1. Basic reporting

English is acceptable (but it is not my mother language). Literature is not suficiently and adequately explored. Article structure is deficient. Relevant results are hiddend by not original data.

2. Experimental design

Manuscript meets Aims and Scope of the Journal. Research question is well stated. Methods are not fully with sufficient detail.

3. Validity of the findings

Impact and novelty are obscured by many secondary informative information obtained from anectodal observations and from literature, sometimes both sources are mixed and confounded with each other. Data are partially robust. Conclusion is not fully supported by the results.

In this manuscript the authors provide a number of new records of exotic bipaliine species introduced into France and overseas French territories. Part of the records were gathered with the collaboration of citizens during the last four years. Identification of the specimens from up to five species was conducted through examination of pictures of the specimens and COI gene fragment sequences, and compared against DNA sequences from GenBank. Since I am not expert in the molecular methods my comments on this point should be considered anecdotal.

Regarding the general aspects of the manuscript, I noticed main deficiencies as follows: (a) part of the headings and subheadings are out of place, (b) statements in Results are not based on evidences, (c) methods on which behavioural aspects (predation, reproduction) reported in Results and Discussion are not presented, and (d) behavioural aspects bring no novelty and are anecdotal. The whole manuscripts needs a profound revision and many sections should be deleted:

MATERIAL AND METHODS (M&M)

• It is not stated how the descriptions of the species were done. It is not presented how behavioural and reproductive observations were conducted (although they should be deleted since they are out of the scope of the manuscript).

RESULTS

Description of B. kewense (lines 225-237) is a practically a copy-pasted version of Winsor's
 (1983) description (as acknowledged by the authors). This is wrong. In this context, the
 purpose of the description is to provide evidence that the examined specimens do belong to
 kewense. Authors should have described their own specimens, and eventually conclude that

- the description matches that of B. kewense. The same is valid for the remaining known species.
- "Predation of earthworms", and "Morphological evidences of reproduction by scissiparity"
 of B. kewense (lines 242-249) are out of the scope of the work and Methods followed by the
 authors for obtaining associated data are not provided. These two subheadings should be
 deleted (out of scope).
- Line 264: This statement has no support from the data and M&M (How did authors obtained these results? Did they offer non-earthworm prey?). These lines should be deleted.
- After having read the description of B. kewense, I am not sure whether the description of
 external aspect of Bipalium multilineatum (lines 269-288) is original or it is a version of a
 description found elsewhere. Only descriptions based on collected specimens are valid for
 identification purposes. This should be stated unequivocally in M&M and the Results
 presented accordingly.
- Lines 289-293 are not "Morphology" but comparative discussion and should be placed in a separate subheading. The same for every "Morphology" section of all species.
- Source of the description of Bipalium vagum (lines 310-322) is uncertain. At least body dimensions are from the holotype found in Jones & Sterrer (2005) not from the specimens (or pictures) collected by the authors; this is wrong (see comment for B. kewense). It seems that the remaining description is based on the observation of the French specimens collected by the authors.
- "Remarks" (lines 345-348; 363). Authors state that "The COI barcode of this specimen is clearly different from all other known sequences" and from this difference they conclude that it represents a different species. As presente, this is a weak argument. For instance, see the two terminals of B. venosum on Fig. 2; they are clearly different, but also considered to belong to just one species. The statement should be rewritten.

DISCUSSION

- Supporting references should be given (lines 390-393).
- Subheadings "Possible specific identity of the "black" and "blue" species", and "A note about taxonomy of Diversibipalium" should be moved to the "Remarks" of either species.
- Subheading "Predation in Bipaliines" (lines 408-431) should be deleted. The statements presented are purposeless and not derived from the M&M nor from Results.
- Lines 438-444: It is concluded that "clearly, B. kewense is an invasive species". Further in the same paragraph the authors declare to have examined the literature and citizen-science

information to show that B. kewense is not confined to indoors but it may also be found outdoors. From having recorded B. kewense outdoors, authors conclude that this species is invasive. The meaning of is term, however, is not free from univocal meaning. What does it mean invasive for the authors? For a discussion, see Coulatti & MacIsaac (A neutral terminology to define 'invasive' species. Diversity and Distributions, (2004) 10, 135–141). Most records shown do no report the environs: indoors, outdoors, in natural habitats, natural forest areas, planted forests? There are indeed no reports on possible detrimental effects on the native fauna or rural activities (earthworm farms, grasslands...) apart from the two references provided (Winsor, 1983a, 1888b) in which it is reported that B. kewense is "a minor pest in earthworm farms". To the best of my knowledge, this species has not been found in natural habitats outside its original distributional range area. The title of the manuscript is revealing in showing it: "flatworms [...] in *metropolitan* France [...]". By reading the manuscript, I do not see reason for considering the species as invasive, independently from the habitats it is found. In the same rationale, the House Sparrow *Passer* domesticus could also be considered an invasive species. Author should make it clear what does 'invasive' mean, as suggested above.

• It is unclear whether distributional data presented on lines 445-454 and Fig. 38 are the same shown in Fig. 1.

CONCLUSIONS

- Some conclusions are not supported by the Results.
- Last sentence is a belief (as they said) since no data are provided.

I would make additional minor comments to the manuscript, but at this stage, the text should best be rewritten. Nevertheless, I made minor suggestion in the PDF version of the manuscript. I recomend Major revision.

Fernando Carbayo



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

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 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

¹ ISYEB - Institut de Systématique, Évolution, Biodiversité, Muséum National d'Histoire Naturelle, Paris, France

² College of Science and Engineering, James Cook University of North Queensland, 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



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.



I disagree with the term "hammerhead' for describing the shape of the cephalic region. It is semilunate or fan-shaped.

- 1 Giant worms chez moi! Hammerhead flatworms (Platyhelminthes,
- 2 Geoplanidae, Bipalium spp., Diversibipalium spp.) in metropolitan France and
- 3 overseas French territories
- 4 Jean-Lou Justine 1*, Leigh Winsor 2, Delphine Gey 3, Pierre Gros 4 and Jessica
- 5 Thévenot 5
- 6 1 ISYEB, Institut de Systématique Evolution Biodiversité, UMR7205 CNRS, EPHE,
- 7 MNHN, UPMC, Muséum National d'Histoire Naturelle, Sorbonne Universités, Paris, France
- 8 2 College of Science and Engineering, James Cook University, Townsville, Australia
- 9 3 Service de Systématique moléculaire, Muséum National d'Histoire Naturelle, Paris,
- 10 France
- 11 4 Amateur Naturalist, Cagnes-sur-Mer, France
- 12 5 Coordination technique et scientifique de la stratégie nationale relative aux espèces
- 13 exotiques envahissantes, UMS Patrinat, Muséum National d'Histoire Naturelle, Sorbonne
- 14 Universités, Paris, France
- 15 Correponding author: Jean-Lou Justine, justine@mnhn.fr
- 16 ISYEB, Institut de Systématique Evolution Biodiversité
- 17 UMR7205 (CNRS, EPHE, MNHN, UPMC)
- 18 55 Rue Buffon
- 19 Muséum National d'Histoire Naturelle
- 20 Sorbonne Universités
- 21 Paris, France



22 Abstract

- 23 **Background.** Species of the genera *Bipalium* and *Diversibipalium*, or bipaliines, are giants
- 24 among land planarians (family Geoplanidae), reaching length of 400 mm; they are also easily
- 25 distinguished from other land flatworms by the characteristic hammer shape of their head.
- 26 Bipaliines, which have their origin in warm parts of Asia, are invasive species, now widespread
- 27 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.
- 29 **Methods.** In this paper, on the basis of a 4-year survey based on citizen science, which yielded
- 30 observations from 1999 to 2017, we provide information about the 5 species present in
- 31 Metropolitan France and French overseas territories. We also investigated the molecular
- variability of cytochrome-oxidase 1 (COI) sequences of specimens.
- 33 **Results.** Three species are reported from Metropolitan France: *Bipalium kewense*,
- 34 Diversibipalium multilineatum, and an unnamed Diversibipalium "black" species. We also report
- 35 the presence of *B. kewense* from overseas territories, such as French Polynesia (Oceania), French
- 36 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
- 38 Africa). For B. vagum, observations include French Guiana, Guadeloupe, Martinique, Saint
- 39 Barthélemy, Saint Martin, Montserrat, La Réunion, and Florida (USA). A probable new species,
- 40 Diversibipalium sp. "blue", is reported from Mayotte Island (off South-East Africa). Bipalium
- 41 kewense, B. vagum and D. multilineatum each showed 0% variability in their COI sequences,
- 42 whatever their origin, suggesting that the specimens are clonal, and that sexual reproduction is
- 43 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
- 45 invasive species. In Metropolitan south-west France, a small area located in the Department of
- 46 Pyrénées-Atlantiques was found to be a hot-spot of bipaliine biodiversity and abundance for more
- 47 than 20 years, probably because of the local mild weather.
- 48 **Discussion.** Our numerous records in the open in Metropolitan France raise questions: as
- 49 scientists, we were astonished that these long and brightly coloured worms could escape the
- 50 attention of scientists and authorities in a European developed country for such a long time;
- 51 improved awareness about land planarians is probably necessary.



