Resolving riddles and presenting new puzzles in Chonorinidae Phylogenetics

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HISTORICAL BIOGEOGRAPHY OF THE LAND SNAIL GENUS *CHONDRINA* (GASTROPODA, PULMONATA, CHONDRINIDAE)

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INTRODUCTION

Limestone habitats are of the utmost importance for many species of land snails. The calcium concentrations in such areas are favourable, if not obligatory, for many species. Consequently, species diversity can be 2-10 times higher than in comparable non-calcareous areas (Schilthuizen et al., 2003; Fontaine et al., 2007). Calcareous soils, mostly rocky outcrops, have an island-like geographical distribution. Therefore, local endemism can also be relatively high (Gittenberger, 2007 and other references therein).

There is a strong niche conservatism in the land snail species of the genus *Chondrina* Reichenbach, 1828 (Gastropoda, Chondrinidae). The genus clearly exemplifies a non-adaptive radiation (Gittenberger, 1991, 2004). All species are xerophilous and obligate limestone dwellers, mainly found on more or less vertical, exposed limestone cliffs. Many species or subspecies have relatively small distributional ranges, but some are widely distributed. In Spain for instance, many of the taxa are known from a single 'sierra' only. Nevertheless, their density at any suitable habitat can be very high (fig. 1).

The genus *Chondrina* consists of about 37 extant species. The centres of its diversity are in the Iberian peninsula (including the French part of the Pyrenees) and Morocco, the Italian part of the Alps and the Balkans. In Spain 13 species are currently recognized, with another three in Morocco and one in Portugal. Seven are resticted to the Alps and northern Italy and three can be found in the Balkans, Caucasus and Greece (fig. 2). Two polytypic species include subspecies that have unusually large distributions. *Chondrina avenacea avenacea* (Bruguière, 1792) occurs from Spain (Valencia) in the south to Belgium and southern Germany in the north. The eastermost part of its range is in Thüringen. In southern Germany it is known to occur in sympatry with *Chondrina arcadica clienta* (Westerlund, 1883), which is also widespread, occurring from Öland (Sweden) in the north to Bulgaria and Greece in the southeast.

The monophyly of *Chondrina* has already been verified with DNA sequencing data (Kokshoorn & Gittenberger [Chapter 2]). However, the phylogenetic relationships between the various species were still unclear. Here we present a molecular phylogeny reconstruction of nearly all extant species in *Chondrina*. On the basis of these data, the historical biogeography of the *Chondrina* taxa is dealt with for the first time.

MATERIAL AND METHODS

THE SPECIES

Most *Chondrina* species could be included in the molecular analyses. Exceptions are *C. oligodonta* (Del Prete,1879), *C. bergomensis (Küster, 1850), C. generosensis*

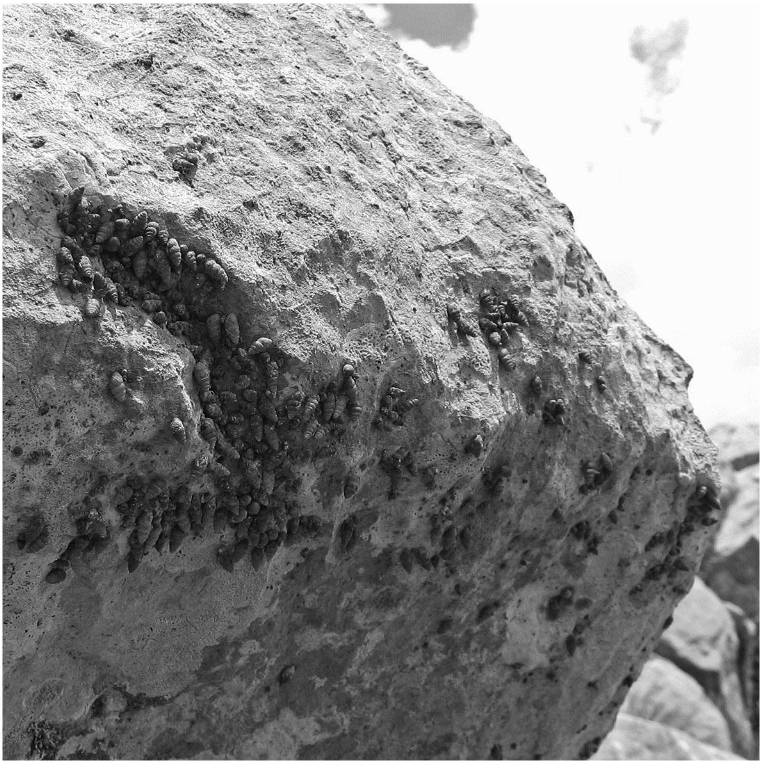


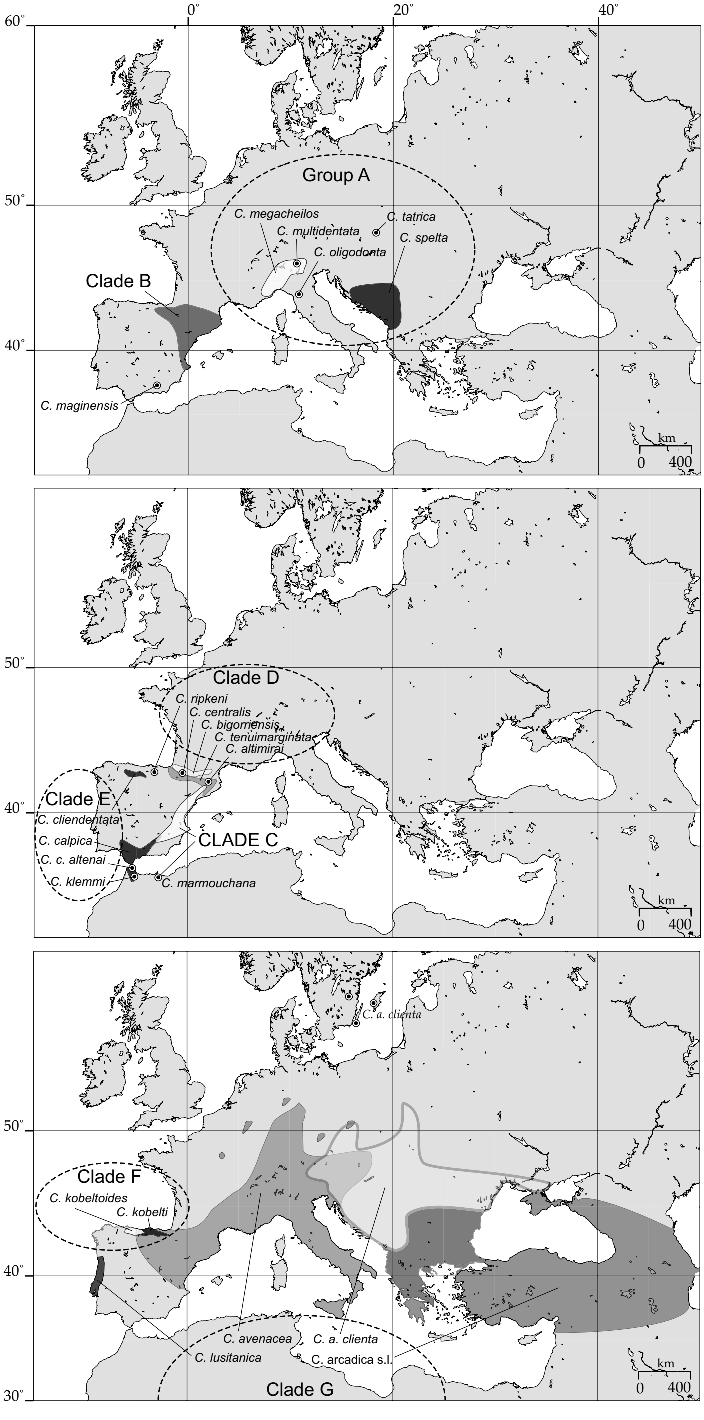
Figure **1**. High density of individuals (and huddling) can for instance be observed in *Chondrina calpica* (Andalucia, Malaga, Antequera). Photo: Inge Erkelens.

