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► **To cite this version:**

Jean-Lou Justine, Delphine Gey, Jessica Thévenot, Clément Gouraud, Leigh Winsor. First report in France of *Caenoplana decolorata*, a recently described species of alien terrestrial flatworm (Platyhelminthes, Geoplanidae). 2020. <hal-03009229>

HAL Id: hal-03009229

<https://hal.science/hal-03009229v1>

Preprint submitted on 18 Nov 2020

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1 **First report in France of *Caenoplana decolorata*, a recently described species**
2 **of alien terrestrial flatworm (Platyhelminthes, Geoplanidae)**

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4 **Jean-Lou Justine ^{1*}, Delphine Gey ², Jessica Thévenot ³, Clément Gouraud ⁴, and Leigh Winsor ⁵**

5

6 1 ISYEB, Institut Systématique Évolution Biodiversité, Muséum National d'Histoire Naturelle, CNRS,
7 Sorbonne Université, EPHE, Université des Antilles, 57 rue Cuvier, CP 51, 75231 Paris Cedex 05,
8 France

9 2 Service de Systématique moléculaire, UMS 2700 CNRS, Muséum National d'Histoire Naturelle,
10 Sorbonne Universités, 43 rue Cuvier, CP 26, 75231 Paris Cedex 05, France

11 Present address: UMR7245 MCAM, Muséum National d'Histoire Naturelle, 61, rue Buffon, CP52,
12 75231 Paris Cedex 05, France

13 3 UMS Patrinat, Muséum National d'Histoire Naturelle, 75231 Paris Cedex 05, France

14 4 Groupe d'étude des invertébrés armoricains (GRETIA), Campus de Beaulieu, 35042 Rennes Cedex,
15 France

16 5 College of Science and Engineering, James Cook University, Townsville, QLD, Australia

17 * Corresponding author: justine@mnhn.fr

18

19 **Abstract**

20 Alien land flatworms (family Geoplanidae) are invading many countries in the world. Some can easily
21 be identified by their morphology and colour pattern, but some are more cryptic and necessitate a
22 molecular approach. *Caenoplana decolorata* Mateos et al., 2020 was recently described, from
23 specimens found in Spain, as a sibling species to *C. coerulea* Moseley, 1877. We found that one
24 specimen collected in Nantes, France in 2014 had a 100% identity of its COI sequence with one
25 specimen of the original description of *C. decolorata*, and thus we record here the species for the
26 first time in France.

27

28 Introduction

29 Alien land flatworms are recorded from many countries worldwide (Sluys, 2016). Some are relatively
30 easy to identify from their morphology and colour pattern. Others belong to species complexes in
31 which the presence of cryptic species make identification by morphology more difficult, necessitating
32 the use of molecular methods. These alien species are generally transported with potted plants, and,
33 as predators of soil animals, can represent a threat to the biodiversity of terrestrial species (Sluys,
34 2016) and soil ecology (Murchie & Gordon, 2013).

35 Winsor considered that *Caenoplana coerulea* Moseley, 1877 was a species complex (Winsor, 1997).
36 Alvarez-Presas et al. (2014) studied various specimens from Spain and considered that specimens
37 identified as “*Caenoplana Ca2*” constituted a distinct species (Álvarez-Presas, Mateos, Tudo, Jones, &
38 Riutort, 2014). Recently, these specimens were described as the new species *Caenoplana decolorata*
39 Mateos et al., 2020 (Mateos, Jones, Riutort, & Álvarez-Presas, 2020). The type-material includes a
40 few specimens found in a plant nursery in Girona Province, Spain, in 2012, and no other occurrence
41 has been reported. By its general colour pattern, the species is close to *C. coerulea* Moseley, 1877.

42 Since 2013, we collected specimens and produced sequence of representatives of the genus
43 *Caenoplana* in France. We checked our sequences to the dataset used by Mateos et al. (2020) and
44 found that one of our sequences matched those of *C. decolorata*, thus showing that the species is
45 present in Metropolitan France.

