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FIRST RECORDS OF THE TRUE SICILIAN SLUG *DEROCERAS PANORMITANUM* (EUPULMONATA: AGRIOLIMACIDAE) IN FRANCE AND MITOCHONDRIAL SEQUENCES OF THREE ADDITIONAL SPECIES OF THE GENUS *DEROCERAS*

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ABSTRACT: The genus *Deroceras* (Eupulmonata: Agriolimacidae) is the richest genus of slugs in terms of described species and as such represents a challenge for taxonomic knowledge, endemic species conservation and invasive species management. Until now, 15 species of *Deroceras* have been recorded in mainland France. We report for the first time in France the presence of the true Sicilian slug, *Deroceras panormitanum* (Lessona et Pollonera, 1882), at three sites. This name had formerly long been used to designate specimens of the tramp slug, *Deroceras invadens* Reise, Hutchinson, Schunack et Schlitt, 2011. In this study, 72 specimens of the genus *Deroceras* collected from 23 French sites were analysed based on anatomical and/or molecular criteria (barcoding fragments of the mitochondrial cytochrome c oxidase subunit I gene and 16S ribosomal RNA gene). The sequences obtained were placed in molecular phylogenies including reference sequences from the literature. Four species were recorded: *D. panormitanum, D. invadens, D. reticulatum* (O. F. Müller, 1774) and *D. agreste* (Linnaeus, 1758). Incongruence between anatomy and mitochondrial sequences in some specimens suggested hybridisation between *D. reticulatum* and *D. agreste*.

KEY WORDS: anatomy; DNA barcoding; France; hybridisation; slug

INTRODUCTION

The genus *Deroceras* Rafinesque, 1820 (Eupulmonata: Agriolimacidae) is the most species-rich genus of slugs, with about 100 species known (WIKTOR 2000). Some species, such as *D. reticulatum* (O. F. Müller, 1774) (grey field slug) and *D. invadens* Reise, Hutchinson, Schunack et Schlitt, 2011 (tramp slug), are widely distributed, often synanthropic and considered harmful to agriculture and horticulture in many parts of the world; others have very restricted distributions and might represent a conservation challenge due to the increasing destruction of their habitats. A total of 15 *Deroceras* species have been recorded in France (GARGOMINY et al. 2011). Some are local endemics (e.g. along the Pyrenees or in Corsica), whereas others are widespread (e.g. *D. reticulatum* and *D. agreste*) or even invasive introduc-

tions (e.g. *D. invadens*). In this article, we report for the first time the presence of the true Sicilian slug, *D. panormitanum* sensu stricto (Lessona et Pollonera, 1882), in France.

Prior to the publication of Reise et al. (2011), the tramp slug was referred to as *D. panormitanum*. These authors showed, however, that *D. panormitanum* sensu stricto (hereafter referred to as *D. panormitanum*) was a species originating from Sicily and/or Malta, which has a more restricted geographic distribution, and morphological characters, mating behaviour and cytochrome c oxidase subunit I (COI) gene sequences that differ from those of the tramp species (hereafter referred to as *D. invadens*).

The two species cannot be reliably distinguished based on external morphological criteria, but genital anatomy does distinguish them. The most obvious feature is the shape of the penial caecum, which is more pointed in *D. panormitanum*. Two other very useful characters for distinguishing the two species are the attachment of the penial retractor muscle and the knobbly appearance of the penial glands. The mating of *D. panormitanum* is also distinctive, for instance in a characteristic behaviour of close contact between the flanks, as the partners crawl past each other antiparallel (Reise et al. 2011). Furthermore, the COI sequences of *D. panormitanum* form a clade clearly distinct from *D. invadens* in phylogenetic analyses (Reise et al. 2011).

Deroceras invadens is an invasive species now widely distributed throughout the world (Hutchinson et al. 2014, 2020), whereas *D. panormitanum* is little reported outside its native range. Currently, *D. panormitanum* is known as an introduction from Bordighera in Italy (Hutchinson et al. 2014), Cãmara de Lobos in Madeira (Hutchinson et al. 2014), and the Balearic Islands (Castillejo & Iglesias 2017), Estremadura and Ribatejo in Portugal (Holyoak et al. 2019), and it was intercepted in a shipment from São Miguel in the Azores (Hutchinson et al. 2014). *Deroceras panormitanum* has also been reported from Whipsnade Zoo (Rowson 2024) and Hampstead, Middlesex (B. Rowson, personal communication) in England, from Cardiff (Rowson et al. 2014, 2016) and Swansea (Rowson et al. 2016) in Wales, and from Kilmacanoge in Ireland (Rowson et al. 2016).

In France, *Deroceras invadens* (then known as *D. caruanae*) was first clearly reported in 1956 (Hameury 1958) but there is evidence of its occurrence already in the 1940s (Hutchinson et al. 2014), and it has been widely distributed in the country since at least 1963 (Reygrobellet 1963). Due to confusion between the two species, *D. panormitanum* may have gone unnoticed in France, and/or samples were incorrectly assigned to *D. invadens*. As part of sampling campaigns to characterise the specific diversity of slugs in France, we set out to distinguish the *Deroceras* species present, using anatomical and molecular tools (mitochondrial COI and 16S ribosomal RNA genes).

We report here the occurrence of *D. panormitanum* at three sites in mainland France: a green avenue in Puteaux and the public garden Parc Floral de Paris in Vincennes, both in the suburbs of Paris, and a private garden in La Rochelle. The occurrence of *D. invadens, D. reticulatum* and *D. agreste* at the sites surveyed is also documented, and COI and 16S sequences are placed in phylogenies including reference sequences from the literature.

Abbreviations (name acronyms): JMCH – John M. C. Hutchinson, LD – Lise Dupont, MV – Mathis Ventura, NM – Nicolas Mazuras, SN – Shanèze Noel, VR – Virginie Roy, XC – Xavier Cucherat.

MATERIAL AND METHODS

SITES AND SAMPLING

Five of the authors (MV, SN, NM, LD and VR) collected slugs of the genus *Deroceras* from November 2021 to April 2023 at 10 sites: 3 sites in Paris and its suburbs (Paris, Puteaux and Limeil-Brévannes), 1 site in La Rochelle, 3 sites near Bordeaux (Biscarosse, Parentis-en-Born and Saint-Médard-en-Jalles) and 3 sites in Brittany (Cléguer, Concarneau and Lampaul-Plouarzel) (Table 1 and Fig. 1). The sites were mainly private gardens, except for one on a busy tree-lined avenue (Puteaux), another on a roof garden (Paris) and a third in a greenhouse of a nursery (Cléguer). Sites were visually inspected by lifting all objects from the ground (pots, bags of potting soil, plastic covers, stones, furniture, and vegetation). Slugs were either directly immersed in absolute ethanol (those slugs collected in 2021 and 2022) or drowned in water over 12 h before being transferred to absolute ethanol (those slugs collected in 2023 and subsequently referred to as "stretched" specimens preserved for anatomical analyses) and stored at 4 °C.

Additionally, JMCH independently sampled multiple sites around France since 2019 mostly focusing on slugs of the genus *Arion*, but slugs externally resembling *D. invadens* were collected when encountered (14 sites, https://doi.org/10.5281/zenodo.14185677). If only juveniles were collected, they were reared to maturity. These slugs were killed by immersion in carbonated water followed by the progressive addition of 70 or 75% ethanol.

