The invasive New Guinea flatworm *Platydemus manokwari* in France, the first record for Europe

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Introduction

An undesirable consequence of globalization, a relatively modern phenomenon, has been an increase in the number of biological invasions that challenge the conservation of biodiversity and natural resources (Secretariat of NOBANIS 2012; Simberloff 2014). Historic biological invasions include the passive dispersal of invasive terrestrial flatworms, also known as land planarians. The main driver for this was probably horticulturalists of the 19th Century using the recently invented Wardian cases to safely transport back to the hothouses and gardens of Europe rare plants, together with soil containing cryptic exotic animal species (Winsor et al. 2004). As a consequence over 30 species of land planarians have established themselves as invasive species in various countries outside their native range (Winsor et al. 2004, Table 5.1).

In human-modified habitat flatworms and their cocoons continue to be associated with rooted and potted plants, rhizomes, and certain types of fresh vegetable produce (Alford et al. 1996). Subsequent secondary dispersal of these invasive flatworm species occurs through the exchange and purchase of plants from nurseries, botanical gardens, garden centres and gardeners (Alford et al. 1996) especially infested nurseries and garden centres (Boag et al. 1994; Moore et al. 1998), active inadvertent dispersal through social traditions of exchanging plants and recycling topsoil (Christensen & Mather 1998), or through the deliberate introduction of flatworms for the purposes of biological control of a pest species such as the giant African snail *Achatina fulica* in the Pacific Region (Muniappan 1987; Waterhouse & Norris 1987).

Land planarians are carnivores, and feed upon a variety of soil organisms such as earthworms, isopods, insects and snails, and some species may pose a threat to local biodiversity (Alford et al. 1996; Cowie 2010; Santoro & Jones 2001; Sugiura 2010) and negatively impact on agriculture, for example through a decline in earthworms species (Murchie & Gordon 2013) resulting in reduced soil fertility (Murchie 2010) and possibly drainage (Jones et al. 2001).

Invasive terrestrial flatworms have been recorded in twelve European countries (Jones 1998; Ogren et al. 1997). These Invasive Alien Species (IAS) can be divided into two broad groups: the "old" and the "new" invasives. The former group includes *Bipalium kewense* and *Dolichoplana striata* that were undoubtedly inadvertently introduced to Europe in the 19th Century by horticulturalists. These species appear to be largely confined to hothouses. One or both these species are present in ten European countries, and are the only invasive flatworms presently recorded in ~~seven~~at least ten of these countries (Austria, Belgium, Czech Republic, Finland, Germany, Ireland, Norway, ~~and~~ Poland, Portugal and Spain). The "new" group of

[Fernando 1] Comentário: There is some debate on terminology to define exotic species (for instance, Coulatti & McIsaac 2004. Diversity and Distributions 10: 135-141).

After the definition of IAS provided by the Convention on Biological Diversity (http://www.cbd.int/idb/2009/about/what/) some land planarians would not macht the definition.

I suggest not to consider all land flatworms as IAS since some species (e.g., B. kewense, D. striata) are seemingly synanthropic at least in the Neotropics. Authors should adopt a definition along the text.

[Fernando 2] Comentário: Please see Filella, E. F. 1983. NOTA SOBRE LA PRESENCIA DE LA PLANARIA TERRESTRE BIPALIUM KEWENSE MOSELEY , 1878 A CATALUNYA. Butll. Inst . Cat. Hist. Nat., 49 (Sec. Zool ., 5): 151.

and

Kawakatsu et al. 2002. Bull. Fuji Women's University 40 (II): 157-177 (http://planarian.net/db/lpindex/ix2002.pdf)

invasive flatworms includes mainly species from the southern hemisphere such as the New Zealand flatworm *Arthurdendyus triangulatus* (United Kingdom; Eire, Denmark – Faroe Islands) and the Australian Blue Garden flatworm *Caenoplana coerulea* (France; and recently Menorca (Breugelmans et al. 2012)). The United Kingdom has some twelve or more IAS most of which are Australian and New Zealand species (Jones 2005). How a species such as *A. triangulatus* was introduced to the British Isles is unknown, though it is suspected that it was introduced by humans, and was associated with the trade between New Zealand and Britain (Boag & Yeates 2001), and spread to the Faroe Islands through non-controlled imports (Christensen & Mather 1998).