46 Material and Methods

47 Specimens were collected as a part of a national project in France involving citizen science and
48 molecular characterization of specimens (Justine, Winsor, Gey, Gros, & Thévenot, 2014). We
49 received, from 2013 to now, about 30 specimens of several species of *Caenoplana*. One specimen,
50 now registered in the collection of the Muséum national d'Histoire naturelle in Paris as MNHN JL150,
51 was collected in a glasshouse in the Jardin des Plantes in Nantes, France, during a survey of ants
52 (Gouraud, 2015). The specimen was not photographed when alive, but the collector, one of us (CG),
53 noted that the specimen, found under a pot, was very slender, about 5 cm in length, with a pink head
54 and a yellow dorsal line on a dark background. The specimen was fixed in ethanol where it showed
55 the general morphological feature of *Caenoplana coerulea* in ethanol, i.e. a well-visible dorsal line.

56 Molecular analysis

57 For molecular analysis, a small piece of the body (1-3 mm³) was taken from the lateral edge of the
58 ethanol-fixed individual. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen). Two
59 sets of primers were used to amplify the COI gene. A fragment of 424 bp was amplified with the

60 primers JB3 (=COI-ASmit1) (forward 5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and JB4.5 (=COI-ASmit2)
61 (reverse 5'-TAAAGAAAGAACATAATGAAAATG-3') (Bowles, Blair, & McManus, 1995; Littlewood,
62 Rohde, & Clough, 1997). The PCR reaction was performed in 20 µl, containing 1 ng of DNA, 1×
63 CoralLoad PCR buffer, 3Mm MgCl₂, 66 µM of each dNTP, 0.15µM of each primer, and 0.5 units of Taq
64 DNA polymerase (Qiagen). The amplification protocol was: 4' at 94 °C, followed by 40 cycles of 94 °C
65 for 30'', 48 °C for 40'', 72 °C for 50'', with a final extension at 72 °C for 7'. A fragment of 825 bp was
66 amplified with the primers BarS (forward 5'-GTTATGCCTGTAATGATTG-3') (Álvarez-Presas, Carbayo,
67 Rozas, & Riutort, 2011) and COIR (reverse 5'-CCWGTYARMCCHCCWAYAGTAAA-3') (Lázaro et al.,
68 2009), following (Mateos, Tudó, Álvarez-Presas, & Riutort, 2013). PCR products were purified and
69 sequenced in both directions on a 3730xl DNA Analyzer 96-capillary sequencer (Applied Biosystems).
70 Results of both analyses were concatenated to obtain a COI sequence of 909 bp in length. Sequences
71 were edited using CodonCode Aligner software (CodonCode Corporation, Dedham, MA, USA),
72 compared to the GenBank database content using BLAST. The final sequence was deposited in
73 GenBank under accession number MW203125.

74 **Trees and distances**

75 Dr Alvarez-Presas kindly provided the matrix used in the characterization of *C. decolorata* (Mateos et
76 al., 2020). In a preliminary analysis (not shown here), we added all our sequences of *Caenoplana* spp.
77 from France (about 30). We found that some our sequences matched *C. variegata*, some matched *C.*
78 *coerulea*, and a single one matched the newly described species *C. decolorata*. We then simplified
79 the original matrix: we deleted unnamed species and kept only the sequences which had no indel
80 and were of the same length or longer than our new sequence. The final matrix thus included 14
81 taxa: 12 sequences from the original dataset (Mateos et al., 2020), an outgroup, *Platydemus*
82 *manokwari* MT081580 (Gastineau, Lemieux, Turmel, & Justine, 2020), and our single sequence of *C.*
83 *decolorata*, MW203125.

84 Using MEGA7 (Kumar, Stecher, & Tamura, 2016), a tree was inferred with the maximum likelihood
85 method, based on the GRT+G model (Nei & Kumar, 2000); all codon positions were used, with 100
86 bootstrap replications. The study did not intend to provide relationships between species but more
87 simply to check whether our sequences matched those of species characterised in the description of
88 *C. decolorata* (Mateos et al., 2020).

