms

Barcoding based on sequences of the mitochondrial gene cytochrome c oxidase I (COI) has been proposed as a solution to the problem of species identification (Hebert et al. 2003). COI-based barcodes have been found to be effective in various groups, including butterflies (Lepidoptera) (Hebert et al. 2003) or fish (Ward et al. 2005). In flatworms (Platyhelminthes), although barcode based only on COI sequences might not be the best choice for some groups (Vanhove et al. 2013), recent studies showed that it efficiently differentiates species in groups such as monogeneans (Ayadi et al. 2017; Chaabane et al. 2016) and various triclads (Álvarez-Presas & Riutort 2014) including land planarians (family Geoplanidae) (Álvarez-Presas et al. 2011; Álvarez-Presas et al. 2014; Álvarez-Presas et al. 2012).

The present study shows that COI short sequences, easily obtained from almost all specimens, have inter-specific distances of 10.9-21.2% (**Table 3**). These interspecific distances are high enough to differentiate species of bipaliines, especially in the absence of intra-species variation. Long sequences provide even higher inter-specific distances, ranging 15.9-25.9% (Table 4) but these are less easily obtained, and the database includes only four species. Of course, it might be objected that the current database (7 species with short sequences) is extremely limited in comparison to the number of species described in the bipaliines – more than 160 (Winsor 1983a). However, the current database includes most invasive world-wide species, inter-specific distances are high, and intra-specific variation was almost inexistent for most species. For these reasons, we believe that identification of common invasive species of bipaliine flatworms can reliably be done from COI barcoding. Barcoding can be done from a very small worm, immature, or even a fragment. Moreover, COI barcoding can probably alert authorities to the presence of species not previously sequenced, if a sequence different from those reported in the present study is found.

The fact that some bipaliines do not reproduce sexually outside their native habitat or tropical and subtropical climates, but only by scissiparity, is probably one reason explaining why no variability was found in specimens, since specimens are cloned, and no or very few mutations can happen. However, this reason is not sufficient, since several populations from various origins, each cloning itself, could be present in the world. In contrast, for *Platydemus manokwari*, COI sequences demonstrated the existence of at least two haplotypes in the world, probably corresponding to two populations and different ways of invasion of the world (Justine et al.

2015). Our current data on bipaliines suggest that one population is at the origin of the invasion for each species. This is particularly striking for *B. kewense*, with identical molecular records from several continents.

Possible specific identity of the "black" and "blue" species

Diversibipalium sp. "Black"

In the absence of detailed data in the literature, it is difficult at present to determine whether *Diversibipalium* sp. 1 "Black" is a new species, or one of the small black species of *Diversibipalium* such as *D.* sp. Kuamoto (Kawakatsu et al. 2005).

Diversibipalium sp. "Blue"

There are no other reports of a bipaliin planarian with this morphology, and the species is thus probably new.

Predation in bipaliines

Land planarians have toxins that may have both a repugnatorial effect on predators, and a toxic effect on prey (Winsor 1998a). Two toxins were identified in *B. kewense*, namely a cardiotoxin localized in the dermal mucus, and a haemolytic toxin that was distributed throughout the planarian body (Arndt 1925). More recently, the presence of tetrodotoxin (TTX), hitherto unknown in terrestrial invertebrates, was demonstrated throughout the body of *Bipalium kewense* with the highest concentration of TTX in the anterior region (Stokes et al. 2014). The headplate and body at the base of the head of *Bipalium* are richly supplied with subepidermal glands that discharge through the ventral surface. Some secretions have an adhesive function (Winsor 1998a) and are possibly responsible to adhering the *Bipalium* to the earthworm prey during the initial contact. Other secretions, of which there are at least three types, may include toxins and enzymes (Winsor 1998a). Severe paralysing effects noted in earthworms touched by *B. kewense* (Johri 1952) suggest a neurotoxic effect possibly due to substances present in the epidermal secretions (Winsor 1998a). Recent results (Stokes et al. 2014) suggest that some release of TTX occurs from the head of *B. kewense* when it uses its body to cover the anterior end of the earthworm (described as capping (Ducey et al. 1999)), and some release of TTX from the mouth during feeding, but did not confirm the release of TTX in the paralysis observed in earthworms in their study; they also suggest that the presence of TTX throughout the planarian body may indicate a possible role in defence.

The pharyngeal region of *B. kewense* contains a collagenolytic enzyme which may function in the digestion of its natural prey, earthworms. The susceptibility of earthworm cuticle collagen, compared to guinea pig collagen, to the collagenase secreted by *B. kewense* may reflect adaptation of the enzyme to its physiological substrate, although the biochemical basis for this observation is not known (Landsperger et al. 1981).

Persistence of *Bipalium kewense* and *Diversibipalium multilineatum* in the open in Metropolitan France

Bipalium kewense was originally described from specimens in the hot-house in Kew, United Kingdom (Moseley 1878). Originally from Vietnam to Kampuchea, the species is currently cosmopolitan (Winsor 1983a). However, distinctions are important between a species which is found only in protected and restricted constructions such as hot-houses, and species which can freely live and reproduce in the open. Clearly, *B. kewense* is an invasive species in the open in countries with tropical moist or humid semitropical climates, and appears to be restricted to anthropogenically-modified habitats; this is the case in the Caribbean, such as Guadeloupe or Martinique from where we obtained specimens. However, until recently (Justine et al. 2014b), it was considered that *B. kewense*, in Europe, was only confined to hot-houses and thus not an invasive species. Examination of literature and citizen-science information (**Figure 1**) now proves otherwise.

In France, the outdoors occurrence of *B. kewense* was reported in Orthez and Bayonne in 2005 (Vivant 2005). Through citizen science, we obtained a movie of the worm filmed in the nearby locality of Urcuit in 1999. Moreover, we obtained information about the presence of the species in Arthez de Béarn, Hasparren, Villefranque, Urt (all in 2014), near Jurançon (2016), Nay (2016) and Saint Jean de Luz (2016), Ustaritz (2017) and, as in the report by Vivant, in Bayonne and Orthez again (2014). We have obtained specimens from Saint-Pée-sur-Nivelle (2013), Ustaritz (2014), Bassussary (2014) and Orthez (2014). All these localities are in the Department of Pyrenées-Atlantiques, and we also have three records from the Department of Landes, north of Pyrenées-Atlantiques, along the Atlantic coast. The remark by Vivant that the animal was collected “five times in the last 20 years”, the record from 1999, and the recent record and specimens in the same locality (Orthez) in 2014 strongly suggests that the species is now established in the open in Orthez and in several localities of the Department of Pyrenées-Atlantiques (**Figure 38**). An alternative hypothesis would be that a single plant nursery near

Bayonne acts as a continuing reservoir of planarians and that all these records are in fact specimens that escaped from recently bought plants, but which subsequently died after being released in the open; this hypothesis is falsified by records over several years in similar localities. The results presented here thus demonstrate that *B. kewense* clearly merits the status of invasive alien species in France, at least in the Department of Pyrenées-Atlantiques. However, all records are from gardens, and it is not known whether the species has invaded non-anthropised areas.

