A new species of alien terrestrial planarian in Spain: *Caenoplana decolorata*

Eduardo Mateos [Corresp., 1] , **Hugh Jones** [2] , **Marta Riutort** [3] , **Marta Álvarez-Presas** [4, 5]

[1] Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals. Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

[2] Life Sciences Department, Natural History Museum, London, United Kingdom

[3] Departament de Genètica, Microbiologia i Estadística. Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

[4] School of Biological Sciences, University of Bristol, Bristol, United Kingdom

[5] Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

Corresponding Author: Eduardo Mateos
Email address: emateos@ub.edu

Terrestrial planarians found in a plant nursery in Spain in 2012 are described as a new species, *Caenoplana decolorata*. Dorsally they are mahogany brown with a cream median line. Ventrally they are pastel turquoise fading to brown laterally. Molecular data indicate that they are a member of the genus *Caenoplana*, but that they differ from other *Caenoplana* species found in Europe. One mature specimen has been partially sectioned, and the musculature and copulatory apparatus is described, confirming the generic placement but distinguishing the species from other members of the genus. It is probable that the species originates from Australia.

A new species of alien terrestrial planarian in Spain: *Caenoplana decolorata*.

Eduardo Mateos[1], Hugh D Jones[2], Marta Riutort[3], Marta Álvarez-Presas[3,4].

[1]Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona, Barcelona 08028, Spain.
[2]Scientific Associate, Life Sciences Department, Natural History Museum, London, SW7 5BD, UK.
[3]Departament de Genètica, Microbiologia i Estadística, Universitat de Barcelona, Barcelona 08028, Spain.
[4]School of Biological Sciences, University of Bristol, Bristol, BS8 1TQ, UK.

Corresponding author: Eduardo Mateos, Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona, Barcelona 08028, Spain. Email: emateos@ub.edu.

Abstract

Terrestrial planarians found in a plant nursery in Spain in 2012 are described as a new species, *Caenoplana decolorata*. Dorsally they are mahogany brown with a cream median line. Ventrally they are pastel turquoise fading to brown laterally. Molecular data indicate that they are a member of the genus *Caenoplana*, but that they differ from other *Caenoplana* species found in Europe. One mature specimen has been partially sectioned, and the musculature and copulatory apparatus is described, confirming the generic placement but distinguishing the species from other members of the genus. It is probable that the species originates from Australia.

Keywords *Geoplana* — molecular identification – alien species – invasive species – land flatworm.

Introduction

Álvarez-Presas et al., (2014*)* recorded several terrestrial planarian species from Spain, some considered native to Europe, others introduced from other continents. Some species were identifiable on the basis of external features such as colour and shape, on anatomical characters and comparative molecular analysis. Molecular results suggested that further species were found but at the time they could not be certainly identified to species, though perhaps to genus. This paper describes specimens (Fig. 1, a-d) listed as "*Caenoplana* Ca2" by *Álvarez-Presas et al.,* (2014*)*. Molecular data (Figure 12 of *Álvarez-Presas et al.,* 2014) indicate that these specimens are of the genus *Caenoplana* Moseley, 1877, but distinct from other *Caenoplana* species. One mature specimen has been partially sectioned, and the musculature and copulatory apparatus is described. It has the characters of the genus *Caenoplana* Moseley, 1877, as amended by *Ogren and Kawakatsu* (1991) and by *Winsor* (1991) but differs from other described species of that genus both in external characteristics and anatomy. Neither do the specimens resemble any species described only on external features such as shape and colour and currently placed in the

46 genus *Australopacifica* Ogren and Kawakatsu 1991, a collective genus containing species "not
47 classifiable into the present taxonomic genera because of insufficient morphological information;
48 geographical distribution largely in Australasia and Indo-Pacific Islands. A collective group for
49 *species inquirendae* and *nomina dubia*". It is described as *Caenoplana decolorata* sp. nov.
50
51

Methods

Sampling

54 Specimens were collected by E. Mateos from a plant nursery named "vivers casa Paraire" in
55 Bordils municipality (Girona province, Spain, WGS84, position: 42.0348°N; 2.8986°E). All
56 were collected by hand from beneath pots (Fig. 1e, f) that contain the plants on 12 January 2012
57 (five specimens: PT426, PT427, PT428, PT430, PT431) and 22 October 2012 (four specimens:
58 PT655, PT657-1, 2 and 3) (Table 1).
59   Specimens from 12-January-2012 and specimens PT655 and PT657-3 were preserved in
60 absolute alcohol for further molecular analyses. Specimens PT657-1 and 2 were killed with
61 boiling water, fixed with 10% formalin and preserved in 70% alcohol. Specimens PT426 and
62 PT657-1 were photographed alive (Fig. 1).
63

