# DNA studies in European Clausiliidae

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## I. Introduction

The molecular studies of European Clausiliidae published until now were carried out by the following working groups (studied taxa groups in brackets):

Leiden group (AICC group, *Balea*);

Hamburg group (*Charpentieria*, *Clausilia*, *Micropontica*, *Alopia*);

Vienna (-Budapest) group (*Alopia*, *Agathylla*, *Montenegrina*);

Greek (Athens + Patras + Iraklion) groups (*Albinaria*);

Urbino (-Sicily) group (*Muticaria*, *Medora*).

Initially, multiple enzymes = allozymes in closely related taxa were used by the Leiden working group until 2001 (GITTENBERGER et al. 2001), then DNA data since1995 (SCHILTHUIZEN et al., DOURIS et al.). The allozyme studies are not discussed in this article.

For DNA studies two methods were applied:

1. Sequence studies (single-locus data):

Used markers:

mtDNA: COI, COII, 12S, 16S, ATPase 8;

nDNA: rRNA gene (with internal transcribed spacers = ITS): - 18S - ITS1 - 5.8S - ITS2 - 28S - or only ITS1 or ITS2, intron of Calmodulin = CaM, Histone genes = H3, H4.

2. Amplified fragment length polymorphism = AFLP studies (multi-locus data):

AFLP is a method to obtain DNA finger-prints (= multi-locus data) with the help of restriction enzymes.

For the authors and dates of the taxa names used in this article see taxa list of Clausiliidae (NORDSIECK 2007: chapter II)..

## II. DNA studies of European Clausiliidae

### General remarks

SAUER (2011: 118, 143, 148) in his thesis (though not on Clausiliidae) has given some important statements for taxonomic DNA studies which are cited here as basic for the comments in this article:

1. Taxonomy in general: „ ... plead for an integrative taxonomy ... combining morphological and molecular approaches to unravel biodiversity."

2. Morphological data: „ ... that most complex morphological characters are influenced by several loci ..., and thus can be understood as a sort of coding of multi-locus data. "

3. Gene trees: „Given the differences between gene trees based on different markers, it is not surprising that there are large disagreements between molecular ... taxonomic units ..., based on different markers. "

4. Taxa trees: „ ... any serious attempt to establish the taxonomy of a group should be based on an analysis of several independent markers. "

5. Single-locus data: „ ... species classifications based exclusively on single-locus data might show idiosyncrasies resulting from incomplete lineage sorting, introgression, random phylogeographic

breaks or pseudogenes. ” , therefore „... taxonomy should not rely exclusively on single-locus markers.”

6. Multi-locus data: „... resulting partitions ... based on single-locus data are less similar to those ... of the AFLP data or to the morphological classification than the partitions obtained by ... the AFLP data ... to the morphological classification. ”

The last statement has been confirmed by following papers of the Hamburg group (SCHEEL & HAUSDORF 2012, HAUSDORF & NÄGELE 2015).

AFLP data are especially useful for phylogenetic studies of nearly related taxa and the analysis of hybridization (allowing admixture analyses of gene flow among populations). Difficult to understand, why this method was used until now only by that working group, though interspecies hybridization plays an important role also in the other studied clausiliid groups from southern Europe (see article on interspecies hybridization, NORDSIECK unpubl.).

**Overview of DNA studies of genus and family taxa**

„Serrulininae”: UIT DE WEERD & GITTENBERGER (2013);

Laminiferinae: UIT DE WEERD & GITTENBERGER (2013);

Alopiinae: UIT DE WEERD & GITTENBERGER (2013);

*Medora*: COLOMBA et al. (2012);

*Agathylla*: FEHÉR et al. (2013);

*Albinaria* = AICC group (sensu NORDSIECK 2007): DOURIS et al. (1995, 1998a, b), SCHILTHUIZEN et al. (1995), VAN MOORSEL (2001, including papers which are also published elsewhere), UIT DE WEERD (2004, including papers which are also published elsewhere), SCHILTHUIZEN et al. (2004), DOURIS et al. (2007), UIT DE WEERD et al. (2009), PALL-GERGELY et al. (2012), KORNILIOS et al. (2015), DIMOPOULOU et al. (2017);