We briefly comment the climate of this region. The department of Pyrenées-Atlantiques is the most southern department on the Atlantic coast of France; it includes a mountainous region and a low altitude region along the ocean. The latter has an Atlantic climate. Within the department, we note that most records (Nay, Urcuit, Urt, Saint-Jean-de-Luz, Saint-Pée-sur-Nivelle, Ustaritz, and Bassussary) are from a small area around Bayonne, along the Atlantic coast (**Figure 38**). The major limiting factor for a tropical species in Europe is, of course, low temperature. For a land planarian which is sensitive to drought and freezing, the numbers of days of drought in summer and the number of days of freezing temperature in winter are also important limiting factors. Detailed meteorological records are available for Biarritz, a locality close to Bayonne (Infoclimat 2017): annual mean temperature is 13.7 °C, annual rain is 1483 mm, even the dryer months (July and August) show a mean of 9-10 days with rain, and days with temperature < to -5°C are only 1.5/year. This suggests that this region is particularly suitable for land planarians. Other localities in the south of France, such as Department of Var and Alpes-Maritimes, and Corsica, both in Mediterranean climate, have higher temperatures and thus could be more suitable for tropical species, but they have longer periods of drought in summer (Infoclimat 2017).

Interestingly, one record of *Diversibipalium multilineatum* is also from the same department, in Bellocq (with records on two years), and the single record of *Diversibipalium* sp. “black” is also from the same department, in Saint-Pée-sur-Nivelle, in a garden where *B. kewense* is also present. Other invasive land planarians found in the Pyrenées-Atlantiques include *Obama nungara*, *Caenoplana bicolor* and *Parakontikia ventrolineata* (unpublished results – (Justine 2017)). With a total of six species of invasive flatworm, clearly the Pyrenées-Atlantiques department is a hot spot of diversity and a small paradise for invasive land planarians!

For *Diversibipalium multilineatum*, we have also two records in the same gardens in two consecutive years (**Table 6**). This suggests that this species also is established in the open in

Metropolitan France, but the total number of records is lower (16 vs 28 for *B. kewense*). One of the records was of hundreds of animals.

A more detailed assessment of the ecoclimatic and other data for the distribution of invasive land planarians in France and French Territories is beyond the scope of this paper.

Bipalium kewense is of marginal medical and veterinary importance because of its implication in cases of pseudoparasitism in humans and domestic animals (Winsor 1980). It has also been implicated in cases of vomiting in cats though the possible role of planarian toxins in this involuntary response is uncertain (Winsor 1983b). *Bipalium kewense* is also of commercial importance as a minor pest in earthworm farms in the U.S. (Winsor 1983a), and Australia (Winsor 1998b).

Additions to the list of invasive species in Metropolitan France and overseas territories

From the observations reported here, we can add new species to the list of invasive land planarians in Metropolitan France and overseas territories, with confirmed identifications (**Figure 39**):

- *Bipalium kewense* in Metropolitan France including Corsica, and in Guadeloupe, Martinique, La Réunion, French Polynesia and French Guiana.
- *Bipalium vagum* in Guadeloupe, Martinique, Saint Barthélemy, Saint Martin and La Réunion. No record from Metropolitan France.
- *Diversibipalium multilineatum* in Metropolitan France. No record from overseas territories.

In addition, two unnamed species, *Diversibipalium* “black” in Metropolitan France and *Diversibipalium* “blue” in Mayotte (Indian Ocean), are recorded.

How could 40-cm long invasive worms escape the attention of the scientists for 20 years?

At the beginning of our study, we were intrigued by the almost total absence of published information about the presence of bipaliines in France. The record by Vivant (2005) was the only one we could find, and since it was published in a rather obscure mycological journal, it certainly did not receive national nor international attention. Moreover, we are still astonished by the complete lack of response from scientific authorities at the presence of these worms. One of the

early records we received (2013) was from a kindergarten in which the children were reportedly scared by hundreds of "small snakes" on the grass (these were later identified as *D. multilineatum*). We also received a report of a citizen who showed a long hammerhead worm found on the fur of her cat to its veterinarian and was told it was a tapeworm (cestode). Other citizens explained that they tried to obtain identifications of land planarians from local universities and were told that the worms were leeches, and/or plain, uninteresting animals. Invasive land planarians were not known in France 10 years ago (Justine et al. 2014a) and the professionals involved in these anecdotes probably were never taught about them. Clearly, more education is needed about land planarians, which, in Europe, will be more and more often encountered by citizens and professionals in agriculture, landscaping, veterinary science and medicine.

It is also astonishing that the presence of such conspicuous animals never provoked a response from scientific authorities, although reports of tiny insect invasives often are followed by appropriate measures; again, the ignorance of professional scientists, science technicians, and amateur naturalists about land planarians was probably the reason. It is significant, in this respect, that the first recent mention of land planarians in France, by one of us (PG) was made public in an internet forum dedicated to insects. We expect that the measures taken at the European level will increase information about land planarians in the future (Tsiamis et al. 2016).

Conclusion

In this paper, we reported five species of Bipaliine worms from Metropolitan France, a few European countries, and overseas French territories in three continents: much remains to be done, including a formal description of the two-unnamed species. Of course, the results recorded here are only a very small part of the spread of these invasive species in the World. Initiatives like ours, including Citizen Science and molecular studies of selected specimens, should be undertaken worldwide. We have shown that molecular barcoding was efficient for the identification of the five species studied here, thus providing tools for future studies. We presented evidence that several species are spreading and that at least one of them is a predator of earthworms, which are important constituents of the soil fauna (Jones et al. 2001; Murchie & Gordon 2013). Recently, a tendency to deny the risks posed by non-native species has emerged

(Ricciardi & Ryan 2017); in opposition to this 'denialism', we strongly believe that invasive flatworms, as active predators, constitute a danger to native fauna wherever they are introduced.

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Figure 1

Map of Metropolitan France (including Corsica) showing records of Bipaliine flatworms.

Most records reported in this paper are outdoor but two are from hothouses. Note the concentration of records in the southern-east region, in the Department of Pyrénées-Atlantiques.

