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1 **Predominance of a single phylogenetic species in colonization events among a sextet of**
2 **decollate land snail, *Rumina decollate* (Mollusca: Pulmonata: Subulinidae), species**

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23 **Abstract:** The hermaphroditic, facultatively selfing, land snail *Rumina decollata* is a common,
24 widespread species that is indigenous to the Mediterranean region and that has been introduced
25 to many other regions of the world. However, recent DNA sequence analyses have indicated that
26 *R. decollata* is a complex of several phylogenetic species, two of which correspond to previously
27 distinguished allozyme strains with different body colors (light vs. dark) and life history
28 characteristics. Against this background, this paper attempts to identify which of these
29 phylogenetic species have been introduced elsewhere in the world. Based on a comparative DNA
30 sequence analysis of putatively introduced populations from South America, North America,
31 Japan and the North Atlantic Islands vs. native Mediterranean populations, it is shown that all
32 putatively introduced populations belong to a single phylogenetic species that was previously
33 recognized as the dark morph. Hence, the colonizing and invasive character of *R. decollata*
34 seems to be due to this phylogenetic species. Nevertheless, in its native area the dark morph is
35 supposed to be outcompeted when sympatric with the light morph of *R. decollata*. This issue is
36 briefly discussed and the Iberian Peninsula is tentatively proposed as an important source for
37 introduced *R. decollata* populations outside Europe.

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39 *Keywords:* *Rumina decollata*, land snail, introduction, color morphs, DNA sequence analysis.

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45 **Introduction**

46 The decollate snail, *Rumina decollata* (Linnaeus, 1758), is a hermaphroditic terrestrial
47 gastropod, capable of both self- and cross-fertilization (mixed breeding) (Selander and Ochman
48 1983). The species is native in the Mediterranean region (e.g. Selander and Kaufman 1973),
49 where it occurs in the Iberian Peninsula, southern France, Italy, the western Balkan and northern
50 Africa (Pilsbry 1905). It has also been introduced in other parts of the world (Figure 1; Table 1,
51 Matsukuma and Takeda 2009).

52 *Rumina decollata* is an efficient colonizer in part due to its ability to self-fertilize (Selander and
53 Kaufman 1973). It is well adapted to many “Mediterranean” soil types and it appears that the
54 temperature and moisture are the limiting factors in its distribution (Batts 1957). Decollate snails
55 are omnivorous and feed on plants, snails, slugs and worms, and may be cannibalistic (Fisher
56 1966). Since *R. decollata* is able to rapidly build up very dense populations and predate on
57 other snails, it may have a negative impact on local native land snail communities in newly
58 colonized areas (Bar-Zeev and Mienis 2007). In the USA *R. decollata* has even been regarded as
59 a biological agent to control *Cornu aspersum*, a widespread agricultural and horticultural pest
60 snail. Therefore, *R. decollata* was intentionally introduced in California in the 1970s (Cowie
61 2001). However, the evidence of its effectiveness as biological control agent is weak (Fisher and
62 Orth 1985), particularly since *R. decollata* does not only prey on *C. aspersum*, but also on many
63 other snail species (Fisher and Orth 1985). Nevertheless, there are no rigorous scientific studies
64 demonstrating that *R. decollata* has any impact on native snail populations (Cowie 2001).
65 Despite *R. decollata* is believed to be a highly sedentary species (Selander and Hudson, 1976), it
66 has been recorded moving as much as 3 to 5 km per year (Matsukuma and Takeda 2009),
67 probably by passive means (e.g. human transport).

