

# First report of the exotic blue land planarian, *Caenoplana coerulea* (Platyhelminthes, Geoplanidae), on Menorca (Balearic Islands, Spain)

Karin Breugelmans<sup>1</sup>, Josep Quintana Cardona<sup>2</sup>, Tom Artois<sup>3</sup>, Kurt Jordaens<sup>4,5</sup>,  
Thierry Backeljau<sup>1,5</sup>

**1** Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium **2** Institut Catala de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, edifici ICP Campus de la UAB, s/n 08193 Cerdanyola del Vallès, Barcelona, Spain **3** Research Group Zoology: Biodiversity & Toxicology, Centre for Environmental Sciences, Hasselt University, Campus Diepenbeek, Agoralaan Building D, B-3590 Diepenbeek, Belgium **4** Joint Experimental Molecular Unit, Royal Museum for Central Africa, Leuvensesteenweg 13, B-3080 Tervuren, Belgium **5** Evolutionary Ecology Group, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium

Corresponding author: *Thierry Backeljau* (Thierry.Backeljau@naturalsciences.be)

---

Academic editor: *David Gibson* | Received 10 April 2012 | Accepted 15 May 2012 | Published 4 June 2012

---

**Citation:** Breugelmans K, Cardona JQ, Artois T, Jordaens K, Backeljau T (2012) First report of the exotic blue land planarian, *Caenoplana coerulea* (Platyhelminthes, Geoplanidae), on Menorca (Balearic Islands, Spain). ZooKeys 199: 91–105. doi: 10.3897/zookeys.199.3215

---

## Abstract

In April 2009 two specimens of a terrestrial flatworm were collected from under a rock in an orchard at Ciutadella de Menorca on the easternmost Balearic island of Menorca (Spain). Their external morphology suggested that both specimens belonged to the invasive blue planarian *Caenoplana coerulea*, a species which is native to eastern Australia. Sequence data of a fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) and of the entire 18S ribosomal RNA confirm its identification. This is one of the first records of the species in Europe where it has only been found in one locality in the United Kingdom, France and NE Spain.

## Keywords

Terrestrial flatworm, 18S rDNA, COI, introduction, molecular identification, Balearic Islands, Spain, Europe

## Introduction

Several species of terrestrial planarian are known as invasive, exotic species in soils of the northern hemisphere. For instance, in North America and the British Isles about a dozen species of exotic terrestrial planarians have been introduced (Jones 1988; Jones and Boag 1996; Ogren and Kawakatsu 1998). Many of these introduced exotic species are predators of earthworms, isopods and snails (e.g. Ogren 1995; Fiore et al. 2004; Sugiura et al. 2006; Iwai et al. 2010; Sugiura 2010). As such, these flatworms may pose a threat to local biodiversity (Santoro and Jones 2001). Because of this, and in view of their rapid dispersal as well as their wide distribution, these introduced exotic terrestrial flatworms are of serious agricultural and nature conservation concern.

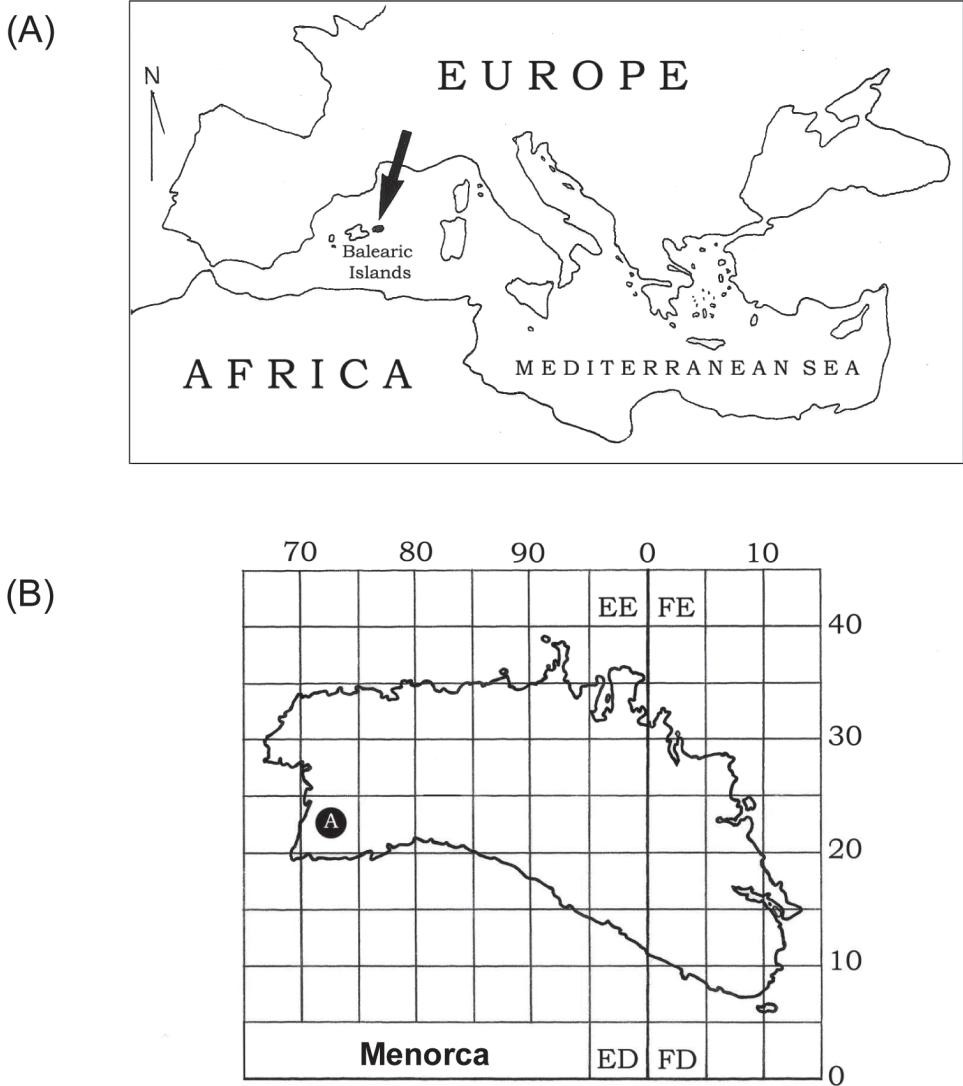
The impacts of introduced exotic terrestrial flatworms may be especially detrimental in islands and archipelagos that support an endemic invertebrate fauna. This is illustrated by the terrestrial flatworm *Platydemus manokwari* De Beauchamp, 1962, which has been introduced in many Pacific islands (e.g. Eldredge and Smith 1995) and is considered a cause of the rapid decline of endemic land snails on these islands (Chiba 2003; Okochi et al. 2004; Ohbayashi et al. 2005; Sugiura et al. 2006; Sugiura 2009; Sugiura and Yamaura 2010). Therefore the species is of serious concern in the conservation of the unique land snail fauna of archipelagos and therefore has been included in the list of the world's 100 worst invasive alien species (see [http://www.issg.org/worst100\\_species.html](http://www.issg.org/worst100_species.html), Lowe et al. 2000). Hence, in order to develop strategies to reduce further spread and to control their impacts on local invertebrates, rapid and accurate identifications of exotic terrestrial flatworms are essential.