65

66

67

79

80

Introduction

53	Land planarians (Platyhelminthes, Geoplanidae) are predatory soil-associated animals. Although
54	small species (generally less than 1 cm in length) such as Microplana spp. or Rhynchodemus spp.
55	are autochthonous in Europe (Álvarez-Presas et al. 2012), large species are not. Reports of
56	invasive alien flatworms in Europe in recent years (Sluys 2016) include Arthurdendyus
57	triangulatus from New Zealand, Platydemus manokwari originally from Papua New Guinea,
58	Obama nungara from Brazil, and Parakontikia ventrolineata, Caenoplana coerulea and
59	Caenoplana bicolor from Australia (see Table 1 for authors of taxa and key references). All these
60	species are conspicuous animals, several centimetres in length. Even larger are the species of I believe fan-shaped / shovel / semilunate best
61	Bipalium (and close genera), or "hammerhead flatworms": these can be longer than 20 tescribes the headplate
62	centimetres (von Graff 1899). In this paper, we focus on these giant species, and we report new
63	A justification of why you only study these species is lacking? Systematic-based, easy of identification? Please add a findings obtained mainly by citizen science in metropolitan France and overseas French sentence
64	territories in the Caribbean (Guadeloupe, Martinique, and Saint Barthélemy), South America

(French Guiana) and Indian Ocean (La Réunion, Mayotte). Five species were found, among

Diversibipalium multilineatum) and two are unnamed.

which three can be attributed to known binomial taxa (Bipalium kewense, Bipalium vagum and

68 Winsor summarized knowledge about the world distribution of *Bipalium kewense*, listing 69 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, 70 Sardinia, and Sicily (Gremigni 2003); Czech Republic and Slovakia (Košel 2002); Cuba (Morffe 71 72 et al. 2016); Ecuador (Wizen 2015); and Pakistan (M. Darley, personal communication to LW). 73 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 74 75 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 76 77 paper), we consider that the major finding of this paper is that several species of hammerhead 78 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

88	Citizen science and collection of information
89	In 2013, one of us (JLJ) organized a citizen science network in France for collecting information
90	about land planarians. This included a blog (http://bit.ly/Plathelminthe) and a twitter account
91	(https://twitter.com/Plathelminthe4). These efforts were advertised through the media (radio,
92	television, and newspapers).
93	Reports of sighting of land planarians were received from citizens, mainly by email, sometimes
94	by telephone. Photographs and details about locality were solicited, and only reports including
95	this information were considered. Wrong records (slugs, myriapods, earthworms, leeches,
96	caterpillars, nematomorphs, and nemerteans) were eliminated. Information collected from citizen
97	science allowed monitoring of several land planarians (Justine et al. 2014a). Photographs were
98	studied, and species were identified whenever possible. Only information relative to bipaliines is
99	reported in this paper). Sometimes citizens provided records dating from before the survey, such
100	as an amateur movie taken in 1999. Most citizens provided an authorisation to use the
101	photographs at the time of the initial contact by email. When we prepared this paper for
102	publication, we sought authorization to use the photographs and to publish them under a Creative
103	Commons Licence; none of the citizens refused to provide the authorization, but some of them
104	did not respond, probably simply because they changed their emails or did not check them. In this
105	case, we provide the scientific information about the presence of species, but we do not include
106	the photograph or the names of the citizen in the paper.
107	Although these efforts were originally aimed at collecting information from Metropolitan France,
108	they unexpectedly reached French territories in other continents and provided additional
109	information and specimens.

Collection of specimens 110 In some cases, after examination of photographs, specimens were solicited from citizens who 111 112 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 113 114 molecular analysis. 115 When specimens were obtained alive, they were fixed in hot water then preserved in 95% 116 ethanol. In some cases, some specimens were also fixed in hot water and preserved in 4% 117 formaldehyde solution. Molecular sequences 118 For molecular analysis, a small piece of the body (1-3 mm³) was taken from the lateral edge of 119 ethanol-fixed individuals. Genomic DNA was extracted using the OIAamp DNA Mini Kit 120 121 (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) 122 123 (forward 5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and JB4.5 (=COI-ASmit2) (reverse 5'-TAAAGAAAGAACATAATGAAAATG-3') (Bowles et al. 1995; Littlewood et al. 1997). The 124 125 PCR reaction was performed in 20 µl, containing 1 ng of DNA, 1× CoralLoad PCR buffer, 3Mm 126 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", 127 48 °C for 40", 72 °C for 50", with a final extension at 72 °C for 7'. A fragment of 825 bp was 128 129 amplified with the primers BarS (forward 5'-GTTATGCCTGTAATGATTG-3') (Álvarez-Presas et al. 2011) and COIR (reverse 5'-CCWGTYARMCCHCCWAYAGTAAA-3') (Lázaro et al. 2009), 130 131 following (Mateos et al. 2013). PCR products were purified and sequenced in both directions on a 132 3730xl DNA Analyzer 96-capillary sequencer (Applied Biosystems). Results of both analyses 133 were concatenated to obtain a COI sequence of 909 bp in length (designated in this text as "long" 134 sequence"). Sequences were edited using CodonCode Aligner software (CodonCode Corporation, 135 Dedham, MA, USA), compared to the GenBank database content using BLAST and deposited in 136 GenBank under accession number xxxx-xxxx. For several specimens only "short" sequences 137 were obtained (Table 2). **Trees and distances** 138 MEGA7 (Kumar et al. 2016) was used to estimate genetic distances (kimura-2 parameter 139 140 distance) and the evolutionary history was inferred from the kimura-2 parameter distance using



141	the Neighbour-Joining method (Saitou & Nei 1987); all codon positions were used, with 1000
142	bootstrap replications. The evolutionary history was also inferred using Maximum Likelihood
143	(ML) method. The best evolutionary model for the data set was estimated in MEGA7 (Kumar et
144	al. 2016) under the Bayesian Information Criterion (BIC) to be Hasegawa-Kishino-Yano model
145	(Hasegawa, Kishino & Yano, 1985) with a discrete Gamma distribution and some sites
146	invariables (HKY + G +I). The ML tree was computed in MEGA7, with 100 bootstrap
147	replications. Both NJ and ML trees showed comparable topologies, but the bootstrap values of
148	branches, in both trees, were contrasted: 100% for all branches representing species, and very low
149	Ambiguous. Do you mean 'most basal' / 'supraspecific'? for upper nodes. We thus considered that the trees were informative for showing the genetic
150	identity of all specimens within a species, but not for inferring relationships between taxa; thus,
151	no further comment about interspecies relationships are given in the rest of this text.
152	A note about taxonomy of <i>Diversibipalium</i>
153	Morphology-based taxonomy of land planarians is based on a suite of characters, especially those
154	afforded by internal anatomy, and in particular those of the reproductive system (Winsor et al.
155	1998). Reproductive organs are only available in sexually mature specimens and require
156	extensive histological preparations for their description. Unfortunately, many species of land
157	planarians have been described from external morphology only. Some species can use only
158	asexual reproduction and thus do no show mature organs; this is especially the case of some
159	invasive species when they are not in their region of origin. However, the bipaliines represent a
160	special case because the external morphology, i.e. the presence of a "hammer" head is distinctive
161	of the subfamily, which thus can be easily differentiated if a photograph of the head is available.
162	The genus Diversibipalium Kawakatsu et al., 2002 is a collective group created to temporarily
163	accommodate species of the subfamily Bipaliinae whose anatomy of the copulatory apparatus is
164	still unknown (Kawakatsu et al. 2002). For this reason, we attribute our two undescribed species,
165	"black" and "blue" to this genus. We insist that attribution of species to the genus
166	Diversibipalium does not mean that these species have characters in common – the only feature
167	they share is our ignorance of their internal anatomy.



168	Results Rewrite first sentence. Important is not initial finding but lauching the citizen program. as it is written, it seems that Gros's finding launched the reasearch
169	Collection of information from citizen science
170	After the initial finding in June 2013 of two species in his garden by Pierre Gros, an amateur
171	entomologist and photographer, more than 600 reports were received over 4 years (June 2013-
172	September 2017). Most records were from citizens, some from scientists or other professionals.
173	Unexpectedly, these reports included mentions of more than 8 species of land planarians (Justine
174	et al. 2014a), the most recent being Marionfyfea adventor. Among these, 106 reports concerned
175	bipaliines. Figure 1 is a map of these records in Metropolitan France.
176	Results are presented here as follows: after an assessment of the identification of specimens from
177	both morphology and molecules, separate paragraphs provide, for each species, a brief
178	description and its range in Metropolitan France and overseas French territories, from both
179	sampled specimens and photographs obtained through citizen science.
180	Molecular identification of sampled specimens
181	Sequences were obtained from specimens belong to five species (Table 2), including three named
182	species, <i>Bipalium kewense</i> (specimens from 13 localities, 17 sequences including replicates),
183	Diversibipalium multilineatum (specimens from 4 localities, 8 sequences including replicates),
184	Bipalium vagum (specimens from 3 localities, 5 sequences including replicates) and two
185	unnamed species, Diversibipalium "black" (1 specimen from 1 locality, 1 sequence) and
186	Diversibipalium "blue" (specimens from 2 localities, 6 sequences including replicates).
187	inferred A tree was constructed from an analysis of our new COI sequences and sequences from
188	GenBank. Each of the three-named species belonged to a clade with high (100%) bootstrap
189	support (Figure 2). As explained in Materials and Methods, we do not comment upon the
190	relationships between nodes because of very low bootstrap values.
191	Açores For <i>Bipalium kewense</i> , the clade includes GenBank sequences from Spain, Acores Islands, and
192	Cuba; our 13 new sequences (excluding replicates) are from 7 localities in metropolitan France, 3
193	overseas French territories (Guadeloupe, Martinique, French Guiana) and 2 other countries,
194	Monaco and Portugal. All COI sequences were strictly identical.
195	For Diversibipalium multilineatum, the clade includes GenBank sequences from Italy and France
196	(sequence from specimen MNHN JL177, already published (Mazza et al. 2016)), and our 6 new



