Giant worms *chez moi!* Hammerhead flatworms (Platyhelminthes, Geoplanidae, *Bipalium* spp., *Diversibipalium* spp.) in metropolitan France and overseas French territories (#21767)

First revision

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

Jean-Lou Justine Corresp., [1] , **Leigh Winsor** [2] , **Delphine Gey** [3] , **Pierre Gros** [4] , **Jessica Thévenot** [5]

[1] Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, Paris, France
[2] College of Science and Engineering, James Cook University, Townsville, Queensland, Australia
[3] Service de Systématique Moléculaire, Museum national d'Histoire naturelle, Paris, France
[4] Amateur Naturalist, Cagnes-sur-Mer, France
[5] UMS Patrinat, Museum national d'Histoire naturelle, Paris, France

Corresponding Author: Jean-Lou Justine
Email address: justine@mnhn.fr

Background. Species of the genera *Bipalium* and *Diversibipalium*, or bipaliines, are giants among land planarians (family Geoplanidae), reaching length of 1 m; 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 and a total of 111 records, 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.** The present findings strongly suggest that the species present in Metropolitan France and overseas territories should be considered Invasive Alien Species. Our numerous records in the open in Metropolitan France raise questions: as scientists, we were amazed 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 certainly necessary.

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Jean-Lou Justine [1*], Leigh Winsor [2], Delphine Gey [3], Pierre Gros [4] and Jessica Thévenot [5]

1 Institut Systématique Évolution Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, 57 rue Cuvier, CP 51, 75005 Paris, France

2 College of Science and Engineering, James Cook University, Townsville, Australia

3 Service de Systématique Moléculaire, Muséum National d'Histoire Naturelle, Paris, France

4 Amateur Naturalist, Cagnes-sur-Mer, France

5 UMS Patrinat, Muséum National d'Histoire Naturelle, Paris, France

Corresponding author: Jean-Lou Justine, justine@mnhn.fr

Institut Systématique Évolution Biodiversité (ISYEB),

Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE,

57 rue Cuvier, CP 51, 75005 Paris, France

Abstract

Background. Species of the genera *Bipalium* and *Diversibipalium*, or bipaliines, are giants among land planarians (family Geoplanidae), reaching length of 1 m; 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 and a total of 111 records, 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.** The present findings strongly suggest that the species present in Metropolitan France and overseas territories should be considered Invasive Alien Species. Our numerous records in the open in Metropolitan France raise questions: as scientists, we were amazed 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 certainly 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) and one species even attains a length of 1 metre in elongated state (Kawakatsu et al. 1982). 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.

Land planarians are dispersed between countries, and within countries, through the transport of plants. Winsor (1983a) summarized knowledge about the world distribution of *Bipalium kewense,* listing the occurrence of the species in 39 territories; 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.

external aspect only is usually not reliable, but the morphology of copulatory organs provides should be.

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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; only one 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 these cases, we provide the scientific information about the presence of species, but we do not include the photograph of the worm or the name 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′-TTTTTGGGCATCCTGAGGTTTAT-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 MG655587- MG655618. 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.

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 only reproduce asexually (scissiparity) 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. These two species will be histologically examined and fully described by the authors elsewhere.

Results

Collection of information from citizen science

After the initial finding in June 2013 of two species of land planarians 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, 111 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 (**Figure 2**) was constructed from an analysis of our new COI sequences and sequences from GenBank. 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; in that we follow the general principles of COI barcoding (Hebert & Gregory 2005): "we emphasize that DNA barcodes do not aim to recover phylogenetic relationships; they seek instead to identify known species and to aid the discovery of new ones". We remarked, but do not comment, probable misidentification of certain sequences deposited in GenBank, such as *Novibipalium venosum* or the "*D. multilineatum"* HM346600.

Each of the three named species belonged to a clade with high (100%) bootstrap support (**Figure 2**).

For *Bipalium kewense*, the clade includes GenBank sequences from Spain, Azores 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 bipaliine 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 bipaliine 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%.

Taxonomy and Geographic Distribution

~~Information for each species~~ Replace

***Bipalium kewense* Moseley, 1878**

Morphology and colour pattern (Figures 3-9)

The specimens which were sent to us or for which we received photographs corresponded to published morphological descriptions of the species (Winsor 1983a). Living specimens are long and thin and ranged in length from 100 mm – 270 mm **(Table 5)**. Preserved specimens from which COI results were obtained, measured 170 mm (MNHN JL224), 120 mm (MNHN JL308) and 65 mm (MNHN JL270) in length, with the relative mouth: body length 41.2%, 41.7% and 32.3% respectively. None of the preserved specimens examined had a gonopore and thus they were considered to be non-sexual. The anterior end is expanded into a transversely semi-lunate-shaped headplate with recurved lappets (falciform). The dorsal ground colour is usually a light – mid 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 dorsal headplate **(Figure 4, 5)** 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 ventral headplate is a greyish colour with a light ochre margin. The ventral surface **(Figure 6)** 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 **Figure 7**, we present evidence of predation on an unidentified native European earthworm, and in **Figures 8-9** evidence of reproduction by scissiparity where the shed fragment is immediately motile but does not possess the characteristic hammer-shaped head.

Differentiation from other species

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 (Winsor 1983a).