Against this background, we here report for the first time the occurrence of the invasive blue land planarian *Caenoplana coerulea* Moseley, 1877 in the Balearic Islands (Menorca, Spain). Its identification was confirmed by DNA sequence analysis of the entire nuclear 18S ribosomal RNA (18S rDNA) gene and of a portion of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene.

## Materials and methods

In April 2009 two specimens of a terrestrial flatworm were collected by hand under a rock in an orchard at Ciutadella de Menorca on the easternmost Balearic island of Menorca (Spain, 39°57'00"N, 03°51'00"E; Figures 1 and 2). Both specimens (labelled '1957' and '1958') were stored in 100% ethanol.

Genomic DNA was extracted using the NucleoSpin® Tissue Kit (Machery-Nagel, Düren, Germany). A 424 bp fragment of the COI gene was amplified using the primer pair flatCOIL and flatCOIH (modified from Bessho et al. 1997; Table 1). PCR was performed in a total volume of 25 µl, containing 2 µl of DNA and 0.2 µM of each primer, and using the Qiagen® Multiplex PCR Kit with HotStarTaq® DNA polymerase and a final concentration of 3 mM MgCl<sub>2</sub>. The PCR profile was 15 min at 95 °C followed by 35 cycles of 45 s at 95 °C, 45 s at 50 °C and 1 min at 72 °C, and with a final



**Figure 1.** (A) Location of the Balearic Islands in the Mediterranean Sea. Menorca is in black and indicated by an arrow. (B) Detailed map of Menorca: the locality where *Caenoplana coerulea* was found is indicated with the letter A.

extension step of 10 min at 72 °C. The entire 18S rDNA gene was amplified using the primer pair 4F18S and 16R18S (Winnepenninckx et al. 1994, Table 1). PCR was performed in a total volume of 25 µl containing 2 µl of DNA, 0.2 µM of each primer, 200 µM of each dNTP, 0.62 units of Taq DNA polymerase (Qiagen) and mQ-H<sub>2</sub>O. Triclad flatworms are known to have two types of 18S rDNA genes (Type I and II) (Carranza et al. 1996, 1999). Therefore, 18S rDNA PCR products were cloned using



**Figure 2.** One of the two specimens of *Caenoplana coerulea* collected on Menoca.

**Table 1.** Forward (F) and reverse (R) primers used for amplification and sequencing of the mitochondrial cytochrome *c* oxidase subunit I (COI) and the nuclear 18S ribosomal RNA (18S rDNA) genes of the two *Caenoplana* specimens in this study.

| Name        | Sequence 5'-3'            | Source                             |
|-------------|---------------------------|------------------------------------|
| COI:        |                           |                                    |
| F: flatCOIL | GCAGTTTTTGGTTTTTTGGACATCC | modified from Bessho et al. (1997) |
| R: flatCOIH | GAGCAACAACATAATAAGTATCATG | modified from Bessho et al. (1997) |
| 18S rDNA:   |                           |                                    |
| F: 4F18s    | CTGGTTGATYCTGCCAGT        | Winnepenninckx et al. (1994)       |
| R: 10R18S   | TTGGYRAATGCTTTTCGC        | Winnepenninckx et al. (1994)       |
| F: 9F18S    | CGCGGTAATTCCAGCTCCA       | Winnepenninckx et al. (1994)       |
| R: 3R18S    | GACGGGCGGTGTGTRC          | Winnepenninckx et al. (1994)       |
| F: 14F18S   | ATAACAGGTCTGTGATGCC       | Winnepenninckx et al. (1994)       |
| R: 16R18S   | CYGCAGGTTACCTACRG         | Winnepenninckx et al. (1994)       |

TOPO TA Cloning® Kit for Sequencing (Invitrogen) following the suppliers' instructions. Fifteen colonies of each specimen were amplified as described above.