Nordsieck, 1962, *C. falkneri* Gittenberger, 2002, *C. gerhardi* Gittenberger, 2002, and *C. amphorula* Schileyko, 1984. Therefore, these species of uncertain affinities cannot be dealt with in the biogeographical considerations.

SAMPLES AND DNA EXTRACTION

Material of the majority of the Iberian species and subspecies was sampled during three fieldwork periods in 2004-2006. Additional material of *Chondrina* species from elsewhere was obtained from the collection of the National Museum of Natural History (Naturalis) in Leiden, The Netherlands, from the private collection of Mr. W.J.M. Maassen, Duivendrecht, The Netherlands, and from the collection of

Figure **2**. Distribution of the *Chondrina* species used in this study grouped by clade (see fig. 3).


0°
20°
40°
60°
50°
40°
Group A
C. megacheilos
C. multidentata
C. tatrica
C. spelta
C. oligodonta
Clade B
C. maginensis
km
0
400
50°
40°
Clade D
C. ripkeni
C. centralis
C. bigorriensis
C. tenuimarginata
C. altimirai
Clade E
C. cliendentata
C. calpica
C. c. altenai
C. klemmi
CLADE C
C. marmouchana
km
0
400
C. a. clienta
50°
40°
30°
Clade F
C. kobeltoides
C. kobelti
C. avenacea
C. a. clienta
C. arcadica s.l.
C. lusitanica
Clade G
km
0
400

Mr. G. Falkner, deposited in the Staatliches Museum für Naturkunde in Stuttgart, Germany.

Up to 5 individuals of each sample were preserved in 96% ethanol for DNA extraction (table 1). The remaining snails were either relaxed and preserved in 70% ethanol for anatomical study, or dried for shell-morphological analyses.

The shells were broken in two parts to extract the snail tissue. Shell remains, including the undamaged aperture, which carries key features for identification, were stored as vouchers. Then the snail tissue was fragmented and genomic DNA was extracted using the Qiagen tissue kit for recently collected, alcohol stored material. Dry snail remains were extracted using the E.Z.N.A. Mollusc DNA kit (Omega Bio-tek). Final elution of the DNA was done in 200 ul of provided buffer.

A 637 bp fragment of the Cytochrome Oxidase I (COI) gene was amplified using primers H2198-alb (5′-ACT CAA CGA ATC ATA AAG ATA TTG G-3′) and L1490-alb (5′-TAT ACT TCA GGA TGA CCA AAA AAT CA-3′) (Uit de Weerd et al., 2004). The PCR products were cleaned using the Nucleospin Extract II kit (Clontech). Sequences were subsequently obtained by direct sequencing on a Megabace 1000, 96 capillary sequencer. Some products were sequenced by Macrogen, Seoul, Korea.

All *Chondrina* sequences were deposited with GenBank (for accession codes see table 1).

Phylogenetic analyses

Both forward and reverse strands of the DNA were sequenced. The chromatograms were aligned using the Chromas Pro package (Technelysium, Australia). In most cases ambiguous positions could easily be solved. If not, IUPAC ambiguity codes were used. Consensus sequences were exported as fasta file and individual sequences were aligned using Clustal W (Chenna et al, 2003) with default parameters, as incorporated in BioEdit (Hall, 1999). The alignment was saved in fasta format and subsequently imported in MacClade 4.04 (Maddison & Maddison, 2002). All variable base positions were manually checked and compared with the chromatograms to rule out editing errors. Primer sites were omitted from the dataset.

Codon positions were calculated by minimizing the number of stop codons, using the *Drosophila* mitochondrial code. The final alignment was then translated and checked. A test for saturation (Xia et al., 2003) was performed for the entire COI dataset and for the individual codon positions. The proportion of invariable sites estimate was obtained from MrModeltest. Uncorrected pairwise distances for transition and transversion substitutions were plotted to visualise the amount of saturation. Additionally base frequencies were checked for the individual codon positions and for the entire dataset using chi-square statistics implemented in PAUP* (Swofford, 2002). A G1-skewness test (Hillis & Huelsenbeck, 1992) based on 1,000 random trees was used to test for phylogenetic signal. The best fitting model for substitution was calculated using MrModeltest (Nylander, 2004). Phenetic and parsimony phylogenetic analyses were performed using PAUP*. A Neighbor Joining tree was created and subsequently bootstrapped with 1,000 replicates, both using the model of evolution suggested by MrModeltest. A heuristic search was performed and followed by a parsimony bootstrap using 1,000 replicates with ACCTRAN optimization and full heuristic search. The model suggested by MrModeltest was applied in MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) for Bayesian inference. Here 5 incrementally heated chains were used next to a cold one. The program was run until the average standard deviation between the two simultane runs was below 0.01 for a minimum of 1,000,000 generations. An additional 1 million generations were then run with a sample frequency of 100. The initial trees were discarded as burnin and a consensus of the 20,000 remaining trees from both runs was created using the SUMT command. To facilitate both the presentation of the results and the discussion, the tree has been subdivided in species group A and clades B-G (see fig. 3).