Unknown worms recently found in a hothouse in Caen (France) were identified as the New Guinea flatworm *Platydemus manokwari* de Beauchamp, 1963. Their identity was subsequently confirmed by molecular analysis of COI sequences. *Platydemus manokwari* is among the "100 World's Worst Invader Alien Species" (Lowe et al. 2000). In this paper, we present evidence for the identification of the species in France, the first record in Europe, and provide a brief review of the records of the species in the world, a list of its known prey, and possible control options.

Material and Methods

Material

Specimens were found in a hothouse in the Jardin des Plantes in Caen (France); according to witnesses, it is likely that similar specimens were present in the hothouse for months. Specimens were collected by hand and sent alive to Paris by postal service. They were photographed alive, then killed in hot water and stored in ethanol or formalin. A small piece of the body was taken from two ethanol-fixed individuals for molecular analysis. Photographs were forwarded to one of us (LW) for identification. Histological anatomical investigations were not undertaken at this time. Specimens are deposited in the collections of the Muséum National d'Histoire Naturelle, Paris, under registration number MNHN JL81.
Limited prey experiments were undertaken with the few living available specimens; very simply, flatworms were put in a small plastic container with snails. We report a single preying event.

Molecular sequences

Genomic DNA was extracted from a small piece of the worm, using the QIAamp DNA Mini Kit (Qiagen). A fragment of 424 bp of COI gene was amplified with the primers COI-ASmit1

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[Fernando 6] Comentário: How many specimens?

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[Fernando 8] Comentário: Does this number referr to just only one specimen or to a set?

[Fernando 9] Comentário: Which one? After the Section "Molecular identification"the DNA of two worms were sequenced

(forward 5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and COI-ASmit2 (reverse 5′-TAAAGAAAGAACATAATGAAAATG-3′) (Littlewood et al. 1997). The PCR reaction was performed in 20 µl, containing 1 ng of DNA, 1× CoralLoad PCR buffer, 3Mm MgCl2, 66 µM of each dNTP, 0.15µM of each primer, and 0.5 units of Taq DNA polymerase (Qiagen). The amplification protocol was: 4' at 94 °C, followed by 40 cycles of 94 °C for 30'', 48 °C for 40'', 72 °C for 50'', with a final extension at 72 °C for 7'. PCR products were purified and sequenced in both directions on 3730xl DNA Analyzer 96-capillary sequencer (Applied Biosystems). Sequences were edited using CodonCode Aligner software (CodonCode Corporation, Dedham, MA, USA), compared to the GenBank database content using BLAST and deposited in GenBank under accession number KF887958. Sequences were compared using MEGA5 (Tamura et al. 2011).

Results

Morphology

The flatworm was broadest in the middle, tapering evenly anteriorly but more abruptly posteriorly (Figure 1). Two large prominent eyes were situated back from the tip of the elongate snout-like head (Figure 2). In cross section the flatworm was convex dorsally and flat ventrally. The figured live mature specimen was 50mm long and 5mm wide. The mouth was located just behind the midpoint of the ventrum, with gonopore about half way between the mouth and posterior end. The dorsum was a dark olive brown colour, which under a lens showed a fine pale brownish graininess. A pale cream median dorsal longitudinal stripe, some 0.3 mm wide, began just behind the eyes and continued to the posterior tip (Figure 3). The olive brown colour graded to grey at the anterior tip. A thin submarginal cream stripe with fine lower greyish margin ran laterally from the anterior end along the length of the body (Figure 3). The ventral surface was a pale finely mottled light brown (Figures 4-5), slightly paler mid ventrally. These features are consistent with those of *Platydemus manokwari* de Beauchamp, 1963 (Platyhelminthes, Continenticola, Geoplanidae, Rhynchodeminae) (de Beauchamp 1962; Kawakatsu et al. 1992; Winsor 1990). The pharynx, protruding from the ventral surface, was visible when the flatworm was preying on a snail (Figure 5).