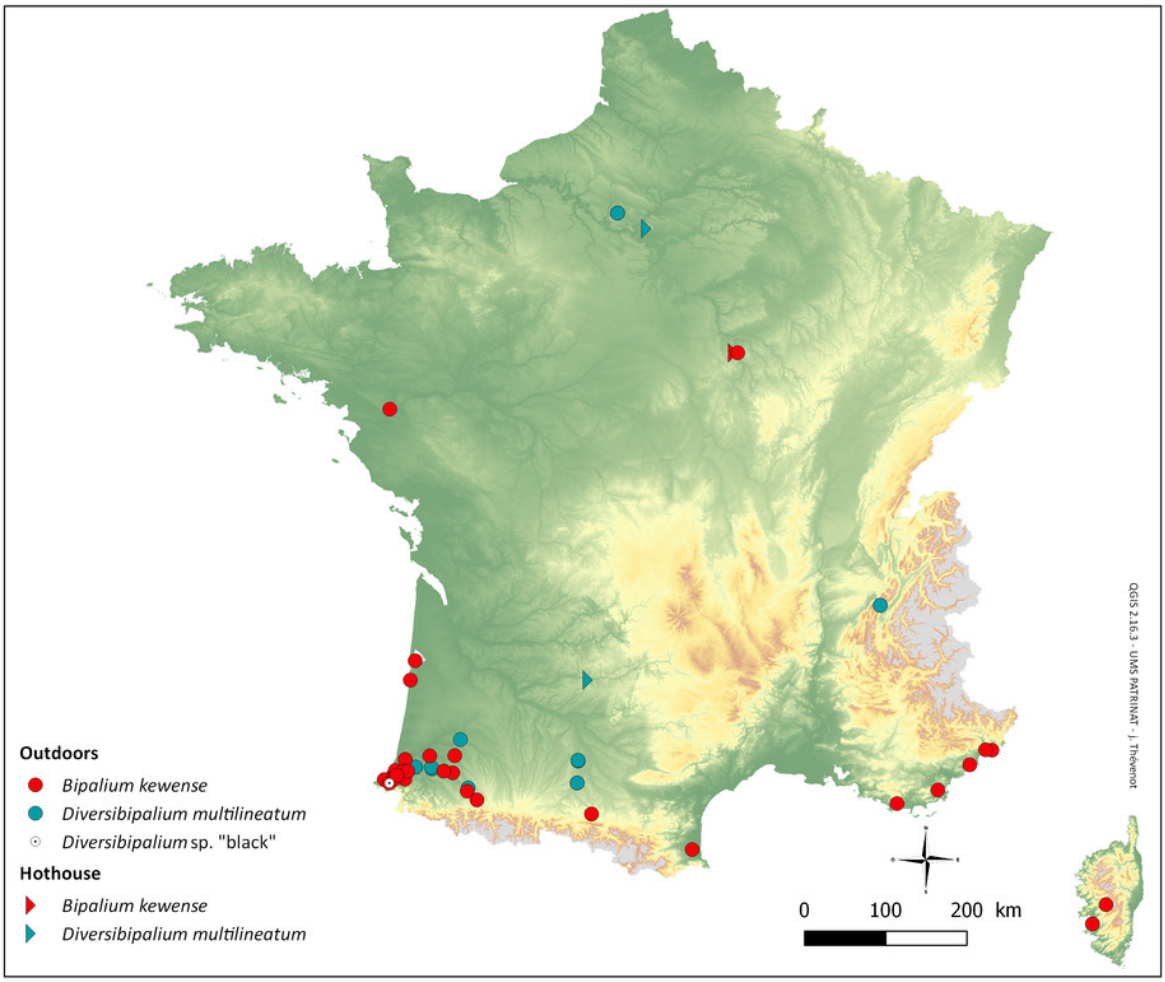

Figure 2

Evolutionary relationships of taxa.

The tree shown was inferred using the Neighbour-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches, only when >70. The evolutionary history inferred by Maximum Likelihood method had similar topology. In both trees, branches representing the four species with several samples (*Bipalium kewense*, *Bipalium vagum*, *Diversibipalium multilineatum* and *Diversibipalium* 'Blue') all had 100% bootstrap values, but bootstrap values for upper nodes were very low. We consider that the tree is informative for showing the genetic identity of all specimens within a species, but not for inferring relationships between taxa. New records with molecular information are indicated by *. For records in Metropolitan France, the number indicates the department code (i.e. 64: Pyrénées-Atlantiques).