DISTRIBUTIONAL DATA

Distributional data for the various taxa were obtained from the collections of the National Museum of Natural History, Leiden. Additional records were taken from the literature (Gittenberger, 1973; Kerney et al., 1983).

RESULTS AND CONCLUSIONS

Minor saturation was found in ingroup vs. outgroup comparison. The ingroup was not saturated. Of the 597 characters, 311 were constant and 286 variable, of which 253 were parsimony informative.

The hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC) gave different solutions as to the best fitting model of nucleotide substitution. The first suggested the symmetrical model with the proportion of invariant sites and the gamma shape parameter (SYM+I+G), while the AIC favoured the general time reversable model with the same parameters (GTR+I+G). It was decided to implement the SYM+I+G model to prevent over-parametrization of the data in the analyses.

The monophyly of the genus *Chondrina* is supported by the molecular data (fig. 3). As a sistergroup to the combined other *Chondrina* species, *C. maginensis* comes out at a basal position in the tree.

Group A is poorly supported and forms a basal polytomy in the clade containing all *Chondrina* species except *C. maginensis*. It is formed by species found in the

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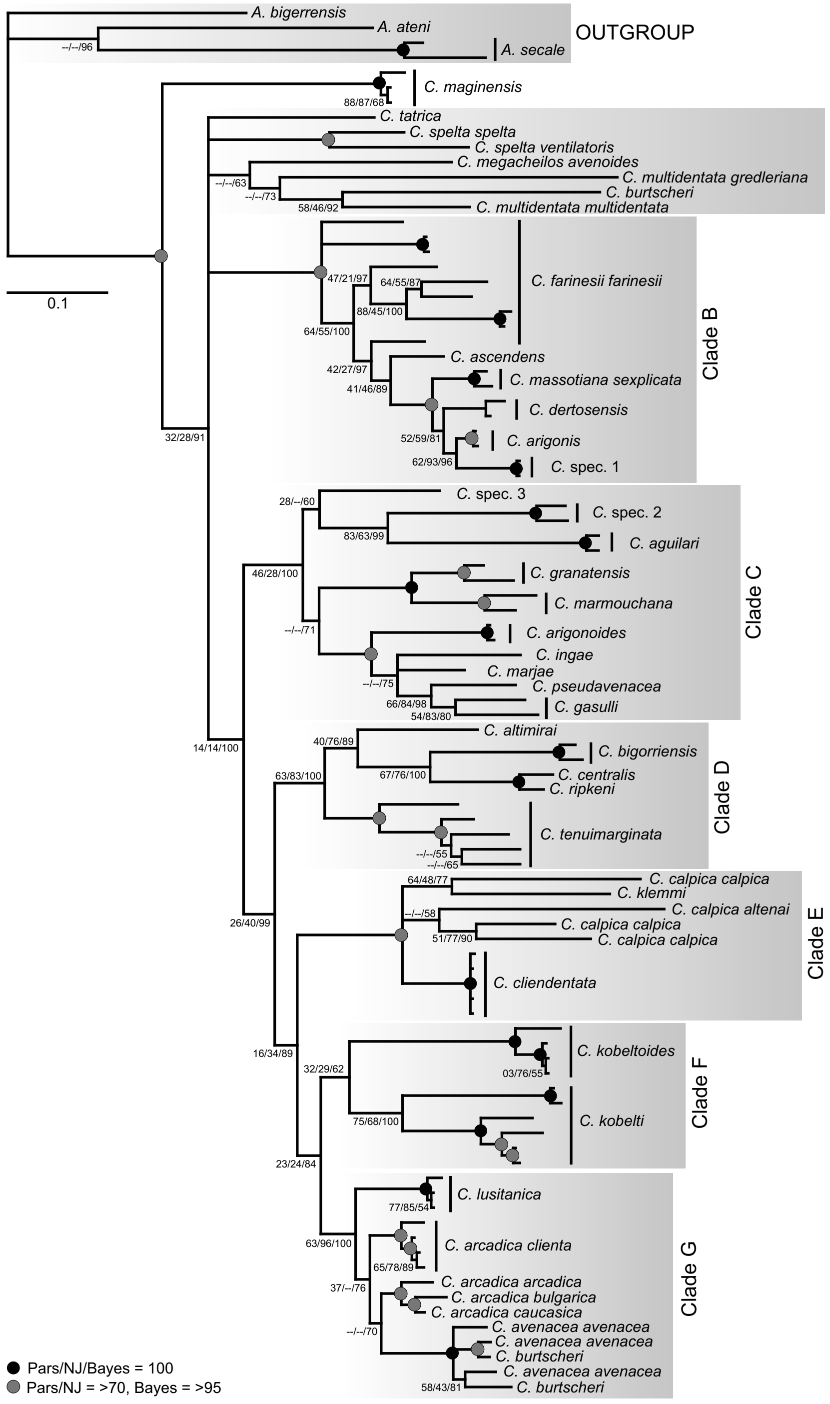
Table **1** (pages 48-51). Samples used in this study.
Samples collected by the authors, unless stated otherwise.