Records obtained from citizen science

We obtained 50 records of *B. kewense*, including 14 confirmed by molecules (**Table 2**) and 36 from photographs only (**Table 6**). Localities where bipaliines were found in the open, generally in gardens, include Portugal (1 record), Martinique (3), Guadeloupe (6), French Guiana (1), French Polynesia (1), La Réunion (1), Monaco (1), i.e. from 7 territories in 5 continents (Europe, North America, South America, Africa, Oceania), and 36 from Metropolitan France (**Figure 1**), from 9 departments: Corse-Sud (Corsica) (2), Var (2), Gironde (1), Loire-Atlantique (1), Landes (3), Alpes-Maritimes (5), Yonne (2), Hautes-Pyrénées (1) and Pyrénées-Atlantiques (16). In addition, we received two reports in hothouses in the Department of Yonne. Among the 34 records in the open in Metropolitan France, 16, i.e. more than half, were from the department of Pyrénées-Atlantiques (**Tables 2, 6**). The distribution of our records is shown in **Figure 1** for Metropolitan France (including Corsica). Dates of records ranged 1999-2017; the oldest record (1999) was in the Pyrénées-Atlantiques.

Molecular results

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

Diversibipalium multilineatum (Makino and Shirasawa, 1982)

Morphology and colour pattern (Figures 10-14)

The specimens which were sent to us or for which we received photographs corresponded to the published morphological description of the species (Makino & Shirasawa 1983; Mazza et al. 2016). Living specimens ranged in length from 150 mm (MNHN JL 177) to 210 mm (MNHN JL059). Representative preserved specimens from which COI results were obtained measured 85 mm (MNHN JL210), 65 mm (MNHN JL161A), and 60 mm (MNHN JL142A) in length (**Table 5**), with the relative mouth: body length 29.4%, 38.5%, and 41.7% respectively. None of the specimens examined had a gonopore and thus they were considered to be non-sexual. The body is elongated (**Figure 10**) with the anterior end expanded into a transversely semi-lunate-shaped headplate with rounded lappets (**Figure 11-13**). Immediately behind the neck the body narrows to form a “neck”, subsequently it gradually broadens to the maximum width over the pharyngeal region, and tapers slightly to a rounded posterior end. 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 lenticulate shape beginning at the anterior third of the headplate, 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 14**) 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 at the ventral

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termination of the collar, and extending the entire body length. A finer, generally discontinuous mid ventral dark stripe extends from the base of the headplate to the posterior end.

Differentiation from other species

Diversibipalium multilineatum is differentiated externally from similar striped species by the presence of the lenticulate-shaped 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.

Records obtained from citizen science

We obtained a total of 19 records. One record was from Switzerland and 16 from outdoor locations 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, 7**).

Molecular results

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 and colour pattern (Figures 15-18)

The specimens which were sent to us or ~~for~~ of which we received photographs corresponded to the published morphological description of the species (Jones & Sterrer 2005). Living specimens are medium sized, with one measuring around 36 mm (**Table 5**, observation V04, from a scaled photo). Preserved specimens, from which COI results were obtained, measured 27.5 mm (MNHN JL164), 25.6 mm (MNHN JL163) and 15 mm (MNHN JL307) in length, with the relative mouth: body length 60.7%, 50.4% and 49% respectively, and gonopore: body length 70.7% (MNHN JL163) and 72% (MNHN JL307).

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 (**Figures 15-18**).

Differentiation from other species

Bipalium vagum is distinguished externally from species of similar morphology by the combination of characters, especially its relatively small size, the transverse neck band that is continuous dorsally, from which the broad median black stripe originates, and the relative position of the body apertures.

Records obtained from citizen science

No record was obtained from Metropolitan France. We obtained 39 records (**Tables 2, 8**), 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 (10), Martinique (3), 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 15-18**). Dates of records ranged 2005-2017; the oldest record (2005) was from French Guiana (**Tables 2, 7**).

Molecular results

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

Diversibipalium sp. "black" from Metropolitan France

Morphology and colour pattern (Figures 19-21)

The living specimen attains a length of 20 – 25 mm. A preserved sexual specimen (MNHN JL090) is 20 mm long and 3.2 mm wide, with the mouth situated ventrally 6 mm (mouth: body length 30 %), and gonopore 7.8 mm (gonopore: body length 9%) from the anterior end.

The dorsal ground colour of this small planarian is black, with no evidence of dorsal stripes (**Figures 19-21**). The ventral surface is a light grey colour with paler creeping sole.

Differentiation from other species

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 *Diversibipalium* sp. Kuamoto (Kawakatsu et al. 2005).

What does it mean?

Possible origin of this species

We do not propose any hypothesis concerning the geographic origin of this species, apart the fact that it is obviously not European, since no bipaliines are known from this continent.

Molecular ~~results~~ identification

Replace (here and elsewhere)

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.

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

Morphology and colour pattern (Figures 22-26)

Unfortunately, scaled photos of this planarian are unavailable and the length of the living specimen could not be determined. The preserved sexual specimen is 9 mm long and 1 mm wide, with the mouth situated ventrally approximately 3.5 mm (mouth: body length 39%), and gonopore 6.5 mm (gonopore: body length 72.2%) from the anterior end.

The headplate in this beautiful, 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"), 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 (**Figures 22-26**).

Differentiation from other species

There are no other reports of a bipaliine planarian with this morphology.

Possible origin of this species

Mayotte and the Comoros are small volcanic islands which experienced intense human trade from centuries with the close island and Madagascar and more distant territories including Asia. Any of these could be the origin of this species.

Records obtained from citizen science

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, 9**).

Molecular results

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.

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 scientists 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 (Winsor 1983a), is probably one reason explaining why no variability was found in specimens, since specimens are clones, and no or very few mutations can happen. However, this explanation 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.