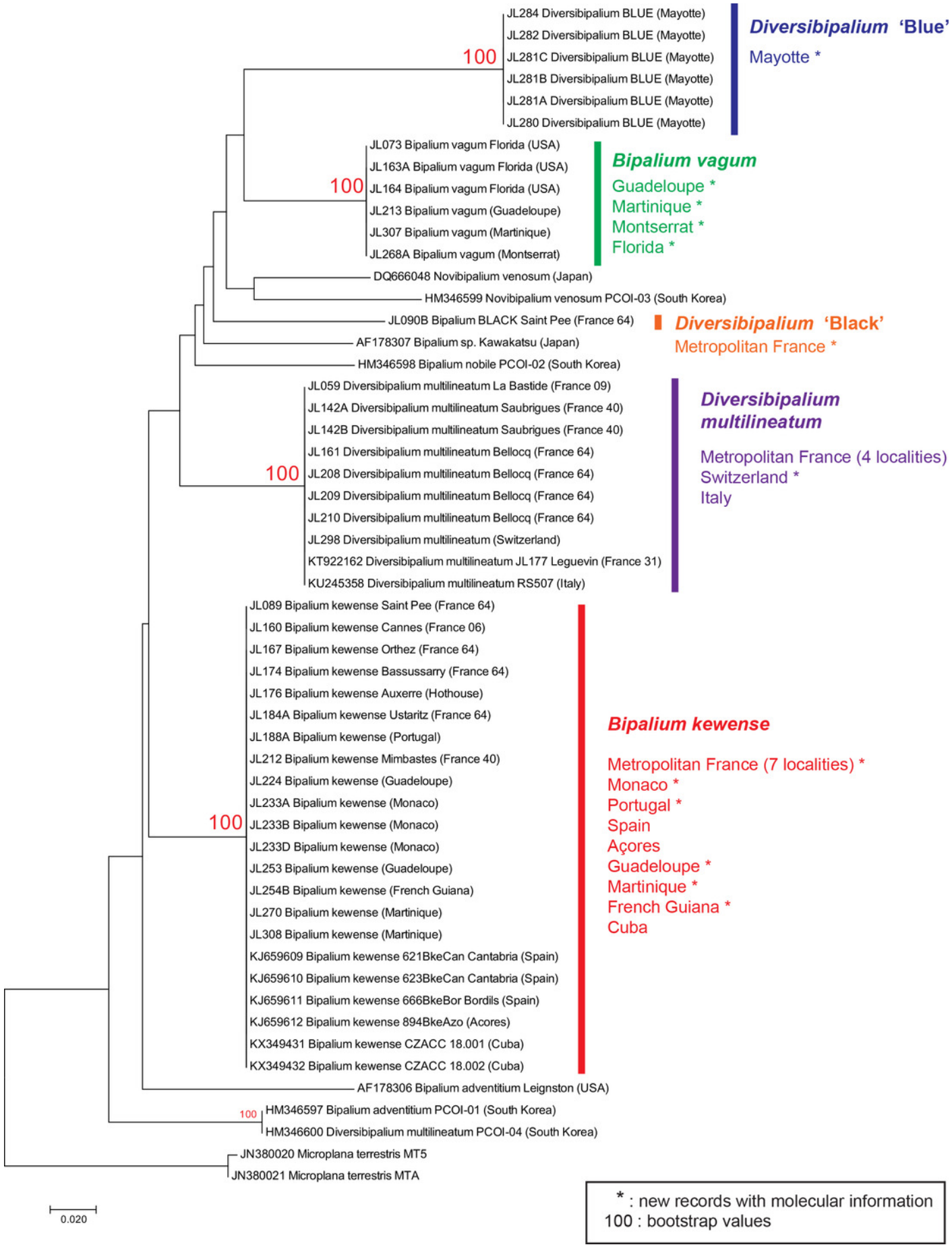
JL284 Diversibipalium BLUE (Mayotte)
JL282 Diversibipalium BLUE (Mayotte)
100
JL281C Diversibipalium BLUE (Mayotte)
JL281B Diversibipalium BLUE (Mayotte)
JL281A Diversibipalium BLUE (Mayotte)
JL280 Diversibipalium BLUE (Mayotte)
Diversibipalium 'Blue'
Mayotte *
JL073 Bipalium vagum Florida (USA)
JL163A Bipalium vagum Florida (USA)
100
JL164 Bipalium vagum Florida (USA)
JL213 Bipalium vagum (Guadeloupe)
JL307 Bipalium vagum (Martinique)
JL268A Bipalium vagum (Montserrat)
Bipalium vagum
Guadeloupe *
Martinique *
Montserrat *
Florida *
DQ666048 Novibipalium venosum (Japan)
HM346599 Novibipalium venosum PCOI-03 (South Korea)
JL090B Bipalium BLACK Saint Pee (France 64)
Diversibipalium 'Black'
Metropolitan France *
AF178307 Bipalium sp. Kawakatsu (Japan)
HM346598 Bipalium nobile PCOI-02 (South Korea)
JL059 Diversibipalium multilineatum La Bastide (France 09)
JL142A Diversibipalium multilineatum Saubrigues (France 40)
JL142B Diversibipalium multilineatum Saubrigues (France 40)
JL161 Diversibipalium multilineatum Bellocq (France 64)
100
JL208 Diversibipalium multilineatum Bellocq (France 64)
JL209 Diversibipalium multilineatum Bellocq (France 64)
JL210 Diversibipalium multilineatum Bellocq (France 64)
JL298 Diversibipalium multilineatum (Switzerland)
KT922162 Diversibipalium multilineatum JL177 Leguevin (France 31)
KU245358 Diversibipalium multilineatum RS507 (Italy)
Diversibipalium multilineatum
Metropolitan France (4 localities)
Switzerland *
Italy
JL089 Bipalium kewense Saint Pee (France 64)
JL160 Bipalium kewense Cannes (France 06)
JL167 Bipalium kewense Orthez (France 64)
JL174 Bipalium kewense Bassussarry (France 64)
JL176 Bipalium kewense Auxerre (Hothouse)
JL184A Bipalium kewense Ustaritz (France 64)
JL188A Bipalium kewense (Portugal)
JL212 Bipalium kewense Mimbastes (France 40)
JL224 Bipalium kewense (Guadeloupe)
JL233A Bipalium kewense (Monaco)
100
JL233B Bipalium kewense (Monaco)
JL233D Bipalium kewense (Monaco)
JL253 Bipalium kewense (Guadeloupe)
JL254B Bipalium kewense (French Guiana)
JL270 Bipalium kewense (Martinique)
JL308 Bipalium kewense (Martinique)
KJ659609 Bipalium kewense 621BkeCan Cantabria (Spain)
KJ659610 Bipalium kewense 623BkeCan Cantabria (Spain)
KJ659611 Bipalium kewense 666BkeBor Bordils (Spain)
KJ659612 Bipalium kewense 894BkeAzo (Acores)
KX349431 Bipalium kewense CZACC 18.001 (Cuba)
KX349432 Bipalium kewense CZACC 18.002 (Cuba)
Bipalium kewense
Metropolitan France (7 localities) *
Monaco *
Portugal *
Spain
Açores
Guadeloupe *
Martinique *
French Guiana *
Cuba
AF178306 Bipalium adventitium Leignston (USA)
100
HM346597 Bipalium adventitium PCOI-01 (South Korea)
HM346600 Diversibipalium multilineatum PCOI-04 (South Korea)
JN380020 Microplana terrestris MT5
JN380021 Microplana terrestris MTA
0.020
* : new records with molecular information
100 : bootstrap values

Figure 3

Bipalium kewense, general morphology.

Dorsal aspect of the planarian with a partial view of the ventral surface. Note the rounded posterior end indicating reproduction by scissiparity. Photo by Pierre Gros.

Figure 4

Bipalium kewense, general morphology of the dorsal anterior end.

The expanded headplate, transverse black band (“collar”) at the neck, and the median, paired lateral and marginal dorsolateral dark longitudinal stripes are evident. Note that the median dorsal stripe does not pass onto the headplate. Photo by Pierre Gros.

Figure 5

Bipalium kewense, general morphology, ventral surface.

The dark transverse neck band is incomplete ventrally, and the paired diffuse grey-purplish stripes delineate the off-white creeping sole. The position of the mouth is indicated by *, and the approximate position of the plicate protrusible pharynx within the body is evident as the pale area either side of the mouth. Photo by Pierre Gros.

Figure 6

Bipalium kewense, side view of the headplate.

Bipalium kewense hunts its earthworm prey using mechanoreceptors and chemoreceptors located along the leading margin of the headplate. These receptors are exposed when the papillae around the headplate are distended and moved like stubby fingers in an undulating motion to sense the environment, seen in this image. The under surface of the headplate is richly endowed with a variety of glands that include secretions with adhesive, lubricating and probably toxin-related functions. Photo by Pierre Gros.

Figure 7

Bipalium kewense, hunting its earthworm prey.

Having located its prey, the planarian has bunched its body ready to rapidly extend along the earthworm. Photo by Pierre Gros.

Figure 8

Bipalium kewense, attacking its earthworm prey.

Having found its prey, the planarian rapidly glides along the earthworm's body towards the head. Photo by Pierre Gros.

Figure 9

Bipalium kewense, capping its earthworm prey

Bipalium kewense then initiates the process of “capping” the anterior end of the earthworm. Observed reactions of the prey suggest that it is at this stage that the planarian secretes a toxin to reduce prey mobility. The planarian also produces secretions from its headplate and body that adhere it to the prey, despite often sudden violent movements of the latter during this stage of capture. Photo by Pierre Gros.

Figure 10

Bipalium kewense, initiating envelopment of the earthworm.

The planarian rapidly overlies the earthworm, covering the body of the prey with folds of its own body very close to each other. This provides protection for the delicate exposed plicate pharynx during the subsequent pre-digestion process. Note the flattening of that part of the planarian's body on the substratum that anchors the prey, further reducing movement. After a while the earthworm ceases to move. The process of attack and capture can take less than a minute. Photo by Pierre Gros.

Figure 11

Bipalium kewense, feeding on the prey.

The plicate pharynx is extended like a thin translucent veil over the body of the earthworm. The pharyngeal secretions include a collagenase that is particularly active against earthworm cuticle. The resulting pre-digested slurry is transferred to the planarian gut by action of the cilia lining the inner pharynx. Photo by Pierre Gros.

Figure 12

Bipalium kewense, continuing digestion of the earthworm.