Molecular methods

65 All the sequences used in the present work were obtained in previous studies with the exception
66 of some *Cytochrome Oxidase I* (herein Cox1) sequences that were obtained from individuals
67 collected at the Real Jardín Botánico de Córdoba (Spain) by Mónica López (Table 1). In all
68 cases, a small section of the anterior end of specimens preserved in absolute ethanol was used for
69 DNA extraction. The new sequences were obtained following the same protocol as in *Álvarez-*
70 *Presas et al.,* (2014).
71   A nucleotide alignment was obtained for the Cox1 sequences based on the AA translation
72 as a guide using BioEdit software (*Hall, 1999*) and the echinoderm mitochondrial genetic code
73 (9). A Maximum Likelihood (ML) phylogeny was inferred using the IQtree software (*Nguyen et*
74 *al., 2015*) with the MFP+MERGE implementation and 10000 replicates for ultrafast bootstrap
75 search (-bb option). Then two single locus molecular species delimitation methods were applied
76 in order to check the validity of the new species presented here and the ones already described
77 and included in the phylogeny. Automatic Barcode Gap Discovery (ABGD) (*Puillandre et al.,*
78 *2012*) was the first method performed, implemented in the webpage:
79 http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html. The default parameters were used,
80 selecting initial partitions as they are supposed to be more stable and generally give as a result a
81 closer number of groups described by taxonomists than recursive partitions. The second method
82 applied was the multi-rate Poisson Tree Process (mPTP) analysis (*Kapli et al., 2017*). The
83 newick tree obtained in the ML phylogenetic inference was used as input in the website
84 http://mptp.h-its.org/#/tree.
85

Anatomical methods

87 Specimens PT657-1 and 2 were sent to HDJ and are deposited in the Natural History Museum
88 London, accession numbers NHMUK.2014.5.13.12-13. The larger specimen had a visible
89 gonopore, was assumed to be mature and selected for partial sectioning. It was divided into four
90 portions: anterior portion about 2 cm long not sectioned, in alcohol; pre-pharyngeal section, TS,
91 five slides, two at 15 μm, three at 10 μm; posterior portion including pharynx and copulatory

92   apparatus, LS, 16 slides (pharynx separated, HLS) at 15 μm. Slides stained in haematoxylin and
93   eosin and mounted in Canada balsam. The second specimen, about 3.4 cm long, had no visible
94   gonopore and remains in alcohol.
95   Colours are expressed as RAL colours (www.ralcolor.com).
96   The electronic version of this article in Portable Document Format (PDF) will represent a
97   published work according to the International Commission on Zoological Nomenclature (ICZN),
98   and hence the new names contained in the electronic version are effectively published under that
99   Code from the electronic edition alone. This published work and the nomenclatural acts it
100 contains have been registered in ZooBank, the online registration system for the ICZN. The
101 ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed
102 through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The
103 LSID for this publication is: urn:lsid:zoobank.org:pub:B2636DF8-4372-405C-8A8C-
104 4FBEC7396276. The LSID for the new species described is: *Caenoplana decolorata* sp. nov.
105 urn:lsid:zoobank.org:act:C0CEE92F-A51E-4EDD-B18B-E7F021338667. The online version of
106 this work is archived and available from the following digital repositories: PeerJ, PubMed
107 Central and CLOCKSS.
108
109 Figure abbreviations: 1-7, arbitrary regions of the ejaculatory duct (see text); cf, common female
110 duct; gp, gonopore; clm, cutaneous longitudinal muscle; ml, median dorsal line; nc, nerve cord;
111 od, ovovitelline duct; od-cf, opening of ovovitelline ducts to common female duct; plm,
112 parenchymal muscle; rh, rhabdites; sd, sperm duct; t, testis.
113

114   Results
115   Molecular results

116 The final dataset comprises 43 Cox1 sequences (including 3 outgroups, Table 1), with a final
117 length of 822 bp. The resulting ML tree (Fig. 2) shows monophyletic groups comprising 7
118 putative *Caenoplana* species. Although the bootstrap values (bb) are not high enough to give
119 support to the relationships between these clades, the monophyly of the new species described
120 here, *C. decolorata*, harbor maximum support. The results of the molecular species delimitation
121 analyses (both mPTP and ABGD) match the same clades present in the phylogeny (Fig. 2) giving
122 rise to 7 putative *Caenoplana* species. Among them, we find the subject of this study,
123 *Caenoplana decolorata*.
124

125   Taxonomic section

126 Order Tricladida Lang, 1884
127   Suborder Continenticola Carranza, Littlewood, Clough, Ruiz-Trillo, Baguñà and Riutort,
128   1998
129   Family Geoplanidae Stimpson, 1857
130   Subfamily Rhynchodeminae Graff, 1896
131   Tribe Caenoplaninae Ogren and Kawakatsu, 1991
132   Genus *Caenoplana* Moseley, 1877
133

134 *Caenoplana decolorata* new species**.**
135   *Caenoplana* Ca2 Álvarez-Presas et al., 2014.
136