Table 1. *Deroceras* individuals analysed morphologically and molecularly (for additional *D. invadens* collected by JOHN M. C. HUTCHINSON see https://doi.org/10.5281/zenodo.14185677). Collectors' acronyms: JMCH – JOHN M. C. HUTCHINSON, LD – LISE DUPONT, MV – MATHIS VENTURA, NM – NICOLAS MAZURAS, SN – SHANÈZE NOEL, VR – VIRGINIE ROY, XC – XAVIER CUCHERAT; Preservation method: E – specimens directly immersed in ethanol (not preserved for anatomical determination), S – specimens drowned in water and stretched and preserved in ethanol, C – specimen killed by immersion in carbonated water and preserved in ethanol; COI-Hap and 16S-Hap: COI and 16S haplotype numbers for this study; COI and 16S ID: molecular identification resulting from phylogenetic analyses of COI and 16S rRNA genes; Dp – *D. panormitanum*, Di – *D. invadens*, Dr – *D. reticulatum*, Da – *D. agreste*

Specimen ID	Date	Collectors	Site	Habitat type	Preservation	COI Hap	Genbank AN	COI ID	16S Hap	Genbank AN	16S ID	Anatomical ID
A21 G2B L1	15/10/2021	MV, SN, NM, LD, VR	La Rochelle	Private garden	E	COI-Hap P1	PQ066670	Dp				
A21 G2B L3	15/10/2021	MV, SN, NM, LD, VR	La Rochelle	Private garden	E	COI-Hap P1	PQ066671	Dp				
A21 G2B L25	15/10/2021	MV, SN, NM, LD, VR	La Rochelle	Private garden	E	COI-Hap P1	PQ066672	Dp				
A21 G2B L26	15/10/2021	MV, SN, NM, LD, VR	La Rochelle	Private garden	E	COI-Hap P1	PQ066673	Dp				
A22 G2B M36	20/10/2022	MV, SN, NM, LD, VR	La Rochelle	Private garden	E	COI-Hap P1	PQ066674	Dp				
P23 G2B L4	17/04/2023	MV, SN, NM, LD, VR	La Rochelle	Private garden	S	COI-Hap P1	PQ066675	Dp	16S-Hap P1	PQ066738	Dp	Dp
P23 G2B L6	17/04/2023	MV, SN, NM, LD, VR	La Rochelle	Private garden	S	COI-Hap P1	PQ066676	Dp	16S-Hap P1	PQ066739	Dp	Dp
A21 G1B L22	26/11/2021	MV, SN, NM, LD, VR	Puteaux	Planted avenue	E	COI-Hap P1	PQ066677	Dp				
A21 G1B L23	26/11/2021	MV, SN, NM, LD, VR	Puteaux	Planted avenue	E	COI-Hap P1	PQ066678	Dp				
A21 G1B L33	26/11/2021	MV, SN, NM, LD, VR	Puteaux	Planted avenue	E	COI-Hap P1	PQ066679	Dp	16S-Hap P1	PQ066740	Dp	
A21 G1B L34	26/11/2021	MV, SN, NM, LD, VR	Puteaux	Planted avenue	E	COI-Hap P1	PQ066680	Dp				
A21 G1B L36	26/11/2021	MV, SN, NM, LD, VR	Puteaux	Planted avenue	E	COI-Hap P1	PQ066681	Dp				
P22 G1B L1	08/04/2022	MV, SN, NM, LD, VR	Puteaux	Planted avenue	E	COI-Hap P1	PQ066682	Dp	16S-Hap P1	PQ066741	Dp	
P22 G1B L5	08/04/2022	MV, SN, NM, LD, VR	Puteaux	Planted avenue	E	COI-Hap P1	PQ066683	Dp				
SMNG p23845	26/09/2019	JMCH	Paris	Parc Floral de Paris, Vincennes	C	COI-Hap P1	PQ285439	Dp				Dp
				Total				15			4	3
A21 G4B L11	27/10/2021	MV, SN, NM, LD, VR	Cléguer	Greenhouse nursery	E	COI-Hap I1	PQ066684	Di				

Table 1 continued

A21 G4B L12	27/10/2021	MV, SN, NM, LD, VR	Cléguer	Greenhouse nursery	E	COI-Hap I1	PQ066685	Di				
A22 G4B M5	02/11/2022	MV, SN, NM, LD, VR	Cléguer	Greenhouse nursery	E	COI-Hap I1	PQ066686	Di				
P22 G4B L20	20/04/2022	MV, SN, NM, LD, VR	Cléguer	Greenhouse nursery	E	COI-Hap I3	PQ066687	Di				
P23 G4B M8	03/05/2023	MV, SN, NM, LD, VR	Cléguer	Greenhouse nursery	S	COI-Hap I4	PQ066688	Di	16S-Hap I2	PQ066742	Di	Di
P23 G4C M9	04/05/2023	MV, SN, NM, LD, VR	Concarneau	Private garden	S	COI-Hap I1	PQ066689	Di	16S-Hap I1	PQ066743	Di	Di
A21 G2B L2	15/10/2021	MV, SN, NM, LD, VR	La Rochelle	Private garden	E	COI-Hap I1	PQ066690	Di				
P22 G2B L13	13/04/2022	MV, SN, NM, LD, VR	La Rochelle	Private garden	E	COI-Hap I1	PQ066691	Di				
P23 G2B L5	17/04/2023	MV, SN, NM, LD, VR	La Rochelle	Private garden	S	COI-Hap I1	PQ066692	Di	16S-Hap I1	PQ066744	Di	Di
A21 G4E L18	29/10/2021	MV, SN, NM, LD, VR	Lampaul-Plouarzel	Private garden	E	COI-Hap I1	PQ066693	Di				
A22 G4E M16	04/11/2022	MV, SN, NM, LD, VR	Lampaul-Plouarzel	Private garden	E	COI-Hap I1	PQ066694	Di				
P23 G4E M5	05/05/2023	MV, SN, NM, LD, VR	Lampaul-Plouarzel	Private garden	S	COI-Hap I1	PQ066695	Di	16S-Hap I1	PQ066745	Di	Di
P22 G4E L33	22/04/2022	MV, SN, NM, LD, VR	Lampaul-Plouarzel	Private garden	E	COI-Hap I6	PQ066696	Di	16S-Hap I1	PQ066746	Di	
P23 G1C M10	19/04/2023	MV, SN, NM, LD, VR	Limeil-Brévannes	Private garden	S	COI-Hap I7	PQ066697	Di	16S-Hap I3	PQ066747	Di	Di
A21 G3B L6	18/10/2021	MV, SN, NM, LD, VR	Parentis-en-Born	Private garden	E	COI-Hap I1	PQ066698	Di				
P22 G3B L17	14/04/2022	MV, SN, NM, LD, VR	Parentis-en-Born	Private garden	E	COI-Hap I5	PQ066699	Di				
P23 G1A M1	13/04/2023	MV, SN, NM, LD, VR	Paris	Planted roof	S	COI-Hap I1	PQ066700	Di	16S-Hap I1	PQ066748	Di	Di
A22 G1A M24	18/10/2022	MV, SN, NM, LD, VR	Paris	Planted roof	E	COI-Hap I4	PQ066701	Di				
P23 G1A M2	13/04/2023	MV, SN, NM, LD, VR	Paris	Planted roof	S	COI-Hap I4	PQ066702	Di	16S-Hap I2	PQ066749	Di	Di
A22 G1A M25	18/10/2022	MV, SN, NM, LD, VR	Paris	Planted roof	E	COI-Hap I5	PQ066703	Di	16S-Hap I1	PQ066750	Di	
A21 G1B L24	26/11/2021	MV, SN, NM, LD, VR	Puteaux	Planted avenue	E	COI-Hap I1	PQ066704	Di				