As the earthworm is digested, the planarian progressively gathers the body of the prey into an increasingly tight mass to facilitate envelopment of the latter by the pharynx and the feeding process. Photo by Pierre Gros.

Figure 13

Bipalium kewense, continuing feeding.

Feeding can take from about half an hour up to four hours (Barnwell et al. 1965). Photo by Pierre Gros.

Figure 14

Bipalium kewense, concluding feeding.

After feeding, little remains of the prey. Photo by Pierre Gros.

Figure 15

Bipalium kewense, conclusion of feeding.

Having finished feeding, *Bipalium* crawls off over the bolus of prey remains. Photo by Pierre Gros.

Figure 16

Bipalium kewense, remains of the earthworm prey.

The scant remains of the earthworm following the attack, capture and feeding by the planarian. Photo by Pierre Gros.

Figure 17

Bipalium kewense, reproduction by scissiparity.

Some 1-2 days following feeding, the fission process is first manifested by a slight pinching of the body, some 1-2 cm. from the tail tip. Severance occurs when the tail tip adheres to the substratum and the rest of the planarian pulls away. Sexual reproduction outside their native habitat is restricted to individuals occupying outdoor situations in tropical or subtropical climates. Elsewhere they reproduce asexually. The links between sexuality and climate, and switching between scissiparity and egg cocoon production, indicate that several interacting factors are involved, not least the availability of food and climatic variability (Winsor et al. 2004). Photo by Pierre Gros.

Figure 18

Bipalium kewense, reproduction by scissiparity – the shed tail fragment.

The free tail fragment is immediately motile. It develops a head and pharynx within 7-10 days, and within 2-3 weeks it is adult in form and behaviour (Connella & Stern 1969). Asexual reproduction in *B. kewense* and some other land planarians is considered to underlie the colonizing success of these species (Hyman 1951, p. 163). Photo by Pierre Gros.

Figure 19

Diversibipalium multilineatum, general morphology.

Dorsal aspect with a partial view of the ventral surface. The dark dorsal median stripe extends onto the headplate, and the headplate is more rounded than the falciform headplate of *Bipalium kewense*. Note the rounded posterior end of the body indicating reproduction by scissiparity. Photo by Pierre Gros.

Figure 20

Diversibipalium multilineatum, headplate.

On the headplate, the dark median dorsal stripe begins at the anterior third of the headplate and has a pronounced characteristic oblanceolate shape. Photo by Pierre Gros.

Figure 21

Diversibipalium multilineatum, general morphology, ventral surface.

The three dark longitudinal stripes begin at the “neck” and extend the length of the body. The position of the mouth is indicated by *, and the approximate position of the plicate protrusible pharynx within the body is evident by the diffuse line of the median stripe in this region. Photo by Pierre Gros.

Figure 22

Diversibipalium multilineatum, general morphology, anterior end.

The lateral dorsal stripes begin immediately behind the headplate. A transverse dark band ("Collar") is absent. Photo by Pierre Gros.

Figure 23

Diversibipalium multilineatum, ventral headplate morphology.

The fine, generally discontinuous mid ventral dark stripe extends from the anterior third of the headplate to the posterior end. There are also faint indications of the beginnings of the ventral paired lateral stripes on the headplate. Photo by Pierre Gros.

Figure 24

Bipalium vagum. Specimen from French Guiana.

The dorsal marking on this specimen are typical of the species. Note the dark patches on the headplate, continuous neckband, black median stripes, brown paired lateral stripes, and caudal black tip. Photo by Sébastien Sant, Parc Amazonien de Guyane.

Figure 25

Bipalium vagum. Specimen from Guadeloupe, West Indies.

This specimen exhibits very light pigmentation, especially on the headplate, the indistinct brown paired lateral stripes and the caudal tip. Photo by Pierre and Claudine Guezennec.

Figure 26

Bipalium vagum. Specimen from Martinique, West Indies.

In this specimen the headplate exhibits marked pigmentation so that it appears almost black. Photo Mathieu Coulis.

Figure 27

Bipalium vagum. Specimen from La Réunion, Indian Ocean.

This specimen exhibits typical markings of the species. The paired dark patches on the headplate, and the dark pigmented caudal tip are clearly shown. Photo by Dominique Martiré.

Figure 28

Diversibipalium sp. ‘black’ from Metropolitan France.

The dorsal ground colour of this small planarian is black, with no evidence of dorsal stripes – what appear to be light coloured stripes are reflected highlights. Photo Marc Gauthier.

Figure 29

Diversibipalium sp. ‘black’ from Metropolitan France, dorsal aspect.

Photo Marc Gauthier.

Figure 30

Diversibipalium sp. ‘black’ from Metropolitan France, dorsal aspect.

What appears to be a darker median dorsal stripe is a lighting artefact. Photo Marc Gauthier.

Figure 31

Diversibipalium sp. ‘black’ from Metropolitan France, preserved specimen.

Specimen MNHN JL090. Dorsolateral aspect showing the partly protruded pharynx. Photo Jean-Lou Justine.

Figure 32

Diversibipalium sp. ‘black’ from Metropolitan France, preserved specimen.

Specimen MNHN JL090. Ventral aspect. The ventral ground colour is grey, with the creeping sole a lighter tone. The pharynx is slightly protruded from the mouth, and the gonopore is evident as a small transverse white slit on the creeping sole some 2 mm below to the mouth. Scale is in mm. Photo Jean-Lou Justine.

Figure 33

Diversibipalium sp. ‘blue’ from Mayotte, Indian Ocean, dorsal aspect.

The headplate of this small planarian is a brown colour, with a blue dorsum. This living specimen is approximately 45 mm long. Photo by Benoît Duperron.

Figure 34

Diversibipalium sp. ‘blue’ from Mayotte, Indian Ocean, dorsal aspect.

Specimen MNHN JL282. The headplate of this small planarian is a rusty-brown colour that extends to some irregular patches on the “neck”. The dorsal ground colour is an iridescent blue-green (“dark turquoise glitter”). Photo by Laurent Charles.

Figure 35

Diversibipalium sp. ‘blue’ from Mayotte, Indian Ocean, dorsal aspect.

Specimen MNHN JL282. As for Figure 34. Photo by Laurent Charles.

Figure 36

Diversibipalium sp. ‘blue’ from Mayotte, Indian Ocean. Dorsal aspect of a regenerating specimen with a damaged anterior end.

Specimen MNHN JL280. Under appropriate lighting, the colour of the specimen takes on a beautiful, almost metallic green colour. The iridescence and blue-green colour are lost on fixation, leaving the specimen a dark brown. Photo by Laurent Charles.

Figure 37

Diversibipalium sp. ‘blue’ from Mayotte, Indian Ocean. Dorsal aspect of a regenerating specimen with a damaged anterior end.

Specimen MNHN JL280. A small portion of the brown-pigmented ventral surface with the median pale creeping sole, can be seen. Photo by Laurent Charles.

Figure 38

Map of the south-eastern part of France, showing numerous new Bipaliine records.

Names of communes are indicated. Most records are from the Department of Pyrénées-Atlantiques, especially its lower part near the Atlantic Ocean.