Genbank Accession	RMNH	(sub)species	Locality	Collector	alc/ dry
EU395325	102414	*Abida secale ateni*	France, Pyrénées Atlantiques, Escot	2005	a96
EU395308	103374	*Abida biggerensis*	Spain, Arágon, Candanchu	2006	a96
EU395401	102472	*Abida secale secale*	Belgium, Limburg, St. Pieter mountain	2006	a96
EU395409	106691	*Abida secale secale*	Austria, Nieder-Oesterreich, Weissenbach a/d Triesting	P. Reischütz, 2006	a96
FJ171530	102484	*Chondrina altimirai*	Spain, Catalunya, St Privat d'en Bas	2005	a96
FJ171531	82103	*Chondrina arcadica arcadica*	Greece, Macedonia, Nomos Grevena	Gittenberger & Maassen, 2001	d
FJ171532	104434	*Chondrina arcadica bulgarica*	Bulgaria, Plovdiv, Bachkovski Manastir	Kalkman, 2006	d
FJ171533	x	*Chondrina arcadica caucasica*	Krim, 1 km W of Sudak, N-slope of Mount Bolvan	Kuznetsov, 1992	d
FJ171534	107582	*Chondrina arcadica clienta*	Yugoslavia, Kosovo I Metohija, Vata	Gittenberger, 1986	d
FJ171535	x	*Chondrina arcadica clienta*	Sweden, Öland, Möckelmossen	G. Falkner, 1992	d
FJ171536	x	*Chondrina arcadica clienta*	Sweden, Öland, Möckelmossen	G. Falkner, 1992	d
FJ171537	x	*Chondrina arcadica clienta*	Sweden, Öland, Eketorp	G. Falkner, 1992	d
FJ171538	103231	*Chondrina arigonis*	Spain, Valencia, Marquesa	2006	a96
FJ171539	103231	*Chondrina arigonis*	Spain, Valencia, Marquesa	2006	a96
FJ171540	103300	*Chondrina arigonis*	Spain, Valencia, Calp	2006	a96
FJ171541	103300	*Chondrina arigonis*	Spain, Valencia, Calp	2006	a96
FJ171542	103407	*Chondrina ascendens*	Spain, Pais Vasco, Sobron	2006	a96
FJ171543	103275	*Chondrina avenacea avenacea*	Spain, Valencia, Morella	2006	a96
FJ171544	103404	*Chondrina avenacea avenacea*	Spain, Castilla y Léon, Puerto de Orduña	2006	a96
FJ171545	105217	*Chondrina avenacea avenacea*	France,	2006	a96
FJ171546	102487	*Chondrina bigorriensis*	France, Aude, Axat	2005	a96
FJ171547	103366	*Chondrina bigorriensis*	France, Pyrénées Atlantiques, Arrens	2006	a96
FJ171548	x	*Chondrina burtscheri*	Austria, Vorarlberg, Bludenz, Montikel mountain	G. Falkner, 1982	d

FJ171549	x	*Chondrina burtscheri*	Austria, Vorarlberg, Bludenz, Montikel mountain	2007	d
FJ171550	x	*Chondrina burtscheri*	Austria, Vorarlberg, Bludenz, Montikel mountain	2007	d
FJ171551	102344	*Chondrina calpica altenai*	Morocco, Ceuta, Jebel Musa	J.A.J.H. Nienhuis, 1981	a70
FJ171552	103188	*Chondrina calpica calpica*	Spain, Andalucia, Ronda	2006	a96
FJ171553	103191	*Chondrina calpica calpica*	Spain, Andalucia, Ronda	2006	a96
FJ171554	103214	*Chondrina calpica calpica*	Spain, Andalucia, Malaga, Ventas de Zafarraya	2006	a96
FJ171555	103374	*Chondrina centralis*	Spain, Aragon, Candanchu	2006	a96
FJ171556	102434	*Chondrina farinesii farinesii*	Spain, Catalunya, Castellar de Nuch	2005	a96
FJ171557	102444	*Chondrina farinesii farinesii*	Spain, Catalunya, Saldes	2005	a96
FJ171558	102450	*Chondrina farinesii farinesii*	Spain, Catalunya, La Roca	2005	a96
FJ171559	102472	*Chondrina farinesii farinesii*	Spain, Catalunya, Sadernes	2005	a96
FJ171560	102481	*Chondrina farinesii farinesii*	France, Aude, Gorge de Galamus	2005	a96
FJ171561	102485	*Chondrina farinesii farinesii*	France, Pyrénées Orientales, Coustouges	2005	a96
FJ171562	102486	*Chondrina farinesii farinesii*	Spain, Catalunya, La Pobleta de Bellvei	2005	a96
FJ171563	103217	*Chondrina farinesii farinesii*	Spain, Murcia, Ricote	2006	a96
FJ171564	103220	*Chondrina farinesii farinesii*	Spain, Valencia, Alicante, Biar	2006	a96
FJ171565	103248	*Chondrina farinesii farinesii*	Spain, Valencia, Xativa	2006	a96
FJ171566	103256	*Chondrina farinesii farinesii*	Spain, Valencia, Beijis	2006	a96
FJ171567	103259	*Chondrina farinesii farinesii*	Spain, Valencia, Aranuel	2006	a96
FJ171568	103259	*Chondrina farinesii farinesii*	Spain, Valencia, Aranuel	2006	a96
FJ171569	103278	*Chondrina farinesii farinesii*	Spain, Aragon, Valderobes Beceite	2006	a96
FJ171570	103297	*Chondrina farinesii farinesii*	Spain, Catalunya, Amer	2006	a96
FJ171571	103317	*Chondrina farinesii farinesii*	Spain, Catalunya, La Pobla de Lillet	2006	a96
FJ171572	x	*Chondrina farinesii farinesii*	Spain, Catalunya, Congost de Collegats	2007	d
FJ171573	x	*Chondrina farinesii farinesii*	Spain, Catalunya, Congost de Collegats	2007	d

FJ171574	x	*Chondrina farinesii farinesii*	France, Pyrénées Orientales, La Preste	2007	d
FJ171575	99086	*Chondrina farinesii sexplicata*	Spain, Catalunya, road from Sort to Enviny	2004	a96
FJ171576	103222	*Chondrina gasulli*	Spain, Valencia, Calp	2006	a96
FJ171577	103228	*Chondrina gasulli*	Spain, Valencia, Guadalest	2006	a96
FJ171578	103208	*Chondrina granatensis*	Spain, Andalucia, Tiscar	2006	a96
FJ171579	103211	*Chondrina granatensis*	Spain, Andalucia, Tiscar	2006	a96
FJ171580	102331	*Chondrina klemmi*	Morocco, Chechaouen	J.A.J.H. Nienhuis, 1978	a70
FJ171581	103419	*Chondrina kobelti cliendentata*	Spain, Castilla y Léon, Ventanilla	2006	a96
FJ171582	103422	*Chondrina kobelti cliendentata*	Spain, Castilla y Léon, Santibanez	2006	a96
FJ171583	103425	*Chondrina kobelti cliendentata*	Spain, Castilla y Léon, Espiguëte	2006	a96
FJ171584	103425	*Chondrina kobelti cliendentata*	Spain, Castilla y Léon, Espiguëte	2006	a96
FJ171585	103425	*Chondrina kobelti cliendentata*	Spain, Castilla y Léon, Espiguëte	2006	a96
FJ171586	103410	*Chondrina kobelti kobelti*	Spain, Cantabria, Ramales de la Victoria	2006	a96
FJ171587	103413	*Chondrina kobelti kobelti*	Spain, Cantabria, Aredondo	2006	a96
FJ171588	103416	*Chondrina kobelti kobelti*	Spain, Cantabria, Puerto Alisas	2006	a96
FJ171589	103433	*Chondrina kobelti kobelti*	Spain, Cantabria, Desfiladero de la Hermida	2006	a96
FJ171590	103444	*Chondrina kobelti kobelti*	Spain, Cantabria, Desfiladero de los Beyos	2006	a96
FJ171591	103447	*Chondrina kobelti kobelti*	Spain, Cantabria, Desfiladero de la Sella	2006	a96
FJ171592	103431	*Chondrina kobeltoides*	Spain, Cantabria, Desfiladero de la Hermida	2006	a96
FJ171593	103439	*Chondrina kobeltoides*	Spain, Cantabria, Lago de Ercina	2006	a96
FJ171594	103439	*Chondrina kobeltoides*	Spain, Cantabria, Lago de Ercina	2006	a96
FJ171595	103439	*Chondrina kobeltoides*	Spain, Cantabria, Lago de Ercina	2006	a96
FJ171596	103459	*Chondrina lusitanica*	Portugal, Estremadura, Óbidos	2006	a96
FJ171597	103462	*Chondrina lusitanica*	Portugal, Estremadura, Serra da Arrábida	2006	a96
FJ171598	103462	*Chondrina lusitanica*	Portugal, Estremadura, Serra da Arrábida	2006	a96
FJ171599	103205	*Chondrina maginensis*	Spain, Andalucia, Jean, Sierra de Magina	2006	a96