89 **Results**

90 The tree showed three main branches, each one representing one of the three species of
91 *Caenoplana*, namely *C. variegata*, *C. coerulea* and *C. decolorata*. The two taxa with several
92 sequences, *C. coerulea* and *C. decolorata*, had 100% bootstrap value, but relationships between taxa

93 showed low bootstrap value and are no more commented here. Our new sequence MW203125 from
94 specimen MNHN JL150 belonged to the *C. decolorata* clade. The similarity between our new
95 sequence and sequence MN990644 was 100% along 781 bp.

96

97 Discussion

98 Species of *Caenoplana*, which originate from Australia, are often recorded as alien in various
99 countries. *Caenoplana variegata* has been recorded from Italy (Dorigo, Dal Lago, Menchetti, & Sluys,
100 2020) and Greece (Crete) (Vardinoyannis & Alexandrakis, 2019) (in both cases as *C. bicolor*).
101 *Caenoplana coerulea* has been recorded from Spain (Álvarez-Presas et al., 2014; Mateos et al., 2013),
102 the Canary Islands (Suárez, Martín, & Naranjo, 2018), the Balearic Islands (Breugelmans, Quintana
103 Cardona, Artois, Jordaens, & Backeljau, 2012), and Argentina (Luis-Negrete, Brusa, & Winsor, 2011).

104 The identity of species belonging to *Caenoplana* in Europe is now clearer than a few years ago,
105 thanks to several recent works. The presence of *Caenoplana coerulea* has been confirmed with
106 molecular data (Álvarez-Presas et al., 2014). Specimens previously attributed to *C. bicolor* (Graff,
107 1899) are now attributed to *C. variegata* (Fletcher & Hamilton, 1888) (Jones, Mateos, Riutort, &
108 Alvarez-Presas, 2020). The recent description of the new species *C. decolorata* has added a third
109 binomial species (Mateos et al., 2020).

110 Our single specimen had a 100% match with one of the sequences of *C. decolorata* from Spain, thus
111 ascertaining that it belongs to the same species. Interestingly, the original description mention that
112 the species was found from beneath pots in a plant nursery, which is exactly where our specimen
113 was found in Nantes, France. Nantes is about 500 km north of the Atlantic Spanish border and about
114 800 km away from Bordils, the type-locality. However, these distances have no biogeographical
115 signification since the propagation of land flatworms occurs by human transportation of plants and
116 pots. In both cases, specimens were not from the wild but from protected environments, i.e. a plant
117 nursery and a glasshouse. The Spanish specimens were collected in 2012 and our French specimens
118 were collected in 2014; the species is thus present in Europe for at least 8 years, and probably more.
119 For the recently described species *Marionfyfea adventor* Jones & Sluys, 2016, the authors found that
120 the species was already present in several European countries when they became aware of its
121 presence (Jones & Sluys, 2016).

122 The presence of *C. decolorata* adds one species to the list of alien land flatworms (family
123 Geoplanidae) in Metropolitan France. Currently, the list includes 10 species:

- 124 • *Platydemus manokwari*, found only in a hothouse in Caen (Justine et al., 2015; Justine,
125 Winsor, et al., 2014)
- 126 • *Bipalium kewense*, widespread in the open, mainly in the South (Justine, Winsor, Gey, Gros,
127 & Thévenot, 2018)
- 128 • *Diversibipalium multilineatum*, widespread in the open, mainly in the South (Justine et al.,
129 2018)
- 130 • *Diversibipalium* “black”, an undescribed species, found in a single location (Justine et al.,
131 2018)
- 132 • *Parakontikia ventrolineata*, with a wide distribution (Justine, Thévenot, & Winsor, 2014)
- 133 • *Obama nungara*, with a wide distribution on more than 75% of Metropolitan France (Justine,
134 Winsor, Gey, Gros, & Thévenot, 2020)
- 135 • *Marionfyfea adventor*, found in a single location in the wild (Jones & Sluys, 2016)
- 136 • *Caenoplana coerulea*, with several locations in the open (Justine, Thévenot, et al., 2014)
- 137 • *Caenoplana variegata*, with several locations in the open, locally very abundant (Justine,
138 Thévenot, et al., 2014)
- 139 • *Caenoplana decolorata*, found in a single location in a hothouse in Nantes (this paper)

140 In addition to specimens for which we could obtain sequences, we received a number of records of *C.*
141 *coerulea* from Metropolitan France based only on photographs obtained by citizen science; since
142 both species are close in colour pattern, it cannot be excluded that some of these are in fact *C.*
143 *decolorata*.