68 Although *R. decollata* is common, widespread and (supposedly) well-known, recent
69 phylogenetic analyses of DNA sequence data have shown that in fact it is a complex of several
70 phylogenetic species (PS), six of which are currently referred to as clades A to F (Prévot et al.
71 2013a). Two of these PS occur sympatrically in southern France, where their population genetics
72 have been studied in the Montpellier area (Selander and Kaufman 1973; Selander and Hudson
73 1976; Selander and Ochman 1983; Prévot et al. 2013b). Both PS were previously regarded as
74 color morphs of *R. decollata* that, through maintained self-fertilization, appeared as fixed
75 homozygous multilocus genotypes, differing at 13 out of 26 allozyme loci (Selander and Hudson
76 1976). Although these allozyme data also indicate that both color morphs occasionally hybridize,
77 subsequent mitochondrial and nuclear DNA sequence data confirmed that the two taxa behave as
78 highly divergent, well-supported PS (i.e. clades A and Eb) (Prévot et al. 2013a). As such, the two
79 PS can be easily distinguished by (i) their body color (black body with a dull olive-gray foot for
80 clade A vs. gray body with a pale yellow foot for clade Eb), (2) several diagnostic allozymes
81 (Selander and Hudson 1976; Prévot et al. 2013b), (3) species-specific esterase profiles resolved
82 by isoelectric focusing (Prévot et al. 2013b) and (4) their haplotype composition (and
83 divergence) at several mitochondrial (12S rDNA, 16S rDNA, COI, CytB) and nuclear (ITS1,
84 ITS2) genes (Prévot et al. 2013a). Clade A has been recorded in the Iberian Peninsula, Southern
85 France and Tunisia, while clade Eb seems to be restricted to Southern France and Eastern Spain
86 (Figure A1). Prévot et al. (2013b) also confirmed that in Montpellier both morphs have a mixed
87 breeding system, which may be an advantage for colonization since selfing gastropods appear
88 better colonizers (Baur and Bengtsson 1987). The other PS within *R. decollata* are currently only
89 diagnosable by DNA sequence data (Prévot et al. 2013a), and their geographic distribution so far
90 known is summarized in Figure A1. In view of the new taxonomic interpretation of *R. decollata*
91 the question arises as to which of the six PS have established non-indigenous populations outside

92 the Mediterranean area. Therefore, the present contribution uses DNA sequence data to identify
93 *R. decollata* in various regions of the world, where the species complex is supposed to have been
94 introduced.

95

96 **Materials and Methods**

97 Specimens of *R. decollata* ($n = 107$), representing 12 putatively introduced populations from
98 South America, North America, Japan, and several North Atlantic islands, were surveyed (Table
99 2).

100 Snails were hand-picked in the field and either kept alive or stored in 70% or 90% ethanol.
101 Whenever possible, living specimens were photographed and scored for foot and body color.

102 A rarefaction curve of the mtDNA from the native populations studied by Prévot et al. (2013a) (n
103 = 458 specimens) was inferred using EstimateS v. 8.2 (<http://viceroy.eeb.uconn.edu/estimates>) in
104 order to assess to what extent sampling effort yielded a representative coverage of the haplotype
105 richness in *R. decollata*.

106 Genomic DNA was extracted from a small piece of the foot using the NucleoSpin®Tissue Kit
107 (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. PCR
108 amplification and nucleotide sequencing of mitochondrial 12S rDNA, 16S rDNA, COI and CytB
109 gene fragments in 107 specimens and complete nuclear ribosomal internal transcribed spacers
110 ITS1 and ITS2 in 98 specimens were done according to Prévot et al. (2013a).

111 The sequences of the 12 putatively introduced populations were added to the concatenated
112 mtDNA (12S, 16S, COI, CytB) and the concatenated ITS (ITS1, ITS2) alignments of Prévot et
113 al. (2013a), yielding a mtDNA data set involving $458 + 107 = 565$ specimens and an ITS data set
114 involving $430 + 98 = 528$ specimens representing all currently recognized PS in the complex.

