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

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**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, 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 MgCl₂. 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
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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-H₂O. 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 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.
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 | TTGYRAATGCTTTTCGC     | Winnepenninckx et al. (1994)         |
| F: 9F18S  | CCGGTTAATTCAGCTTCCA    | Winnepenninckx et al. (1994)         |
| R: 3R18S  | GACGGGCGGTGTGTRC       | Winnepenninckx et al. (1994)         |
| F: 14F18S | ATACACAGGTCTGTGATGCC    | Winnepenninckx et al. (1994)         |
| R: 16R18S | CYGCAGGTTTCACCTACRG     | 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 (Katoh 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 Bdellouriidae). 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 ≥ 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 ≥ 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.
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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 ≥ 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 ± 0.02, whereas the P-distance with other Geoplanid species was higher (0.16 ± 0.03) and comparable to what we found among Geoplanidae taxa (0.17 ± 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 ± 0.002), whereas those of 18S rDNA type II formed a strongly supported clade with *Caenoplanana* sp.1 AF048765 (unknown origin) and *Caenoplanana* sp. ‘Armidale’ AJ270156 (from Australia) (mean P-distance: 0.003 ± 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 ± 0.003 and 0.058 ± 0.005, respectively.

*Caenoplanana 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/Caenoplanana-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. coerulaea* 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, Baguñà 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, Baguñà J, Riutort M (1999) Origin and evolution of paralogous rRNA gene clusters within the flatworm family Dugesiidae (Platyhelminthes, Tricladida). Journal of Molecular Evolution 49: 250–259. doi: 10.1007/PL00006547

Carranza S, Giribet G, Ribera C, Baguñà 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-Subirà E (1983) Nota sobre la presència de la planària terrestre *Bipalium kewense* Mosoley [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 *Caenoplanca 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 Rynchodemidae, 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, Túdó 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 *Caenoplanca 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;amp;amp;mode=topic&amp;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 Beau-champ, 1962 (Tricladiida: Terricola: Rhyynchodemidae), known as a predatory flatworm of land snails in the Ogasawara (Bonin) Islands, Japan. Applied Entomology and Zoology 40: 609–614. doi: 10.1303/acz.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:BIROC.0000021334.39072.2d

Olewine DA (1972) Further observations in Georgia on the land planarians, *Bipalium kewense* and *Geoplan a 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

Sliys R, Kawakatsu M, Riutort M, Baguñà 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/msn092

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 |
|---------|----------|-----|-------------------|
| **Maricola** | | | |
| **Family Bdellouridae** | | | |
| Subfamily Bdellourinae | | | |
| *Bdelloura candida* | Z99947 | AJ405983 | |
| **Continenticola** | | | |
| **Family Planariidae** | | | |
| *Polycelis tenuis* | Z99949 | AF178321 | Spain |
| **Family Dendrocoelidae** | | | |
| *Dendrocoelum lacteum* | AJ312271 | AF178312 | France |
| **Family Dugesiidae** | | | |
| *Cara pinguis* | AF033043 | AF178309 | New Zealand |
| *Dugesia etrusca* | | AF178310 | Italy |
| *Dugesia gonocephala* | DQ666002 | DQ666033 | The Netherlands |
| *Dugesia japonica* | AF013153 | D83382 | DQ666034 | Japan |
| *Dugesia ryakyuensis* | AF050433 | AF178311 | Japan |
| *Dugesia sícula* | | DQ666035 | Spain |
| *Dugesia subtentaculata* | AF013155 | DQ666036 | Spain |
| *Girardia anderlani* | DQ666013 | DQ666038 | Brasil |
| *Girardia dorotocephala* | | AF178314 | USA |
| *Girardia schubarti* | DQ666015 | DQ666041 | Brasil |
| *Girardia tigrina* | AF013157 | AF178319 | |
| *Neppia montana* | AF050432 | AF178319 | |
| *Romankenkius libidinosus* | Z99951 | | |
| *Schmidtea mediterranea* | U31084 | U31085 | AF178322 | Spain |
| *Schmidtea lugubris* | | AF290022 | |
| *Schmidtea nova* | | AF290023 | |
| *Schmidtea polychroa* | AF013152 | AF178323 | Spain |
| *Spathula alba* | DQ666006 | | New Zealand |
| *Spathula sp.* | DQ666007 | | New Zealand |
| **Family Geoplanidae** | | | |
| **Subfamily Bipaliinae** | | | |
| *Bipalium adventitium* | DQ666000 | AF178306 | USA |
| | | HM346597 | |
| *Bipalium kewense* | AF033039 | | Japan |
| | | EU589209 | |
| *Bipalium multilineatum* | | HM346600 | Japan / South Korea |
| Species                  | 18S rDNA COI | Sampling locality |
|-------------------------|--------------|-------------------|
|                         | Type I       | Type II           |
| **Bipalium nobile**     | DQ666001     | Japan             |
| **Bipalium sp. 'Kawakatsu'** | X91402       | AF178307 Japan    |
| **Novibipalium venosum**| DQ666048     | Japan             |
|                         | HM346599     | South Korea       |