137 Etymology: "decolorata" indicating that live specimens resemble *C. coerulea* but are
138 comparatively pale and discolored.
139
140 **NHMUK.2014.5.13.12-13**
141 E. Mateos collection code PT657-1 and PT657-2. Locality: Bordils (Girona, Spain), position
142 N42.0348049 E2.8986153, date 22 October 2012.
143   Preserved dimensions: holotype (PT657-1): length 46 mm; width 2 mm; height 1 mm;
144 anterior to mouth 28 mm (61% of body length); mouth to gonopore 11 mm (85% of body
145 length); gonopore to posterior 7 mm; co-type (PT657-2): length 34 mm; width 2.1 mm; anterior
146 to mouth 17 mm (50%); apparently immature.
147   All other specimens (with a small section of the anterior end removed) are deposited in
148 the collection of M. Riutort at the University of Barcelona.
149
150 **External characters**
151 Live specimens are "mahogany brown" (RAL 8106) with a narrow "cream" (RAL 9001) mid-
152 line dorsally, merging to "beige brown" (RAL 8024) laterally. The anterior end is "copper
153 brown" (RAL 8004). The ventral mid-line is "pastel turquoise" (RAL 6034) merging into the
154 lateral "beige brown".
155   Eyes in a sparse uniserial row round the anterior end, biserial for a short distance behind
156 the anterior end and sparse staggered uniserial to the posterior end. Sole nearly the whole of the
157 ventral surface.
158
159 **Anatomy**
160 Transverse sections (Fig. 3a) are about 1.3 mm high and 2 mm wide. The ciliated creeping sole is
161 about 80% of the width. The cilia are about 5 μm long. The ventral epidermis is a monolayer
162 about 30 μm thick and has few rhabdites. Ventral sub-epidermal muscle consists of a layer of
163 circular muscle fibres about 10 μm thick and longitudinal muscle in bundles about 30 μm thick.
164 Dorsal to the longitudinal muscle bundles is a ventral nerve plexus. There is a distinct, compact
165 layer of parenchymal longitudinal muscle ventrally, 40-50 μm thick, 150 μm in from ventral
166 surface. Ventral nerve cords are about 750 μm centre to centre, about 120 μm in diameter, with
167 transverse commissures. Laterally and dorsally the parenchymal muscle is less compact and 10-
168 20 μm thick. Dorsal epidermis is 45 μm thick, non-ciliated and has numerous rhabdites. Dorsal
169 and lateral sub-epidermal circular muscle is about 10 μm thick, and longitudinal muscle in
170 bundles about 35 μm thick. Rhabdites are numerous dorsally and laterally ental to the sub-
171 epidermal muscle, but in the mid-dorsal region, the rhabdites layer is slightly deeper (Fig. 3a, b),
172 presumed to be coincident with the pale midline visible in the living animal.
173   The pharynx is cylindrical about 2.5 mm long and 0.9 mm in diameter. The pharyngeal
174 aperture is about half way along the pharyngeal pouch. Pharyngeal musculature consists of an
175 outer layer of circular muscle about 10 μm thick, a layer of mixed longitudinal and radial muscle
176 about 360 μm thick and an inner layer of circular muscle about 30 μm thick.
177   The anterior portion containing the ovaries has not been sectioned. Ovovitelline ducts are
178 about 500 μm apart on the inner dorsal surface of the ventral nerve cords (Fig. 3a, d). Their outer
179 and inner diameters are about 25 μm and 7 μm respectively. They run to about 800 μm behind
180 the gonopore, turn dorsally and open into the common female duct about 800 μm long which
181 extends forwards with little differentiation to open into the common antrum above the gonopore
182 Fig. 4a, c, e). There is little or no glandular tissue surrounding the common female duct.

183   Testes are numerous, ventral, ovate, about 200 µm wide and 300 µm high (Fig. 4a, d, e)
184 and run almost to the copulatory apparatus. The sperm ducts cannot be certainly distinguished in
185 transverse sections. They enter the anterior end of the muscular bulb of the penis, widen slightly
186 and have small amounts of stored sperm (Fig. 4b). They separately enter the anterior end of the
187 ejaculatory duct which is complex, long and sinuous, about 1.5 mm from its anterior end to the
188 gonopore (Fig. 4a, b, d, f). It has several regions, for ease of reference they are here arbitrarily
189 numbered 1-7 from anterior to posterior as follows. 1, a small chamber which extends
190 transversely through 10 x 15 µm sections, thus about 150 µm wide, the two sperm ducts entering
191 on either lateral extremity. 2, a narrow duct extending posteriorly and turning ventrally and
192 opening into, 3, a sinus-like duct wide laterally, 23 x 15 µm thus 345 µm wide, but only 35 µm
193 in the antero-posterior direction. This duct initially turns ventrally then narrows and curves
194 posteriorly to be almost U-shaped (second arm shorter). The ejaculatory duct continues into, 4, a
195 narrow sinus-like lumen surrounded by strongly eosinophilic cells forming a structure roughly
196 spherical in outline about 400 µm in diameter. The cells of this region appear to be elongate with
197 nuclei mostly adjacent to the lumen (Fig. 4g). This in turn opens into, 5, a portion about 400 µm
198 long with sinuous margins, which in turn seems to open via, 6, a small papilla-like opening into a
199 longer and wider duct, 7, about 600 µm long with sinuous walls which in turn opens to the
200 common antrum above the gonopore.
201

Discussion

203 The previous molecular results (*Álvarez-Presas et al., 2014*) analyzing only *Caenoplana*
204 sequences (and an outgroup) indicated that *C. decolorata* specimens are closely related to
205 *Caenoplana variegata* (Fletcher and Hamilton 1888) (named as *C. bicolor* (Graff 1899) in that
206 work, see *Jones et al., 2020*) although without support. In the present work, the tree shows a
207 closer relationship between *C. decolorata* and *C. coerulea,* while *C. variegata* is sister to the
208 clade formed by these two species (plus some putative unknown species), which will be an
209 expected result having into account the more similar external coloration pattern of the first two
210 species. However, the bb values do not support the relationships among species in the present
211 work neither and make impossible to validate this hypothesis.
212   The sectioned specimen has multiple eyes, ventral testes, a layer of parenchymal
213 longitudinal muscle, stronger ventrally, a long and fairly elaborate copulatory apparatus, the
214 ejaculatory duct particularly so, and other anatomical characters of the genus *Caenoplana*
215 Moseley 1877 as amended by *Ogren and Kawakatsu* (1991) and by *Winsor* (1991). Thus we are
216 confident of the generic placement.
217   However, comparison with other *Caenoplana* species is problematic. *Ogren and*
218 *Kawakatsu* (1991) list 11 described species of *Caenoplana*, that is species with an anatomical
219 description. *Winsor* (1991) lists 19 species, seven "provisionally placed", within *Caenoplana*.
220 None of those has a similar external colouration to the present specimens, and the ejaculatory
221 duct of the present specimens is distinctly different to that of any of those 11. They also differ
222 from *C. variegata* (Fletcher and Hamilton 1888) (synonymous with *C. bicolor* (Graff 1899), see
223 *Jones et al.,* (2020)).
224   *Winsor* (1997) lists a further six numbered, unnamed, *Caenoplana* species in addition to
225 two named species, *C. coerulea coerulea* (Moseley 1877), and *C. variegata* (as *C. bicolor* (Graff
226 1899)). *Winsor (1998)* states that 22 *Caenoplana* species were present in Australia, with no other
227 details. Presumably this total includes the six numbered, unnamed, species above. *Álvarez-*