115 Bayesian Inference (BI) and Maximum Likelihood (ML) trees were derived in exactly the same

116 way and under the same conditions as in Prévot et al. (2013a), except for the number of
117 generations (5 000 000 for mtDNA and 500 000 for ITS) and the sample frequency (500 for
118 mtDNA and 50 for ITS) in BI. Trees were rooted with the land snail *Subulina octona* (Bruguière,
119 1798) (Subulinidae). Only tree branches supported by posterior probabilities of ≥ 0.95 for BI
120 (Huelsenbeck and Rannala 2004) and/or bootstrap values of $\geq 70\%$ for ML (Hillis and Bull
121 1993) were considered to be meaningful. MEGA v. 4.0 (Tamura et al. 2007) was used to
122 calculate p-distances and their standard error (excluding gaps and missing data).

123 All specimens used in this study have been deposited in the collections of the Royal Belgian
124 Institute of Natural Sciences, Brussels, under catalogue number IG 31 791. DNA sequences have
125 been deposited in GenBank (Accession numbers: KC833684-KC833749).

126

127 **Results**

128 Of the 107 specimens surveyed in the putatively introduced populations, 33 (from the Azores
129 and the Canary Islands) were photographed and scored for their color. They all had the typical
130 black body and dull olive-gray foot of *R. decollata* from clade A as defined in Prévot et al.
131 (2013a).

132 After incorporation of the new sequences in the sequence alignment of Prévot et al. (2013a), the
133 resulting concatenated mtDNA dataset comprised 1719 bp, 768 of which were polymorphic and
134 741 were parsimony informative. This alignment comprised 90 different haplotypes, nine of
135 which occurred in the 107 specimens from the putatively introduced populations. Eight
136 haplotypes of these latter specimens were not yet recorded by Prévot et al. (2013a). Only
137 haplotype Ca from Riverside (California, USA) was previously detected and was identical to
138 haplotype A13 of clade A, from the Iberian Peninsula (Portuguese populations) (Prévot et al.
139 2013a). In three localities two haplotypes co-occurred, viz. São Paulo (Brazil: Br1.a and Br1.b),

140 Vila Baleira (Porto Santo: PS.a and PS.b), and Gran Canaria (Can.a and Can.b) (Table 3).
141 Haplotype Br1.b also occurred in the Brazilian populations from Porto Alegre (Br1.b = Br2, Br3)
142 and Curitiba City (Br1.b = Br4), while haplotype PS.b (= Az1, Az2 and Az3) was the only one
143 observed in the three Azorean populations (Table 2). The rarefaction curve (Figure 2) suggested
144 that the current sampling still underestimates the actual haplotype diversity in native populations
145 of *R. decollata*.

146 The concatenated ITS sequences of the putatively introduced specimens were incorporated in the
147 ITS alignment of Prévot et al. (2013a). The resulting alignment comprised 957 bp, 269 of which
148 were polymorphic and 32 were parsimony informative. As such the ITS alignment contained 36
149 distinct haplotypes, five of which occurred in the 98 putatively introduced specimens scored for
150 ITS (Table 2). Two of these latter haplotypes (Argentina and Brazil: Arg and Br1, Br2, Br3, Br4)
151 were not observed by Prévot et al. (2013a), the three others were identical to haplotypes nA1,
152 nA3 and nA8 of clade A, that were recorded in the Iberian Peninsula (Prévot et al. 2013a). All
153 putatively introduced populations consisted of single ITS haplotypes, which were sometimes
154 shared by very distant populations, e.g. haplotype "Jap, Az2, Az3, Can" from Japan, Azores and
155 Gran Canaria clustered with the Maltese haplotype nA9 from Prévot et al. (2013a).

156 Both the mtDNA and ITS trees (Figs 3-4) illustrate that all haplotypes from putatively
157 introduced populations were unambiguously nested with high support within *R. decollata* clade
158 A of Prévot et al. (2013a). Yet, the haplotypes from putatively introduced populations themselves
159 did not constitute a monophyletic unit and did not show any particular pattern in their branching
160 order, except for the surprisingly deep divergence and monophyly of the two mtDNA haplotypes
161 from Gran Canaria (Can.a and Can.b). These two haplotypes were separated from the other
162 haplotypes of clade A by a mean p-distance of 0.050 ± 0.005 , while the mean p-distance among
163 the other clade A haplotypes was 0.013 ± 0.002 .