All PCR products were purified using NucleoFast 96 PCR plates (Macherey-Nagel, Düren, Germany) and bidirectionally sequenced using the BigDye Terminator

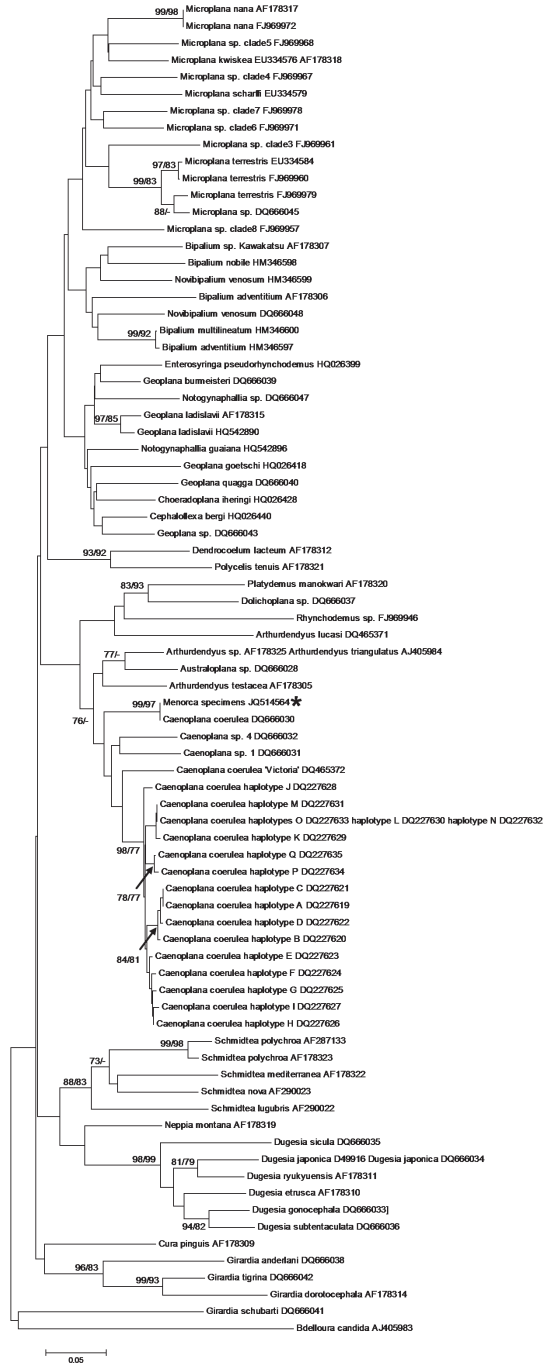
v1.1 chemistry on an ABI 3130xl automated capillary DNA sequencer (Life Technologies). For the sequencing of 18S rDNA several internal primers were used (Table 1). Sequences were visually inspected and aligned in SeqScape v2.5 (Life Technologies). COI and 18S rDNA sequences from other flatworm species of the Continenticola (see e.g. Álvarez-Presas et al. 2008, Sluys et al. 2009) were imported from GenBank (See Appendix). Sequence data sets were aligned in MAFFT v6.861 (Kato and Toh 2008) and trimmed at 296 bp for the COI and at 1765 bp for the 18S rDNA fragment. From the Menorca specimens only 18S rDNA clones that yielded sequences without ambiguous positions were retained for further analyses.

Two tree reconstruction methods were implemented: Neighbor-Joining (NJ) (Saitou and Nei 1987) and Maximum Likelihood (ML). The most appropriate nucleotide substitution models for ML were selected using JMODELTEST v0.1.1 (Posada 2008). These were the GTR+G model for the COI fragment and the GTR+I+G model for the 18S rDNA fragment. NJ trees were made in MEGA v5.0 (Tamura et al. 2007) using K2P distances and with complete deletion of indels. ML trees were made in PAUP\* v4.0b10 (Swofford 2002) using a heuristic search with the tree-bisection-reconnection branch-swapping algorithm and random addition of taxa. Trees were rooted with *Bdelloura candida* (Girard, 1850) (Maricola, family Bdellouridae). Branch support was assessed via nonparametric bootstrapping using 1000 bootstrap replicates for NJ or 200 bootstrap replicates for ML (Felsenstein 1985). Only nodes with bootstrap values of  $\geq 70\%$  were retained and considered meaningful (Hillis and Bull 1993). P-distances were calculated with MEGA v5.0.