Table 1 continued

A21 G1B L32	26/11/2021	MV, SN, NM, LD, VR	Puteaux	Planted avenue	E	COI-Hap I2	PQ066705	Di	16S-Hap I1	PQ066751	Di	
A22 G3C M42	08/11/2022	MV, SN, NM, LD, VR	Saint-Médard-en-Jalles	Private garden	E	COI-Hap I1	PQ066706	Di				
A22 G3C M43	08/11/2022	MV, SN, NM, LD, VR	Saint-Médard-en-Jalles	Private garden	E	COI-Hap I1	PQ066707	Di				
P23 G3C M6	25/04/2023	MV, SN, NM, LD, VR	Saint-Médard-en-Jalles	Private garden	S	COI-Hap I1	PQ066708	Di	16S-Hap I1	PQ066752	Di	Di
				Total				25			11	8
P23 G3A M2	24/04/2023	MV, SN, NM, LD, VR	Biscarosse	Private garden	S	COI-Hap R3	PQ066709	Dr	16S-Hap R1	PQ066753	Dr	Dr
P23 G4B M9	03/05/2023	MV, SN, NM, LD, VR	Cléguer	Greenhouse nursery	S	COI-Hap R6	PQ066710	Dr	16S-Hap R1	PQ066754	Dr	Dr
P23 G4C M1	04/05/2023	MV, SN, NM, LD, VR	Concarneau	Private garden	S	COI-Hap R1	PQ066711	Dr	16S-Hap R1	PQ066755	Dr	Dr
P23 G4C M8	04/05/2023	MV, SN, NM, LD, VR	Concarneau	Private garden	S	COI-Hap R7	PQ066712	Dr				Dr
P22 G1C L8	06/04/2022	MV, SN, NM, LD, VR	Limeil-Brévannes	Private garden	E	COI-Hap R2	PQ066713	Dr	16S-Hap R2	PQ066756	Dr	
A22 G1C M30	26/10/2022	MV, SN, NM, LD, VR	Limeil-Brévannes	Private garden	E	COI-Hap R4	PQ066714	Dr				
P22 G3B L18	14/04/2022	MV, SN, NM, LD, VR	Parentis-en-Born	Private garden	E	COI-Hap R3	PQ066715	Dr				
A21 G1A L19	18/11/2021	MV, SN, NM, LD, VR	Paris	Planted roof	E	COI-Hap R1	PQ066716	Dr	16S-Hap R1	PQ066757	Dr	
P23 G3C M5	25/04/2023	MV, SN, NM, LD, VR	Saint-Médard-en-Jalles	Private garden	S	COI-Hap R3	PQ066717	Dr	16S-Hap R1	PQ066758	Dr	Dr
A22 G3C M45	08/11/2022	MV, SN, NM, LD, VR	Saint-Médard-en-Jalles	Private garden	E	COI-Hap R5	PQ066718	Dr	16S-Hap R2	PQ066759	Dr	
				Total				10			7	5
P23 G4B M5	03/05/2023	MV, SN, NM, LD, VR	Cléguer	Greenhouse nursery	S	COI-Hap A1	PQ066719	Da				Dr
P23 G4E M6	05/05/2023	MV, SN, NM, LD, VR	Lampaul-Plouarzel	Private garden	S	COI-Hap A1	PQ066720	Da				Dr
A21 G4E L17	29/10/2021	MV, SN, NM, LD, VR	Lampaul-Plouarzel	Private garden	E	COI-Hap A1	PQ066721	Da	16S-Hap A1	PQ066760	Da	
A22 G4E M18	04/11/2022	MV, SN, NM, LD, VR	Lampaul-Plouarzel	Private garden	E	COI-Hap A1	PQ066722	Da	16S-Hap A1	PQ066761	Da	
P22 G4E L32	22/04/2022	MV, SN, NM, LD, VR	Lampaul-Plouarzel	Private garden	E	COI-Hap A1	PQ066723	Da	16S-Hap A2	PQ066762	Da	
				Total				5			3	2