164

165 **Discussion**

166 Our results indicate that all putatively introduced populations of *R. decollata* surveyed here
167 belong to clade A as defined by Prévot et al. (2013a). This is the phylogenetic species with the
168 black body and dull olive-gray foot, i.e. the dark morph of Selander and Hudson (1976) and
169 Selander and Ochman (1983). For all these populations the status of being introduced seems
170 undisputed except perhaps for the population from Gran Canaria (Canary Islands: Can). Indeed,
171 although as far as we know there are no fossil records of *R. decollata* from Gran Canaria, the
172 species has been repeatedly reported from Quaternary fossil beds in Lanzarote, Fuerteventura,
173 La Graciosa, and Montaña Clara (Yanes et al. 2004, 2008). Hence, at least in the eastern islands
174 of the Canary Archipelago *R. decollata* may be native. Therefore, it remains to be decided
175 whether or not this also applies to Gran Canaria. Anyway, it appears that the colonizing ability of
176 *R. decollata* is largely, if not entirely, due to the PS of clade A. This is in line with the
177 observation that all putatively introduced specimens of which the color could be recorded, had
178 the dark morphotype typical of clade A. This result is surprising since Selander and Ochman
179 (1983) claimed that the dark morph has a lower reproductive potential than the light morph of *R.*
180 *decollata* [= clade Eb from Prévot et al. (2013a)]. Indeed, lab experiments suggested that the
181 light morph has a faster embryonic and posthatching development than the dark morph, so that it
182 starts reproducing at a much younger age (Selander and Ochman 1983). Moreover, Selander and
183 Ochman (1983) observed an increase of the proportion of the light morph, and a decrease of the
184 dark morph, in 12 colonies in Montpellier (southern France) (where both morphs co-occur) that
185 had been studied earlier by Selander and Hudson (1976). This suggests that the light morph
186 outcompetes the dark morph. Hence, one would expect that the taxon with the higher
187 reproductive potential (i.e. the light morph of clade Eb) should be the better colonizer. Therefore,

188 the apparent colonizing success of the PS of clade A is probably due to other features of its
189 biology that outweigh its alleged lower reproductive potential. For example, Selander and
190 Hudson (1976) stated about the dark morph that “*although the frequency of laying is roughly*
191 *equal, clutch size is consistently larger in the dark strain. The data also suggest that the number*
192 *and total weight of eggs laid per day are larger in the dark strain. Individual egg weight does*
193 *not differ between the strains.*” Obviously, if this claim is correct, then the higher daily egg
194 production of the dark morph could counteract or even overwhelm the effects of its slower
195 development and older age at reproduction, so that it may become the more efficient colonizer.
196 Moreover, still other, unknown, biological features of the dark morph may be at the base of its
197 colonizing ability (e.g. vulnerability to parasites and diseases, ecological plasticity, climatic
198 tolerance, active dispersal capacity, breeding system, etc.). Hence, further studies on the life-
199 history and breeding system of the dark and light morphs of *R. decollata* are needed. For
200 example, it is often suggested that r-strategists are better colonizers (e.g. Parsons 1982), even if
201 the relation between life-history traits and colonization success is not straightforward (Baur and
202 Bengtsson 1987). Similarly, self-fertilizing species appear to be disproportionately represented
203 among colonizing terrestrial slugs and snails (Foltz et al. 1984). However, as both the dark (clade
204 A) and the light (clade Eb) morphs of *R. decollata* in the Montpellier area (France) are capable
205 of self-fertilization (Selander and Hudson 1976; Prévot et al. 2013b) it seems unlikely that this
206 would be the main cause of their different colonization success. Nevertheless, this issue requires
207 further scrutiny since Prévot et al. (2013b) suggested that the degree of self-fertilization in *R.*
208 *decollata* is less than that inferred from the allozyme data of Selander and Kaufman (1973) and
209 Selander and Hudson (1976). As such, it may be possible that the dark and the light morphs
210 differ in their propensity to self-fertilize and therefore have different colonization capacities.
211 However, the current mtDNA data provide no information on this issue.