228 *Presas et al.,* (2014*)* list two further unnamed *Caenoplana* species, one the subject of this paper.
229 Whether either of these is similar to any of *Winsor's* (1997) unnamed species is unknown.
230   In comparing this species to other *Caenoplana* species or to species placed in the
231 collective genus *Australopacifica*, particular attention should be made to those with a broadly
232 similar pigment distribution, that is those with, dorsally, a narrow mid-dorsal pale line on an
233 otherwise uniform dark colour (any dark colour) and ventrally with a more or less uniform, but
234 different, colour. The only two species with such a distribution are *C. coerulea* Moseley 1877
235 and *C. purpurea* (Dendy 1895).
236   *Caenoplana coerulea* Moseley 1877, originally found in New South wales, Australia,
237 was described as follows: "Entire body of a dark Prussian blue colour, somewhat lighter on the
238 under surface … with a narrow, mesial, dorsal, longitudinal stripe of white"; 5 cm long. *H*yman
239 (1943, 1954) and *Ogren* (1989) have described the anatomy of similar specimens found in the
240 USA. This species has distinctly different coloration from the present specimens and the
241 ejaculatory duct has a different structure *(Ogren, 1989).* It has subsequently been found in New
242 Zealand *(Dendy, 1895),* several European countries *(Álvarez-Presas et al., 2014)* and North and
243 South America (*Ogren, 1989*; *Luis-Negrete et al., 2011*).
244   *Geoplana purpurea* Dendy 1895, originally from South Island, New Zealand, was
245 described as follows: "dorsal surface rather dark reddish-purple … a very narrow median band of
246 nearly white", "ventral surface paler purple, under a lens appearing very finely mottled in two
247 shades"; 3.5 cm long. *Dendy* (1895*)* comments: "It is perhaps doubtful whether this species
248 ought to be separated from the Australian *G. coerulea*, from which it differs only in colour". But
249 in the same paper Dendy also records *C. coerulea*. *Geoplana purpurea* was placed by *Ogren and*
250 *Kawakatsu* (1991) in the collective genus *Australopacifica*, with the note that "this probably
251 belongs to *Caenoplana* on basis of external similarities to *Caenoplana coerulea*". *Winsor* (1991)
252 "provisionally placed" it within *Caenoplana*. There has been no anatomical description of
253 specimens under that species name. However, the coloration is different to the specimens from
254 Spain and it seems unlikely that the latter are of the same species.
255   None of the other species listed by *Ogren and Kawakatsu* (1991) under *Australopacifica*
256 has a colouration similar to the present species.
257   Thus the specimens do not match the description of any species previously described and
258 are described as a new species, *Caenoplana decolorata*.
259   One possible confusing factor is that the colour of some species has been shown to vary
260 over time and between individuals due to feeding (*Jones et al. 2020*; *McDonald and Jones*
261 *2007*). Only prolonged observations on live animals before and after feeding could clarify if that
262 might be the case with this species. Such observations would also indicate its preferred prey.
263   The ejaculatory duct of the new species is distinctive. The structure here numbered 4 is
264 unlike anything present in any other described species of *Caenoplana* or for that matter any other
265 terrestrial planarian. The function of this structure is not obvious; it does not appear to be either
266 glandular or muscular.
267   It is fortuitous for taxonomists that the reproductive system of terrestrial planarians varies
268 between genera and between species within a genus. But why should it be so variable?
269   Since at least one of the specimens was mature, it is presumed that this species
270 reproduces by sexual reproduction, though it is entirely possible that it may also reproduce by
271 partial fission, as in *C. variegata* (see *Jones at al., 2020*) and several other land planarian
272 species.

