At home at least: the taxonomic position of some north African Xerocrassa species (Pulmonata, Geomitridae)

Issaad Kawther Ezzine¹, Beat Pfarrer², Najet Dimassi¹, Khaled Said¹, Eike Neubert²³

¹ LR génétique, biodiversité et valorisation des bio-ressources, Institut Supérieur de Biotechnologie de Monastir, Avenue Täher Hadded (B.P 74) Monastir 5000, Tunisia ² Natural History Museum Bern, Bernastr. 15, CH-3005 Bern, Switzerland ³ Institute of Ecology and Evolution, University of Bern, 3012 Bern, Switzerland

Corresponding author: Issaad Kawther Ezzine (issaadkawther.ezzine@gmail.com)

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Abstract
In order to clarify the systematic position of Helix latastei Letourneux in Letourneux & Bourguignat, 1887, and Helix latasteopsis Letourneux & Bourguignat, 1887, a comprehensive approach using morphological and molecular methods is presented. The investigation of the genital organs of both species showed that they belong to the genus Xerocrassa Monterosato, 1892 with two very small dart sacs and a few tubiform glandulae mucosae. In our phylogenetic analysis using the mitochondrial markers COI, 16S and the nuclear cluster 5.8S-ITS2-28S, the results of the anatomical research were confirmed. Thus, the genus Ereminella Pallary, 1919, which is based on H. latastei, becomes a junior synonym of Xerocrassa. A review of the genus-level taxa Xerobarcana Brandt, 1959, and Xeroregima Brandt, 1959, showed that these should also be considered as synonyms of Xerocrassa. A third species, Helix lacertara Bourguignat, 1863 from Algeria was found to be closely related to X. latastei based on its shell morphology. A map showing the distribution of the three species treated is supplied.

Résumé
Une étude basée sur des approches morphologiques et moléculaires a été réalisée dans le but de clarifier la position systématique de deux espèces Helix latastei Letourneux 1887 et Helix latasteopsis Letourneux & Bourguignat, 1887. L’examen des organes génitaux a montré des critères typiques du genre Xerocrassa Monterosato, 1892 avec la présence de deux petits “Dart Sac” et des glandes digitiformes à mucus. Les résultats de l’analyse phylogénétique de deux gènes mitochondriaux (COI et 16S) et un gène nucléaire 5.8S-ITS2-28S ont confirmé les résultats de l’étude anatomique. Par conséquent, le genre Ereminella Pallary, 1919, qui a
été basé sur *Helix latastei* est donc un synonyme du genre *Xerocrassa*. La révision de deux genres *Xerobarcana* Brandt, 1959 et *Xeroregima* Brandt, 1959, suggère que ces deux genres sont aussi des synonymes du genre *Xerocrassa*. L’examen de la coquille de l’espèce Algérienne *Helix lacertara* Bourguignat, 1863 a montré une forte ressemblance avec *X. latastei*, ce qui nous a permis, ainsi, de la classer dans le genre *Xerocrassa*. Une carte montrant la distribution des trois espèces a été fournie.

**Keywords**
Algeria, anatomy of genital organs, systematics, Tunisia, *Xerocrassa latastei*, *Xerocrassa latasteopsis*, COI, 16S, 5.8S-ITS2-28S

**Mots clés**
Algérie, anatomie de l’appareil génital, systématique, Tunisie, *Xerocrassa latastei*, *Xerocrassa latasteopsis*, COI, 16S, 5.8S-ITS2-28S

**Introduction**
The systematic position of most taxa described by Letourneux and Bourguignat, 1887 in their “Prodrome” on the Tunisian malacofauna is under debate since their description. This holds true for *Helix latastei* as well as for *Helix latasteopsis*. Their generic status was maintained until Pallary (1919) erected the new genus *Ereminella* based on *H. latastei*, but without giving any descriptive characters that could discriminate this taxon from others. The first researcher intensively dealing with *H. latastei* was Brandt (1959: 113), who, deducing from an anatomical drawing by Bisacchi (1932: 363–364, figs 2–4), perceived *H. latastei* to be a member of his *Trochoidea* sensu lato (which at that time included what is separated today as *Xerocrassa*). Bisacchi erroneously identified the Libyan specimens he dissected as *Helix* (*Xerophila*) *pseudosimulata* Germain, 1921 from Alexandria, Egypt. However, Forcart (1976: 152) recognized this Egyptian taxon as a synonym of *Xerocrassa simulata* (Ehrenberg 1831) (for further discussion of this name refer to Forcart, loc. cit.). Jaeckel (1963) repeated Brandt’s generic affiliation while recording the species from Djerba. Finally, Frank (1988) mentioned *X. latastei* from northern Tunisia, a record which is out of the recently known range of this species and needs to be verified. A comparison of Tunisian species with a selection of *Xerocrassa* species from the radiation of this genus on the Island of Crete (Sauer and Hausdorf 2009) and from western Europe including Spain and the Balearic Islands (Chueca et al. 2017) is provided.

**Materials and methods**

**Sampling**
Living specimens were collected from several localities in Tunisia during two periods: spring 2014, and winter 2015/2016. Geographic coordinates were recorded using GPS (see Table 1). For subsequent molecular analysis, specimens were preserved and stored in 80% ethanol until dissection and DNA extraction.
Empty shells were also collected (see section material under the species description) in order to complete the distributional record of the species. Specimens used in this study (both shells and preserved animals) are housed in the voucher collections of the High Institute of Biotechnology of Monastir and the Natural History Museum Bern; all sequenced specimens are deposited in the museum’s collection.

**Morphological and anatomical studies**

First assessments of the shell morphological characters were done by using simple magnifying glasses. Preserved animals were dissected under LEICA M212 stereo-microscope using thin tweezers. The genital organs of the specimens were removed from the body, the genital situs (i.e. the outer morphology of the complete hermaphroditic genital organ) and further morphological details were investigated. After that, shells, genital situs, and details of the genital organs were photographed with a LEICA DFC 425 camera combined with a LEICA M205 C. The multifocal images were processed by using an imaging software (Imagic Switzerland).

**Abbreviation of museum’s acronyms**

| Acronym   | Description                                           |
|-----------|-------------------------------------------------------|
| MVHN      | Museu Valencià d’Historia Natural;                    |
| MHNG-MOLL | Museum d’Histoire Naturelle de Genève, malacological collection; |
| NMBE      | Naturhistorisches Museum der Burgergemeinde Bern;     |
| ZMH       | Zoological Museum of the University of Hamburg.       |

**Abbreviations of shell measurements**

D: shell diameter; H: shell height; PD: peristome diameter; PH: peristome height; W: number of whorls.
Molecular study

Fourteen specimens of *Xerocrassa* from southern Tunisia could be used in this study, originating from five localities. Sequenced specimens are housed in the voucher collection of the NMBE (Table 2). In the analysis, sequences of four Cretan *Xerocrassa* species were also included (Sauer and Hausdorf 2009), and eleven Spanish and Balearic *Xerocrassa* species from the work recently published by Chueca et al. (2017).