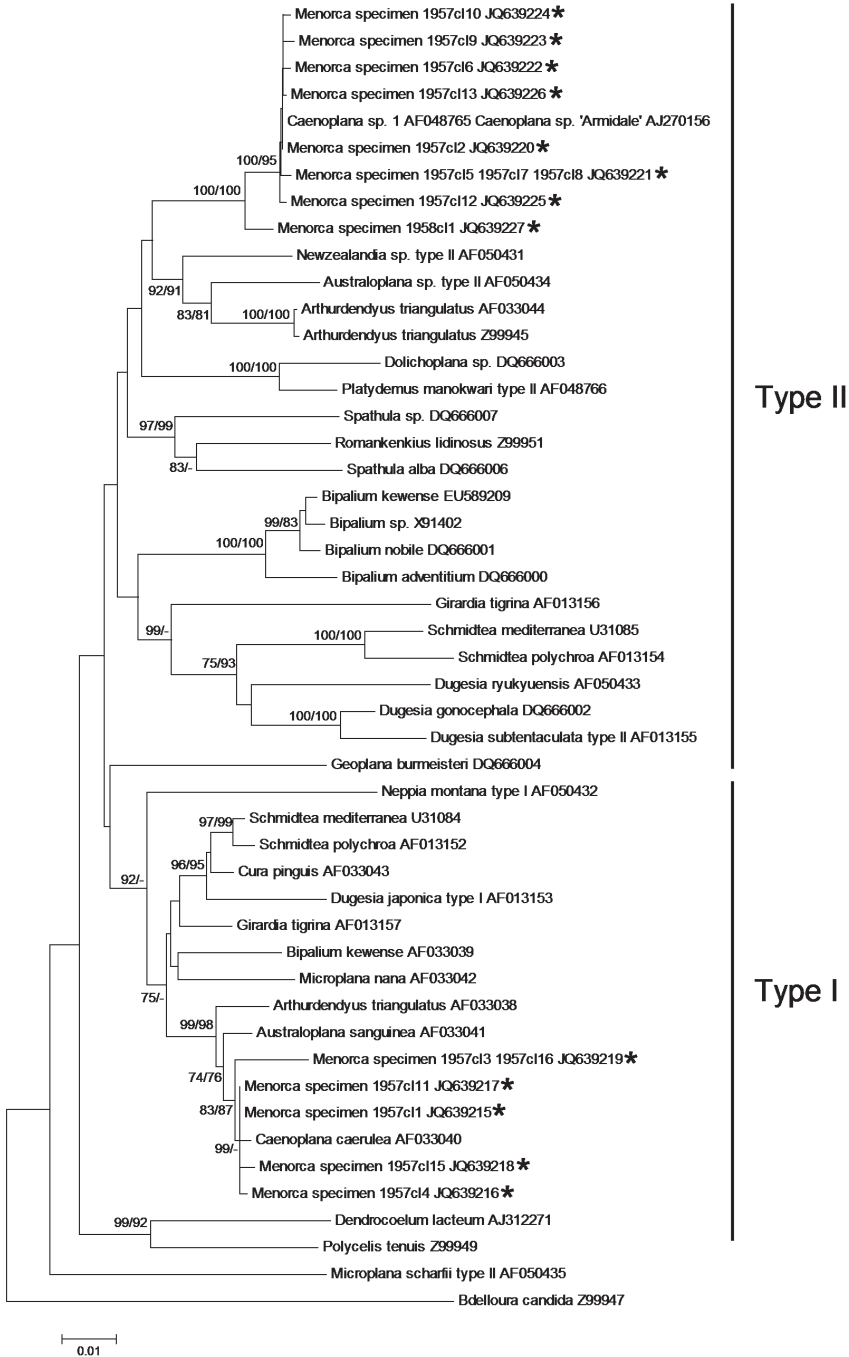
Both specimens have been deposited in the collections of the Royal Belgian Institute of Natural Sciences, Brussels, under catalogue number IG.32062. DNA sequences have been deposited in GenBank under accession numbers JQ639215–JQ639227 (for 18S rDNA) and JQ514564 (for COI).

## Results and discussion

The dorsal dark blue ground-colour with a thin median dorsal stripe, the intense blue colour of the ventral side, and eyes that are arranged in a single row around the anterior tip and which do not extend dorsally, suggest that the two specimens belong to the species of blue land planarian, *Caenoplana coerulea* Moseley, 1877 (Geoplanidae). This is corroborated by our phylogenetic analysis of the COI and 18S rDNA genes. Both individuals had the same COI haplotype; as in other triclads, there were two different intra-individual types of 18S rDNA (Carranza et al. 1996, 1999). We found five type I and eight type II 18S rDNA variants. Figures 3–4 show the phylogenetic trees inferred from the COI and 18S rDNA data, respectively. The COI haplotype of the Menorcan specimens clustered with strong support with a haplotype of *C. coerulea* from the UK (GenBank accession number DQ666030), from which it differed by only one, ambiguous position (i.e. a G for DQ666030, while 'N' for the Menorcan haplotype). The mean P-distance between the COI haplotype from Menorca and the other *C. coerulea* haplo-



**Figure 3.** Neighbor-Joining and ML tree of the 296 bp dataset of the mitochondrial cytochrome *c* oxidase subunit I gene (COI). The haplotype of the Menorcan specimens is indicated with an asterisk. Bootstrap values  $\geq 70\%$  for the NJ and ML trees are given as NJ/ML and are shown at the nodes. – indicates that the node was not supported by the analysis.



**Figure 4.** Neighbor-Joining and ML tree of the 1765 bp dataset of the nuclear 18S rDNA gene. The clones (cl) of the Menorcan specimens '1957' and '1958' are indicated with an asterisk. Bootstrap values  $\geq 70\%$  for the NJ and ML trees are given as NJ/ML and are shown at the nodes. – indicates that the node was not supported by the analysis. Note that the clades of the type I and type II 18S rRNA variants are not supported.

types was  $0.10 \pm 0.02$ , whereas the P-distance with other Geoplanid species was higher ( $0.16 \pm 0.03$ ) and comparable to what we found among Geoplanidae taxa ( $0.17 \pm 0.03$ ). The 18S rDNA type I sequences from the Menorcan specimens formed a strongly supported clade with *C. coerulea* AF033040 (from the UK) (mean P-distance:  $0.008 \pm 0.002$ ), whereas those of 18S rDNA type II formed a strongly supported clade with *Caenoplana* sp.1 AF048765 (unknown origin) and *Caenoplana* sp. 'Armidade' AJ270156 (from Australia) (mean P-distance:  $0.003 \pm 0.001$ ). The mean P-distance between the Menorcan type I and type II sequences and sequences from the other geoplanid species was substantially higher, viz.  $0.019 \pm 0.003$  and  $0.058 \pm 0.005$ , respectively.

*Caenoplana coerulea* is native to eastern Australia but, as a result of human activities, it has been introduced to New Zealand, the United States, the United Kingdom, Norfolk Island (Australia), and France (Ogren 1989; Winsor 1998; Jones 1998, 2005), and more recently in Argentina (Luis-Negrete et al. 2011) and NE Spain (Mateos et al. 2012). After introduction, the species may expand its range rapidly. For example, since its accidental introduction into the USA prior to 1943, it has spread rapidly over a large part of the country (California: 1943, Florida: 1961, Georgia: 1972, Texas: 1978, Iowa: 1999, North Carolina: 2001) (Ogren 2001). Whether this fast expansion is due to its high intrinsic dispersal capacity or due to repeated, independent introductions, is unknown.