*Muticaria*: COLOMBA et al. (2010, 2012);

*Alopia*: FEHÉR et al. (2013), KOCH et al. (2017);

*Montenegrina*: FEHÉR et al. (2018);

*Cochlodina*:SZALONTAYOVA (2013);

*Charpentieria:* SCHEEL & HAUSDORF (2012);

„Mentissoideinae“: UIT DE WEERD & GITTENBERGER (2013);

*Clausilia*: JAKSCH (2012), HAUSDORF & NÄGELE (2015);

Baleinae: GITTENBERGER et al. (2006), UIT DE WEERD & GITTENBERGER (2013), KOCH et al. (2016).

The unpublished results of DNA studies of several genera, which I got as written communications from some working groups, are not mentioned here.

**Annotated list of DNA studies**

In the annotations the main results of the study (abbreviated = R) and comments (= C) are given.

European Clausiliidae:

UIT DE WEERD & GITTENBERGER (2013): 28S, H3 + H4. BEAST analysis tree fig. 3.

R: Four clades: Serrulininae within Phaedusinae sensu lato paraphyletic; Laminiferinae basal to Phaedusinae and other extra-European Clausiliidae; Alopiinae; Clausiliinae s. l., both latter sister groups.

Phaedusinae s. l.: *Pontophaedusa* basal, remaining Serrulininae sister group of Phaedusinae s. s., with *Caspiophaedusa* basal.

Alopiinae: *Macedonica* basal (contrast to UIT DE WEERD 2004: 32-33, figs. 2.6-2.7), Medorini + *Montenegrina* + Alopiini and *Cochlodina* + Delimini sister groups. Within the first group Medorini and Alopiini + *Montenegrina* sister groups, *Medora* group (*Agathylla*) + *Lampedusa* group monophyletic, AICC group paraphyletic. Within second group *Cochlodina* and Delimini sister groups. Delimini monophyletic; within Delimini *Siciliaria* group and *Delima* group sister groups, *Charpentieria* basal.

Clausiliinae s. l.: *Strumosa* and Boettgeriini basal, remaining Mentissoideinae and Clausiliinae sensu stricto + Baleinae sister groups, with *Olympicola* basal. Baleinae paraphyletic, Clausiliinae s. s. monophyletic.

C: 28S multi-copy gene, Histone genes rather conservative. Number of examined species low (< 10%); several genera, among them some of phylogenetic interest (*Filosa, Euxinella, Graciliaria*), not included. Relationships of genus taxa in part not corresponding with other analyses. For further discussion see NORDSIECK (2017: chapter 4).

*Medora*:

COLOMBA et al. (2012): 16S, COI, ITS2, trees figs. 1-3.

R: *Medora* taxa from Italy (only in 16S tree all taxa except *M. i. kobelti* considered) forming seven groups. Only in mtDNA trees species separate from *M. italiana*, *M. dalmatina* (*pollinensis*) and *M. garganensis*, basal.

C: Morphological differences not discussed, no species limits (of *M. italiana* and *M. dalmatina*) recognizable. Evaluation of some groups as species overdone; closely related taxa from Balkan peninsula (except *M. m. macascarensis*) not considered.

*Agathylla*:

FEHÉR et al. (2013): COI, II, 16S, concatenated tree fig. 5.

R: Subgeneric division into *A.* (*Agathylla*) and *A.* (*Agathyllina*) confirmed. *A. biloba* and *A. neutra* separated as species, in spite of the hybrid zone in Drin valley. *A. neutra* + *A. biloba* monophyletic, also *A. biloba*, *A. neutra* paraphyletic with respect to *A. biloba*.

C: Only mtDNA markers. Consideration of shell characters within Albanian species incomplete (development of lunellar and clausilium plate). Outcoming of *A. sulcosa* as two clades in contrast to the morphological characters. Some species (*A. regularis*, *A. narentana*, *A, viperina*) not included.

*Albinaria* and other AICC groups:

SCHILTHUIZEN et al. (1995): *Albinaria-Isabellaria*. ITS1, tree fig. 5.