FJ171600	108829	*Chondrina maginensis*	Spain, Andalucia, Jean, Sierra de Magina	2006	a96
FJ171601	108829	*Chondrina maginensis*	Spain, Andalucia, Jean, Sierra de Magina	2006	a96
FJ171602	102332	*Chondrina marmouchana*	Morocco, Moyen atlas, Qued Meskedal	J.A.J.H. Nienhuis, 1980	a70
FJ171603	102337	*Chondrina marmouchana*	Morocco, Moyen atlas, Talzemt	J.A.J.H. Nienhuis, 1981	a70
FJ171604	102874	*Chondrina megacheilos avenoides*	Italy, Brescia, Toscolano	2005	a96
FJ171605	72606	*Chondrina multidentata gredleriana*	Italy, Belluno, Peron	W.H. Neuteboom, 1968	d
FJ171606	102859	*Chondrina multidentata multidentata*	Italy, Bergamo, Val Taleggio	2005	a96
FJ171607	103394	*Chondrina ripkeni*	Spain, Castilla y Léon, Puerto de Orduña	2006	a96
FJ171608	103303	*Chondrina spec. 1*	Spain, Valencia, Calp	2006	a96
FJ171609	103286	*Chondrina spec. 2*	Spain, Catalunya, Pratdip	2006	a96
FJ171610	103289	*Chondrina spec. 2*	Spain, Catalunya, Llaberia	2006	a96
FJ171611	107583	*Chondrina spelta spelta*	Yugoslavia, Hrvatska, Osojnik	Gittenberger, 1986	d
FJ171612	72717	*Chondrina spelta ventilatoris*	Yugoslavia, Croatia, Krcic	W.H. Neuteboom, 1974	d
FJ171613	94838	*Chondrina tatrica*	Slovakia, Zadielski dolina, Slovak karst	J. Gregor	d
FJ171614	102428	*Chondrina tenuimarginata*	Spain, Catalunya, Coll de Sta Maria de Finestres	2005	a96
FJ171615	102433	*Chondrina tenuimarginata*	Spain, Catalunya, Castellar de Nuch	2005	a96
FJ171616	102443	*Chondrina tenuimarginata*	Spain, Catalunya, Pedraforca	2005	a96
FJ171617	102449	*Chondrina tenuimarginata*	Spain, Catalunya, Camprodon	2005	a96
FJ171618	102453	*Chondrina tenuimarginata*	Spain, Huesca, Broto	2005	a96


A. bigerrensis
A. ateni
A. secale
OUTGROUP
--/--/96
C. maginensis
88/87/68
C. tatrica
C. spelta spelta
C. spelta ventilatoris
C. megacheilos avenoides
C. multidentata gredleriana
C. burtscheri
C. multidentata multidentata
--/--/63
--/--/73
58/46/92
Group A
0.1
C. farinesii farinesii
47/21/97
64/55/87
88/45/100
64/55/100
C. ascendens
42/27/97
C. massotiana sexplicata
41/46/89
C. dertosensis
52/59/81
C. arigonis
62/93/96
C. spec. 1
32/28/91
Clade B
C. spec. 3
28/--/60
C. spec. 2
83/63/99
C. aguilari
46/28/100
C. granatensis
C. marmouchana
--/--/71
C. arigonoides
C. ingae
C. marjae
--/--/75
C. pseudavenacea
66/84/98
C. gasulli
54/83/80
Clade C
C. altimirai
40/76/89
C. bigorriensis
63/83/100
67/76/100
C. centralis
C. ripkeni
14/14/100
C. tenuimarginata
--/--/55
--/--/65
Clade D
64/48/77
C. calpica calpica
C. klemmi
C. calpica altenai
--/--/58
C. calpica calpica
51/77/90
C. calpica calpica
26/40/99
C. cliendentata
Clade E
C. kobeltoides
03/76/55
16/34/89
32/29/62
C. kobelti
75/68/100
Clade F
23/24/84
C. lusitanica
77/85/54
63/96/100
C. arcadica clienta
65/78/89
C. arcadica arcadica
37/--/76
C. arcadica bulgarica
C. arcadica caucasica
--/--/70
C. avenacea avenacea
C. avenacea avenacea
C. burtscheri
C. avenacea avenacea
58/43/81
C. burtscheri
Clade G
● Pars/NJ/Bayes = 100
● Pars/NJ = >70, Bayes = >95

southern Alps, viz. *C. megacheilos* and *C. multidentata*, the Balkans, with *C. spelta*, and the the Tatra in the Carpathian mountains, with *C. tatrica*. One of three specimens of *C. burtscheri* is also found here (compare clade G). Due to the low resolution at the basis of the tree, the relations between these taxa are not clear.

Clade B is retrieved in the parsimony, Neighbor Joining and Bayesian analyses, but with exception of the Bayesian analysis is poorly supported (fig. 3). This clade consists of specimens, referred to as *C. farinesii* s.l., i.e. *C. ascendens*, *C. massotiana sexplicata*, *C. dertosensis*, *C. arigonis* and *C.* spec. 5, that are distributed in the French Pyrenees and the northeastern part of the Iberian peninsula.