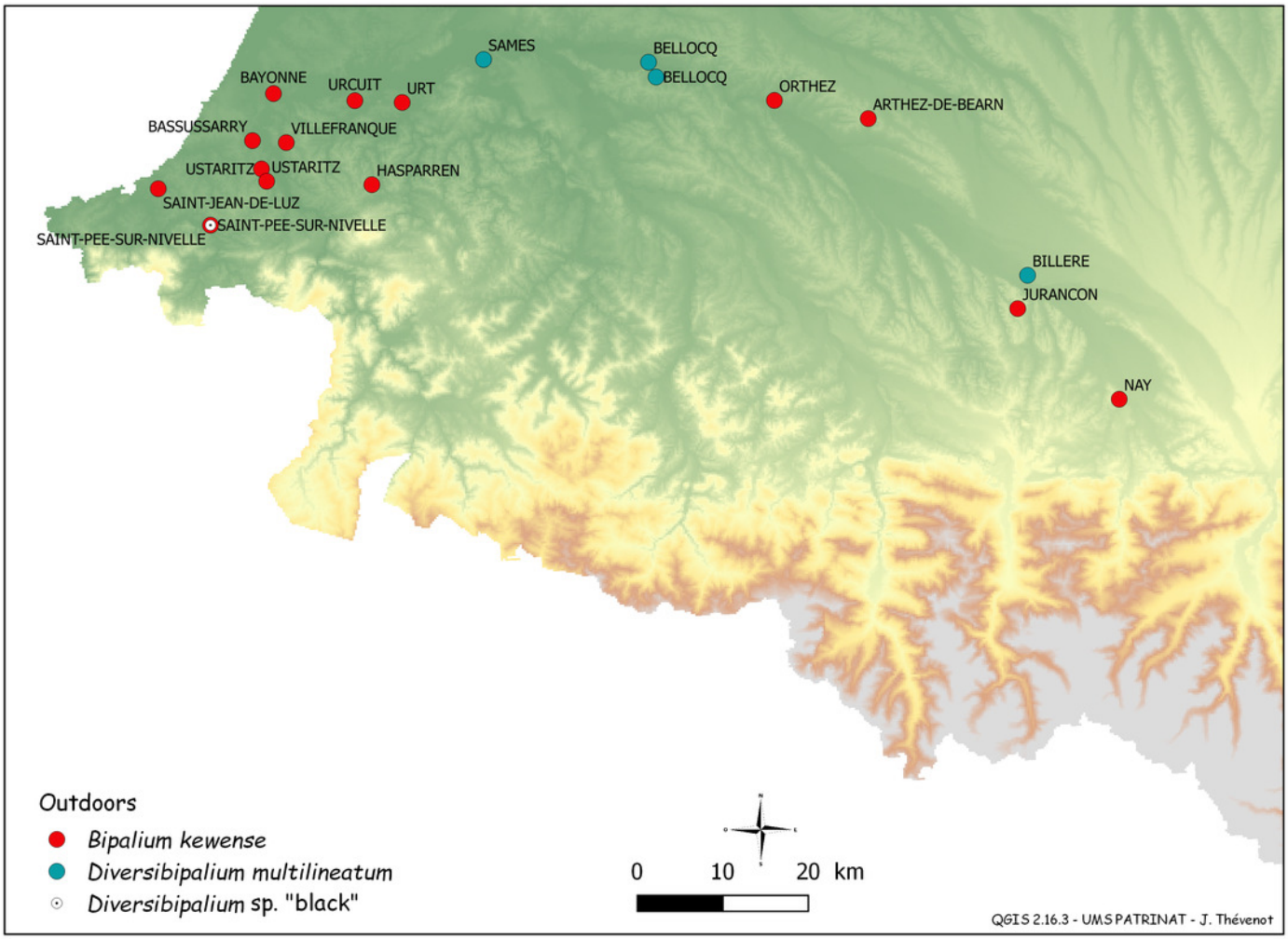

Figure 39

Map of the World, showing new records of Bipaliine from French territories.

New records are from three continents.

Table 1(on next page)

Invasive land planarians found in Europe, authors of taxa and key references

This table provides complete information about authors and taxa and combination, thus making the general text lighter. Sluys (2016) listed additional species with limited records and information: *Artioposthia exulans* Dendy, 1901, *Australoplana sanguinea* (Moseley, 1877), *Dolichoplana striata* Moseley, 1877, *Kontikia andersoni* Jones, 1981.

Taxon and authors	Synonyms	References for taxon	Main references for presence in Europe
Arthurdendyus triangulatus (Dendy, 1896) Jones, 1999	*Artioposthia triangulata*	Dendy 1895, Jones 1999	Boag et al. 1994
Platydemus manokwari De Beauchamp, 1963		de Beauchamp 1962	Justine et al. 2014b
Obama nungara Carbayo, Álvarez-Presas, Jones & Riutort, 2016	*Obama marmorata*	Carbayo et al. 2016	Carbayo et al. 2016
Parakontikia ventrolineata (Dendy, 1892) Winsor, 1991	*Kontikia ventrolineata*	Dendy 1891, Winsor 1991	Álvarez-Presas et al. 2014
Caenoplana coerulea Moseley, 1877		Moseley 1877	Álvarez-Presas et al. 2014; Breugelmans et al. 2012
Caenoplana bicolor (Graff, 1899) Winsor, 1991	Geoplana bicolor	von Graff 1899, Winsor 1991	Álvarez-Presas et al. 2014
Marionfyfea adventor Jones & Sluys, 2016		Jones & Sluys 2016	Jones & Sluys 2016
Diversibipalium multilineatum (Makino & Shirasawa, 1983) Kubota & Kawakatsu, 2010	*Bipalium multilineatum*	Makino & Shirasawa 1983, Kubota & Kawakatsu 2010	Mazza et al. 2016, This paper
Bipalium kewense Moseley, 1878		Moseley 1878	This paper

Table 2(on next page)

Specimens with molecular identification

* JL177 already published (Mazza et al. 2016); ** specimen from hot house, all others are from the open; *** Specimen MCSN 719.990/77.590 kept in Museo Cantonale di Storia Naturale, Lugano, Switzerland, forwarded by Jean Mariaux (Geneva, Switzerland). BK: *Bipalium kewense*; BV: *Bipalium vagum*; DM: *Diversibipalium multilineatum*; Dblue: *Diversibipalium* sp. 'blue'; Dblack: *Diversibipalium* sp. 'black'.