R: G-type closing apparatus = CA evolved several times; *Isabellaria* not monophyletic.

C: Only two examples.

DOURIS et al. (1998a): *Albinaria-Isabellaria*. 16S, tree fig. 3B.

R: G-type CA evolved several times; *Isabellaria* not monophyletic. With conchological analysis (trees figs. 3A, C); more broadly based than SCHILTHUIZEN et al. 1995. *Albinaria* + Peloponnese *Isabellaria* species forming one clade.

C: Only one mtDNA marker. Informative and uninformative shell characters used. G-type CA underestimated (G-type species classified as subspecies of N-type species!).

DOURIS et al. (1998b): *Albinaria* from Crete and Peloponnese. 16S, trees figs. 1, 3, 4.

R: *Albinaria* taxa from Crete (+ *A. lerosiensis*) except two species forming three clades (1. western and central species, 2. eastern species with *A. lerosiensis*, 3. species from Dia island with *A. teres*). If Peloponnese species are added (tree fig. 4), Cretan species representing no monophyletic group.

C: Only one mtDNA marker. Grouping of species without considering shell morphology.

VAN MOORSEL, SCHILTHUIZEN, PIEL & GITTENBERGER in VAN MOORSEL (2001): *Albinaria*, Alopiinae. ITS1, 2, trees figs. 4-5.

R: In comparison with other genera of Alopiinae *Albinaria* (+ *A. haessleini* with G-type CA) monophyletic, also Alopiinae with respect to Clausiliinae subfamily group.

C: Only multi-copy gene.

VAN MOORSEL, DIJKSTRA & GITTENBERGER in VAN MOORSEL (2001): *Albinaria-Isabellaria*. ITS1, 2, tree fig. 3.

R: G-type CA evolved several times; *Isabellaria* not monophyletic. *Albinaria* and Peloponnese *Isabellaria* taxa forming one clade (but only G-type *I. confusa* coming out as sister species of N-type *A. grisea*). Transformation N-type to G-type as probable as vice versa (figs. 5); parallel evolution and character reversal of CA-type possible.

C: Only multi-copy gene. G-type CA as ancestral state and reversal G-type to N-type unlikely.

VAN MOORSEL, VAN NES, GITTENBERGER & MEGENS in VAN MOORSEL (2001): *Albinaria* phylogeny. ITS 1, 2, consensus tree fig. 3.

VAN MOORSEL, SCHILTHUIZEN & GITTENBERGER in VAN MOORSEL (2001): *Albinaria* phylogeny. ITS1, 2, intron of Calmodulin = CaM, 16S, trees figs. 1-3.

R (both papers): *Albinaria* monophyletic. No relations between *A.* subgroups recognizable; only subspecies and closely related or neighbouring species clustering together. If trees of different markers are compared, they support different phylogenies, no improvement by consensus tree of these markers (fig. 3). Only in CaM trees well-supported subgroups. Cretan *A.* species no monophyletic group.

C: Grouping of species without considering shell morphology. Use of multi-copy genes, but also of other single-locus markers not sufficient, especially in a case of rapid speciation. Possible interspecies hybridization not or nearly not considered.

SCHILTHUIZEN et al. (2004): *Albinaria* from Crete. ITS1, trees figs. 2-3.

R: Two clades: *A. hippolyti* clustering with western species (and *A. caerulea*), *A. arthuriana* with eastern species, the latter therefore regarded as separate species. Subspecific division of *A. hippolyti* confirmed, but some subspecies (*A. h. aphrodite*, *A. h. holtzi*) polyphyletic.

C: Only one multi-copy gene.

UIT DE WEERD & GITTENBERGER in UIT DE WEERD (2004): Classification of *Carinigera*. ITS1, 2, 12S, consensus tree fig. 2.6.

UIT DE WEERD & GITTENBERGER in UIT DE WEERD (2004): Relationships of eastern *Albinaria* species. Same markers, trees figs. 3.5-3.6.

UIT DE WEERD, GITTENBERGER & PIEL in UIT DE WEERD (2004): Polyphyly of some genera of *Albinaria* group. Same markers, COI added, trees figs. 4.7-4.8.