[Fernando 10] Comentário: Since cryptic species are known. It would be welcome to refer here to voucher coded specimens, not just to a flatworm.

Molecular identification

The two COI sequences we obtained from two individuals were identical. They were compared to the only available COI sequences of a member of the genus *Platydemus* in GenBank (*Platydemus manokwari*; Accession number: AF178320.1). The p-distance between our new sequence and the GenBank sequence of *Platydemus manokwari* was 4%. Although this is not mentioned in the GenBank record, we know that this specimen was collected in Australia by one of us (LW). The population from which the GenBank example of *Platydemus manokwari* (AF178320.1) was taken had previously been confirmed histologically (by LW) as *P. manokwari*.

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Nomenclatural clarification

There is some variation in the literature about the date of description of *P. manokwari*, 1962 or 1963. We carefully examined the original publication. The paper was presented at a meeting in December 1962 and is included in the volume dated 1962, but the actual date of publication was April 18, 1963. In accord with Article 21.1 (International Commission on Zoological Nomenclature 1999)~~-~~, the date of the taxon is 1963. The bibliographical date of the publication remains 1962, but the taxon is *Platydemus manokwari* de Beauchamp, 1963.

Discussion

Molecular identification

The p-distance between our two sequences and the GenBank sequence of *Platydemus manokwari* was 4%. This genetic distance roughly corresponds to the genetic distances generally found between closely related species or distant populations within a single species. Á~~A~~lvarez-Presas et al. (2012) studied variation of COI in species belonging to the same family ~~-~~as *Platydemus manokwari*, i.e. the Geoplanidae. In this study of the European species *Microplana terrestris*, specimens were studied from two localities, East and West of Northern Spain. Variation ranged from 0% to 3% within the western localities, and from 0% to 1.6% in the eastern localities; West and East presented a difference of 2.4% to 4%. The between-species difference (*M. terrestris* vs *M. robusta*) was about 19%. Therefore, we consider that the difference of 4% found between our French specimens of *P. manokwari* and the Australian specimen in GenBank is compatible with intraspecific variation. The molecular data thus confirm the morphological identification.

Previous records of land planarians in France

Previous records of Invasive Alien Species (IAS) of land planarians in France include a *Pelmatoplana* sp. from a greenhouse in Saint Max, a suburb of Nancy (identified by de Beauchamp (Remy 1942)); *Bipalium kewense* Moseley, 1878 and *Caenoplana coerulea* Moseley, 1877 from an urban garden in Villeneuve de la Raho in the Languedoc-Roussillon region, France (mentioned as "France" in (Winsor et al. 2004)), where potted plants purchased from a local plant supermarket were believed to be the source of the flatworms (Gérard Peaucellier, *in litt*); *Bipalium kewense* in Orthez (Vivant 2005) and a *Bipalium* sp. in Bayonne (Vivant 2005), with both locations in the department of Pyrenées-Atlantiques (south western France).

[Fernando 14] Comentário: Are these species in France really invasive or just exotic?

Other species, often unidentified, have been recorded recently in France in newspapers (Gasiglia 2013; Guyon 2014; Heyligen 2013), magazines (Groult & Boucourt 2014) and blogs (Justine 2013) and mentioned in governmental documents (Placé 2013), but not in published scientific publications.

The occurrence of the invasive flatworm *Platydemus manokwari* in the Jardin des Plantes, Caen, in the department of Basse-Normandie (Normandy, France), is the first record of the species in Europe.

Previous records of *Platydemus manokwari*

Platydemus manokwari occurs naturally in relatively undisturbed sub-alpine forest at Pindaunde station, Mt Wilhelm at 3625 metres altitude, and at Kainantu at 1558 metres altitude in the eastern highlands of New Guinea. The natural range of this upland species has yet to be determined.

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Until now, *Platydemus manokwari* was confined to the Indo-Pacific region within the bounds of the Ogasawara Islands, Japan in the north; near Mackay in Queensland, Australia to the south; French Polynesia to the east; with the most westerly extent of the flatworm in the Maldives. The Caen record of this highly invasive species is a significant westerly extension of the occurrence of *P. manokwari* from the Indo-Pacific region to Europe.