197 198	sequences (excluding replicates) are from 3 localities in metropolitan France. All COI sequences were strictly identical.
199	For Bipalium vagum, no sequence was found in GenBank. Our 5 new sequences are from 1
200	overseas French territory (Guadeloupe) and 2 other countries, Montserrat (West Indies) and
201	Florida, USA. All COI sequences were strictly identical.
202	For Diversibipalium "black" from Metropolitan France and Diversibipalium "blue" from
203	Mayotte, each sequence was found to have no close match in GenBank sequences or our new
204	sequences, suggesting that they each belong to a species which has never been sequenced for COI
205	gene.
206	Distances between taxa
207	"Short" sequences were obtained from all specimens and "long" sequences" were obtained from
208	only some of them. Distances between species of bipaliines were computed from two sets of
209	sequences, "short" sequences and "long" sequences.
210	The first set included "short" sequences and 7 bipaliin taxa were available. Distances varied from
211	10.9% to 21.2% (Table 3). The closest taxa were B . kewense – D . multilineatum with an
212	interspecific distance of 10.9%, and the most distant were <i>Diversibipalium</i> "blue" and <i>B</i> .
213	adventitium with 21.2%.
214	The second set included only "long" sequences and 4 bipaliin taxa were available. Distances were
215	higher than with short sequences and varied from 15.9% to 25.9% (Table 4). The closest taxa
216	were, again, B. kewense – D. multilineatum with an interspecific distance of 15.9%, and the most
217	distant were <i>Diversibipalium</i> "blue" and <i>D. multilineatum</i> with 25.9%. Biological?/Taxonomic? Make it clear
218	Information for each species
219	I disagree with copy-paste Winsor's description of B. kewense. Important here is to provide diagnostic features observed in the specimens collected for this study so as to provide their identification.
220	Morphology
221	Italics A long, thin bipaliine, living specimens of B. kewense may attain a length of up to 350 mm. The
222	dorsal ground colour is usually a light ochre (Figure 3), with five black to grey-coloured
223	longitudinal stripes: a median, paired lateral, and paired marginal stripes which begin at or near
	Tongeration outpost a moduli, panea interior, and panea marginar outpost which degin at of hour



224	the base of the headplate where it joins the body the neck. The headplate (Figure 4) is usually
225	the same colour as the body, or slightly darker, with recurved posterior margins. The median
226	stripe is black, narrow, with sharp margins, extending caudally from below the neck over the
227	entire body length, and is broadest over the pharyngeal area. Paired dark to pale brown coloured
228	lateral stripes with diffuse margins, constant over the entire body length, are separated from the
229	median and marginal stripes by an equal width of ground colour. The paired black, fine, marginal
230	stripes, with sharp margins, extend the entire body length. The paired lateral and marginal stripes
231	unite just behind the neck to form an incomplete black transverse neck band, interrupted dorsally
232	by a small median gap, and ventrally by the creeping sole. The headplate is a greyish colour with
233	a light ochre margin. The ventral surface (Figure 5) is a light ochre colour, with a distinct off-
234	white creeping sole, delineated by paired, narrow, longitudinal diffuse grey-violet stripes
235	beginning at the ventral termination of the collar, and extending the entire body length. In a
236	preserved sexual specimen, the mouth is situated ventrally at 31.2 mm (39.7 % of the body
237	length) from the anterior end, and gonopore 6 mm (7.7% of the body length) posterior to the
238	mouth (Winsor 1983a). Bipalium kewense is differentiated externally from similar striped species
239	by the incomplete black transverse band at the neck (the "collar"), the thin dorsal median
240	longitudinal stripe that begins at or below the transverse neck band, the pattern and form of the
241	dorsal and ventral stripes, and the relative position of body apertures.
242	Predation of earthworms
243	The main stages in <i>B. kewense</i> hunting, attacking, capturing, and consuming an earthworm are
244	illustrated in Figures 6-16.
245	Morphological evidences of reproduction by scissiparity
246	Some 1-2 days following feeding, the fission process in <i>B. kewense</i> is first manifested by a slight
247	pinching of the body, some 1-2 cm. from the tail tip (Figures 17-18). Severance occurs when the
248	tail tip adheres to the substratum and the rest of the planarian pulls away. The free tail fragment is
249	immediately motile.
250	Remarks
251	We obtained 44 records of <i>B. kewense</i> , including 14 confirmed by molecules and 30 from
252	photographs only (Tables 2, 5). Localities where bipaliines were found in the open, generally in
253	gardens, include Portugal (1 record), Martinique (3), Guadeloupe (7), French Guiana (1), La



254 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 255 (1), Landes (3), Alpes-Maritimes (2), Yonne (1), and Pyrénées-Atlantiques (15). In addition, we 256 257 received two reports in hothouses in the Department of Yonne. Among the 28 records in the open 258 in Metropolitan France, 15, i.e. more than half, were from the department of Pyrénées-259 Atlantiques. The distribution of our records is shown in Figure 5 for Metropolitan France 260 (including Corsica). Dates of records ranged 1999-2017; the oldest record (1999) was in the 261 Pyrénées-Atlantiques. 262 The COI sequences were strictly identical for specimens from all localities where specimens were 263 sequenced. 264 Earthworms appear to be the sole natural prey of B. kewense. The hunting, capture and 265 consumption of this prey have been vividly described (Barnwell et al. 1965; Johri 1952; Lehnert 266 1891). 267 Diversibipalium multilineatum (Makino and Shirasawa, 1982) Morphology 268 269 A long thin bipaliine, living specimens of D. multilineatum may attain lengths of more than 200 270 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 271 paired marginal longitudinal stripes. The median stripe is black, and narrow with sharp margins. 272 273 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 274 length, broadest over the pharyngeal area. Either side of the median stripe, each separated by an 275 276 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 277 278 lateral stripes are a black to dark brown colour with diffuse margins, approximately 2-3 times the 279 width of the median stripe; the narrow, brown paired marginal stripes are approximately the same 280 thickness as the median stripe. The ventral surface (Figure 21) is a light brown ochre colour, 281 generally slightly paler than that dorsally, with a distinct white creeping sole, delineated by 282 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

283



284	dark stripe extends from the anterior third of the ventral headplate to the posterior end (Figure
285	23). The posterior margins of the headplate are not recurved, giving the head a club-shape.
286	Preserved specimens are 24-138 mm long. In a preserved specimen (MNHN JL161A) 71 mm
287	long and 2 mm wide, the mouth is situated ventrally at 71 mm (36.6% of the body length) from
288	the anterior end. All specimens found to date are non-sexual and have no gonopore. They
289	reproduce by scissiparity, evidenced by the puckered, or bluntly rounded posterior tip./ New paragraph
290	Diversibipalium multilineatum is differentiated externally from similar elongate striped species
291	by the oblanceolate-shape of the beginning of the median stripe on the headplate, presence of
292	distinct dark paired ventral median stripes, the thin, dark, generally incomplete midventral
293	longitudinal stripe, and the relative position of the mouth.
294	Remarks
295	We obtained 1 record of D. multilineatum from Switzerland and 16 records in the open in
296	Metropolitan France, in the departments of Ariège (1), Haute-Garonne (3), Isère (2), Landes (2),
297	Val d'Oise (2), and Pyrénées-Atlantiques (6). One record was confirmed two years in a row
298	(2014-2015) in the same garden in Bellocq (Pyrénées-Atlantiques). In addition, two records were
299	from hot-houses, in the Department of Lot (1) and Val d'Oise (1). Among the 16 records in the
300	open in Metropolitan France, more than one third (6) are from the department of Pyrénées-
301	Atlantiques. The distribution of our records is shown in Figure 5 for Metropolitan France
302	(including Corsica). Dates of records ranged 2010-2017; the oldest record (2010) was in the
303	Pyrénées-Atlantiques (Tables 2, 6).
304	As for B. kewense, the COI sequences of D. multilineatum were strictly identical for specimens
305	from all localities.
306	Bipalium vagum Jones and Sterrer, 2005
307	Same comment as for B. kewense: weher are these morphological data from? Are they observations by the authors on the collected specimens? Morphology
308	A relatively small bipaliine, living specimens of this species attain a length of only some 35 mm.
309	Figures 24-27 show specimens from four localities, French Guiana, Guadeloupe, Martinique and
310	La Réunion, with varied colour patterns. The dorsal ground colour is a pale brown, with three
311	black to brown dorsal longitudinal stripes: a median sharply demarcated broad black stripe, and
312	two lateral dark brown stripes, less sharply delineated, all beginning at the transverse neck band,