As outgroup species *Cernuella virgata*, *Trochoidea elegans*, and *Trochoidea pyramidata* were used. All three species are each represented by one specimen from Tunisian localities, and complemented by one specimen of *Hygromia limbata*, one *Xerosecta adolfi*, and one *T. elegans* (Razkin et al. 2014). All specimens used to produce phylogenetic trees are listed in Table 2. Specimens where nuclear markers are not available were excluded from the analysis of the concatenated mitochondrial // nuclear dataset. Thus, all Cretan *Xerocrassa* specimens, except two specimens of *X. cretica* (recently collected by Neubert), and the Tunisian *Trochoidea* and *Cernuella* species were not used in this type of analysis.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from the foot muscle tissue using the standard phenol chloroform method (Estoup et al. 1996). Two mitochondrial gene fragments and one rDNA region were chosen to be analysed in the current study. Mitochondrial markers were consisting of Cytochrome c oxidase subunit I (COI) and the 16S ribosomal RNA subunit (16S) gene. The nuclear marker was formed by the 3’ end of the 5.8s ribosomal RNA, the complete ITS2 region and the 5’ end of the large subunit of the 28S rRNA. Polymerase chain reactions (PCR) were performed in a reaction mixture, containing 15 ng of DNA template, 1×1.5 mM buffer reaction, 0.1 mM of each selected couple primers, 0.2 mM dNTPs, Taq polymerase (1.25U) and adjusted till a total volume of 25 µl with DNAase free water/sterilized water (UNIMED) (H2O). PCR reactions were run under following conditions: 3 min at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 40°C and 1 min at 72°C and finally, 5 min at 72°C for COI. For 16S the amplification conditions were: 3 min at 95°C, followed 35 cycles of 1 min at 95°C, 1 min at 50°C and 1 min at 72°C. To amplify the ribosomal cluster, two pairs of primers were used to get a sequence of 1300 bp: the standard LSU1/LSU3 and the 28SF/28SR (see Table 3). PCR reactions were run under the following conditions: 3 min at 96°C, followed by 35 cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C and finally, 5 min at 72°C for LSU1/LSU3 and 5 min at 95°C, followed by 35 cycles of 1 min at 95°C, 30 s at 62°C and 1 min at 72°C and finally, 10 min at 72°C for 28SF/28SR. PCR products were sequenced using automated and standardised ABI 3730 XL sequencing run with a read length up to 1100 bp (PHRED20 quality) and using the same primers as for the PCR (Table 3).
Table 2. Taxa used: Species, localities, and voucher and GenBank accession numbers for the mitochondrial genes COI and 16S and the nuclear ribosomal 5.8S-ITS2-28S region.