In the Iberian Peninsula and Balearic Islands, at present ten autochthonous species of the family Geoplanidae have been reported (Mateos et al. 1998, 2009; Vila-Farré et al. 2008, 2011). In addition, two introduced species, *Bipalium kewense* Moseley, 1878 (Bipaliidae; recorded from Barcelona) (Filella-Subirá 1983) and *Platydemus* sp. (Geoplanidae; recorded from Benamargosa, Málaga) (Vila-Farré et al. 2011), have been reported from the Iberian Peninsula but not from the Balearic Islands where only *Microplana terrestris* (O.F. Müller, 1774) (Geoplanidae) has been found (Minelli 1977). Hence, the present record of two specimens of *C. coerulea* implies the first introduced species of Geoplanidae in the Balearic Islands. Very recently, the species was also found on the Iberian Peninsula (La Garrotxa, Girona province) (Mateos et al. 2012). Also, pictures of the species that were taken in Spain (Boadilla del Monte, October 2010 and Girona, 22 December 2011) can be found at <http://www.flickr.com/photos/51708886@N03/6351086047/> and <http://www.biodiversidadvirtual.org/insectarium/Caenoplana-coerulea-img293381.html>, respectively. In Europe, the species is further only known from a hothouse in Liverpool (Jones 1998, 2005) and one locality in France (Ogren 1989; Winsor 1998; Winsor et al. 2004).

We do not know when exactly this exotic species arrived in the Balearic Islands. The first specimens of *C. coerulea* were found in an orchard in April 2009. In 2011 the species had spread to a nearby garden, where it was found at shaded places. As is the case in other land planarians, its spread and distribution in newly colonized areas is probably mainly determined by moisture (Fraser and Boag 1998). Even in its native region (Australia), the distribution of *C. coerulea* is restricted to areas with a high humidity (Luis-Negrete et al. 2011). Even though the impact of *C. coerulea* on earthworm and terrestrial gastropod populations is not known, the species is at least reported to



feed on isopods, diplopods, earwigs, and snails (Olewine 1972; Barnwell 1978; Terrace and Baker 1994; Jones 2005). Its broad food spectrum might facilitate the establishment and possible spread of the species in Spain and, eventually, elsewhere in Europe.

## Acknowledgements

We would like to thank Dr. Leigh Winsor (Condon, Australia) for providing part of the literature and for information on the distribution of *C. coerulea* and two anonymous referees for their valuable comments. This work was supported by FWO grant G.0208.08N and the Belgian Network for DNA Barcoding (FWO contract number W0.009.11N).

## References

- Álvarez-Presas M, Baguña J, Riutort M (2008) Molecular phylogeny of land and freshwater planarians (Tricladida, Platyhelminthes): From freshwater to land and back. *Molecular Phylogenetics and Evolution* 47: 555–568. doi: 10.1016/j.ympev.2008.01.032
- Barnwell GM (1978) *Geoplana vaga*: a sexually-reproducing terrestrial planarian in San Antonio. *Southwestern Naturalist* 23: 151–152. doi: 10.2307/3669990
- Bessho Y, Tamura S, Hori H, Tanaka H, Ohama T, Osawa S (1997) Planarian mitochondria sequence heterogeneity: relationships between the type of cytochrome *c* oxidase subunit I gene sequence, karyotype and genital organ. *Molecular Ecology* 6: 129–136. doi: 10.1046/j.1365-294X.1997.00162.x
- Carranza S, Baguña J, Riutort M (1999) Origin and evolution of paralogous rRNA gene clusters within the flatworm family Dugesidae (Platyhelminthes, Tricladida). *Journal of Molecular Evolution* 49: 250–259. doi: 10.1007/PL00006547
- Carranza S, Giribet G, Ribera C, Baguña J, Riutort M (1996) Evidence that two types of 18S rDNA coexist in the genome of *Dugesia (Schmidtea) mediterranea* (Platyhelminthes, Turbellaria, Tricladida). *Molecular Biology and Evolution* 13: 824–832. doi: 10.1093/oxfordjournals.molbev.a025643
- Chiba S (2003) Species diversity and conservation of *Mandarina*, an endemic land snail of the Ogasawara Islands. *Global Environmental Research* 7: 29–37.
- Eldredge LG, Smith BD (1995) Triclad flatworm tours the pacific. *Aliens* 2: 11.
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791. doi: 10.2307/2408678
- Filella-Subirá E (1983) Nota sobre la presència de la planària terrestre *Bipalium kewense* Mosley [sic], 1878 a Catalunya. *Butlletí de la Institució Catalana d'Història Natural* 49: 151.
- Fiore C, Tull JL, Zehner S, Ducey PK (2004) Tracking and predation on earthworms by the invasive terrestrial planarian *Bipalium adventitium* (Tricladida, Platyhelminthes). *Behavioural Processes* 67: 327–334.
- Fraser PM, Boag B (1998) The distribution of lumbricid earthworm communities in relation to flatworms: a comparison between New Zealand and Europe. *Pedobiologia* 42: 542–553.

- Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192.
- Iwai N, Sugiura S, Chiba S (2010) Predation impacts of the invasive flatworm *Platydemus manokwari* on eggs and hatchlings of land snails. *Journal of Molluscan Studies* 76: 275–278. doi: 10.1093/mollus/eyq007
- Jones HD (1988) The status and distribution of British terrestrial planarians. *Progress in Zoology* 36: 511–516.
- Jones HD (1998) The African and European land planarian faunas, with an identification guide for field workers in Europe. *Pedobiologia* 42: 477–489.
- Jones HD (2005) British land flatworms. *British Wildlife* 16: 189–194.
- Jones HD, Boag B (1996) The distribution of New Zealand and Australian terrestrial flatworms (Platyhelminthes: Turbellaria: Tricladida: Terricola) in the British Isles – the Scottish survey and MEGALAB WORMS. *Journal of Natural History* 30: 955–975. doi: 10.1080/00222939600770511
- Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298. doi: 10.1093/bib/bbn013
- Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the world's worst invasive alien species: a selection from the global invasive species database. The Invasive Species Specialist Group (ISSG), a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), Auckland, Australia.
- Luis-Negrete LH, Brusa F, Winsor L (2011) The blue land planarian *Caenoplana coerulea*, an invader in Argentina. *Revista Mexicana de Biodiversidad* 82: 287–291.
- Mateos E, Giribet G, Carranza S (1998) Terrestrial planarians (Platyhelminthes, Tricladida, Terricola) from the Iberian Peninsula: first records of the family Rhynchodemidae, with the description of a new *Microplana* species. *Contributions to Zoology* 67: 267–276. doi: 10.1111/j.1463-6409.2009.00398.x
- Mateos E, Cabrera C, Carranza S, Riutort M (2009) Molecular analysis of the diversity of terrestrial planarians (Platyhelminthes, Tricladida, Continenticola) in the Iberian Peninsula. *Zoologica Scripta* 38: 637–649.
- Mateos E, Tudó A, Álvarez-Presas M, Riutort M (2012) Una nova invasió biològica a la Garrotxa. VI Seminari de Patrimoni Natural de la comarca del la Garrotxa, Institució Catalana d'Història Natural (Olot, Girona): 12.
- Minelli A (1977) A taxonomic review of the terrestrial planarians of Europe. *Bollettino di Zoologia* 44: 399–419. doi: 10.1080/11250007709429278
- Ogren RE (1989) Redescription and a new name for the blue land planarian *Geoplana vaga* Hyman now considered conspecific with *Caenoplana coerulea* Moseley from Australia (Turbellaria: Tricladida: Geoplanidae). *Journal of the Pennsylvania Academy of Sciences* 63: 135–142.
- Ogren RE (1995) Predation behavior of land planarians. *Hydrobiologia* 305: 105–111. doi: 10.1007/BF00036370
- Ogren RE (2001) The Blue planarian: A new locality. <http://course.wilkes.edu/REOgren/discuss/msgReader195722?d=6&m=8&amp;mode=topic&y=2005>
- Ogren RE, Kawakatsu M (1998) American Nearctic and Neotropical planarian (Tricladida: Terricola) faunas. *Pedobiologia* 42, 441–451.

- Ohbayashi T, Okochi I, Sato H, Ono T (2005) Food habit of *Platydemus manokwari* De Beauchamp, 1962 (Tricladida: Terricola: Rhynchodemidae), known as a predatory flatworm of land snails in the Ogasawara (Bonin) Islands, Japan. *Applied Entomology and Zoology* 40: 609–614. doi: 10.1303/aez.2005.609
- Okochi I, Sato H, Ohbayashi T (2004) The cause of mollusk decline on the Ogasawara Islands. *Biodiversity and Conservation* 13: 1465–1475. doi: 10.1023/B:BIOC.0000021334.39072.2d
- Olewine DA (1972) Further observations in Georgia on the land planarians, *Bipalium kewense* and *Geoplana vaga* (Turbellaria: Tricladida: Terricola). *Association of Southeastern Biologists Bulletin* 19: 88.
- Posada D (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256. doi: 10.1093/molbev/msn083
- Saitou N, Nei M (1987) The Neighbor-Joining method – a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425.
- Santoro G, Jones HD (2001) Comparison of the earthworm population of a garden infested with the Australian land flatworm (*Australoplana sanguinea alba*) with that of a non-infested garden. *Pedobiologia* 45, 313–328. doi: 10.1078/0031-4056-00089
- Sluys R, Kawakatsu M, Riutort M, Baguña J (2009) A new higher classification of planarian flatworms (Platyhelminthes, Tricladida). *Journal of Natural History* 43: 1763–1777. doi: 10.1080/00222930902741669
- Sugiura S (2009) Seasonal fluctuations of invasive flatworm predation pressure on land snails: Implications for the range expansion and impacts of invasive species. *Biological Conservation* 142: 3013–3019. doi: 10.1016/j.biocon.2009.07.032
- Sugiura S (2010) Prey preference and gregarious attacks by the invasive flatworm *Platydemus manokwari*. *Biological Invasions* 12: 1499–1507. doi: 10.1007/s10530-009-9562-9
- Sugiura S, Yamaura Y (2010) Potential impacts of the invasive flatworm *Platydemus manokwari* on arboreal snails. *Biological Invasions* 11: 737–742. doi: 10.1007/s10530-008-9287-1
- Sugiura S, Okochi I, Tamada H (2006) High predation pressure by an introduced flatworm on land snails on the oceanic Ogasawara Islands. *Biotropica* 38: 700–703. doi: 10.1111/j.1744-7429.2006.00196.x
- Swofford DL (2002) PAUP\* Phylogenetic analysis using parsimony (\*and other methods) Version 4b10. Sinauer Associates, Sunderland, Massachusetts.
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596–1599. doi: 10.1093/molbev/msm092
- Terrace TE, Baker GH (1994) The blue land planarian, *Caenoplana coerulea* Moseley (Tricladida: Geoplanidae), a predator of *Ommatoiulus moreleti* (Lucas) (Diplopoda: Julidae) in Southern Australia. *Journal of the Entomological Society of Australia* 33: 371–372. doi: 10.1111/j.1440-6055.1994.tb01250.x
- Vila-Farré M, Mateos E, Sluys R, Romero R (2008) Terrestrial planarians (Platyhelminthes, Tricladida, Terricola) from the Iberian Peninsula: new records and description of three new species. *Zootaxa* 1739: 1–20.
- Vila-Farré M, Sluys R, Mateos E, Jones HD, Romero R (2011) Land planarians (Platyhelminthes: Tricladida: Geoplanidae) from the Iberian Peninsula: new records and description