**Subfamily Microplaninae**

| Species                  | 18S rDNA COI | Sampling locality |
|-------------------------|--------------|-------------------|
| **Microplana kwiskea**   | EU334576     | Spain             |
|                         | AF178318     |                   |
| **Microplana nana**      | AF033042     | Spain             |
|                         | AF178317     | Spain             |
|                         | FJ969972     |                   |
| **Microplana scharffi**  | AF050435     | EU334579 UK       |
| **Microplana terrestris**| FJ969960     | Spain             |
|                         | FJ969979     | Spain             |
| **Microplana sp.**       | DQ666045     | Spain             |
| **Microplana sp. clade 3**| FJ969961     | Spain             |
| **Microplana sp. clade 4**| FJ969967     | Spain             |
| **Microplana sp. clade 5**| FJ969968     | Spain             |
| **Microplana sp. clade 6**| FJ969971     | Spain             |
| **Microplana sp. clade 7**| FJ969978     | Spain             |
| **Microplana sp. clade 8**| FJ969957     | Spain             |

**Subfamily Rhynchodeminae**

| Species                  | 18S rDNA COI | Sampling locality |
|-------------------------|--------------|-------------------|
| **Arthurdendyus lucasi**| DQ465371     |                   |
| **Arthurdendyus testacea**| AF178305     | Australia         |
| **Arthurdendyus sp.**   | AF178325     | Australia         |
| **Arthurdendyus triangulatus**| AF033038     | Z99945 AJ405984  |
| **Australoplana sanguinea**| AF033041     | Australia         |
| **Australoplana sp.**   | AF050434     | DQ666028 Australia|
| **Caenoplana coerulea** | 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 Type I | 18S rDNA Type II | COI                        | Sampling locality |
|-----------------------------|-----------------|-----------------|---------------------------|-------------------|
| haplotype N                 |                 |                 | DQ227632                  | Australia         |
| haplotype O                 |                 |                 | DQ227633                  | Australia         |
| haplotype P                 |                 |                 | DQ227634                  | Australia         |
| haplotype Q                 |                 |                 | DQ227635                  | Australia         |
| 1957 clone1                 |                 |                 | JQ514564                  | Spain (Menorca)   |
| 1957 clone4                 |                 |                 | JQ514564                  | Spain (Menorca)   |
| 1957 clone11                |                 |                 | JQ514564                  | Spain (Menorca)   |
| 1957 clone15                |                 |                 | JQ514564                  | Spain (Menorca)   |
| 1957 clone3-16              |                 |                 | JQ514564                  | Spain (Menorca)   |
| 1957 clone2                 |                 |                 | JQ514564                  | Spain (Menorca)   |
| 1957 clone5-7-8             |                 |                 | JQ514564                  | Spain (Menorca)   |
| 1957 clone6                 |                 |                 | JQ514564                  | Spain (Menorca)   |
| 1957 clone9                 |                 |                 | JQ514564                  | Spain (Menorca)   |
| 1957 clone10                |                 |                 | JQ514564                  | Spain (Menorca)   |
| 1957 clone12                |                 |                 | JQ514564                  | Spain (Menorca)   |
| 1957 clone13                |                 |                 | JQ514564                  | Spain (Menorca)   |
| 1958 clone1                 |                 |                 | JQ514564                  | Spain (Menorca)   |
| Caenoplana sp. 'Armidale'   | AJ270156        |                 |                           | Australia         |
| Caenoplana sp. 1            | AF048765        | DQ666031        |                           |                   |
| Caenoplana sp. 4            |                 | DQ666032        |                           |                   |
| Dolichoplana sp.            |                 | DQ666003        | DQ666037                  |                   |
| Newzealandia sp.            | AF050431        |                 |                           |                   |
| Platydemus manokwari        | AF048766        | FJ969946        |                           |                   |
| Rhyynchodemus sp.           |                 |                 |                           |                   |
| Subfamily Geoplaninae       |                 |                 |                           |                   |
| Cephaloflexa bergi          |                 |                 | HQ026440                  | Brasil            |
| Choeradoplana iheringi      |                 |                 | HQ026428                  | Brasil            |
| Enterosyringa pseudorhynchodemus |             |                 | HQ026399                  | Brasil            |
| Geoplana burmeisteri        | DQ666004        | DQ666039        | Brasil                    |                   |
| Geoplana goetschi           |                 |                 | HQ026418                  | Brasil            |
| Geoplana ladislavii         |                 |                 | AF178315                  | Brasil            |
| Geoplana quagga             |                 |                 | HQ542890                  | Brasil            |
| Geoplana sp.                |                 |                 | DQ666040                  | Brasil            |
| Notogynaphallia guaiiana    |                 |                 | HQ542896                  | Uruguay           |
| Notogynaphallia sp.         |                 |                 | DQ666047                  | Brasil            |