Like clade B, clade C is retrieved by all three reconstruction methods, but it is only well supported by the bayesian analysis. It contains taxa from the eastern and southern part of the Iberian peninsula, viz. *C.* spec. 2, *C.* spec. 1, *C. aguilari*, *C. granatensis*, *C. arigonoides*, *C.* spec. 3, *C.* spec. 4, *C. pseudavenacea* and *C. gasulli*, and *C. marmouchana* from Morocco. In this clade, *C. granatensis* from southern Spain and the Moroccan *C. marmouchana* turn out to be sisterspecies.

The relatively large species found in the Pyrenees, viz. *C. tenuimarginata*, *C. altimirai* and *C. bigorriensis* form the well supported clade D, together with the Pyrenean *C. centralis* and the Cantabrian *C. ripkeni*. The shells of these species have a more or less clearly reflexed apertural lip.

In the strongly supported clade E, *C. cliendentata* from the southern slope of the Cantabrian mountains, is placed as the sisterspecies of *C. calpica*, which is known from southern Spain and Morocco. In a single sequence of *C. cliendentata* (RMNH 103425 A) a gap of 48 basepairs was observed. This gap corresponds to a deletion of 16 triplets from position 73-120 in the alignment.

The Cantabrian species *C. kobelti* and *C. kobeltoides*, which are often found in sympatry, form clade F.

The well supported clade G contains the isolated, Portuguese *C. lusitanica*, and the two conspicuously widespread *Chondrina* species *C. avenacea* and *C. arcadica*, which are sometimes found in sympatry. Two specimens of *C. burtscheri* are also placed here.

DISCUSSION

The three analyses (neighbor joining bootstrap, parsimony bootstrap and bayesian likelihood) that were applied all yielded largely the same topology of the tree. Figure 3 shows the result with the highest likelihood from the bayesian analysis; the support values of this- and the neighbor joining and parsimony analyses are indicated at the nodes. The deeper nodes in the phylogenetic reconstruction are poorly supported by both the parsimony and neighbor joining bootstrap analyses. They do

Figure **3**. Phylogeny reconstruction for *Chondrina*.The consensus of the Bayesian analyses is shown with at the nodes the support values from the parsimony, neighbor joining and Bayesian analyses respectively.

give, however, the same topology as the bayesian analysis when compatible groups are allowed in the 50% majority rule consensus tree. Allthough the support is thus poor, the fact that all three analyses give the same topology in itself supports this reconstruction, which is proposed here as a working hypothesis.

The monophyly of the genus *Chondrina*, including *C. maginensis*, is once again (see Kokshoorn & Gittenberger [Chapter 2]) supported by the molecular data (fig. 3). *Chondrina maginensis* reminds of certain *Rupestrella* species in shell characters, but its radula is diagnostic for *Chondrina* (Kokshoorn & Gittenberger [Chapter 8, p. 149]). Thus, the value of this morphological character is confirmed by the molecular analysis. As the sistergroup of a clade with all other *Chondrina* species *C. maginensis* is placed at a basal position in the tree. It is an endemic species of the small Sierra de Magina in the Spanish province of Jaèn, in the southern part of the Iberian peninsula. In the southern and eastern part of the Iberian peninsula a large part of the species diversity of the genus is concentrated. Therefore and for reasons mentioned further on, we hypothesize that that region has acted as an area of origin for some waves of dispersal into central and eastern Europe.

A co-occurrence of two *Chondrina* species is not uncommon (fig. 4), but nowhere more than two species are found in sympatry. Across its range *C. avenacea* occurs sympatrically with 12 *Chondrina* species, but in only four cases two species that belong to the same clade may occur sympatrically. In all other instances of sympatry, the species involved are less closely related. *Chondrina klemmi* and *C. calpica* have been reported from the same locality but it is uncertain whether they were found sympatrically indeed (Gittenberger, 1973). The Cantabrian *C. kobeltoides* is always found with its sisterspecies *C. kobelti*. Also in this case there is a clear difference in size, with a shell height of 5.6-8.5 mm for the former species versus 3.7-6.7 mm for the latter one. *Chondrina tenuimarginata* and *C. centralis* have been found together in the Pyrenees (Gittenberger, 1973); they are not sister species and their shells are also quite different in size, with a shell height of 7.0-14.0 mm for the former species and 6.1-7.0 mm for the latter one. The size difference may reflect adaptation of these sympatric species to different (micro-) niches. However, this size difference does not apply to all sympatric species that belong to the same clade. *Chondrina tenuimarginata* also occurs sympatrically with *C. altimirai*. They do belong to the same clade and their general shell sizes are more or less identical at those localities. The shell sizes of *Chondrina avenacea* and *C. arcadica* are also quite similar. They may be found sympatrically in southern Germany and NE Austria (Ehrmann, 1933; Kuiper, 1953).

The species that are here united in group A, occurring in the southern Alps (*C. megacheilos* and *C. multidentata*), the Balkans (*C. spelta*) and the Tatra (*C. tatrica*) might represent a first wave of dispersal outside the Iberian peninsula. Possibly these species or their ancestors (and maybe extinct relatives) once occupied larger ranges. Their current distribution coincides with glacial refugia (Hewitt, 2000; Hausdorf & Hennig, 2004). This suggests that their ranges may have been reduced