- of two new species, with a discussion on ecology. *Journal of Natural History* 45: 869–891. doi: 10.1080/00222933.2010.536267
- Winnepenninckx B, Backeljau T, De Wachter R (1994) Small ribosomal-subunit RNA and the phylogeny of the Mollusca. *The Nautilus* 108: 98–110.
- Winsor L (1998) The Australian terrestrial flatworm fauna (Tricladida: Terricola). *Pedobiologia* 42: 457–463.
- Winsor L, Johns PM, Barker GM (2004) Terrestrial planarians (Platyhelminthes: Tricladida: Terricola) predaceous on terrestrial gastropods. In: Barker GM (Ed.) *Natural Enemies of Terrestrial Molluscs*. CAB International, London, 227–278. doi: 10.1079/9780851993195.0227

## Appendix

List of samples used in this study with GenBank accession numbers and sampling locality (if known). The classification follows Sluys et al. (2009).

| Species                         | 18S rDNA |           | COI      | Sampling locality   |
|---------------------------------|----------|-----------|----------|---------------------|
|                                 | Type I   | Type II   |          |                     |
| <b>Maricola</b>                 |          |           |          |                     |
| <b>Family Bdellouridae</b>      |          |           |          |                     |
| Subfamily Bdellourinae          |          |           |          |                     |
| <i>Bdelloura candida</i>        | Z99947   |           | AJ405983 |                     |
| <b>Continenticola</b>           |          |           |          |                     |
| <b>Family Planariidae</b>       |          |           |          |                     |
| <i>Polycelis tenuis</i>         | Z99949   |           | AF178321 | Spain               |
| <b>Family Dendrocoelidae</b>    |          |           |          |                     |
| <i>Dendrocoelum lacteum</i>     | AJ312271 |           | AF178312 | France              |
| <b>Family Dugesiidae</b>        |          |           |          |                     |
| <i>Cura pinguis</i>             | AF033043 |           | AF178309 | New Zealand         |
| <i>Dugesia etrusca</i>          |          |           | AF178310 | Italy               |
| <i>Dugesia gonocephala</i>      |          | DQ666002  | DQ666033 | The Netherlands     |
| <i>Dugesia japonica</i>         | AF013153 | D83382    | DQ666034 | Japan               |
|                                 | D49916   |           |          |                     |
| <i>Dugesia ryukyensis</i>       |          | AF050433  | AF178311 | Japan               |
| <i>Dugesia sicula</i>           |          |           | DQ666035 | Spain               |
| <i>Dugesia subtentaculata</i>   |          | AF013155  | DQ666036 | Spain               |
| <i>Girardia anderalani</i>      |          | DQ666013  | DQ666038 | Brasil              |
| <i>Girardia dorotocephala</i>   |          |           | AF178314 | USA                 |
| <i>Girardia schubarti</i>       |          | DQ666015  | DQ666041 | Brasil              |
| <i>Girardia tigrina</i>         | AF013157 | AF013156  | DQ666042 | France              |
| <i>Neppia montana</i>           | AF050432 |           | AF178319 |                     |
| <i>Romankenkius libidinosus</i> |          | Z99951    |          |                     |
| <i>Schmidtea mediterranea</i>   | U31084   | U31085    | AF178322 | Spain               |
| <i>Schmidtea lugubris</i>       |          |           | AF290022 |                     |
| <i>Schmidtea nova</i>           |          |           | AF290023 |                     |
| <i>Schmidtea polychroa</i>      | AF013152 | AF0131154 | AF178323 | Spain               |
|                                 | AF287133 |           |          |                     |
| <i>Spathula alba</i>            |          | DQ666006  |          | New Zealand         |
| <i>Spathula sp.</i>             |          | DQ666007  |          | New Zealand         |
| <b>Family Geoplanidae</b>       |          |           |          |                     |
| <b>Subfamily Bipaliinae</b>     |          |           |          |                     |
| <i>Bipalium adventitium</i>     |          | DQ666000  | AF178306 | USA                 |
|                                 |          |           | HM346597 |                     |
| <i>Bipalium kewense</i>         | AF033039 |           |          |                     |
|                                 |          | EU589209  |          | Japan               |
| <i>Bipalium multilineatum</i>   |          |           | HM346600 | Japan / South Korea |