| Species           | Locality                          | Voucher number | GenBank accession numbers      |
|-------------------|-----------------------------------|----------------|-------------------------------|
|                   |                                   |                | COI          | 16S          | 5.8-ITS2-28S |
| X. latastei       | El Djorf, Medenine, Tunisia       | NMBE 541956    | KY706528 | KY747539 | MF687913 |
|                   | Boughrara, Medenine, Tunisia      | NMBE 549851    | KY706529 | KY747540 | MF687914 |
|                   | Boughrara, Medenine, Tunisia      | NMBE 549852    | KY747533 | KY747541 | MF687915 |
|                   | Boughrara, Medenine, Tunisia      | NMBE 549853    | KY706530 | KY747542 | MF687916 |
|                   | Sidi Aich 1, Gafsa, Tunisia       | NMBE 549847    | KY706527 | KY747536 | MF687903 |
|                   | Sidi Aich 1, Gafsa, Tunisia       | NMBE 549848    | KY747531 | KY747537 | MF687904 |
|                   | Sidi Aich 1, Gafsa, Tunisia       | NMBE 548449    | KY747532 | KY747538 | MF687905 |
|                   | Sidi Aich 2, Gafsa, Tunisia       | NMBE 541954    | KY747534 | KY747543 | MF687906 |
|                   | Sidi Aich 2, Gafsa, Tunisia       | NMBE 549846    | KY747535 | KY747544 | MF687907 |
|                   | Henchir el Zitouna, Medenine, Tunisia | NMBE551288 | MF678556 | MF683092 | MF687908 |
|                   | Henchir el Zitouna, Medenine, Tunisia | NMBE551289 | MF678557 | MF683093 | MF687909 |
|                   | Henchir el Zitouna, Medenine, Tunisia | NMBE551290 | MF678558 | MF683095 | MF687911 |
|                   | Henchir el Zitouna, Medenine, Tunisia | NMBE551291 | MF678559 | MF683096 | MF687912 |
| X. frater frater  | Cala Romantica, Baleares, Spain   | EHUMC-1327     | KT968955 | KT969152 | KT969343 |
| [Chueca et al. 2017] | Cala Romantica, Baleares, Spain   | EHUMC-1328     | KT968956 | KT969153 | KT969344 |
|                   | Tossals Verds, Baleares, Spain    | EHUMC-1329     | KT968957 | KT969154 | KT969345 |
| X. majoricensis    | Illetes Calvià, Baleares, Spain   | EHUMC-1317     | KT968945 | KT969142 | KT969333 |
| [Chueca et al. 2017] | Illetes Calvià, Baleares, Spain   | EHUMC-1318     | KT968946 | KT969143 | KT969334 |
|                   | Bunyolí Establiments, Baleares, Spain | EHUMC-1319   | KT968947 | KT969144 | KT969335 |
| X. ferreri ferreri | Path to French’s monument Baleares, Spain | EHUMC-1295  | KT968924 | KT969121 | KT969312 |
| [Chueca et al. 2017] | Peguera Baleares, Spain           | EHUMC-1296     | KT968925 | KT969122 | KT969313 |
| X. prietoi prietoi | Bunyolí, Establiments Baleares, Spain | EHUMC-1399  | KT969024 | KT969221 | KT969392 |
| [Chueca et al. 2017] | Sont Cotoneret Baleares, Spain    | EHUMC-1400     | KT969025 | KT969222 | KT969393 |
|                   | Inca Baleares, Spain              | EHUMC-1401     | KT969026 | KT969223 | KT969394 |
| Species                | Locality                               | Voucher number | GenBank accession numbers |
|------------------------|----------------------------------------|----------------|--------------------------|
|                        |                                        |                | COI          | 16S          | 5.8-ITS2-28S    |
| *X. ponsi* [Chueca et al. 2017] | Path to French’s monument, Baleares, Spain | EHUMC-1387     | KT969012 | KT969209 | KT969386       |
|                        | French’s monument Baleares, Spain      | EHUMC-1388     | KT969013 | KT969210 | KT969387       |
|                        | French’s monument Baleares, Spain      | EHUMC-1390     | KT969015 | KT969212 | KT969388       |
| *X. myeli* [Chueca et al. 2017] | Ses Mongetes, Baleares, Spain          | EHUMC-1361     | KT968987 | KT969184 | KT969374       |
|                        | Ses Mongetes, Baleares, Spain          | EHUMC-1362     | KT968988 | KT969185 | KT969375       |
|                        | Alaior, Baleares, Spain                | EHUMC-1366     | KT968991 | KT969188 | KT969376       |
| *X. cisternasi cisternasi* [Chueca et al. 2017] | Illa de Santa Eulalia Baleares, Spain | EHUMC-1279     | KT968908 | KT969105 | KT969297       |
| *X. caroli caroli* [Chueca et al. 2017] | Cap des Jueu Baleares, Spain          | EHUMC-1259     | KT968888 | KT969085 | KT969277       |
|                        | Cap des Jueu Baleares, Spain          | EHUMC-1260     | KT968889 | KT969086 | KT969278       |
|                        | Cap des Jueu Baleares, Spain          | EHUMC-1261     | KT968890 | KT969087 | KT969279       |
| *X. ebuisitana* [Chueca et al. 2017] | Cap de Barbaria Baleares, Spain       | MVHN-281009TF02 | KT969064 | KT969260 | KT969416       |
|                        | Racó des Forat Baleares, Spain        | EHUMC-1241     | KT968870 | KT969067 | KT969262       |
|                        | Cap de Barbaria Baleares, Spain       | EHUMC-1242     | KT968871 | KT969068 | KT969263       |
| *X. barceloi* [Chueca et al. 2017] | Orihuela, Alicante, Spain             | EHUMC-1413     | KT969038 | KT969235 | KT969406       |
| *X. subrogata* [Chueca et al. 2017] | Serra de la Borja, Tarragona, Spain    | EHUMC-1412     | KT969037 | KT969234 | KT969405       |
|                        | Serra de la Borja, Tarragona, Spain    | EHUMC-1411     | KT969036 | KT969233 | KT969404       |
| *X. amphiconus* [Sauer and Hausdorf 2009; Sauer and Hausdorf 2012] | Kato Zakros, Crete, Greece            | ZMH 36820-606  | FJ627140 | JN701872 | –              |
|                        | Kato Zakros, Crete, Greece            | ZMH 36820-452  | FJ627076 | JN701834 | –              |
|                        | Moni Toplou, Crete, Greece            | ZMH 36606-473  | FJ627090 | JN701848 | –              |
| *X. grabusana* [Sauer and Hausdorf 2009; Sauer and Hausdorf 2012] | Kaliviani, Crete, Greece              | ZMH 29885-465  | FJ627089 | JN701847 | –              |
| *X. mesostena* [Sauer and Hausdorf 2009; Sauer and Hausdorf 2012] | Agia Galini, Crete, Greece            | ZMH 36790-638  | FJ627160 | JN701877 | –              |
|                        | Gerakari, Crete, Greece               | ZMH 29631-636  | FJ627158 | JN701876 | –              |
|                        | Theriso, Crete, Greece                | ZMH 29807-524  | FJ627117 | JN701866 | –              |
| *X. cretica* [Sauer and Hausdorf 2009; Sauer and Hausdorf 2012] | Moni Gorgolani, Crete, Greece         | ZMH 36304-423  | FJ627055 | JN701813 | –              |
|                        | Palekastro, Crete, Greece             | ZMH 50000-671  | FJ627168 | JN701878 | –              |
|                        | Palekastro, Crete, Greece             | ZMH 50121-620  | FJ627150 | JN701874 | –              |
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| Species                  | Locality                                      | Voucher number | GenBank accession numbers |
|--------------------------|-----------------------------------------------|----------------|---------------------------|
|                          |                                               |                | COI           | 16S            | 5.8-ITS2-28S |
| *X. cretica* [coll. Neubert [2017]] | Plateau between Lithines and Perivolarakia, Crete, Greece | NMBE 550935    | MF678560       | MF683097       | MF687917     |
| *X. ripacurcica* [Chueca et al. 2017] | Circo de Armena, Huesca, Spain                | EHUINC-1416    | KT969041       | KT969238       | KT969409     |
|                          | Congost de Ventamillo, Huesca, Spain          | MVHIN-210813FS03 | KT969057     | KT969253       | KT969411     |
| *X. montserratensis* [Chueca et al. 2017] | Monistrol de Montserrat, Barcelona, Spain     | EHUINC-1414    | KT969039       | KT969236       | KT969407     |
|                          | Castellar del Vallès, Barcelona, Spain       | EHUINC-1415    | KT969040       | KT969237       | KT969408     |
| "X. meda" [Chueca et al. 2017] | Mosta, Malta                                  | MVHIN-230412LR01 | KT969058     | KT969254       | –            |
| *T. elegans*             | Ghar el Melh, Bizepte, Tunisia                | NMBE 549908    | KY706532       | KY747546       | –            |
| *T. elegans* [Razkin et al. 2014] | L’Alcudia, Valencia, Spain                   | MVHIN 1310     | KT969047       | KJ458564       | KJ458642     |
| *T. pyramidata*          | Djebal Recas, BenArous, Tunisia               | NMBE 549882    | KY706531       | KY747545       | –            |
| *C. virgata*             | Ain Biter, Bizepte, Tunisia                   | NMBE 549850    | KY706533       | KY747547       | –            |
| *Xerosecta adolfi* [Razkin et al. 2014] | Nijar, Almeria, Spain                        | EHUMC 1036     | KT968868       | KJ458567       | KJ458645     |
| *H. limbata* [Razkin et al. 2014] | Queralbs, Dáíó, Girona, Spain                 | EHUMC 1027     | KT968867       | KJ458529       | KJ458616     |

Table 3. List of primers used for PCR and sequencing.