273   This species almost certainly originates from Australia since most *Caenoplana* species
274   are from there. It is presumed that it has been inadvertently transported to Spain in the course of
275   the trade in horticultural products.
276
Acknowledgements
278   We thank Mónica López, from the Real Jardín Botánico de Córdoba (Spain), for collecting and
279   supplying some flatworm specimens from Córdoba. HDJ would like to thank The School of
280   Biological Sciences, University of Manchester and Peter Walker of the histology laboratory, for
281   access to facilities.
282
References

284   **Álvarez-Presas M, Mateos E, Tudó À, Jones HD, Riutort M. 2014.** Diversity of introduced
285   terrestrial flatworms in the Iberian Peninsula: a cautionary tale, *PeerJ* **2**:e430; DOI
286   10.7717/peerj.430. 35 pp.
287
288   **Carranza S, Littlewood DTJ, Clough KA, Ruiz-Trillo I, Baguñà J, Riutort M. 1998.** A
289   robust molecular phylogeny of the Tricladida (Platyhelminthes: Seriata) with a discussion on
290   morphological synapomorphies. *Proceedings of the Royal Society of London, Series B*
291   **265**:631—640.
292
293   **Dendy A. 1895.** Additions to the cryptozoic fauna of New Zealand. *Annals and Magazine of*
294   *Natural History Series 6* **14**:393–401.
295
296   **Fletcher JJ, Hamilton AG. 1888.** Notes on Australian land-planarians, with descriptions of
297   some new species. *Proceedings of the Linnean Society of New South Wales* **2**:349—374.
298
299   **Graff L von. 1896.** Über die Morphologie des Geschlechtsapparates der Landplanarien.
300   *Verhandlungen der Deutschen Zoologischen Gesellschaft* **6**:75—93.
301
302   **Graff L von. 1899.** *Monographie der Turbellarien. II. Tricladia Terricola (Landplanarien).* W.
303   Engelmann, Leipzig, 574p, + Atlas, taf. I-LVIII.
304
305   **Hall TA. 1999.** BioEdit: a user-friendly biological sequence alignment editor and analysis
306   program for Windows 95/98/NT. *Nucleic Acids Symposium* Series **41**:95–98.
307   https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29.
308
309   **Hyman LH. 1943.** Endemic and exotic land planarians in the United States with a discussion of
310   necessary changes of names in the Rhynchodemidae. *American Museum Novitates* **1241**:1–
311   21.
312
313   **Hyman LH. 1954.** Some land planarians of the United States and Europe, with remarks on
314   nomenclature. *American Museum Novitates* **1667**:1–20.
315
316   **Jones HD, Mateos E, Riutort M, Álvarez-Presas M. 2020.** The identity of the invasive yellow-
317   striped terrestrial planarian found recently in Europe: *Caenoplana variegata* (Fletcher and

318   Hamilton, 1888) or *Caenoplana bicolor* (Graff, 1899)? *Zootaxa* **4731**:193-222.
319   https://doi.org/10.11646/zootaxa.4731.2.2.
320

321 **Kapli P, Lutteropp S, Zhang J, Kobert K, Pavlidis P, Stamatakis A, Flouri T. 2017.** Multi-
322   rate Poisson tree processes for single-locus species delimitation under maximum likelihood
323   and Markov chain Monte Carlo. *Bioinformatics* **33(11):**1630–1638.
324   https://doi.org/10.1093/bioinformatics/btx025.
325

326 **Lang A. 1884.** Die Polycladen (Seeplanarien) des Golfes von Neapel und der angrenzenden
327   Meeresabschnitte. Eine Monographie. *Fauna und Flora des Golfes von Neapel* **11**, R.
328   Friedlander and Sohn, Leipzig, 688 pp. + pls. I—XXXIX.
329

330 **Luis-Negrete LH, Brusa F, Winsor L. 2011.** The blue land planarian *Caenoplana coerulea,* an
331   invader in Argentina. *Revista Mexicana de Biodiversidad* **82**:287–291.
332

333 **McDonald JC, Jones HD. 2007.** Abundance, reproduction, and feeding of three species of
334   British terrestrial planarians: Observations over 4 years. *Journal of Natural History* **41**:293-
335   312. doi: 10.1080/00222930701219149.
336

337 **Moseley HN. 1877.** Notes on the structure of several forms of land planarians, with a description
338   of two new genera and several new species, and a list of all species at present known.
339   *Quarterly Journal of Microscopical Science* **17**:273–292.
340

341 **Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. 2015.** IQ-TREE: A fast and effective
342   stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and*
343   *Evolution* **32(1):**268–274. https://doi.org/10.1093/molbev/msu300.
344

345 **Ogren RE. 1989.** Redescription and a new name for the blue land planarian *Geoplana vaga*
346   Hyman now considered conspecific with *Caenoplana coerulea* Moseley from Australia
347   (Turbellaria: Tricladida: Geoplanidae). *Journal of the Pennsylvania Academy of Science*
348   **63**:135–142.
349

350 **Ogren RE, Kawakatsu M. 1991.** Index to the species of the family (Turbellaria, Tricladida,
351   Terricola) Part II: Caenoplaninae and Pelmatoplaninae. *Bulletin of the Fuji Women's College*
352   **No. 29, Ser. II**:25–102.
353

354 **Puillandre N, Lambert A, Brouillet S, Achaz G. 2012.** ABGD, Automatic Barcode Gap
355   Discovery for primary species delimitation. *Molecular Ecology* **21(8):**1864–1877.
356   https://doi.org/10.1111/j.1365-294X.2011.05239.x.
357

358 **Stimpson W. 1857.** Prodromus descriptiones animalium evertebratum quae in Expeditione ad
359   Oceanum, Pacificum Septentrionalem a Republica Federata missa, Johnne Rodgers Duce,
360   observavit et descripsit. *Proceedings of the Academy of Natural Sciences, Philadelphia* **9**:19–
361   31.
362

363   **Winsor LW. 1991.** A provisional classification of Australian terrestrial geoplanid flatworms
364   (Tricladida: Terricola: Geoplanidae). *The Victorian Naturalist* **108**:42–49.
365
366   **Winsor LW. 1997.** The biodiversity of terrestrial flatworms (Tricladida; Terricola; Terricola) in
367   Queensland: a preliminary report. *Memoirs of the Museum of Victoria* **56 (2):**575–579.
368
369   **Winsor LW. 1998.** The Australian terrestrial flatworm fauna (Tricladida: Terricola).
370   *Pedobiologia* **42**:457–463.