Species	MNHN	GenBank #	date	Locality	Department / State	Country - Continent	COI	Replicates	Collector
BK	JL089		12/11/2013	Saint Pée sur Nivelle	Pyrénées-Atlantiques	Met. France - Europe	short	1	Gauthier, Marc
BK	JL160		23/05/2014	Cannes	Alpes-Maritimes	Met. France - Europe	short	1	Iachia, Valeria
BK	JL167		24/08/2014	Orthez	Pyrénées-Atlantiques	Met. France - Europe	short	1	Rougeux, Christian
BK	JL174		03/09/2014	Bassussary	Pyrénées-Atlantiques	Met. France - Europe	long	1	Mercader, Elisabeth
BK	JL176 **		05/09/2014	Auxerre (hothouse)	Yonne	Met. France - Europe	long	1	Bellina, Arnaud
BK	JL184		Oct. 2014	Ustaritz	Pyrénées-Atlantiques	Met. France - Europe	short	1	Goyheneche, Iker
BK	JL188		08/10/2014	Miramar	Grande Porto	Portugal - Europe	short	1	Soarès, Luciana
BK	JL212		19/12/2014	Mimbastes	Landes	Met. France - Europe	long	1	Jouveau, Séverin
BK	JL224		23/02/2015	Trois Rivières	Guadeloupe	Guadeloupe - C. America	long	1	Van Laere, Guy
BK	JL233		27/09/2014	Monaco	Monaco	Monaco - Europe	long	3	Dusoulier, François
BK	JL253		21/03/2015	Trois Rivières	Guadeloupe	Guadeloupe - C. America	short	1	Van Laere, Guy
BK	JL254		15/05/2015	Matoury	French Guiana	French Guiana - S. America	short	2	Girault, Rémi
BK	JL270		23/04/2015	Ducos	Martinique	Martinique - C. America	long	1	Lucas, Pierre-Damien
BK	JL308		08/09/2016	Morne Vert	Guadeloupe	Guadeloupe - C. America	short	1	Coulis, Mathieu
BV	JL073		Aug. 2013	Sanibel	Florida	USA - North America	short	1	Justine, Jean-Lou
BV	JL163		July 2014	Sanibel	Florida	USA - North America	short	1	Justine, Jean-Lou
BV	JL164		July 2014	Sanibel	Florida	USA - North America	short	1	Justine, Jean-Lou
BV	JL213		29/11/2014	Anse-Bertrand	Guadeloupe	Guadeloupe - C. America	long	1	Charles, Laurent
BV	JL268		Dec. 2014	Montserrat	Montserrat	Montserrat - C. America	short	1	Shoobs, Nathaniel F.
BV	JL307		19/11/2015	Morne Vert	Guadeloupe	Guadeloupe - C. America	short	1	Coulis, Mathieu
DM	JL177 *		30/09/2014	Léguevin	Haute-Garonne	Met. France - Europe	long	1	Chaim, Florence
DM	JL059		15/06/2013	La Bastide de Serou	Ariège	Met. France - Europe	short	1	Brugnara, Sébastien
DM	JL142		22/04/2014	Saubrigues	Landes	Met. France - Europe	long	2	Robineau, Thiérry
DM	JL161		11/06/2015	Bellocq	Pyrénées-Atlantiques	Met. France - Europe	long	1	Audiot, Marie-Claude
DM	JL208		11/06/2014	Bellocq	Pyrénées-Atlantiques	Met. France - Europe	long	1	Audiot, Marie-Claude
DM	JL209		12/06/2014	Bellocq	Pyrénées-Atlantiques	Met. France - Europe	long	1	Audiot, Marie-Claude
DM	JL210		June 2014	Bellocq	Pyrénées-Atlantiques	Met. France - Europe	long	1	Audiot, Marie-Claude
DM	JL298 ***		01/06/2016	Novazzano	Ticino Canton	Switzerland - Europe	long	1	Pollini, Lucia
DBlue	JL280		2015	Mtsamboro	Mayotte	Mayotte - Africa	long	1	Charles, Laurent
DBlue	JL281		29/04/2015	Mtsamboro	Mayotte	Mayotte - Africa	long	3	Charles, Laurent
DBlue	JL282		30/04/2015	Ouangani	Mayotte	Mayotte - Africa	long	1	Charles, Laurent
DBlue	JL284		05/05/2015	Mtsamboro	Mayotte	Mayotte - Africa	long	1	Charles, Laurent
DBlack	JL090		12/11/2013	Saint Pée sur Nivelle	Pyrénées-Atlantiques	Met. France - Europe	short	1	Gauthier, Marc

Table 3(on next page)

Divergences between “short” sequences

There was a total of 266 positions in the final dataset.

	kewense	multilineatum	nobile	“black”	“blue”	vagum
multilineatum	0.109					
nobile	0.131	0.131				
“black”	0.149	0.164	0.163			
“blue”	0.206	0.202	0.164	0.192		
vagum	0.140	0.168	0.163	0.140	0.159	
adventium	0.136	0.178	0.173	0.173	0.212	0.164

Table 4(on next page)

Divergences between “long” sequences

There was a total of 857 positions in the final dataset

	kewense	multilineatum	“blue”
multilineatum	0.159		
“blue”	0.230	0.259	
vagum	0.167	0.179	0.223

Table 5(on next page)

Records of *Bipalium kewense* identified from photographs

Photographs were obtained through citizen science; specimens were identified from photographs by the authors. No molecular identification was possible. There were 31 records, including one from a hothouse. The name of the authors of photographs are indicated only when a formal consent to publish was obtained from the authors. Photographs are in Supplement 2. For the first record, see also Gerlach (2017).

#	Date	Locality	Department / State	Country - Continent	Origin of data
K01	20/08/2017	Bora Bora	French Polynesia	French Polynesia - Oceania	Gerlach, Justin
K02	13/10/2010	Basse-Terre	Guadeloupe	Guadeloupe - C. America	Guezennec, Pierre et Claudine
K03	22/01/2014	Unknown	Guadeloupe	Guadeloupe - C. America	Consent not obtained
K04	14/01/2007	Petit-Bourg	Guadeloupe	Guadeloupe - C. America	Lurel, Félix
K05	19/02/2015	La Trinité	Martinique	Martinique - C. America	Delannoye, Régis
K06	19/04/2016	Saint Joseph	Martinique	Martinique - C. America	Andrebe, Silvio
K07	25/08/2017	Plaine des Cafres	La Réunion	La Réunion - Africa	Pronier, Pascal
K08	16/10/2013	Beaulieu-sur-Mer	Alpes-Maritimes	Met. France - Europe	Pelcer, Jean-Paul
K09	21/07/2014	Nice	Alpes-Maritimes	Met. France - Europe	Gerriet, Olivier *
K10	15/10/2014	Appietto	Corse-Sud (Corsica)	Met. France - Europe	Consent not obtained
K11	17/10/2013	Pietrosella	Corse-Sud (Corsica)	Met. France - Europe	Senee, Patrick
K12	23/08/2014	Arcachon	Gironde	Met. France - Europe	Consent not obtained
K13	21/11/2002	Saint-Jean-de-Vedas	Hérault	Met. France - Europe	Peaucellier, Gérard
K14	27/10/2014	Biscarosse	Landes	Met. France - Europe	Consent not obtained
K15	27/09/2008	Hagetmau	Landes	Met. France - Europe	Jeannotin, Josette
K16	22/09/2016	Nantes	Loire-Atlantique	Met. France - Europe	Consent not obtained
K17	16/10/2014	Grimaud	Var	Met. France - Europe	Bernez, Alain
K18	01/08/2014	Toulon	Var	Met. France - Europe	Consent not obtained
K19	29/07/2014	Sens (Hothouse)	Yonne	Met. France - Europe	Burel, Jonathan **
K20	17/12/2014	Arthez de Béarn	Pyrénées-Atlantiques	Met. France - Europe	Sillard, Dominique
K21	20/09/2014	Bayonne	Pyrénées-Atlantiques	Met. France - Europe	Bonnefous, François
K22	18/08/2014	Hasparren	Pyrénées-Atlantiques	Met. France - Europe	Voise, Mireille
K23	22/04/2016	Jurançon (near)	Pyrénées-Atlantiques	Met. France - Europe	Pauchet, Marjolaine
K24	29/04/2016	Nay	Pyrénées-Atlantiques	Met. France - Europe	Lamaille, Corinne
K25	28/09/2014	Orthez	Pyrénées-Atlantiques	Met. France - Europe	Rougeux, Christian
K26	22/08/2016	Saint Jean de Luz	Pyrénées-Atlantiques	Met. France - Europe	Centelles, Ruben
K27	01/01/1999	Urcuit	Pyrénées-Atlantiques	Met. France - Europe	Esposito, Mario
K28	14/09/2014	Urt	Pyrénées-Atlantiques	Met. France - Europe	Chanderot, Vincent
K29	12/08/2017	Ustaritz	Pyrénées-Atlantiques	Met. France - Europe	Lescourret, Monique & Bernard
K30	14/09/2014	Villefranque	Pyrénées-Atlantiques	Met. France - Europe	Consent not obtained