212 Alternatively, given that the dark and the light morphs both co-occur in a large part of their
213 supposedly native range (southern France and the eastern part of the Iberian peninsula), one
214 could assume that both taxa have the same chance to be passively introduced (e.g. human
215 activities). In the latter case, introduced populations either should more often be mixtures of both
216 morphs or should at least show a more balanced representation of dark and light populations.
217 Yet, the fact that this was not observed again suggests that the dark morph may have a number of
218 unrecognized features that preadapt it better as colonizing species than the light morph. Finally,
219 one might speculate that the introduction of clade A, but not Eb, specimens outside the native
220 area of *R. decollata* is simply due to the possibility that during the Portuguese and Spanish
221 colonization period, snails were transported by ship from places where only clade A occurs, i.e.
222 the Atlantic coasts of the Iberian Peninsula. Obviously, these speculations on the relative
223 colonizing ability of the PS of *R. decollata* emphasize the need for comparative ecological
224 studies of invasive and co-occurring non-invasive animal taxa in their native regions (e.g.
225 Schlaepfer et al. 2010; van Kleunen et al. 2010, 2011).

226 Although the present data do not allow to accurately pinpoint the areas of origin of the putatively
227 introduced populations, they do suggest that: (1) the single mtDNA and the three ITS haplotypes
228 from the native European area that were recorded elsewhere, all occur in the Iberian Peninsula,
229 so that the Iberian Peninsula may be an important source area; (2) the positioning of the
230 Brazilian and one Porto Santo mtDNA haplotypes among Iberian haplotypes, also points to the
231 Iberian Peninsula as a possibly important source area; (3) the association of the Argentinian
232 mtDNA haplotype with a majority of haplotypes from southern France indicates that this may be
233 another source area; (4) the peculiar association between the single mtDNA haplotype from
234 Malta, with the mtDNA haplotype of the Azores (+ PS.b from Porto Santo) and between the
235 single ITS haplotype from Malta, with the single ITS haplotype in the Azores, Gran Canaria and

236 Japan, tentatively suggests that the Maltese haplotype could be the result of an introduction as
237 well, even if the origin of the ancestral haplotype cannot be inferred from the current data and
238 even if *R. decollata* is well-represented in Quaternary deposits of Malta (e.g. Giusti et al. 1995);
239 (5) the relatively large number of supposedly introduced mtDNA haplotypes, which were not
240 (yet) recorded in the native range of *R. decollata*, may involve either rapid local evolutionary
241 divergences or undersampling of the native range, though the rarefaction curve strongly supports
242 the latter suggestion; (6) finally, the deep, monophyletic branching of the two “endemic” mtDNA
243 haplotypes in the Canary Islands (with their relatively high p-distance compared to the other
244 haplotypes in clade A) could either reflect a rapid local divergence after a recent introduction or,
245 and this may be more likely, an endemic clade derived from native populations in some of the
246 eastern islands of the Canary Archipelago (e.g. Backhuys 1975), where *R. decollata* has been
247 recorded in Quaternary fossil beds (e.g. Yanes et al. 2004, 2008). Yet, as the rarefaction curve
248 shows that the sampling of native populations is too limited to represent accurately overall
249 haplotype diversity, it is impossible to dismiss either possibility.

250 Obviously, both the tracing of the source areas of introduced populations and the occurrence of
251 rapidly diverging “endemic” haplotypes of *R. decollata* need further scrutiny based on a far more
252 intensive sampling of haplotypes in introduced and native populations over the entire range of
253 this species complex. Such an endeavor will also have to focus on the closely related
254 morphospecies *R. saharica* Pallary, 1901 [clades Sa and Sb in Prévot et al. (2013a)], a species
255 that is native in the eastern part of the Mediterranean, but that recently has been reported from
256 e.g. Madeira (Seddon 2008), the Balearic Islands (Quintana Cardona 2006), France (Mienis
257 2008), and Sicily (Liberto et al. 2012).