R (all three papers): *Carinigera* belonging to Medorini (belonging to Montenegrinini unlikely, fig. 2.7). *Albinaria* species from Lebanon belonging to *Cristataria*. Species pairs with N- and G-type CA sister species of recurrent origin. Whole *Albinaria* group (= AICC group sensu NORDSIECK 2007) monophyletic, forming seven clades: 1 *Carinigera* (+ *Sericata albicosta*), 2 *S. stussineri* + *S. dextrorsa* + *Isabellaria praecipua*, 3 *I. thessalonica* + *I.* species of northern Sporades (+ *S. liebegottae*), 4 *I. vallata*, 5 *S.* and *I.* species of eastern continental Greece, 6 *C. haussknechti* + *S. inchoata*, *Cristataria*,

7 *Albinaria* (+ Peloponnese *I*. species). 1-3 named northern group (GITTENBERGER & UIT DE WEERD 2006: Genus *Carinigera*, with subgenera *Nymphogena* and *Sporadhia*), 4 western group (Genus *Vallatia*), 5 southern group (Genus *Isabellaria*), 6 (except *Cristataria*) Epirus-Pindos group (Genus *Inchoatia*). G-type CA assumed to have evolved in four groups (2, 3, 4, 5, *S. liebegottae* hypothesized as reversal!), transformation N- to G-type CA to be a simple change in CA ontogeny.

C: Only one multi-copy gene and one or two mtDNA markers. Possible interspecies hybridization not considered. Xenologous gene transfer (GITTENBERGER 1998) no more discussed.

Following questions are unanswered (see NORDSIECK 2007: chapter VII):

1. high frequency of transformation N- to G-type CA in the AICC group;

2. restriction of G-type species of AICC group (except *I. vallata*) to a small part of the whole range (eastern Greece);

3. complexity of transformation of N-type to G-type CA within the AICC group (several stages present, see NORDSIECK 1997);

4. restriction of species with penial papillae evolved from inverted caeca (not from protruding epiphallus openings as in some *Albinaria* species) to two small parts of the range (northern Greece with adjacent countries, Pindos).

Genera of GITTENBERGER & UIT DE WEERD (2006) not characterized morphologically, thus not comparable with other genera of Alopiinae from the Balkan peninsula; species can only be classified by DNA examination.

*A. sericata* and *A. liebegottae* classified with different genera, though very similar in shell morphology. Genetical similarity of *A. liebegottae* to *Isabellaria* species from northern Sporades probably due to hybridization; reversal of CA type from G- to N-type very unlikely.

UIT DE WEERD, SCHNEIDER & GITTENBERGER in UIT DE WEERD (2004): *Carinigera buresi*. COI, trees figs. 5.4-5.5.

R: *C. pharsalica* in the trees nesting among *C. buresi* subspecies, closely related to *C. b. conciliatrix*. Mitochondrial lineages not coinciding with morphologically defined subspecies, because of hybridization.

C: Only one mtDNA marker.

UIT DE WEERD, GROENENBERG, GITTENBERGER & SCHILTHUIZEN in UIT DE WEERD (2004): *Sericata dextrorsa, Isabellaria lophauchena*. COI, tree fig. 6.5.

R: *I. lophauchena* monophyletic, *S. dextrorsa* paraphyletic with respect to *S. torifera*. Inverse coiling of *S. dextrorsa* possibly evolved twice. No proof of reproductive character displacement by inversion of coiling.

C: Only one mtDNA marker. The examined species are no enantiomorphs; thus it is not a simple inversion of coiling. Diphyly of *S. dextrorsa* unlikely.

DOURIS et al. (2007): *Albinaria* from Aegean islands. 16S, ATPase8, trees fig. 3.

R: *A. caerulea* + *A. brevicollis* coming out as a monophyletic group, *A. turrita* basal, group of its own. Within *caerulea-brevicollis* group two clades (with subgroups on certain islands): *A. caerulea*, *A. brevicollis*; both vicariant, on some islands (Tilos, Anafi) haplotypes of both clades present, explained by dispersal.

C: Only two mtDNA markers. *A. c. kosensis* not belonging to *A. brevicollis* .