313	continuing the length of the run body, and often terminating in a wen-defined black up. The
314	longitudinal stripes are separated from each other by an equal width of ground colour. Typically,
315	the headplate ground colour is a dark brown marginally with two large grey-brown to black
316	patches separated by an interval of lighter ground colour. The posterior margins of the headplate
317	are slightly recurved. In a preserved sexual specimen 24 mm long and 1.5 mm wide, the mouth is
318	situated ventrally at 12 mm (50% of the body length) from the anterior end, and the gonopore 5
319	mm (21% of the body length) posterior to the mouth (Jones & Sterrer 2005). Bipalium vagum is
320	distinguished externally from species of similar morphology by the combination of characters,
321	especially the transverse neck band that is continuous dorsally, from which the broad median
322	black stripe originates.
323	Remarks
324	No record was obtained from Metropolitan France. We obtained 37 records, all in the open, from
325	French Guiana (4 records) and from 5 islands in the West Indies, including Montserrat (1) and 4
326	French territories, namely Guadeloupe (8), Martinique (2), Saint Barthélemy (2), and Saint
327	Martin (1), and, from the Indian Ocean island of La Réunion (15); specimens from Florida, USA,
328	were also sequenced. Unfortunately, in spite of the many photographic records from La Réunion,
329	no specimen was received for sequencing, but the morphology and colour pattern were similar to
330	other localities (Figures 24-27). Dates of records ranged 2005-2017; the oldest record (2005) was
331	from French Guiana (Tables 2, 7).
332	The COI sequences were strictly identical for specimens from all localities.
333	Diversibipalium sp. "black" from Metropolitan France
334	Morphology
335	The dorsal ground colour of this small bipaliine is black, with no evidence of dorsal stripes
336	(Figures 28-30). The ventral surface is a light grey colour with paler creeping sole. The posterior
337	margins of the headplate are not recurved, giving the headplate a shovel-shape. The living
338	specimen attains a length of 20 – 25 mm. A preserved sexual specimen (Figures 31-32) is 20 mm
339	long and 3.2 mm wide, with the mouth situated ventrally 6 mm (30 % of the body length) from
340	the anterior end, and gonopore 1.8 mm (9% of the body length) posterior to the mouth.





341	This species was recorded in 2013 from a single garden in Saint-Pée-sur-Nivelle (Pyrénées
342	Atlantiques) in which B. kewense was also present. According to the owner, the species was
343	present for years in the garden and is still present in 2017.
344	Very informal and vague sentence. For example, although sequence data from specimens of N. venosum are not shown, they are also clearly different from all other known sequences, it does not mean they belong to different species.
345	The COI barcode of this specimen is clearly different from all other known sequences. We can
346	safely claim that this species has never been sequenced before. Whether the species is already
347	described or not is not an easy question to answer, and would require examination of mature
348	specimens; in the discussion we list a few species which have the same black colour pattern.
349	Diversibipalium sp. "blue" from Mayotte (Indian Ocean)
350	Morphology
351	The headplate in this beautiful, small planarian is a rusty-brown colour that extends to some
352	irregular patches on the "neck" (Figures 33-37). The dorsal ground colour is an iridescent blue-
353	green ("dark turquoise glitter"), and the ventral surface a very pale brown colour, with the
354	creeping sole white to pale green. The iridescence and blue-green colour are lost on fixation,
355	leaving a dark brown ground colour. The posterior margins of the headplate are not recurved, but
356	rounded, giving the headplate a club-shape. The living specimens are about 45 mm in length. The
357	preserved sexual specimen is 9 mm long and 1 mm wide, with the mouth situated ventrally
358	approximately 3.5 mm (39% of the body length) from the anterior end, and gonopore 3 mm (33%
359	of the body length) posterior to the mouth.
360	Remarks
361	We obtained records of this species only from Mayotte, from two independent observers, one
362	who provided specimens and photographs and one who provided only photographs (Tables 2,
363	8). The COI barcode of this specimen is clearly different from all other known sequences. We can
364	safely claim that this species has never been sequenced before. Whether the species is already
365	described or not is not an easy question to answer; see the discussion.



Discussion

367	Validity of COI for barcoding of bipaliine flatworms
368	Barcoding based on sequences of the mitochondrial gene cytochrome c oxidase I (COI) has been
369	proposed as a solution to the problem of species identification (Hebert et al. 2003). COI-based
370	barcodes have been found to be effective in various groups, including butterflies (Lepidoptera)
371	(Hebert et al. 2003) or fish (Ward et al. 2005). In flatworms (Platyhelminthes), although barcode
372	based only on COI sequences might not be the best choice for some groups (Vanhove et al. 2013),
373	recent studies showed that it efficiently differentiates species in groups such as monogeneans
374	(Ayadi et al. 2017; Chaabane et al. 2016) and various triclads (Álvarez-Presas & Riutort 2014)
375	including land planarians (family Geoplanidae) (Álvarez-Presas et al. 2011; Álvarez-Presas et al.
376	2014; Álvarez-Presas et al. 2012). Álvarez-Presas et al. 2011, 2012, 2014
377	The present study shows that COI short seguences, easily obtained from almost all specimens
	The present study shows that COI short sequences, easily obtained from almost all specimens,
378	have inter-specific distances of 10.9-21.2% (Table 3). These interspecific distances are high
379	enough to differentiate species of bipaliines, especially in the absence of intra-species variation.
380	Long sequences provide even higher inter-specific distances, ranging 15.9-25.9% (Table 4) but
381	these are less easily obtained, and the database includes only four species. Of course, it might be
382	objected that the current database (7 species with short sequences) is extremely limited in
383	comparison to the number of species described in the bipaliines – more than 160 (Winsor 1983a).
384	However, the current database includes most invasive world-wide species, inter-specific distances
385	are high, and intra-specific variation was almost inexistent for most species. For these reasons,
386	we believe that identification of common invasive species of bipaliine flatworms can reliably be
387	done from COI barcoding. Barcoding can be done from a very small worm, immature, or even a
388	fragment. Moreover, COI barcoding can probably alert authorities to the presence of species not
389	previously sequenced, if a sequence different from those reported in the present study is found.
390	The fact that some bipaliines do not reproduce sexually outside their native habitat or tropical and
390 391	subtropical climates, but only by scissiparity, is probably one reason explaining why no
392	variability was found in specimens, since specimens are cloned, and no or very few mutations can
393	happen. However, this reason is not sufficient, since several populations from various origins,
394	each cloning itself, could be present in the world. In contrast, for <i>Platydemus manokwari</i> , COI
395	sequences demonstrated the existence of at least two haplotypes in the world, probably
396	corresponding to two populations and different ways of invasion of the world (Justine et al.



397	2015). Our current data on bipallines suggest that one population is at the origin of the invasion
398	for each species. This is particularly striking for B. kewense, with identical molecular records
399	from several continents.
400	Possible specific identity of the "black" and "blue" species
401	Diversibipalium sp. "Black"
402	In the absence of detailed data in the literature, it is difficult at present to determine whether
403	Diversibipalium sp. 1 "Black" is a new species, or one of the small black species of
404	Diversibipalium such as D. sp. Kuamoto (Kawakatsu et al. 2005). Unclear
405	Diversibipalium sp. "Blue"
406 407	There are no other reports of a bipaliin planarian with this morphology, and the species is thus probably new.
408	Predation in bipaliines
409	Land planarians have toxins that may have both a repugnatorial effect on predators, and a toxic
410	effect on prey (Winsor 1998a). Two toxins were identified in B. kewense, namely a cardiotoxin
411	localized in the dermal mucus, and a haemolytic toxin that was distributed throughout the
412	planarian body (Arndt 1925). More recently, the presence of tetrodotoxin (TTX), hitherto
413	unknown in terrestrial invertebrates, was demonstrated throughout the body of Bipalium kewense
414	with the highest concentration of TTX in the anterior region (Stokes et al. 2014). The headplate
415	and body at the base of the head of Bipalium are richly supplied with subepidermal glands that
416	discharge through the ventral surface. Some secretions have an adhesive function (Winsor 1998a)
417	and are possibly responsible to adhering the Bipalium to the earthworm prey during the initial
418	contact. Other secretions, of which there are at least three types, may include toxins and enzymes
419	(Winsor 1998a). Severe paralysing effects noted in earthworms touched by B. kewense (Johri
420	1952) suggest a neurotoxic effect possibly due to substances present in the epidermal secretions
421	(Winsor 1998a). Recent results (Stokes et al. 2014) suggest that some release of TTX occurs from
422	the head of B. kewense when it uses its body to cover the anterior end of the earthworm
423	(described as capping (Ducey et al. 1999)), and some release of TTX from the mouth during
424	feeding, but did not confirm the release of TTX in the paralysis observed in earthworms in their
425	study; they also suggest that the presence of TTX throughout the planarian body may indicate a
426	possible role in defence.