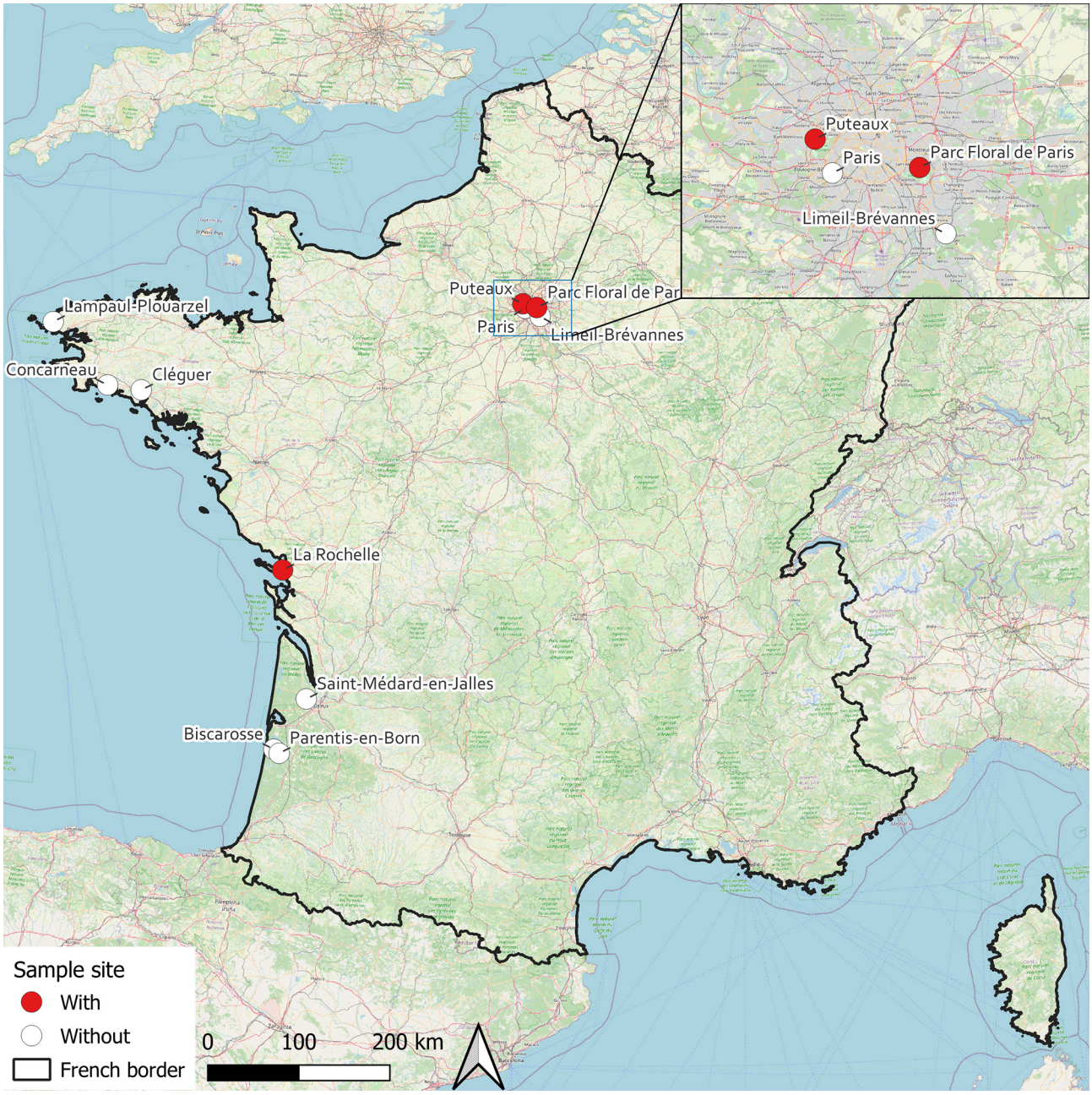


Fig. 1. Map of the sampling sites for sequenced *Deroceras* samples. Red circles indicate sites where *D. panormitanum* was found. See https://doi.org/10.5281/zenodo.14185677 for additional *D. invadens* samples collected by JMCH

Specimens collected by MV, SN, NM, LD and VR are preserved at University Paris-Est Créteil and specimens collected by JMCH are preserved in the Senckenberg Museum of Natural History Görlitz.

ANATOMICAL DETERMINATION

Anatomical determination was conducted on 17 individuals of the 54 sampled by MV, SN, NM, LD and VR, i.e. all "stretched" specimens (Table 1) and on 18 specimens collected by JMCH (Table 1 and https://doi.org/10.5281/zenodo.14185677). Prior to molecular identification, the individuals were dissected by cutting the integument just above the left margin of the foot and folding the dorsal integument over onto the right side. Differentiation between *D. invadens* and *D. panormitanum* was made using the following diagnostic characters for the genitalia: the shape of the penial caecum, the knobbliness of the penial glands and the attachment of the penial retractor muscle. The distinction between *D. reticulatum* and *D. agreste* was made on the basis of the shape of the penial glands (Table 2).

MOLECULAR ANALYSIS

DNA extraction

DNA extraction was performed on 54 individuals i.e., all specimens collected by MV, SN, NM, LD and VR (Table 1). Each individual was rinsed with 95% ethanol to avoid contamination prior to extraction, and a fragment of the mantle of approximately 4 mm² was collected using a sterile blade. Total genomic DNA was isolated according to the protocol recommendations of the DNeasy® Blood & Tissue Kit (Qiagen, France), with final elution in 50 µL of buffer.

PCR amplification and sequencing

The standard DNA barcoding region (HEBERT et al. 2003) was amplified by PCR for the 54 specimens collected by MV, SN, NM, LD and VR (Table 1). It consists of a fragment of 655 bp at the 5' end of the COI mitochondrial gene. PCR reactions were performed in a final volume of 25 µL containing 5 µL of 1 × colourless GoTaq® Buffer Master Mix (Promega, France), 0.5 µL of 0.2 µM of each primer, 1.25 µL of 0.5 mM dNTPs, 0.125 µL of 0.025 U GoTaq® G2 DNA polymerase (Promega, France), 2 µL of about 20ng/µL extracted DNA and 15.625 µL of ultrapure water. Forward and reverse primers were LCO1490-GGTCAACAAATCATAAAGATTGG and HCO2198-TAAACTTCAGGGTGACCAAAAAATCA (FOLMER et al. 1994). An initial denaturation step at 94 °C for 3 min was followed by 35 cycles at 94 °C for 30 s, an annealing step at 50 °C for 45 s and an extension step at 72 °C for 1 min, and a final extension step at 72 °C for 10 min (T100 thermal Cycler Bio-Rad, France).

For 25 of the 54 specimens collected by MV, SN, NM, LD and VR (Table 1), amplification of a fragment of the mitochondrial 16S ribosomal RNA gene of approximately 400 bp was carried out after identification based on COI sequence, using the same protocol but with primers 16sar-CGCCTGTTTATCAAAAACAT and 16sbr-CCGGTYTGAACTCAGATCAYGT (PALUMBI 1991) and an annealing temperature of 51 °C.

PCR products were sent to Eurofins Genomics for sequencing with the primers used for PCR amplification.

Specimens collected by JMCH were not sequenced except for one individual identified anatomically as *D. panormitanum*, for which the same region of the COI gene was sequenced using the protocol described in HUTCHINSON et al. (2020).

MOLECULAR IDENTIFICATION

Sequences were manually corrected using Chromas 2.6.6 software (https://technelysium.com.au), aligned using the Muscle program in SeaView (GOUY et al. 2010) and submitted to GenBank. Accession numbers are listed in Table 1. Haplotype data files were generated using DNAsp v6.12.03 (ROZAS et al. 2017).

The sequences obtained in this study were placed in phylogenetic trees that included the COI sequences from the reference studies that morphologically and molecularly distinguished *D. panormitanum* and *D. invadens* (REISE et al. 2011), and *D. agreste* and *D. reticulatum* (ROWSON et al. 2014, ZAJĄC & STEC 2020). To discuss the origin of the lineages present in France, COI sequences used in previous phylogeographic studies were also included (GREGORIC et al. 2013, COLGAN 2017, ARAIZA-GÓMEZ et al. 2017, HUTCHINSON et al. 2020). The origin, expansion pathways and genetic diversity of *D. invadens* have been extensively studied using the COI gene (HUTCHINSON et al. 2020), and two widespread clades of COI haplotypes, called the red and blue haplogroups, were proposed by these authors. We use this designation to integrate our data with those of HUTCHINSON et al. (2020) in a readable manner.

Because the reference studies did not use the 16S rRNA gene, *D. invadens*, *D. panormitanum*, *D. reticulatum* and *D. agreste* sequences from the literature were not included in the phylogenetic analysis for this gene, to avoid introducing incorrectly assigned specimens. The SMS (Smart Model Selection in PhyML) software (LEFORT et al. 2017) was used to select the best nucleotide substitution model with an AIC criterion.

Table 2. Diagnostic features in the genital tract used to differentiate *D. invadens* and *D. panormitanum,* and *D. reticulatum* and *D. agreste*

Character	*D. invadens*	*D. panormitanum*
Penial caecum	Smooth in outline, rounded at the tip	Bulky base on which inserts a long, thin and somewhat bent pocket that tapers towards the tip; usually with swellings along one side
Penial glands	Fingers with no, or only weak, swellings	Fingers with prominent papillae
Penial retractor muscle	Insertion between the lobe and the caecum	Insertion on the side of the lobe
Character	***D. reticulatum***	***D. agreste***
Penial glands	Variable shape, long, usually a few branches, occasionally a single branch	Finger-shaped, hooked, short, never branched, smooth, without glandular papillae

Phylogenetic trees were reconstructed by maximum likelihood using PhyML (Guindon et al. 2010) with 1000 bootstrap replicates. Trees were rooted either with *D. laeve* (COI tree for *D. invadens/D. panormitanum*), *D. turcicum* (*D. reticulatum/D. agreste*) or on the midpoint (16S tree). The uncorrected p-distance – the proportion of nucleotide sites differing between two sequences – was calculated using MEGA version 11 (Tamura et al. 2021).