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269

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382 235–256.

385 **Table 1** Areas where *R. decollata* as been reported as introduced species.

Place	Introduction history
Argentina	First recorded in 1988, in the northeastern Pampas of Buenos Aires Province (Miquel 1988), subsequently in the region of La Pampa and Mendoza provinces in central-western Argentina (De Francesco and Lagiglia 2007)
Azores	Nobre (1930); Backhuys (1975)
Brazil	Simone (2006)
Bermuda	Possibly introduced prior to 1876 (Simmonds and Hughes 1963, Bieler and Slapcinsky 2000)
Britain	Found in March 2005 at Caerphilly, South Wales (Seddon and Pickard 2005)
Canary Islands	Maybe introduced in some islands (Backhuys 1975) even if Quaternary fossils were recorded in the eastern islands of the Canary (e.g. Yanes et al. 2004; Yanes et al. 2008)
Cape Verde	Morelet (1873); Nobre (1930); Panelius (1958)
P.R. China	Reported in Shangai in 1978 (Chen and Gao 1987 as <i>Tortaxis trunciformis</i>) and in 1989 (Beckmann 1989), and in Xian in 2007 (Bar-Zeev and Mienis 2007)
Cuba	Jaume (1953); Selander and Kaufman (1973); Espinosa and Ortea (2009)
Israel	Reported in the garden of the Terra Sancta Monastery in Jerusalem (Bar-Zeev and Mienis 2002; Singer and Mienis 1993)
Japan	Reported the first time in 1982 (Azuma 1982), followed by Mashino (1992, 2001) and Matsukuma and Takeda (2009)
Madeira	Nobre (1930); Seddon (2008)
Mexico	Thompson(2011)

South Africa	Introduced before 1897 (Melvill and Ponsonby, 1898) but has been eradicated since (Herbert 2010)
Uruguay	Reported in 1995 (Miquel et al. 1995)
USA	<i>R. decollata</i> was first reported in North America in 1813; by 1915 it had spread westward from South Carolina and northern Florida through Texas and southern Oklahoma (Fisher 1966, Dundee 1970, Selander and Kaufman 1973). It was first reported in Arizona in 1952 and in California in 1966 (Fisher 1966, Selander and Kaufman 1973). <i>R. decollata</i> was intentionally introduced in California as biological control in the 1970s (Cowie 2001, Tupen and Roth 2001)

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389 **Table 2** Putatively introduced populations analyzed in this work. Country, locality, population name
 390 (pop), body color of the specimens (Color), mtDNA haplotypes (mtDNA), ITS haplotypes (ITS) and
 391 number of specimens analyzed (*n*) for mtDN and for ITS. ns – not scored. Haplotypes that were
 392 previously scored from the native area by Prévot et al. (2013a) are in bold.
 393

Country	Locality	Pop	Color	mtDNA	<i>n</i>	ITS	<i>n</i>
Argentina	Mendoza	Arg	ns	Arg	7	Arg	7
Brazil	São Paulo	Br1	ns	Br1.a	14	Br1 ^c	14
				Br1.b ^a			
	Porto Alegre	Br2	ns	Br2 ^a	4	Br2 ^c	4
				Br3	4	Br3 ^c	4
	Curitiba City	Br4	ns	Br4 ^a	4	Br4 ^c	4
Japan	Hakozaki, Fukuoka	Jap	ns	Jap	15	Jap^d	15
Portugal	Livramento, São Miguel, Azores	Az1	Dark	Az1 ^b	9	-	-
	Ponta Delgada, São Miguel, Azores	Az2	Dark	Az2 ^b	11	Az2^d	11
	Livramento, São Miguel, Azores	Az3	Dark	Az3 ^b	8	Az3^d	8
	Vila Baleira, Porto Santo	PS	ns	PS.a	16	PS	16
				PS.b ^b			
Spain	Jardin Botanico, Gran Canaria, Canary Islands	Dark	Can.a	Can.a	5	Can^d	5
			Can.b	Can.b			
U.S.A.	Riverside, California	Ca	ns	Ca	10	Ca	10