| Gene   | Name | Sequence                                      | Reference                   |
|--------|------|-----------------------------------------------|-----------------------------|
| COI    | COIF | 5’-ACTCAACGAATCTATAAGATATCTGG-3’             | Folmer et al. 1994          |
|        | COIR | 5’-TATACCTTCAAGGGATGA CGAAAAATCA-3’          | Folmer et al. 1994          |
| 16S    | 16Sar| 5’-CGCCCTTTATCAAAAACAT-3’                    | Palumbi et al. 1991         |
|        | 16Sbr| 5’-CCGGTCGACATCGTGATCAT-3’                   | Palumbi et al. 1991         |
| 5.8S-ITS2 | LSU-1 | 5’-CTAGTCGGGAGAATTATGTGA-3’               | Wade et al. 2000            |
|        | LSU-3 | 5’-ACTTTCCCCTCCACGGTGACTTG-3’               | Wade et al. 2000            |
| 28S    | 28SF | 5’-AACGCAATGGCCCGCTCGG-3’                    | Koene and Schulenburg 2005  |
|        | 28SR | 5’-GAAGACGGGTGCTGGAATG-3’                    | Koene and Schulenburg 2005  |

Sequence alignment

Forward and reverse sequences were assembled, checked for ambiguities and aligned using default settings of “Clustal W” implemented in Bioedit V 7.2.5 (Hall 1999). Aligned sequences of Tunisian Xerocrassa species were analysed using DnaSP v5.10.01 software (Librado and Rozas 2009) to estimate number of informative sites and nucleotide diversity for each marker used. The p-distance values within Tunisian samples were estimated using Mega v.6 (Tamura et al. 2013). The relationships of inferred haplotypes of mitochondrial nuclear and concatenated dataset of Tunisian Xerocrassa species were estimated using the TCS method (Clement et al. 2002) implemented with Popart software v1.7 (Leigh et al. 2015).
Phylogenetic analysis

Our data consist of two mitochondrial markers and one nuclear ribosomal cluster. The data was partitioned using the PartitionFinder software v1.1.1 (Lanfear et al. 2012), in six partitions: three codon positions of the COI, the 16S the rRNA 5.8S and 28S were considered as a single partition and finally the ITS2.

For the mitochondrial dataset as well as for the concatenated data, we produced two phylogenetic trees within the Mediterranean Xerocrassa species using the Maximum Likelihood (ML) and the Bayesian inference (BI). The ML analyses were conducted using RAxML v7.2.6 (Boc et al. 2012, Stamatakis 2006) under the GTRGAMMA model, with 1000 nonparametric bootstrap replicates to estimate node support. For the Bayesian Inference, we used Mr Bayes v3.2.2 (Ronquist and Huelsenbeck 2003) using partition scheme and substitutions models suggested by PartitionFinder v1.1.1 (Lanfear et al. 2012). Four independent runs were conducted for $10^6$ generations, sampling every 1000. The first 25% trees were discarded as default burn-in and a majority rule consensus tree was calculated from the remaining trees. The topology obtained, and the posterior probabilities of each node were displayed on Figtree V1.4.0 (Rambaut 2012).

Results

Taxonomy

Both, the results of our morphological research on the genital organs as well as the molecular study, prove the affiliation of *Helix latastei* and *Helix latasteopsis* to the genus *Xerocrassa* Monterosato, 1892. For the subgeneric placement refer to the chapter “Discussion.”

*Xerocrassa (Xerocrassa) latastei* (Letourneux in Letourneux & Bourguignat, 1887)
Figs 1, 2, 3

1887 *Helix latastei* Letourneux in Letourneux & Bourguignat, Prodrome de la malacologie terrestre et fluviatile de la Tunisie: 63 [Ketenna et dans le vallon de l’Oued El-Fbour, ainsi qu’à l’oasis du Hammam de Gabès. Plaine entre Ras-el-Aïn et Sidi-Salem-Bouguerara. Bir-el-Ahmar. Bords de l’Oued Medzesar et de l’Oued Taferma entre Aïn-Magroun et Fratis. Ras-ed-Djerf, vis-à-vis de Djerba; Zarzis, etc. (Let.). — En Algérie: Ouled Nâil près de Biskraou, à Aïn-Gussera, à Bou-Ghezoul sur les hauts plateaux, entre Boghar et Laghouat et entre cette ville et Djelfa].

Type specimens. Brandt (1959: 113) considered four taxa of hygromiid species described by Letourneux and Bourguignat, 1887 to constitute the species *H. latastei*. Our investigation of the type specimens of these taxa revealed that the species *Helix fratisiana*
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Figure 1. Anatomy of genital organs of Xerocassa latastei; Jorf, 6.12.2015, leg. Ezzine, NMBE 549907/1; A situs B atrium. Abbreviations: a = atrium; al = atrial lumen; ds = dart sac(s); ep = epiphallus; fl = flagellum; gm = glandulae mucosae; itc = internal tissue cone; mrp = penial retractor muscle; p = penis; ped = pedunculus; po = pore of penial papilla; pp = penial papilla; sod = spermoviduct; sti = stimulator (?); vd = vas deferens.

and Helix tafermica, which had been listed by him in the synonymy of H. latastei, belong to species of the Hygromiidae living in Tunisia. In order to stabilize nomenclature, we herewith select MHNG-MOLL 115121 as lectotype for Helix latastei [hic!]. Thus,
the type locality of this species is herewith restricted to Ketenna [= Kettana]: mouth of Oued El Ferd, Gouv. Gabés, at 33.7575 10.2047; paralecotypes MHNG-MOLL 115121b/4, MHNG-MOLL 115128/2.

**Additional specimens examined.** Bou Hedma, 29.3.1997, leg. J. Gugel, 34.4958°N 9.488°E, NMBE 516753/1; Boughrara, Medenine, 6.12.2015, leg. Ezzine, NMME 541952/3, ditto, NMME 547176/3; ditto, NMME 541955/7 (preserved); Jorf (El Djorf), Mednine, 6.12.2015, leg. Ezzine, NMME 541956/1 (preserved), NMME 549907/1 (anat.); “plaine entre Ras-el-Aïn et Sidi-Salem-Bouguerara”, MHNG-MOLL 115118/3, MHNG-MOLL 115126/6, MHNG-MOLL 115127/6, MHNG-MOLL 115129/4; Bir-el-Ahmar MHNG-MOLL 115119/1; Zarzis MHNG-MOLL 115120/2; “Oued el Four près de Gabès” MHNG-MOLL 115124/6; “Ras-ed-Djerf, vis-à-vis de Djerba” MHNG-MOLL 115125/1. — Specimens recorded from literature: ruins of Gighti close Djorf (Djerba) (Jaeckel 1963).

**Diagnosis.** Shell small to medium sized, thick, basic colour white; protoconch brownish to blackish; three first whorls with granulations; whorls ribbed; suture moderately deep; umbilicus very small, conical.