| Species                           | 18S rDNA |                    | COI      | Sampling locality |
|-----------------------------------|----------|--------------------|----------|-------------------|
|                                   | Type I   | Type II            |          |                   |
| <i>Bipalium nobile</i>            | DQ666001 |                    |          | Japan             |
|                                   | HM346598 |                    |          |                   |
| <i>Bipalium</i> sp. 'Kawakatsu'   |          | X91402             | AF178307 | Japan             |
| <i>Novibipalium venosum</i>       |          |                    | DQ666048 | Japan             |
|                                   |          |                    | HM346599 | South Korea       |
| <b>Subfamily Microplaninae</b>    |          |                    |          |                   |
| <i>Microplana kwiskea</i>         |          |                    | EU334576 | Spain             |
|                                   |          |                    | AF178318 |                   |
| <i>Microplana nana</i>            | AF033042 |                    | AF178317 | Spain             |
|                                   |          |                    | FJ969972 | Spain             |
| <i>Microplana scharffi</i>        |          | AF050435           | EU334579 | UK                |
| <i>Microplana terrestris</i>      |          |                    | EU334584 |                   |
|                                   |          |                    | FJ969960 | Spain             |
|                                   |          |                    | FJ969979 | Spain             |
| <i>Microplana</i> sp.             |          |                    | DQ666045 | Spain             |
| <i>Microplana</i> sp. clade 3     |          |                    | FJ969961 | Spain             |
| <i>Microplana</i> sp. clade 4     |          |                    | FJ969967 | Spain             |
| <i>Microplana</i> sp. clade 5     |          |                    | FJ969968 | Spain             |
| <i>Microplana</i> sp. clade 6     |          |                    | FJ969971 | Spain             |
| <i>Microplana</i> sp. clade 7     |          |                    | FJ969978 | Spain             |
| <i>Microplana</i> sp. clade 8     |          |                    | FJ969957 | Spain             |
| <b>Subfamily Rhynchodeminae</b>   |          |                    |          |                   |
| <i>Arthurdendyus lucasi</i>       |          |                    | DQ465371 |                   |
| <i>Arthurdendyus testacea</i>     |          |                    | AF178305 | Australia         |
| <i>Arthurdendyus</i> sp.          |          |                    | AF178325 | Australia         |
| <i>Arthurdendyus triangulatus</i> | AF033038 | AF033044<br>Z99945 | AJ405984 |                   |
| <i>Australoplana sanguinea</i>    | AF033041 |                    |          | Australia         |
| <i>Australoplana</i> sp.          |          | AF050434           | DQ666028 | Australia         |
| <i>Caenoplana coerulea</i>        | AF033040 |                    | DQ666030 | UK                |
| 'Victoria'                        |          |                    | DQ465372 | Australia         |
| haplotype A                       |          |                    | DQ227619 | Australia         |
| haplotype B                       |          |                    | DQ227620 | Australia         |
| haplotype C                       |          |                    | DQ227621 | Australia         |
| haplotype D                       |          |                    | DQ227622 |                   |
| haplotype E                       |          |                    | DQ227623 | Australia         |
| haplotype F                       |          |                    | DQ227624 | Australia         |
| haplotype G                       |          |                    | DQ227625 | Australia         |
| haplotype H                       |          |                    | DQ227626 | Australia         |
| haplotype I                       |          |                    | DQ227627 |                   |
| haplotype J                       |          |                    | DQ227628 | Australia         |
| haplotype K                       |          |                    | DQ227629 | Australia         |
| haplotype L                       |          |                    | DQ227630 | Australia         |
| haplotype M                       |          |                    | DQ227631 | Australia         |

| Species                                  | 18S rDNA |          | COI      | Sampling locality |
|--|----------|----------|----------|-------------------|
|  | Type I   | Type II  |          |                   |
| haplotype N                              |          |          | DQ227632 | Australia         |
| haplotype O                              |          |          | DQ227633 | Australia         |
| haplotype P                              |          |          | DQ227634 | Australia         |
| haplotype Q                              |          |          | DQ227635 | Australia         |
| 1957                                     |          |          | JQ514564 | Spain (Menorca)   |
| 1958                                     |          |          | JQ514564 | Spain (Menorca)   |
| 1957clone1                               | JQ639215 |          |          | Spain (Menorca)   |
| 1957clone4                               | JQ639216 |          |          | Spain (Menorca)   |
| 1957clone11                              | JQ639217 |          |          | Spain (Menorca)   |
| 1957clone15                              | JQ639218 |          |          | Spain (Menorca)   |
| 1957clone3-16                            | JQ639219 |          |          | Spain (Menorca)   |
| 1957clone2                               |          | JQ639220 |          | Spain (Menorca)   |
| 1957clone5-7-8                           |          | JQ639221 |          | Spain (Menorca)   |
| 1957clone6                               |          | JQ639222 |          | Spain (Menorca)   |
| 1957clone9                               |          | JQ639223 |          | Spain (Menorca)   |
| 1957clone10                              |          | JQ639224 |          | Spain (Menorca)   |
| 1957clone12                              |          | JQ639225 |          | Spain (Menorca)   |
| 1957clone13                              |          | JQ639226 |          | Spain (Menorca)   |
| 1958clone1                               |          | JQ639227 |          | Spain (Menorca)   |
| <i>Caenoplana sp.</i> 'Armidale'         |          | AJ270156 |          | Australia         |
| <i>Caenoplana sp.</i> 1                  |          | AF048765 | DQ666031 |                   |
| <i>Caenoplana sp.</i> 4                  |          |          | DQ666032 |                   |
| <i>Dolichoplana sp.</i>                  |          | DQ666003 | DQ666037 |                   |
| <i>Newzealandia sp.</i>                  |          | AF050431 |          |                   |
| <i>Platydemus manokwari</i>              |          | AF048766 | AF178320 | Australia         |
| <i>Rhynchodemus sp.</i>                  |          |          | FJ969946 |                   |
| <b>Subfamily Geoplaninae</b>             |          |          |          |                   |
| <i>Cephaloflexa bergi</i>                |          |          | HQ026440 |                   |
| <i>Choeradoplana iheringi</i>            |          |          | HQ026428 | Brasil            |
| <i>Enterosyringia pseudorhynchodemus</i> |          | HQ026399 |          |                   |
| <i>Geoplana burmeisteri</i>              |          | DQ666004 | DQ666039 | Brasil            |
| <i>Geoplana goetschi</i>                 |          |          | HQ026418 |                   |
| <i>Geoplana ladislavii</i>               |          |          | AF178315 | Brasil            |
|  |          |          | HQ542890 |                   |
| <i>Geoplana quagga</i>                   |          |          | DQ666040 | Brasil            |
| <i>Geoplana sp.</i>                      |          |          | DQ666043 | Uruguay           |
| <i>Notogynaphallia guaiana</i>           |          |          | HQ542896 |                   |
| <i>Notogynaphallia sp.</i>               |          |          | DQ666047 | Brasil            |