394 ^a – identical mtDNA haplotypes: “Br1.b,Br2,Br3,Br4”

395 ^b – identical mtDNA haplotypes: “Az1,Az2,Az3,PS.b”

396 ^c – identical ITS haplotypes: “Br1,Br2,Br3,Br4”

397 ^d – identical ITS haplotypes that were previously scored by Prévot et al. (2013a): “Jap,Az2,Az3,Can”-

398 Corresponding haplotypes in Prévot et al. (2013a): nA8

399 **Figure Legends**

400 **Fig. 1** Map showing the native distribution of *Rumina decollata* (dark area), the localities where it
401 was reported outside its native range (dark circles), places outside its native range analyzed
402 within this work (white circles), places where fossils were reported outside the native area (x)
403 and places where the species was introduced but has been eradicated(†).

404

405 **Fig. 2** Rarefaction curve plotting the number of individuals sampled against mitochondrial
406 haplotype richness. Dashed lines are 95% confidence intervals.

407

408 **Fig. 3** Phylogenetic relationships of *Rumina* taxa based on Maximum Likelihood and Bayesian
409 inference of concatenated mtDNA (12S, 16S, COI and CytB) haplotypes. Phylogenetic species
410 of *R. decollata* are marked by the letters A to F with their geographic origin in parentheses.
411 Haplotype names are as in Prévot et al. (2013a), followed by the population abbreviations.
412 Population abbreviations start with the country name, i.e, P: Portugal, S: Spain, F: France, T:
413 Tunisia and M: Malta, followed by an initial of the locality where the population was found
414 (Table A1) and additional letters “.a”, “.b”... in case different haplotypes occurred at that
415 locality. Support values (bootstrapping for ML and posterior probabilities for BI) are indicated
416 by circles near the nodes. Black circles: significant support for both ML and BI; circles with left
417 half black: significant support only for ML; circles with right half black: significant support only
418 for BI; white circles: no significant support. Haplotypes from introduced populations are marked
419 in grey. Scale bars show sequence divergence for BI.

420

421 **Fig. 4** Phylogenetic relationships of *Rumina* taxa based on ML and BI of concatenated ITS (ITS1
422 and ITS2) haplotypes. Support values and haplotype names are as in Fig. 1. Haplotypes from

423 introduced populations are marked in grey. Scale bars show sequence divergence for BI.

424 **Table A1** Native populations of *Rumina decollata* from Prévot et al. (2013a). Locality, population name
 425 (pop), number of specimens analyzed (*n*), number of concatenated mtDNA haplotypes (NH_{mtDNA}),
 426 phylogenetic species with the mtDNA data (PS_{mtDNA}), number of concatenated ITS haplotypes (NH_{ITS}) and
 427 phylogenetic species with the ITS data (PS_{ITS}). Between brackets the numbers of specimens per haplotype
 428 in alphabetic order (a, b, c,... as used in the phylogenetic trees).