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

Bipalium kewense was originally described from specimens in ~~the~~ a 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), Billère and 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 including Mimbastes (2014, with molecular information), Hagetmau (2008) and Biscarosse (2014) and one record from the

Department of Hautes-Pyrénées, farther from the coast, in Peyrouse (2017) (**Tables 2, 6**). 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 27**). 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. Recently, one citizen in Billère (Pyrénées-Atlantiques) sent us repeated records in the same garden in September and December 2017 and January 2018, clearly showing numerous specimens alive outdoor, even in Winter; they were found at various ~~depth~~ depths under the soil surface in January, clearly a way for the species to survive the cold season.

Fig. 27 should be either an inset figure of Fig. 1 or should be moved to Fig. 2

Replace

We note that all our records are from gardens and that none were from places away from human presence; this can be expected from citizen science data.

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 27**). 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 Departments 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).

Unclear. Do you mean lower than -5?

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* (data from citizen science). 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 7**). This suggests that this species also is established in the open in Metropolitan France, but the total number of records is lower (16 vs 34 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.

Do bipaliine land planarians qualify as invasive species in Metropolitan France?

We received several reports by citizens mentioning dozens of specimens in their gardens (**Supplemental Files 1 & 2**); in some cases, citizens repeatedly reported high numbers, even when worms were removed by hand and destroyed. Such reports justify the species as “invasive” in the common, public sense of the word.

However, the term “invasive species” has a more precise meaning in science. Invasive Alien Species (IAS) are defined by both the Convention on Biological Diversity and the International Union for Conservation of Nature as “species whose introduction and/or spread outside their natural past or present distribution threatens biological diversity” (Convention on Biological Diversity ; International Union for Conservation of Nature). Legal definitions are also available in various countries. For the USA, Executive Order 13112 (1999) (Executive Order 13112 1999) defines an invasive species as “an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health.” In Europe, the Institute for European Environmental Policy (Kettunen et al. 2009) uses the following definition: “Invasive alien species (IAS) are non-native species whose introduction and/or spread outside their natural past or present ranges poses a threat to biodiversity". The most recent legal text (European Parliament 2014) reads (a few parts are deleted here for simplification): "(1) The appearance of alien species, whether of animals, plants, fungi or micro-organisms, in new locations is not always a cause for concern. However, a significant subset of alien species can become invasive and have serious adverse impact on biodiversity and related ecosystem services, as well as have other social and economic impact, which should be prevented. […] (2) Invasive alien species represent one of the main threats to biodiversity and related ecosystem services. […](3) The threat to biodiversity and related ecosystem services that invasive alien species pose takes different forms, including severe impacts on native species and the structure and functioning of ecosystems through the alteration of habitats, predation, competition, the transmission of diseases, the replacement of native species throughout a significant proportion of range and through genetic effects by hybridisation."

According to these definitions, bipaliines found in gardens in Metropolitan France and other localities mentioned in this paper should clearly be considered as Invasive Alien Species, because bipaliines are predators, and as such threaten the soil fauna. In absence of detailed ecological studies, we cannot estimate the exact impact of these worms on the fauna; the very large size of bipaliine flatworms, making them the largest terrestrial invertebrate predators, suggests that this impact is not negligible (Zaborski 2002).

A precise classification of alien species based on their environmental impacts has recently been proposed (Blackburn et al. 2014); bipaliines fulfil three of the criteria listed in Table 1 of Blackburn et al. 2014: competition, predation, and poisoning/toxicity. The first two criteria are fulfilled by the predatory character of bipaliines, especially on larger prey (Ducey et al. 1999; Johri 1952; Zaborski 2002); the presence of tetrodotoxin (Stokes et al. 2014) fulfils the criterion of toxicity, and this is reinforced by reports of animals vomiting ingested bipaliines (Winsor 1983b). However, in absence of ecological studies, bipaliines should currently be classified as "data deficient" (Box 1 in Blackburn et al. 2014).

In conclusion, bipaliines are Invasive Alien Species in Europe and the French overseas territories mentioned in this paper (**Figure 28**), but an exact evaluation of their ecological impact requires ecological studies, which are outside the scope of this paper.

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 amazed 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 amazing 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 (**Figures 1, 27, 28**): 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, based on COI, 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). We also demonstrated that bipaliines correspond well with the definition of "Invasive Alien Species" in the European scientific (Kettunen et al. 2009) and legal (European Parliament 2014) documents, but we recognize that a precise assessment of their impact on the local biodiversity is needed – but is outside the scope of this paper. 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.

Acknowledgements

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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.