**Description.** Shell small to medium sized, depressed globular, thick, basic colour creamy white; protoconch very small, brownish to blackish, smooth, consisting of 1½ whors; teleoconch consisting of 5½ slightly flattened whors, sculptured by moderately sized axial ribs; three first whors brown with whitish granules; lower teleoconch whors with up to 5 brown spherical bands; suture moderately deep; underside often white; aperture sub-spherical, slightly descending; columellar peristome thick; umbilicus moderately small, conical.

**Genital anatomy.** The description of the genital organs is taken from an adult and mature specimen collected in El Djorf. Figure 1B shows the lumen of the atrium with its internal structures.

Male part. Penis club-shaped, thick; epiphallus longer than penis; penial retractor muscle inserting at the boundary between penis and epiphallus, with a strong fascia enveloping the genitals; flagellum short; penial papilla subdivided in a simple basal shaft and a subsequent part characterised by deep perpendicular grooves, terminal part of the penial papilla strongly kinked, with central pore at its tip.

Genital atrium. Considerably thickened, lumen filled by two structures: 1) a strong crest of fleshy tissue (here called stimulator), auricle-shaped, the interior side (i.e. opposite to the penial papilla) with zigzag-shaped longitudinal pilasters becoming smooth when entering the interior wall of the atrium, and 2) a longitudinal spoon- or tongue-shaped tissue plate (here called internal tissue cone), with the outer rims bent upwards forming a hollow structure.

Female part. Two very small, almost spherical dart sacs in opposite position; glandulae mucosae simple, tubes randomly attached on the vaginal wall between dart sacs and pedunculus; vagina moderately long, pedunculus formed by a quite strong tube.

**Measurements.** Lectotype *lattastei*: D: 15.9 mm; H: 12.39 mm; PD: 8.58 mm; PH: 6.72; W: 6.25.
At home at least: the taxonomic position of some north African Xerocrassa species...

Distribution (Fig. 2). This species is currently known from the coastal and neighbouring inland areas of central and southern Tunisia. It occurs almost in sympatry with H. latasteopsis in some areas of the province Medenine and Sidi Bouzid.

The Senckenberg Museum houses a considerable number of dry shells under the name H. latastei from Libya, based on the collections of Brandt (1959: 112 ff.). They were examined by Neubert during the last years, and they in fact are very similar to X. latastei from Tunisia. However, all these shells were collected in the Cyrenaica and its hinterland with the westernmost locality being Marsa Brega (ca. 200 km SSW of Bengasi). So far we have not seen any shells from the Sirte nor the Tripolitanian area towards Tunisia, which embraces almost half of the coastal stripe of Libya. The gap to the Tunisian populations is more than 800 km as the crow flies. This area was visited several times by Kaltenbach (Kaltenbach 1950a; 1950b), but there are no records for X. latastei from this area in his rich collection, which is also housed in SMF. As long as no preserved specimens from the Cyrenaica are available, we consider these populations as not conspecific.

Remark. Specimens of this species are characterized by a globose shell with a quite small umbilicus if compared to the large Cernuella species, which live sympatrically in southern Tunisia.

The internal structures in the genital atrium are poorly understood. However, when dissecting the atrium, the internal tissue cone is always found to almost completely envelop the penial papilla; the situation shown in Fig. 1B is the result of pulling the penial papilla out of the internal tissue cone. Spreading the opened atrium then leads to a position of this organ on the right side.
Xerocrassa latasteopsis (Letourneux & Bourguignat, 1887)
Figs 2, 3, 5–6

1887 Helix latasteopsis Letourneux & Bourguignat, Prodrome de la malacologie terrestre et fluviatile de la Tunisie: 63 [Foum-Hallouf et à Ras-ed-Djerf, vis-a-vis de Djerba].

Type specimens. latasteopsis: Foum Hallouf MHNG-MOLL 115131/1 here selected as lectotype [hic!]. paralectotype: Ras-ed-Djerf MHNG-MOLL 115130/1.

Additional specimens. Oasis NE of Tozeur, 10.12.2015, leg. Ezzine, 33.9672°N 8.0421°E, NMBE 541953/1; Bou Hedma, 3.3.2006, leg. I. Abbes, NMBE 551321/X; Oued Medzesar MHNG-MOLL 115122/1; Ksar Sidi Aich 1, Gafsa, 29.4.2014, leg. Ezzine, NMBE 549849/1, 549848/1, 549857/1; Ksar Sidi Aich 2, Gafsa, 34.7061°N 8.7972°E, 9.12.2015, leg. Ezzine, NMBE 549906/1, 549846/1, 547177/1, 541954/1; (Ksar Sidi Aich 1 is located ca. 200 m east of Ksar Sidi Aich 2); Henchir el Zitouna, Medenine, 10.2016, leg. Ezzine, NMBE 551301/9, 551293/6, 551291/1, 551290/1, 551289/1, 551288/1, 549854/1. — Additional specimens in coll. Ezzine/Monastir.

Diagnosis. Shell creamy white throughout, upper teleoconch whorls with fine axial riblets, last whorl almost smooth, umbilicus open, narrow.

Description. Shell medium sized, depressed, creamy white with irreguarly dispersed opaque spots, shell walls thick; protoconch very small, brownish to blackish, smooth, consisting of 1½ whorls; teleoconch consisting of up to 6 whorls, upper teleoconch whorls with fine axial riblets and a regular pattern of brownish axial flames fading out as subsutural dots; riblets becoming obsolete on the median teleoconch whorls, last whorl almost smooth with irregular rugosities; suture deep; aperture sub-spherical, slightly descending; umbilicus open, narrow, conical.

Genital anatomy. The genital anatomy of two adults specimens collected in Henchir el Zitouna and Sidi Aich 2 are illustrated.

Male part. penis club-shaped, thick, with a solid ring-like structure formed by the basis of the penial papilla; epiphallus longer than penis; penial retractor muscle inserting somewhat distal to the boundary between penis and epiphallus, muscle fascia weak; flagellum very short; penial papilla cone shaped, simple, with 2-3 small folds with a central pore at its tip.

Genital atrium. Expanded sac-like structure, with a strongly developed stimulator tissue. The stimulator consists of a thick and tightly upfolded part, connected to the internal tissue cone. The internal tissue cone is fleshy, solid, formed like a stick, and not fully separated from the stimulator.

Female part. Dart sacs in opposite position, very small; glandulae mucosae simple, tubes randomly attached on the vaginal wall between dart sacs and pedunculus; vagina long, pedunculus not strongly developed.

Measurements. Lectotype: H = 14.5 mm; D = 18.34 mm; PH = 9.93 mm; PD = 9.4 mm; W = 6.