RESULTS

DEROCERAS PANORMITANUM AND *D. INVADENS*

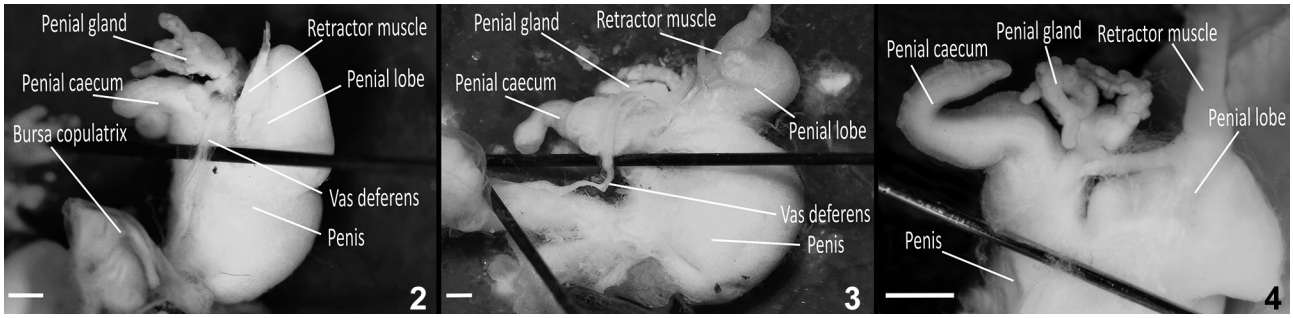
Three of the specimens dissected had the diagnostic genital characters of *D. panormitanum*, two collected by MV, SN, NM, LD and VR, and one collected by JMCH (Table 1). All had a penial caecum which was long, thin and tapering to a point, in contrast to the shorter, rounded penial lobe. Penial glands had prominent papillae. The penial retractor muscle was inserted on the side of the penial lobe (Figs 2–4).

Fifteen specimens that we sequenced had COI sequences corresponding to a single haplotype (COI-Hap P1, Table 1) included in a clade grouping all reference COI sequences of *D. panormitanum* (Fig. 5). Considering all *D. panormitanum* COI sequences from France and from the literature, the maximum distance was p = 0.04 (between haplotypes from Malta and Sicily).

The three specimens anatomically identified as *D. panormitanum* belonged to this clade and were thus confirmed as having the mitochondrial genome of *D. panormitanum*.

Four identical 16S sequences (haplotype 16S-Hap P1, Table 1) were obtained from the individuals with COI sequences of *D. panormitanum*. They clearly differed from the *D. invadens* clade in the 16S ML tree (Fig. 6). Since no 16S sequence for *D. panormitanum* is available in nucleotide databases, this haplotype represents the first for this species.

The anatomical characters diagnostic for *D. invadens* were observed in eight specimens collected by MV, SN, NM, LD and VR (Table 1) and in 17 specimens collected by JMCH (https://doi.org/10.5281/zenodo.14185677). COI sequences from 25 specimens collected by MV, SN, NM, LD and VR, including the eight specimens anatomically identified as *D. invadens*, were included in a clade of published *D. invadens* sequences (Fig. 5). These 25 sequences consisted of seven distinct COI haplotypes (Table 1). The most common haplotype (COI-Hap I1) was carried by 16 individuals and was found at 8 out of 10 sites sampled. It corresponds to haplotype 0 from the red haplogroup in the study by Hutchinson et al. (2020). COI-Hap I5 and I6 correspond to haplotype 2 and haplotype 1 from the red haplogroup (Fig. 5). The second most common haplotype (COI-Hap I4), carried by three individuals, corresponds to haplotype 3 from the blue haplogroup. Haplotype COI-Hap I3 is a new haplotype, also within the blue haplogroup. Two haplotypes were not included in either of the haplogroups: haplotype I2, corresponding to haplotype 6 of Hutchinson et al. (2020), and the new haplotype I7.



Figs 2–4. Male genitalia of *D. panormitanum*: 2 – specimen P23 G2B L4 from La Rochelle, France; 3 – specimen P23 G2B L6 from La Rochelle, France; 4 – specimen SMNG p23845 from the Parc Floral de Paris in Vincennes, France. Photos: Xavier Cucherat (2–3) and John M.C. Hutchinson (4). Scale bars 0.6 mm

Fig. 5. Phylogenetic tree reconstructed by maximum likelihood based on the cytochrome c oxidase I gene fragment for *D. panormitanum* (in blue) and *D. invadens* (in green) samples from this study and from the literature, for which the Genbank accession numbers are given. For each sample, the origin, the haplotype number and the haplogroup referring to Hutchinson et al. (2020) are mentioned. Samples with the annotation *** were anatomically determined, and in this case the species indicated corresponds to the morphology. For the others, the identification is based on the sequence. The tree is rooted with sequences of *D. laeve*. Bootstrap values greater than 70 are indicated at the nodes. The scale bar represents the number of substitutions per site