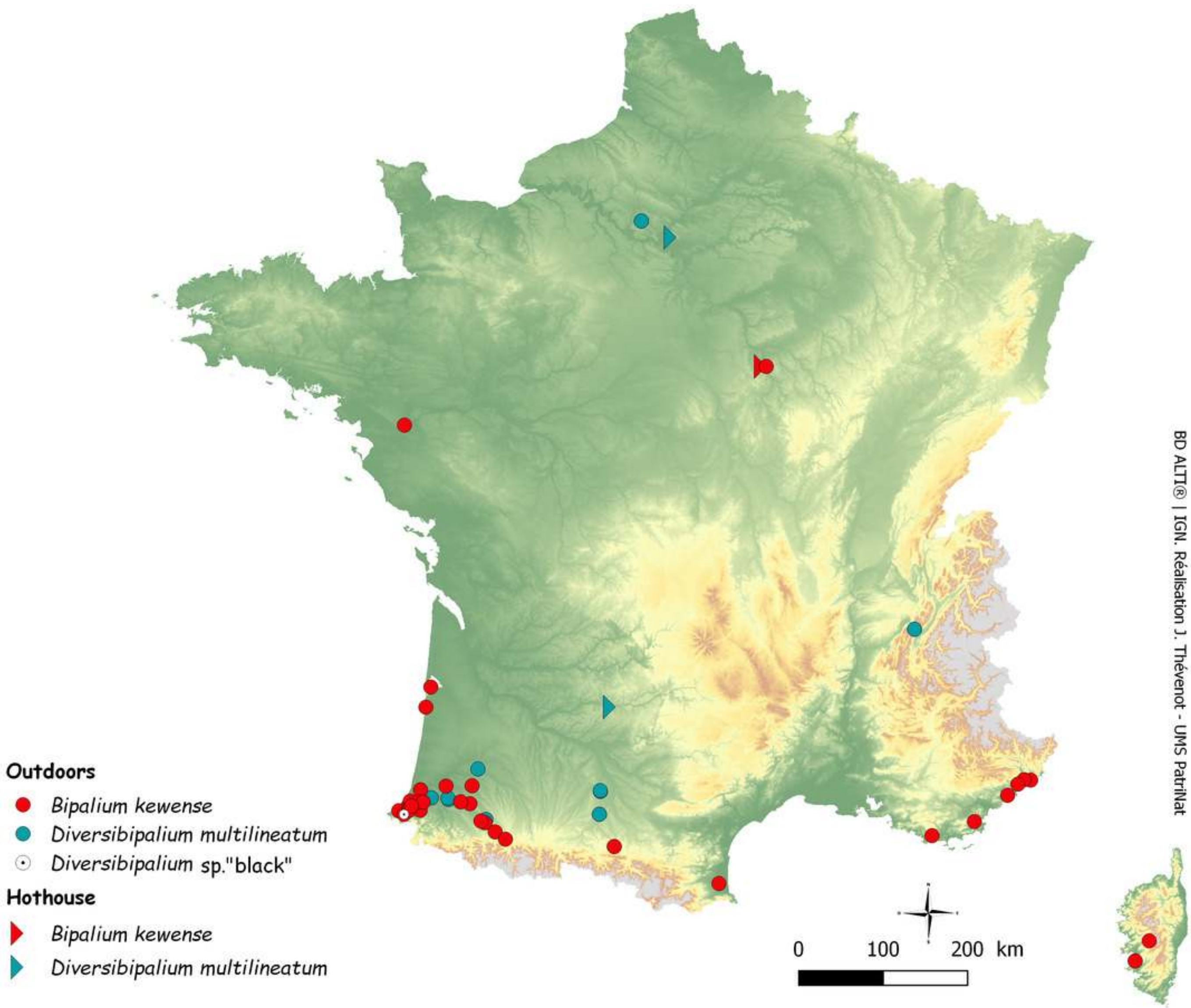

Figure 2

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).

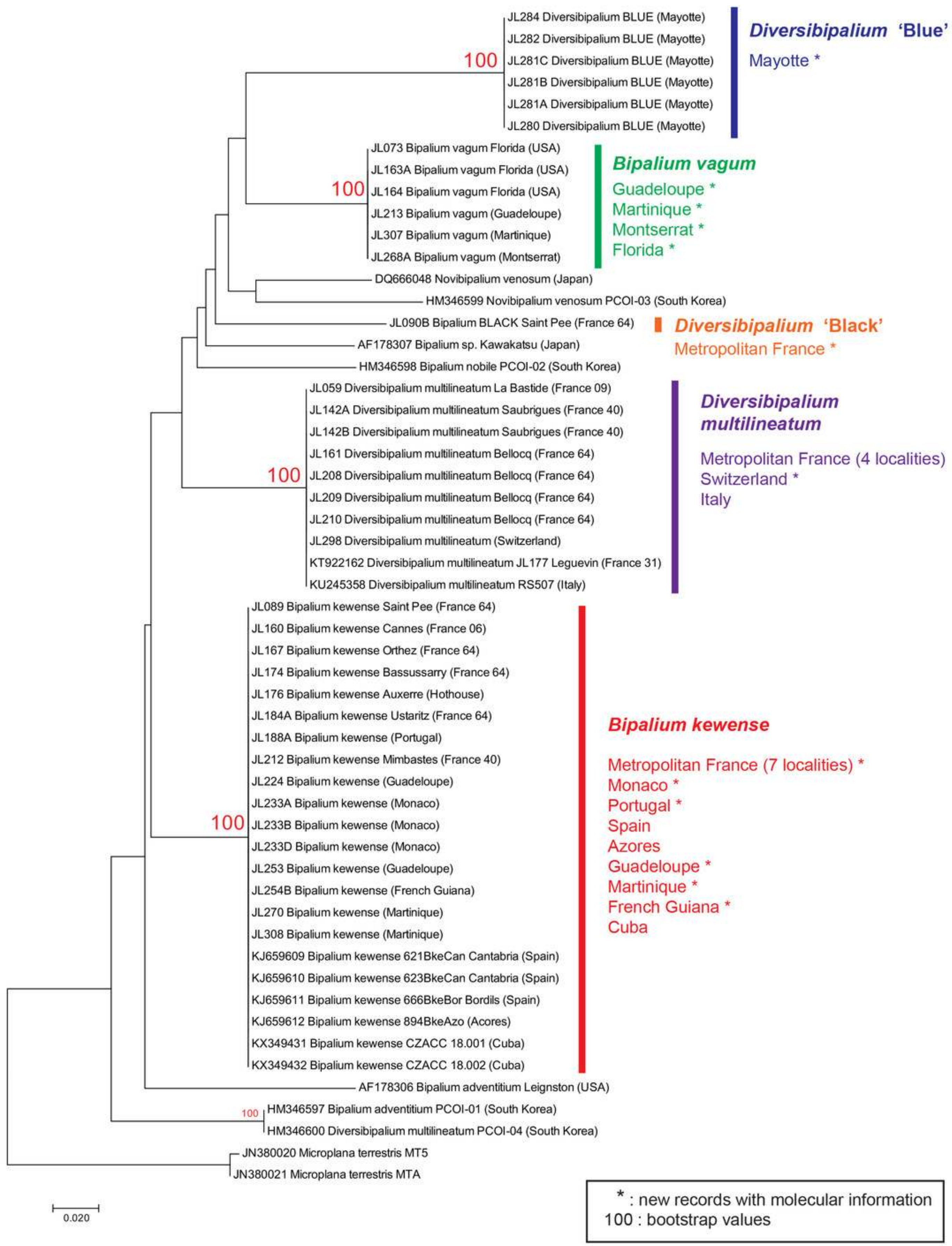
JL284 Diversibipalium BLUE (Mayotte)
JL282 Diversibipalium BLUE (Mayotte)
JL281C Diversibipalium BLUE (Mayotte)
JL281B Diversibipalium BLUE (Mayotte)
JL281A Diversibipalium BLUE (Mayotte)
JL280 Diversibipalium BLUE (Mayotte)
100
Diversibipalium 'Blue'
Mayotte *
JL073 Bipalium vagum Florida (USA)
JL163A Bipalium vagum Florida (USA)
JL164 Bipalium vagum Florida (USA)
JL213 Bipalium vagum (Guadeloupe)
JL307 Bipalium vagum (Martinique)
JL268A Bipalium vagum (Montserrat)
100
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)
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)
100
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)
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)
100
Bipalium kewense
Metropolitan France (7 localities) *
Monaco *
Portugal *
Spain
Azores
Guadeloupe *
Martinique *
French Guiana *
Cuba
AF178306 Bipalium adventitium Leignston (USA)
HM346597 Bipalium adventitium PCOI-01 (South Korea)
HM346600 Diversibipalium multilineatum PCOI-04 (South Korea)
100
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, 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 6