Distribution (Fig. 2). This species is known from southeastern Tunisia in the areas north and south of the Chott el Jerid. It also occurs in the Bou Hedma National Park.
in central Tunisia, where it obviously comes close to *H. latastei*. Our records from Bou Hedma National Park originate from two different sources, and the exact collecting sites are not known. A sympatric occurrence cannot be excluded. The type locality Foum Hallouf as given by Letourneux and Bourguignat is also imprecise, this term is used for a larger area east of the small hill chain between Dkhilet Toujane and Beharya;
the locality Henchir el Zitouna is situated in the centre of this area, so these specimens can be considered as topotypes (Fig. 2).

**Remarks.** Besides the genetic difference observed (see Figs 8, 9), there are also slight differences found in the morphology of both, shells, and genital organs. The shell of *X. latasteopsis* is always white (with up to five brown spiral bands in *X. latastei*), the riblets are fine (much stronger in *X. latastei*), the lower whorls are smooth and a bit wrinkled (ribbed throughout in *X. latastei*), and the umbilicus is narrow (somewhat larger in *X. latastei*). The penial papilla is short conical in *X. latasteopsis* (elongate in *X. latastei*), and the flagellum is short if compared to the epiphallus (longer in *X. latastei*).

When describing their *Helix latastei*, Letourneux and Bourguignat mentioned several localities for this species from Algeria. However, it turned out that these localities had been mentioned earlier by Bourguignat in his description of *Helix lacertarum* in 1863. Obviously, Letourneux and Bourguignat in 1887 considered both nominal species to be conspecific without clearly stating this opinion. After examination of all specimens in the collection of Bourguignat we come to the conclusion that, for the time being, the Algerian shells have to be considered as a separate species.

**Xerocrassa (Xerocrassa) lacertara (Bourguignat, 1863)**

Figs 2, 7

1863 *Helix lacertarum* Bourguignat Malacologie de l’Algérie, I: 209 [Plaines entre Djelfa et El-Aghouat (de la Péraudière)].

1863 *Helix lacertarum* var. *minor* Bourguignat Malacologie de l’Algérie, I: 209 [collines d’Ogla-Zemera, à 10 lieues nord-ouest de Bou-Sààda (Marès)].

1898 *Helix lacertarum*, Pallary, Comptes rendu de l’association française pour l’avancement des Sciences [Saint-Etienne], 26 (2) [1897]: 557.

**Type specimens.** *lacertarum*: Syntypes MHNG-MOLL 114001/5; *minor*: syntype MHNG-MOLL 114006/1.

**Additional specimens.** “Djebel Sahara près de Djelfa (34.6743°N 3.2552°E) MHNG-MOLL 114003/10; “entre le rocher du Sel et Mesram” (34.8375°N 3.0921°E) MHNG-MOLL 114004/8; “entre Ain Ouessera et Bou Ghezoul” (35.5819°N 2.8992°E) MHNG-MOLL 114005/11; “Aïn-Seba, près de Boussaoud” (35.2118°N 4.1763°E) MHNG-MOLL 114007/1. — Localities mentioned in the synonymy of *X. latastei*, but not represented in Bourguignat’s collection: “Ouled Nâil près de Biskraou” (34.8370°N 5.75104°E); “à Aïn-Gussera” (= Ain Oussera 35.4495°N 2.9045°E); “à Bou Ghezoul sur les hauts plateaux” (= Boughezoul 35.6992°N 2.8482°E); “entre Boghar et Laghouat” (34.7554°N 3.1747°E) “et entre cette ville et Djelfa” (34.2577°N 3.0998°E). — unclear: MHNG-MOLL 114002/1, Saida (pr. Oran); MHNG-MOLL 114008/1 Sebdou (pr. Oran).

**Description.** Shell small, globular, basic colour creamy-whitish; protoconch very small, brownish, consisting of two whorls; teleoconch with many axial riblets, surface
submalleate; whorls well rounded, with a moderately deep suture; last whorl with a single brown band at the periphery, often dissolved to a string of brown stripes; dark spots may occur usually irregularly spread all over the teleoconch, sometimes arranged in axial stripes; aperture semioval, with a small white lip; peristome small, sharp; umbilicus narrow, nearly completely obscured by a reflection of the columellar callus.

Measurements (syntype). D: 11.8 mm; H: 10.1 mm; PD: 6.7 mm; PH: 5.63 mm; W: 5.75.
Figure 8. Haplotype Network showed the number of variable sites. A Haplotypes and numbers of variables sites based on mitochondrial markers B Haplotypes numbers of variables sites based on concatenated mitochondrial and nuclear data.

**Distribution** (Fig. 2): *Xerocrassa lacertara* is hitherto only known from the collection of Bourguignat, and seems to live restricted to the arid areas in eastern Algeria.

**Remark.** Deduced from its shell morphology, this species is close to *X. latastei*. Both species share the globular shell form, the glossy shell surface, the ribbing mode, and the colouration pattern. In the absence of preserved specimens, we used these criteria to classify this species within the genus *Xerocrassa*. It differs from *X. latastei* in size (smaller in *X. lacertara*), in the umbilicus, which is more strongly covered in *X. lacertara* than in *X. latastei*, in the more pronounced ribbing pattern of the teleoconch whorls, and the missing granulation of the upper teleoconch (in *X. latastei*), which is malleate in *X. lacertara*.

When describing *X. latastei*, Bourguignat mentioned some of the localities, where he recorded *X. lacertara* 34 years before. This proves that he had no clear concept of
these two species. Looking to the distribution patterns, both species are separated by a large area (ca. 300 km as the crow flies) without any record of the one or the other species. This is not simply an artefact due to undersampling, because the southern part of the province of Constantine is relatively well represented in his collection. For this reason and the pronounced differences in shell morphology we keep these two taxa as separate species until preserved animals from Algeria can be studied.

There are two records for this species from western Algeria south of Oran in MHNG, but their presences in the area needs reconfirmation in order to avoid any mis-labelling in the museum. Pallary (1898) records the species from “sur les berges de l’O. Souag (= O. el Hammam), à 12 kilomètres S.-O. d’Aïn Fekan”. These specimens were not seen by the authors, and thus their identity remains uncertain.

**Molecular analysis**

This dataset consists of two mitochondrial markers (COI and 16S) and one nuclear cluster (5.8S-ITS2-28S). The mitochondrial data was analysed first and afterwards the nuclear marker was added to confirm the results.