KF894277 I47 D. laeve Ireland South-east Galway
KF894229 13L3 D. laeve Ireland South-east Galway
KF894262 13L4 D. laeve Ireland South-east Galway
KX959491 D. laeve Mexico
KX959496 D. laeve Mexico
KX959494 D. laeve Mexico
KX959501 D. laeve Mexico
KF894311 M33 D. laeve United Kingdom West Gloucestershire
KX959495 D. laeve Mexico
KX959499 D. laeve Mexico
KX959492 D. laeve Mexico
KX959498 D. laeve Mexico
KX959493 D. laeve Mexico
KX959500 D. laeve Mexico
KX959497 D. laeve Mexico
KF894364 SN7 D. laeve UK Caernarvonshire
KF894348 S42 D. laeve United Kingdom East Inverness-shire
KF894261 12m5 D. laeve United Kingdom Co. Antrim
KF894228 13L2 D. laeve United Kingdom Co. Armagh
KF894344 S1 D. laeve United Kingdom West Ross & Cromarty
KF894381 Y36 D. laeve United Kingdom Mid-west Yorkshire
KF894260 K6 D. laeve United Kingdom East Sussex
MH830187 D. invadens United Kingdom North Berwick Hap 33
JN248315 D. invadens USA Salt Lake City Hap 5
MH830235 D. invadens Switzerland Kirchenfel, Hap 83
JN248314 D. invadens France Dunquerque Hap 1
KF894252 GB2 D. invadens UK Lanarkshire Hap 1
P22 G4E L33 D. invadens France Lampaul-Plouarzel Hap I6
KF894244 D. invadens UK Nottinghamshire Hap 1
MH830182 D. invadens Australia Mortlake Hap 28
KF894385 Y41 D. invadens UK Northeast Yorkshire Hap 28
MH830170 D. invadens France Angouleme Hap 16
MH830221 D. invadens France Etaples Hap 69
MH830226 D. invadens South Africa Hogsback Hap 74
JQ743070 D. invadens Argentina Isla Victoria Hap 0
A22 G4B M5 D. invadens France Cléguer Hap I1
A22 G3C M42 D. invadens France Saint-Médard-en-Jalles Hap I1
A21 G1B L24 D. invadens France Puteaux Hap I1
A21 G2B L2 D. invadens France La Rochelle HapI1
A21 G3B L6 D. invadens France Parentis-en-Born Hap I1
MH830223 D. invadens Ireland Killarney Hap 71
MH830185 D. invadens Canada Boundary Bay Hap 31
JN248295 D. invadens Germany Hap 2
P22 G3B L17 D. invadens France Parentis-en-Born Hap I5
A22 G1A M25 D. invadens France Paris Hap I5
A22 G4E M16 D. invadens France Lampaul-Plouarzel Hap I1
A22 G3C M43 D. invadens France Saint-Médard-en-Jalles Hap I1
A21 G4B L11 D. invadens France Cléguer Hap I1
P23 G1A M1 D. invadens France Paris Hap I1***
P22 G2B L13 D. invadens France La Rochelle HapI1
KF894319 M51 D. invadens UK Co. Antrim Hap 0
P23 G4C M9 D. invadens France Concarneau Hap I1***
MH830237 D. invadens Belgium Bertrix Hap 85
KX959489 D. invadens Mexico Hidalgo Hap 0
KX959488 D. invadens Mexico Mexico Hap 0
MH830225 D. invadens New Zealand Canterbury Hap 73
KX959490 D. invadens Mexico Tlaxcala Hap 0
JN248297 D. invadens Canada Vancouver Island Hap 0
KF894370 WF3 D. invadens UK Worcestershire Hap 0
KF894259 DN3 D. invadens UK Denbighshire Hap 0
P23 G2B L5 D. invadens France La Rochelle HapI1***
P23 G4E M5 D. invadens France Lampaul-Plouarzel Hap I1***
P23 G3C M6 D. invadens France Saint-Médard-en-Jalles Hap I1***
A21 G4E L18 D. invadens France Lampaul-Plouarzel Hap I1
A21 G4B L12 D. invadens France Cléguer Hap I1
JN248296 D. invadens UK Banstead Hap 0
MH830181 D. invadens Italy Ariano nel Polesine Hap 27
MH830165 D. invadens Italy Bordighera Hap 11
MH830195 D. invadens Italy Refugio de Gaspari Hap 41
MH830232 D. invadens Italy Bellafemmina Hap 80
MH830199 D. invadens Italy Bellafemmina Hap 45
MH830204 D. invadens Italy Lazzaro Hap 50
MH830196 D. invadens Italy Campotenese Hap 42
MH830174 D. invadens Italy Pianillo Hap 20
MH830191 D. invadens Italy Matera Hap 37
MH830193 D. invadens Italy Potenza Hap 39
MH830192 D. invadens Italy Matera Hap 38
MH830231 D. invadens Italy Caparico Valle Hap 79
MH830209 D. invadens Italy Picerno Hap 55
MH830194 D. invadens Italy Prato Comune Hap 40
MH830197 D. invadens Italy San Benedetto Hap 43
MH830198 D. invadens Italy San Benedetto Hap 44
P23 G1C M10 D. invadens France Limeil-Brévannes Hap I7***
KF894343 ROY6 D. invadens Greece Crete Hap 67
MH830172 D. invadens United Kingdom Belford Hap 18
A21 G1B L32 D. invadens France Puteaux Hap I2
JN248300 D. invadens Italy Sardinia Hap 6
JN248299 D. invadens Italy Sardinia Hap 6
JN248298 D. invadens Italy Sardinia Hap 6
MH830218 D. invadens United Kingdom Cardiff Hap 64
MH830161 D. invadens United Kingdom Bath Hap 7
MH830186 D. invadens Italy Chioggia Hap 32
MH830212 D. invadens Italy Marina di Caulonia Hap 58
MH830205 D. invadens Italy Marina di Caulonia Hap 51
MH830201 D. invadens Italy Falcone Hap 47
MH830234 D. invadens Italy Santa Marina Salina Hap 82
MH830164 D. invadens Italy Monte Rivi Hap 10
MH830180 D. invadens Italy Monte Argentario Hap 26
MH830203 D. invadens Italy Caronia Hap 49
MH830167 D. invadens Italy Isola di Volcano Hap 13
MH830168 D. invadens Italy Lipari Hap 14
MH830163 D. invadens Italy Acquacalda Hap 9
MH830215 D. invadens Italy Acquacalda Hap 61
MH830220 D. invadens Italy Caronia Hap 66
MH830178 D. invadens Italy Porto Ercole Hap 24
MH830227 D. invadens New Zealand Grafton Hap 75
MH830175 D. invadens Italy Palazzolo Acreide Hap 21
MH830189 D. invadens Australia Armidale Hap 55
KX977425 D. invadens Australia Norfolk Island Hap 68
MH830222 D. invadens New Zealand Waihi Hap 70
MH830211 D. invadens Italy Alcara li Fusi Hap 57
MH830166 D. invadens Italy Alcara li Fusi Hap 12
MH830206 D. invadens Italy Crucoli Hap 52
MH830169 D. invadens Italy Rocche del Crasto Hap 15
MH830216 D. invadens Italy Falcone Hap 62
MH830233 D. invadens Italy Contrada Ridente Hap 81
MH830214 D. invadens Italy Palazzolo Acreide Hap 60
MH830202 D. invadens Italy Contrada Ridente Hap 48
MH830208 D. invadens Italy Pescara Hap 54
MH830176 D. invadens Italy Scalo dei Saraceni Hap 22
JN248301 D. invadens Germany Goerlitz Hap 3
P23 G4B M8 D. invadens France Cléguer Hap I4***
JN248303 D. invadens UK Bristol Hap 3
MH830236 D. invadens Germany Siegen Hap 84
KF894241 DF11 D. invadens UK Cambridgeshire Hap 3
KF894245 DN6 D. invadens UK Denbighshire Hap 3
P23 G1A M2 D. invadens France Paris Hap I4***
A22 G1A M24 D. invadens France Paris Hap I4
MH830238 D. invadens Italy Montecristo Hap 86
MH830188 D. invadens Germany Stuttgart Hap 34
MH830228 D. invadens United Kingdom Pontefract Hap 76
MH830210 D. invadens Italy Porto Ercole Hap 56
KX9774268 D. invadens Australia Norfolk Island Hap 3
JN248302 D. invadens Germany Senzig Hap 4
MH830219 D. invadens Germany Sangerhausen Hap 65
MH830190 D. invadens France Metz Hap 36
P22 G4B L20 D. invadens France Cléguer Hap I3
MH830217 D. invadens United Kingdom Portsmouth Hap 63
MH830229 D. invadens Italy Elba Lacona Hap 77
MH830230 D. invadens Austria Innsbruck Hap 78
MH830224 D. invadens New Zealand Wellington Hap 72
MH830179 D. invadens Italy Chiusa di Ginestreto Hap 25
MH830183 D. invadens Germany Koeln Hap 29
MH830171 D. invadens Germany Berlin Hap 17
MH830177 D. invadens Italy Stignano Hap 23
MH830162 D. invadens Italy Lake Garda Hap 8
MH830184 D. invadens United Kingdom Bicester Hap 30
MH830173 D. invadens Italy Tivoli Hap 19
MH830213 D. invadens isolate Italy Gioia Tauro Hap 59
MH830200 D. invadens Italy Gioia Tauro Hap 46
MH830207 D. invadens Italy Fragagnone Hap 53
JN248311 D. panormitanum Malta
JN248313 D. panormitanum Italy Sicily
P22 G1B L1 D. panormitanum France Puteaux Hap P1
JN248309 D. panormitanum Malta Gozo
JN248305 D. panormitanum Italy Sicily
P23 G2B L4 D. panormitanum France La Rochelle Hap P1***
P23 G2B L6 D. panormitanum France La Rochelle Hap P1***
A21 G1B L22 D. panormitanum France Puteaux Hap P1
A21 G1B L23 D. panormitanum France Puteaux Hap P1
A21 G1B L33 D. panormitanum France Puteaux Hap P1
A21 G1B L34 D. panormitanum France Puteaux Hap P1
A21 G1B L36 D. panormitanum France Puteaux Hap P1
A21 G2B L1 D. panormitanum France La Rochelle Hap P1
A21 G2B L25 D. panormitanum France La Rochelle Hap P1
A21 G2B L26 D. panormitanum France La Rochelle Hap P1
A21 G2B L3 D. panormitanum France La Rochelle Hap P1
A22 G2B M36 D. panormitanum France La Rochelle Hap P1
D808 D. panormitanum France, Vincennes Hap P1
JN248307 D. panormitanum Malta Gozo
JN248308 D. panormitanum Malta Gozo
JN248312 D. panormitanum Italy Sicily
P22 G1B L5 D. panormitanum France Puteaux Hap P1
JN248310 D. panormitanum Malta
JN248304 D. panormitanum Italy Sicily
KF894327 NF1 D. panormitanum UK Glamorganshire
JN248306 D. panormitanum Italy Sicily
JN248292 D. golcheri Malta
JN248293 D. golcheri Malta
JN248291 D. golcheri Malta
red haplogroup
blue haplogroup
D. invadens
D. panormitanum
0.02