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 7

Bipalium kewense, predation on earthworm.

The flatworm initiates here 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 (Stokes et al. 2014). 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 8

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 9

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 10

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 11

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 12

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 13

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 14

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 15

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 16

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 17

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 18

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 19

Diversibipalium sp. ‘black’ from Metropolitan France.

Drawings from photographs of three living specimens in dorsal view. The dorsal ground colour of the specimens is black, with no evidence of dorsal stripes. The scale (10 mm) is valid for the two specimens on the left, the specimen on the right has no scale.

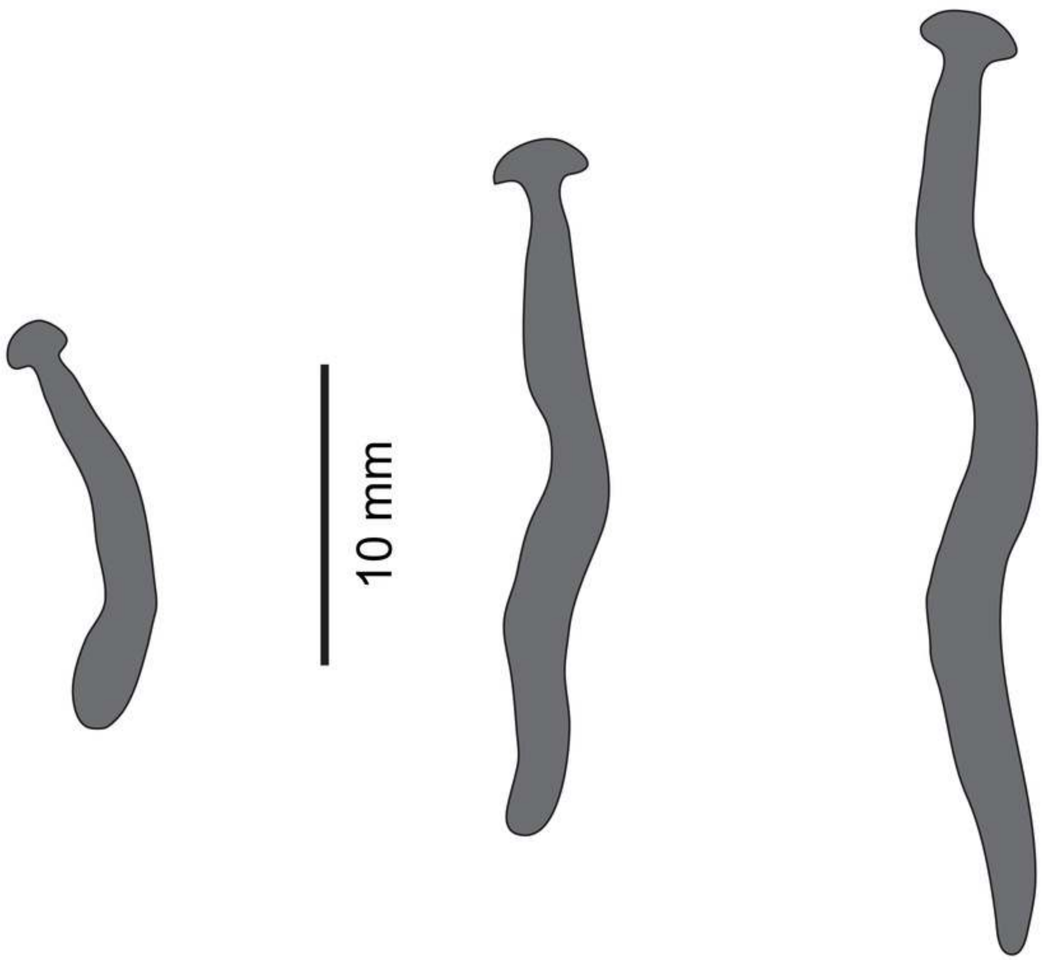

Figure 20

Diversibipalium sp. 'black' from Metropolitan France, preserved specimen.