UIT DE WEERD et al. (2009): Epirus-Pindos group. COI, tree fig. 1.

R: Two clades: *S. inchoata* (+ *regina*), *C. haussknechti* + *C. megdova*. Genital differences (presence of penial caecum or penial papilla, respectively) confirmed. Within *haussknechti* clade *A. megdova* polyphyletic.

C: Only one mtDNA marker. Some taxa (*C. h. refuga*, *C. h. semilaevis*, *C. m. palatalifera*) not included.

PÁLL-GERGELY et al. (2012): *A. lycica*. COI, 12S, combined tree fig. 5.

R: *A. lycica* with two clades (*A. l. lycica*, *A. l. phaselis*, the latter with subgroups), differing like species.

C: Only mtDNA markers.

KORNILIOS et al. (2015): *Albinaria* from Taygetos mountains and Mani peninsula. ITS1, 16S, COI, trees figs. 2-3.

R: Dextral *A.* species (all samples determined as *A. voithii*) not coming out as monophyletic group. Clades: 1. *voithii* from southernmost part of Mani; 2. *A. nivea* (+ *ithomensis*); 3. *A. maculosa*, in mtDNA tree monophyletic, but diphyletic in ITS1 tree, other *A. voithii* samples clustering with *A. maculosa*. Multiple dextral lineages by parallel evolution or introgression, the latter assumed to be less probable (only one dextral specimen apparent hybrid).

C: Shell characters not considered (except for coiling direction). Differences of dextral species of *voithii* group ignored (clearly more than one species, see article on *Albinaria*, NORDSIECK unpubl.). Affiliation of the dextral samples examined to species according to the given localities can only be speculative. Possible interspecies hybridization insufficiently examined. Judging from shell characters, unlikely that species of *voithii* group (all with non-emerging subcolumellar lamella) have evolved from *A. maculosa* (with emerging subcolumellar lamella).

DIMOPOULOU et al. (2017): *Albinaria* from Crete, especially from Dia island. ITS1, 16S, concatenated tree fig.2.

R: *Albinaria* taxa from Crete forming five clades (1. *A. tenuicostata*, 2. eastern species, 3. western and central species, 4. *A. teres* with related species, 5. species from Dia island). Within *A. torticollis* two lineages, differing like species.

C: Grouping of species without considering shell morphology.

*Muticaria*:

COLOMBA et al. (2010): *Muticaria* from Sicily. 16S, COI, trees figs. 1-2.

COLOMBA et al. (2012): *Muticaria brancatoi* n. sp.. COI, tree fig. 15.

R (both papers): One clade; *M. syracusana* from Siracusa (Teatro Romano) and *M. neuteboomi* coming out as two subclades. *M. brancatoi* from Cugno Lungo within the *syracusana-neuteboomi* clade, but as a basal subclade. Therefore, in spite of only slight differences to *M. syracusana* (sculpture), regarded as separate species.

C: Only one or two mtDNA markers. Possible interspecies hybridization (examined *M. syracusana* probably hybrids, NORDSIECK unpubl.) not considered.

*Montenegrina*:

FEHÉR et al. (2018): COI, 16S, 12S, concatenated tree fig. 2.

R: Nearly all species and subspecies examined. Tree shows three clades, with 15 subclades. Mostly, but not entirely congruent with morphology-based system. 25% of populations without DNA data, allocated according to positions of morphologically related taxa. Fewer than expected co-occurrences among species and intrageneric clades. Species divergence preceding niche partitioning = non-adaptive speciation.

C: Only mtDNA markers. Possible interspecies hybridization not considered. Discrepancies of system and tree not discussed. For details and further comments on the used system and the results of the DNA study see article on *Montenegrina* (NORDSIECK unpubl.).

*Alopia*:

FEHÉR et al. (2013): COI, unrooted tree fig. 2.