Country	Locality	Pop	<i>n</i>	NH _{mtDNA}	PS _{mtDNA}	NH _{ITS}	PS _{ITS}
Algeria	Unknown	Alg1	8	1	C	ns	-
	Unknown	Alg2	8	1	Ea	1	Ea
Croatia	Komiza, Vis Island	Cro2	11	1	F	1	F
	Milna, Brac Island	Cro1	4	1	F	1	F
France	Cotignac	FM1	11	1	A	1	A
		FM2	5	1	A	3(2,2,1)	A,A,A
	Marseille	FM3	10	2(3,7)	Eb,A	2(3,7)	Eb,A
	Montpellier Arceaux 1	FmtA1	10	2(3,7)	Eb,Eb	2(3,7)	Eb,Eb
	Montpellier Arceaux 2	FmtA2	10	1	Eb	1	Eb
	Montpellier Botanique 1	FmtB1	13	3(4,1,8)	Eb,Eb,A	hetero	A/Eb
	Montpellier Botanique 2	FmtB2	26	2(25,1)	A,Eb	1(25), ns(1)	A,-
	Montpellier Cimetière 1	FmtC	20	3(16,2,2)	Eb,A,Eb	3(16,2,2)	Eb,A,Eb
	Montpellier Lycée de Joffre	FmtL	13	1	Eb	1	Eb
	Montpellier Spot 1	FmtS	12	1	Eb	hetero	A/Eb
Italy	Gargano	ItG	7	1	F	1	F
	Napoli	ItNp1	11	1	F	1	F
	Roma	ItRo1	2	1	F	1	F
Malta	Dingli	M1	19	1	A	1	A
Morocco	Aazanèn	Mrc3	2	2(1,1)	D	2(1,1)	D

	Beni Snassen	Mrc1	3	3(1,1,1)	D	1	D
	Marrakech	Mrc4	14	1	B	2(9,5)	B
	Tafersite	Mrc2	1	1	D	1	D
<hr/>							
	Carvoeiro	PCa1	7	1	A	1	A
	Carvoeiro	PCa2	8	1	A	1	A
	Laranjeiro	PL2	5	1	A	2(3,2)	A,A
	Laranjeiro	PL3	3	1	A	ns	-
	Laranjeiro	PL4	2	1	A	ns	-
	Lousã	PLo	8	1	A	ns	-
Portugal	Luz de Tavira	PLt1	3	1	A	1	A
	Luz de Tavira	PLt2	2	1	A	1	A
	Moncarapacho	PM1	2	2(1,1)	A,A	2(1,1)	A,A
	Moncarapacho	PM2	10	1	A	1	A
	São braz de Alportel	PSB	7	1	A	1	A
	Silves	PSi	8	4(4,1,2,1)	A,A,A,A	1	A
	Tavira	PTv	11	2(6,5)	A,A	2(6,5)	A,A
<hr/>							
	Almuñécar	SAl1	2	2(1,1)	A,A	1	A
	Almuñécar	SAl2	4	1	A	1	A
	Artziniega	SAr	16	1	A	1	A
	Ayamonte	SA	3	2(1,2)	A,A	1	A
Spain	Benalmadena (Malaga)	SBe	9	2(4,5)	A,A	1	A
	La Herradura	SHe	3	1	A	1	A
	Los Mases, Castellón	SC2	5	1	Eb	2(1,4)	Eb,Eb
	Mas de Borràs, Castellón	SC1	6	2(3,3)	Eb,Eb	1	Eb
	Torrenueva de Motril	STo	3	1	A	1	A

	Valencia	SV2	8	1	Eb	1	Eb
	Beja	T6	4	1	C	1	C
	Beni Metir	T9	6	2(1,5)	C,C	1	C
	Bir El Bey	T5	2	2(1,1)	Ea,F	1,ns	Ea,-
	Dar chichou	T8	1	1	C	1	C
	Oudhna	T7	2	2(1,1)	C,C	1	C
Tunisia	Oued sejnene	T10	3	3(1,1,1)	C,C,C	1	C
	Oasis Tozeu	T12	6	1	C	1	C
	Tunis	T4	7	1	A	1	A
	Utique	T11	3	2(2,1)	C,C	2(2,1)	C,C
	Zaghouan	T1	5	2(4,1)	Ea,C	2(4,1)	Ea,C
	Zaghouan2	T2	2	1	Ea	1	Ea
	Zaghouan3	T3	3	2(1,2)	Ea,Ea	2(1,2)	F,Ea