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

Figure 21

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 2mm below to the mouth. Scale is in mm. Photo by Jean-Lou Justine.

Figure 22

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 23

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 24

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

Specimen MNHN JL282. As for Figure 25. Photo by Laurent Charles.

Figure 25

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 26

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 27

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.

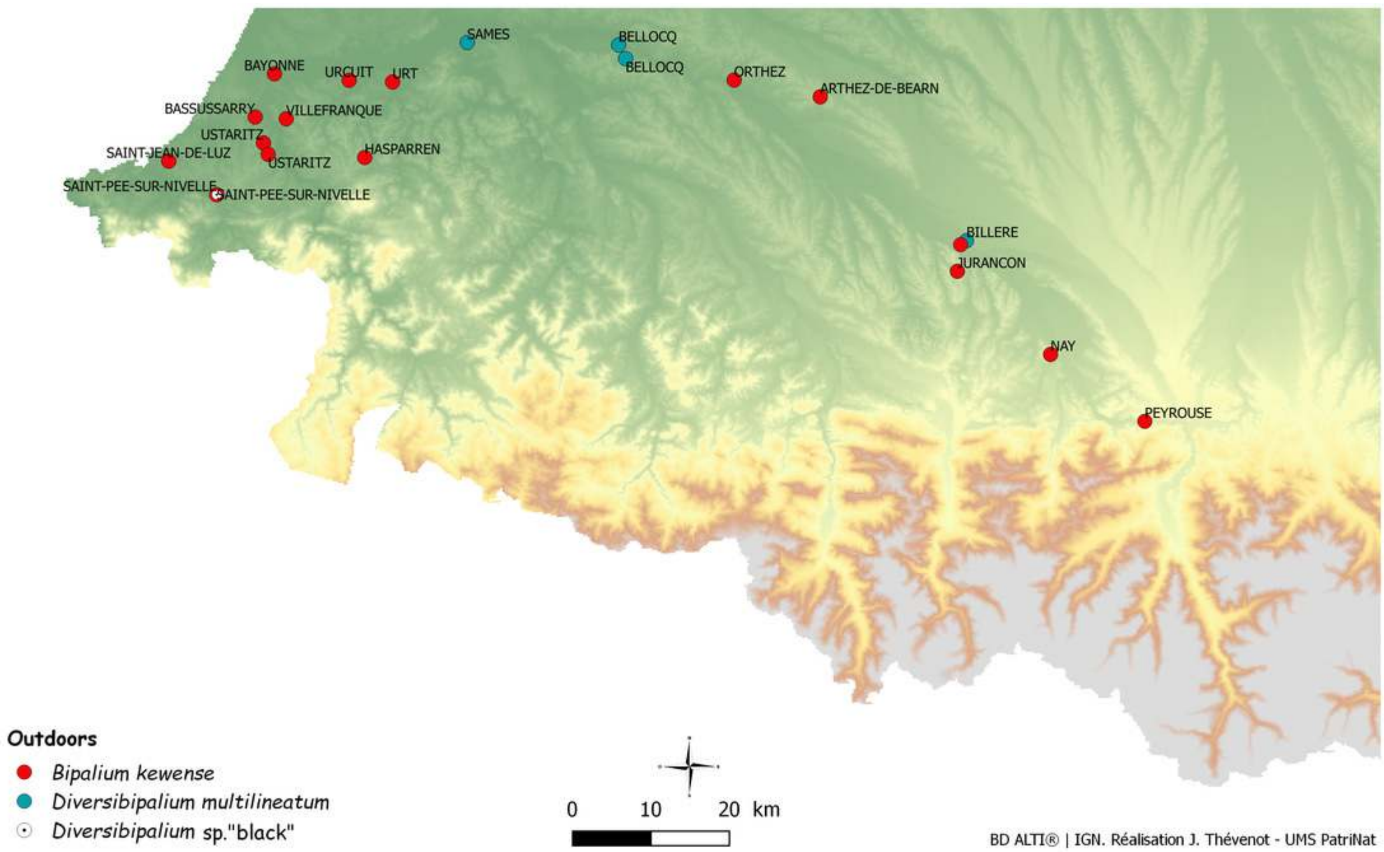

Figure 28

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

New records are from four continents (North America, South America, Polynesia, Africa).

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

In italics

Table 2(on next page)

Specimens of bipaliines 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'.

Most collectors were citizens; these collectors are professional: Arnaud Bellina, FREDON Bourgogne; Laurent Charles, Muséum Science et Nature, Bordeaux; Mathieu Coulis, CIRAD Martinique; Pierre-Damien Lucas, FREDON Martinique; Guy Van Laere, Parc National de Guadeloupe.