R: Tree including all *Alopia* species, coming out as six clades, marked with letters A-F: A including A1 = *A. bielzii* (dextral) and A2 = *A. bogatensis* (sinistral); B including B1 *A. subcosticollis* (sinistral, + dextral *A. h. fortunata*), B2 *A. grossuana* (sinistral and dextral), *A. plumbea* (sinistral), and B3 *A. lischkeana* (dextral, + sinistral *A. g. boettgeri*); C including C1 *A. regalis* (sinistral) and C2 *A. straminicollis* + *A. mafteiana* (both sinistral); D including D1 *A. canescens* (sinistral) + *A. nefasta* and *A. helenae* (sinistral and dextral), *A. livida* (dextral), *A. alpina* (sinistral) + *A. nixa* (sinistral) and *A. fussi* (dextral), D2 *A. glorifica* (sinistral, + dextral *A. l. lischkeana* = *A. deceptans*), D3 *A. hildegardae* (dextral, + sinistral *A. mariae*), E including *A. meschendorferi* (dextral), F including *A.* (*Kimakowiczia*) species. Several enantiomorphs with similar or identical haplotypes. Dextrality evolved several (at least 13) times, with divergence of different depth. Relationships of far distantly distributed *A.* taxa revealed. Early *A.* divergence dated with 1-2 Mya.

C: Only one mtDNA marker. Possible interspecies hybridization not considered. Shell morphology insufficiently considered. Dating of early divergence too young. For details and further comments see article on *Alopia* (NORDSIECK unpubl.).

KOCH et al. (2017): Relationship *Alopia straminicollis* / *livida*. COI, tree fig. 3, AFLP, network fig. 4.

R: Localities under consideration: Bucegi mountains, Cheile Tătarului and Valea Velicanului. In each locality some populations, among them one mixed population. In COI tree two clades, *A. straminicollis* = s and *A. livida* = l. In Cheile Tătarului haplotypes like coiling direction, but one s-haplotype within l, one l-haplotype within s. In mixed population 80% of both s and l had s-haplotypes. In Valea Velicanului in mixed population most individuals l-haplotype, though l is rarer. In pure s 20% l-haplotypes. In AFLP network not s and l, but Cheile Tătarului and Valea Velicanului individuals forming separate clusters, including s and l. Admixtured genotypes in both hybrid zones very frequent. Species without strong barriers against gene flow.

C: No information on shell characters of hybrids. Hybridization in *Alopia* insufficiently discussed.

*Cochlodina*:

SZALONTAYOVA (2013): 16S, COI, trees figs. 4-5.

R: *C. triloba* basal, two clades: *C. costata* + (*C.orthostoma* + *C. cerata*) and *C. fimbriata* + (*C. laminata* + *C. dubiosa*). *C. laminata* with four clades (I-IV), III = *C. liburnica*. *C. dubiosa* two clades, representing the two subspecies. *C. fimbriata* four clades.

C: Only mtDNA markers. Individual numbers per clade very different. As concerns *C. triloba*, relationships based on morphology not considered (see article on *Cochlodina*, NORDSIECK unpubl.). *C. liburnica* wrongly determined as *C. laminata*. Infraspecific clades overestimated as taxa, though within species hybridization has to be expected..

*Charpentieria*:

SCHEEL & HAUSDORF (2012): 16S, tree fig. 3, AFLP, tree fig. 4, network fig. 5.

R: Relationships of stenzioid taxa = *C. clavata* to *C. itala*. 16S tree: *C. stenzii* basal, *C. ornata* sister group of *C. itala* + stenzioids. Within the latter clade one *C. c. lorinae* sample basal, *C. c. variscoi*, *C. c. balsamoi* and *C. c. clavata* forming separate clades, *C. c. lorinae* (+ *trepida* + transitional form = *allatollae*) in clades together with subspecies of *C. itala*. AFLP tree and network same result, but *C. c.*

*lorinae* with *C. c. trepida* in a separate clade. Network showing ten clusters: western stenzioids three distinct clusters, but two *clavata* individuals in an *itala* cluster, *C. itala* four clusters, eastern stenzioids and transitional form (*allatollae*) three clusters. Separate species *C. clavata* not accepted because of gene flow, but stenzioids with partial reproductive barriers. Stenzioids have common origin, diverged early from the *C. itala* stem.