**Haplotype network and genetic diversity**

The results of the anatomical and morphological studies of the Tunisian samples show that there are two *Xerocrassa* species existing: *X. latasteopsis* and *X. latastei*. The nucleotide divergence of these two morphological groups is studied, and a haplotype network is produced. Among fourteen sequences of 1090 bp (655 bp of COI and 435 bp of 16S) of Tunisian *Xerocrassa*, six haplotypes were identified using both markers, suggesting a high haplotype diversity (Hd=0.8022). The haplotypes obtained cluster in two divergent haplo-groups: the first is formed by samples collected from SidiAich1, SidiAich2, and Henchir El Zitouna, and the second was formed by samples collected from El Djorf and Boughrara (Fig. 8A). A high number of variable sites could be found in-between the groups (190 sites: COI: 118 and 16S: 72), but only a low number within the groups (maximum of 60 sites within the group of Boughrara_El Djorf). Thus, the nucleotide divergence was of 17% between haplo-groups, and 5.5% within the haplo-group of Boughrara-El Djorf and a low divergence within Gafsa-Henchir El Zitouna (<1%).

The analyses of each mitochondrial separately showed some differences between COI and 16S. The nucleotide divergence of COI sequences reached 18% between groups, and varied between 0.4% and 6% within haplo-groups. Additionally, the amino acid composition of the partial COI sequence (218 amino acids) displayed eight different amino acids between haplo-groups of which two are of different polarity.

The ribosomal gene 16S showed a high nucleotide divergence between haplo-groups (16%) and a low divergence with a maximum of 2% of nucleotide divergence
Figure 9. Phylogenetic trees obtained by Bayesian inference (BI) and Maximum Likelihood (ML) methods. A Tree inferred based on partial mitochondrial sequences of COI and 16S B Tree inferred based on mitochondrial data, partial sequences of 5.8S, complete sequence of ITS2 and partial sequence of 28S rRNA. Posterior probability (PB) obtained from Bayesian analysis and bootstrap values obtained from Maximum likelihood (ML) were presented on each node (*: BS= 100).

within the haplo-group of Boughrara-El Djorf and a monomorphic haplo-group formed by the sequences of Gafsa and Henchir el Zitouna.

The assessment of the nuclear ribosomal cluster 58S-ITS2-28S (1320 bp) showed that all 5.8S and 28S sequences used were identical. The sequences of ITS2 displayed only a single insertion/deletion mutation and one substitution between *X. latasteopsis* and *X. latastei* suggesting an extreme conservation of nuclear sequences in Tunisian *Xerocrassa* species. Adding the ribosomal cluster did not affect the haplotype diversity obtained using the mitochondrial data. In fact, we observed six haplotypes grouped in two haplo-groups with a haplotype diversity of 0.8022, a nucleotide divergence of 8% between *X. latasteopsis* and *X. latastei* (Fig. 8B).
Both topologies of the mitochondrial (mt) data from the ML and BI analyses are identical. The tree obtained is rooted by two *Trochoidea* species, *C. virgata*, *Xerosecta adolfi* and *H. limbata*. Mediterranean *Xerocrassa* species were divided in two groups following the geographical distribution pattern (Fig. 9A): 1) An East-Mediterranean group formed by the Tunisian and the Cretan *Xerocrassa* species. 2) A West-Mediterranean group composed by three clades: one clade comprising the Balearic radiation, and two continental Spanish clades. Both groups are supported by high bootstrap values (BS=100%) and posterior probability (PP=0.83). The East-Mediterranean group shows three highly supported clades: one formed by Tunisian species, one composed by the Cretan *X. cretica* and one formed by the rest of the Cretan *Xerocrassa* radiation. In Tunisia, the *Xerocrassa* species split into two well supported (BS=100%, PP=1) monophyletic clades. In *X. latasteopsis* samples, which were collected from two distinct areas, were grouped in rake proving a low mitochondrial diversity within species.

The position of “*Xerocrassa meda*” close to *Trochoidea* is quite unexpected. In case it is not a mix-up with a specimen from the highly polymorphic *Trochoidea spratti*-group, then the mitochondrial sequences are not informative. Inclusion of nuclear markers in the analysis will probably yield a better result.
For the concatenated tree, all Cretan \textit{Xerocrassa} species except two samples of \textit{X. cretica} had to be excluded because of nuclear data deficiency. Both trees based on Ml and BI analyses show identical topologies; they are rooted by \textit{T. elegans}, \textit{Xerosecta adolfi} and \textit{H. limbata}. Again, two main Mediterranean clades appear, their node is well supported (Fig. 9B): all Spanish \textit{Xerocrassa} species cluster together forming a single clade (BS=100%, PP=1), which in itself is divided in the three groups, one insular and two continental. Here, the Cretan \textit{X. cretica} clusters with Tunisian \textit{Xerocrassa} species composing a well-supported group (BS=100%, PP=1). The two Tunisian species are well separated and strongly supported (BS=100%, PP=1).

**Discussion**

The research approach followed here is according to DeSalle (2005) and Hirano et al. (2014), who argue that a biological classification is only valid when using the efforts of a combined study of morphological, anatomical, and molecular characters. Thus, the type areas of \textit{Helix latastei} and \textit{Helix latasteopsis} in Tunisia were visited and living animals and dry shells from the respective localities listed by Bourguignat were collected in order to work with topotypic specimens.

**Taxonomic considerations**

The type species of \textit{Xerocrassa} Monterosato, 1892 is the east Mediterranean species \textit{Helix seetzeni} L. Pfeiffer, 1847 (by monotypy). \textit{Xerocrassa} is currently characterized by a symmetrical dart apparatus consisting of two small accessory sacs (= appendicula sensu auct.) and usually four branched glandulae mucosae around the vagina, irregular folds at the inner side of the wall of the vagina and the lack of a well-developed appendix at the atrium; the penis is innervated from the right cerebral ganglion (Hausdorf and Sauer 2009: 375). The absence of the atrial appendix is basically the only character state that separates \textit{Xerocrassa} from \textit{Trochoidea} Brown, 1827. Hausdorf and Sauer (2009) report the presence of an atrial “bulge-like stimulatory structure” in some \textit{Xerocrassa} species such as \textit{X. cretica}, \textit{X. franciscoi} and \textit{X. heraclea}, which can be seen as a small protuberance at the side of the atrium in the Tunisian \textit{X. latastei} and \textit{X. latasteopsis} (see Figs 1, 3), the atrium is much wider, and bulge considerably more pronounced. The homology of this organ with the atrial appendix seen in \textit{Trochoidea} is not clear, and there is no other evidence than the similar position at the atrium. The internal tissue crest is here called a stimulator referring to the similarly shaped stimulator found in the atrium of many helicid genera. It seems to consist of two parts, a massive block of tissue, and a separate tongue- or cone-shaped stylus. A similar structure is illustrated by Giusti et al. (1995) for \textit{Trochoidea} species (CAA in their nomenclature). Thus, a morphological separation of the two genera remains difficult, and our results show that the two genera are closely related.
Pallary (1919) based his monotypic genus *Ereminella* on *Helix latastei* Letourneux in *Letourneux & Bourguignat*, 1887 without delivering any discriminating characters. Brandt (1959) recognized that the species recorded by Bisacchi (1932: 361, Figs 2–4) under *Helicella (Xerocrassa) pseudosimulata* (Germain 1921) from El Agheila and Soluch-Agedabia (Libya) was a misinterpretation, and identified it with *H. latastei*, which he subsequently affiliated to *Trochoidea (Ereminella)*. In the same publication, Brandt introduced the monotypic subgenus *Trochoidea (Xerobarcana)* (based on *Xerobarcana huggani* Brandt, 1959), and *Trochoidea (Xeroregima)* (based on *Trochoidea (Xeroregima) regimaensis* Brandt, 1959). Both subgenera show the same principal construction of their genital organs and are congruent with what is considered *Xerocrassa* today. *Xerobarcana* is defined as “differing from all other subgenera of this genus [*Trochoidea*] by the rudimentary wart-like flagellum and the conspicuously strong vas deferens” (translated from the original German text). Today, the relative length of the flagellum is considered a character state that encodes on species level, and is widely used in hygromiidi and geomitrid taxonomy (Hausdorf 2000: 62); thus, the reduced flagellum reported by Brandt simply constitutes a character state of *Xerocrassa huggani*. The definition of *Xeroregima* is as follows: “Anatomically differentiated from *Trochoidea (Trochoidea)* s. str. by the lack of the vaginal appendix [sic!] and the penis, which is club-like swollen at the transition between penis and epiphallus” translated from the original German text). Apparently, Brandt confused the terms vaginal with atrial, and thus exactly described the situation as known in *Xerocrassa*. Even the club-shaped transition between penis and epiphallus is perfectly seen in the majority of Cretan *Xerocrassa* species as well as in *X. latastei*. Consequently, we consider *Xeroregima* as a junior synonym of *Xerocrassa*.