List of samples used in the molecular analysis with GenBank accession numbers

*Sequences obtained in this study

1 **Table 1.** List of samples used in the molecular analysis with GenBank accession numbers.

Species/Morphotype	Locality	GenBank Code *Cox1*
Family Geoplanidae		
Subfamily Rhynchodeminae		
Tribe Caenoplanini		
Artioposthia sp.	Australia	MN990642
Arthurdendyus testaceus	-	MN990643
Caenoplana coerulea	New Zealand	DQ665961
	Menorca (Spain)	JQ514564
	Liverpool, UK	DQ666030
	El Prat de Llobregat (Barcelona, Spain)	KJ659617
	Vall de'n Bas (Girona, Spain)	KJ659618
		KJ659619
		KJ659620
		KJ659622
		KJ659623
		KJ659624
		KJ659626
	Badalona (Barcelona, Spain)	KJ659633
		KJ659634
	Adelaide (Australia)	KJ659642
	-	KJ659645
	Granollers (Barcelona, Spain)	KJ659647
PT1304	Real Jardín Botánico de Córdoba (Córdoba, Spain)	MT727076*
PT1305		MT727077*
PT1307		MT727078*
PT1310		MT727079*
Caenoplana sp. 1	-	DQ666031
Caenoplana sp. 2	Tallaganda (Australia)	DQ227621
		DQ227625
		DQ227627
		DQ227634
Caenoplana sp. 3	Victoria (Australia)	DQ465372
Caenoplana sp. 4	-	DQ666032
Caenoplana variegata	Bordils (Girona, Spain)	KJ659648
	Southampton, UK	MN990646
	Cardiff, UK	MN990647
		MN990648
Caenoplana decolorata sp. nov.	Bordils (Girona, Spain)	KJ659628
		KJ659629
		KJ659630
		KJ659631
		KJ659632
		MN990644
		KJ659649
OUTGROUP: Tribe Rhynchodemini		
Dolichoplana sp.	-	DQ666037
D. striata	Igreginha (Brazil)	KC608226
Rhynchodemus sylvaticus	Canyamars (Barcelona, Spain)	FJ969946

3 *Sequences obtained in this study

Figure 1

Caenoplana decolorata sp. nov.

(a–d) Photographs of live specimens, anterior to the right. (a) Dorsal view of specimen PT426 showing the "mahogany brown" colour and "cream" median line. Scale bar 10 mm. (b) A twisted specimen PT657-1 showing the "pastel turquoise" ventral surface. Scale bar 10 mm. (c) Specimen PT657-1 and (d) specimen PT426, anterior end showing anterior "copper brown" colour and the eyes (the two white lines in (d) are reflections from the lighting). Scale bars 4 mm. (e & f) pots under which the specimens were found, in a greenhouse (e) and outdoors (f).
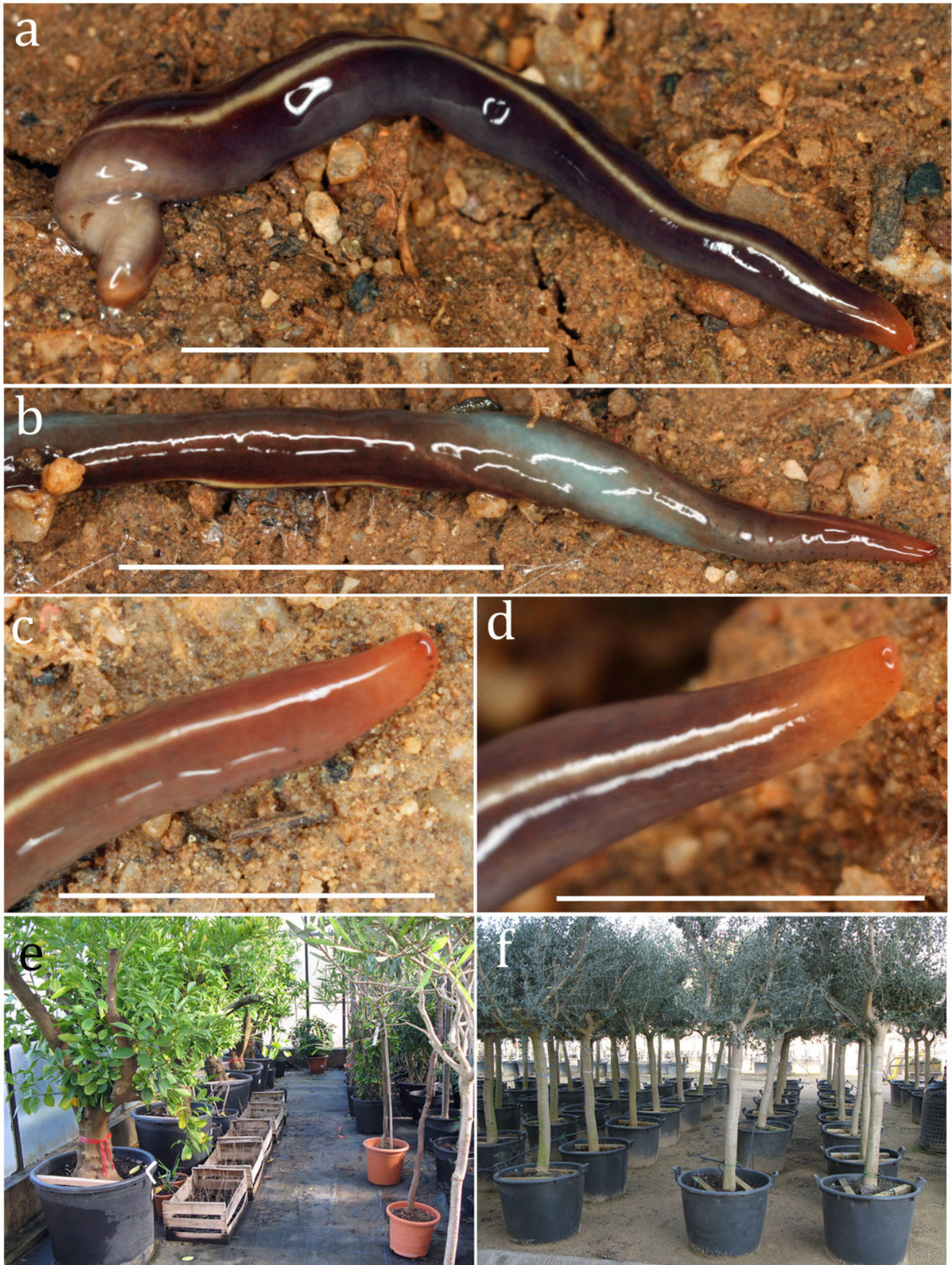
Figure 2