Since it was first discovered in the Agricultural Research Station in Manokwari, Irian Jaya in 1962 where it was credited with the decline of the Giant African Snail *Achatina fulica*, an invasive pest species of coconut plantations and other crops (Mead 1963; Mead 1979; Muniappan et al. 1986; Schreurs 1963; van Driest 1968; Waterhouse & Norris 1987), *Platydemus manokwari* has progressively spread throughout the Indo-Pacific (Table 1). The flatworm has been accidently introduced, probably together with plants and soil, to various

islands in the Pacific region including Australia, Guam, Palau, Hawaii, Federated States of Micronesia, French Polynesia, and Samoa. The most recent report of *P. manokwari* in the Pacific region is its occurrence in Rotuma in the Fiji archipelago (Brodie et al. 2013). The flatworm was also deliberately introduced as a bio-control agent for the Giant African Snail *Achatina fulica* to Bugsuk in the Philippines (Muniappan et al. 1986; Waterhouse & Norris 1987), Yokohama, Japan (Eldredge & Smith 1995), and the Maldives (Muniappan 1987). The rate of secondary dispersal of *Platydemus manokwari* is low and depends upon transport of infected plants and soil, or the flatworms themselves, by humans. The flatworms are incapable of travelling long distances on their own.

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Reproduction

Under experimental conditions the optimum temperature for rearing *P. manokwari* in terms of pre-oviposition period and cocoon production is 24^{o}C, with a mean post-oviposition developmental period for the young to hatch from the cocoon of 7.8± 1.2 days (Kaneda et al. 1992). Cocoons contain an average of 5.2 juveniles (3-9) each. The flatworm begins oviposition within 3 weeks of hatching (Kaneda et al. 1992). The temperature threshold for oviposition lies between 15^{o}C and 18^{o}C, and for cocoon and juvenile stages 10^{o}C and 11.7^{o}C respectively (Kaneda et al. 1992). The flatworm normally reproduces sexually, and does not appear to reproduce by fission (Kaneda et al. 1990).

Biology

P. manokwari prefers wet humid conditions and is unable to survive in completely dry habitats; high humidity and adequate precipitation are essential for the survival of the flatworm (Kaneda et al. 1990; Sugiura 2009). The flatworm is diurnal if the moisture conditions are right (Kaneda et al. 1990). Temperature appears to influence predation rate by the flatworm in field and laboratory experiments, and also its survival. Sugiura (Sugiura 2009) considers that 10^{o}C is a possible threshold temperature for the establishment of *P. manokwari*, and speculates that low winter temperatures may have restricted the invasion and establishment of *P. manokwari* in temperate countries.

Platydemus manokwari, like a number of other platydemids of the Australia-New Guinea region, is an upland species that naturally range from alpine through to sub-alpine, cool temperate and warm temperate zones to tropical climates. At the Pindaunde station on Mt Wilhelm, New Guinea the mean daily temperature is 11.6^{o}C, mean minimum of 4^{o}C, absolute maximum of 16.7^{o}C, absolute minimum of -0.8^{o}C, and precipitation of some 3450 mm per

year (Corlett 1984), though it is expected that the microclimate on the forest floor would be milder. The climate at Pindaunde has been described as "wintery at night, (and) has days which seem to belong to a chilly spring or autumn" (McVean 1968). Were it introduced to temperate countries and escape hothouse or similar containment, the flatworm may well survive winters and become established. High frequencies of warm winters in temperate zones may also facilitate the establishment of the flatworm in these places (Sugiura 2009). The flatworm has survived in the hothouse at Caen, and it is expected that it would also survive outdoors in this region. An assessment of the global potential distribution of *Platydemus manokwari*, based on ecoclimatic data has not yet been undertaken.