C: Downgrading of the stenzioids to subspecies of *C. itala* unsatisfactory; stenzioids behave in part like species, in part like subspecies. No information on morphological differences of *C. c. trepida* and *C. c. lorinae* or within *C. c. lorinae* given (see article on Delimini, NORDSIECK unpubl.).

*Clausilia*:

JAKSCH (2012): *Clausilia dubia*. COI, trees figs. 19A-B, networks figs. 20-22.

R: As result of examination of shell morphology instead of current subspecies morpho-groups 1-4 proposed. Tree showing five clades, but without congruence with subspecies or morpho-groups defined by shell morphology or with geographical regions.

C: Only one mtDNA marker. Incongruence of clade division probably because of hybridization which has to be expected within species. Uninformative and informative shell characters used, some informative ones (e. g., clausilium plate) not. Determination of more than one subspecies at one and the same locality not acceptable; nomenclature not up to date.

HAUSDORF & NÄGELE (2015): *Strobeliella*. 16S, tree fig. 1A, AFLP, tree fig. 1B, network fig. 2.

R: 16S tree: *Clausilia* one clade, with subclades *Clausilia* s. s. and *Strobeliella* + *Neostyriaca*, *N. strobeli* = *Lombardiella* basal, *N. c. styriaca* basal within *Strobeliella* -*Neostyriaca* clade , subclades *N. corynodes*, *C. brembina* (+ *klemmi*), *C. b. alanica* + *C. umbrosa* (+ *gardonensis*), *C. whateliana* + *C. exoptata*, both latter mixed. AFLP tree: same clades, but *C. b. alanica* in *brembina* clade, *C. exoptata* separate within *whateliana* clade (except one transitional individual). AFLP network : *C. b. alanica* between *C. brembina* and *C. umbrosa*, *C. exoptata* and *C. whateliana* separated, but slight divergence like *brembina* subspecies. *Strobeliella* divided into two species, *C. brembina* and *C. whateliana*. AFLP indicating extensive admixture between *C. whateliana* and *C. exoptata*. *C. alanica* sister group of *C. umbrosa* in 16S tree, more related to *C. brembina* in AFLP tree. C. *umbrosa* and *C. brembina* not differing in morphology; therefore *C. umbrosa* classified as subspecies of *C. brembina*.

C: Downgrading of *C. exoptata* to a subspecies of *C. whateliana* in contrast to the differences in morphology. *C. umbrosa* differing from *C. brembina* in some shell and genital characters; therefore uniting of both taxa within a species unsatisfactory. For details see article on *Clausilia* (NORDSIECK unpubl.)..

*Balea*:

GITTENBERGER et al. (2006): COI, tree fig. 2b.

R: *Tristania* from Tristan-Gough archipelago belonging to *Balea*. Tree showing two clades: *B. perversa* and clade with other *B.* species + *Tristania*. Ancestral species reached Azores, from there Tristan-Gough archipelago, probably by passive long-distance dispersal by birds. One of the Azores species returned to the continent (*B. heydeni* = *sarsii*). ITS tree not discussed.

C: Only one mtDNA marker. *Balea* species from Bulgaria not considered (see article on Baleinae, NORDSIECK unpubl.).

*Micropontica*:

KOCH et al. (2016): 16S, COI, concatenated tree fig. 6, AFLP, admixture of populations fig. 5.

R: Tree including several Caucasian Baleinae: *Quadriplicata-Mucronaria* sp. (*Q. quadriplicata* + *M. acuminata*, *Q. dipolauchen*, *Q. aggesta* + *Q. pumiliformis*) basal, *Micropontica* one clade, *M. closta*

basal, *M. circassica* (+ one *M. interjecta* population) paraphyletic, remaining *M. interjecta* and *M. caucasica* sister groups. AFLP: *M. interjecta* with different portions of *caucasica* and *circassica* alleles. Exhibiting intermediate shell characters, having a special haplotype and forming a separate AFLP cluster. Hybrid speciation in the past; *M. interjecta* incipient species adapted to a special habitat (high altitudes). Including *M. annae* (and syntopic *M. caucasica*).

C: Information on shell characters, especially of the hybrid species, insufficient. *M. interjecta* obviously only based on DNA data, because *M. annae* in shell morphology much different.

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