427	The pharyngeal region of B. kewense contains a collagenolytic enzyme which may function in the
428	digestion of its natural prey, earthworms. The susceptibility of earthworm cuticle collagen,
429	compared to guinea pig collagen, to the collagenase secreted by B. kewense may reflect
430	adaptation of the enzyme to its physiological substrate, although the biochemical basis for this
431	observation is not known (Landsperger et al. 1981).
432	Persistence of Bipalium kewense and Diversibipalium multilineatum in the open
433	in Metropolitan France
434	Bipalium kewense was originally described from specimens in the hot-house in Kew, United
435	Kingdom (Moseley 1878). Originally from Vietnam to Kampuchea, the species is currently
436	cosmopolitan (Winsor 1983a). However, distinctions are important between a species which is
437	Against what? found only in protected and restricted constructions such as hot-houses, and species which can
438	freely live and reproduce in the open. Clearly, B. kewense is an invasive species in the open in
439	countries with tropical moist or humid semitropical climates, and appears to be restricted to
440	anthropogenically-modified habitats; this is the case in the Caribbean, such as Guadeloupe or
441	Martinique from where we obtained specimens. However, until recently (Justine et al. 2014b), it
442	was considered that <i>B. kewense</i> , in Europe, was only confined to hot-houses and thus not an
443	invasive species. Examination of literature and citizen-science information (Figure 1) now
444	proves otherwise.
445	In France, the outdoors occurrence of <i>B. kewense</i> was reported in Orthez and Bayonne in 2005
446	(Vivant 2005). Through citizen science, we obtained a movie of the worm filmed in the nearby
447	locality of Urcuit in 1999. Moreover, we obtained information about the presence of the species
448	in Arthez de Béarn, Hasparren, Villefranque, Urt (all in 2014), near Jurançon (2016), Nay (2016)
449	and Saint Jean de Luz (2016), Ustaritz (2017) and, as in the report by Vivant, in Bayonne and
450	Orthez again (2014). We have obtained specimens from Saint-Pée-sur-Nivelle (2013), Ustaritz
451	(2014), Bassussary (2014) and Orthez (2014). All these localities are in the Department of
452	Pyrenées-Atlantiques, and we also have three records from the Department of Landes, north of
453	Pyrenées-Atlantiques, along the Atlantic coast. The remark by Vivant that the animal was
454	collected "five times in the last 20 years", the record from 1999, and the recent record and
455	specimens in the same locality (Orthez) in 2014 strongly suggests that the species is now
456	established in the open in Orthez and in several localities of the Department of Pyrenées-
457	Atlantiques (Figure 38). An alternative hypothesis would be that a single plant nursery near



458	Bayonne acts as a continuing reservoir of planarians and that all these records are in fact
459	specimens that escaped from recently bought plants, but which subsequently died after being
460	released in the open; this hypothesis is falsified by records over several years in similar localities.
461	The results presented here thus demonstrate that B. kewense clearly merits the status of invasive
462	alien species in France, at least in the Department of Pyrenées-Atlantiques. However, all records
463	are from gardens, and it is not known whether the species has invaded non-anthropised areas.
464	We briefly comment the climate of this region. The department of Pyrenées-Atlantiques is the
465	most southern department on the Atlantic coast of France; it includes a mountainous region and a
466	low altitude region along the ocean. The latter has an Atlantic climate. Within the department, we
467	note that most records (Nay, Urcuit, Urt, Saint-Jean-de-Luz, Saint-Pée-sur-Nivelle, Ustaritz, and
468	Bassussary) are from a small area around Bayonne, along the Atlantic coast (Figure 38). The
469	major limiting factor for a tropical species in Europe is, of course, low temperature. For a land
470	planarian which is sensitive to drought and freezing, the numbers of days of drought in summer
471	and the number of days of freezing temperature in winter are also important limiting factors.
472	Detailed meteorological records are available for Biarritz, a locality close to Bayonne (Infoclimat
473 474	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
475	1.5/year. This suggests that this region is particularly suitable for land planarians. Other localities
476	in the south of France, such as Department of Var and Alpes-Maritimes, and Corsica, both in
477	Mediterranean climate, have higher temperatures and thus could be more suitable for tropical
478	species, but they have longer periods of drought in summer (Infoclimat 2017).
479	Interestingly, one record of <i>Diversibipalium multilineatum</i> is also from the same department, in
480	Bellocq (with records on two years), and the single record of <i>Diversibipalium</i> sp. "black" is also
481	from the same department, in Saint-Pée-sur-Nivelle, in a garden where B. kewense is also present.
482	Other invasive land planarians found in the Pyrenées-Atlantiques include Obama nungara,
483	Caenoplana bicolor and Parakontikia ventrolineata (unpublished results – (Justine 2017)). With
484	a total of six species of invasive flatworm, clearly the Pyrenées-Atlantiques department is a hot
485	spot of diversity and a small paradise for invasive land planarians! Delete
486	For Diversibipalium multilineatum, we have also two records in the same gardens in two
487	consecutive years (Table 6). This suggests that this species also is established in the open in



489	the records was of hundreds of animals.
490	A more detailed assessment of the ecoclimatic and other data for the distribution of invasive land
491	planarians in France and French Territories is beyond the scope of this paper.
492	Bipalium kewense is of marginal medical and veterinary importance because of its implication in
493	cases of pseudoparasitism in humans and domestic animals (Winsor 1980). It has also been
494	implicated in cases of vomiting in cats though the possible role of planarian toxins in this
495	involuntary response is uncertain (Winsor 1983b). Bipalium kewense is also of commercial
496	importance as a minor pest in earthworm farms in the U.S. (Winsor 1983a), and Australia
497	(Winsor 1998b).
498	Additions to the list of invasive species in Metropolitan France and overseas
499	territories
500	From the observations reported here, we can add new species to the list of invasive land
501	planarians in Metropolitan France and overseas territories, with confirmed identifications (Figure
502	39):
503	• Bipalium kewense in Metropolitan France including Corsica, and in Guadeloupe,
504 505	 Martinique, La Réunion, French Polynesia and French Guiana. Bipalium vagum in Guadeloupe, Martinique, Saint Barthélemy, Saint Martin and La
506 507	Réunion. No record from Metropolitan France.Diversibipalium multilineatum in Metropolitan France. No record from overseas
508	territories.
509	In addition, two unnamed species, Diversibipalium "black" in Metropolitan France and
510	Diversibipalium "blue" in Mayotte (Indian Ocean), are recorded.
511	How could 40-cm long invasive worms escape the attention of the scientists for
512	20 years?
513	At the beginning of our study, we were intrigued by the almost total absence of published
514	information about the presence of bipaliines in France. The record by Vivant (2005) was the only
515	one we could find, and since it was published in a rather obscure mycological journal, it certainly
516	did not receive national nor international attention. Moreover, we are still astonished by the
517	complete lack of response from scientific authorities at the presence of these worms. One of the





518	early records we received (2013) was from a kindergarten in which the children were reportedly
519	scared by hundreds of "small snakes" on the grass (these were later identified as D .
520	multilineatum). We also received a report of a citizen who showed a long hammerhead worm
521	found on the fur of her cat to its veterinarian and was told it was a tapeworm (cestode). Other
522	citizens explained that they tried to obtain identifications of land planarians from local
523	universities and were told that the worms were leeches, and/or plain, uninteresting animals.
524	Invasive land planarians were not known in France 10 years ago (Justine et al. 2014a) and the
525	professionals involved in these anecdotes probably were never taught about them. Clearly, more
526	education is needed about land planarians, which, in Europe, will be more and more often
527	encountered by citizens and professionals in agriculture, landscaping, veterinary science and
528	medicine.
529	It is also astonishing that the presence of such conspicuous animals never provoked a response
530	from scientific authorities, although reports of tiny insect invasives often are followed by
531	appropriate measures; again, the ignorance of professional scientists, science technicians, and
532	amateur naturalists about land planarians was probably the reason. It is significant, in this respect,
533	that the first recent mention of land planarians in France, by one of us (PG) was made public in
534	an internet forum dedicated to insects. We expect that the measures taken at the European level
535	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 No novel data
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





556

548	flatworms, as active predators, constitute a danger to native fauna wherever they are introduced.
	No evidence provided
549	Acknowledgements
550	We thank all the citizens who participated in the survey; those who sent specimens are
551	particularly thanked. Names of citizens, and sometimes scientists, who provided photographs
552	and/or specimens are indicated in Tables 2 and Tables 5-8. We apologize for not mentioning the
553	names of citizens who kindly provided information but could not be contacted later for obtaining
554	a formal consent. The support of various Fédérations Régionales de Défense contre les

Organismes Nuisibles (FREDON), in Metropolitan France and overseas departments, is

acknowledged. LW thanks Martin Darley for the specimen of Bipalium kewense from Pakistan.