Nothing appears to be known about natural enemies of *Platydemus manokwari*. Parasitization of *P. manokwari* by nematodes, gregarines or mycetophilid flies, known in other land planarians, has not yet been reported. *P. manokwari* has a most unpleasant astringent taste (LW *pers. obs*.), just as has been noted for other species (Dendy 1891). Bellwood (pers. comm. to LW, 13.11.1997) in his private urban garden, remarked that free-range domestic bantams that noticed *P. manokwari* on an upturned log pecked at, took the flatworms into their mouths, then immediately rejected them; when at a much later time *P. manokwari* was subsequently noticed by the bantams they refused to peck at the flatworms. This is similar to behaviour of domestic fowls offered *Caenoplana spenceri* (Dendy 1891). It is probable that most vertebrates would find the flatworm distasteful.

Prey

Terrestrial molluscs form the principal prey upon which *Platydemus manokwari* has been observed to feed in the field and under laboratory and experimental conditions, though the flatworm will feed upon other soil-dwelling invertebrates including annelids, arthropods, nemerteans, and flatworms (Table 2). The flatworm does not appear to be cannibalistic (Kaneda et al. 1990; Ohbayashi et al. 2005; Sugiura 2010).

A number of species of land flatworms will, when moisture conditions are right, seek prey above the ground. *P. manokwari* has been observed feeding on both juvenile and adult partulid snails at heights above one metre in trees, and in captivity the flatworm fed on specimens of *Partula* sp. and *Pythia* sp. (Eldredge & Smith 1995; Hopper & Smith 1992). Experimentally, *P. manokwari* has been shown to track artificially created snail scent trails on the ground (Iwai et al. 2010), and up trees, supporting the hypothesis that the introduction of *P. manokwari* is an important cause in the rapid decline or extinction of native arboreal snails as well as ground-dwelling snails on Pacific Islands (Sugiura & Yamaura 2008).

Where there are sufficient individuals of *P. manokwari* following sensory cues of the same prey the flatworms can overwhelm their prey by sheer numbers in a gregarious or "gang" attack (Mead 1963; Ohbayashi et al. 2005; Sugiura 2010).

Waterhouse and Norris (Waterhouse & Norris 1987) considered that *P. manokwari* appeared to be an opportunistic carnivore and generally unselective in the choice of prey. Success of *Platydemus manokwari* as a biological control agent for *Achatina fulica* can be attributed to its polyphagy, resistance to starvation, ability to survive and reproduce on alternative prey and potential to reproduce rapidly in synchrony with prey populations (Winsor et al. 2004).

Invasion of a site by *Platydemus manokwari* may directly and indirectly impact on native and introduced arboreal, terrestrial soil and to a much lesser extent semi-aquatic slow-moving invertebrate fauna.

Impacts

From an agricultural perspective *Platydemus manokwari* is not a direct plant pest. In fact it has been and probably will continue to be used by plant protection agencies in the Pacific region as a Bio-agent in the control of outbreaks of the Giant African snail *Achatina fulica* (Food and Agriculture Organization Sub-regional officer for the Pacific Islands (FAO-SAPA) 2002; Winsor et al. 2004), though other factors apart from flatworm predation may contribute to the decline in pest snail populations (Lydeard et al. 2004).

Examined from an environmental perspective *P. manokwari* has demonstrably had a serious negative impact on the biodiversity of native snail populations in the Pacific region (Cowie 2010) and wherever it is deliberately or accidentally introduced it will continue to pose a threat not only to native molluscs, but possibly to other slow-moving soil invertebrates (Sugiura 2010). It may also indirectly have a negative impact on vertebrate species dependent upon these soil invertebrates.

An environmental pest risk assessment along the lines of that in the International Standards for Phytosanitary Measures (International Plant Protection Convention (IPPC) 2004) may need to be undertaken for *P. manokwari*: an assessment of the probability of spread of the flatworm, considered here to be low; whether the population is actively reproducing and is viable; an assessment of economic consequences, for example potential threats to commercial snail farming; and environmental consequences, for example negative impacts on soil invertebrate biodiversity in France and elsewhere. The extent of this incursion, and whether or not it is limited to the hothouse in Caen, the likely primary dispersal source of

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the current incursion, and secondary dispersal through plant exchanges between botanic gardens, and garden centres or plant supermarkets should also be considered.