**Molecular analysis**

As with the morphological and anatomical investigation, the results of our molecular approach show that, independently which maker is considered, Tunisian samples divided into two species and cluster together with the selected *Xerocrassa* species from Crete, the Balearic Islands and Spain, and thus our generic affiliation of the species is correct. There are several remarkable findings, which require deeper examination.

**Haplotype network and genetic diversity**

The divergence of the COI sequences between Tunisian species (18%) widely exceeded the threshold of 3% as suggested by Hebert et al. (2003) to characterize animal species in general and the threshold of 4% to identify land snails (Davison et al. 2009). In Tunisia, Chott el Jerid is widely described as a geographical barrier for many taxa (Millington et al. 1989; Ben Othmen et al. 2009; Abdallah et al. 2012; Farjallah et al. 2012). Such a barrier may restrict the gene flow between geographically isolated
populations resulting in independent evolution and increase the genetic divergence within species (Funk 2003). In this case, *X. latasteopsis* shows a low divergence between the northern (Sidi Aich 1, Sidi Aich 2) and the southern (Henchir el Zitouna) populations, which share one haplotype. This result disproves this hypothesis for the snail species concerned, suggesting that Chott el Jerid does not restrict the gene flow. It cannot be considered as a barrier for this species. In contrast, *X. latastei* shows a quite high divergence within the population of Boughrara (6%) which could be interpreted as individual diversity.

This high divergence between the two Tunisian *Xerocrassa* species (16%) was also demonstrated by analysis of the 16S marker. High values of genetic divergence were reported for the land slug *Phyllocaulis* (13.1%) (Gomes et al. 2010) and between congeneric species of Ariophantidae and Dyakiidae (4.3 to 10.1%) (Abu-Bakar et al. 2014). Moreover, Liew et al. (2009) reported divergence values of 5% to 25% within *Everettia* spp. (Dyakiidae). The divergence of the 16S between Tunisian *Xerocrassa* species is higher than the divergence of 11.8% between Cretan *Xerocrassa* species as shown by Sauer and Hausdorf (2009). The divergence seen here is quite remarkable but not completely outstanding.

The nuclear cluster 5.8S-ITS2-28S widely confirms the results obtained from the mitochondrial markers. Both, the 5.8S and 28S sequences seem to be conserved within the Tunisian species, and the ITS2 shows only one nucleotide substitution and one insertion/deletion mutation. Thus, the genetic variability is focused on the mitochondrial markers, while the nuclear markers investigated seem to be highly conserved.

**Phylogeny**

This is the first time that a combined phylogeny for this widespread genus has been shown. As could be expected, the clades follow the distribution pattern of a west and an east Mediterranean group. Each cluster includes an island radiation and a continental radiation. The latter fall in two groups for Europe, and one of them, which includes *Helix montserratensis* Hidalgo, 1870 as its type species [by monotypy] may bear the subgeneric name *Amandana* Fagot, 1891. The results of these combined markers proved the results obtained by mitochondrial markers and confirmed the split between geographical *Xerocrassa* groups. Our results suggest that Tunisian *Xerocrassa* species are more closely related to the Cretan species than to the Spanish and Balearic species. However, within the east Mediterranean clade, the relationship between *X. cretica* and the rest of the Cretan radiation is not that close with a low support of 0.75 in the mitochondrial tree. A direct comparison with the Tunisian species is problematic. The eastern Mediterranean area, especially Libya, and Egypt, is heavily undersampled, and including more species from this area and the Middle East will certainly change the relative position of Tunisian species to the Cretan species as well as the position of *X. cretica* on the tree.
The shell morphology of land snails is extremely affected by environmental conditions (Alonso et al. 1985; Chiba 1999; Pfenninger et al. 2006). The use of these characters in the taxonomic analysis of land snail species were severely criticized (Giusti and Manganelli 1992; Schilthuizen and Gittenberger 1996; Uit de Weerd et al. 2004; Holland and Hadfield 2007). As shown by (Elejalde et al. 2008), a comparison between shell morphological and molecular characters result in incongruent data. However, the integrative approach as used here results in a distinct network of character states enabling to interpret the morphology even of the shells, and to formulate distinctive shell traits. Here, the morphological and anatomical disparity between X. latasteopsis and X. latastei has been confirmed by phylogenetic analysis. In the reverse conclusion we now can pinpoint the significance of relative flagellum length and form of penial papilla as well as the ribbing mode of the shell, its colouration and other structural details as relevant and useful for species identification within Tunisian Xerocrassa species (Hausdorf and Sauer 2009).

Conclusions

This study, based on morphological, anatomical and molecular characters allows the placement of the Tunisian species Helix latastei Letourneux, 1887, and Helix latasteopsis Letourneux & Bourguignat, 1887 to Xerocrassa. This investigation of relationships among species within the genus demonstrates that Tunisian Xerocrassa species are more closely related to the Cretan radiation than to the Balearic and Spanish radiation.

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