547 (Ricciardi & Ryan 2017); in opposition to this 'denialism', we strongly believe that invasive



References

558 559 560 561	Alvarez-Presas M, Carbayo F, Rozas J, and Riutort M. 2011. Land planarians (Platyhelminthes) as a model organism for fine-scale phylogeographic studies: understanding patterns of biodiversity in the Brazilian Atlantic Forest hotspot. <i>Journal of Evolutionary Biology</i> 24:887-896.
562 563	Álvarez-Presas M, Mateos E, Tudo A, Jones H, and Riutort M. 2014. Diversity of introduced terrestrial flatworms in the Iberian Peninsula: a cautionary tale. <i>PeerJ</i> 2:e430.
564 565 566 567 568	Álvarez-Presas M, Mateos E, Vila-Farré M, Sluys R, and Riutort M. 2012. Evidence for the persistence of the land planarian species <i>Microplana terrestris</i> (Müller, 1774) (Platyhelminthes, Tricladida) in microrefugia during the Last Glacial Maximum in the northern section of the Iberian Peninsula. <i>Molecular Phylogenetics and Evolution</i> 64:491-499.
569 570	Álvarez-Presas M, and Riutort M. 2014. Planarian (Platyhelminthes, Tricladida) diversity and molecular markers: a new view of an old group. <i>Diversity</i> 6:323-338.
571 572	Arndt W. 1925. Ober die Gilfte der Plattwurmer. Verhandlungen der Deutschen Zoologischen Gesellschaft:135-145.
573 574 575	Ayadi ZEM, Gey D, Justine J-L, and Tazerouti F. 2017. A new species of <i>Microcotyle</i> (Monogenea: Microcotylidae) from <i>Scorpaena notata</i> (Teleostei: Scorpaenidae) in the Mediterranean Sea. <i>Parasitology International</i> 66:37-42.
576 577 578	Barnwell GM, Peacock LJ, and Taylor RE. 1965 Feeding behavior of a land planarian, Bipalium kewense. Unpublished manuscript presented to the Southern Society for Philosophy and Psychology, Atlanta, GA, April, 15-17, 1965.
579 580 581	Boag B, Palmer LF, Neilson R, and Chambers SJ. 1994. Distribution and prevalence of the predatory planarian <i>Artioposthia triangulata</i> (Dendy) (Tricladida: Terricola) in Scotland. <i>Annals of Applied Biology</i> 124:165-171.
582 583	Bowles J, Blair D, and McManus DP. 1995. A molecular phylogeny of the human schistosomes. <i>Molecular Phylogenetics and Evolution</i> 4:103-109.



585 586	the exotic blue land planarian, <i>Caenoplana coerulea</i> (Platyhelminthes, Geoplanidae), on Menorca (Balearic Islands, Spain). <i>Zookeys</i> 199:91-105.
587 588 589	Carbayo F, Alvarez-Presas M, Jones HD, and Riutort M. 2016. The true identity of <i>Obama</i> (Platyhelminthes: Geoplanidae) flatworm spreading across Europe. <i>Zoological Journal of the Linnean Society</i> 177:5–28.
590 591 592 593	Chaabane A, Neifar L, Gey D, and Justine J-L. 2016. Species of <i>Pseudorhabdosynochus</i> (Monogenea, Diplectanidae) from groupers (<i>Mycteroperca</i> spp., Epinephelidae) in the Mediterranean and Eastern Atlantic Ocean, with special reference to the "beverleyburtonae group" and description of two new species. <i>PLoS ONE</i> 11:e0159886.
594 595	Connella JV, and Stern DH. 1969. Land planarians: Sexuality and occurrence. <i>Transactions of the American Microscopical Society</i> 88:309-311.
596 597	de Beauchamp P. 1962. <i>Platydemus manokwari</i> n. sp., planaire terrestre de la Nouvelle-Guinée Hollandaise. <i>Bulletin de la Societe Zoologique de France</i> 87:609-615.
598 599	Dendy A. 1891. Short descriptions of new Land Planarians. <i>Proceedings of the Royal Society of Victoria</i> :pp. 35-38.
600 601	Dendy A. 1895. Notes on New Zealand Land Planarians: Part II. <i>Transactions of the Royal Society of New Zealand</i> 28:210-214.
602 603 604	Ducey PK, Messere M, Lapoint K, and Noce S. 1999. Lumbricid prey and potential herpetofaunal predators of the invading terrestrial flatworm <i>Bipalium adventitium</i> (Turbellaria: Tricladida: Terricola). <i>American Midland Naturalist</i> 141:305-314.
605 606 607	Gerlach J. 2017. Partula survival in 2017, a survey of the Society islands. Published by the author (29pp) - available from https://islandbiodiversitycom/; dowloaded 10 November 2017.
608 609	Gremigni V. 2003. Turbellaria. In: Stoch F, ed. <i>Checklist of the species of the Italian fauna Online Version 20 http://www.faunaitaliait/checklist/index.html</i> .
610 611 612	Hebert PDN, Cywinska A, Ball SL, and deWaard JR. 2003. Biological identifications through DNA barcodes. <i>Proceedings of the Royal Society of London Series B: Biological Sciences</i> 270:313-321.



513 514	Hyman LH. 1951. <i>The Invertebrates: Platyhelminthes and Rhynchocoela</i> . New York: MacGraw-Hill.
515	Infoclimat A. 2017. Normes et records 1961-1990.
616 617 618	Johri LN. 1952. A report on a Turbellarian <i>Placocephalus kewense</i> , from Delhi State and its feeding behaviour on the live earthworm <i>Pheretima posthume</i> . <i>Science and Culture</i> (Calcutta) 18:291.
519 520 521	Jones HD. 1999. A new genus and species of terrestrial planarian (Platyhelminthes; Tricladida; Terricola) from Scotland, and an emendation of the genus <i>Artioposthia</i> . <i>Journal of Natural History</i> 33:387-394.
522 523 524	Jones HD, Santoro G, Boag B, and Neilson R. 2001. The diversity of earthworms in 200 Scottish fields and the possible effect of New Zealand land flatworms (<i>Arthurdendyus triangulatus</i>) on earthworm populations. <i>Annals of Applied Biology</i> 139:75-92.
525 526	Jones HD, and Sluys R. 2016. A new terrestrial planarian species of the genus <i>Marionfyfea</i> (Platyhelminthes: Tricladida) found in Europe. <i>Journal of Natural History</i> 50:2673-2690.
527 528	Jones HD, and Sterrer W. 2005. Terrestrial planarians (Platyhelminthes, with three new species) and nemertines of Bermuda. <i>Zootaxa</i> 1001:31-58.
529 530	Justine J-L. 2017. Plathelminthes terrestres invasifs. Blog (in French). https://sites.google.com/site/jljjustine/plathelminthe-terrestre-invasif.
531 532	Justine J-L, Thévenot J, and Winsor L. 2014a. Les sept plathelminthes invasifs introduits en France. <i>Phytoma</i> :28-32.
633 634 635 636	Justine J-L, Winsor L, Barrière P, Fanai C, Gey D, Han AWK, La Quay-Velazquez G, Lee BPY-H, Lefevre J-M, Meyer J-Y, Philippart D, Robinson DG, Thévenot J, and Tsatsia F. 2015. The invasive land planarian <i>Platydemus manokwari</i> (Platyhelminthes, Geoplanidae): records from six new localities, including the first in the USA. <i>PeerJ</i> 3:e1037.
537 538 539	Justine J-L, Winsor L, Gey D, Gros P, and Thévenot J. 2014b. The invasive New Guinea flatworm <i>Platydemus manokwari</i> in France, the first record for Europe: time for action is now. <i>PeerJ</i> 2:e297.



540 541 542	Kawakatsu M, Ogren RE, Froehlich EM, and Sasaki G-Y. 2002. Additions and corrections of the previous land planarian indices of the world (Turbellaria, Seriata, Tricladida, Terricola). Bulletin of the Fuji Women's College (Series 2) 40:157-177.
643 644 645 646	Kawakatsu M, Sluys R, and Ogren RE. 2005. Seven new species of land planarian from Japan and China (Platyhelminthes, Tricladida, Bipaliidae), with a morphological review of all Japanese bipaliids and a biogeographic overview of Far Eastern species. <i>Belgian Journal of Zoology</i> 135:53-77.
647 648	Košel V. 2002. Checklist of turbellaria in Slovakia. <i>Acta Zoologica Universitatis Comenianae</i> 44:37-40.
649 650 651 652	Kubota S, and Kawakatsu M. 2010. Distribution record of a single species of the collective group <i>Diversibipalium</i> (Plathelminthes, Tricladida, Continenticola, Geoplanidae, Bipaliinae) in Wakayama Prefecture, Honshu, Japan, with a taxonomic note of new higher classification of the Tricladida <i>Nanki Seibutsu</i> 52:97-101.
653 654	Kumar S, Stecher G, and Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. <i>Molecular Biology and Evolution</i> 33:1870-1874.
555 556	Landsperger WJ, Peters EH, and Dresden MH. 1981. Properties of a collagenolytic enzyme from <i>Bipalium kewense. Biochimica et Biophysica Acta (BBA) - Enzymology</i> 661:213-220.
357 358 359 360	Lázaro EM, Sluys R, Pala M, Stocchino GA, Baguñà J, and Riutort M. 2009. Molecular barcoding and phylogeography of sexual and asexual freshwater planarians of the genus <i>Dugesia</i> in the Western Mediterranean (Platyhelminthes, Tricladida, Dugesiidae). <i>Molecular Phylogenetics and Evolution</i> 52:835-845.
561	Lehnert GH. 1891. Beobachtungen an Landplanarien. Berlin: Nicolaische.
562 563 564	Littlewood DTJ, Rohde K, and Clough KA. 1997. Parasite speciation within or between host species? - Phylogenetic evidence from site-specific polystome monogeneans. International Journal for Parasitology 27:1289-1297.
565 566 567	Makino N, and Shirasawa Y. 1983. Morphological and ecological comparison with two new species of elongatedslender land planarians have several stripes and their new scientific names. <i>Bulletin of Tokyo Medical College</i> :69-83 [In Japanese, English summary].