Table 6(on next page)

Records of *Diversibipalium multilineatum* identified from photographs

Photographs were obtained through citizen science; specimens were identified from photographs by the authors. No molecular identification was possible. There were 11 records, including 2 from hothouses. The name of the authors of photographs are indicated only when a formal consent to publish was obtained from the authors. Photographs are in Supplement 2.

#	Date	Locality	Department / State	Country - Continent	Origin
M01	27/06/2010	Longages	Haute-Garonne	Met. France - Europe	Lombard, Yoann
M02	22/03/2011	Longages	Haute-Garonne	Met. France - Europe	Lombard, Yoann
M03	06/07/2016	Saint-Egrève	Isère	Met. France - Europe	Tuaillon, Jean-Louis
M04	17/05/2017	Saint-Egrève	Isère	Met. France - Europe	Tuaillon, Jean-Louis
M05	27/06/2016	Benquet	Landes	Met. France - Europe	Broustaut, François
M06	28/03/2014	Cahors (Hothouse)	Lot	Met. France - Europe	Consent not obtained
M07	04/07/2014	Andilly (Hothouse)	Val d'Oise	Met. France - Europe	Burel, Jonathan *
M08	27/04/2015	Magny-en-Vexin	Val d'Oise	Met. France - Europe	Mellac, Céline
M09	29/05/2016	Magny-en-Vexin	Val d'Oise	Met. France - Europe	Mellac, Céline
M10	19/04/2010	Sames	Pyrénées-Atlantiques	Met. France - Europe	Grenier-Falaise, Nadine
M11	07/04/2017	Billère	Pyrénées-Atlantiques	Met. France - Europe	Vincent, Jean-François

Table 7 (on next page)

Records of *Bipalium vagum* identified from photographs

Photographs were obtained through citizen science; specimens were identified from photographs by the authors. No molecular identification was possible. There were 30 records. The name of the authors of photographs are indicated only when a formal consent to publish was obtained from the authors. Photographs are in Supplement 2.

#	Date	Locality	Department / State	Country - Continent	Origin
V01	21/06/2005	Cayenne	French Guiana	French Guiana – S. America	Girault, Rémi
V02	15/05/2017	Macouria	French Guiana	French Guiana – S. America	Boutin, Élodie
V03	12/05/2017	Saint-Laurent-du-Maroni	French Guiana	French Guiana – S. America	Muraine, François Xavier
V04	26/07/2017	Saül	French Guiana	French Guiana – S. America	Sant, Sébastien
V05	21/08/2017	Petit-Bourg	Guadeloupe	French Guiana – S. America	De Tienda, Marine
V06	24/11/2013	Gosier	Guadeloupe	Guadeloupe - C. America	Consent not obtained
V07	30/10/2016	Gosier	Guadeloupe	Guadeloupe - C. America	Brisson, Bernard
V08	22/11/2013	Petit Bourg	Guadeloupe	Guadeloupe - C. America	Oettly, Olivier
V09	22/11/2014	Petit Bourg	Guadeloupe	Guadeloupe - C. America	Marques, Maryvonne
V10	29/04/2011	Petit-Bourg	Guadeloupe	Guadeloupe - C. America	Guezennec, Pierre et Claudine
V11	25/07/2010	La Trinité	Martinique	Martinique - C. America	Delannoye, Régis
V12	18/11/2015	Morne Vert	Martinique	Martinique - C. America	Coulis, Mathieu
V13	01/04/2014	Saint Barthélemy	Saint Barthélemy	Saint Barthélemy - C. America	Moulard, Grégory
V14	01/05/2014	Saint Barthélemy	Saint Barthélemy	Saint Barthélemy - C. America	Consent not obtained
V15	11/05/2014	Saint Martin	Saint Martin	Saint Martin – C. America	Yokoyama, Mark
V16	21/11/2015	Avirons	La Réunion	La Réunion - Africa	Consent not obtained
V17	23/03/2017	Bras Panon	La Réunion	La Réunion - Africa	Saman-Latchimy, Teddy
V18	29/03/2017	Le Tampon	La Réunion	La Réunion - Africa	Consent not obtained
V19	26/10/2014	Petite Ile	La Réunion	La Réunion - Africa	Abonnenc, José
V20	12/03/2016	Petite Ile	La Réunion	La Réunion - Africa	Le Gars, René
V21	16/05/2014	Saint Louis	La Réunion	La Réunion - Africa	Faujour, Anne
V22	08/04/2014	Saint Paul	La Réunion	La Réunion - Africa	Consent not obtained
V23	16/03/2016	Saint Pierre	La Réunion	La Réunion - Africa	Collet, Jean
V24	10/03/2013	Sainte Marie	La Réunion	La Réunion - Africa	Fontaine, Romuald
V25	06/03/2016	Sainte Marie	La Réunion	La Réunion - Africa	Fontaine, Romuald
V26	12/02/2009	unknown	La Réunion	La Réunion - Africa	Gilson, Michel
V27	03/03/2010	unknown	La Réunion	La Réunion - Africa	Gilson, Michel
V28	01/05/2011	unknown	La Réunion	La Réunion - Africa	Martiré, Dominique
V29	28/10/2013	unknown	La Réunion	La Réunion - Africa	Martiré, Dominique
V30	17/08/2015	unknown	La Réunion	La Réunion - Africa	Lacoste, Marie

Table 8(on next page)

Records of *Diversibipalium* sp. “blue” identified from photographs

1 record.

Date	Locality	Department / State	Country - Continent	Origin
07/03/2014	unknown	Mayotte	Mayotte - Africa	Duperron, Benoît