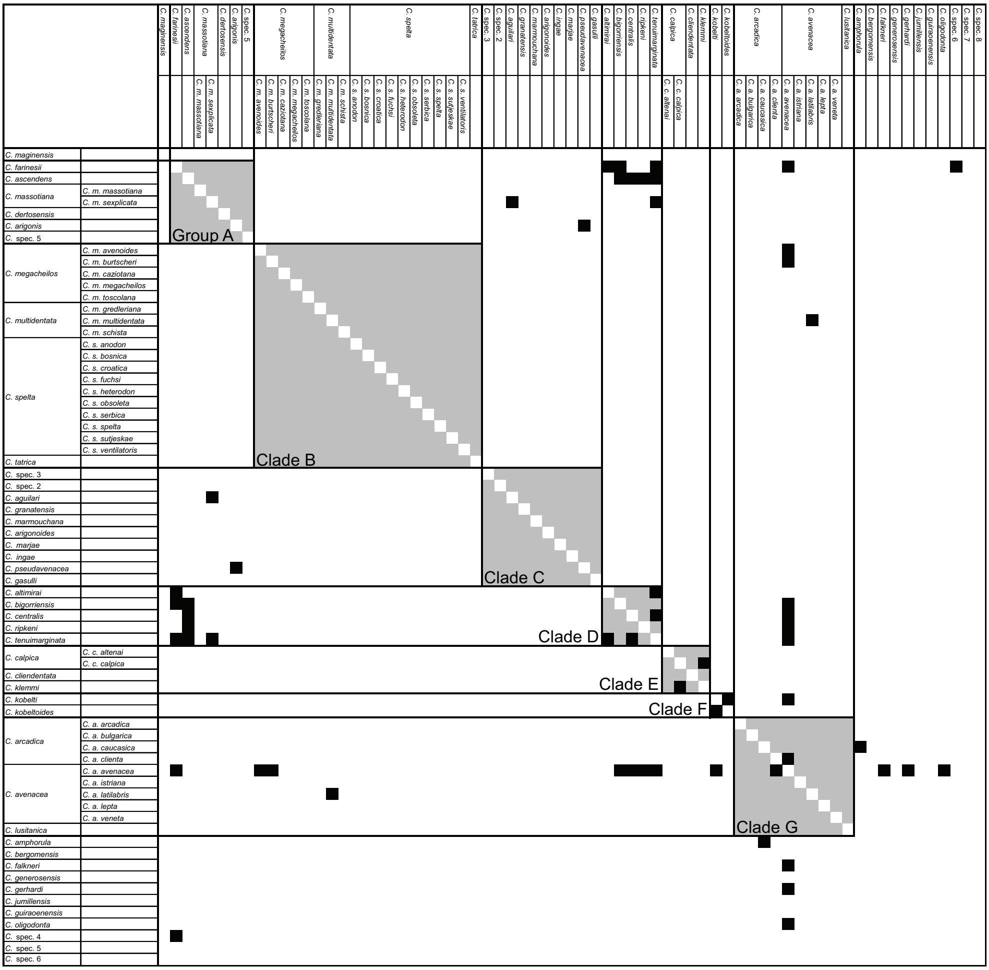


Table **2**. Co-occurrance table for *Chondrina* species. Grey clusters indicate clades as defined in figure 3.

by the major, pleistocene glaciations. Due to the low resolution at the basis of the tree, the relations between these taxa are unclear.

When we assume that the clades B-G represent separate radiations, a picture of iterative, partly overlapping waves of dispersal and speciation emerges. In that case, clade B represents a radiation that remained restricted to the eastern Pyrenees and the northeastern Iberian peninsula, partly overlapping the radiation of clade C. The latter radiation took place in a larger area, reaching as far south as Morocco. The Pyrenean-Cantabrian clade D partly overlaps with species from clades B and C. In this clade, the ranges of the relatively widespread *C. bigorriensis* and *C. tenuimargina-*

ta are clearly determined by the Pyrenean watershed, whereas the ranges of *C. altimirai* and the sisterspecies *C. centralis* and *C. ripkeni* are less clearly related to environmental factors. Clade E is a geographically problematic combination of species, with *C. cliendentata* from a limestone zone south of the Picos de Europa, together with the still poorly inderstood *C. calpica* from southern Spain and Morocco and *C. klemmi* from Morocco. In clades C and E Morocco was reached. In the Cantabrian mountains, the clearly delimitated range of clade F, with the largely sympatric sisterspecies *C. kobelti* and *C. kobeltoides*, *C. ripkeni* occurs as a single species of clade D. Clade G contains the Portuguese *C. lusitanica* as the sistergroup of the two combined, most widespread *Chondrina* species, viz. *C. arcadica* and *C. avenacea*.

Chondrina (megacheilos) burtscheri, shows up at different places, in group A and clade B, with one and two specimens, respectively. On the basis of its conchological characters, i.e. the similarity with *C. megacheilos*, *C. burtscheri* might be considered a member of group A.

In *C. maginensis*, the shells show a reduction in the apertural teeth, when we assume that the chondrinid teeth did not evolve repeatedly in parallel. Assuming that to be correct, the teeth became obsolete or absent, several times independently, viz. in group A (only *C. spelta anodon* [Nordsieck, 1970], and *C. tatrica*), clade B (most species, but in *C. arigonis* and *C. massotiana sexplicata* only partially), clade C (most species, but see *C. pseudavenacea*), clade D (only *C. altimirai*), and clade E (most extreme in *C. klemmi*). Obviously, *C. farinesii*, as defined by Gittenberger (1973), is not a natural entity but a combination of taxa from clades B and C. In clades F and G there is no reduction in apertural teeth. Among the Chondrinidae, shells with reduced apertural teeth are only known for the species inhabiting exposed rock-faces. Maybe this character has an adaptive significance in that kind of habitat, or the chondrinid apertural teeth do not enhance fitness for snails occurring on rock-faces.

For the moment being we consider *C. farinesii* a paraphyletic species with a large intraspecific variation. The molecular data indicate that the genetic distances between some population are comparable to the distances found in *Chondrina* between 'good' species. We refrain from introducing additional new taxa, however. To do so more convincingly, more data should be available, especially also because the populations involved are very similar in shell characters.

The gap of 48 basepairs which was found in a single sequence of *C. k. cliendentata* (RMNH 103425 A) corresponds to a deletion of 16 triplets from position 73-120 in the alignment. It is unlikely that such a large deletion has happened in this individual without consequences for its survival (cut short by being collected for a musuem collection). We therefore assume that this deletion is due to an in vitro hairpin coil in the template.

By far the largest part of the total range of *Chondrina* is currently occupied by only two species, i.e. *C. avenacea* and *C. arcadica*. The low genetic variability (Armbruster et al., 2007; Szarowska et al., 2003) across their relatively large ranges (i.e. *C. arcadica clienta* from Öland, Sweden, to Greece, fig. 3) suggests that these species have occupied their actual ranges only after the Last Glacial Maximum (LGM).

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REFERENCES

ARMBRUSTER, G.F.J., M. HOFER, & B. BAUR, 2007. Effect of cliff connectivity on the genetic population structure of a rock-dwelling land snail species with frequent self-fertilization. – Biochemical Systematics and Ecology 35(6): 325-333.

BAUR, A., & B. BAUR, 1991. The effect of hibernation position on winter survival in the rock-dwelling land snails *Chondrina clienta* and *Balea perversa* on Öland, Sweden. –Journal of Molluscan Studies 57: 331–336.