669	Garrotxa. Annals de la Delegació de la Garrotxa de la Institució Catalana d'Història
670	Natural 6:67-73.
671 672 673 674	Mazza G, Menchetti M, Sluys R, Solà E, Riutort M, Tricarico E, Justine J-L, Cavigioli L, and Mori E. 2016. First report of the land planarian <i>Diversibipalium multilineatum</i> (Makino & Shirasawa, 1983) (Platyhelminthes, Tricladida, Continenticola) in Europe. <i>Zootaxa</i> 4067:577–580.
675 676 677	Morffe J, García N, Adams BJ, and Hasegawa K. 2016. First record of the land planarian Bipalium kewense Moseley, 1878 (Tricladida: Geoplanidae: Bipaliinae) from Cuba. BioInvasions Records 5:127-132.
678 679 680	Moseley H. 1877. Notes on the structure of several forms of land planarians, with a description of two new genera and several new species, and a list of all species at present known. *Quarterly Journal of Microscospical Science 17:273-292.*
681 682	Moseley HN. 1878. Description of a new species of land-planarian from the hothouses at Kew Gardens. <i>Annals and Magazine of Natural History</i> 1:237-239.
683 684	Murchie AK, and Gordon AW. 2013. The impact of the "New Zealand flatworm", <i>Arthurdendyus triangulatus</i> , on earthworm populations in the field. <i>Biological Invasions</i> 15:569-586.
685 686	Ricciardi A, and Ryan R. 2017. The exponential growth of invasive species denialism. <i>Biological Invasions</i> in press. doi: 101007/s10530-017-1561-7.
687 688	Saitou N, and Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. <i>Molecular Biology and Evolution</i> 4:406-425.
689	Sluys R. 2016. Invasion of the Flatworms. American Scientist 104:288.
690 691 692 693	Stokes AN, Ducey PK, Neuman-Lee L, Hanifin CT, French SS, Pfrender ME, Brodie ED, III, and Brodie Jr ED. 2014. Confirmation and distribution of Tetrodotoxin for the first time in terrestrial invertebrates: Two terrestrial flatworm species (<i>Bipalium adventitium</i> and <i>Bipalium kewense</i>). <i>PLoS ONE</i> 9:e100718.
694 695 696	Tsiamis K, Gervasini E, D'Amico F, Deriu I, Katsanevakis S, Crocetta F, Zenetos A, Arianoutsou M, Backeljau T, Bariche M, Bazos I, Bertaccini A, Brundu G, Carrete M, Çinar ME, Curto G, Faasse M, Justine J-L, Király G, Langer MR, Levitt Ya, Panov VE, Piraino S, Pahitach W, Baguas A, Saelara P, Shenkar N, Sârby L, Triceriae E, Vennini A, Vallestad
697	Rabitsch W, Roques A, Scalera R, Shenkar N, Sîrbu I, Tricarico E, Vannini A, Vøllestad



698 699 700	LA, Zikos A, and Cardoso AC. 2016. The EASIN Editorial Board: quality assurance, exchange and sharing of alien species information in Europe. <i>Management of Biological Invasions</i> 7:321–328.
701 702 703	Vanhove MP, Tessens B, Schoelinck C, Jondelius U, Littlewood DT, Artois T, and Huyse T. 2013 Problematic barcoding in flatworms: A case-study on monogeneans and rhabdocoels (Platyhelminthes). <i>Zookeys</i> :355-379.
704 705	Vivant J. 2005. <i>Bipalium kewense</i> Moseley, ver tropical terricole, existe à Orthez (Pyr. atl.). <i>Bulletin de la Société Mycologique Landaise</i> :46-48.
706 707	von Graff L. 1899. <i>Monographie der Turbellarien. II. Tricladida, Terricola (Landplanarien)</i> . Leipzig: Englemann.
708 709 710	Ward RD, Zemlak TS, Innes BH, Last PR, and Hebert PD. 2005. DNA barcoding Australia's fish species. <i>Philosophical Transactions of the Royal Society of London B Biological Sciences</i> 360:1847-1857.
711 712	Winsor L. 1980. Pseudoparasitism of domestic and native animals by geoplanid land planarians. Australian Veterinary Journal 56:194-196.
713 714 715	Winsor L. 1983a. A revision of the Cosmopolitan land planarian <i>Bipalium kewense</i> Moseley, 1878 (Turbellaria: Tricladida: Terricola). <i>Zoological Journal of the Linnean Society</i> 79:61-100.
716 717	Winsor L. 1983b. Vomiting of land planarians (Turbellaria: Tricladida: Terricola) ingested by cats. <i>Australian Veterinary Journal</i> 60:282-283.
718 719	Winsor L. 1991. A provisional classification of Australian terrestrial geoplanid flatworms (Tricladida: Terricola: Geoplanidae). <i>Victorian Naturalist (Blackburn)</i> 108:42-49.
720 721	Winsor L. 1998a. Aspects of the taxonomy and functional histology in terrestrial flatworms (Tricladida: Terricola). <i>Pedobiologia</i> 42:412-432.
722 723	Winsor L. 1998b. The Australian terrestrial flatworm fauna (Tricladida: Terricola). <i>Pedobiologia</i> 42:457-463.
724 725 726	Winsor L, Johns PM, and Barker GM. 2004. Terrestrial planarians (Platyhelminthes: Tricladida: Terricola) predaceous on terrestrial gastropods. In: Barker GM, ed. <i>Natural enemies of terrestrial molluscs</i> . Oxfordshire, UK: CAB International, 227-278.



PeerJ

727	Winsor L, Johns PM, and Yeates GW. 1998. Introduction, and ecological and systematic
728	background, to the Terricola (Tricladida). Pedobiologia 42 389-404.
729	Wizen G. 2015. Photograph. Caption: Huge terrestrial flatworm (Bipalium kewense), Mindo,
730	Ecuador, March. Nature Picture Library, Image number 01504312. Available from:
731	https://www.naturepl.com/search/preview/huge-terrestrial-flatworm-bipalium-kewense-
732	mindo-ecuador-march/0_01504312.html. Consulted on 06 Nov 2017.



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.

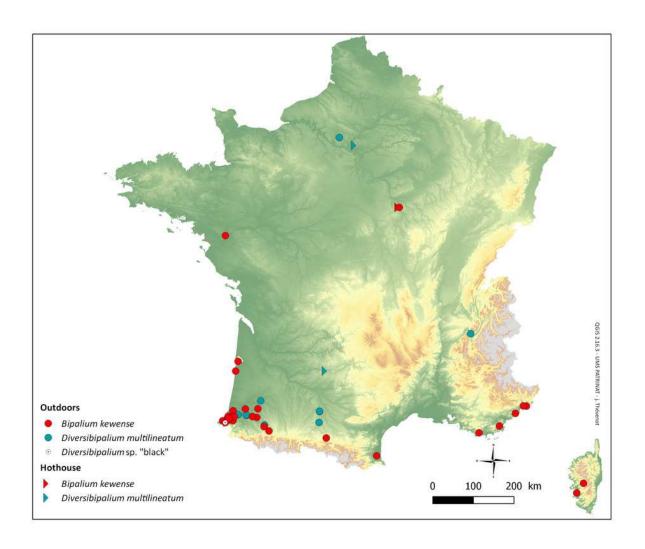




Figure 2

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).



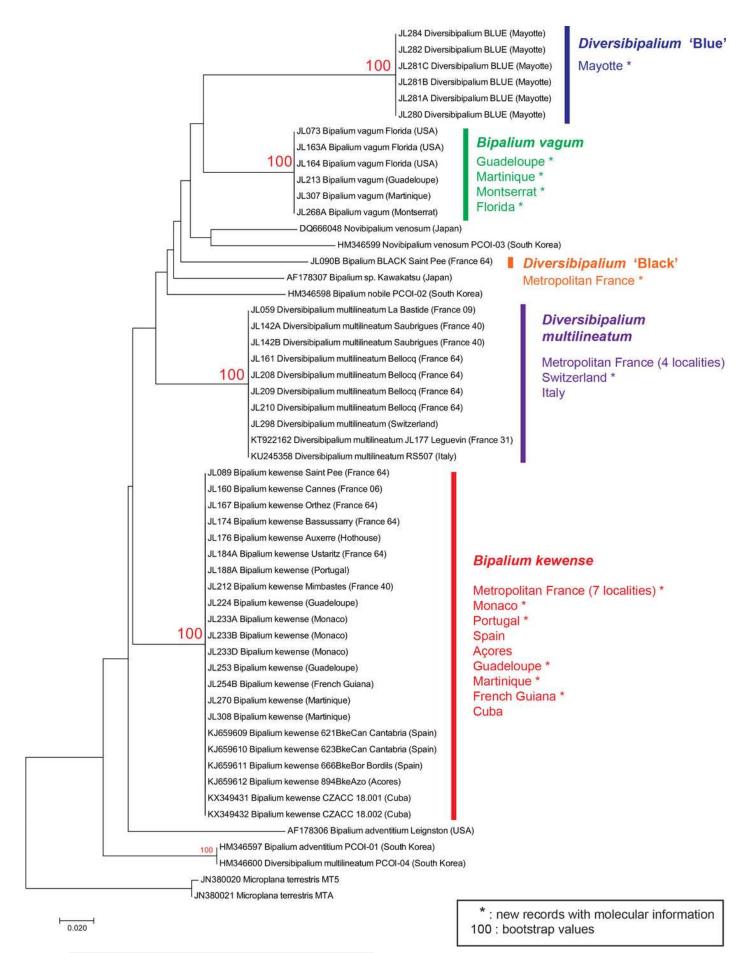


Figure 3

Figures should be restricted to the aim of the MS, i.e, species identification.