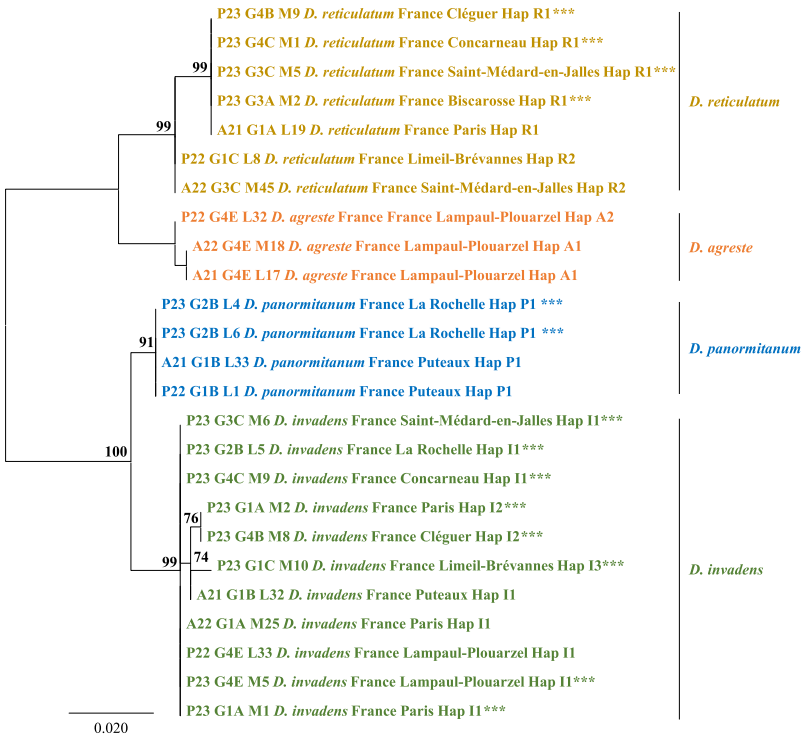


Fig. 6. Phylogenetic tree reconstructed by maximum likelihood based on the 16S rDNA fragment of the *Deroceras* samples from this study. Samples with the annotation *** were anatomically determined, and in these cases the species indicated corresponds to the anatomy. For the others, identification is based on the sequence. The origin of the samples is included. The tree is rooted on midpoint. Bootstrap values greater than 70 are indicated at the nodes. The scale bar represents the number of substitutions per site

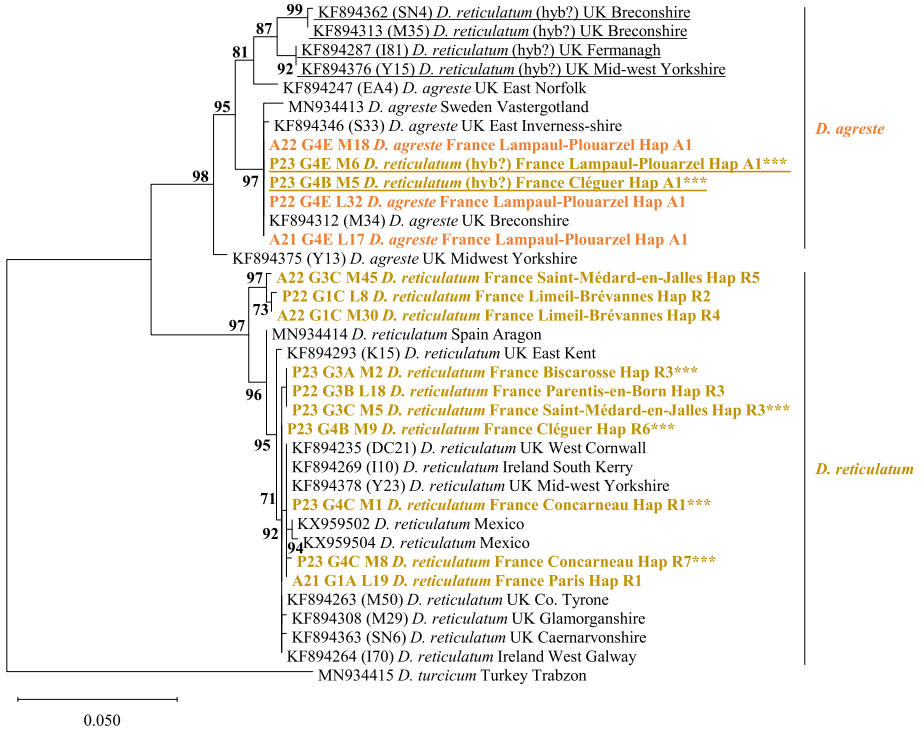


Fig. 7. Phylogenetic tree reconstructed by maximum likelihood based on the cytochrome c oxidase I gene fragment for *D. reticulatum* (in yellow) and *D. agreste* (in orange) samples from this study and from the literature, for which the Genbank accession numbers are given. Samples with the annotation *** were anatomically determined, and in this case the species indicated corresponds to the anatomy. For the others, identification is based on the sequence. Potential hybrids are marked "hyb?" and underlined. The tree is rooted with the sequence of *D. turcicum* (MN934414). Bootstrap values greater than 70 are indicated at the nodes. The scale bar represents the number of substitutions per site

Eleven 16S sequences were obtained for individuals with COI sequences of *D. invadens*. They were represented by three haplotypes (16S-Hap I1-I3) and formed a distinct clade in the ML tree (Fig. 6).