BAUR, A., B. BAUR, & L. FRÖBERG, 1992. The effect of lichen diet on growth rate in the rock-dwelling land snails *Chondrina clienta* (Westerlund) and *Balea perversa* (Linnaeus). – Journal of Molluscan Studies 58: 345–347.

BAUR, A., B. BAUR, & L. FRÖBERG, 1994. Herbivory on calcicolous lichens: different food preferences and growth rates in two co-existing land snails. – Oecologia 98: 313-319.

BAUR, B., & A. BAUR, 1990. Experimental evidence for intra- and interspecific competition in two species of rock-dwelling land snails. – Journal of Animal Ecology 59: 301–315.

BAUR, B., & A. BAUR, 1994. Dispersal in the land snail *Chondrina avenacea* on vertical rock walls. – Malacological Review 27: 53–59.

BAUR, B., & A. BAUR, 1995. Habitat-related dispersal in the land snail *Chondrina clienta*. – Ecography 18: 123-130.

BAUR, B., X. CHEN, & A. BAUR, 1993. Genital dimorphism in natural populations of the land snail *Chondrina clienta* and the influence of the environment on its expression. – Journal of Zoology 231: 275–284.

BAUR, B., L. FRÖBERG, & A. BAUR, 1995. Species diversity and grazing damage in a calcicolous lichen community on top of stone walls in Öland, Sweden. – Annales Botanici Fennici 32: 312-323.

CLEMENTS, R., N.S. SODHI, M. SCHILTHUIZEN, & P.K.L. NG, 2006. Limestone karsts of southeast Asia: Imperiled arks of biodiversity. – Bioscience 56(9): 733-742.

EHRMANN, P., 1933. Mollusken (Weichtiere). In: BROHMER, P., P. EHRMANN, & G. ULMER, eds, Die Tierwelt Mitteleuropas 2(1):1-264. Leipzig.

FALKNER, G., & B. STUMMER, 1996. Isoliertes vorkommen einer südalpinen Kornschnecke in Voralberg: *Chondrina megacheilos burtscheri* n.ssp. (Gastropoda: Chondrinidae). – Nachrichtenblatt der

Ersten Voralberger Malakologischen Gesellschaft 4: 1-8.

FRÖBERG, L., A. BAUR, & B. BAUR, 1993. Differential herbivore damage to calcicolous lichens by snails. – Lichenologist 25: 83-96.

GITTENBERGER, E., 1973. Beiträge zur Kenntnis der Pupillacea III. Chondrinidae. – Zoologische Verhandelingen 127: 1-267.

GITTENBERGER, E., 1984. Vicariantists and dispersalists among the Chondrininae (Gastropoda, Pulmonata). – In: SOLEM, A., & A.C. VAN BRUGGEN (Eds). World-wide Snails: Biogeographical Studies on Non-marine Mollusca. – Brill, Leiden, pp. 56–69.

GITTENBERGER, E., 1991. What about nonadaptive radiation? – Biological Journal of the Linnean Society 43: 263-272.

GITTENBERGER, E., 2004. Radiation and adaptation, evolutionary biology and semantics. – Orgamisms, Diversity & Evolution 4: 135-136.

GOMEZ, B.J., & C.E. PRIETO, 1981. Los generos *Abida, Granaria* y *Granopupa* (Gastropoda, Chondrinidae) em el Pais Vasco y provincias limitrofes. – Cuadernos de Investigación Biológica (Bilbao) 2: 49-61.

GOMEZ, B.J., & E. ANGULO, 1982. El genero *Chondrina* (Mollusca, Gastropoda) en el Pais Vasco. – Iberus 2: 29-40.

GOMEZ, B.J., & A.M. RALLO, 1988. Consideraciones biogeograficas de la subfamilia Chondrininae en las regiones de Pirineos-Occidentales, Cantabrico-Oriental, Valles Alto y Medio del Ebro y N del sistema Iberico. – Iberus 8(2): 103-109.

HESBACHER, S., B. BAUR, A. BAUR, & P. PROKSCH, 1995. Sequestration of lichen compounds by three species of terrestrial snails. – Journal of Chemical Ecology 21: 233-246.

HEWITT, G., 2000. The genetic legacy of the Quaternary ice ages. – Nature 405: 907-913.

KERNEY, M.P., R.A.D. CAMERON, & J.H. JUNGBLUTH, 1983. Die Landschnecken Nord- und Mitteleuropas: 1-384. Paul Parey, Hamburg, Berlin.

KOKSHOORN, B., & E. GITTENBERGER, 200x. *Abida secale* (Mollusca, Gastropoda, Pulmonata): Discrepancies between morphology and molecules. – *In prep*.

KUIPER, J.G.J., 1953. Bemerkungen über *Chondrina avenacea* (Brug.) und *Chondrina clienta* (Westl.) von demselben Fundort in Tirol. – Basteria 17: 51-54.

LOSOS, J.B., & R.E. GLOR, 2003. Phylogenetic comparative methods and the geography of speciation. – Trends in Ecology and Evolution 18(5): 220-227.

NORDSIECK, H., 1962. Die Chondrinen der Südalpen. – Archiv für Molluskenkunde 91(1-3): 1-20.

NORDSIECK, H., 1970. Die *Chondrina*-Arten der dinarischen Länder. – Archiv für Molluskenkunde 100(5-6): 243-261.

RAVEN, J.G.M., 1986. Notes on spanish non-marine molluscs 3. Chondrinidae from the Cantabrian mountains (Gastropoda: Pulmonata). – Zoologische Mededelingen 60(2): 27-37.

SCHILTHUIZEN, M., & E., GITTENBERGER, 1996. Allozyme variation in some Cretan *Albinaria*: paraphyletic species as natural phenomena. – In: TAYLOR, J., Origin and evolutionary radiation of the Mollusca. – Oxford University Press, Oxford, pp. 301-311.

SCHILTHUIZEN, M., H.-N. CHAI, T.E. KIMSIN, & J.J. VERMEULEN, 2003. Abundance and diversity of land-snails (Mollusca: Gastropoda) on limestone hills in Borneo. – The Raffles Bulletin of Zoology 51(1): 35-42.

SZAROWSKA, M., A. FALNIOWSKI, & K. MAZAN-MAMCZARZ, 2003. Genetic structure of isolated selfers: *Chondrina clienta* (Westerlund, 1888) (Gastropoda: Stylommatophora) in Kraków-Czestochowa upland. – Malakologische Abhandlungen 21: 79-89.