144

145 **Acknowledgements.** We thank the authors of the description of *C. decolorata*, and especially Marta
146 Álvarez-Presas, for sending us the original matrix used in their paper, thus saving us time and errors
147 retrieving sequences. We thank Philippe Féraud, botanist, Jardin des Plantes in Nantes, for allowing the
148 collection of specimens. This work was funded by several Actions Thématiques du Muséum between
149 2014 and 2020.

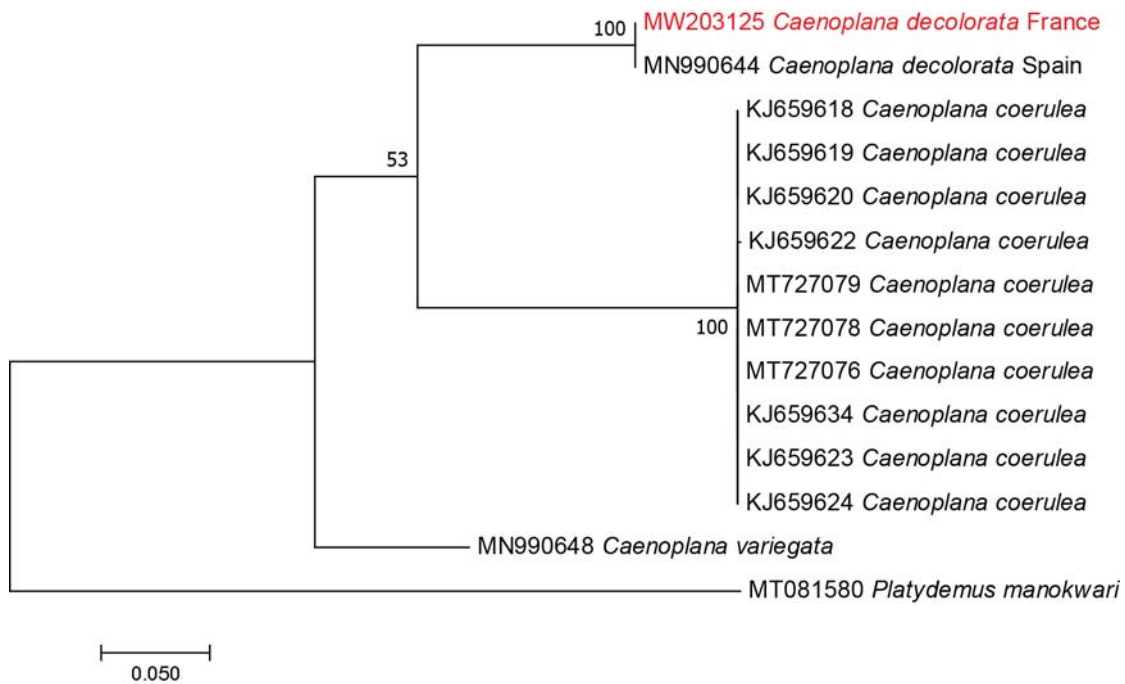
150

151 **Figures**

152

153 **Figure 1.** Tree of relationships between species of *Caenoplana* found in France. The matrix was based
154 on that used by Mateos et al. (Mateos et al., 2020), simplified to keep only sequences without indels
155 and of length similar or longer than our new sequence. The evolutionary history was inferred by
156 using the Maximum Likelihood method based on the General Time Reversible model + Gamma
157 distribution. There was a total of 777 positions in the final dataset.

158



160

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