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.



Bipalium kewense, general morphology of the dorsal anterior end.

Are these features diagnostic? Please make it clear.

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.





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.





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.



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.





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.



Legends are speculative. Unless a reference for each details is given, they should be removed. These llegend text has not been investigated

Bipalium kewense, capping its earthworm prey

Why 'then'? Is is a pictures withn a picture sequence?

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.



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.





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.



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.



Bipalium kewense, continuing feeding.

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





Bipalium kewense, concluding feeding.

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



Bipalium kewense, conclusion of feeding.

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



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.



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.



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.



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.



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.



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.





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.



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.



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.



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.



Bipalium vagum. Specimen from Martinique, West Indies.

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



PeerJ reviewing PDF | (2017:11:21767:0:1:NEW 16 Nov 2017)

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.



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.





Diversibipalium sp. 'black' from Metropolitan France, dorsal aspect.

Photo Marc Gauthier.



Diversibipalium sp. 'black' from Metropolitan France, dorsal aspect.

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



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

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





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.



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.



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.





Diversibipalium sp. 'blue' from Mayotte, Indian Ocean, dorsal aspect.

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





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.





Are data the same as those in Fig. 1? From reading the text, it seems to be data from literature.

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.

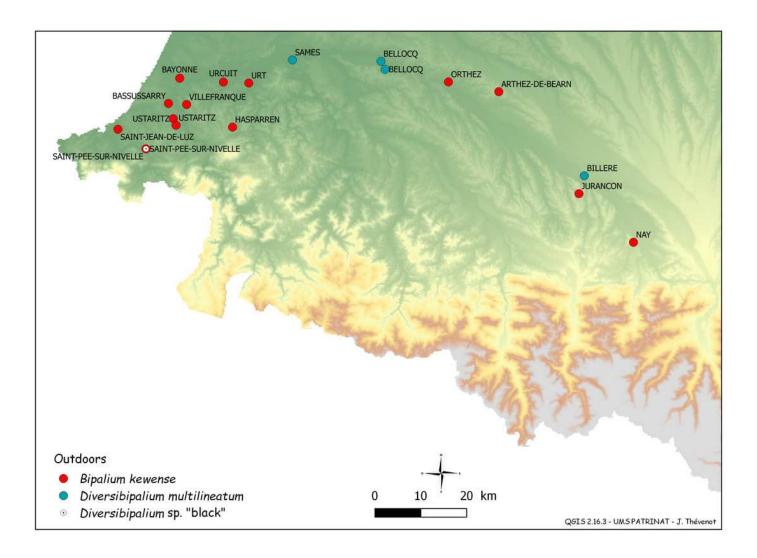




Figure 39 This should be Fig. 2

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

New records are from three continents.

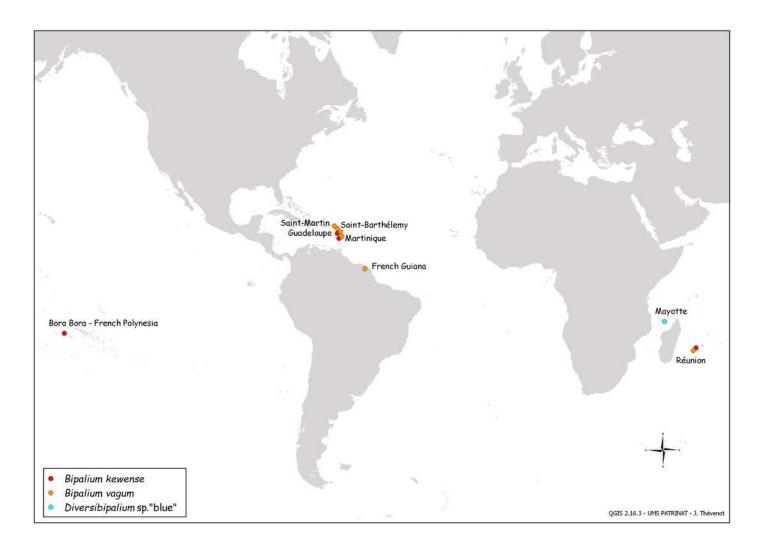
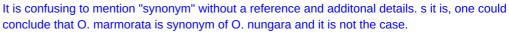




Table 1(on next page)

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.





Manuscript to be reviewed

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	Italics	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'.



Date of collection? Make it clear. "Collector" column should be immediately after 'Locality' Date with capital letter

Specie		GenBank #							Collector
s	MNHN		date	Locality	Department / State	Country - Continent	COI	Replicates	
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
ВК	JL167		24/08/2014	Orthez	Pyrénées-Atlantiques	Met. France - Europe	short	1	Rougeux, Christian
ВК	JL174		03/09/2014	Bassussary	Pyrénées-Atlantiques	Met. France - Europe	long	1	Mercader, Elisabeth
ВК	JL176 **		05/09/2014	Auxerre (hothouse)	Yonne	Met. France - Europe	long	1	Bellina, Arnaud
3K	JL184		Oct. 2014	Ustaritz	Pyrénées-Atlantiques	Met. France - Europe	short	1	Goyheneche, Iker
3K	JL188		08/10/2014	Miramar	Grande Porto	Portugal - Europe	short	1	Soarès, Luciana
ЗК	JL212		19/12/2014	Mimbastes	Landes	Met. France - Europe	long	1	Jouveau, Séverin
ЗК	JL224		23/02/2015	Trois Rivières	Guadeloupe	Guadeloupe - C. America	long	1	Van Laere, Guy
3K	JL233		27/09/2014	Monaco	Monaco	Monaco - Europe	long	3	Dusoulier, François
ЗК	JL253		21/03/2015	Trois Rivières	Guadeloupe	Guadeloupe - C. America	short	1	Van Laere, Guy
ЗК	JL254		15/05/2015	Matoury	French Guiana	French Guiana - S. America	short	2	Girault, Rémi
ЗК	JL270		23/04/2015	Ducos	Martinique	Martinique - C. America	long	1	Lucas, Pierre-Damien
ЗК	JL308		08/09/2016	Morne Vert	Guadeloupe	Guadeloupe - C. America	short	1	Coulis, Mathieu
3V	JL073		Aug. 2013	Sanibel	Florida	USA - North America	short	1	Justine, Jean-Lou
3V	JL163		July 2014	Sanibel	Florida	USA - North America	short	1	Justine, Jean-Lou
3V	JL164		July 2014	Sanibel	Florida	USA - North America	short	1	Justine, Jean-Lou
3V	JL213		29/11/2014	Anse-Bertrand	Guadeloupe	Guadeloupe - C. America	long	1	Charles, Laurent
3V	JL268		Dec. 2014	Montserrat	Montserrat	Montserrat - C. America	short	1	Shoobs, Nathaniel F.
3V	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
MC	JL059		15/06/2013	La Bastide de Serou	Ariège	Met. France - Europe	short	1	Brugnara, Sébastien
MC	JL142		22/04/2014	Saubrigues	Landes	Met. France - Europe	long	2	Robineau, Thiérry
MC	JL161		11/06/2015	Bellocq	Pyrénées-Atlantiques	Met. France - Europe	long	1	Audiot, Marie-Claude
MC	JL208		11/06/2014	Bellocq	Pyrénées-Atlantiques	Met. France - Europe	long	1	Audiot, Marie-Claude
MC	JL209		12/06/2014	Bellocq	Pyrénées-Atlantiques	Met. France - Europe	long	1	Audiot, Marie-Claude
MC	JL210		June 2014	Bellocq	Pyrénées-Atlantiques	Met. France - Europe	long	1	Audiot, Marie-Claude
M	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

adventitium



Table 4(on next page)

Divergences between "long" sequences

There was a total of 857 positions in the final dataset

PeerJ

	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).

Is this record that indicated with "#1"? Rewrite the sentence



What does "orign of data" mean? In Tab 2 you use "Collector". Is it the same? If yes, replace "Orign of data" for "Collector" writsince it is unambigous.

#	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.



Same comment

#	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.



Same comment

#	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