Species	MNHN	GenBank #	date	Locality	Department / State	Country - Continent	COI	Replicates	Collector
BK	JL089	MG655587	12/11/2013	Saint Pée sur Nivelle	Pyrénées-Atlantiques	Met. France - Europe	short	1	Consent not obtained
BK	JL160	MG655605	23/05/2014	Cannes	Alpes-Maritimes	Met. France - Europe	short	1	Iachia, Valeria
BK	JL167	MG655615	24/08/2014	Orthez	Pyrénées-Atlantiques	Met. France - Europe	short	1	Rougeux, Christian
BK	JL174	MG655616	03/09/2014	Bassussary	Pyrénées-Atlantiques	Met. France - Europe	long	1	Mercader, Elisabeth
BK	JL176 **	MG655617	05/09/2014	Auxerre (hothouse)	Yonne	Met. France - Europe	long	1	Bellina, Arnaud
BK	JL184	MG655603	Oct. 2014	Ustaritz	Pyrénées-Atlantiques	Met. France - Europe	short	1	Goyheneche, Iker
BK	JL188	MG655604	08/10/2014	Miramar	Grande Porto	Portugal - Europe	short	1	Soarès, Luciana
BK	JL212	MG655592	19/12/2014	Mimbastes	Landes	Met. France - Europe	long	1	Jouveau, Séverin
BK	JL224	MG655607	23/02/2015	Trois Rivières	Guadeloupe	Guadeloupe - C. America	long	1	Van Laere, Guy
BK	JL233	MG655608	27/09/2014	Monaco	Monaco	Monaco - Europe	long	3	Dusoulier, François
BK	JL253	MG655609	21/03/2015	Trois Rivières	Guadeloupe	Guadeloupe - C. America	short	1	Van Laere, Guy
BK	JL254	MG655610	15/05/2015	Matoury	French Guiana	French Guiana - S. America	short	2	Girault, Rémi
BK	JL270	MG655594	23/04/2015	Ducos	Martinique	Martinique - C. America	long	1	Lucas, Pierre-Damien
BK	JL308	MG655602	08/09/2016	Morne Vert	Guadeloupe	Guadeloupe - C. America	short	1	Coulis, Mathieu
BV	JL073	MG655611	Aug. 2013	Sanibel	Florida	USA - North America	short	1	Justine, Jean-Lou
BV	JL163	MG655613	July 2014	Sanibel	Florida	USA - North America	short	1	Justine, Jean-Lou
BV	JL164	MG655614	July 2014	Sanibel	Florida	USA - North America	short	1	Justine, Jean-Lou
BV	JL213	MG655593	29/11/2014	Anse-Bertrand	Guadeloupe	Guadeloupe - C. America	long	1	Charles, Laurent
BV	JL268	MG655595	Dec. 2014	Montserrat	Montserrat	Montserrat - C. America	short	1	Shoobs, Nathaniel F.
BV	JL307	MG655601	19/11/2015	Morne Vert	Guadeloupe	Guadeloupe - C. America	short	1	Coulis, Mathieu
DM	JL177 *	KT922162	30/09/2014	Léguevin	Haute-Garonne	Met. France - Europe	long	1	Chaim, Florence
DM	JL059	MG655618	15/06/2013	La Bastide de Serou	Ariège	Met. France - Europe	short	1	Brugnara, Sébastien
DM	JL142	MG655612	22/04/2014	Saubrigues	Landes	Met. France - Europe	long	2	Robineau, Thiérry
DM	JL161	MG655606	11/06/2015	Bellocq	Pyrénées-Atlantiques	Met. France - Europe	long	1	Audiot, Marie-Claude
DM	JL208	MG655589	11/06/2014	Bellocq	Pyrénées-Atlantiques	Met. France - Europe	long	1	Audiot, Marie-Claude
DM	JL209	MG655590	12/06/2014	Bellocq	Pyrénées-Atlantiques	Met. France - Europe	long	1	Audiot, Marie-Claude
DM	JL210	MG655591	June 2014	Bellocq	Pyrénées-Atlantiques	Met. France - Europe	long	1	Audiot, Marie-Claude
DM	JL298 ***	MG655600	01/06/2016	Novazzano	Ticino Canton	Switzerland - Europe	long	1	Pollini, Lucia
DBlue	JL280	MG655596	2015	Mtsamboro	Mayotte	Mayotte - Africa	long	1	Charles, Laurent
DBlue	JL281	MG655597	29/04/2015	Mtsamboro	Mayotte	Mayotte - Africa	long	3	Charles, Laurent
DBlue	JL282	MG655598	30/04/2015	Ouangani	Mayotte	Mayotte - Africa	long	1	Charles, Laurent
DBlue	JL284	MG655599	05/05/2015	Mtsamboro	Mayotte	Mayotte - Africa	long	1	Charles, Laurent
DBlack	JL090	MG655588	12/11/2013	Saint Pée sur Nivelle	Pyrénées-Atlantiques	Met. France - Europe	short	1	Consent not obtained

Table 3(on next page)

Divergences between “short” sequences of bipaliine flatworms.

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 of bipaliine flatworms.

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 36 records (35 from outdoor and 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).

* Muséum d’Histoire Naturelle, Nice, France; ** FREDON Île de France.

#	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	03/11/2013	Cagnes-sur-Mer	Alpes-Maritimes	Met. France - Europe	Gros, Pierre
K09	19/01/2014	Cagnes-sur-Mer	Alpes-Maritimes	Met. France - Europe	Gros, Pierre
K10	05/11/2014	Cagnes-sur-Mer	Alpes-Maritimes	Met. France - Europe	Gros, Pierre
K11	16/10/2013	Beaulieu-sur-Mer	Alpes-Maritimes	Met. France - Europe	Pelcer, Jean-Paul
K12	21/07/2014	Nice	Alpes-Maritimes	Met. France - Europe	Gerriet, Olivier *
K13	15/10/2014	Appietto	Corse-Sud (Corsica)	Met. France - Europe	Consent not obtained
K14	17/10/2013	Pietrosella	Corse-Sud (Corsica)	Met. France - Europe	Senee, Patrick
K15	23/08/2014	Arcachon	Gironde	Met. France - Europe	Consent not obtained
K16	21/11/2002	Saint-Jean-de-Vedas	Hérault	Met. France - Europe	Peaucellier, Gérard
K17	27/10/2014	Biscarosse	Landes	Met. France - Europe	Consent not obtained
K18	27/09/2008	Hagetmau	Landes	Met. France - Europe	Jeannotin, Josette
K19	22/09/2016	Nantes	Loire-Atlantique	Met. France - Europe	Consent not obtained
K20	16/10/2014	Grimaud	Var	Met. France - Europe	Bernez, Alain
K21	01/08/2014	Toulon	Var	Met. France - Europe	Consent not obtained
K22	29/07/2014	Sens (Hothouse)	Yonne	Met. France - Europe	Burel, Jonathan **
K23	23/10/2017	Peyrouse	Hautes-Pyrénées	Met. France - Europe	Tremosa, Clémence
K24	17/12/2014	Arthez de Béarn	Pyrénées-Atlantiques	Met. France - Europe	Sillard, Dominique
K25	17/09/2017	Billère	Pyrénées-Atlantiques	Met. France - Europe	Rolland, Geneviève
K26	28/01/2018	Billère	Pyrénées-Atlantiques	Met. France - Europe	Rolland, Geneviève
K27	20/09/2014	Bayonne	Pyrénées-Atlantiques	Met. France - Europe	Bonnefous, François
K28	18/08/2014	Hasparren	Pyrénées-Atlantiques	Met. France - Europe	Voise, Mireille
K29	22/04/2016	Jurançon (near)	Pyrénées-Atlantiques	Met. France - Europe	Pauchet, Marjolaine
K30	29/04/2016	Nay	Pyrénées-Atlantiques	Met. France - Europe	Lamaille, Corinne