Possible control options

Statutes, Standards and Guidelines. As it is not a plant pest *Platydemus manokwari* is not listed in the European and Mediterranean Plant Organization (European and Mediterranean Plant Protection Organization 2000a) A1 or A2 List of pests recommended for regulation as quarantine pests (version 2013-09), nor listed by (European Alien Species Information Network (EASIN) 2014-). In Europe countries participating in the NOBANIS network have established a simple early warning system (Secretariat of NOBANIS 2012). When a participating country becomes aware that a new alien species has been found in their country, a warning is sent to the other participating countries and posted on the NOBANIS website. This early warning enables countries to be alerted that a new species has been observed in the region.

Depending upon the outcome of an environmental risk assessment and related investigations, threats from *Platydemus manokwari* may need to be responded to in a similar manner to the invasive New Zealand flatworm *Arthurdendyus triangulatus*. This species is now subject to a European and Mediterranean Plant Protection Organisation (EPPO) Standard regarding import requirements (European and Mediterranean Plant Protection Organization 2000a) and nursery inspection, exclusion and treatment (European and Mediterranean Plant Protection Organization 2000b) for the flatworm (Murchie 2010). The problem with *P. manokwari* is that even though it is primarily an environmental threat, it does not 'indirectly affect plants through the effects on other organisms". Consequently there is the possibility that responsibility for managing this invasive species may fall between the remits of agricultural and environmental regulatory bodies. This could delay effective management of *P. manokwari*.

Chemical control

Although a range of commercial pesticides were tested against *Arthurdendyus triangulatus* only gamma-HCH (Lindane), a broad spectrum, organochlorine insecticide gave significant control but was considered unsuitable for the widespread control of the flatworm (Cannon et al. 1999). There may be limited scope for the use of chemicals within an integrated approach

to control of invasive alien flatworms combining chemical, physical and cultural methods (Blackshaw 1996; Cannon et al. 1999).

Plant sanitization

Heat or hot water treatment of containerised plants that would kill invasive alien species has been investigated for *Arthurdendyus triangulatus* and *Platydemus manokwari.* Specimens of *A. triangulatus* were killed after immersion in a vial for 5 minutes in water at a temperature of 34^{o}C (Murchie & Moore 1998). This method showed great promise (Cannon et al. 1999) but it does not appear to have been used extensively; rather, the current advice to some amateur composters who had flatworm infestation was to place their compost in glasshouses to get the temperatures as high as possible before disseminating the compost (Murchie, *pers. com*). Similar experiments were undertaken on four invertebrate soil taxa that included *Platydemus manokwari*, using immersion in hot water at higher temperatures (Sugiura 2008). It was found that exposure of the animals to hot water at $\geq$ 43^{o}C to 50^{o}C for 5 minutes resulted in 100% mortality for all species tested. In both sets of experiments the flatworms were tested in plastic vials. The ability of the hot water treatment to kill animals in potted soil masses was not examined. Depending upon its porosity and wetting ability the soil may act as a thermal buffer. A more promising method of hot-water treatment is the drenching method of Tsang (Tsang et al. 2001) developed to sanitise potted plants of burrowing nematodes and potential other pest species. The treatment in which potted plants were drenched with hot water at 50^{o}C for 15-20 minutes was more effective at killing burrowing nematodes than dipping potted plants in hot water for the same temperature-time regime. Based upon Sugiura's (Sugiura 2008) data the temperature-time regime of the hot-water drench would kill *Platydemus manokwari*. The drench apparatus may be amenable to commercial development and use.

Biological control

There are no known biological control methods for *Platydemus manokwari* and little is known of its natural enemies.

Conclusion

The serious negative environmental impacts of *Platydemus manokwari* on the biodiversity of native land snails in the Indo-Pacific are well documented. The risks posed by the incursion of this species in France have not yet been assessed. The European Union has recently proposed

new legislation to prevent and manage the rapidly growing threat to biodiversity from invasive species (European Commission (EC) 2013). The proposal centres on a list of invasive alien species of Union concern, which will be drawn up with the Member States using risk assessments and scientific evidence. Whether or not *Platydemus manokwari* will be included on this list remains to be seen.

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