Maximum Likelihood (ML) phylogeny inferred with Cox 1 sequences.

Values at nodes correspond to ultrafast bootstrap replicates (bb) obtained with IQtree software. Vertical bars to the right of the phylogeny correspond to the molecular species delimitation methods results (mPTP, left bar and ABGD, right bar). Scale bar represents number of substitutions per site. Photograph of specimen PT426 in dorsal view (anterior to the right).

Tree scale: 0.01 ⊢━━⊣
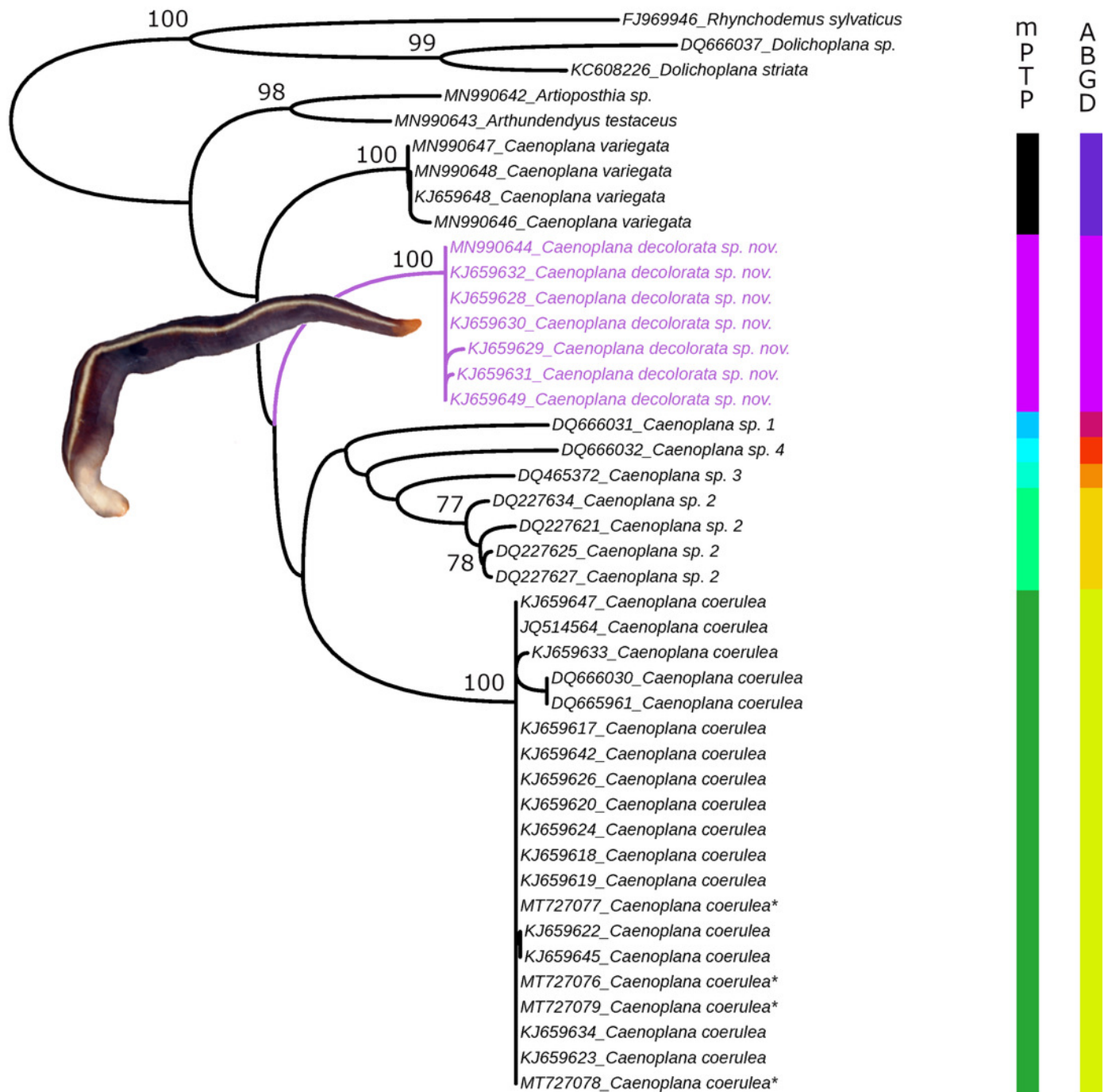
Figure 3

Caenoplana decolorata specimen PT657-1 (NHMUK2014.5.13.12).