DEROCERAS RETICULATUM AND *D. AGRESTE*

Of the seven specimens of these two species examined, all had the diagnostic anatomical characteristics of *D. reticulatum*, that is penial glands consisting of a few branches; none of the specimens examined had the diagnostic characteristics of *D. agreste* (Table 1).

COI sequences were obtained from 15 specimens. Ten individuals, including five from the seven specimens anatomically identified as *D. reticulatum*, were placed either within, or basally to, the *D. reticulatum* clade formed by the reference sequences (Fig. 7). Seven COI haplotypes were found (COI-Hap R1-R7, Table 1). The other two individuals anatomically identified as *D. reticulatum* (P23 G4B M5 and P23 G4E M6) and three other individuals without anatomical identification (i.e. not preserved for this purpose) were placed in a clade including reference sequences affiliated to *D. agreste* from the UK and Sweden and were represented by only one haplotype (COI-Hap A1, Table 1). The same result was obtained when extraction, amplification and sequencing procedures were repeated a second time independently to rule out potential contamination.

Seven 16S sequences were obtained for specimens with COI sequences of *D. reticulatum*, represented by two haplotypes (16S-Hap R1 and R2, Table 1), and three 16S sequences were obtained for specimens with COI sequences of *D. agreste*, represented by two haplotypes (16S-Hap A1 and A2, Table 1). Phylogenetic results confirm that French samples belong to two distinct clades (Fig. 6). Unfortunately, the specimens with incongruent morpho-molecular results (P23 G4B M5 and P23 G4E M6) did not yield 16S sequences.

DISCUSSION

In this study, we sought to distinguish the *Deroceras* species present in ten urban gardens and green spaces in mainland France, using the mitochondrial COI and 16S ribosomal RNA genes, and genital anatomy of preserved specimens. Additionally, we confirmed the presence of *D. invadens* from 14 further sites around France. The presence of *D. panormitanum*, the true Sicilian slug, was confirmed both anatomically and molecularly at three sites: Puteaux and Vincennes, both in the suburbs of Paris, and in La Rochelle, on the Atlantic coast. *Deroceras panormitanum* was found in plant beds at the edge of an avenue, under dead leaves or in a mulch substrate in Puteaux, and in flower beds in the large public garden Parc Floral de Paris in Vincennes. In La Rochelle, samples were collected in a private garden, with most found under tiles laid on the ground.

For this species only one haplotype was found for each gene, and the COI sequence grouped with those of *D. panormitanum* from France, Sicily, Malta and the UK. These results are consistent with the post-2014 records of this species in Europe outside its native range (Rowson et al. 2014, 2016, Hutchinson et al. 2014). Indeed, genetic bottlenecks have been observed following introductions and can be explained by the establishment of new populations by a few founding individuals (founder effect) (Dlugosch & Parker 2008).

The presence of *D. invadens*, the tramp slug, was molecularly confirmed at nine of the ten sites sampled by MV, SN, NM, LD and VR. This species was one of the most common and abundant in our samples. It also showed great haplotypic richness for the COI gene, with seven haplotypes found in this study. Most of these haplotypes belong to the red and blue haplogroups defined by Hutchinson et al. (2020). The most common haplotype in our study (COI-Hap I1 = haplotype 0 of the red haplogroup, found in 16/25 *D. invadens* specimens) is also the most common in the study by Hutchinson et al. (2020), being observed in 39% of their sites. The second most common haplotype in our study (COI-Hap I4 = haplotype 3 of the blue haplogroup, found in 3/25 specimens) is found in 18% of the sites in the Hutchinson et al. (2020) study. The blue haplogroup was identified in our study from further west (Paris and Brittany) than it had so far been found in France. Previous studies have shown star-shaped genealogies for the two haplogroups, suggesting rapid population expansion. Indeed, the colonisation of Europe by *D. invadens* may have occurred in two steps from southern Italy (Hutchinson et al. 2020). It has been proposed that the red haplogroup first colonised Great Britain and France and then spread to Spain, Central Europe and northern Italy. The blue haplogroup may have spread to northern Italy, then colonised Central Europe, Britain, France, Spain and Portugal (Hutchinson et al. 2020).

Deroceras panormitanum and *D. invadens* were found together at Puteaux, Vincennes and La Rochelle. Previously, *D. panormitanum* and *D. invadens* had been found together only in a single garden in Bordighera, Italy (Hutchinson et al. 2014) and at Kilmacanoge, Ireland (Rowson et al. 2016). In matings between the species in the laboratory, Reise et al. (2011) noted that *D. invadens* was never observed to trans-

fer sperm to *D. panormitanum*, while the reverse occurred repeatedly. It would be worthwhile to monitor *D. panormitanum* populations in France, to confirm whether both species continue to coexist at a site, or whether one outcompetes the other, or whether they introgress.

Our study confirms that presently *D. invadens* is much commoner in France than *D. panormitanum*, but nevertheless the latter is common enough that anatomical determination should be required before reporting the presence of *D. invadens*. In all, at 23 sites we confirmed the presence of *D. invadens* (i.e. we distinguished it from *D. panormitanum*) to which we can add the 12 samples from France listed in HUTCHINSON et al. (2020). The three sites where *D. panormitanum* were also found represents 8% of those sites. Routine anatomical identifications of *D. invadens* is also likely to detect further occurrences of *D. sturanyi*, another introduced species that is externally indistinguishable.

The grey field slug *D. reticulatum* was identified at seven of the ten sites sampled by MV, SN, NM, LD and VR, while only at the Lampaul-Plouarzel site in Brittany was there evidence of the field slug *D. agreste*, and that was based only on COI sequences. The pattern found in France for these two species is similar to what was observed in the UK (ROWSON et al. 2014) since all individuals with a *D. reticulatum* mitochondrial sequence had *D. reticulatum* anatomy, but some individuals with *D. reticulatum* anatomy had a *D. agreste* mtDNA sequence. Potentially, contamination during the molecular analysis might explain this pattern, but an independent repetition unaccompanied by samples from *D. agreste* has ruled out this explanation. The most probable explanation for such a pattern of discordance between morphological and molecular data is that these specimens are introgressed interspecific hybrids whose mitochondrial DNA was inherited from a *D. agreste* ancestor within the maternal line. An alternative explanation is incomplete lineage sorting. However, rigorously distinguishing introgression from incomplete lineage sorting would require sequencing nuclear loci from a representative range of individuals of both species, which is beyond the scope of this work. ROWSON et al. (2014) mentioned four individuals which displayed the characteristic genital morphology of *D. reticulatum* but had COI sequences characteristic of *D. agreste*. Since the reverse case was never found, these results were explained as an example of one-way introgression of *D. reticulatum* genes into *D. agreste* populations.

In conclusion, this study revealed the presence of the true Sicilian slug, *D. panormitanum*, and three other *Deroceras* species in French urban gardens and green spaces. Using anatomical and mitochondrial data, our analysis suggested that some specimens were hybrids between D. *reticulatum* and *D. agreste*. This result deserves further investigation with more specimens adequately preserved to study anatomy and additional molecular markers, including nuclear markers (e.g. microsatellites, HUTCHINSON et al. 2021). This study highlights the need for population monitoring of species of which introductions are already recurrent or are likely to be in the future, in order to understand their dynamics and potential impacts.

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