K31	28/09/2014	Orthez	Pyrénées-Atlantiques	Met. France - Europe	Rougeux, Christian
K32	22/08/2016	Saint Jean de Luz	Pyrénées-Atlantiques	Met. France - Europe	Centelles, Ruben
K33	01/01/1999	Urcuit	Pyrénées-Atlantiques	Met. France - Europe	Esposito, Mario
K34	14/09/2014	Urt	Pyrénées-Atlantiques	Met. France - Europe	Chanderot, Vincent
K35	12/08/2017	Ustaritz	Pyrénées-Atlantiques	Met. France - Europe	Lescourret, Monique & Bernard
K36	14/09/2014	Villefranque	Pyrénées-Atlantiques	Met. France - Europe	Consent not obtained

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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.

* FREDON Île de France.

#	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 (no molecular identification).

Photographs were obtained through citizen science; specimens were identified from photographs by the authors. No molecular identification was possible. There were 33 records, all from outdoor. 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	21/10/2017	Petit-Canal	Guadeloupe	Guadeloupe - C. America	Charles, Laurent
V12	29/11/2016	Le Moule	Guadeloupe	Guadeloupe - C. America	Consent non obtained
V13	25/07/2010	La Trinité	Martinique	Martinique - C. America	Delannoye, Régis
V14	18/11/2015	Morne Vert	Martinique	Martinique - C. America	Coulis, Mathieu
V15	05/01/2018	Trois Ilets	Martinique	Martinique - C. America	Consent non obtained
V16	01/04/2014	Saint Barthélemy	Saint Barthélemy	Saint Barthélemy - C. America	Moulard, Grégory
V17	01/05/2014	Saint Barthélemy	Saint Barthélemy	Saint Barthélemy - C. America	Consent not obtained
V18	11/05/2014	Saint Martin	Saint Martin	Saint Martin – C. America	Yokoyama, Mark
V19	21/11/2015	Avirons	La Réunion	La Réunion - Africa	Consent not obtained
V20	23/03/2017	Bras Panon	La Réunion	La Réunion - Africa	Saman-Latchimy, Teddy
V21	29/03/2017	Le Tampon	La Réunion	La Réunion - Africa	Consent not obtained
V22	26/10/2014	Petite Ile	La Réunion	La Réunion - Africa	Abonnenc, José
V23	12/03/2016	Petite Ile	La Réunion	La Réunion - Africa	Le Gars, René
V24	16/05/2014	Saint Louis	La Réunion	La Réunion - Africa	Faujour, Anne
V25	08/04/2014	Saint Paul	La Réunion	La Réunion - Africa	Consent not obtained
V26	16/03/2016	Saint Pierre	La Réunion	La Réunion - Africa	Collet, Jean
V27	10/03/2013	Sainte Marie	La Réunion	La Réunion - Africa	Fontaine, Romuald
V28	06/03/2016	Sainte Marie	La Réunion	La Réunion - Africa	Fontaine, Romuald
V29	12/02/2009	unknown	La Réunion	La Réunion - Africa	Gilson, Michel
V30	03/03/2010	unknown	La Réunion	La Réunion - Africa	Gilson, Michel
V31	01/05/2011	unknown	La Réunion	La Réunion - Africa	Martiré, Dominique
V32	28/10/2013	unknown	La Réunion	La Réunion - Africa	Martiré, Dominique
V33	17/08/2015	unknown	La Réunion	La Réunion - Africa	Lacoste, Marie

Table 8 (on next page)

Records of *Diversibipalium* “blue” identified from photographs (no molecular identification).

1 record.

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

Table 9(on next page)

Measurements of living specimens of bipaliines.

Measurements were estimated from photographs with scales obtained from citizen science (Supplemental Files 1 & 2).

Species	MNHN Specimen or photograph from Citizen Science	Locality	Body length (cm)
Bipalium kewense	MNHN JL089	France	21
	MNHN JL184	France	16
	MNHN JL188	Portugal	25
	MNHN JL224	Guadeloupe	21
	MNHN JL270	Martinique	11
	K04	Guadeloupe	13
	K05	Martinique	20
	K07	La Réunion	10
	K24	France	20
	K25	France	27
	K28	France	15
	K35	France	17
Diversibipalium multilineatum	MNHN JL177	France	15
	MNHN JL059	France	21
Bipalium vagum	V04	French Guiana	3.6