(a) Entire transverse section (* * indicate the width of the ventral creeping sole; scale line 1 mm). (b) Enlarged mid-dorsal (scale line 100 µm). (c) Enlarged mid-ventral (scale line 100 µm). (d) The testis, ventral nerve cord and ovovitelline duct on one side (scale line 200 µm). (e) Longitudinal section showing several testes (scale line 250 µm).
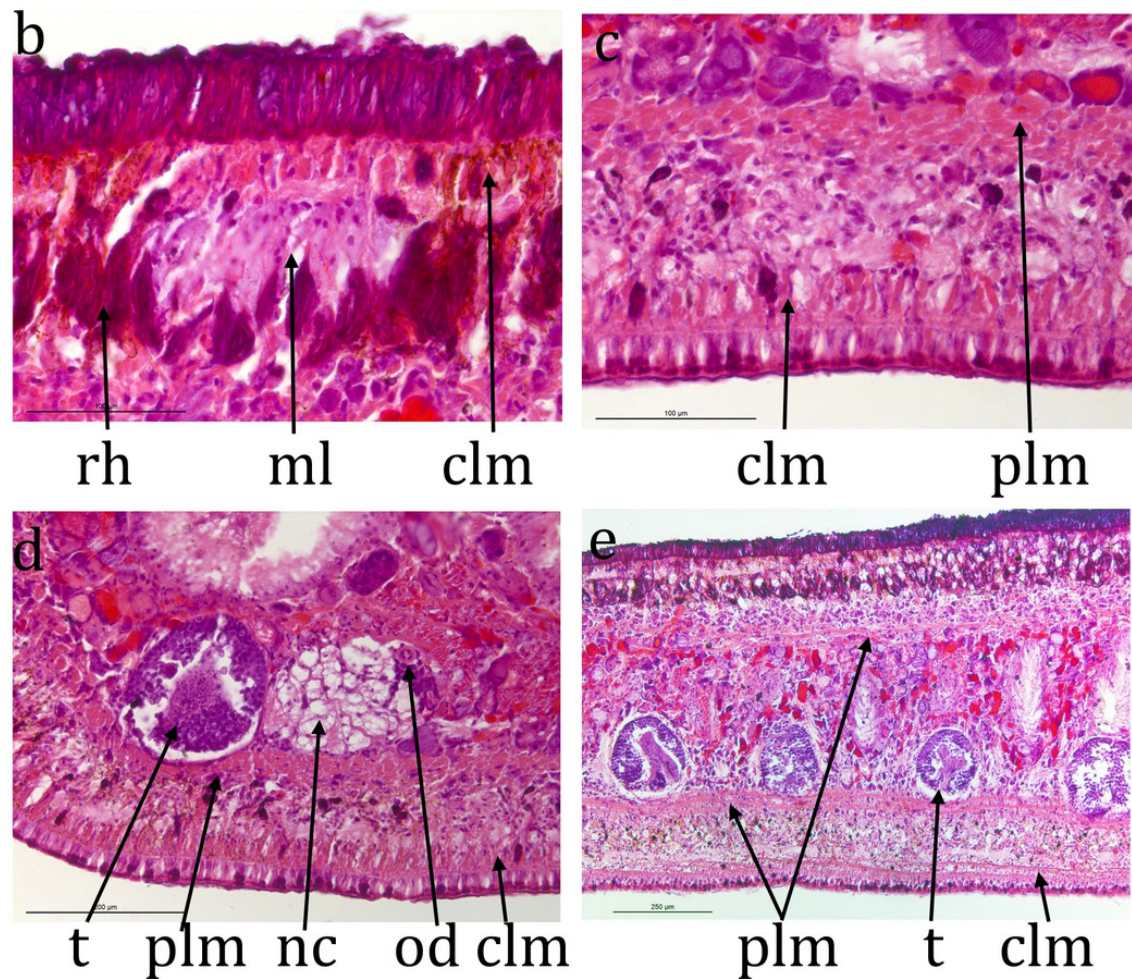
Figure 4

Caenoplana decolorata specimen PT657-1 (NHMUK2014.5.13.12).

(a) Reconstruction diagram and (d–g) longitudinal sections of the copulatory apparatus (anterior to the left); a, b and c are to the same scale. Micrographs: (b & c) Mid-sections through the male and female portions respectively (both folded sections) (scale lines 1000 µm). (d & f) Further sections through the proximal portion of the male ducts (scale lines 500 µm). (e) Section showing the approach of an ovovitelline duct to the common female duct (scale line 500 µm). (g) Enlargement of region 4 of the male duct (scale line 200 µm). The nuclei (cyanophylic) are mostly adjacent to the lumen.